



Improved IgG antibody diagnostics of hypersensitivity pneumonitis and pulmonary mycoses by means of newly evaluated serum antibody ranges and frequencies using IgG ImmunoCAP™

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Summary

Background The ranges of most human IgG antibodies against avian, microbial, and chemical antigens between their cut off values and their maximum values detected by IgG ImmunoCAP™ (Thermo Fisher Diagnostics, Freiburg, Germany) are not well known in pulmonary immune-mediated disorders. In addition, for many antigens it is not yet known how frequently their IgG antibodies appear in patients with these lung diseases. Therefore, we evaluated ranges and frequencies of these IgG antibodies.

Methods The sera of 47,200 patients with suspected hypersensitivity pneumonitis (HP, extrinsic allergic alveolitis) or bronchopulmonary mycoses (mainly allergic bronchopulmonary aspergillosis [ABPA]), which were examined for 32 various IgG antibodies against birds, bacteria, molds, yeasts, and chemicals using the IgG ImmunoCAP™ assay, were evaluated retrospectively.

Results We found a large spreading of the specific IgG ranges with maximum values from 26 mgA/l for *Rhizopus nigricans* up to 4640 mgA/l for pigeon. When the maximum values in the literature are also taken into account, the ranges of avian antibodies reach values up to 7280 mgA/l, the ranges of molds and yeasts up to 1707 mgA/l, of bacterial thermoactinomycetes up to 206 mgA/l, and of chemicals up to 139 mgA/l. The evaluated antibody ranges of the individual antigens can be used to decide whether a detected IgG antibody value is weakly, moderately or strongly positive. According to consistent evidence from numerous

studies, a strongly positive antibody indicates HP or pulmonary mycosis more likely than a weakly positive antibody. It was found that the antigens of the highest maximum antibody levels—pigeon, budgerigar, parrot, *Aspergillus fumigatus*—are the causative agents of the most common immune-mediated lung diseases: bird breeder's lung and pulmonary aspergillosis. Evaluation of the frequencies of eight major IgG antibodies of HP revealed the following rates: pigeon 28%, *Aspergillus fumigatus* 25%, budgerigar 23%, *Penicillium chrysogenum* 11%, *Saccharopolyspora rectivirgula* 7%, *Acremonium kiliense* 6%, *Aureobasidium pullulans* 5%, and *Thermoactinomyces vulgaris* 2%.

Conclusion This study is the first to evaluate the ranges not only of avian and *Aspergillus fumigatus* antigens, as has been done up to now, but also of antibodies against 24 other environmental antigens. Quantification of IgG antibodies regarding their specific ranges can help to improve the serodiagnostics of immune-mediated lung diseases. In the lower ranges IgG antibodies are mainly physiological, while higher ranges correlate with the mentioned diseases as HP and ABPA/allergic bronchopulmonary mycoses (ABPM). The determined frequencies of the eight HP antibodies can be helpful in establishing HP screening tests.

Keywords Antigen-specific IgG · Enzyme-linked immunosorbent assay · Extrinsic allergic alveolitis · Allergic bronchopulmonary aspergillosis · Immune-mediated lung disease

Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
ABPM	Allergic bronchopulmonary mycosis
CF	Cystic fibrosis
ELISA	Enzyme-linked immunosorbent assay
HD	Hexamethylene diisocyanate

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HP	Hypersensitivity pneumonitis
IgG	Immunoglobulin G
MDI	Diphenylmethane diisocyanate
TDI	Toluene diisocyanate
TMA	Trimellith anhydride

Introduction

IgG antibodies against avian, plant, and chemical antigens, bacteria, molds, and yeasts are increasingly used today for diagnosing immune-mediated and infectious lung diseases [1–5]. Originally, the IgG antibodies were detected by immunoprecipitation techniques, in particular the Ouchterlony test [3, 6]. Today, immunoassays, particularly the very sensitive and highly reproducible ImmunoCAP™ assay, are widely used [2–8] and recommended for the diagnosis of hypersensitivity pneumonitis (HP), allergic bronchopulmonary aspergillosis (ABPA) and other bronchopulmonary mycoses (ABPM) [2, 3, 7, 8].

Several studies demonstrated that strongly positive IgG antibodies in immunoassays correlate better with the diagnosis of HP than do weakly positive IgG antibodies [7–12].

In acute bird-related hypersensitivity pneumonitis and in the recurrent type of chronic bird-related hypersensitivity pneumonitis, very high and high specific IgG antibody levels are predominantly seen. However, in the insidious type of chronic HP, lower IgG antibody levels occur, which overlap with the levels found in subjects without HP [2].

In chronic pulmonary aspergillosis (CPA), strongly positive *Aspergillus* IgG antibodies in the IgG ImmunoCAP™ or IgG-ELISA are typical [3, 4, 13, 14]. In allergic bronchopulmonary mycoses (ABPM), these IgG antibodies can be anything from normal to strongly positive [15].

In healthy individuals these IgG antibodies appear most commonly in low concentrations [2, 16–19].

These facts demonstrate that immune-mediated lung diseases are associated with different IgG antibody levels. Therefore, it is essential for serological diagnostics to be able to judge whether a measured value is negative, questionable, weak, medium or strong positive. This requires knowledge of the ranges between the cut offs and the maximum limits of these antibodies.

Looking at the literature, one will discover that only the ranges for IgG antibodies against pigeon, budgerigar, parrot, and *Aspergillus fumigatus* have been identified on IgG ImmunoCAP™ by large quantities of serum samples [6–8, 13, 20].

Since we have a large number of ImmunoCAP™ IgG antibody assay results of patients with suspected lung diseases, predominantly HP, pulmonary mycosis, and cystic fibrosis (CF), we were able to analyze the ranges of 32 human IgG antibodies against environmental aeroantigens within the IgG ImmunoCAP™.

In addition, we evaluated the frequencies of some antibodies in patients with these suspected pulmonary diseases. The frequencies of the antibodies could be helpful in selecting tests for diagnosis, as

Table 1 Medians and percentiles of IgG antibodies from Fig. 1

Antigen	Median (mgA/l)	75th percentile (mgA/l)	90th percentile (mgA/l)	95th percentile (mgA/l)	97.5th percentile (mgA/l)
Pigeon	25.5	48.3	91.6	200.0	200.0
Budgerigar	39.0	91.0	200.0	200.0	200.0
Canary	42.5	94.0	200.0	200.0	200.0
Parrot	26.5	91.3	200.0	200.0	200.0
Finch	31.5	80.3	196.5	200.0	200.0
Duck	27.0	51.3	73.3	116.8	150.4
Goose	35.5	58.5	118.2	154.3	177.5
Chicken	32.5	74.3	119.1	154.8	177.5
<i>Aspergillus fumigatus</i>	93.0	116.0	146.0	166.0	179.0
<i>Acremonium kiliense</i>	31.0	40.3	56.2	91.2	101.1
<i>Penicillium chrysogenum</i>	90.0	117.3	150.4	173.5	200.0
<i>Fusarium proliferans</i>	61.5	81.3	106.1	118.1	128.6
<i>Cladosporium herbarum</i>	62.0	93.0	143.1	157.4	190.5
<i>Aureobasidium pullulans</i>	32.5	46.3	76.4	96.0	116.1
<i>Alternaria alternata</i>	18.0	25.0	38.3	48.2	70.2
<i>Mucor racemosus</i> <i>n</i> = 21	13.0	16.0	24.0	26.0	26.0
<i>Rhizopus nigricans</i> <i>n</i> = 37	12.0	15.0	23.8	26.2	27.1
<i>Candida albicans</i>	105.0	139.3	195.5	200.0	200.0
<i>Thermoactinomyces vulgaris</i>	38.5	56.0	83.3	115.2	149.1
<i>Sacharopolyspora rectivirgula</i>	20.5	29.3	53.1	97.2	119.9

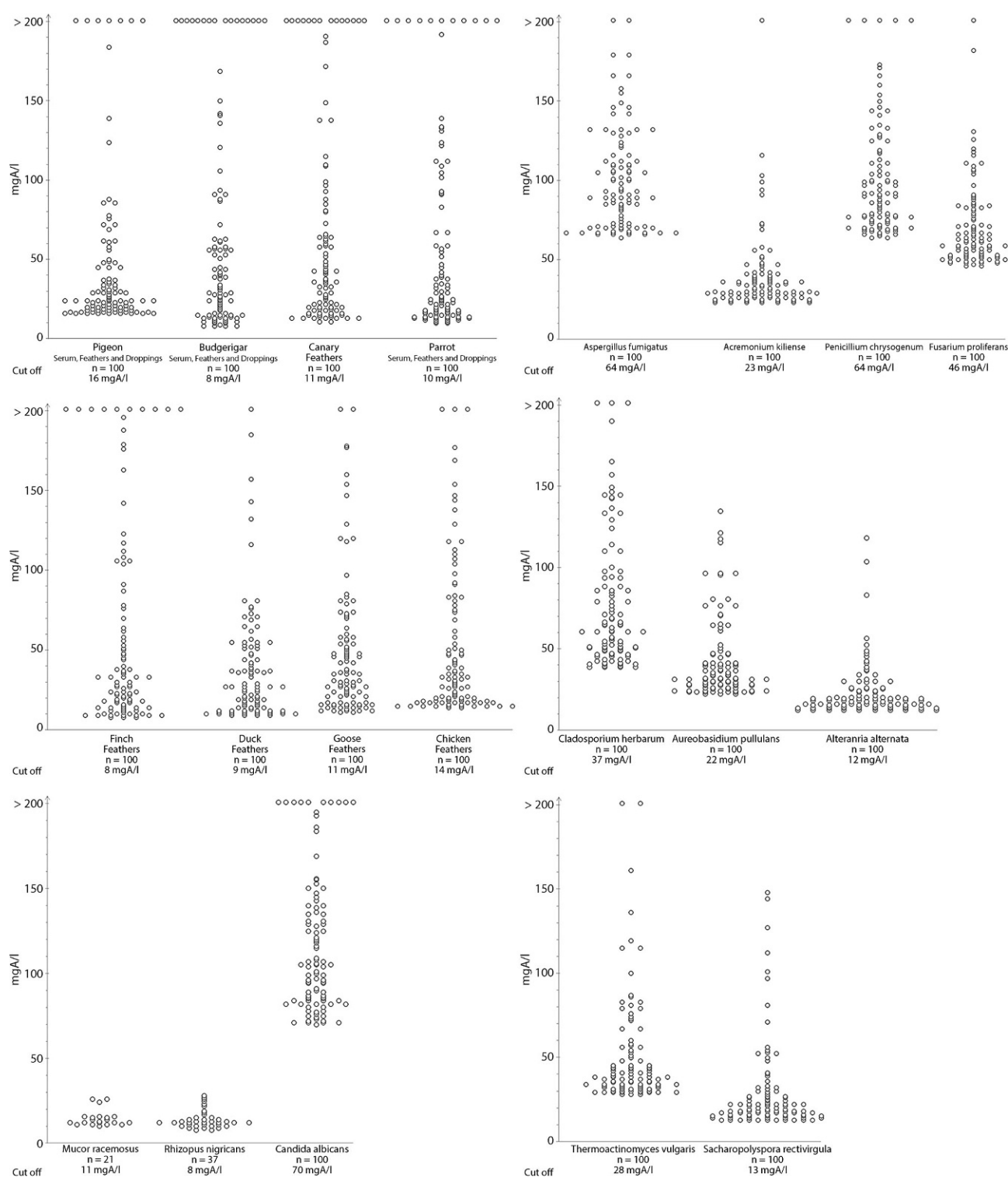


Fig. 1 Graphical representation of 100 individual values (Mucor and Rhizopus with lower numbers) for each 20 IgG antibodies from sera loaded undiluted into the ImmunoCAP™ analyzer

they give an indication of the probability with which a positive result can be expected.

Materials and methods

The test results of IgG antibodies of 47,200 patients' sera with suspected hypersensitivity pneumonitis (extrinsic allergic alveolitis), pulmonary mycoses, and cystic fibrosis were evaluated retrospectively. The sera

had been sent to us together with the submission sheet of our laboratory, on which is listed that these IgG antibodies will be analyzed for the diagnosis of immune-mediated lung diseases. With the exception of a few individual cases, the senders did not provide us with information on allergen exposures and diagnoses.

The test results were recorded anonymously by evaluating only the consecutive numbers of the sera without awareness of patients' names. For the values of Table 1 and Fig. 1, the evaluation of values was performed sequentially starting with the year 2014.

The sera had been sent to our laboratory by physicians from all over Germany ("senders"), mostly by pneumologists. The evaluation covers the period of 2014–2020. Usually several IgG antibodies were tested in the serum. Some antibodies (against cockatiel, finch, *Aspergillus flavus*, *Aspergillus glabrum*, *Trichoderma viride*, and *Stachybotrys chartarum*) were not analyzed over the complete period because they had been added first over the years.

Our senders had the option to choose individual IgG antibodies of their choice. Additionally, we offered a HP screening test with a panel of eight IgG antibodies against pigeon, budgerigar, *Aspergillus fumigatus*, *Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*, *Penicillium chrysogenum*, *Acremonium kiliense* (*Cephalosporium acremonium*) and *Aureobasidium pullulans*.

This most frequently used screening test was requested when there was no anamnestic information on exposure to definite antigens in case of clinical suspicion of HP. 400 screening tests were counted for the individual antigens and their frequencies were evaluated in percentages starting with the year 2014.

In addition, we offered our senders lists of IgG antibodies for the diagnostics of allergic bronchopulmonary mycoses, farmer's lung, humidifier's lung, indoor HP, machine worker's lung, and other rare HP for their antibody selection.

The IgG antibodies were measured with the Phadia ImmunoCAP™ system (Thermo Fisher Scientific) using the Phadia™ 250 analyzer. The IgG antibodies in the patients' sera react in small vessels, called CAPs, with the corresponding antigens, bound to the walls of the CAPs. By means of an enzyme-labeled anti-IgG the amount of human IgG antibodies bound to the antigens is quantitatively measured by fluorescence optics [7].

IgG antibodies against the antigens we studied are also detectable in healthy people, usually in low amounts which are not disease-relevant. In medical diagnostics of immune-mediated human diseases, the IgG antibodies usually exceed thresholds, so-called "cut offs". These cut offs are usually determined using percentiles in sera from healthy persons [16, 19]. We had selected the frequently used 95% percentile to determine the cut offs for IgG antibodies against the above-mentioned antigens in sera from 20 healthy

individuals in a previous study [5]. These control persons were not exceptionally exposed to avian and moldy dusts and did not suffer from lung disease, had no allergies, tumors or chronic inflammation. For the antibodies against chemicals (HDI, MDI, TDI, TMA, phthalic acid), we applied the cut off of 5 mgA/l recommended by Pronk et al. [21]. For the remaining antibodies, we used the cut offs recommended by the manufacturer Thermo Fisher Scientific. The cut off values are shown on Table 2.

The measurement ranges in ImmunoCAP™ extends up to 200 mgA/l. In the period from 2014–2016, the patient sera were filled undiluted into the analyzer. From 2016–2020 sera with values 200 mgA/l were diluted 1:10 and—where appropriate—also 1:100 before loading into the analyzer, so values up to 20,000 mgA/l were recorded.

The frequencies of Fig. 2 were determined from 400 HP screening tests. For each of the eight antigens, the ratio of antibody levels above the cut off compared to antibody levels below the cut off was calculated as a percentage and the percentages were plotted in a vertical column chart.

Results

Ranges of IgG antibodies

Levels of IgG antibodies

Fig. 1 shows 100 sequentially measured readings between the cut offs and ≥ 200 mgA/l of 18 different IgG antibodies. Additionally, the levels of *Mucor racemosus* ($n=21$) and *Rhizopus nigricans* ($n=37$) are reported with lesser sample numbers.

As in many sera with values above 200 mgA/l, no further serum dilution had been performed, only values up to 200 mgA/l were included in Fig. 1. The antibody concentrations above 200 mgA/l are included in the >200 values regardless of whether their sera were titrated out or not.

Most high values of >200 mgA/l appear with the birds budgerigar, canary, and parrot together with similarly low cut off values. However, for antibodies against molds, the yeast *Candida albicans* and thermophilic bacteria, the cut-offs are more different.

As a supplement to Fig. 1, Table 1 presents the median values and the 75th, 90th, 95th, and 97.5th percentiles of the antibody values.

In order to be able to assess whether an IgG antibody is to be classified as weak, medium or strong positive, the range within which the IgG antibody is known to fluctuate must be known for each antigen. This is the range between the cut off and the maximum value.

Maximal antibody values and ranges of variation of the IgG antibodies

Since maximum values in large serum collectives have so far only been determined for a few antigens, as

Table 2 Evaluated maximum values of all detected IgG antibodies, quantities of analyses, published maximum values of other authors, and ranges of these antibodies. We consider the range values in bold letters to be applicable for diagnostics nowadays; the others still require further evaluation. No ranges are given for antigens with less than 20 antibodies above the cut off

	Antigen	Cut off (mgA/l)	Maximum value (mgA/l)	Number of analyses ^e (n^+ = positive results with less than 100 detected antibodies)	Published maximum values (mgA/l) [Reference]	Range (mgA/l)
Avian Antigens	Pigeon Serum, feathers and droppings	16 ^a	4640	27 225	7280 [8]	16–7280
	Budgerigar Serum, feathers and droppings	8 ^a	2770	25 783	3540 [8]	8–3540
	Cockatiel Droppings	10 ^a	1550	230 (n^+ = 76)	–	10–1550
	Canary Feathers	11 ^a	1440	756	–	11–1440
	Parrot Serum, feathers and droppings	10 ^a	1150	885	1230 [8]	10–1230
	Finch Feathers	8 ^b	790	735	–	8–790
	Chicken Feathers	14 ^a	423	1665	–	14–423
	Goose Feathers	11 ^a	880	2550	174 [23]	11–880
	Duck Feathers	9 ^a	209	2435	97 [23]	9–209
Molds	Aspergillus fumigatus	64 ^a	1120	31 035	1707 [20]	64–1707
	Aspergillus niger	45 ^a	409	482 (n^+ = 88)	–	45–409
	Aspergillus flavus	61 ^a	274	42 (n^+ = 11)	–	–
	Penicillium chrysogenum	64 ^a	850	20 435	–	64–850
	Penicillium glabrum	21 ^b	176	43 (n^+ = 7)	–	–
	Acremonium kiliense	23 ^a	1390	19 245	–	23–1390
	Aureobasidium pullulans	22 ^a	187	19 233	–	22–187
	Cladosporium herbarum	37 ^a	453	631	–	37–453
	Alternaria alternata	12 ^a	118	626	–	12–118
	Fusarium proliferatum	46 ^a	477	2537	–	46–477
	Trichoderma viride	17 ^b	83	147 (n^+ = 13)	–	–
	Stachybotrys atra (chartarum)	11 ^b	43	163 (n^+ = 12)	–	–
	Mucor racemosus	8 ^a	26	331 (n^+ = 22)	–	8–26
	Rhizopus nigricans	11 ^a	28	353 (n^+ = 38)	–	11–28
Bacteria	Saccharopolyspora rectivirgula (Micropolyspora faeni)	13 ^a	206	19 430	–	13–206
	Thermoactinomyces vulgaris	28 ^a	200	19 332	–	28–200
Yeasts	Candida albicans	70 ^a	504	467	–	70–504
	Saccharomyces cerevisiae	17 ^a	249 ^d	72 (n^+ = 7)	–	–
Chemicals	HDI (Hexamethylene diisocyanate)	5 ^c	87 ^d	252 (n^+ = 21)	–	5–87
	MDI (Diphenyl methane diisocyanate)	5 ^c	139 ^d	252 (n^+ = 21)	–	5–139
	TDI (Toluene diisocyanate)	5 ^c	47	252 (n^+ = 25)	–	5–47
	TMA (Trimellitic acid)	5 ^c	38	121 (n^+ = 8)	–	–
	Phthalic acid	5 ^c	26	112 (n^+ = 9)	–	–

^a In house cut-off [5]

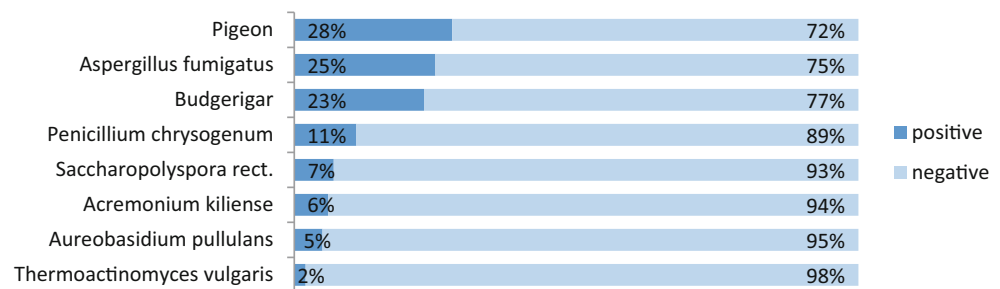
^b Cut-off recommended by Phadia ImmunoCAP IgG Kit

^c Cut-off from [21]

^d Sera from patients with definite HP

^e Most sera were tested for several antibodies

Fig. 2 Frequencies of the IgG antibodies of the HP screening test. Evaluation of 400 screening tests



outlined in the introduction, we evaluated maximum values for additional IgG antibodies and present these for a total of 32 aeroantigens in Table 2.

The highest maximum values in our sera with more than 1000 mgA/l are reached by the antibodies against birds (pigeon, budgerigar, cockatiel, canary, and parrot) and molds (*Aspergillus fumigatus* and *Acremonium kiliense*). Similar values for pigeon, budgerigar, parrot, and *Aspergillus fumigatus* were reported in the literature. These similarities between our values and the values of references provide a good basis for the ranges derived from them.

The antibodies against bacteria and chemicals lie on a considerably lower level.

It is noteworthy that the ranges of these IgG antibodies are extremely different for the various antigens, with a maximum range for pigeon antibodies (16–7280 mgA/l) to the narrowest range for *Rhizopus nigricans* (11–26 mgA/l).

Frequencies of IgG antibodies

The frequencies of the eight antibodies of our hypersensitivity screening test are shown in Fig. 2. The antibodies against avian antigens and *Aspergillus fumigatus* are most prevalent, whereas antibodies against *Thermoactinomyces vulgaris* are least frequent.

Discussion

Levels of IgG antibodies

The antibody levels differ considerably (Fig. 1). For instance, 16% of the budgerigar antibodies exceed 200 mgA/l but no *Alternaria* antibody reaches this level. Therefore, a uniform scoring of these IgG antibodies, as for IgE antibodies with a universal cut off at 0.35 kU/l, is not applicable. Each antigen-specific IgG antibody requires a unique cut off value. For our region, Central Europe, cut offs have been evaluated by Kränke, Pronk, Sennekamp, and Raulf [5, 16, 19, 21]. Since Raulf's results were not yet available at the beginning of our evaluation, we used our own cut offs [5] as well as for additional antigens those recommended by the manufacturer and those of Pronk et al. for the chemical antigens [21].

Maximal antibody values and ranges of variation of the IgG antibodies

The ranges of IgG antibodies against different antigens (Table 2) vary considerably from 8–26 mgA/l for *Mucor racemosus* to 16–7280 mgA/l for pigeon (Table 2). This exceeds by far the ranges of the cut offs (e.g. for chemical antigens 5 mgA/l on the lower end and for *Candida albicans* 70 mgA/l on the upper end). Knowledge of these ranges could improve the interpretation of IgG results for each individual antigen. Sera with 200 mgA/l values should be titrated by means of additional dilution analyses.

Higher antibody levels for pigeon, budgerigar and parrot than ours were reported by Lopata et al. ([8]; Table 2). Additionally, three high values of IgG antibodies against pigeon serum on ImmunoCAP™ in the range between 3500 mgA/l and 4000 mgA/l were reported by van Hoeyveld et al. for patients affected by pigeon breeder's lung [6].

Koschel et al. examined IgG antibodies against goose and duck feathers in the IgG ImmunoCAP™ in patients with proved feather duvet lungs; they found maximum values of 174 mgA/l for goose feathers and 97 mgA/l for duck feathers [22].

For *Aspergillus fumigatus*, Page et al. identified a maximum value of 1707 mgA/l ([20]; Table 2), while van Hoeyveld et al. and Fujiuchi et al. reported lower maximum values around 1000 mgA/l [6, 13].

For other molds we could not find representative maximum values of analyses done by IgG ImmunoCAP™ in literature for pulmonary mycoses and HP. Thus, the maximum values reported here (Table 2) will be of particular scientific importance. Apart from *Penicillium chrysogenum* and *Acremonium kiliense*, the IgG maximum values and the ranges are considerably lower than for *Aspergillus fumigatus*. However, some of our maximum values are likely to be too low because the quantities of sera examined are small (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium glabrum*, *Trichoderma viride*, *Stachybotrys atra*, *Mucor racemosus*, *Rhizopus nigricans*).

The bacteria (thermoactinomycetes) *Saccharopolyspora rectivirgula* and *Thermoactinomyces vulgaris* induce only HP (farmer's lung, humidifier's lung, bagassosis), not any other diseases [23, 24]. The maximum values of their antibodies around 200 mgA/l are mod-

erately high. For these we could not find representative maximum values in literature.

The yeast *Candida albicans* is one of the antigens causing the seldomly diagnosed allergic bronchopulmonary mycoses (ABPM). Besides other criteria, the serologic diagnosis is done by specific *Candida* IgE and IgG antibodies [25].

Candida albicans is also a rare cause of HP [23, 26]. However, we found no *Candida* ImmunoCAP™ IgG analyses in these patients in literature.

The maximum IgG value of *Saccharomyces cerevisiae* in Table 2 marked with ^d (249 mgA/l) belongs to a HP patient with occupational inhalation of dry yeast powder [27]. The food-induced *Saccharomyces cerevisiae* IgG antibodies occur with lower levels in Crohn's and in celiac disease [27].

For the isocyanate IgG antibodies additional ImmunoCAP™ values were reported in literature [28]. The highest values were for TDI (toluene diisocyanate) 8 mgA/l, for MDI (diphenylmethane diisocyanate) 13 mgA/l, and for HDI (hexamethylene diisocyanate) 15 mgA/l. We obtained some particularly high IgG antibody values in our sera which belonged to patients with documented HP [27, 29]. This supports the phenomenon reported for other antigens in that high levels of IgG are typical for patients with acute HP [7–12].

If a uniform scale were chosen for all aerogenic IgG antibodies, for example from 5–10,000 mgA/l, these values of our isocyanate patient from 42 mgA/l up to 87 mgA/l (Table 2) would be classified as low. In contrast, using the HDI isocyanate range of 5–87 mgA/l and the TDI range of 5–47 mgA/l in Table 2, they are judged to be high-titer antibodies indicating probable HP.

An effective way to assess the value of an IgG antibody is to add a semiquantitative rating to the measured value, for example with the categories: negative—questionable—weak—moderate—strong [5, 16]. Another way to judge a reading is to add a scale of the range of variation to it. For this purpose, authors used logarithmic graduations [3, 6, 13].

The top IgG maximum value levels of pigeon (7280 mgA/l), budgerigar (3540 mgA/l), parrot (1230 mgA/l), and *Aspergillus fumigatus* (1707 mgA/l) (Table 2) are seen in the most common immune-mediated lung diseases: in bird-related HP [23, 24, 30, 31] and in the two most common *Aspergillus*-related lung diseases: ABPA with high prevalences (e.g. in Germany up to 150 cases per 100,000 people [32]) and chronic pulmonary aspergillosis (CPA) with a prevalence of 2.5/100,000 people. The number of CPA cases worldwide was estimated to be at least 4.8 million [1, 33]. Obviously, these antigens with high maximum specific IgG values are more potent to induce extrinsic immune-mediated lung diseases than antigens which only induce a weaker IgG response.

Frequencies of IgG antibodies

In analyzing the frequencies of the antibodies, we have assessed the eight antigens of our HP screening test (Fig. 2), which was done exclusively in sera from patients with suspected HP.

The most frequent IgG antibodies of Fig. 2 are the antiavian IgGs. Epidemiological studies have consistently shown that bird fancier's lung is the most common HP-entity, accounting for 40% of all HPs in the United States and in Germany [24, 30, 31]. Similarly, the antibodies against the avian antigens, especially against pigeons and budgerigars, reach the highest maximum values and the highest ranges in this study (Table 2). Therefore, the antibodies against the avian antigens are highly relevant for diagnostics of HP.

Aspergillus fumigatus IgG antibodies are present in different HP entities but also play a role in pulmonary mycoses (infectious and allergic), which are—taken together—more common than HP [3, 4, 24, 33].

The antibodies against the bacterial antigens *Saccharopolyspora rectivirgula* and *Thermoactinomyces vulgaris* occur least frequently in our examined patients (Fig. 2). They are the main antigens in farmer's lung, which has become rare in Germany with the change of storing hay in silages and better methods of drying the hay [23]. Sometimes thermoactinomycetes are the trigger of humidifier lung, also a rare type of HP. We could not find any human infections or colonisations caused by these thermobacteria in the literature.

The determined frequencies of the eight HP antibodies can be helpful for rational IgG antibody diagnostics and in establishing new HP screening tests [34].

IgG antibodies in other conditions

Aspergillus fumigatus, *Candida albicans*, and *Acremonium kiliense* not only cause allergies in humans, but infections or colonisations of mucus membranes too, which also induce IgG antibodies [3, 4, 35, 36]. *Penicillium chrysogenum* antibodies can be elevated as a result of cross-reactivity with *Aspergilli* even without the patient having had contact with the *Penicillium* fungus [37, 38].

In addition, these antibodies related either to infection, colonisation or cross-reaction are also likely to increase the median values (Table 1; [19]). IgG antibodies are particularly frequently induced by *Candida* yeasts, colonizing the mucous membranes of the oral cavity, intestine, vagina, and anus [39, 40].

Aspergillus fumigatus grows particularly in tuberculous caverns, bronchiectasis and on the bronchial mucosa of patients with cystic fibrosis [3, 4, 36]. Such colonisations are asymptomatic and usually there is no need for treatment.

One can expect that among the subjects in large control collectives for the evaluation of cut offs there

Table 3 Maximum, median, and mean values of IgG antibodies in healthy persons evaluated by Kränke et al. [16], Raulf et al. [19], and Tan et al. [17]

IgG antibody against		Kränke et al. n= 48		Raulf et al. n= 121		Tan et al. n= 120
		Maximum value (mgA/l)	Median (mgA/l)	Maximum value (mgA/l)	Median (mgA/l)	Mean value (mgA/l)
Avian Antigens	Pigeon Serum, feathers and droppings	53	2	43	9	12
	Budgerigar Serum, feathers and droppings	26	3	18	3	6
	Canary Feathers	18	5	14	2	–
	Parrot Serum, feathers and droppings	41	12	32	4	14
	Chicken Feathers	–	–	27	6	–
	Goose Feathers	–	–	19	5	–
	Duck Feathers	–	–	15	4	–
Molds	Aspergillus fumigatus	86	14	726	21	31
	Penicillium chrysogenum (notatum)	42	8	143	14	–
	Acremonium kiliense	–	–	39	5	–
	Aureobasidium pullulans	47	7	26	3	5
	Cladosporium herbarum	72	12	65	11	–
	Alternaria alternata	–	–	37	3	–
	Fusarium proliferatum	–	–	92	9	–
	Trichoderma viride	–	–	32	3	–
	Stachybotrys atra	–	–	53	2	–
	Mucor racemosus	–	–	12	3	–
	Rhizopus nigricans	–	–	14	2	–
Bacteria	Saccharopolyspora rectivirgula (Micro- polyspora faeni)	15	3	14	2	–
	Thermoactinomyces vulgaris	47	11	30	7	–
Yeasts	Candida albicans	201	33	112	26	47
Chemicals	Isocyanates	–	–	7	1	–

are such asymptomatic individuals with IgG antibodies, especially in large control collectives. They complicate the determination of the cut off values, especially for *Candida albicans*, Aspergilli, and the cross-reacting Penicillia molds [16, 19].

Other antigens, in particular the avian proteins, the thermoactinomycetes *Saccharopolyspora rectivirgula* and *Thermoactinomyces vulgaris*, *Aureobasidium pullulans*, *Stachybotrys atra* and the chemicals (Table 2), do not colonize human mucous membranes and cause no human infections. This might be the reason for their low cut off levels (Table 2). Positive IgG antibodies against these antigens are therefore better qualified for diagnosing immune-mediated lung diseases. Otherwise, Aspergilli, Penicillia, *Acremonium kiliense*, and *Candida albicans* IgG antibodies have a broader screening potential for diverse respiratory diseases including infectious disorders of the lung.

IgG antibodies in healthy persons

The maximum values in healthy persons in Austria [16] and in Germany ([19]; Table 3) are markedly lower than in our sera from patients with suspected lung disease (Table 2). Particularly high maximum values in these healthy people however were recorded for *Penicillium chrysogenum*, *Aspergillus fumigatus*, and *Candida albicans* (Table 2). As mentioned above, these germs can infect human tissues or colonize mucous membranes in clinically asymptomatic persons [36, 39, 40].

For the first two collectives of healthy individuals in Table 3, Kränke et al. and Raulf et al. [16, 19] had selected only healthy individuals who had no obvious contact with birds, molds, and bacteria. Tan et al. [17] did not perform this selection.

Diagnostic interpretation of the IgG antibodies

Healthy individuals exposed to antigens may well also have clearly positive IgG antibodies to these antigens

in their sera, so that the diagnosis of these lung diseases cannot be made on the basis of antibody levels alone. Only together with the other diagnostic criteria of HP [41–43], pulmonary mycoses [1, 3, 4, 15, 43, 44], CF [36, 37, 43] or pulmonary infections [3, 4, 14, 39] the IgG antibodies are a valuable tool for diagnosing these diseases.

The most specific for the diagnostics of HP are the IgG antibodies against avian antigens, thermoactinomycetes (*Saccharopolyspora rectivirgula* and *Thermoactinomyces vulgaris*), *Aureobasidium pullulans*, *Stachybotrys atra*, and the chemicals (Table 2) because these antigens do not colonize human mucous membranes and cause no human infections.

Otherwise, IgG antibodies against Aspergilli, Penicillia and *Candida albicans* have a broader screening potential for diverse lung diseases as ABPA, ABPM, and infectious disorders of the lung, and sometimes also indicate cross-reactions with other microorganisms [3, 4, 23, 36, 37, 39, 40].

Limitations

This is a retrospective study where we had no influence on the choice of antibody selection by the senders. A further disadvantage of our study is certainly, that we have not titered the maximum values ≥ 200 mgA/l in the period 2014–2016.

The most frequently analyzed IgG antibodies against antigens as pigeon, budgerigar, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Acremonium kiliense*, *Aureobasidium pullulans*, *Saccharopolyspora rectivirgula* and *Thermoactinomyces vulgaris* had a greater chance of getting high maximum levels than the less frequent analyzed other antibodies (Table 2). This also applies to the maximum values of the literature. There are more studies of IgG antibodies against the antigens pigeon [6–8, 12] and *Aspergillus fumigatus* [6, 13, 15–20] and thus higher chances for particularly high values than for other antigens as goose and duck feathers [22, 34], isocyanates [21, 28, 29] or *Saccharomyces cerevisiae* [27]. For the maximum values and ranges of these rare antibodies further evaluations will be required in future.

Concerning the frequencies, we would have liked to evaluate more than the described eight antibodies of the HP screening test. However, an evaluation attempt revealed extremely high frequencies of antibodies against birds and bed feathers. On the “visible” birds (meaning anamnesticly recognizable), the hit rate is of course higher than on the “invisible” bacteria and molds. Therefore, such an evaluation would distort the results.

An additional limitation of our study is the dependence of our results on the chosen cut-offs. The decision to include only IgG values over the individual cut-offs of each antigen was taken because graduation is only needed above the cut offs; below the cut offs all values are graded as negative. For some of

the evaluated antigens, especially *Aspergillus fumigatus*, *Penicillium chrysogenum*, and *Candida albicans*, the cut-offs differ considerably in the cited studies [5, 16, 19]. This has to be taken into account when interpreting our results. Fortunately, there were major differences only for these three antigens mentioned above, whereas the cut offs of nearly all other antigens lay close together.

Conclusion

This study is the first to evaluate the ranges of the most relevant IgG antibodies against inhalant antigens using ImmunoCAP™. Knowledge of these different ranges may be helpful to graduate an IgG antibody reading as a questionable, weak, medium or strong positive IgG antibody. A grading of the specific IgGs is of clinical relevance, as HP and pulmonary mycoses, particularly CPA, are more likely with higher IgG antibodies.

IgG antibodies against birds, thermoactinomycetes and chemicals are the most specific for diagnosing HP. Mold and yeast antibodies are less specific because they also occur in bronchopulmonary mycoses, in infections, cystic fibrosis, mucosal colonisations and can be the result of cross-reactions with other microorganisms.

Taken together, we consider the evaluated IgG antibody ranges and antibody frequencies described here may help to improve serologic diagnostics of these immune-mediated lung diseases.

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Declarations

Conflict of interest J. Sennekamp, E. Lehmann and M. Joest declare that they have no competing interests.

Ethical standards This is a retrospective study with anonymized data.

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