Accuracy of Inhalation Challenge Test for Bird-related Fibrotic Hypersensitivity Pneumonitis: a Case Control Study

Ryo Okuda (✉ b980013@yahoo.co.jp)  
Kanagawa Cardiovascular and Respiratory Center

Eri Hagiwara  
Kanagawa Cardiovascular and Respiratory Center

Tomohisa Baba  
Kanagawa Cardiovascular and Respiratory Center

Hideya Kitamura  
Kanagawa Cardiovascular and Respiratory Center

Shigeru Komatsu  
Kanagawa Cardiovascular and Respiratory Center

Shota Kaburaki  
Kanagawa Cardiovascular and Respiratory Center

Yu Mikami  
the University of Tokyo

Tamiko Takemura  
Kanagawa Cardiovascular and Respiratory Center

Takashi Ogura  
Kanagawa Cardiovascular and Respiratory Center

Research Article

Keywords: Anti-pigeon IgG antibody, Anti-bird IgG antibody, Bird breeder’s disease, Bird fancier’s lung, Pigeon breeder’s disease, Provocation test, Serum IgG test

Posted Date: December 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1173572/v1

License: ☕️ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Background: The inhalation challenge test is considered to be the “gold standard” for diagnosis of hypersensitivity pneumonitis (HP) and identifying the causative antigen in patients with fibrotic HP. However, the inhalation challenge test is not widely used. This study aimed to examine the value of the inhalation challenge test.

Methods: This was a single-center, case control study. The patients with fibrotic HP were diagnosed pathologically by surgical lung biopsy or transbronchial lung cryobiopsy, and were assumed to be bird-related fibrotic HP if they had a history of obvious avian exposure. The patients with a histopathological diagnosis of fibrotic HP, no history of bird exposure and negative anti-bird antibodies were assumed to be non-bird-related fibrotic HP.

Results: Based on pathological findings and history of avian exposure, 43 of 86 patients were diagnosed with bird-related fibrotic HP. In 43 patients with bird-related fibrotic HP, 15 (35%) were positive for anti-bird IgG antibody, and 36 (81%) were positive for the inhalation challenge test. Patients with both positive inhalation challenge test and anti-bird IgG antibodies had a 2.7% decline in annual FVC before the inhalation (p = 0.029). In patients with positive inhalation challenge test and the negative anti-bird IgG antibodies, the annual FVC decreased by 5.0% (p = 0.047). No significant FVC decline was observed in patients with negative inhalation challenge test and positive anti-bird IgG antibody, and those with both negative tests.

Conclusions: The inhalation challenge test for bird-related fibrotic HP was more sensitive than anti-bird IgG antibodies. Furthermore, the inhalation challenge test was able to find a group of patients with FVC decline.

Introduction

Since the 1980s, pigeon serum, pigeon droppings, and proteins attached to feathers called “bloom” have been used to perform the inhalation challenge tests for diagnosing bird-related hypersensitivity pneumonitis (HP). Although the usefulness of this method has been reported [1-3], it is not too widely adopted because collecting and extracting serum or droppings from causative animals is complicated. In 2020, we reported the use of pigeon eggs for the inhalation challenge test [4].

It is stated in the 2020 ATS/JRS/ALAT guideline for the diagnosis of HP that only specialize facilities with experience can perform the inhalation challenge tests.[5] Additionally, the 2021 CHEST guideline weakly recommended that this test should not be performed because of the lack of test standardization and the well-established positive criteria [6].

Serum tests for anti-pigeon and anti-parrot immunoglobulin G (IgG) antibodies have good sensitivity and specificity for bird-related nonfibrotic HP; however, it shows low sensitivity for bird-related fibrotic HP [7].
Furthermore, notably, anti-bird IgG antibodies only verify exposure to birds at any time from the past to the present, and cannot identify the causative antigen.

As HP is caused by type III and IV allergies, from an allergological point of view, the inhalation challenge test is considered to be the "gold standard" method to diagnose the HP and identify the causative antigen. Its accuracy, however, remains unknown. In this study, we compared the sensitivity of the inhalation challenge test and anti-bird IgG antibodies, and we also investigated the disease progression in positive patients.

Methods

Study design and population

This was a single-center, retrospective study. The patients who underwent the inhalation challenge test at our hospital from August 2018 to August 2021 and met all of the following criteria were included in this study: 1) patients whose serum anti-pigeon IgG antibody, anti-parrot IgG antibody, and anti-Trichosporon asahii antibody were tested; 2) those who underwent surgical lung biopsy or transbronchial lung cryobiopsy and with pathological diagnosis of fibrotic HP by a pathologist (T.T.) specializing in HP; 3) those in whom fibrotic HP was the first diagnosis in multi-disciplinary discussion (MDD); and 4) those with a history of bird-keeping (excluding chickens) for ≥6 months from the past to the present or those with a history of using chicken manure fertilizer for ≥2 years or those with no history of bird-keeping (including chicken) and chicken manure fertilizer use. Patients with a history of bird exposure during a certain period of time and a histopathological diagnosis of fibrotic HP were assumed to have bird-related fibrotic HP, whereas patients with a histopathological diagnosis of fibrotic HP, no history of bird exposure and negative anti-bird antibodies were assumed to have non-bird-related fibrotic HP. The study was approved by the ethics committee of our hospital (KCRC-21-0027). As this was a retrospective study, informed consent was waived, and information of each patient was anonymized before analyses.

Purification method of pigeon eggs

Surface dirt was removed from pigeon eggs with tap water, and they were then washed with a sodium hypochlorite solution of at least 150 ppm. Only egg whites were used, which were heated at 58°C for 30 min twice. Following pasteurization, the whites were frozen and stored at −80°C for further analyses [4].

Test method

Frozen egg whites were thawed at room temperature and diluted with saline solution to prepare the inhalation solution. Using an ultrasonic nebulizer, 3–4 mL of this solution was dissolved in a 16–17 mL saline solution and a total of 20 mL was inhaled. Up to 24 h after inhalation, worsening of cough, body temperature, arterial blood gas, and white blood cell count were evaluated, and a pulmonary function test was conducted. If there was no significant change was found up to 24 h after inhalation, a second inhalation was performed; the same subsequent process was repeated post-inhalation.
Positive criteria for the inhalation challenge test

If either of the following criteria was met, the inhalation challenge test was considered positive.

A. A positive result was determined when >2 of the following criteria were met: 1) worsening of cough or appearance of chills, 2) increase in body temperature of ≥0.5°C, 3) worsening of forced vital capacity (FVC), 4) worsening of alveolar-arterial oxygen difference by >10 Torr, 5) increase of >20% in white blood cell count or increase of >0.2 mg/dL in C-reactive protein levels, and 6) appearance of reticular shadows or ground-glass opacity around upper lobe ground-glass opacity or existing interstitial lesions on high-resolution CT.

B. Patients with a positive predictive value of ≥70% based on binomial logistic regression analysis which we created before this study. The variable items were the changes of FVC, C-reactive protein, alveolar-arterial oxygen difference and CT findings.

Antibody measurement

A commercialized IgG antibody kit for pigeon and parrot method were performed using the ImmunoCAP method (Thermo Fisher Scientific, USA). In this method, extracts of pigeon serum, bloom, and droppings are used as antigens; however, details about antigens have not been disclosed. A positive result was determined when levels of either of the two antibodies were more than the threshold. Although such threshold values of anti-pigeon and anti-parrot IgG antibodies were set to 0–24.0 and 0–14.0 mg A/L, respectively [7]. Anti-Trichosporon asahii antibody was measured also using a commercialized enzyme-linked immune-sorbent assay method (Falco Biosystems, Japan).

Statistical analysis

For comparison, the Fisher’s exact test or unpaired/paired t-test was used; p < 0.05 indicated statistical significance. All analyses were performed with BellCurve for Excel (Social Survey Research Information Co., Ltd.).

Results

Totally, 86 patients with clinically suspected bird-related fibrotic HP underwent the inhalation challenge test with pigeon eggs. Based on pathological findings and history of avian exposure, 52 of the 86 patients were included in this study (Figure 1). Patients had birds as pets for a mean duration of 5.9 years, and only 3 (7%) had birds as pets at the time of diagnosis. Most patients kept small ornamental birds, such as parakeets, bengalese finch, and java sparrow, in their homes, and some were keepers of medium-sized birds, such as pigeons and wild ducks, which should have been kept outdoors (Table1).

Among the 43 patients with bird-related fibrotic HP, 15 (35%) were positive for anti-pigeon IgG or anti-parrot IgG antibodies, and 36 (81%) were positive for the inhalation challenge test (Figure 2). Those who were positive for anti-pigeon or parrot IgG antibodies showed a significantly higher titer of anti-
Trichosporon ashii antibody when compared with those who were negative for both antibodies ($p = 0.001$) (Table 3). Of the nine patients assumed to have non-bird-related fibrotic HP, four were positive for the inhalation challenge test, and thus the specificity of the test was 56%.

Patients with both positive inhalation challenge test and anti-pigeon IgG antibody showed a 2.7% decline in annual FVC ($p = 0.029$). Furthermore, in patients with positive inhalation challenge test and the negative anti-bird IgG antibodies, annual FVC was decreased by 5.0% ($p = 0.047$). By contrast, no significant decline in annual FVC was observed in patients with negative inhalation challenge test and positive anti-bird IgG antibodies, or in patients with negative for both (Table 4). The annual changes in C-reactive protein and Krebs von den Lungen-6 were not significantly different in any group.

None of the 86 patients showed worsening of HP after the inhalation challenge test, which required additional treatment with, for example, prednisolone or immunosuppressants, or oxygen administration.

**Discussion**

In this study, we investigated the sensitivity of the inhalation challenge test in patients with pathological findings and reliable history of avian exposure, assuming that they had bird-related fibrotic HP. The sensitivity of the inhalation challenge test was better than that of the anti-bird IgG antibodies (81% vs. 35%). Regardless of the titer of anti-bird IgG antibodies, patients with positive inhalation challenge test showed a significant difference in the rate of annual FVC decline, and inhalation challenge test results revealed a trend of annual FVC decline.

In both the 2020 ATS/JRS/ALAT guideline for HP diagnosis and the CHEST guideline, the inhalation challenge test was omitted from the diagnostic matrix table on the basis that it is a non-standardized test. Furthermore, antigen avoidance test, environmental exposure test, and seasonal change in interstitial pneumonia markers, such as Krebs von den Lungen-6 and surfactant protein-D, do not contribute to the diagnostic confidence levels of HP diagnostic guidelines [5, 6]. According to a concept, all available information should be integrated to perform MDD [8]; nevertheless, this concept and the method of matrix diagnosis in the guidelines contradict each other.

In 2016, Walsh et al. investigated the concordance rate of 70 patients diagnosed by multiple MDD teams; the diagnostic concordance rate of idiopathic pulmonary fibrosis and connective tissue disease-associated interstitial lung diseases among MDD teams was slightly high (Kappa value = 0.71–0.73). Nevertheless, the diagnostic concordance rate of HP was extremely low (Kappa value = 0.29) [9]. Since certain criteria have been established for high-resolution CT imaging and pathology in recent guidelines for diagnosis of HP, the diagnostic concordance rate is expected to improve. Because the definitive diagnosis of HP is the reproduction of symptoms by the inhalation challenge test or reduction of symptoms by the antigen avoidance test, it needs to be confirmed whether an improvement in the diagnostic concordance rate of fibrotic HP is associated with an improvement in diagnostic accuracy.
Identifying causative antigens is vital to potentially improve prognosis [10, 11]; however, in the patients with fibrotic HP, causative antigen identification is challenging. In this study, a trend of annual FVC decline in patients with positive inhalation challenge test was observed, regardless of the results of anti-bird IgG antibodies. Conversely, FVC showed no consistent in patients with positive anti-bird IgG antibodies. The inhalation challenge test could extract the similar disease progression; in other words, the inhalation challenge test may have a high diagnostic accuracy. The FVC change before rather than after the inhalation challenge test was used to avoid the intervention of factors related to changes in FVC, such as the inhalation challenge test, and treatment.

According to numerous studies, anti-pigeon and anti-parrot IgG antibodies can effectively diagnose HP [7, 12]. In Japan, the ImmunoCAP method to measure anti-bird IgG antibodies has been commercialized, and it is widely applied for measuring IgG antibodies. In the case of nonfibrotic HP, anti-bird IgG antibodies show a sensitivity of 70%–91% and a specificity of 77%–86%. By contrast, in the case of fibrotic HP, anti-bird IgG show a sensitivity of 33%–61% and a specificity 77%–86% [7]. In this study, the similar result was obtained with a sensitivity of 35%. In addition, to investigate the specificity of the inhalation challenge test, we focused on the relatively high specificity of anti-bird IgG antibodies, and assumed that fibrotic HP patients with negative anti-bird IgG antibodies had non-bird-related fibrotic HP, after first confirming that there was no contact with birds by questionnaires.

Regardless of common antigenicity or cross-reactivity, cases with positive IgG antibody to one antigen was likely to be positive in IgG antibodies to other antigens [13]. In this study, patients who were positive for anti-bird IgG antibodies showed a significantly higher rate of positive anti-\textit{Trichosporon ashii} antibody. Whether some people are more likely to IgG test positive than others, or whether the positive results were due to chance, is an issue for further study.

Questionnaires or even anti-IgG antibodies are not conclusive for identifying antigens. For example, a study attempted to identify causative antigens in three 70-year-old men suspected of having fibrotic HP on the basis of histopathological and high-resolution CT findings, the first had raised five pigeons in an outdoor environment for 10 years starting at the age of 10 years; the second kept one parakeet in an indoor environment for 1 year starting at the age of 50 years; the third kept 20 ducks away from his home starting at the age of 69 years. It was unable to assess a difference in the amount of exposure among these three subjects from questionnaires.

In this study, poultry farmers were excluded. Poultry farmers have a low incidence of bird-related HP, and flightless and domesticated birds are considered to be less antigenic because they have less “bloom” [14-16]. Conversely, as bird droppings show the strongest antigenicity; hence, those who use chicken manure fertilizer were determined to be intensively exposed [17].

When using pigeon eggs for the inhalation challenge test, the eggs only need to be boiled in hot water at 58°C. Pasteurization destroys viruses, such as avian influenza virus, as well as bacteria, including spore-forming bacteria [18, 19]. Additionally, pigeon eggs are more hygienic than pigeon serum or droppings extract. Nevertheless, the response to the inhalation challenge test on using pigeon eggs was usually
weak, so careful observation was needed [4]; additionally, because of the response is weak, the possibility of acute exacerbation after the inhalation challenge test was considered to be low.

This study had some limitations. First, this was a single-center, retrospective study which introduced significant bias in patient selection and statistical analyses. As the inhalation challenge test becomes more widespread, a multicenter prospective study will be conducted. Second, the possibility that the causative antigen was not avian cannot be completely ruled out. In this study, cases with a pathological diagnosis and a definite history of avian contact were assumed to have bird-associated fibrotic HP. According to the current guidelines, the best method to identify the causative antigens is using a questionnaire; thus, the causative antigen in cases with a definite history of bird keeping was considered to be avian. Finally, the specificity of the inhalation challenge test may have come out lower than the true specificity in this study, because performing the inhalation challenge test in patients with interstitial lung diseases other than bird-related HP, such as idiopathic pulmonary fibrosis, and connective tissue disease-associated interstitial lung disease is ethically difficult. The inhalation challenge test is not feasible for patients with definite interstitial lung diseases other than HP, because it requires CT imaging and arterial blood test. Since non-bird-related fibrotic HP was diagnosed using a questionnaire and anti-bird IgG antibodies in this study, further investigation of the causative antigen in patients with no history of avian exposure, negative anti-bird IgG antibodies, and positive inhalation challenge test is necessary.

In summary, for diagnosing bird-related fibrotic HP, the inhalation challenge test with pigeon eggs was much more sensitive than with anti-bird IgG antibodies. Additionally, patients with positive inhalation challenge test show a trend of annual FVC decline, suggesting the presence of fewer false positives. This indicated that the precision of the inhalation challenge test was high.

List Of Abbreviations

HP; hypersensitivity pneumonitis, IgG; immunoglobulin, MDD; multi-disciplinary discussion, FVC; forced vital capacity,

Declarations

Ethics approval and consent to participate

The ethics committee of Kanagawa cardiovascular and respiratory center approved this retrospective study. (KCRC-21-0027)

Acknowledgements

T.O. and his institution have received a research grant from the Diffuse Lung Diseases Research Group from the Ministry of Health, Labour and Welfare, Japan. The authors would like to thank Enago (www.enago.jp) for the professional English language review.
Competing interests

The authors declare no conflict of interest.

Authors' contributions

RO, EH, YM and TO proposed the concept. RO, SK, TT and TO collected the patients and data. RO, TB, and SK evaluated and analyzed the data. RO conceived of the study design and drafted the manuscript. EH, TB, HK, SK, YM, TT and TO supervised the study. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

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

Consent for publication

Not applicable.

References

8. Wells AU. "Any fool can make a rule and any fool will mind it". BMC Med. 2016;14(23).

Tables

Table 1 History of avian exposure
<table>
<thead>
<tr>
<th>Subject</th>
<th>Bird-related fHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>43</td>
</tr>
<tr>
<td>Total years of bird keeping</td>
<td>5.9 ± 8.1</td>
</tr>
<tr>
<td>Number of bird keepers at diagnosis</td>
<td>3</td>
</tr>
<tr>
<td>Pigeon breeding</td>
<td>10</td>
</tr>
<tr>
<td>Parakeet breeding</td>
<td>14</td>
</tr>
<tr>
<td>Bengalese finch breeding</td>
<td>6</td>
</tr>
<tr>
<td>Java sparrow breeding</td>
<td>5</td>
</tr>
<tr>
<td>Canary breeding</td>
<td>2</td>
</tr>
<tr>
<td>White-eye breeding</td>
<td>2</td>
</tr>
<tr>
<td>Parrot breeding</td>
<td>1</td>
</tr>
<tr>
<td>Wild duck breeding</td>
<td>1</td>
</tr>
<tr>
<td>Chicken manure fertilizer</td>
<td>2</td>
</tr>
</tbody>
</table>

*Note: In several cases, >1 species has been kept, so one species with the longest breeding history is listed. Values are presented as n or mean ± standard deviation. fHP, fibrotic HP.*

**Table 2** Baseline characteristics of study subjects
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Bird-related fHP (n = 43)</th>
<th>Non-bird-related fHP (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>69 (64–73)</td>
<td>69 (68–75)</td>
</tr>
<tr>
<td>Sex (M/F), n</td>
<td>33 / 10</td>
<td>6 / 3</td>
</tr>
<tr>
<td>Smoking history (Y/N), n</td>
<td>35 / 8</td>
<td>7 / 2</td>
</tr>
<tr>
<td>Surgical lung biopsy, n</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Cryobiopsy, n</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>WBC (/μL)</td>
<td>5920 (5230–6750)</td>
<td>5790 (4900–5970)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.16 (0.07–0.23)</td>
<td>0.11 (0.04–0.23)</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>14 (10–25)</td>
<td>20 (11–25)</td>
</tr>
<tr>
<td>KL-6 (U/mL)</td>
<td>920 (580–1490)</td>
<td>660 (410–1180)</td>
</tr>
<tr>
<td>SP-D (ng/mL)</td>
<td>290 (200–380)</td>
<td>440 (200–510)</td>
</tr>
<tr>
<td>Anti-pigeon IgG antibody (mg A/L)</td>
<td>9.7 (14.0–25.5)</td>
<td>12.4 (10.0–20.4)</td>
</tr>
<tr>
<td>Anti-parrot IgG antibody (mg A/L)</td>
<td>10.5 (6.3–21.3)</td>
<td>9.0 (7.6–11.6)</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>2.87 (2.51–3.36)</td>
<td>3.22 (2.33–3.38)</td>
</tr>
<tr>
<td>FVC %pred (%)</td>
<td>82 (77–92)</td>
<td>100 (84–105)</td>
</tr>
<tr>
<td>PaO$_2$ (Torr)</td>
<td>91 (82–96)</td>
<td>92 (87–97)</td>
</tr>
<tr>
<td>BAL lymphocytes (%)</td>
<td>24 (14–44)</td>
<td>22 (13–35)</td>
</tr>
<tr>
<td>Prednisolone, n</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Immunosuppressant, n</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Antifibrotic agents, n</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: Values represent as n or mean (IQR). The reference ranges of CRP, and anti-pigeon and anti-parrot IgG antibodies are 0–0.3 mg/dL, 0–24.0 mg A/L, and 0–14.0 mg A/L respectively. fHP, fibrotic HP; WBC, white blood cell; 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; PaO$_2$, partial pressure of arterial oxygen; BAL, bronchoalveolar lavage.

Table 3 Anti-*Trichosporon asahii* antibody according to the results of anti-bird IgG antibodies and the inhalation challenge test.
A. IgG antibodies in fungi and birds

<table>
<thead>
<tr>
<th>Anti-pigeon or anti-parrot IgG antibodies</th>
<th>Positive for either (n = 15)</th>
<th>Negative for both (n = 28)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-\textit{Trichosporon asahii} IgG antibody</td>
<td>0.14 ± 0.15</td>
<td>0.04 ± 0.10</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\textit{Note:} Data are presented as mean ± standard deviation. The unpaired t test was used to compare.

B. Anti-\textit{Trichosporon asahii} IgG antibody and the inhalation challenge test

<table>
<thead>
<tr>
<th>Inhalation challenge test</th>
<th>Positive (n = 35)</th>
<th>Negative (n = 8)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-\textit{Trichosporon asahii} IgG antibody</td>
<td>0.07 ± 0.13</td>
<td>0.10 ± 0.14</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

\textit{Note:} Data are presented as mean ± standard deviation. The unpaired t test was used to compare.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
& Subjects & delta FVC %pred & delta CRP (mg/dL) & delta KL-6 (U/mL) \\
\hline
Both positive & 12 & $-2.7 ± 3.5^*$ & 0.02 ± 0.18 & $-81 ± 635$ \\
\hline
Positive-ICT and negative-IgG test & 23 & $-4.5 ± 10.1^*$ & 0.05 ± 0.16 & $-89 ± 301$ \\
\hline
Negative-ICT and positive-IgG test & 3 & 0.3 ± 6.3 & $-0.33 ± 0.18$ & $-268 ± 349$ \\
\hline
Both negative & 5 & $-0.3 ± 3.2$ & 0.02 ± 0.07 & $-349 ± 491$ \\
\hline
\end{tabular}
\caption{Annual changes before the inhalation challenge test}
\end{table}

\textit{Note:} Data are presented as mean ± standard deviation. The paired t test was used to compare the changes in the year before the inhalation challenge test. FVC, forced vital capacity; %pred, % predicted; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; ICT, inhalation challenge test; *, p < 0.05.

\section*{Figures}
Figure 1.

86 patients underwent the inhalation challenge test between August 2018 - August 2021.

82 patients underwent pathological examination. 4 patients did not undergo pathological examination.

Nonfibrotic HP (n = 5)  
A history of bird keeping (n = 43)

Fibrotic HP (n = 71)  
No history of bird keeping and negative anti-bird antibodies (n = 9)

Diagnosis other than HP on histological findings (n = 6)  
Others (n = 19)

Figure 1

Flowchart showing patient inclusion criteria.
Figure 2

Relationship between anti-bird IgG antibodies and the inhalation challenge test using Chode diagram.