Screening and diagnosis of acute and chronic bird-related hypersensitivity pneumonitis by serum IgG and IgA antibodies to bird antigens with ImmunoCAP®

Short title: Utility of ImmunoCAP® for bird-related hypersensitivity pneumonitis.

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Y.M. designed this concept originally. Y. Tanino, T.N., Y.T., S.H., Y. Taguchi, T.B., T.O., K.K., M.N., Y.Y., and S.M. were involved in the concept of the research, selected patients and collected blood samples. T.S. selected patients, collected blood samples, analysed data, and wrote the manuscript. S.N. curated data and performed statistical analysis. All authors have read and approved the final manuscript.

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Abstract

Background: Bird antigens are some of the most relevant antigens in hypersensitivity pneumonitis (HP). Possible sources of bird antigens are bird breeding, feather products and fertilizer with fowl droppings. For the screening and diagnosis of HP, the measurement of bird-specific antibodies should be standardized.

Objective: The aim of this study was to clarify the utility of serum IgG (sIgG) and serum IgA (sIgA) antibodies to bird antigens in screening and diagnosing acute/chronic bird-related HP with ImmunoCAP® in multi-centre clinical research.

Method: We performed a clinical performance test by conducting a multi-institutional study to measure the levels of sIgG/sIgA against pigeon, parrot and budgerigar antigens by the ImmunoCAP® system in 29 acute and 46 chronic bird-related HP patients.

Results: The levels of sIgG/sIgA against the bird antigens of the three species were significantly higher in subjects with acute bird-related HP and chronic bird-related HP with acute episodes (recurrent type) than in the control subjects. For sIgG, the optimal cutoff values by ROC analysis were 24.6 mgA/L for pigeon, 14.0 mgA/L for parrot, and 8.7 mgA/L for budgerigar. By measuring multiple bird antigens and combining sIgG values of two species, the sensitivity and specificity for acute and recurrent-type chronic bird-related HP patients were 85-91% and 73-80%, respectively. For recurrent and insidious types of chronic bird-related HP, the sensitivity and specificity were 48-61% and 73-80%, respectively.

Conclusion: The measurement of the levels of sIgG/sIgA against pigeon, budgerigar and parrot antigens by ImmunoCAP® was useful for screening and diagnosis in bird-related HP.

Key words:
Hypersensitivity pneumonitis, specific antigen, bird antigen, ImmunoCAP®

Introduction

Hypersensitivity pneumonitis (HP) is an immune-mediated interstitial lung disease (ILD) with Gell and Coomb type III (immunocomplex-mediated) and type IV (cell-mediated) reactions to specific antigens. HP is classified into acute and chronic according to the clinical presentation, and chronic HP is further subgrouped into the recurrent type and insidious type. Patients with recurrent chronic HP have recurrent but gradually diminished acute episodes, including mild dyspnoea on exertion, cough, and low-grade fever; however, patients with insidious chronic HP have a slowly progressive course without acute episodes.[1,2]

Causative antigens include animal-derived proteins, such as bird antigens; fungi/bacteria; and inorganic materials, such as isocyanate. Bird antigens can exist in places close to humans because bird-related HP develops by not only direct exposure due to bird breeding but also the use of feather products, such as duvets and down jackets;[3] the use of chicken fertilizer in gardens;[4] bird breeding by neighbours;[5] and other indirect exposures to bird antigens. Therefore, among the HP antigens, bird antigens are some of the most frequent inciting antigens in patients with chronic bird-related HP.[6,7]

The diagnosis of patients with acute bird-related HP is relatively simple when they breed birds and have typical high-resolution computed tomography (HRCT) findings. However, in some cases, patients have two or more causative environmental factors in their occupations, home environments or hobbies.[8,9] In those cases, the detection of specific antibodies is useful for identifying antigen exposure.

It is extremely difficult to separate chronic HP from idiopathic pulmonary fibrosis (IPF)[10] and other fibrosing interstitial lung disease because of similar clinical, radiological and pathological features among them. It is important to discriminate between these ILDs because the treatment strategies are completely distinct. When the titres of specific antibodies against bird antigens are high, it could be a clue to suspect a bird-related HP. Furthermore, disease progression could be decelerated by discontinuing bird breeding, avoiding the bird-colonized environment, discarding feather products, etc.

In summer-type HP, the measurement of sIgG by antigen-conjugated ELISA against *Trichosporon asahii*, the causative antigen, began being covered by health insurance in

2013 and is widely used clinically in Japan.[11] sIgG against various bird antigens can also be measured by Thermo Fisher Scientific, Uppsala, Sweden, using a kit for measuring sIgG/serum IgA (sIgA) against pigeon, parrot and budgerigar antigens with the ImmunoCAP® system.[12] We analysed the usefulness of antibody measurement by ImmunoCAP® for acute and chronic bird-related HP in a single-centre study. In chronic HP, the sensitivity and specificity of sIgG against pigeon and budgerigar antigens were 27% and 100%, respectively, indicating its usefulness for confirming diagnosis in patients with presumed chronic bird-related HP.[13].

Based on this information, the measurement of bird-specific antibodies is expected to be standardized in the screening and diagnosis of HP. Next, we conducted clinical performance testing of sIgG and sIgA antibodies against pigeon, parrot, and budgerigar antigens in a multi-institutional study.

Material and methods

Subjects

Seventy-five patients with bird-related HPs (29 with acute HP, 46 chronic with HP; 17 cases of recurrent type and 29 cases of insidious type), 64 patients with control diseases, and 146 healthy subjects were included in this study from 9 tertiary hospitals (Tokyo Medical and Dental University, Fukushima Medical University, Saitama Cardiovascular and Respiratory Center, Tenri Hospital, Kanagawa Cardiovascular and Respiratory Center, Tosei General Hospital, Jichi Medical University, JR Tokyo General Hospital, and Hamamatsu University). The study was approved by the Ethics Committee of Tokyo Medical and Dental University (approval date, Oct 3, 2012; approval no. M2000-1271). We obtained consent from participants, including patients by making public information concerning the implementation of this study to ensure the opportunities to withdraw such consent. All participants signed an informed consent form for participation in the study.

Acute bird-related HP was diagnosed upon meeting all of the following criteria. (1) Exposure to bird antigens is evident. (2) Serum levels of CRP, KL-6, and SP-D are increased. (3) Chest CT images show diffuse micronodular or ground-glass opacities on both lungs.[14]

Chronic bird-related HP was diagnosed, in addition to the above (1) and (2), based on (4) chest CT images showing fibrosis. Chronic bird-related HP was grouped into two

subgroups: recurrent type and insidious type. The recurrent type was characterized by progressive pulmonary fibrosis with recurrent symptoms, including mild exertional dyspnoea, cough, and low-grade fever, reproduced by environmental provocation. The insidious type was characterized by progressive pulmonary fibrosis without symptoms. Patients receiving treatment with steroids and HP patients with unidentified causative antigens were excluded from the study.[15]

Measurement of serum IgG and IgA antibodies

Serum was collected by venous blood sampling and stored at -80°C. sIgG and sIgA antibodies against pigeon, parrot and budgerigar antigens were measured by the ImmunoCAP® system from Thermo Fisher Scientific.[12 393]

Statistics

Statistical significance tests and ROC analyses were performed with IBM SPSS Statistics (ver. 25). The differences in titres of antibodies among groups were analysed by the Mann-Whitney U test and the Kruskal-Wallis test, and less than 5% was determined to be significant. The sensitivity, specificity, positive rate and correlation were analysed using Microsoft Excel 2013.

Results

Patient characteristics

The patient characteristics are shown in Table 1. Of the 64 patients with control diseases, 15 had idiopathic interstitial pneumonias, 4 had IPF, 24 had collagen vascular disease-related interstitial pneumonia, 10 had summer-type HP, 1 had humidifier lung, and 10 had other interstitial pneumonia. In the acute and chronic bird-related HP groups, 8 out of 29 cases (27.5%) in the acute bird-related HP group and 3 out of 46 cases (6.5%) in the chronic bird-related HP group had a history of raising birds. There were no significant differences in median age between the acute and chronic bird-related HP groups and the control disease group. Median serum levels of KL-6 were significantly higher in the acute bird-related HP group than in the control disease group; however, there was no difference compared with that in the chronic bird-related HP group. The levels of CRP did not show significant differences among the groups.

Distribution of antibody titres

sIgG and sIgA antibodies in the acute bird-related HP group and the chronic recurrent HP group were significantly higher for the pigeon, parrot and budgerigar antigens than in the other groups, including 15 cases of idiopathic interstitial pneumonia, 4 cases of IPF, 24 cases of collagen vascular disease-related interstitial pneumonia, 10 cases of summer-type HP, one case of humidifier lung, and 10 cases of other ILD. ($p < 0.05$). Furthermore, in the chronic insidious HP group, neither sIgG nor sIgA showed any difference from those in the control disease group for any of the three antigens (Figure 1).

Cutoff values

Based on ROC analyses between the bird-related HP with acute episode group (acute and chronic recurrent bird-related HP) and the control disease group, the area under the curve (AUC) of sIgG was 0.8 or more (0.85-0.90) for any antigen, but that of specific IgA was less than 0.8 (0.71-0.76) (Figure 2). The optimal cutoff value of sIgG for pigeon was 24.6 mgA/L, the sensitivity was 85%, the specificity was 67%. For parrot; the optimal cutoff value was 14.0 mgA/L, the sensitivity was 96%, the specificity was 65%. Budgerigar was set to 8.7 mgA/L, the sensitivity was 74%, and the specificity was 91% (Table 2). sIgA optimal cutoff values were 4.2 mgA/L for pigeon, 4.3 mgA/L for parrot and 5.0 mgA/L for budgerigar; the sensitivity was 46-65%, and the specificity was 71-98%. The AUC from the ROC analysis between chronic insidious bird-related HP and the control disease group was less than 0.59; therefore, the optimal cutoff could

not be defined. The sensitivity for the insidious type of chronic bird-related HP by the cutoff from the ROC analysis between acute and chronic recurrent bird-related HP and control disease was 21-45%.
In both types of chronic bird-related HP regardless of the presence or absence of acute episodes, the sensitivity and specificity were 48-61% and 73-80%, respectively (Supplemental Table S1). When the 97.5th percentile sIgG titre of the control disease group was used, the cutoff values were 36.6 mgA/L for pigeon, 41.7 mgA/L for parrot, and 19.5 mgA/L for budgerigar, and the sensitivity and specificity were 15-24% and 98%, respectively.

Utility of the measurement of the three bird antigens

The correlations of the sIgG titres among the three bird antigens were evaluated for the acute bird-related HP group, the chronic bird-related HP group (recurrent and insidious), and the control disease group (Figure 3). sIgG showed a high correlation for the pigeon, parrot and budgerigar antigens (correlation coefficient 0.92-0.97).
A positive result was defined as when either one of any two species of antigens from the three species was positive. When both were negative, the result was defined as negative. Based on this definition, the sensitivity according to the optimal cutoff value calculated by ROC analysis was 85-91% for the acute and recurrent chronic bird-related HP groups, and the specificity was 73-80% (Table 3). Both types of chronic bird-related HP were evaluated based on the same definition, and the sensitivity and specificity were 48-61% and 73-80%, respectively (Supplemental Table S1). For sIgA, a high correlation (correlation coefficient 0.92-0.97) was observed among the three antigens. When performing the same evaluation for acute and recurrent-type chronic bird-related HP, the sensitivity was 65-74%, and the specificity was 69-78%.

Utility of the measurement of the sIgG and sIgA classes

The titres of sIgG and sIgA against the same antigen had a relatively high correlation for the three antigens (correlation coefficient 0.70-0.77). The evaluation was determined as positive when either sIgG or sIgA was positive, and the evaluation was determined as negative when both sIgG and sIgA were negative. Based on the evaluation, the sensitivity and specificity were 74-96% and 65-91%, respectively. Using the sum of the sIgG and sIgA titres against the same antigen, ROC analysis of the acute bird-related HP group and chronic recurrent-type bird-related HP showed an AUC of 0.85-0.89 (95% confidence interval (CI): 0.78-0.95). The results were similar to those of specific IgG alone (AUC: 0.85-0.89, 95% CI: 0.76-0.96).

Discussion

In the present study, we conducted multi-institutional research to evaluate the clinical performance of measurement of the levels of sIgG and sIgA antibodies against pigeon, parrot and budgerigar antigens by the ImmunoCAP® system.

In the acute bird-related HP group and in the chronic recurrent-type bird-related HP group, sIgG and sIgA levels were significantly higher for all three bird antigens than in the other groups. The results implied that measurement of sIgG/sIgA was an excellent perception method for not only acute bird-related HP[13] but also chronic bird-related HP with acute episodes (recurrent type), discriminating from other ILDs. Based on the cutoff values of sIgG from ROC analysis between these groups and by measuring multiple bird antigens and combining the results of two species, the diagnostic values of sIgG showed high sensitivity and specificity, 85-91% and 73-80%, respectively, indicating that this approach is useful for screening and diagnosing bird-related HP with acute episodes.

Considering the superiority between sIgG and sIgA, the titre of sIgG was higher and had better performance regarding both sensitivity and specificity than sIgA for all three bird antigens, as well as the specific IgG antibody in the acute bird-related HP group and the chronic recurrent bird-related HP group. The correlation between the sIgA and sIgG antibody titres was good (correlation coefficient 0.70-0.77), and the AUCs obtained by ROC analysis using the sum of sIgG and sIgA titres were comparable to the results for the titre of sIgG alone. Therefore, the clinical significance of measuring sIgA in addition to sIgG was considered to be reduced.

On the other hand, there was no significance in any results for the chronic insidious bird-related HP group compared to the control disease group. For chronic insidious bird-related HP, our previous report revealed that the positive rate of sIgG against bird antigens was only 35% (6 out of 17 cases),[1] and in this study, the positive rate of sIgG was relatively low, at 21% for pigeon, 45% for parrot and 28% for budgerigar. This result may be explained by the involvement of the immune response in HP without specific antibodies, i.e., the role of cellular immunity in the pathophysiology.

However, for both types of chronic bird-related HP regardless of the presence or absence of acute episodes, the sensitivity of sIgG was increased to 48-61% by

combining the results of two bird species. This approach is not sufficient for a single screening test of chronic bird-related HP, though it is the only examination available in clinical practice. Furthermore, the sensitivity can be increased by combining other screening methods, such as questionnaires or interviews focused on exposure to birds. Additionally, based on the 97.5th percentile titre of the control groups, the specificity and positive predictive value of sIgG for both types of chronic bird-related HP were high, at 98% and 88-92%, respectively.

In bird-related HP, it is known that an antigen-specific immune response is produced even for different species of birds. On the other hand, it has been suggested that cross-antigenicity exists[12,16] because the amino acid sequence is conserved among bird species.[17] In our previous study comparing sIgG/sIgA against various bird species, pigeons and budgerigars, and both sIgG and IgA showed a high correlation between the two bird antigens.[13] In this study, both sIgG and sIgA had a high correlation with pigeon, parrot, and budgerigar antigens, indicating cross-antigenicity among bird species. Furthermore, measuring sIgG against multiple species simultaneously is useful, as single-positive cases were found in the individual data of the study. Actually, the sensitivity of sIgG using the optimal cutoff from the ROC analysis by single antigen was 70-91% for the acute episode group, but that for multiple antigens was 85-91%.

In conclusion, measurement of the levels of sIgG and sIgA against pigeon, parrot and budgerigar antigens by ImmunoCAP® is useful for screening and diagnosing acute/chronic bird-related HP with acute episodes. Measuring multiple antigens could be useful for screening for both types of chronic bird-related HP.

References

1. Ohtani Y, Saiki S, Sumi Y, et al. Clinical features of recurrent and insidious chronic bird fancier's lung. Ann Allergy Asthma Immunol. 2003;90(6):604-610.
2. Miyazaki Y, Tsutsui T, Inase N. Treatment and monitoring of hypersensitivity pneumonitis. Expert Rev Clin Immunol. 2016;12(9):953-962.
3. Inase N, Ohtani Y, Endo J, Miyake S, Yoshizawa Y. Feather duvet lung. Med Sci Monit. 2003;9(5):CS37-40.
4. Vasakova M, Morell F, Walsh S, Leslie K, Raghu G. Hypersensitivity Pneumonitis: Perspectives in Diagnosis and Management. Am J Respir Crit Care Med. 2017;196(6):680-689.
5. Saltoun CA, Harris KE, Mathisen TL, Patterson R. Hypersensitivity pneumonitis resulting from community exposure to Canada goose droppings: when an external environmental antigen becomes an indoor environmental antigen. Ann Allergy Asthma Immunol. 2000;84(1):84-86.
6. Lacasse Y, Selman M, Costabel U, et al. Clinical diagnosis of hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2003;168(8):952-958.
7. Okamoto T, Miyazaki Y, Ogura T, et al. Nationwide epidemiological survey of chronic hypersensitivity pneumonitis in Japan. Respir Investig. 2013;51(3):191-199.
8. Koike K, Sekiya M, Yae T, et al. [Two cases of familial summer-type hypersensitivity pneumonitis requiring differentiation from bird breeder's lung]. Nihon Kokyuki Gakkai Zasshi. 2009;47(10):947-952.
9. Matsushima H, Oda T, Yamamoto M, et al. [A case of chronic bird fancier's lung in which VATS biopsy was diagnostically useful]. Nihon Kokyuki Gakkai Zasshi. 2007;45(12):982-986.
10. Morell F, Villar A, Montero MA, et al. Chronic hypersensitivity pneumonitis in patients diagnosed with idiopathic pulmonary fibrosis: a prospective case-cohort study. The lancet Respiratory medicine. 2013;1(9):685-694.
11. Miyake S, Hamaoka A, Yoshizawa Y. [Clinical usefulness of antigen-captured ELISA method using mouse anti-Trichosporon asahii monoclonal antibody D-8 for diagnosis of summer-type hypersensitivity pneumonitis]. Nihon Kokyuki Gakkai Zasshi. 2001;39(1):7-11.
12. Rodrigo MJ, Postigo I, Wangensteen O, Guisantes JA, Martinez J. A new application of Streptavidin ImmunoCAP for measuring IgG antibodies against non-available commercial antigens. Clin Chim Acta. 2010;411(21-22):1675-1678.
13. Inase N, Unoura K, Miyazaki Y, Yasui M, Yoshizawa Y. [Measurement of bird specific antibody in bird-related hypersensitivity pneumonitis]. Nihon Kokyuki Gakkai

Zasshi. 2011;49(10):717-722.
14. Ando M, Arima K, Yoneda R, Tamura M. Japanese summer-type hypersensitivity pneumonitis. Geographic distribution, home environment, and clinical characteristics of 621 cases. Am Rev Respir Dis. 1991;144(4):765-769.
15. Yoshizawa Y, Ohtani Y, Hayakawa H, Sato A, Suga M, Ando M. Chronic hypersensitivity pneumonitis in Japan: a nationwide epidemiologic survey. J Allergy Clin Immunol. 1999;103(2 Pt 1):315-320.
16. Sennekamp J, Lange G, Nerger K, Berdel D, Meier-Sydow J. Human antibodies against antigens of the sparrow, blackbird, weaver finch, canary, budgerigar, pigeon and hen using the indirect immunofluorescent technique. Clin Allergy. 1981;11(4):375-384.
17. Shirai T, Furusawa H, Furukawa A, et al. Protein antigen of bird-related hypersensitivity pneumonitis in pigeon serum and dropping. Respir Res. 2017;18(1):65.

Table 1. Demographic and biological characteristics of patients and controls.

		Acute BRHP	Chronic BRHP		Control
			Recurrent type	Insidious type	
Number of patients		29	17	29	64
Male sex, *n* (%)		10 (34)	8 (47%)	18 (62%)	35 (55%)
Age, years, median (IQR)		60 (51-72)	72 (55-74)	71 (64-77)	66 (60-73)
KL-6, U/ml, median (IQR)	*n*	28	17	29	57
		3285 (1495-6193)	2580 (784-4891)	823 (594-1470)	1091 (675-1816)
SP-D, ng/ml, median (IQR)	*n*	22	13	27	45
		519 (191-713)	281 (129-433)	176 (101-387)	171 (103-330)
CRP, mg/dl, median (IQR)	*n*	27	15	29	60
		0.3 (0.1-1.2)	0.7 (0.0-2.0)	0.1 (0.0-0.6)	0.3 (0.1-1.5)

BRHP: bird-related hypersensitivity pneumonitis. HP: hypersensitivity pneumonitis. IQR: interquartile range.

Table 2. Diagnostic accuracy of serum IgG antibody against three bird antigens for acute/chronic bird-related hypersensitivity pneumonitis with acute episodes.

	Cutoff value, mgA/L	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Pigeon	24.6	70	86	78	80
Parrot	14.0	91	77	74	92
Budgerigar	8.7	74	89	83	83

The cutoff value was calculated by ROC analysis between acute/recurrent-type chronic BRHP (n=46) and control diseases (n=64).
NPV: negative predictive value. PPV: positive predictive value.

Table 3. Diagnostic accuracy of serum IgG antibody with each bird antigen combination for acute/chronic bird-related hypersensitivity pneumonitis with acute episodes.

	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Pigeon/Budgerigar	85	80	75	88
Pigeon/Parrot	91	73	72	92
Parrot/Budgerigar	91	73	71	92

A positive result was defined as when either one of the two species antigens was positive. When both were negative, the result was defined as negative. LHR: likelihood ratio. NPV: negative predictive value. PPV: positive predictive value.

Figure legends

Figure 1. Titres determined by ImmunoCAP® of serum IgG (A) and IgA (B) against pigeon, parrot and budgerigar antigens in acute bird-related hypersensitivity pneumonitis patients (n=46), recurrent-type chronic bird-related hypersensitivity pneumonia patients (n=19), insidious-type chronic bird-related hypersensitivity pneumonia patients (n=29), control disease subjects (n=64), and healthy donors (n=146). BRHP: bird-related hypersensitivity pneumonitis.

Figure 2. ROC curves for titres of serum IgG (A) and IgA (B) against pigeon, parrot and budgerigar antigens for acute and recurrent-type chronic bird-related hypersensitivity pneumonitis.

Figure 3. Correlation of the titres of serum IgG determined by ImmunoCAP® between pigeon and budgerigar (A), parrot and budgerigar (B), and parrot and pigeon (C). BRHP: bird-related hypersensitivity pneumonitis. C-BRHP: chronic bird-related hypersensitivity pneumonitis/

Appendices

Supplementary Table S1.

Figure 1

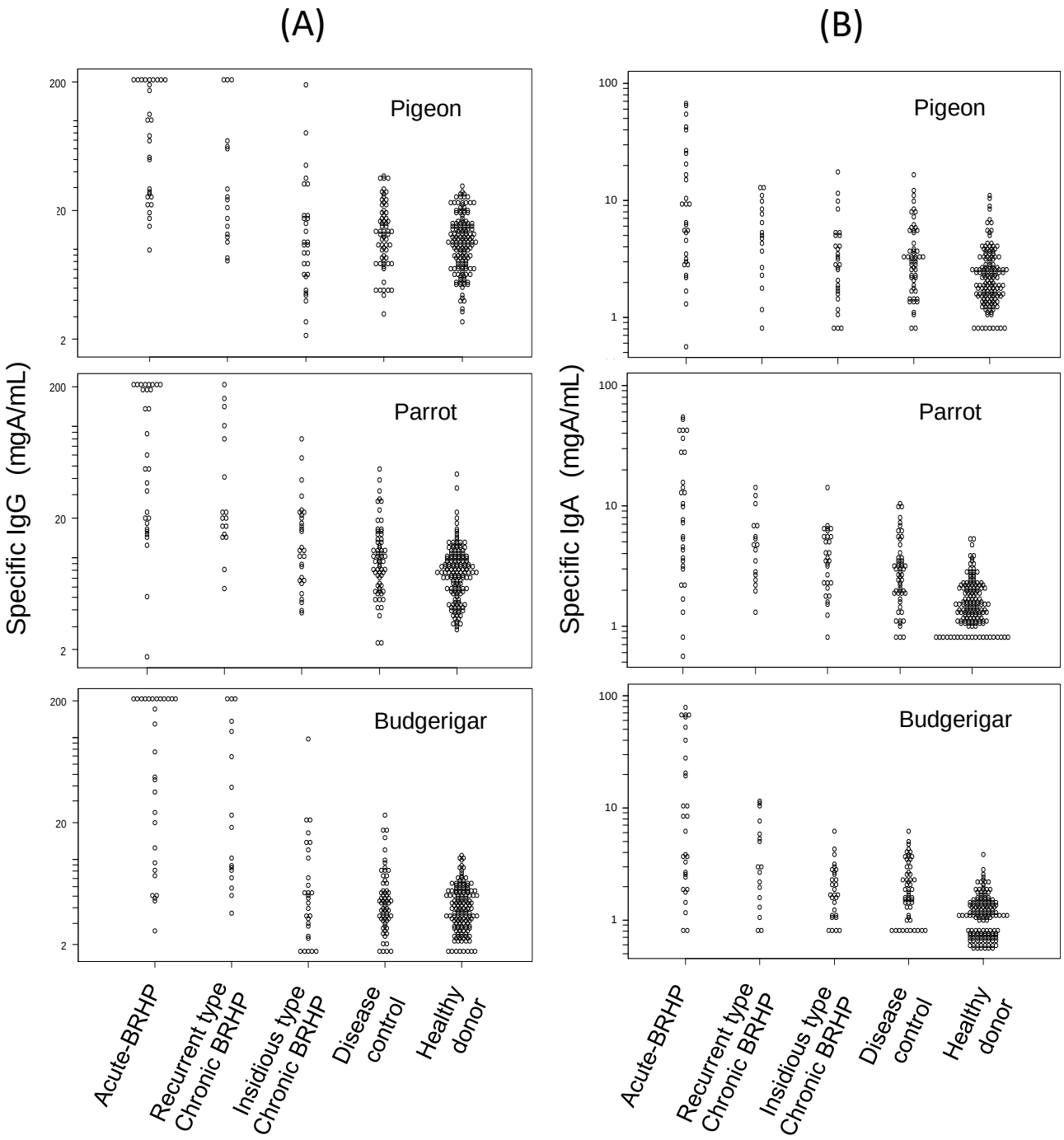
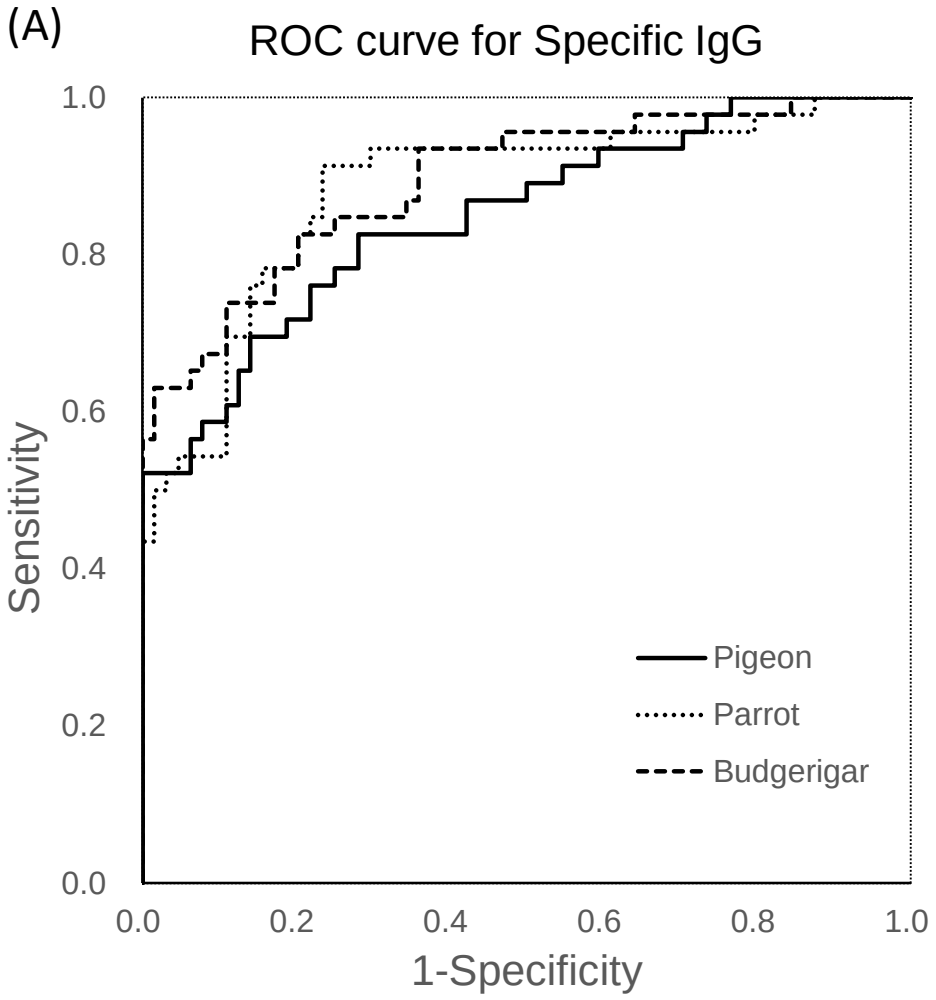

(A)
(B)
Pigeon
Parrot
Budgerigar
Specific IgG (mgA/mL)
Specific IgA (mgA/mL)
200
20
2
100
10
1
Acute-BRHP
Recurrent type Chronic BRHP
Insidious type Chronic BRHP
Disease control
Healthy donor

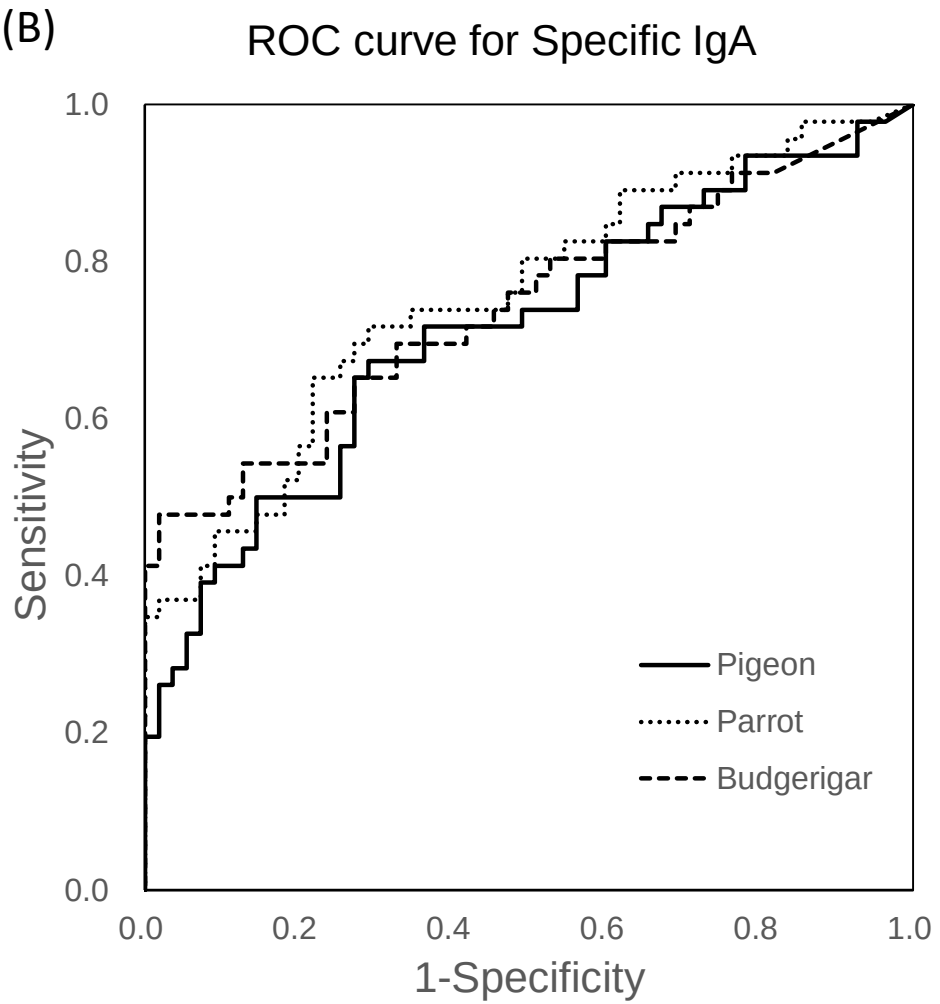
Figure 2

(A)



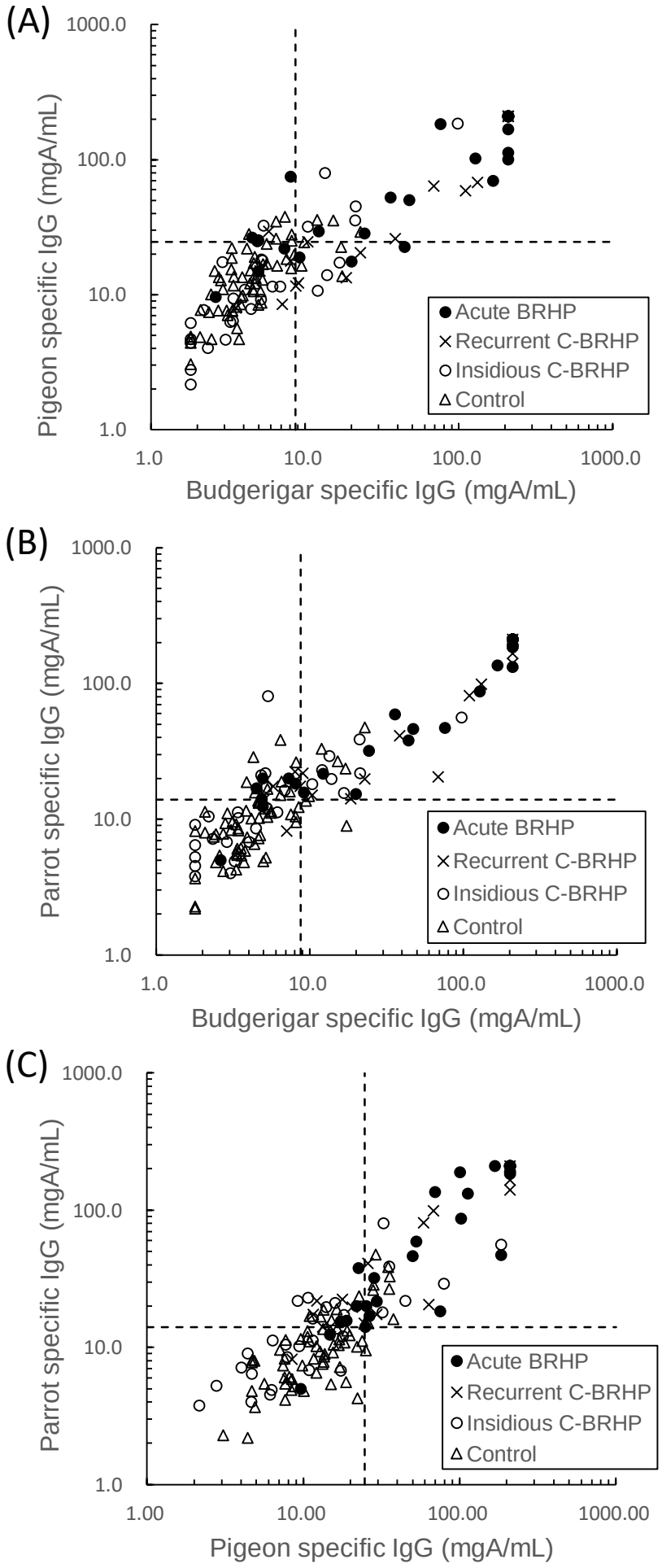
	AUC	95% CI	P-value
Pigeon	0.85	0.78-0.92	<0.001
Parrot	0.88	0.82-0.95	<0.001
Budgerigar	0.89	0.83-0.96	<0.001

(B)



	AUC	95% CI	P-value
Pigeon	0.71	0.61-0.82	<0.001
Parrot	0.76	0.66-0.85	<0.001
Budgerigar	0.74	0.64-0.84	<0.001

Figure 3


(A)
1000.0
100.0
10.0
1.0
Pigeon specific IgG (mgA/mL)
1.0
10.0
100.0
1000.0
Budgerigar specific IgG (mgA/mL)
Acute BRHP
Recurrent C-BRHP
Insidious C-BRHP
Control
(B)
1000.0
100.0
10.0
1.0
Parrot specific IgG (mgA/mL)
1.0
10.0
100.0
1000.0
Budgerigar specific IgG (mgA/mL)
Acute BRHP
Recurrent C-BRHP
Insidious C-BRHP
Control
(C)
1000.0
100.0
10.0
1.0
Parrot specific IgG (mgA/mL)
1.00
10.00
100.00
1000.00
Pigeon specific IgG (mgA/mL)
Acute BRHP
Recurrent C-BRHP
Insidious C-BRHP
Control