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Abstract

Background: Chronic hypersensitivity pneumonitis (CHP) remains a diagnostic challenge. The process of collecting and extracting serum and droppings from causative animals for the inhalation challenge test is complicated and the risk of inducing disease progression exists.

Objective: To investigate the utility and safety of an inhalation challenge test using pigeon eggs.

Methods: Pigeon eggs were pasteurized and mixed with a saline solution to produce an inhalation fluid. An inhalation challenge test was conducted on 19 patients with bird-related CHP and 17 patients with interstitial lung disease other than bird-related CHP. To identify antigens in pigeon eggs, the antigen–antibody responses of the pigeon eggs and serum from patients were evaluated using western blotting.

Results: The mean changes in C-reactive protein, alveolar-arterial oxygen difference, erythrocyte sedimentation rate and lactate dehydrogenase significantly increased by 0.32 mg/dL ($p = 0.014$), 7.8 Torr ($p = 0.002$), 1.4 mm/h ($p = 0.012$) and 5.4 U/mL ($p = 0.0019$), respectively, in bird-related CHP group compared to the control 24 hours after the inhalation challenge test. Furthermore, within 24 hours of the inhalation test, the mean forced vital capacity decreased by 2.3% in the bird-related CHP group compared with a decline of 0.05% in the control group ($p = 0.035$). Serum collected from seven bird-related CHP patients who underwent the inhalation challenge test and reacted to antigens with molecular weights of 37–75 KDa, and these molecular weights were consistent with egg albumin and globulin.

Conclusion: Since a mild response was observed after the inhalation challenge test using pigeon eggs, this test was an obvious candidate for diagnosing bird-related CHP.

KEYWORDS: Bird fancier's lung; hypersensitivity pneumonitis; inhalation provocation test; pigeon breeder's lung.

1. INTRODUCTION

Hypersensitivity pneumonitis is type III and IV allergic lung diseases induced by inhalation antigens. The causative antigens are diverse in nature, such as fungi, bacteria, animal proteins, and chemicals.¹ On the basis of the clinical disease type, the disease is classified as acute or chronic hypersensitivity pneumonitis (CHP). In Japan, 60% of patients with CHP have been diagnosed as bird-related CHP, and 15% have been diagnosed as chronic summer-type hypersensitivity pneumonitis, which is mainly caused by the *Trichosporon* species.² Since the elimination of the causative antigen affects the prognosis, complete antigen avoidance is crucial when a definite causative antigen can be identified.^{3,4}

Both humoral and cellular immunities are involved in the disease mechanism.^{5,6} In acute hypersensitivity pneumonitis, specific antibodies and inhaled antigens produce a localized immune response in the lungs, resulting in the formation of granulomas. CHP is predominantly a reaction between sensitized lymphocytes and antigens. The formation of granulomas is relatively rare.^{7 8 9} Although specific serum IgG tests are tools for identifying the causative agent, they are less useful than questionnaires as diagnostic methods for CHP,¹⁰ and the presence of specific IgG antibodies does not imply the cause of CHP.

To identify the causative antigens, it is effective to validate the improvement of symptoms with antigen avoidance and reproduce the aggravation of symptoms with antigen exposure. In acute hypersensitivity pneumonitis, isolation and environmental provocation tests are useful for diagnosis; however, in CHP, the improvement and reproduction of symptoms are challenging because of difficulties in isolation and conducting environmental provocation tests. Inhalation challenge tests using pigeon serum and purified droppings are useful in diagnosing bird-related CHP.¹¹⁻¹⁴ However, a large volume of pigeon serum is relatively difficult to obtain because it is often collected by inserting a needle into the pigeon's heart.¹² In inhalation challenge tests using

pigeon droppings, the sterilization process is complicated.¹⁵ Additionally, protein classification is difficult owing to proteolysis by digestive juice enzymes, and nonspecific biological contamination with bacteria and fungi cannot be excluded. Furthermore, a risk of inducing disease progression exists after the inhalation challenge test. Because of all these reasons, the conduction of inhalation challenge tests is limited in clinical settings.

Serum from patients with bird-related hypersensitivity pneumonitis reportedly contains antibodies to various components, including avian serum, droppings, feathers, and egg yolk and white. In particular, serum albumin, serum globulin, proteins on feathers (bloom), and intestinal mucins are possible antigens.¹⁶ Extracting intestinal mucins requires dissection of the birds and collection of the bloom, which is technically difficult; albumin and globulin were not commonly found in bloom, saliva, and gastric juice. The crop and oviduct contained albumin and globulin; however, the birds had to be dissected to collect them. Furthermore, egg albumin (ovalbumin), egg globulin (ovoglobulin), and egg mucin (ovomucin) may also be antigens.¹⁷ Because pigeon eggs were considered easier to collect and the extraction procedure was not complicated, we conducted an inhalation challenge test using pigeon eggs on patients with suspected bird-related CHP.

2. METHODS

2.1. Study population

The study population comprised patients with interstitial lung disease (ILD) who underwent the inhalation challenge test between August 2018 and February 2020. Patients who met the following criteria were included in the target population to improve the diagnostic accuracy for ILD, especially CHP: patients who underwent bronchoalveolar lavage; those who underwent surgical lung biopsy and/or transbronchial lung cryobiopsy; and those whose ILD diagnosis was made on the basis of a multidisciplinary discussion of ILD experts. Patients who were diagnosed with CHP

had a recurrent exposure to birds based on clinical information were diagnosed with bird-related CHP. CHP patients whose cause could not be identified without any history of bird exposure were diagnosed with unidentifiable CHP. The patients with unidentifiable CHP, CHP caused by reasons other than birds, and ILD other than CHP were used as controls. Some articles reported that the patients with low PaO₂ or low forced vital capacity (FVC) should be contraindicated for the inhalation challenge test.^{18,19} Patients with %FVC <50% or PaO₂ <60 Torr were excluded because acute respiratory failure may occur after the inhalation challenge test. This study was conducted in accordance with the amended Declaration of Helsinki and was approved by the institutional review board of Kanagawa Cardiovascular and Respiratory Center (KCRC-0026). Written informed consent was obtained from all patients.

2.2. Preparation for inhalation challenge test

Pigeon eggs, which were received from pigeon breeders, were stored at 8 °C or below. The surfaces of the egg shells were cleaned with tap water and subsequently washed with a solution of sodium hypochlorite at ≥150 ppm. Subsequently, the eggs were cracked, the egg yolk and white were mixed, and the egg fluid was heated at 58 °C for 30 min, cooled for 30 min, and reheated at 58 °C for 30 min. After completing two rounds of low-temperature disinfection, the egg fluid was cryopreserved at −80 °C.²⁰

To perform the inhalation challenge test, the frozen egg liquid was thawed at room temperature, and 3 mL of egg fluid was dissolved in 10–17 mL of saline solution and utilized for inhalation using an ultrasonic nebulizer (NE-U17, Omron, Tokyo). If a compressor nebulizer was used, 3 mL of egg fluid was dissolved in 3–5 mL of saline solution (NE-C29, Omron, Tokyo). Prior to this study, one patient with bird-related acute hypersensitivity pneumonitis and four patients with bird-related CHP were tested to determine the amount of eggs to use. The eggs were titrated from

0.1 mL to 4 mL. The response after inhalation was strongest at 3 mL and above. Therefore, the dose used in this study was set at 3 mL of eggs. The viscosity of the inhalation solution became so high at 4 mL that the inhalation solution occasionally did not atomize.

2.3. Data collection

The inspection items and timing of the inspection were determined based on previous articles.¹¹⁻¹⁴ The following were evaluated before and after the inhalation: body temperature, arterial blood gas, inflammatory and interstitial pneumonia markers, pulmonary function test, and chest high-resolution CT (HRCT). Body temperature was measured every hour from the end of inhalation until 8 h later and was also measured 24 h after the end of inhalation. Arterial blood gas test, blood sampling, FVC, and chest X-ray were performed at 6 and 24 h after inhalation. A second inhalation test was conducted in cases with no clear response within 24 h to the first inhalation test. Initially, the interval between the first and second tests was set at 48 h; however, because little change was observed at 24 and 48 h, it was decided that a second test would be performed after 24 h. A chest HRCT was performed 24 h after the first or second inhalation. Anti-pigeon IgG was quantified using the commercial ImmunoCAP method (BML, Tokyo) in which the extracts of serum proteins, feathers, and droppings of pigeons were used as antigens. The anti-pigeon IgG was measured in all patients before and after inhalation challenge test. Histopathological specimens obtained using surgical lung biopsy or cryobiopsy were semi-quantitatively (grades 0–3) evaluated by a pulmonary pathologist for CHP-related findings.

2.4. Immunoblotting

To identify antigens in pigeon eggs, the antigen–antibody responses between the pigeon eggs and serum from patients undergoing the inhalation challenge tests were evaluated using western

blotting. Serum from a patient with bird-related acute hypersensitivity pneumonitis, a healthy volunteer, and an asymptomatic volunteer who used to raise pigeons were also investigated. The whites of pigeon eggs were diluted 300-fold with phosphate-buffered saline. Subsequently, 1M dithiothreitol + 10% sodium dodecyl sulfate + western blot stock buffer, a reducing agent, was added and heated to 95°C. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 12% Mini-PROTEAN TGX precast gels and transferred onto a nitrocellulose membrane. Moreover, 5% solution of skim milk (2.5 g of skim milk + 50 mL of tris-phosphate-buffered saline) was used as a blocking buffer. A 100-fold dilution of the human serum in the blocking buffer was used as the primary antibody, which was added to the membrane and allowed to react at room temperature for 2 h. The secondary antibody, goat anti-human IgG (R&D Systems Inc., Minneapolis), was diluted 5000-fold and developed in 3,3',5,5'-tetramethylbenzidine solution.

2.5. Statistical analysis

For comparison, the Mann–Whitney U test, Fisher's exact test or paired t test was used, and p-values <0.05 were considered statistically significant. The analysis was performed with the BellCurve for Excel (SSRI CO., Ltd).

3. RESULTS

Nineteen patients were diagnosed with bird-related CHP based on the clinical information, histopathological and radiological surveys and were willing to undergo the inhalation challenge test. The participants' history of prior contact with birds has been shown in Table 1. The patients with bird-related CHP often had pigeon breeding, parakeet breeding, or chicken breeding. Total of 17 chronic ILD patients were used as controls: 11 with unidentifiable CHP, 5 with IPF, and 1 with

summer type CHP. No unidentifiable CHP cases had a history of contact with birds.

The baseline characteristics of patients with bird-related CHP and controls have been summarized in Table 2. The mean %FVC and PaO₂ values were relatively preserved as the severe cases were excluded from this study. Lymphocytes in bronchoalveolar lavage were elevated at 36.6%, consistent with chronic hypersensitivity pneumonia in patients with bird-related CHP. There were no significant differences in age, sex, anti-pigeon IgG antibody, FVC, alveolar-arterial oxygen difference (A-aDO₂), and lymphocytes in bronchoalveolar lavage between the two groups.

Most bird-related CHP patients presented with centrilobular fibrosis, bronchiolitis, granulomas, giant cells, and peribronchiolar metaplasia which were the typical characteristics of CHP (Table 3). The histopathology patterns were classified into four types according to the 2018 Diagnosis of IPF Guidelines:²¹ usual interstitial pneumonia (UIP) (0 patients), probable UIP (1 patient), indeterminate for UIP (15 patients), and alternative diagnosis (3 patients). On chest HRCT, typical findings of CHP including air trapping, ground-glass opacity and centrilobular nodules were observed in most patients with bird-related CHP (Table 4). For the four diagnostic categories of HRCT, there were 10 patients classified as indeterminate for UIP and 9 patients as alternative diagnosis.

The changes were compared between the bird-related CHP and the control groups 6 and 24 h after the inhalation challenge test (Table 5). In the bird-related CHP group, lactate dehydrogenase (LDH), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) elevation, and A-aDO₂ increase were significantly observed 24 h after the inhalation challenge test in comparison with the control group.

When the value of lower FVC 6 or 24 h after inhalation was selected, 2.3% and 0.05% decline in the bird-related CHP and control groups ($p = 0.035$), respectively, were observed. Post-inhalation HRCT revealed mild exacerbation of bilateral diffuse reticular shadows and/or ground-glass

opacities around the existing interstitial shadows in 11/18 patients (61%) with bird-related CHP. In the control group, 4 out of 16 cases (25%) had worse shadows after inhalation. Compared with the control group, the rate of worsening interstitial shadows was significantly higher in the bird-related CHP group using Fisher's exact test ($p = 0.045$).

Depending on the degree of bronchiolitis, patients with bird-related CHP were divided into two groups: a stronger group (grade 2 or 3) and a weaker group (grade 0 or 1). Patients in the weaker group had lower WBC counts at the initiation of the inhalation test and a higher WBC elevation thereafter. Although there was no significant difference, the CRP change after the inhalation challenge test was larger in the weaker group (Figure 1).

Receiver operating characteristic curve analyses were used to establish cut-off values for each variable, and the sensitivity and specificity of each were calculated. The cut off values of CRP, LDH, ESR, and A-aDO₂ were generally bordering on zero, whereas a 40-mL FVC decline was the benchmark (Table 6). No significant differences were found when comparing the areas under the curves for each variable (Figure 2).

The anti-pigeon IgG antibody was measured before and after inhalation in patients with bird-related CHP. The mean values of IgG antibody were 21.0 mgA/L before inhalation and 12.3 mgA/L after inhalation, indicating a significant decrease after inhalation challenge test ($p < 0.001$) (Figure 3).

Western blotting was performed to investigate the presence of antibodies to pigeon eggs using serum from seven patients with bird-related CHP who underwent the inhalation challenge test, one patient with bird-related acute hypersensitivity pneumonitis, one healthy volunteer with no history of bird exposure, and one healthy volunteer with a history of raising pigeons. All patients with bird-related CHP had multiple bands at 37–75 kDa, whereas no bands were observed in the healthy volunteer without exposure to birds (Figure 4).

To exclude the possible bacterial and fungal contamination in the pasteurized egg fluid, the pasteurized egg fluid was cultured on blood agar and deoxycholate hydrogen sulfide lactose agar media. The subsequent culture results were negative.

None of the patients who underwent the inhalation challenge test required additional treatment, such as prednisolone, immunosuppressants, or oxygen therapy due to the inhalation challenge test. In the patients with bird-related CHP, the values of WBC, LDH, and CRP measured at 1 month showed a tendency to be lower than those measured at 24 h after the inhalation challenge test (Table 7).

4. DISCUSSION

In this study, to increase the diagnostic accuracy of bird-related CHP, only patients who underwent histopathological examinations and those diagnosed with CHP based on a multidisciplinary discussion who had a clear history of bird exposure were included. Mild changes, such as increased CRP, LDH, ESR, and A-aDO₂, decreased FVC, and deterioration of interstitial shadows were observed after the inhalation challenge test using pigeon eggs.

FVC declines of 10–15% were positive criteria in previous studies.^{11,12,14} However, little decline in vital capacity (0.6%) was reported.¹³ In this study, the largest decline in FVC was 2.3%, which was significantly different from the control group, and the slight FVC decline may not have caused respiratory failure after the inhalation challenge test. The threshold of WBC elevation after inhalation was 20% in a report,¹⁴ whereas the WBC elevation was 15.3% after 24 h of inhalation in this study, which was a slightly weaker reaction. In contrast, a decrease of >3 Torr PaO₂ was a positive criterion in the Ramirez-Venegas study. The mean PaO₂ decreased by 7.7 Torr in our patients with bird-related CHP.¹² Although there were no reports on ESR, it was also found to be elevated after the inhalation challenge test. These mild changes were not usually considered as

minimally important differences in clinical practice. However, many changes such as CRP, ESR, LDH, A-aDO₂, and HRCT findings, were observed after the inhalation challenge test, and these mild changes were considered minimally important differences caused by allergic reactions. Therefore, careful observation is required because the reaction following inhalation may be overlooked. The combined use of HRCT findings was very effective for detecting changes after inhalation. The reaction was mild because the antigenicity of serum albumin, serum globulin, and mucin in the intestinal fluid might have been stronger than that of eggs containing ovalbumin, ovoglobulin, or ovomucin. The acute exacerbation of CHP caused by the inhalation challenge test is one of the main reasons that the inhalation provocation test is not widespread. After the inhalation challenge test using pigeon dropping extracts, 2 (1.5%) out of 130 patients required steroid pulse therapy.¹⁹ In a report on avian serum extracts, oral steroid administration was required in 3 (2.7%) out of 113 cases.¹⁸ Since the response after the inhalation challenge test using pigeon eggs was mild, no additional treatment was required in all of our 36 patients. Moreover, the parameters that were elevated after the inhalation challenge test were measured again 1 month later; none was significantly elevated after 1 month of inhalation, and there was no suggestion of the development of an acute exacerbation of CHP after 1 month of inhalation.

In the inhalation challenge test of the group with bird-related CHP, the WBC count is known to be elevated after inhalation.¹⁹ WBC elevation has also been found in acute hypersensitivity pneumonitis,²² suggesting an association between WBC elevation and acute exposure to the causative antigen. The group with stronger bronchiolitis had a higher WBC count at the initiation of the inhalation test than the weaker bronchiolitis group, and the WBC elevation was lower after exposure to pigeon eggs. These results were consistent with the hypothesis that the patients with stronger bronchiolitis had already been exposed to a large amount of antigen before the inhalation challenge test.

To date, numerous studies of causative antigens for bird-related acute hypersensitivity pneumonitis and CHP have been reported. The molecular weight of IgA in pigeons is 200–220 kDa, and antigen–antibody reactions were observed at 220 kDa.^{23,24} Proteins at 50 kDa, 29–32 kDa, 26 kDa, and 21 kDa in pigeon droppings are reportedly associated with disease onset.^{5,25–27} In this study, multiple bands were found in the serum of patients with bird-related CHP at 37–75 kDa. The molecular weight of ovalbumin is 45 kDa, and that of ovoglobulin is 49 kDa, indicating that antibodies against ovalbumin and ovoglobulin may be present.

Hypersensitivity pneumonitis is caused by types III and IV allergies;¹ type III allergies generally take 3–8 h to respond and type IV allergies taking 24–72 h to respond. Hendrick et al. reported changes during 36 h from inhalation initiation,¹¹ whereas Ramirez-Venegas et al. reported the changes at 24 h,¹² and Ohtani et al. confirmed changes after 6 and 24 h.¹³ In our few cases, the changes after 48 h was confirmed; however, there was little difference between 24 h and 48 h. Considering previous studies and the reaction time of the allergies, the survey was conducted at 6 and 24 h after inhalation in this study.

The surfaces of eggs may be contaminated with bacteria, such as *Salmonella*. Thus, we pasteurized the eggs based on a report showing that the complete sterilization of egg liquid could be achieved.²⁰ The results of the egg fluid culture performed prior to the study initiation were negative. Additionally, none of the patients presented with egg-derived infectious diseases after inhalation.

Since it is ethically difficult to conduct the inhalation challenge test in IPF patients with highly confident diagnoses, the inhalation challenge test was conducted in patients with suspected CHP or IPF with a bird exposure. The following factors were considered for the necessity and usefulness of this inhalation challenge test. First, it is controversial whether a case of CHP with a history of bird exposure can be diagnosed as bird-related CHP. The observation of an allergic

reaction on performing the inhalation challenge test may lead to a more reliable diagnosis. Second, many CHP cases are diagnosed with low confidence because of the inability to perform histopathological examinations and multidisciplinary discussions by specialists in clinical practice. The inhalation challenge test of pigeon eggs in patients with low diagnostic confidence may contribute to increased diagnostic confidence.

Tests for specific antibodies to avian antigens using the ImmunoCAP method have been commercialized in Japan. In a previous report, many patients with bird-related CHP did not have higher anti-pigeon IgG antibody levels than patients with connective tissue disease-associated interstitial lung diseases and healthy volunteers.²⁸ Moreover, the values of anti-pigeon IgG antibody using the ImmunoCAP method did not differ between bird-related CHP and other respiratory diseases, and it may be difficult to diagnose bird-related CHP using specific antibodies alone.²⁹ The values of anti-pigeon antibody did not increase after the inhalation challenge test, which seemed unsuitable as an indicator of a positive response to the inhalation challenge test. Since there are various methods for measuring the specific antibodies, such as ELISA and precipitin, other methods need to be examined in the future.

There were some limitations to this study. The bird-related CHP group had a bias; because patients who underwent surgical lung biopsy or cryobiopsy were included in this study, most patients had mild disease and were highly motivated to undergo the examinations. Another possible limitation was that patients with unidentifiable CHP were included in the control group. Therefore, it is possible the patients with bird-related CHP were assigned to the control group. It would be preferable for the control group to be composed of healthy volunteers or patients with IPF. However, it was ethically difficult to conduct the inhalation challenge test on healthy volunteers or patients without suspected hypersensitivity pneumonitis.

In this study, western blotting was performed to investigate the presence or absence of antibodies

to pigeon eggs. If there were no antibodies for pigeon eggs, the changes after the inhalation challenge test may be caused by inhalation stimuli rather than an allergic reaction. The presence of antibodies to eggs was confirmed in certain patients with bird-related CHP, suggesting that the changes in the inhalation test using pigeon eggs may be due to allergic reactions. However, because western blotting was performed few cases, further investigation is important for identifying the causative antibody. Finally, cross-reactivity occurs among birds, and allergic reactions may occur even if eggs from birds other than pigeons are inhaled.^{30,31} Although the incidence of bird-associated CHP is reportedly low in contact with poultry, such as chicken and turkey which produce very little bloom, it is unclear whether chicken eggs can be used in inhalation challenge tests. Therefore, cross-reactivity should be investigated further.^{32,33}

In conclusion, the inhalation challenge test using pigeon eggs was effective for bird-related CHP and the risk of inducing disease progression was very low. Additionally, pigeon eggs are relatively available and can be used for inhalation challenge tests simply by pasteurizing the eggs. The pigeon egg could be a candidate for inhalation challenge test.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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TABLE 1 The history of prior contact with birds

	Bird-related CHP	Control	
		Unidentifiable and summer type CHP	IPF
Subjects	19	12	5
Pigeon breeding	4	0	2
Parakeet breeding	5	0	0
Bengalese finch breeding	2	0	1
Chicken breeding	5	0	1
Wild duck breeding	1	0	0
Wild bird contact	1	0	0
Chicken manure	1	0	0
Java sparrow breeding	0	0	1

Note: Data are presented as n or mean $\pm$ standard deviation. Abbreviations: *CHP*, chronic hypersensitivity pneumonitis; *IPF*, idiopathic pulmonary fibrosis.

TABLE 2 Baseline characteristics of the study subjects

Subjects	Bird-related CHP (n=19)	Control (n=17)	p-values	U-statistic	<i>Note:</i> Data are presented as n or mean $\pm$ standard deviation. Mann–Whitney U test and Fisher’s exact test were used to compare the values. The reference ranges of LDH,
Age (years)	67.4 $\pm$ 8.1	67.2 $\pm$ 8.0	0.84	155	
Sex (M/F), n	17 / 2	12 / 5	0.22		
Smoking history (Y/N), n	18 / 1	13 / 4	0.17		
Surgical lung biopsy, n	12	8	1.0		
Cryobiopsy, n	14	15	0.41		
WBC (/ μ L)	6105 $\pm$ 1356	6225 $\pm$ 1554	0.65	147	
LDH (U/L)	218 $\pm$ 32	218 $\pm$ 40	0.85	155.5	
CRP (mg/dL)	0.16 $\pm$ 0.15	0.23 $\pm$ 0.25	0.59	144.5	
ESR (mm/h)	14.4 $\pm$ 9.2	24.5 $\pm$ 19.0	0.13	114	
KL-6 (U/mL)	1477 $\pm$ 785	1253 $\pm$ 678	0.48	139	
SP-D (ng/mL)	461.2 $\pm$ 329.7	529.2 $\pm$ 628.5	0.44	137	
Anti-pigeon IgG antibody (mg A/L)	21.0 $\pm$ 11.4	18.1 $\pm$ 8.5	0.69	150	
FVC (L)	3.06 $\pm$ 0.57	2.72 $\pm$ 0.83	0.06	102	
FVC %pred (%)	87.8 $\pm$ 13.1	79.9 $\pm$ 16.4	0.051	100	
PaO ₂ (Torr)	88.2 $\pm$ 8.0	85.6 $\pm$ 11.2	0.33	131	
A-aDO ₂ (Torr)	13.2 $\pm$ 9.4	13.5 $\pm$ 8.4	0.61	145.5	
BAL lymphocytes (%)	36.6 $\pm$ 28.3	21.1 $\pm$ 18.5	0.08	123.5	
Prednisolone, n	0	0			
Immunosuppressant, n	0	0			
Anti-fibrotic agents, n	2	1			

CRP, and anti-pigeon IgG antibody are 124–222 U/L, 0–0.3 mg/dL, and 0–14.0 mg A/L respectively. Abbreviations: *WBC*, white blood cell; *LDH*, lactate dehydrogenase; *CRP*, C-reactive protein; *ESR*, erythrocyte sedimentation rate; *KL-6*, Krebs von den Lungen-6; *SP-D*, surfactant protein D; *FVC*, forced vital capacity; *%pred*, % predicted; *PaO₂*, partial pressure of arterial oxygen; *A-aDO₂*, alveolar-arterial oxygen difference; *BAL*, bronchoalveolar lavage.

TABLE 3 Pathological investigations in 19 patients with bird-related CHP

Histological findings	Grade 3	Grade 2	Grade 1	Grade 0
Centrilobular fibrosis	2	8	5	4
Bronchiolitis	1	6	10	2
Granulomas	8	3	5	3
Giant cells	4	2	9	4
Peribronchiolar metaplasia	6	4	6	3
Fibroblastic foci	2	6	9	2
Organizing pneumonia	1	3	12	3
Bridging fibrosis (n = 12)	1	4	7	0

Note: Data are presented as n. Grade 0 = no finding, and grade 3 = finding was most prevalent.

The degree of bridging fibrosis was only evaluated in patients with surgical lung biopsy.

Abbreviations: *CHP*, chronic hypersensitivity pneumonitis.

TABLE 4 HRCT findings in patients with bird-related CHP and control

	Bird-related CHP		Control	
	(n = 19)		(n = 17)	
Radiological findings	Yes	No	Yes	No
Mosaic attenuation	18	1	16	1
Ground glass opacities	19	0	17	0
Centrilobular nodules	19	0	16	1
Honeycomb	6	13	8	9

Note: Data are presented as n. Abbreviations: *HRCT*, high-resolution CT; *CHP*, chronic hypersensitivity pneumonitis.

	After 6 hours				After 24 hours			
Parameters	Bird-related CHP (n=19)	Control (n=17)	p-values	U-statistic	Bird-related CHP (n=19)	Control (n=17)	p-values	U-statistic
Δ Body temperature	0.09 ± 0.4	-0.01 ± 0.36	0.656	147.5	0.07 ± 0.44	-0.06 ± 0.29	0.265	126.5
Δ WBC (%)	8.4 ± 14.0	6.1 ± 14.0	0.739	151	15.3 ± 29.2	3.9 ± 19.3	0.457	138
Δ LDH (U/L)	4.5 ± 27.2	-7.1 ± 17.7	0.310	129.5	5.4 ± 26.1	-12.7 ± 13.3	0.019	87.5
Δ CRP (mg/dL)	0.02 ± 0.13	-0.06 ± 0.09	0.047	99	0.32 ± 0.83	-0.05 ± 0.11	0.019	87.5
Δ ESR (mm/h)	1.4 ± 5.2	0.4 ± 5.6	0.382	134	1.9 ± 3.7	-1.4 ± 4.6	0.012	83
Δ KL-6 (U/mL)	-19 ± 109	-57 ± 110	0.375	133.5	-10 ± 130	-31 ± 93	0.558	143
Δ SP-D (ng/mL)	-29.4 ± 82.0	-16.6 ± 68.4	0.692	149	-3.6 ± 83.1	-18.8 ± 85.2	0.601	145

ΔPaO_2 (Torr)	-3.8 ± 14.0	0.1 ± 8.3	0.140	95	-7.7 ± 10.0	0.6 ± 10.4	0.051	87.5	TABLE 5 Mean changes in parameters after 6 and 24
$\Delta A-aDO_2$ (Torr)	3.4 ± 11.8	1.2 ± 7.3	0.296	107	6.3 ± 10.3	-1.2 ± 8.3	0.034	88	
ΔFVC (mL)	-48 ± 126	-2 ± 105	0.222	123	-45 ± 137	14 ± 101	0.084	107	
ΔFVC %pred (%)	-1.1 ± 3.4	0.7 ± 3.4	0.117	112	-0.8 ± 3.9	1.0 ± 2.9	0.062	102.5	

h from baseline

Note: Data are presented as mean $\pm$ standard deviation. Mann–Whitney U test was used to compare the values. Abbreviations: *CHP*, chronic hypersensitivity pneumonitis; *WBC*, white blood cell; *LDH*, lactate dehydrogenase; *CRP*, C-reactive protein; *ESR*, erythrocyte sedimentation rate; *KL-6*, Krebs von den Lungen-6; *SP-D*, surfactant protein D; *PaO₂*, partial pressure of arterial oxygen; *A-aDO₂*, alveolar-arterial oxygen difference; *FVC*, forced vital capacity; %pred, % predicted.

TABLE 6 Sensitivity and specificity of inhalation challenge test in patients with bird-related CHP

Note: Δ CRP, Δ LDH, Δ ESR, and Δ A-aDO₂ were the differences between the baseline and 24 h after inhalation. For Δ FVC, the lower FVC at 6 or 24 h after inhalation was compared to the

Parameters	Cut-off values	Sensitivity (%)	Specificity (%)	AUC	95% CI	baseline FVC.
Δ CRP (mg/dL)	0.01	57.9	81.3	0.739	0.571–0.906	Abbreviat
Δ LDH (U/L)	– 5	79.0	75.0	0.725	0.544–0.907	ions:
Δ ESR (mm/h)	0.275	79.0	62.5	0.711	0.536–0.885	CHP,
Δ A-aDO ₂ (Torr)	– 0.275	73.7	62.5	0.711	0.536–0.885	chronic
Δ FVC (mL)	– 40	79.0	62.5	0.648	0.457–0.839	hypersens
						itivity

pneumonitis; *AUC*, area under the curve; *CI*, confidence interval; *CRP*, C-reactive protein; *LDH*, lactate dehydrogenase; *ESR*, erythrocyte sedimentation rate; *A-aDO₂*, alveolar-arterial oxygen difference; *FVC*, forced vita capacity.

TABLE 7 Mean changes from 24 h after the inhalation challenge test to approximately 1 month after the inhalation challenge test

Parameters	Bird-related CHP	Control	p-values	U-statistic	<i>Note:</i>
Δ WBC (%)	-9.3 ± 8.8	2.5 ± 15.2	0.080	100	Abbrevia
Δ LDH (U/L)	-5.4 ± 20.1	16.5 ± 47.0	0.069	98	tions:
Δ CRP (mg/dL)	-0.33 ± 0.86	0.03 ± 0.16	0.137	108	<i>WBC</i> ,
					white

blood cell; *LDH*, lactate dehydrogenase; *CRP*, C-reactive protein; *ESR*, erythrocyte sedimentation rate; *KL-6*, Krebs von den Lungen-6; *SP-D*, surfactant protein D

Figure legends

FIGURE 1

Mean WBC and CRP changes in bird-related chronic hypersensitivity pneumonitis patients with weaker (grades 0 and 1) and stronger (grades 2 and 3) bronchiolitis. A: The WBC count in the weaker bronchiolitis group was significantly lower at the initiation of inhalation test (5662/ μ L vs. 6866/ μ L). There was no significant difference in the WBC count after 6 and 24 h between the two groups. B: Although there was no significant difference between the two groups, CRP increased after inhalation test in the weaker bronchiolitis group. Mann–Whitney U test was used. Data are presented as mean $\pm$ standard error. WBC, white blood cell; CRP, C-reactive protein.

FIGURE 2

Receiver operating characteristic curves for positive judgment of inhalation challenge test (A: Δ CRP, Δ A-aDO₂, and Δ FVC, B: Δ LDH and Δ ESR). CRP, C-reactive protein; A-aDO₂, alveolar-arterial oxygen difference; FVC, forced vital capacity; LDH, lactate dehydrogenase; ESR, erythrocyte sedimentation rate.

FIGURE 3

Comparison of anti-pigeon IgG antibody values before and after the inhalation challenge test in 19 patients with bird-related chronic hypersensitivity pneumonitis. The mean values of anti-pigeon IgG antibody before the inhalation challenge test were significantly higher than those after the test using paired t test. Post-inhalation IgG values were measured 24 h after the last inhalation was completed.

FIGURE 4

IgG immunoblotting patterns with pigeon eggs and human serum. Lanes 1–5 and 7–8, patients with bird-related chronic hypersensitivity pneumonitis; lane 6, a patient with acute bird-related hypersensitivity pneumonitis; lane 9, a healthy volunteer; lane 10, an asymptomatic volunteer who used to be a pigeon breeder. Multiple bands of 37–75 kDa (arrows) were observed in patients with acute and chronic bird-related chronic hypersensitivity pneumonitis.

FIGURE 1
(A)

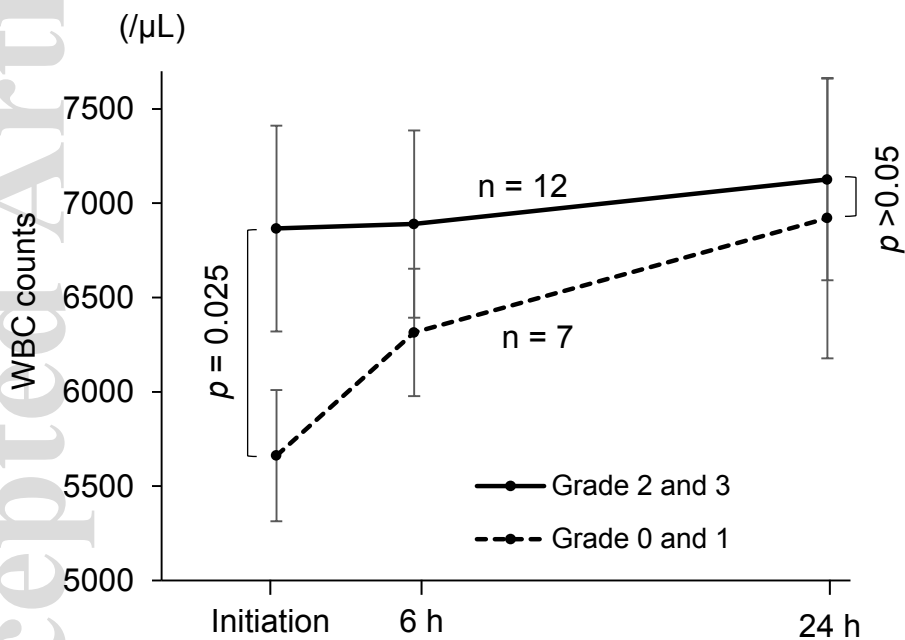


FIGURE 1
(B)

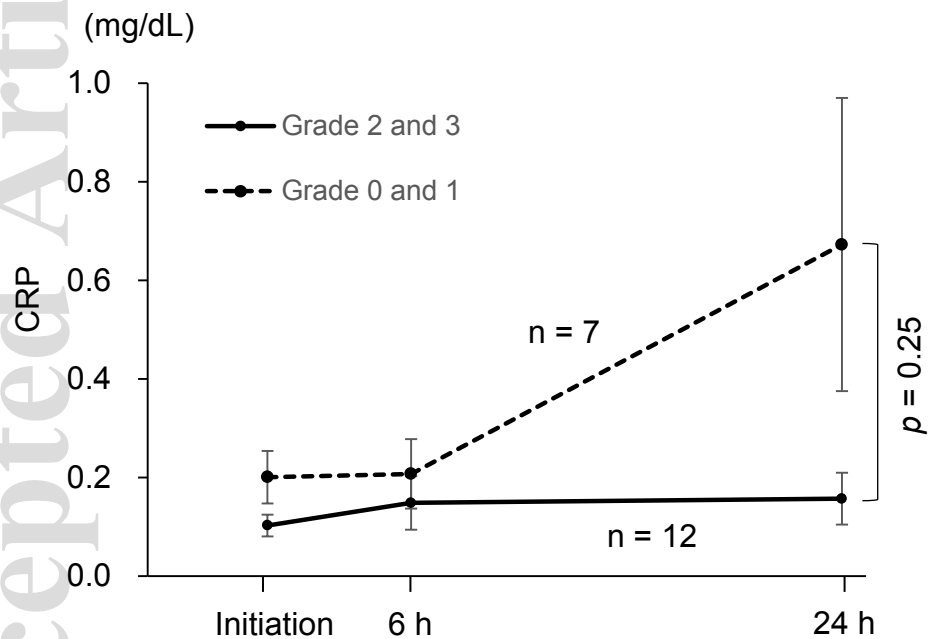


FIGURE 2

(A)

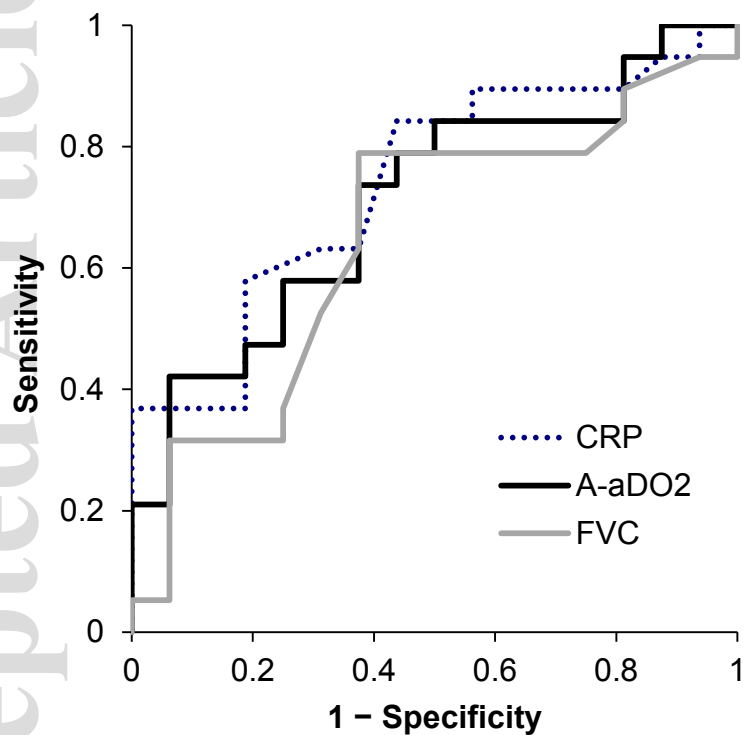


FIGURE 2

(B)

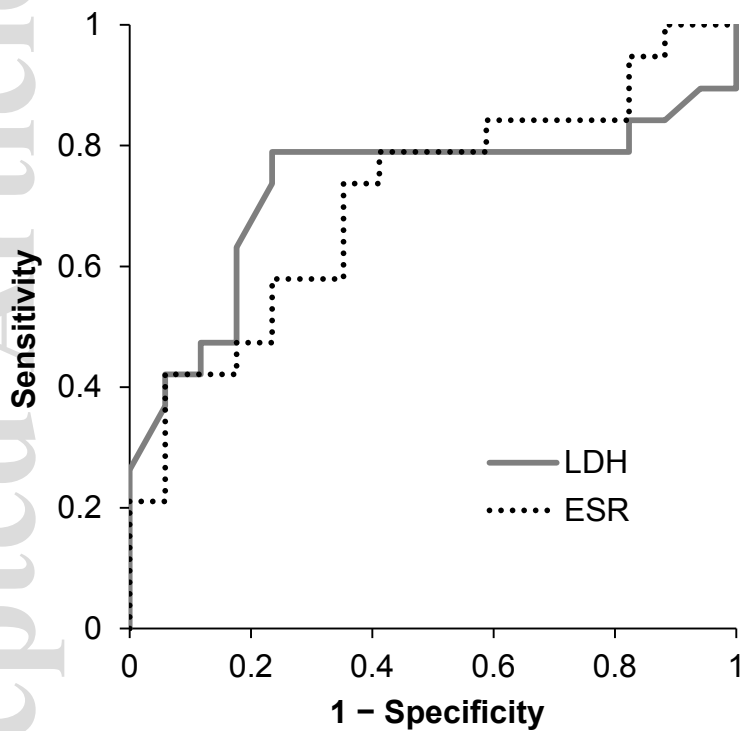


FIGURE 3

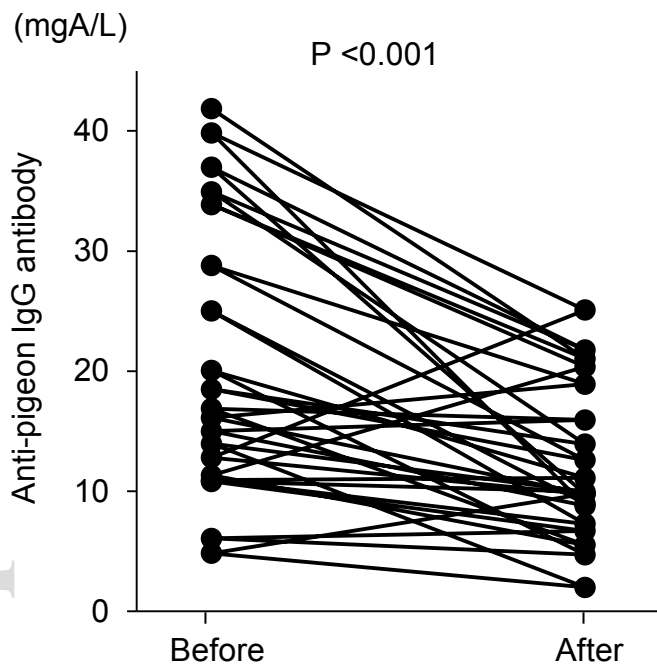


FIGURE 4

