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Quantification of IgG antibodies to mold antigens in the diagnosis of allergic alveolitis

Abstract

Objective: The primary objective of this study was to explore the potential of a quantitative IgG antibody assay for identifying and monitoring patients with suspected allergic alveolitis (AA).

Material & Methods: Serum samples from healthy blood donors (n=60) and patients with (n=63) and without (n=40) precipitins submitted to the Department of Clinical Immunology, Karolinska Hospital suspected of AA were tested. ImmunoCAP™ technology was used to quantify IgG antibodies specific to *Penicillum notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Mucor racemosus*, *Alternaria tenius*, *Thermoactinomyces vulgaris* and *Micropolyspora faeni*. These are common molds primarily seen in "farmers lung" disease, which is one of the most common types of allergic alveolitis. Two new mold mixes including most of these antigens were also tested.

Results: The distribution of IgG antibodies to mold antigens in the healthy population was compared to that of patients with suspected and proven AA. Cut-off levels could be defined for each included mold or mix as the geometric mean ± 2 SD of the reference population.

Conclusions: The ImmunoCAP™ technology was found to have several important advantages in comparison to the precipitin technology, such as allowing antigen specific diagnosis, evaluation of the intensity of exposure and follow-up testing, as well as being fast to perform and giving highly reproducible, quantitative results.

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Introduction

Intense and prolonged or repeated exposure to large amounts of inhaled organic dust can cause allergic alveolitis (AA), previously termed extrinsic allergic alveolitis, which is an inflammation of the lung parenchyma in the terminal bronchioles and alveoli. Prolonged intense exposure often leads to emphysema and progresses to irreversible pulmonary fibrosis. Farmer's lung (FL) belongs to this group of immunologic lung disorders and represents the most common type of AA (1, 2).

Exposure to offending antigens elicits the formation of IgG antibodies to these antigens. The antibodies appear in sera from exposed AA patients, but may also be found in sera from exposed asymptomatic subjects. Thus, the presence of IgG antibodies in the serum of an AA patient is a sign of exposure rather than a sign of disease. The absence of IgG antibodies to the antigen does not exclude the disease. However, high levels of IgG antibodies in a symptomatic patient strongly support the diagnosis (2). Patients with FL often have very high levels of IgG antibodies to the inhaled antigen in their serum. These antibodies can be detected by precipitation techniques (1).

The mold that mainly induces FL in Europe and North America is *Micropolyspora faeni* (MF), and in Britain *Thermoactinomyces vulgaris* (TV) together with MF. *Aspergillus fumigatus* (AF) seems to be the most important *Aspergillus* species associated with FL. Antibodies to *Cladosporium herbarum* (CH) and *Penicillium notatum* (PN) have also been found in sera from FL patients. *Aspergillus* is an airborne fungus that causes diseases mainly in the respiratory tract. Repeated exposure to inhaled *Aspergillus* antigens leads to interstitial alveolar inflammation and AA. AF may grow in the bronchial mucus of patients with complicated asthma and gives rise to a disorder known as allergic bronchopulmonary aspergillosis (ABPA). Aspergilloma, a fungus ball, may develop with chronic lung disease (2).

Objective

The objective of this study was to evaluate an automated and quantitative test system to quantify IgG antibodies to mold antigens in human serum. This quantitative method would fulfill a great need to replace the qualitative precipitation technique used in routine and research clinical laboratories. The distribution of IgG antibodies to mold antigens was determined in a healthy population and compared to those of patients with suspected and proven AA. The aim was to explore the diagnostic potential of a quantitative IgG antibody assay for identifying and monitoring patients with suspected AA.

Material and methods

ImmunoCAP™ technology

ImmunoCAP™ (Phadia AB, Uppsala, Sweden), the most widely used technology for serum IgE measurement, was adapted for the detection of IgG antibodies by exchanging the anti-IgE conjugate by an anti-IgG-conjugate. The UniCAP® 100 instrument automatically performs all necessary steps, from incubation, washing and measuring to calculation of results. UniCAP 100 finally evaluates the data and prints the requested patient and laboratory reports.

The antigen is covalently coupled to a solid phase, ImmunoCAP, which is a cellulose carrier, and reacts with the IgG antibodies in the patient serum. Samples were prediluted 1:100 with specific IgA/IgG Sample diluent (Phadia AB). Non-reactive IgG is washed away and enzyme-labeled antibodies (conjugate) to IgG are added. Unbound conjugate is washed away and the ImmunoCAP carrying bound antibody complex is then incubated with a developing reagent, resulting in the formation of a fluorescent product that is measured. The fluorescence generated is proportional to the concentration of IgG antibodies to the antigen in question present in the specimen. The results are then compared with a 6-point calibration curve. The measuring range for a diluted sample is 2-200 mg_A/l. The results are expressed quantitatively in mg_A/l. Samples higher than 200 mg_A/l were further diluted and re-tested. The intra-assay variation was between 3.5-6.1 % (coefficient of variation, CV) and the interassay variation was 14 % (CV).

ImmunoCAP™ antigens

Seven different mold ImmunoCAP were used in the investigation: *Penicillum notatum* (PN), *Cladosporium herbarum* (CH), *Aspergillus fumigatus* (AF), *Mucor racemosus* (MR), *Alternaria tenius* (AT), *Thermoactinomyces vulgaris* (TV), and *Micropolyspora faeni* (MF) (Phadia AB). Two new mixes of mold antigens were prepared and coupled to ImmunoCAP on request through Phadia Special Allergen Service. Mix 1 was composed of PN, CH, MR and AT antigens; Mix 2 of TV and MF antigens.

Collection of sera

Serum samples from three different groups of individuals were collected. The first group consisted of 60 healthy blood donors (Group BD) without any precipitating antibodies to mold antigens. They represented the healthy, reference population for comparison with AA patients. The second group consisted of 63 patients with clinically suspected AA and precipitates against one or several mold antigens in the panel (Group PP-patients, i.e. with precipitates). The third group consisted of 40 individuals clinically similar to group PP, but lacking precipitates against all seven molds in the panel (Group Non-PP). Ten samples with strong intensive precipitates against AF and MF obtained from UKNEQAS (United Kingdom National External Assessment Schemes for autoimmune serology and special immunochemistry, Department of Immunology, United Kingdom) were also included together with five positive control samples, used for the routine precipitation technique of the laboratory. These sera all had clear precipitates against CH, MR, AT, TV, AF and PN.

Statistical analysis

The distributions of IgG antibody values were skewed towards high values. However, since the distribution were normalized in all three populations after logarithmic transformation the data were expressed as the geometric mean and ± 2 standard deviation confidence intervals were used to describe the population.

Results

BD-group

The serum samples of the 60 blood donors (BD) were tested against the seven different mold antigens in UniCAP 100 and with the precipitation technique. Three of the 60 BD samples (sample 9, 11 and 40) formed precipitates against one or several of the molds in the panel and were therefore excluded from the statistical analysis. Low concentrations of IgG antibodies against the majority of the molds were detected in most of the BD group sera. However, somewhat higher values were seen against AF.

The levels of IgG antibodies to the different antigens varied considerably in the BD group; from <2 to 120 mg_A/l. Only 2.5% showed concentrations higher than 50 mg_A/l and two samples had antibody concentrations >100 mg_A/l, both to AF. If AF was excluded the upper range value dropped to 46 mg_A/l. The mean IgG antibody level to AF (26 mg_A/l) was the highest observed (Table 1a).

Table 1a. IgG antibodies in the BD group (mg_A/l).

	PN	CH	AF	MR	AT	TV	MF
n:	57	57	57	57	57	57	57
Range:	<2-27	7.3-46	5.5-120	<2-17	2.4-24	3.5-32	<2-11
Geom. mean:	9.5	18	26	4.8	9.1	11	3.8
±2 SD interval:	2.5-33	5.1-56	5.0-126	1.5-15	3.0-25	2.8-37	1.5-8.9

Table 1b. IgG antibodies in the Non-PP group (mg_A/l).

	PN	CH	AF	MR	AT	TV	MF
n:	39	39	39	39	39	39	39
Range:	<2-36	<2-42	<2-151	<2-10,9	<2-21	<2-20	<2-7,9
Geom. mean:	12	11	17	3,9	4,7	7,2	2,9
±2 SD interval:	3.0-47	2.5-48	2.3-132	1.6-6.3	1.5-6.9	2.1-25	1.4-4.2

Table 1c. IgG antibodies in the PP group (mg_A/l).

	PN	CH	AF	MR	AT	TV	MF
n:	22	19	28	9	8	9	13
Range:	26-810	43-508	56-1250	3.5-90	37-181	31-87	2.7-691
Geom. mean:	135	106	297	21	76	48	54
±2SD interval:	26-708	28-398	47-1862	2.2-200	24-240	28-83	1.4-4169

Non-PP group

The 40 serum samples of the Non-PP group had almost the same concentration of IgG antibodies to the seven molds, <2 to 151 mg_A/l, as the BD-group and the levels were even lower for some antigens (Table 1b). If AF was excluded the range varied from 2 to 42 mg_A/l. One of the samples in the Non-PP group appeared to have precipitating antibodies to AF upon re-testing and was therefore excluded. Within this group of sera there were only 2.2 % with concentrations above 50 mg_A/l, all against AF. The mean value for each mold was very similar to the corresponding value in the BD-group.

PP group

The samples from the PP group had much higher IgG antibody levels than the BD and Non-PP groups. The range varied from 2.7 to 1250 mg_A/l (Table 1c). The concentrations of antibodies to MR and MF were generally lower than for the other mold species. A total of 76 % had concentrations above 50 mg_A/l and half of all samples tested had concentrations above 100 mg_A/l. Most of the low values found were against MR, TV and MF. Apart from these, generally 10-fold higher values were found in the PP group than in the BD or Non-PP groups.

Mixes with mould antigens

Because of great similarities between the different antigens and in order to reduce the number of tests required for screening, mixes of molds were coupled to the solid phase (ImmunoCAP™) and tested. Mix 1 was composed of PN, CH, MR and AT; Mix 2 of TV and MF. All 57 samples from the BD group and the 39 samples from the Non-PP group were tested against the two mixes. Very low concentrations of IgG antibodies were observed in these two groups against both mixes (Table 2).

Furthermore, 52 samples from the PP-group which contained IgG antibodies to one or several antigens were analyzed with Mix 1 and Mix 2. These samples showed much higher concentrations of IgG to the mixes compared to the BD and Non-PP groups (Table 2). The range varied from <2 to 30 mg_A/l among BD and Non-PP, compared to 5.5 to 573 mg_A/l among the PP-population. Since AF is specifically linked to Aspergilloma we did not include this mold antigen in Mix 1 or Mix 2.

The logarithmic distribution of IgG antibody values to the two mixes and AF in the three patient groups is illustrated in Fig. 1-3. Group BD and group Non-PP showed almost identical distributions. There is some minor overlap

Table 2. IgG antibodies to Mix 1 and Mix 2 in the BD, Non-PP and PP groups (mg_A/l).

	BD group		Non-PP group		PP group	
	Mix 1	Mix 2	Mix 1	Mix 2	Mix 1	Mix 2
n:	57	57	39	39	37	20
Range:	<2-30	<2-20	<2-26	<2-16	19-437	7.7-573
Geom. mean:	8.4	5.6	9.6	5.0	72	52
±2SD interval:	1.7-38	1.1-22	3.0-30	1.5-17	18-347	2.4-661

between group BD on the one hand and group PP on the other for Mix 2 (Fig. 1-2), but in general the distribution in group PP is well above that of group BD. If the upper 2 SD confidence limit of the BD group is used as a cut-off value between healthy individuals and patients, acceptable sensitivities and specificities are achieved.

The IgG antibody concentrations to Mix 1 and Mix 2 were compared with those of the single allergens from each mix. For most of the antigens a good correlation, rs = 0.98, was found between the IgG antibody value to the single antigen and that to the respective mix. However, the correlation between MR and Mix 1 was moderate, rs = 0.76. Furthermore, the individual patients also varied considerably. In no single case, however, was a serum negative to a mix but positive to any of the corresponding single allergens, or vice versa.

Discussion

Most of the ImmunoCAP™ results were in agreement with the results of the precipitation technique. Thus, samples with precipitins also had a high IgG antibody level and samples

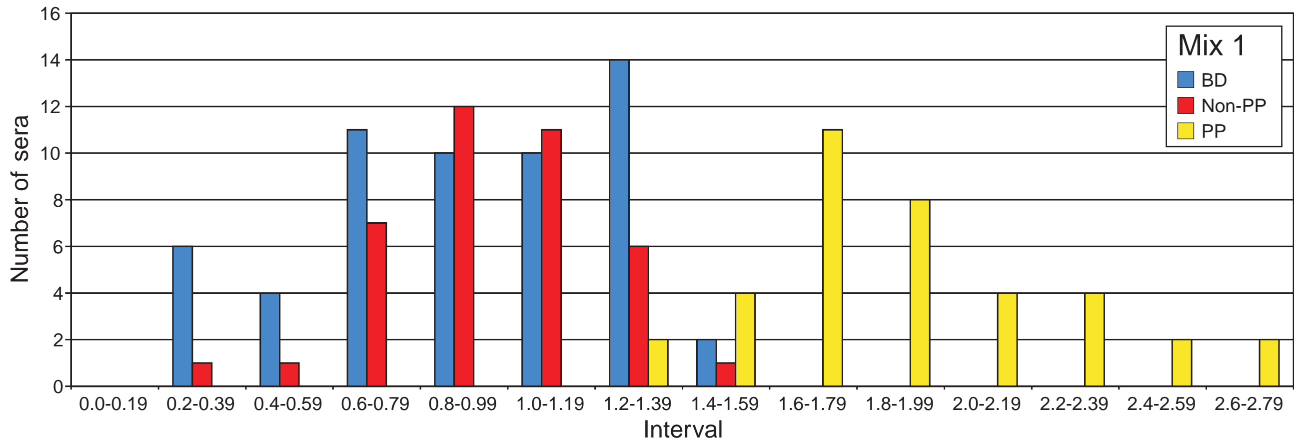

Figure 1. The logarithmic distribution of IgG antibody values (ln mg_A/l) to Mix 1 in the BD, Non-PP and PP groups.

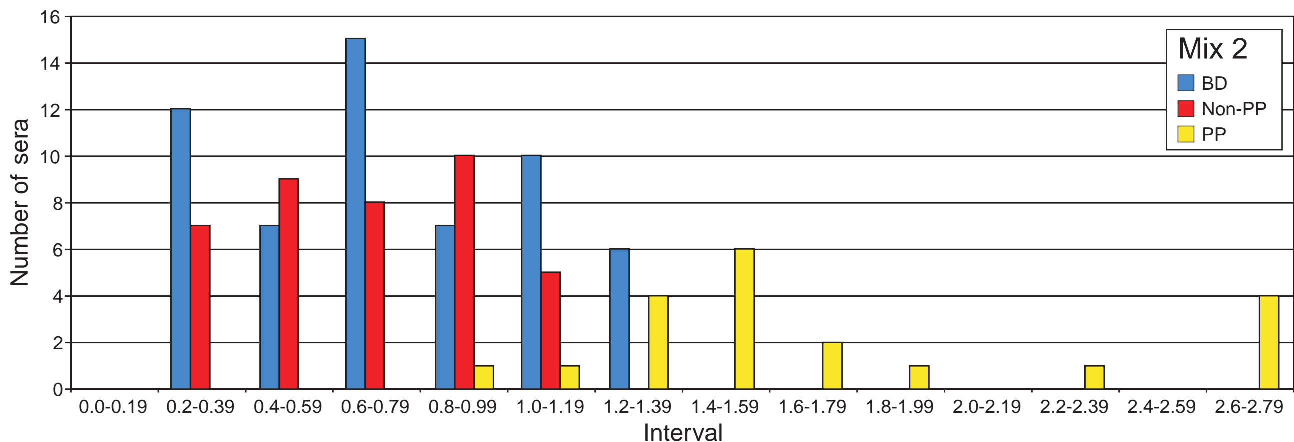

Figure 2. The logarithmic distribution of IgG antibody values (ln mg_A/l) to Mix 2 in the BD, Non-PP and PP groups.

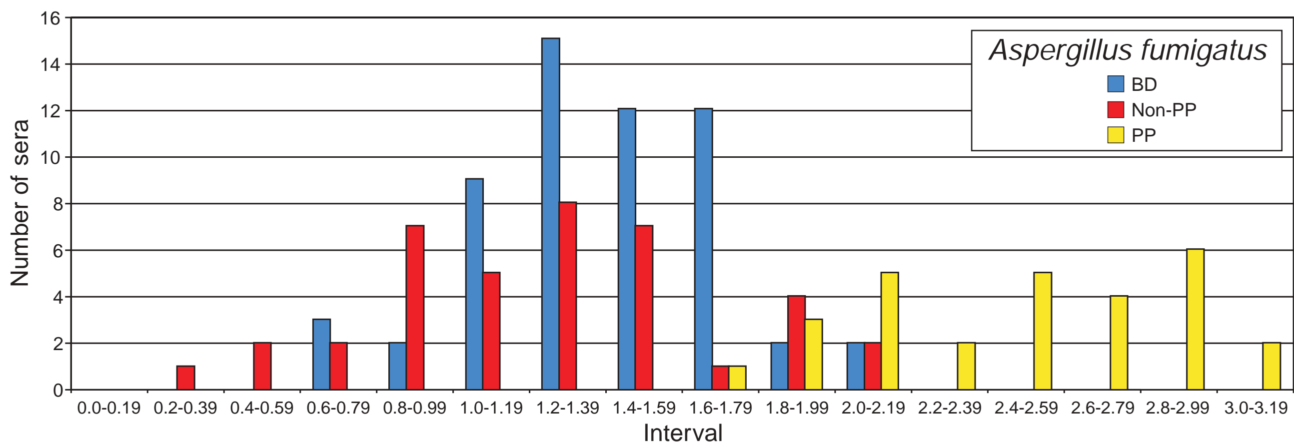

Figure 3. The logarithmic distribution of IgG antibody values (ln mg_A/l) to AF in the BD, Non-PP and PP groups.

that lacked precipitates had low IgG antibody values with ImmunoCAP. Some samples without precipitating IgG antibodies had rather high IgG values, which may be due to the fact that ImmunoCAP is a more sensitive technology. Samples with precipitates and low IgG values were also found, which could depend on the quality of the antigen extract. A good correlation was obtained between the single antigens and the two new mixes, which included the same antigens. In no single case was a serum negative to a mix but positive to the corresponding single allergen, or vice versa. The mixes can thus be valuable tools for identification of AA. A serum positive to a mix can, if considered relevant, easily be tested against the individual antigens of the mix.

The ImmunoCAP technology gives reproducible, quantitative values that make it possible to follow the patient's IgG antibody concentrations over time, which could facilitate the treatment. Another great advantage with this test system is the automated and fast instrument. The results are ready after a few hours, which is very attractive in comparison to the precipitation technique.

The aim of the study was to determine the distribution of antigen-specific IgG antibodies among healthy individuals and in the sera of patients with suspected clinical AA. Based on the results it is recommended to use the geometric mean plus the upper 2 SD as a cut-off value between a healthy, non-sensitized population and patients with suspected AA.

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