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

Joachim Sennekamp · Emilia Lehmann · Marcus Joest

Received: 27 September 2021 / Accepted: 24 January 2022 / Published online: 17 May 2022
© The Author(s) 2022

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
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 (HP), allergic bronchopulmonary aspergillosis (ABPA) and other bronchopulmonary mycoses (ABPM) [2, 3, 7, 8].

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

| 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                    |
| Aspergillus fumigatus    | 93.0           | 116.0                   | 146.0                   | 166.0                   | 179.0                    |
| Acremonium kiiense       | 31.0           | 40.3                    | 56.2                    | 91.2                    | 101.1                    |
| Penicillium chrysogenum  | 90.0           | 117.3                   | 150.4                   | 173.5                   | 200.0                    |
| Fusarium proliferans    | 61.5           | 81.3                    | 106.1                   | 118.1                   | 128.6                    |
| Cladosporium herbarum    | 62.0           | 93.0                    | 143.1                   | 157.4                   | 190.5                    |
| Aureobasidium pullulans  | 32.5           | 46.3                    | 76.4                    | 96.0                    | 116.1                    |
| Alternaria alternata     | 18.0           | 25.0                    | 38.3                    | 48.2                    | 70.2                     |
| Mucor racemosus n = 21   | 13.0           | 16.0                    | 24.0                    | 26.0                    | 26.0                     |
| Rhizopus nigricans n = 57| 12.0           | 15.0                    | 23.8                    | 26.2                    | 27.1                     |
| Candida albicans         | 105.0          | 139.3                   | 195.5                   | 200.0                   | 200.0                    |
| Thermoactinomyces vulgaris| 38.5          | 56.0                    | 83.3                    | 115.2                   | 149.1                    |
| Sacharopolyspora rectiv. | 20.5           | 29.3                    | 53.1                    | 97.2                    | 119.9                    |
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 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™ extend 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

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

° Most sera were tested for several antibodies

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

* Most sera were tested for several antibodies
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 off [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-
IgG antibodies occur with lower levels in Crohn's and in celiac disease [27].

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 Cow'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, and 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 antialian 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]

| Avian Antigens | 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) |
| 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 atrothecia | – | – | 53 | 2 | – |
| Mucor racemosus | – | – | 12 | 3 | – |
| Rhizopus nigricans | – | – | 14 | 2 | – |
| Bacteria       | Saccharopolyspora rectivirgula (Micropolyspora faeni) | 15 | 3 | 14 | 2 | – |
| Thermactinomycetes vulgarensis | 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 Thermactinomycetes vulgaris, Aureobasidium pullulans, Stachybotrys atrothecia 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 Aspergillus, 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 senders. A further disadvantage of our study is certainly that we have not titered the maximum values senders. A further disadvantage of our study is certainly that we have not titered the maximum values senders.

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 anamnestically 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.

**Acknowledgements** We are grateful to Sabine Schmidt, Brigitte Hünke, Manuela Schmitz-Gerhardt and Barbara Kläge for their perfect analyses of the IgG antibodies.

**Funding** Open Access funding enabled and organized by Projekt DEAL.

**Declarations**

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

**Ethical standards** This is a retrospective study with anonymized data.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.
References

1. Denning DW, Cadranel J, Beigelman-Aubry C, Ader E, Chakrabarti A, Blot S, et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. Eur Respir J. 2016;47:45–68.

2. Shirai T, Tanino Y, Nikaido T, Takaku Y, Hashimoto S, Taguchi Y, et al. Screening and diagnosis of acute and chronic bird-related hypersensitivity pneumonia by serum IgG and IgA antibodies to bird antigens with ImmunoCAP. Allergol Int. 2021;70:208–14.

3. Page ID, Richardson M, Denning DW. Antibody testing in aspergillosis—quod vadis? Med Mycol. 2015;53:417–39.

4. Richardson MD, Page ID. Aspergillus serology: have we arrived yet? Med Mycol. 2017;55:48–55.

5. Sennekamp J, Lehmann E, Joest M. Work-related extrinsic allergic alveolitis. ASU Int. 2015;56:38–52.

6. Van Hoeveld E, Dupont L, Bossuyt X. Quantification of IgG antibodies to aspergillus fumigatus and pigeon antigens by ImmunoCAP® technology: an alternative to the precipitation technique? Clin Chem. 2006;52:1785–93.

7. McSharry C, Dye GM, Ismail T, Anderson K, Spiers EM, Boyd G. Quantifying serum antibody in bird fanciers’ hypersensitivity pneumonia. BMC Pulm Med. 2006;6:16.

8. Lopata AL, Schinkel M, Potter PC, Jeebhay MF, Hashemi C, Jansson SG, et al. Qualitative and quantitative evaluation of bird-specific IgG antibodies. Int Arch Allergy Immunol. 2004;134:173–8.

9. Erkinjunti-Pekkanen R, Reiman M, Kokkarinen JI, Tukiainen HO, Terho EO. IgG antibodies, chronic bronchitis, and pulmonary function values in farmer’s lung patients and matched controls. Allergy. 1999;54:1181–7.

10. Lewis C, McSharry C, Anderson K, Speekenbrink A, Kemeny DM, Durnin S, et al. Quantifying serum antibody class and subclass responses by enzyme immunoassay in humidifier-related disease. Clin Exp Allergy. 1991;21:601–7.

11. Woge MJ, Ryu JH, Moua T. Diagnostic implications of positive avian serology in suspected hypersensitivity pneumonia. Respir Med. 2017;129:173–8.

12. Khan S, Chowdhury SR, Ghosh S, Sengupta A, Ramasubban S, Sen D. Quantitation of avian IgG antibodies with clinico-radiological tests in the diagnosis of bird fancier’s hypersensitivity pneumonia. PulmFace. 2015;15:48–54.

13. Fujiiuchi S, Fujiita H, Suzuki H, Doushita K, Kuroda H, Takahashi M, et al. Evaluation of a quantitative serological assay for diagnosing chronic pulmonary aspergillosis. J Clin Microbiol. 2016;54:1496–9.

14. YuQ, HeJ, Xing B, LiX, Qian H, Zhang H, et al. Potential value of serum aspergillus IgG antibody detection in invasive and chronic pulmonary aspergillosis. BMC Pulm Med. 2020;20:89.

15. Aggarwal AR, Dua D, Choudhary H, Aggarwal AN, Sehgal JS, Dhoraia S, et al. Role of Aspergillus fumigatus-specific IgG in diagnosis and monitoring treatment response in allergic bronchopulmonary aspergillosis. Mycoses. 2017;60:33–9.

16. Kränke B, Wolsche M, Wolsche-Kahr I, Aberer W. IgG-Antikörper gegen EAA-spezifische Umweltantigene. AL. 2001;24:145–54.

17. Tan YH, Nga CC, Huang SW, Loo CM, Low SY. Specific serum immunoglobulin G (IgG) levels against antigens implicated in hypersensitivity pneumonitis in asymptomatic individuals. Ann Acad Med Singap. 2019;48:36–8.

18. Makkonen K, Viitala KI, Parkkila S, Niemelä O. Serum IgG and IgE antibodies against mold-derived antigens in patients with symptoms of hypersensitivity. Clin Chim Acta. 2001;305:89–98.

19. Rauf M, Joest M, Sander J, Hoffmeyer F, Nowak D, Ochmann U, et al. Update of reference values for IgG antibodies against typical antigens of hypersensitivity pneumonia. Allergo J Int. 2019;28:192–203.

20. Page ID, Richardson MD, Denning DW. Comparison of six aspergillus specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). J Infect. 2016;72:240–9.

21. Pronk A, Frellet L, Raulf-Heimsoth M, et al. Respiratory symptoms, sensitization, and exposure—response relationships in spray painters exposed to isocyanates. Ann J Respir Crit Care Med. 2007;176:1090–7.

22. Koschel D, Lützkendorf L, Wiedemann B, Höflken G. Antigen-specific IgG antibodies in leather dust-vulnerable Eur J Clin Invest. 2010;40:797–803.

23. Sennekamp J. Extrinsic allergic alveolitis—hypersensitivity pneumonitis. Munich, Orlando: Dustri-Verlag, 2004.

24. Costabel U, Miyazaki Y, Pardo A, Koschel D, Bonella F, Spagnolo P, et al. Hypersensitivity pneumonitis. Nat Rev Dis Primers. 2020;6:65.

25. Chowdhary A, Agarwal K, Kathuria S, Gaur SN, Randhawa HS, Meis JF. Allergic bronchopulmonary mycosis due to fungi other than aspergillus: a global overview. Crit Rev Microbiol. 2014;40:30–48.

26. Serrano C, Torrego A, Losalí A, Valero A, Picado C. Hypersensitivity pneumonitis after exposure to Candida spp. Arch Bronconeumol. 2010;46:275–7.

27. Gernhold M, Sennekamp J. Exogen-allergische Alveolitis durch Backhefe (Saccharomyces cerevisiae). AL. 2010;33:577–80.

28. Baur X, Fischer A, Budnik IJ. Spotlight on the diagnosis of extrinsic allergic alveolitis (hypersensitivity pneumonitis). J Occup Med Toxicol. 2015:10:15.

29. Schreiber J, Knolle J, Sennekamp J, Schulz KT, Hahn JU, Hering KG, et al. Subacute occupational hypersensitivity pneumonitis due low-level exposure to disocyanates in a scretary. Eur Respir J. 2008;32:807–11.

30. Liebetrau G, Bergmann KC. Morbiditäts- und Mortalitätszahlen für die exogen-allergische Alveolitis (EAA). AL. 1994;17:61–4.

31. Fernández Pérez ER, Swigris JJ, Forssén AV, Tourin O, Solomon JJ, Huie TJ, et al. Identifying an inciting antigen is associated with improved survival in patients with chronic hypersensitivity pneumonitis. Chest. 2013;144:1644–51.

32. Joest M, Sennekamp J. Allergische bronchopulmonale Aspergillose (ABPA) und andere allergische bronchopulmonale Mykosen (ABPM). Munich, Orlando: Dustri-Verlag, 2020.

33. Denning DW, Pleuvry A, Cole D. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. Med Mycol. 2013;51:361–70.

34. Sennekamp J, Lehmann E. Optimierte IgG-Antikörperdiagnostik der Bettfedern-Alveolitis mittels EAA-Suchtest. Pneumologie. 2015;69:638–44.

35. Júnior MC, de Moraes Arantes A, Silva HM, Costa CR, Primers. 2020;6:65.

36. Eschenhagen P, Grehn C, Schwarz C. Prospective evaluation of aspergillus fumigatus specific IgG in patients with cystic fibrosis. Front Cell Infect Microbiol. 2021;10:602836.

37. Brouwer J. Cross-reactivity between aspergillus fumigatus and penicillium. Int Arch Allergy Immunol. 2004;134:173–8.

38. Vojdani A. Cross-reactivity of aspergillus, penicillium, and stachybotrys antigens using affinity-purified antibodies and immunoassay. Ann Occup Hyg. 2004;59:256–65.
39. Richardson JP, Moyes DL. Adaptive immune responses to Candida albicans infections. Virulence. 2015;6:327–37.
40. Huertas B, Prieto D, Pitarch A, Gil C, Pla J, Diez-Orejas R, et al. Serum antibody profile during colonization of the mouse gut by Candida albicans. J Proteome Res. 2017;15:335–45.
41. Petnak T, Moua T. Exposure assessment in hypersensitivity pneumonitis: a comprehensive review and proposed screening questionnaire. ERJ Open Res. 2020;6:230–2020.
42. Quirce S, Vandenplas O, Campo P, Cruz MJ, de Blay F, Koschel D, et al. Occupational hypersensitivity pneumonitis: an EAACI position paper. Allergy. 2016;71:765–79.
43. Samson MH, Vestergaard JM, Knudsen CS, Kolstad HA. Serum levels of IgG antibodies against aspergillus fumigatus and the risk of hypersensitivity pneumonitis and other interstitial lung diseases. Scand J Clin Lab Invest. 2021;81:451–3.
44. Barton RC, Hobson RP, Denton M, Peckham D, Brownlee K, Conway S, et al. Serologic diagnosis of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis through the detection of immunoglobulin G to aspergillus fumigatus. Diagn Microbiol Infect Dis. 2008;62:287–91.