Original article :

Quantitation of avian IgG antibodies with clinico-radiological tests in the diagnosis of Bird Fancier's hypersensitivity pneumonitis

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ABSTRACT

Background : The usefulness of serologic testing remains uncertain in our country for DPLD evolving from chronic hypersensitivity pneumonitis in response to avian antigens.

Methods : We performed a retrospective analysis on patients from Eastern India with history of avian exposure and DPLD diagnosed from HRCT chest. They were classified as either "likely chronic HSP" or 'unlikely HSP" based on their clinico-radiological findings. The avian exposure was tested serologically using Ge91 ImmunoCAP mixture with pigeon serum, feathers and droppings (Phadia 100, Thermo Fisher Scientific). We examined the cut-off value of avian specific IgG level to identify patients with 'likely chronic HSP' related DPLD.

Results : Records of 46 patients (26 females, 20 males) with median age 54 years (range 11-85 years) were analyzed. Twenty patients (43.5%) were identified as 'likely chronic HSP' induced DPLD. Their avian precipitin IgG levels ranged between 32.5 and >200mgA/L (median 71.6, IQR25-75 80.8-106.3 mgA/L) and at a cut-off value of 30mgA/L, there was 100% sensitivity and 84.62 % specificity. The other 26 patients with 'unlikely HSP' had median avian IgG level 20.7 mgA/L (0.01-36.2 mgA/L). The difference in medians between the two groups were highly significant ($p<0.00001$). Seven patients with usual interstitial pneumonia pattern in HRCT chest had very high avian IgG levels (>200 mgA/L)..

Inference : Avian precipitin IgG levels >30 mgA/L with supporting clinical history and HRCT findings can confirm HSP related to avian antigen exposure. The highest level of antibody titer was found in the usual interstitial pneumonia pattern on HRCT.

INTRODUCTION :

Hypersensitivity pneumonitis (HSP), or extrinsic allergic alveolitis (EAA), is an inflammatory reaction in the lung interstitium and terminal bronchioles where the repetitive inhalation of microbes / animal proteins / low-molecular weight organic chemicals leads to intense alveolitis. The long standing exposure or repetitive inflammatory insults presents in some patients leads to diffuse interstitial fibrosis or diffuse parenchymal lung disease (DPLD). HSP involving the pulmonary interstitia in response to avian droppings or antigens on bird feathers is referred to as bird fancier's lung, or pigeon breeder's disease/lung, or pigeon fancier's lung [1]. Unlike the immediate hypersensitivity reaction seen in house dust mite allergy that is mediated through IgE antibodies, the IgG antibody mediated responses to inhaled allergens elicit a variety of inflammatory reactions with involvement of T-cell responses through different cytokines, and therefore involve both cell-mediated (as evident on histopathological samples) and humoral (immunoglobulin IgG antibodies in patient sera) immunopathological processes such as in allergic bronchopulmonary aspergillosis [2].

Although some radiological (high resolution computed tomogram, HRCT) features may suggest HSP, the identification of the causative exposure is

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not possible from radiological evaluation. Since the awareness of HSP is negligible in India and since the exposure to the birds antigens (droppings, feathers, serum proteins) are known to cause HSP that can lead to DPLD, it is important to look for the influence of the exposure (to birds and pigeon in particular) to the development of DPLD through determination of specific humoral immunity in terms of the IgG response to pigeon serum, feathers and droppings. Here we present our experience of measuring IgG antibody levels using the automated Phadia 100 analyzer ImmunoCAP Ge91 (mixture of pigeon serum, feathers and droppings) in cases of DPLD patients with history of exposure to birds.

METHODS

It is a retrospective case series analyses with patients been diagnosed DPLD with history of avian exposure. The objective of the study was to determine the optimum level that could denote avian HSP-related DPLD when we used the combined clinical and radiological data from case notes.The avian exposure effect was measured with antigen specific IgG levels.

The diagnosis of DPLD was suspected first from the history of chronic cough and shortness of breath followed by physical examination and documentation of exercise induced de-saturation. It was confirmed by HRCT chest through obtaining cuts with 1-mm collimation at 10-mm intervals from the lung apices to the bases with patient in supine position at full inspiration and expiration. Next, the patients were divided into two groups as 'probable HSP" and "unlikely HSP" induced DPLD based on the presence of changes that are described in HRCT chest to develop from chronic HSP. This decision was done by more than one pulmonologists and radiologists.

The patients of "probable HSP" were included from the radiological characteristics of presence of ground-glass opacities, poorly defined centrilobular nodules, mosaic attenuation and air trapping, and lack of lower zone or predominant mid zone involvement on HRCT. Cases with usual interstitial pneumonia (UIP) and fibrotic nonspecific interstitial pneumonia (NSIP) pattern not related to connective tissue disease on HRCT were included in "probable/likely HSP induced DPLD" as these two forms are well known in HSP. The rest of the DPLD patients were noted as "unlikely HSP induced DPLD".

The patients were subjected to measurement of Pigeon (Columbia livia) or avian specific IgG antibodies using ImmunoCAP Ge91 mixture of pigeon serum, feathers and droppings according to manufacturer's instructions (Phadia AB, Thermo Fisher Scientific, Uppsala, Sweden). Six-point calibration was carried out using ImmunoCAP IgG calibrators traceable to WHO International Reference Preparation IRP67/86. Serum was analyzed at 1 in 100 dilutions. The fluorescence (OD) is proportional to the concentration of serum IgG antibody, titer being calculated from the six-point calibration curve and curve controls were analyzed at every run. The cut-off for significant exposure determined from previous studies was 10 mgA/L [5], where it refers to arbitrary units given the unavailability of International Reference preparation (IRP) for this serum preparation.

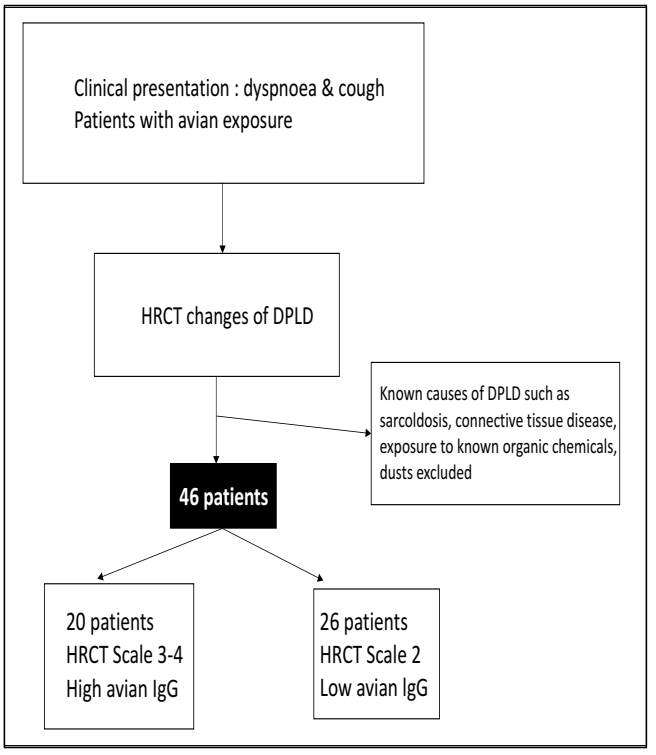
Patients who were found to have evidence of diseases that lead to DPLD such as those with positive collagen vascular disease profile, raised serum angiotensin converting enzyme, or UIP/NSIP pattern on HRCT chest along with history of exposure to several organic and inorganic dusts were excluded in this study. Retrospectively we had taken the details of exposure to birds in most of our patients to determine the actual degree and the circumstances of exposure.

Statistical analysis was done using Microsoft Excel 2013, and non-parametric tests calculated using GraphPad Prism Software Version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Results were expressed as median and inter-quartile range. Student t-test to compare means of the different groups of patients was used and p value <0.01 was considered significant. Different cut-off levels of avian precipitins IgG were analyzed including negative and positive predictive values that would identify significant antigen exposure.

RESULTS

The study layout and the inclusion has been charted below

Flow chart 1:



46 patients with DPLD (20 males, 26 females) with median age of 54 years (range 20-85 years) were studied. Dyspnea and cough were present in 100% of patients on initial evaluation.

Twenty patients (43.5%) were diagnosed or suspected 'probable /likely HSP; they had values of avian precipitin IgG levels between 32.5 and >200 mgA/L with significant radiologic changes on HRCT. The median avian IgG level in these patients was 71.6 mgA/L, interquartile range 25-75 (IQR25-75) at 80.8-106.3 mgA/L. This group of 'probable / likely HSP' were thus diagnosed to have HSP secondary to avian antigens.

26 patients also exposed to avian antigens had low HRCT suggestion for HSP ('unlikely HSP') had shown a median avian IgG level 20.7 mgA/L. The difference in medians between 'probable / likely HSP' and 'unlikely HSP' was highly significant (Student's t-test 2-tailed, equal variance, $p<0.00001$) [Table 1].

Of the "probable /likely HSP" patients, eleven patients had avian IgG levels between 71.2 to >200 mgA/L, of which 7 with extensive honey combing on HRCT suggesting UIP pattern had very high avian IgG levels at >200 mgA/L. All seven patients with levels >200 mgA/L had a history of significant exposure to pigeons (20-200 birds). Two patients with radiological findings of interstitial infiltrates and decreased resting oxygen saturation (SaO2 between 84-95%) had median avian IgG levels 105 mgA/L.

Table 1

n=46	**Number**	**Age range**	**MedianAge (years)**
Males	20	32 – 81 years	53
Females	26	11 – 85 years	55.5
	Number	**Median Avian IgG level(mgA/L)**	**Trimmed Mean (mgA/L)**
Unlikely HSP	26*	20.7	20.2
Possible / likely HSP**	20	71.6	99.0
Very high precipitins (>200 mgA/L)	7		
High value (100-200 mgA/L)	2		
Intermediate range (30-99 mgA/L)	11		

*4 patients had high HRCT Scale at 3 but low avian IgG levels

**All patients had high HRCT Scale 3-4

Using avian IgG levels, the two categories of patients were analyzed using Mann-Whitney U-test (rank 1-3). The low probability of EAA/HSP (n=26, confidence rating 1) patients differed from the moderate to high probability of EAA/HSP (n=20, confidence rating 2 and 3) with median avian IgG values 20.7 and 71.55 mgA/L respectively ina highly significant way (p<0.0001) [Figure 1, values >200 were trimmed at 200]. Using avian IgG cut-off value at 30mgA/L, we derived a yield sensitivity (Se) at 100%, specificity (Sp) at 84.62% with positive predictive value (PPV) and negative predictive value (NPV) as 83.33 and 100% [Table 2].

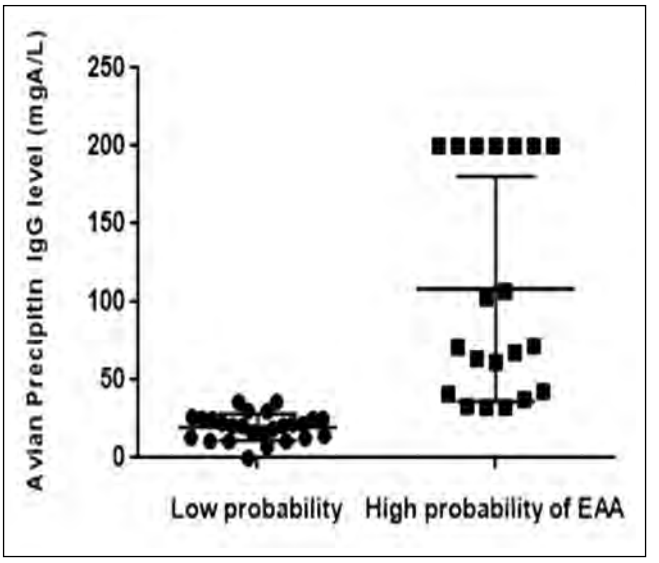


Figure 2

Dot plot of patients with probability of extrinsic allergic alveolitis / hypersensitivity pneumonitis (EAA/HSP) and avian IgG levels Mann-Whitney U- test was used with patients ranked 1-3; low probability of EAA (n=26, confidence rating 1) vs moderate to high probability of EAA (n=20, confidence rating 2 and 3) with median avian IgG values 20.7 and 71.6 mgA/L respectively (two-tailed P value, highly significant, p<0.0001)

Table 2

Predictive value of avian precipitin IgG at different cut-off levels measured using ImmunoCAP 100 (Sensitivity = (true positive/true positive + false negative) x 100; Specificity = (true negative/true negative + false positive) x 100; positive predictive value, PPV = (true positive/true positive + false positive) x 100; negative predictive value, NPV = (true negative/true negative + false negative) x 100)

Predictive value with cut-off at 10 mgA/L			
	Positive avian IgG	Negative avian IgG	
Probable/likely HSP	20	0	Sensitivity = 100% Specificity =7.69% PPV = 45.45 NPV = 100
Unlikely HSP	24	2	
Predictive value with cut-off at 20 mgA/L			
	Positive avian IgG	Negative avian IgG	
Probable/likely HSP	20	0	Sensitivity = 100% Specificity = 42.31% PPV = 57.14 NPV = 100
Unlikely HSP	15	11	
Predictive value with cut-off at 30 mgA/L			
	Positive avian IgG	Negative avian IgG	
Probable/likely HSP	20	0	Sensitivity = 100% Specificity = 84.62% PPV = 83.33 NPV = 100
Unlikely HSP	4	22	

DISCUSSION

This retrospective case series analysis provides an insight into the prevalence of extrinsic allergic alveolitis in this part of India, and the usefulness of the ImmunoCAP technology (fluoroenzyme immunoassay, FEIA) in the management of this condition.

The British Thoracic Society recommended in its publication in 1999 of the requirement of testing for antibody against HSP-associated antigens in the evaluation of interstitial lung disease [3]. Part of the difficulty in establishing a laboratory diagnosis lies in the absence of robust reproducible assays due to (1) the requirement of validated allergen source that is critical for the assay to be successful; and (2) the 'quality' or in-vivo 'function' of the antibody identified in the assays. The original 'precipitin' test utilizes the Ouchterlony double diffusion technique in agar, where sufficient time is given for the antigen and antibody to diffuse in the cut-out agar wells, and the number of antigen-antibody precipitation lines ('precipitin') are read or counted using indirect lighting and microscope eyepiece. This test relies on good quality antigen source, agar gel preparation and training of the observer to identify the correct lines (excluding lines of non-identity).Hence, there is lowering the sensitivity and high inter-observer variability [4]. A viable alternative is the quantitative automated fluorimetry method which utilizes a solid-phase indirect enzyme immune-assay (Phadia, Thermo Fisher Scientific) where antigens are immobilized onto cellulose allowing them to retain their 3-dimensional configuration (ImmunoCAP), increasing the sensitivity and specificity. Several different avian ImmunoCAPs such as pigeon serum(Ge93), droppings (e7), feathers (Re215), budgerigar serum (e79), droppings (e77) and feathers (e78) have been evaluated on this platform.

The British Thoracic Society guidelines do not discuss in details the technical or the laboratory quality control aspects required to accurately identify patients with HSP, and heavily rely on clinical/radiological parameters. This study also demonstrates that the acumen for clinico-radiologic suspicion of HSP is very high in our pulmonologists and radiologists on evaluation of HRCT jointly. However, in the absence of a universal 'cut-off' makes for IgG antibody to avian antigen, it remained difficult for clinicians to differentiate the between 'idiopathic' ILD and definite HSP. This cut-off will invariably vary between geographical locations due to amount of antigen exposure (i.e., bird keepers versus daily bird feeders), quality of air (i.e., air pollution) and on other factors. The effect of air pollution on IgG response to such antigens remains unknown. Previous studies have sought 10 mg/L as the optimal cut-off of avian precipitin IgG using immunoassays for the diagnosis of HSP. The 10 mg/L value was based on the upper quartile levels of 8.9 mg/L from pathological control samples (n=17), and 11.1 mg/L in the pigeon fanciers (n=50) who were unlikely to have HSP [5]. However, individual case management and exposure profiles in our clinical experience seemed 10 mg/L as a very low cut-off (i.e., several false positives) particularly without significant lung changes on HRCT or typical clinical symptoms profiles (either severe persistent asthma or a history atypical of HSP/EAA).

This study was therefore undertaken to identify whether (1) a cut-off value can be identified in avian HSP, and (2) the usefulness of quantitative ImmunoCAP (FEIA) technology in helping establish this cut-off in our patients exposed to varying amounts of avian antigens. Patients with severe dyspnea and lung damage on HRCT suggesting UIP pattern had very high avian IgG levels >200 mgA/L. It indicated towards probably a constant and significant amount of antigen exposure. Therefore, to our mind, patients with UIP pattern should be kept on high priority in the consideration evaluation for chronic HSP in our country. Incidentally, the patients with UIP pattern in HRCT chest tend to receive a diagnosis of idiopathic pulmonary fibrosis (IPF) and escape further evaluation as open lung biopsy, they no longer receive the benefit of a re-look at the diagnosis and treatment for HSP.

In our series, apart from the revelations as

mentioned above, we have noticed that the younger patients typically gave a history of more intimate exposure to birds and the symptoms started to develop within 6 months of exposure. The slightly older patients had often a history of birds staying outside on the air-conditioning units or pigeons used to come to be fed on the windows for short periods regularly; they used to developed symptoms after a few hours of exposure as described in acute hypersensitivity pneumonitis. These were not considered significant exposure by patients and therefore not communicated to doctors during their regular 'asthma' visits. In some of them the antigen exposure history was not taken by doctors and sometimes a diagnosis of allergic bronchopulmonary aspergillosis was thought of. Incidentally, in none of such patients in our record had positivity for Aspergillus fumigatus precipitin IgG (i.e., above the threshold of 40mgA/L)(data not shown). It may be worthwhile to note that many of these patients were not considered DPLD outside and they communicated to the doctors with an impression of suffering from 'asthma'. Furthermore, the treatment history was also largely unknown as many of them could have taken steroid before visiting the hospital.

Our study has been able to establish that a higher cut-off at 30mgA/L shows a better assay characteristics with sensitivity and specificity at 100% and 84.62% respectively. Previous studies had shown that patients with possible HP had a median level of 70 mgA/L, and those with probable HP had a median level of 135 mgA/L [5], the latter tallies to our observation (patients with moderate to high probability of EAA/HSP had with median avian IgG value of 71.6 mgA/L). Although pigeon serum has been noted to be the cleanest and most comprehensive avian antigen for testing sensitivity to most common pet bird species [6], we used a mixture of all three antigen sources in our study. This assay has been robustly standardized against an international IgG standard and it can be claimed to be more sensitive than the more commonly used precipitin formation test for which the threshold for the presence of antibody being approximately 40 ug/ml. Therefore, the increased sensitivity of our assay may better identify the patients with antibody level below this mentioned one, with subacute disease or with disease of insidious onset. Possibly, all of these patients would have been missed on agar gel-based precipitin formation assays. It is not impossible that we might have missed some real HSP from the avian exposure with insidious onset. Lung biopsy and histo-pathological examinations would have certainly been the ideal for us to do. However, a keen follow up of the 'unlikely HSP' patients with continuation of the exposure with repetition of the same IgG estimation periodically may make us wise in the face of the lack of invasive diagnosis. Other tests such as serum C-reactive protein, TNF-α and total IgG or bronchoalveolar lavage fluid cellular assessments may be helpful as raised values would indicate active systemic inflammation, or physiological evidence of lung inflammation including increased alveolar permeability and bronchial wall thickness [7-11].

The study has several limitations such as (1) being retrospective in nature, only selected patients were included in the study; (2) the exact prevalence of chronic HSP as a percentage of DPLD patients cannot be ascertained; (3) details of the quantity and duration of exposure was not documented even though exposure was universal; (4) avian specific IgG levels were not monitored longitudinally. The strong suspicion of chronic HSP was actually forthcoming from the history on routine questioning in out-patient practice. We believe that a stringent protocol based approach should be used in future studies. It is possible that some patients could escape inclusion as all the clinicians concerned were possibly not equally aware or inclined to suspect chronic HSP or have not given attention to the history of exposure to birds. The referral for the test was largely individual decision and not out of observation of a protocol. Longitudinal studies are required in all 'at-risk' patients or those with UIP pattern and low-titre antibodies on initial testing (avian IgG levels <30 mgA/L).

CONCLUSIONS:

Measurement of avian specific IgG antibody should be considered as an important investigation in the evaluation algorithm of diffuse pulmonary lung disease in India especially when there remains a history of exposure to birds.

The UIP pattern on HRCT had the highest titer of antibody and this radiologic finding should prompt clinicians to enquire into the possibility of hypersensitivity pneumonitis-induced diffuse pulmonary lung disease.

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