Quantification of IgG Antibodies to Aspergillus fumigatus and Pigeon Antigens by ImmunoCAP Technology: An Alternative to the Precipitation Technique?

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**Background:** We evaluated the ImmunoCAP technique for measurement of IgG specific to Aspergillus fumigatus and pigeon antigens.

**Methods:** We used ImmunoCAP and precipitation technique to measure concentrations of IgG to A. fumigatus or pigeon antigens in sera from 265 patients and 42 controls. We also evaluated linearity, interference, imprecision, concordance, and diagnostic accuracy of the measuring techniques.

**Results:** The precipitation and ImmunoCAP technique showed moderate concordance ($\kappa = 0.46$ for both A. fumigatus and pigeon antibodies). Specific IgG results for A. fumigatus and pigeon were linear ($r = 0.98$ and 0.97, respectively), with interrun reproducibility rates of 23% and 14% and maximal interference of 36.5% and 8% by lipid and 24% and 21% by hemolysis, respectively. A. fumigatus antibody concentrations were higher in patients with aspergillosis and allergic bronchopulmonary aspergillosis (ABPA) (median, 103 and 70.1 mgA/L, respectively) than in patients with other pulmonary diseases (median, 18.15–33.40 mgA/L). Antibodies to pigeon antigens were high in patients with hypersensitivity pneumonitis (median, 1024 mgA/L) but also in patients with other pulmonary diseases (median, 445 mgA/L). Antibody titers were substantially higher in patients with other pulmonary diseases and contact with pigeons (median, 1060 mgA/L) than in patients without antigen contact (median, 27.35 mgA/L) ($P < 0.004$).

**Conclusions:** Agreement between the precipitation and ImmunoCAP technique was 86% for A. fumigatus and 70% for pigeon antigens. Highest concentrations of specific IgG to A. fumigatus were found in patients with aspergillosis and ABPA. Our results suggest that antigen contact was the most important variable affecting the presence of antibodies to pigeon antigen.

Organic dust is known to be involved in the pathogenesis of hypersensitivity pneumonitis. Diagnosis is based on characteristic clinical symptoms and demonstration of antigen-specific IgGs (1–4). Antigen exposure also elicits production of IgGs in other pulmonary diseases, such as invasive aspergillosis (5, 6), allergic bronchopulmonary aspergillosis (ABPA) (7), and ABPA in cystic fibrosis (8).

A widely used method to detect specific IgG in clinical laboratories is the immunoprecipitation technique. However, detection of precipitating antibodies has disadvantages. Specificity (9) and sensitivity (10, 11) are limited, reproducibility is poor, and antibody concentrations cannot be measured (11). Moreover, this method is time-consuming and therefore not recommended for routine purposes (11, 12).

Other immunodiffusion assays, such as counterimmunoelectrophoresis (5) and radioimmunoprecipitation (13, 14), are more rapid tests with lower detection limits than the precipitation technique. Although ELISA was shown to be even more sensitive and reliable than immunodiffusion (9, 10, 15), its suboptimal specificity can lead to false-positive results, identifying clinically irrelevant IgG responses.

Recently, an automated technique for detecting specific IgG has become available on the ImmunoCAP system (Sweden Diagnostics). Agreement between specific IgG determination on ImmunoCAP and the precipitation tech-
nique has been reported in a limited number of selected patients (12, 16), but data on the performance of ImmunoCAP in a routine clinical setting are lacking.

In this study, we evaluated the diagnostic performance of ImmunoCAP for detecting antigen-specific antibodies. The distributions of IgG specific to A. fumigatus and pigeon antigens was determined in a healthy nonexposed control population and in different groups of patients with well-defined pulmonary diseases. The results were compared with results obtained with the precipitation technique.

Materials and Methods

Study Population
We collected sera from 42 healthy female laboratory technicians, ages 25–61 years, who had no history of allergy to A. fumigatus or avian antigens and no contact with birds. Over a 1-year period, (January 2004 to January 2005), we also collected sera from 223 consecutive patients from the Departments of Internal Medicine and Pediatrics to ensure a random sampling. For these serum samples, we determined serum precipitins against A. fumigatus and/or pigeon serum antigens. Also included in the study were 42 stored patient sera, collected over a 2-year period (January 2003 to December 2004), with a high number of precipitation lines (≥4) for one or both antigens. Samples from 56 patients were excluded because medical data on the patients were not available. For all patients included in the study, there were no indeterminate or missing results. We tested 219 patient sera for A. fumigatus antibodies [98 males (ages 2–91 years; median, 24 years) and 92 females (ages 4–83 years; median, 24.5 years)], and 46 sera for pigeon antibodies [9 females and 37 males (ages 21–81 years; median, 66 years)]. Performing the study had no adverse effects on the participants.

Precipitation Technique

Extracts. A. fumigatus strain BC 192.65 was obtained from the Centraal Bureau voor Schimmelcultures (Baarn, the Netherlands) and grown on Sabouraud agar (Sanofi Diagnostics Pasteur) for 5 days. Afterward, the fungus was grown by shaking in 250 mL of Czapek-Dox broth with an initial pH 5.0 in 1-L flasks at 37 °C for 3–4 weeks (17). The culture filtrate was separated from mycelia by passage through Whatman no. 4 paper. Filtrates from different flasks were pooled and dialyzed against distilled water at 4 °C (relative molecular mass cutoff, 10,000). The medium was freeze-dried and reconstituted at a concentration of 250 g/L in distilled water.

Lyophilized pigeon serum was obtained from Hal Laboratories. Before use, it was dissolved in 1 mL of distilled water at a concentration of 30 g/L.

Procedure. The test was performed by standard Ouchterlony technique (18) in 1.5% agarose. Undiluted patient serum (275 μL) was placed in the central well and antigens (35 μL) were placed in the peripheral wells. Plates were incubated for 5 days at room temperature, rinsed with 5% trisodium citrate to remove aspecific S-polysaccharide-C-reactive protein lines, washed with 0.9% NaCl, dried, and stained with Amido Black. Results were expressed as number of precipitation lines. The assay was performed by 2 experienced technologists who were blinded for the results of the reference standard.

ImmunoCAP Technology

Specific IgG was determined on the same frozen samples as the precipitating antibodies. Quantification was done in one center with one UniCAP 100 system (Sweden Diagnostics), according to the manufacturer’s instructions. Results were expressed as milligrams of antigen-specific antibodies (mg/L) per liter. Concentrations for 1/100 diluted samples were 2–200 mg/L. Samples with concentrations >200 mg/L were further diluted and retested. Sera were investigated for the presence of A. fumigatus (gm3) and/or pigeon (ge91) IgG. The assay was performed by 2 experienced technologists who were blinded for the results of the reference standard.

Evaluation Details

Specific IgG was determined on frozen material after collection of all samples. Within-assay and between-assay variability were calculated according to CLSI (formerly NCCLS) guidelines (19). For both A. fumigatus and pigeon, 1 sample was assayed 20 times within the same run, and 2 samples were assayed in 20 different runs.

To investigate interference by lipids, samples were supplemented with different dilutions of a serum with high triglyceride concentration (9.09 g/L) as described previously (20). The effect of hemoglobin was studied by adding increasing concentrations (up to 4.6%) of human hemolysate, as described previously (20).

The reference standard was the clinical diagnosis. Data on diagnosis and exposure were collected retrospectively from the medical records. All medical records were reviewed by an experienced pneumologist. Diagnoses were based on criteria described by Denning (21) for aspergillosis, by Greenberger et al. (22) for ABPA, by the 2004 NIH report (23) for bronchial asthma, by Rosenstein et al. (24) for cystic fibrosis, and by Stevens et al. (25) for ABPA in cystic fibrosis (cystic fibrosis and ABPA). Another group consisted of patients with bronchiectases that were not the result of CF or ABPA. Other pulmonary diseases for which A. fumigatus antibodies were ordered by the physician were classified as “other pulmonary diseases”. Diagnosis of pigeon breeder’s disease was based on criteria of Richerson et al. (26).

Statistical Analysis

The distribution of IgG concentrations was described by continuous summary descriptives (Analyze-it, version 1.62; Smart Software).
Differences in median concentrations of specific IgG were tested by the Mann–Whitney U-test. For differences in the number of precipitation lines, the χ² test was used; \( P < 0.05 \) was considered significant.

ROC plot analyses were performed with Analyze-it.

**Results**

**Linearity**

Dilution of a sample with a high concentration of IgG to *A. fumigatus* (161 mgA/L) with sample diluent (0%–100% with increments of 10%) was linear \( (r = 0.98) \), as was dilution of a sample with a high concentration of IgG to pigeon (178 mgA/L) \( (r = 0.97) \).

**Imprecision**

Within-run CV \( (n = 20) \) was 3% for *A. fumigatus* IgG (concentration, 56.5 mgA/L) and 10% for antipigeon IgG (concentration, 386 mgA/L). Between-run CVs \( (n = 20) \) for *A. fumigatus* IgG were 23% for an antibody concentration of 42.7 mgA/L and 15% for an antibody concentration of 146 mgA/L. Between-run CVs for antipigeon IgG were 14% for an antibody concentration of 484 mgA/L and 10% for an antibody concentration of 1612 mgA/L.

**Interference**

To evaluate interference by hemoglobin and triglycerides, increasing amounts of these substances were added to a sample containing 20.2 and 24.1 mgA/L *A. fumigatus* IgG, respectively. The specific antibody concentrations were 2.88, 5.75, 11.5, 23, and 46 g/L, respectively, and 25.2, 23.1, 27.8, 27.2, 32.1, and 32.9 mgA/L for final triglyceride concentrations of 0.454, 0.908, 1.816, 2.72, 3.63, and 4.54 g/L, respectively.

The same amounts of hemoglobin and triglycerides were added to a sample containing, respectively, 7.32 and 92.1 mgA/L pigeon IgG. The IgG concentrations were 6.74, 6.37, 6.64, 6.89, and 6.03 mgA/L for final hemoglobin concentrations of 2.88, 5.75, 11.5, 23, and 46 g/L, respectively, and 87.2, 90.1, 92.1, 90.4, 94.5, and 99.3 mgA/L for final triglyceride concentrations of 0.454, 0.908, 1.816, 2.72, 3.63, and 4.54 g/L, respectively.

**Control Group**

Low concentrations of IgG to *A. fumigatus* and pigeon antigens were detected in sera of healthy controls. The median values were 13.75 mgA/L for *A. fumigatus* and 6.64 mgA/L for pigeon antigen; the 97.5th percentile values were 70.1 and 19.5 mgA/L, respectively (Table 1).

**Correlation Between Specific IgG Determination and the Precipitation Technique**

The correlation between specific IgG concentrations and the number of precipitation lines is shown in Fig. 1. Agreement between tests was 86% for *A. fumigatus* and 70% for pigeon antibodies \( (κ, 0.46) \), for both *A. fumigatus* and pigeon.

**IgG Antibody Values Among Different Patient Groups**

The median concentrations of IgG to *A. fumigatus* and pigeon in healthy controls and in different patient groups are given in Table 1.

IgG to *A. fumigatus* was determined in 219 patients with the following diagnoses: aspergillosis \( (n = 10) \), comprising 9 patients with invasive aspergillosis and 1 with aspergillosa), ABPA \( (n = 10) \), bronchial asthma \( (n = 20) \), cystic fibrosis \( (n = 112) \), cystic fibrosis and ABPA \( (n = 11) \), bronchiectases not caused by cystic fibrosis or to ABPA \( (n = 8) \), and miscellaneous pulmonary diseases, including chronic obstructive pulmonary disease (COPD) and pneumonia \( (n = 48) \).

High concentrations of IgG to *A. fumigatus* were found in patients with aspergillosis (median, 103 mgA/L; 25th–75th percentiles, 90.95–147 mgA/L) and in patients with ABPA (median, 70.1 mgA/L; 25th–75th percentiles, 41.83–113.45 mgA/L) (Table 1). Differences were highly significant compared with healthy controls \( (P < 0.0001) \). In other pulmonary diseases, median values were 18.15–33.40 mgA/L (Table 1). Except in patients with bronchial asthma, IgG concentrations were significantly higher than in controls but substantially lower than in patients with aspergillosis and ABPA \( (P ≤ 0.001) \) for aspergillosis and \( P ≤ 0.02 \) for ABPA). IgG concentrations in patients with aspergillosis were not substantially different from those in patients with ABPA \( (P = 0.19) \).

We measured IgG to pigeon antigens in samples from 46 patients, including patients with pigeon breeder’s disease \( (n = 20) \), COPD \( (n = 15) \), bronchial asthma \( (n = 1) \), idiopathic lung fibrosis \( (n = 5) \), pneumonia \( (n = 1) \), infection \( (n = 2) \), and other diseases \( (n = 2) \). IgG was extremely high in patients with pigeon breeder’s disease (median, 1024 mgA/L; 25th–75th percentiles, 484-1720). High concentrations of pigeon antibodies were also found in patients without pigeon breeder’s disease (median, 445 mgA/L; 25th–75th percentiles, 25.75–1150) (Table 1). Differences were highly significant compared with healthy controls \( (P < 0.0001) \) for pigeon breeder’s disease patients and \( P < 0.001 \) for patients without pigeon breeder’s disease.

Patients with pigeon breeder’s disease tended to have higher antipigeon IgG values than patients without pigeon breeder’s disease \( (P = 0.07) \).

**Distribution of IgG Antibody Concentrations and Diagnostic Performance**

The logarithmic distribution of *A. fumigatus* IgG in healthy controls, in patients with aspergillosis and ABPA, and in diseased controls (bronchial asthma, cystic fibrosis, cystic fibrosis and ABPA, bronchiectases, and other pulmonary diseases) is illustrated in Fig. 2. Because IgG values in patients with aspergillosis and ABPA were comparable (Table 1) and because these are the medical conditions typically associated with the presence of antigen-specific antibodies, these 2 groups were taken together. All other patient groups were considered as diseased controls.
Table 1. Distribution and number of patients above and below cutoff values for specific IgG antibody concentrations to *Aspergillus fumigatus* in healthy controls, in diseased controls (patients with AB, CF, CF + ABPA, BRECT, and OPDs), and in patients with ASP and ABPA; distribution of specific IgG antibody concentrations to pigeon antigens in healthy controls, in diseased controls (non-PBD) and in PBD

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*Abbreviations: AB, bronchial asthma; CF, cystic fibrosis; BRECT, bronchiectasis; OPDs, other pulmonary diseases; ASP, aspergillosis; PBD, patients with pigeon breeder’s disease; non-PBD, patients without pigeon breeder’s disease.*
There was a minor overlap between healthy controls and patients (aspergillosis and ABPA), but the majority of the patients had substantially higher antibody concentrations than the controls. Values obtained in diseased controls overlapped those for healthy controls and patients, with the greatest overlap with healthy controls. ROC curve analysis revealed areas under the curve of 0.957 and 0.859 for differentiating aspergillosis and ABPA patients from healthy individuals and diseased controls, respectively. The cutoff points with the highest sum of detection limit and specificity were 35.9 and 35.2 mgA/L, respectively, when healthy controls or diseased controls were taken as reference. At these cutoff points, the detection limit for healthy or diseased controls was 35 and 70 mgA/L, respectively, when healthy controls or diseased controls were taken as reference. At these cutoff points, the detection limit for healthy or diseased controls was 35 and 70 mgA/L, respectively, when healthy controls or diseased controls were considered.

The number of precipitation lines and IgG concentrations in sera from patients without pigeon breeder’s disease patients with and without current exposure (Fig. 4A). Four of the 5 pigeon breeder’s disease patients without active contact with pigeons had 1 or 0 precipitating lines. In pigeon breeder’s disease patients with actual exposure, >80% had ≥4 precipitation lines. Fig. 4B shows the IgG concentrations to pigeon antigens in the same patient groups. The differentiation between IgG concentrations in pigeon breeder’s disease patients with or without actual antigen contact was highly significant (P < 0.002). All diseased patients without contact with pigeons had antibody concentrations <500 mgA/L, whereas patients with contact had concentrations ≥500 mgA/L, except for 1 patient receiving steroid therapy.

The number of precipitation lines and IgG concentrations in sera from patients without pigeon breeder’s disease patients with and without antigen contact (median, 1060 mgA/L; n = 10) was significantly lower (P < 0.004) than in patients with antigen contact (median, 1060 mgA/L; n = 13). Of the patients without pigeon breeder’s disease and without antigen contact (n = 10), 4 were former pigeon breeders. IgG concentrations in these patients were higher than in healthy controls. The antibody concentration in the other nonexposed patients without pigeon breeder’s disease was, except for 1 patient, comparable to the concentration in nonexposed controls. Finally, we compared the number of precipitation lines and the concentration of IgG to pigeon between exposed pigeon breeder’s disease patients and exposed

**Influence of Antigen Contact on Antibody Production**

To further explore the diagnostic utility of antipigeon IgG determination, we subdivided patients with and without pigeon breeder’s disease on the basis of presence or absence of antigen contact.

The number of precipitation lines to pigeon serum antigens was clearly different between pigeon breeder’s disease patients with and without current exposure (Fig. 4A). Four of the 5 pigeon breeder’s disease patients without active contact with pigeons had 1 or 0 precipitating lines. In pigeon breeder’s disease patients with actual exposure, >80% had ≥4 precipitation lines. Fig. 4B shows the IgG concentrations to pigeon antigens in the same patient groups. The differentiation between IgG concentrations in pigeon breeder’s disease patients with or without actual antigen contact was highly significant (P < 0.002). All diseased patients without contact with pigeons had antibody concentrations <500 mgA/L, whereas patients with contact had concentrations ≥500 mgA/L, except for 1 patient receiving steroid therapy.

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patients with other pulmonary diseases. IgG titers to pigeon antigens in exposed pigeon breeder’s disease patients (median, 1280 mgA/L; n = 11) were comparable to the titers found in exposed patients with other pulmonary diseases (median, 1060 mgA/L; n = 13) (P = 0.41). Similarly, a comparable number of precipitation lines was found in both groups of patients (data not shown).

Discussion

We evaluated the ImmunoCAP technique for measuring IgG antibodies to A. fumigatus and pigeon antigens. Evaluation was done by comparison with the well-established precipitation technique and with clinical diagnosis. The ImmunoCAP test demonstrated acceptable technical performance.

Samples with a high number of precipitation lines had high IgG antibody concentrations with ImmunoCAP, and vice versa. However, a few samples without precipitating antibodies had rather high IgG values, and some samples with precipitating antibodies had low IgG values. Similar observations have been reported (11, 12, 27–29) and may be partly explained by differences in assay detection limit and quality and composition of the antigen extract. Indeed, pigeon serum antigen was used in the precipitation assay, whereas the antigen coupled to the ImmunoCAP contained proteins from serum, feathers, and droppings.
Precipitating antibodies to *A. fumigatus* were reported as highly reliable in patients with aspergillosis (9). Specific IgG is considered a major criterion for diagnosis of ABPA (8, 30). We confirmed that aspergillosis and ABPA patients had high numbers of precipitating antibodies and high concentrations of anti-*A. fumigatus* IgG.

Previous reports also described colonization with *A. fumigatus* in patients with cystic fibrosis (13, 31, 32), COPD, and pulmonary infection (33–35). All patient groups, except those with bronchial asthma, had substantially higher concentrations of specific IgG to *A. fumigatus* than did nondiseased controls but significantly lower concentrations than the aspergillosis and ABPA patient groups (Table 1). At a cutoff point of 70 mgA/L, corresponding to the 97.5th percentile of the values obtained in 42 healthy, nonexposed controls, detection limit was 70% and specificity was 97.6% with healthy controls as a control group and 85.9% when diseased controls were considered. Hashemi (16) proposed a cutoff value of 126 mgA/L. The difference in cutoff values might be related to differences in exposure to *A. fumigatus*, a ubiquitous antigen, as also suggested by Makkonen et al. (36), who found moderate IgG titers in healthy controls that indicated exposure to mold-derived antigens.

Precipitating antibodies and IgG to pigeon antigens were found in patients with pigeon breeder’s disease. However, these antibodies were also found in many diseased patients without pigeon breeder’s disease who had been exposed to pigeon antigen. Other investigators also found antibodies in sera of asymptomatic but exposed individuals (10). Contact with antigen led to the production of high concentrations of anti-pigeon serum antibodies, meaning that no differentiation was possible between exposed individuals without pigeon breeder’s disease and individuals with pigeon breeder’s disease.

We collected data on exposure for every patient and found that contact with antigen was the most important variable related to antibody production. Patients with antigen contact had specific IgG values >500 mgA/L. Nonexposed control individuals had values <20 mgA/L. In comparison, Lopata et al. (12) reported a cutoff of 9.8 mg/L. Most patients without pigeon breeder’s disease...
with values of 20–500 mg/L, including patients with
dyspnea and COPD, had a history of contact with pigeons.

By the use of clinically well-defined patient groups in
combination with data on antigen exposure, our study
demonstrated agreement between the precipitation tech-
nique and the automated ImmunoCAP technique for the
detection of antibodies to *A. fumigatus* and pigeon anti-
gens. High concentrations of specific antibodies to *A.
fumigatus* were found in patients with aspergillosis and
ABPA. The presence of IgG antibodies to pigeon antigens
indicates exposure to pigeons in individuals with or
without pigeon breeder’s disease.

We are grateful to Sweden Diagnostics, Brussels, Belgium
for providing the reagents for this study. We are also
grateful to Jacqueline L’Heureux and Lieve Godefridis for
expert technical assistance.

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