Specific Serum Immunoglobulin G (IgG) Levels Against Antigens Implicated in Hypersensitivity Pneumonitis in Asymptomatic Individuals

Dear Editor,

Hypersensitivity pneumonitis (HP) is a complex syndrome resulting from repeated exposure to a variety of organic particles. It was previously thought to be uncommon, with an incidence of 0.9 per 100,000 person-years,[1] but a recent retrospective case-cohort study demonstrated that in a population of patients initially diagnosed with idiopathic pulmonary fibrosis, up to 43% may be reclassified as HP.[2] This highlights the challenges in making a diagnosis of HP in patients who have presented with an undifferentiated interstitial lung disease, particularly when the clinical history of allergen exposure cannot be elicited. There is currently no universally agreed upon diagnostic criteria for HP. Vasoka et al has proposed a novel classification system and diagnostic algorithm wherein the diagnosis of HP may be made confidently if there is positive exposure history to a known allergen along with typical radiological and bronchoalveolar lavage findings. In the setting of negative or uncertain history of exposure to a known allergen, serum specific immunoglobulin G (IgG) may be used as a surrogate for said exposure history to support a diagnosis of probable or possible HP.[3]

Serum specific IgG remains the main method of demonstrating causality in HP, and therefore has a role to play in patient counselling and antigen avoidance. Worldwide, the most commonly implicated antigens are thermophilic actinomycetes species, fungi, and bird proteins.[4-7]

Identification of positive serum specific IgG facilitates performance of a specific inhalation challenge if the diagnosis of HP remains to be in doubt. Normal individuals may have elevated levels of specific IgG directed against common causes of HP without significant disease or long-term sequelae.[8] Conversely, in patients who present with unclear exposure history, the absence of elevated serum specific IgG directed against particular exposures may help to reduce the diagnostic probability of HP being due to those exposures. Diagnostic certainty and antigen identification is paramount as early diagnosis and exposure avoidance are cornerstones to disease management.

In Singapore, there was previously no laboratory that performed specific serum IgG directed against antigens commonly implicated in HP. A commercial kit, ImmunoCAP Specific IgG (Phadia Laboratory Systems, Thermo Scientific, Sweden), is available for testing of some antigens implicated in HP. Detection limits of 2.0 to 200 mgA/L are furnished in the product manual, but reference ranges specific for particular IgG directed against specific antigens are not provided.

This study aimed to test for these specific serum IgG levels in asymptomatic individuals, thereby enabling the establishment of operationally ready reference ranges for a panel of specific serum IgG antibodies directed against antigens commonly implicated in HP for use within our institutional laboratory.

Materials and Methods

Healthy volunteers who work in, and are employees of the Department of Respiratory and Critical Care Medicine in a tertiary care university-affiliated academic hospital, as well as inpatients and outpatients seen by the same department, who did not have a clinically suspected diagnosis of HP were included in the study.

Patients requiring intensive care unit or high dependency admission were excluded. The study was approved by the local Institutional Review Board (CIRB Ref 2016/2735). Vulnerable persons including intellectually impaired and incarcerated persons, as well as pregnant patients were excluded from the study.

Five millilitres of blood was prospectively drawn from each of the study participants by routine venipuncture after obtaining informed consent. The blood specimens were centrifuged at 3000 rpm for 10 minutes and sera separated into sterile serum tubes and kept at -20°C until processing. Sera were then tested for a panel of 9 specific IgG antigens implicated in HP using the commercial kit ImmunoCAP Specific IgG (Phadia Laboratory Systems, Thermo Scientific, Sweden).

ImmunoCAP assays were fluoroenzymeimmunoassays. Specific IgG antibodies from study subjects' sera bind to antigens that had been fixed on a cellulose derivative. After washing away non-specific IgG antibodies, fluorescent enzyme-labelled antibodies directed against IgG were added to form a complex. Excess enzyme anti-IgG antibodies were washed away, and the bound complex was incubated with a developing agent. At the end of the reaction, the fluorescence of the final complex was measured in order

to quantify the amount of specific IgG that were present in the sample.

Demographic information including age, gender and ethnicity were collected from each of the study participants.

The authors' approach to sample size calculation followed recommendations from EP28-A3C Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, 3rd Edition.[9]

As there were no pre-existing local studies of serum specific IgG levels in asymptomatic individuals, mean reference values and standard deviations are not available for further power calculations. This study is hence intended as a pilot study for larger population studies to determine a range of normal values, or for future case-control studies to determine a range of possibly abnormal values. An estimated sample size of 120 was taken to achieve a 90% confidence limit.

Results

The mean age of the study participants was 42.6 years (standard deviation 15.3). Forty-two (35%) of them were male. The study participants were distributed amongst Chinese (50.8%), Malay (9.2%), Indian (25.8%) and other ethnic groups (10.0%).

Levels of IgG directed against various antigens implicated in HP are presented in Table 1 as mean with standard deviation.

Analysis for statistical significance was conducted using paired 2 tailed t-tests. The levels of IgG directed against *Aspergillus fumigatus* and *Candida albicans* were significantly higher than levels of IgG antibodies directed against other antigens (P <0.01). Levels of IgG directed against *Aspergillus fumigatus* were also significantly higher than those directed against *Candida albicans* (P <0.01).

Table 1. Mean Levels of IgG Directed Against Antigens Implicated in Hypersensitivity Pneumonitis

Antigen	Serum Level of Specific IgG (mg/L)
Penicillium chrysogenum, Cladosporium herbarum (homodendrum), Mucor racemosus, Alternaria alternata	9.59 + 7.33
Micropolyspora faeni, Thermoactinomyces vulgaris	7.31 + 5.11
Stachybotrys atra	4.80 + 5.34
Aspergillus fumigatus	30.9 + 31.7
Candida albicans	47.3 + 43.0
Aureobasidium pullulans	5.46 + 5.13
Budgerigar serum proteins, feathers and droppings	6.40 + 4.62
Parrot serum proteins, feathers and droppings	14.4 + 9.26
Pigeon serum proteins, feathers and droppings	12.3 + 9.63

Data is presented as mean + standard deviation.

Discussion

There is currently no published data on the prevalence and incidence of HP in Singapore. International data and experience suggests that HP is underdiagnosed and undertreated, especially the chronic form of the disease.[10] This has been postulated to reflect the lack of unified diagnostic criteria and variations in practice,[11] as well as challenges in the overall diagnostic process.

Serum specific IgG antibodies can provide objective evidence of sensitisation of individuals to certain allergens, but cannot be used in isolation to diagnose HP, as sensitised individuals may have elevated levels of serum specific IgG without developing clinically significant disease.

'Normal' levels of IgG against different antigens depend on the prevailing level of exposure within a population, and are likely to vary in different populations in accordance with different environmental exposures. In our study population of 120 subjects not known or suspected to have HP, levels of IgG antibodies directed against *Aspergillus fumigatus* and *Candida albicans* were significantly higher than levels of IgG antibodies directed toward other antigens commonly implicated in HP. This study was intended to sample individuals without clinical suggestion of HP in order to provide operationally ready reference ranges for use within the institutional laboratory, but has highlighted that significant variations in sensitisation and hence exposure exist even within an asymptomatic population. It is, hence, vital for individual laboratories to establish reference ranges within their own populations.

Our study has several limitations. Firstly, this was a single centre study where a large proportion of the study volunteers were employees within a single healthcare institution. This may contribute a significant source of selection bias as these individuals are likely to share a significant number of environmental exposures. Study subjects were also not age-matched, and there were no patients with HP to form a control group. We are, therefore, unable to determine the sensitivity or the specificity of our findings. Secondly, the mean age of the study population was 42.6 years old. Experience from the HP study by Lacasse et al in 2003 suggests that patients with HP present at a mean age of 46 to 50 years old. As such, our study population is younger than the at-risk population and may not be fully representative.[12] Thirdly, there were no criteria for selecting or excluding subjects with any particular hobbies that would predispose them to exposure to antigens relevant to HP such as bird keeping or agricultural work.

Nevertheless, with the completion of our study, testing for serum specific IgG directed against a panel of antigens commonly implicated in HP is now available locally. Alongside the international call to greater consideration and recognition of HP amongst patients presenting with undifferentiated diffuse interstitial lung disease, we hope this is an important step towards facilitating the diagnostic process for patients suspected of having HP in Singapore.

Conclusion

Our study demonstrated that significant variations in sensitisation and exposure exist even within an asymptomatic population and highlighted the importance for individual laboratories to establish reference ranges within their own populations. As this was a pilot study, the standard deviations for the reference values generated are large, implying that the true mean of the parametric or non-parametric distribution of normal values remains uncertain. Larger studies are therefore required to determine the 95% confidence intervals for normal values of normal ranges in the general population.

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