



Communication

The Role of Serum IgG Precipitins against Six Typical Organic Antigens Involved in Hypersensitivity Pneumonitis: A 10-Year Retrospective Study of a Referral Interstitial Lung Disease Centre

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Abstract: Hypersensitivity pneumonitis (HP) represents the third common interstitial lung disease caused by an exaggerated immune response following the inhalation of organic and/or chemical environmental antigens. The aim of this study was to determine the cut-off values of specific IgG antibodies (named precipitins) and their association with clinical data in the diagnosis of HP. In this 10-year retrospective study, the IgG concentrations against six antigens, *Penicillium chrysogenum/notatum*, *Aspergillus fumigatus*, *Alternaria alternata*, *Aspergillus niger*, *Micropolyspora faeni*, and pigeon droppings, were retrieved. The controlled group was made of 1516 healthy subjects without diagnosis of lung pathologies, while the case group consisted of 54 individuals affected by HP. Considering all six IgG antibodies together and the 97.5% percentiles determined in the control group, 30 of 54 subjects (56%) had one or more positive precipitins. In these patients, the major frequencies found were IgG antibodies against pigeon droppings, followed by *Penicillium chrysogenum/notatum* and *Aspergillus niger*. Although the sensitivity of serum precipitins depends on the population enrolled and the method used, the cut-off values determined in this study can be a valuable tool for clinicians in the diagnosis of HP, in eliminating the antigens responsible from the environment, and in establishing more specific IgG panels.

Keywords: hypersensitivity pneumonitis; IgG antibodies; fungal antigens; precipitin; extrinsic allergic alveolitis



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1. Introduction

Hypersensitivity pneumonitis (HP) is a complex immune-mediated disease that involves the lung parenchyma and respiratory tract of susceptible and sensitised subjects in response to a repeated and prolonged inhalation of several different antigens or mixtures of antigens. Actually, there are more than 200 antigens in the workplace, at home, and in recreational activities known to be a possible cause of HP, and they can be divided into two broad groups: (I) organic antigens, such as from bacteria, fungi, animals, or plant proteins; (II) inorganic antigens, such as low-molecular-weight chemicals or metals [1–3]. In HP, following antigen exposure, inflammation is mediated by humoral and cellular mechanisms, particularly by T-helper cells and antigen-specific immunoglobulin (IgG) antibodies, determining an accumulation of lymphocytes and the formation of granulomas [4]. Recently, the American Thoracic Society (ATS), the Japanese Respiratory Society

(JRS), and the Asociación Latinoamericana del Tórax (ALAT), together with European and Australian experts in HP, and the American College of Chest Physicians (CHEST), published two guidelines that propose to categorise the patients as having non-fibrotic HP (purely inflammatory state) or fibrotic HP (coexisting inflammation and sign of fibrosis or purely fibrotic) [5,6].

The search for exposure using quantitative measurements of immunoglobulin G (IgG) against environmental antigens is important for the diagnosis of HP. The recent guidelines include serum-specific IgG (named precipitins) testing as one of the diagnostic criteria in the algorithm of HP [5]. Different methodologies, such as precipitation, immunoblots, enzyme-linked immunoassays, and agglutination, are used for the determination of specific IgG. However, the lack of standardisation between the different methods makes the comparison between the results difficult, and the absence of well-established cut-off values cannot be useful in the discrimination between normal or pathological conditions. Therefore, in this retrospective study, using a large amount of laboratory data, the aim was to establish reliable population-based cut-off values of IgG antibodies against six typical organic antigens, such as *Penicillium chrysogenum/notatum*, *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Micropolyspora faeni*, and pigeon droppings, and to assess the role of precipitins in the diagnosis of HP.

2. Materials and Methods

2.1. Patients and Study Design

In this 10-year retrospective study, the data of the subjects with immunoglobulin G (IgG) concentrations against six antigens (*Penicillium chrysogenum/notatum*, *Aspergillus fumigatus*, *Alternaria alternata*, *Aspergillus niger*, *Micropolyspora faeni*, and pigeon droppings) that have a potential causative role in hypersensitivity pneumonitis were retrieved from the database of the Clinical Laboratory of Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy, from 1 January 2010 to 31 December 2021. In the case of multiple IgG determinations of an antigen in one subject, only the first one was used for the analysis. The database was divided into two groups: (I) healthy subjects without a diagnosis of interstitial lung disease and/or pulmonary aspergillosis prior to and after the date of IgG tests; (II) patients diagnosed with HP. The patients with no clinical data and/or without a diagnosis were excluded from the analysis.

According to current guidelines, the diagnosis of HP requires a multidisciplinary discussion (MDD) approach based on clinical, radiologic, and, in some cases, bronchoalveolar lavage (BAL) lymphocytosis and histopathologic findings [5,6]. Recently, the clinical practice guidelines (ATS/JRS/ALAT) for the diagnosis and management of HP developed by the American Thoracic Society (ATS), the Japanese Respiratory Society (JRS), and the Asociación Latinoamericana del Tórax (ALAT), and the guidelines released by the American College of Chest Physicians (CHEST), are an extremely important aid for clinicians to improve diagnostic decision-making for new HP and decrease diagnostic variability [5,6]. Based on the CHEST guidelines, a confident diagnosis of HP can be made in subjects who have an identified exposure and a typical HP pattern on a high-resolution computed tomography (HRCT) scan, whereas for ATS/JRS/ALAT guidelines, a confident diagnosis of fibrotic HP also requires evidence of BAL lymphocytosis. In subjects with suspicion of fibrotic HP that did not fulfil the criteria listed above, suggestions were made in favour of obtaining a lung cryobiopsy or a surgical lung biopsy. The typical fibrotic HP pattern is characterised by lung fibrosis with mid-lung zone predominance and at least one abnormality that is indicative of small airway disease (centrilobular nodules or mosaic attenuation). The typical non-fibrotic HP pattern requires the presence of HRCT abnormalities indicative of diffuse parenchymal infiltration (ground glass or mosaic attenuation) and HRCT abnormalities indicative of small airway disease (centrilobular nodules or air trapping).

2.2. Determination of Specific IgG

Sera were collected by venous blood sampling and stored at 2–8 °C. Specific IgG of the six antigens was quantified using the Immulite® 2000/2000XPi Immunoassay system (Siemens Medical Solutions Diagnostics, Malvern, PA, USA), following the manufacturer's instructions. This allergen-specific IgG procedure is a solid-phase, two-step, chemiluminescence immunoassay. There are no cut-off values defined by the manufacturer for each allergen. Concentrations are expressed in milligrams of IgG per litre (mg/L). The range recommended by the manufacturer is 2–200 mg/L, with a limit of detection of 2 mg/L. In cases of concentration values less or greater than the limits of detection, they were reported as <2 and >200 mg/L, respectively, and no dilution was performed.

2.3. Statistical Analysis

IgG results were expressed as median, range minimum/maximum, and 95th and 97.5th percentiles of antibodies values. Statistical analyses were performed using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). A Chi-squared (χ^2) test was used to compare the results obtained in the different groups, and it was performed using MedCalc for Windows, version 19.4 (MedCalc Software, Ostend, Belgium). Concentration values reported as <2 and >200 mg/L were assigned the values 2 and 200 mg/L, respectively. A p -value < 0.05 was considered statistically significant.

3. Results

We retrieved a total of 9420 values, divided into 1614 for *Penicillium chrysogenum/notatum*, 1454 for *Alternaria alternata*, 1904 for *Aspergillus fumigatus*, 1554 for *Aspergillus niger*, 1464 for *Micropolyspora faeni*, and 1430 for pigeon droppings (Table 1). The sera used for the determination of specific IgGs were collected from 1570 subjects, 912 (58.1%) females and 658 (41.9%) males, with a median age of 68 ± 13 years (range: 5–90 years). Of them, 50% were older than 70 years and only 17 (0.01%) subjects were younger than 18 years. The concentrations of specific IgG varied from 2 to 200 mg/L (Table 1).

Table 1. IgG concentrations against six typical environmental organic antigens known to be a possible cause of HP.

Antigen Source	Subjects without a Diagnosis of HP and/or Pulmonary Aspergillosis				Subjects Affected by HP				p -Value *
	Values (n)	Median (mg/L)	95th Percentile (mg/L)	97.5th Percentile (mg/L)	Values (n)	Median (mg/L)	95th Percentile (mg/L)	97.5th Percentile (mg/L)	
<i>Penicillium chrysogenum/notatum</i>	1560	13.6	49.0	71.0	54	52.0	149.0	161.0	<0.001
<i>Aspergillus fumigatus</i>	1850	10.2	38.0	61.8	54	42.0	142.0	157.0	<0.001
<i>Alternaria alternata</i>	1400	9.4	25.7	35.3	54	39.0	71.0	72.0	<0.001
<i>Aspergillus niger</i>	1500	14.3	34.3	44.3	54	59.8	74.0	75.0	<0.001
<i>Micropolyspora faeni</i>	1410	6.3	15.2	20.5	54	14.9	43.0	46.0	<0.001
Pigeon droppings	1376	22.7	50.6	62.4	54	54.0	173.0	184.0	<0.001
Total (n)	9096				324				

* Comparison between the median values between the two groups. A p -value < 0.05 was considered statistically significant; see the *Materials and methods* section for details.

Group I was composed of 1516 healthy subjects, 893 (58.9%) females and 623 (41.1%) males, with a median age of 66 ± 13 years. Group II was composed of 54 subjects diagnosed as being affected by HP, 19 (58.9%) females and 35 (41.1%) males, with a median age of 70 ± 12 years. Non-parametric methods were performed for the analysis due to the differences between raw data and normal distribution, and the results were reported as medians and quantiles.

In group I, low levels of IgG antibodies were found for *Micropolyspora faeni* and *Alternaria alternata* (median concentrations of 6.3 and 9.4 mg/L, respectively), whereas the highest levels were found for the pigeon dropping antigens (median value 22.7 mg/L).

The 97.5th percentiles of IgG antibodies varied from 20.5 mg/L for *Micropolyspora faeni* to 71.0 mg/L for *Aspergillus fumigatus*. The 97.5th percentile of *Aspergillus niger* was significantly different from that determined for *Aspergillus fumigatus* ($p < 0.001$). The 97.5th percentiles of IgG antibodies for *Penicillium chrysogenum/notatum* and pigeon droppings are similar ($p > 0.05$).

In group II, low levels of IgG antibodies were found for *Micropolyspora faeni* and *Alternaria alternata* (median values of 14.9 and 39.0 mg/L, respectively), whereas the highest levels were found for *Aspergillus niger* (median value 59.8 mg/L). The 97.5th percentiles of IgG antibodies varied from 46.0 mg/L for *Micropolyspora faeni* to 184.0 mg/L for pigeon droppings. The 97.5th percentile of *Aspergillus niger* was significantly different from that determined for *Aspergillus fumigatus* ($p < 0.001$). The 97.5th percentiles of IgG antibodies for *Penicillium chrysogenum/notatum* and *Aspergillus fumigatus* are similar ($p > 0.05$).

We examined whether the 97.5th percentile determined in group I could aid in the diagnosis of HP. Taking into account all six IgG antibodies considered together, we found that, out of the 54 subjects in group II, 30 subjects (56%) had one or more IgG antibody concentrations greater than the 97.5th percentile values determined in group I. Out of these 30 patients, 23 had high concentrations of IgG antibodies against pigeon droppings, followed by *Penicillium chrysogenum/notatum* ($n = 14$) and *Aspergillus niger* ($n = 9$). If we used the 97.5th percentile determined in group I, the number of HP-positive subjects increased to 40 (74%).

The data concerning the exposition of the 54 HP subjects were retrospectively retrieved from the medical history of the patients. Out of them, 43 (80%) were smokers, and 20 (37%) documented an exposure to organic and chemical allergens due to the type of work, such as butcher, waiter, cook, mechanic, and painter, or to the presence of moulds in the house. Moreover, 18 subjects reported the use of feather products, such as pillows and down jackets, in agreement with our results obtained.

4. Discussion

The present study showed that in 30 of 54 HP patients, specific IgG values were greater than the determined cut-off values. It is known that a small number of subjects exposed to environmental antigens are affected by HP. In fact, most individuals are only sensitised since the disease occurs when there is an interaction between inducing factors and host genetic and biochemical background, leading to a persistent inflammation state due to an unappropriated and dysregulated immune response [1–3]. Reviewing the literature, different studies tried to establish cut-off or reference values of antigen-specific IgG antibodies in the context of HP diagnosis [7–10]. However, the antigens considered and analysed, the criteria used to define cut-off values, the methods carried out, and the number of control subjects differed between these works, making it difficult to compare and standardise. Raulf and coauthors updated and established, for the first time, reference values of IgG against 32 typical HP antigens, starting from 121 healthy subjects without declared exposure antigens and using a fluorescent enzyme immunoassay methodology. The 97.5th quantiles for *A. fumigatus* was 140.6 mg/L, 12.2 mg/L for *Alternaria alternata*, and 85.6 mg/L for *Penicillium chrysogenum/notatum*. Then, they implemented an online calculator to evaluate specific IgG measurements, but it was useful only in the case of the same method for the determination of antigens [7]. In a retrospective study, Szturmowicz and coauthors compared the results of specific IgG against different organic antigens (bird antigens and thermophilic bacteria) by the double diffusion agar gel technique obtained in 102 subjects considered as the control group and those obtained in 128 HP patients. They observed the presence of specific IgG antibodies in 57% of all HP patients, compared to only 7% in the control group [8]. On the other hand, Sennekamp and coauthors retrospectively evaluated the ranges of specific IgG antibodies against 32 inhalant environmental antigens in 47,200 patients with suspected HP or bronchopulmonary mycosis using a fluorescent enzyme immunoassay methodology. The lower specific IgG ranges were associated with physiological conditions, whereas higher ranges correlated with HP and

bronchopulmonary mycosis. The major IgG antibodies associated with HP were recorded for pigeon's antigens (28%), followed by *Aspergillus fumigatus* (25%), budgerigar's antigens (23%), and *Penicillium chrysogenum/notatum* (11%) [10]. Therefore, using this six-antigen profile, our results of prevalence are in agreement and very similar with those reported in the literature. However, it is also well known that healthy individuals could have positive specific IgG levels and that the identification and quantification of these antigens are limited by their variability among local and regional sites. In a future perspective, increasing the number of specific IgG antibodies tested, including occupational or chemical antigens, bacteria, and other mould antigens, such as *Acremonium kiliense*, *Aureobasidium pullulans*, *Saccharopolyspora rectivirgula*, and *Thermoactinomyces vulgaris*, might aid in the diagnosis of HP in subjects that present clinical signs of this lung disease but low levels of the six precipitins initially considered.

Although our work was based on a large amount of data, which represents a strength, it presents the limitation that this work is a single-centre study, and a multicentre study is needed in order to verify and improve the accuracy of our results. Moreover, we performed our analysis using a solid-phase chemiluminescent immunoassay that, compared to the fluorescence enzyme-linked immunoassay (FEIA), has been reported to be similar, but clinicians have to consider that the methodologies applied are different [11].

5. Conclusions

Collectively, although the sensitivity of specific IgG antibodies depends on the population enrolled and the method used, we determined six cut-off concentrations of IgG antibodies against the most common inhalant antigens, such as 71.0 mg/L for *Penicillium chrysogenum/notatum*, 61.8 mg/L for *Aspergillus fumigatus*, 35.3 mg/L for *Alternaria alternata*, 44.3 mg/L for *Aspergillus niger*, 20.5 mg/L for *Micropolyspora faeni*, and 62.4 mg/L for pigeon droppings, which can aid clinicians in the diagnosis of HP and in eliminating the antigens responsible for lung pathology from the environment.

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