

REFERENCE RANGE FOR SPECIFIC IgG ANTIBODIES TO *ASPERGILLUS FUMIGATUS* IN THE SOUTH AFRICAN ADULT POPULATION

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ABSTRACT

Patients with aspergillosis and allergic bronchopulmonary aspergillosis (ABPA) usually present with high levels of specific IgG antibodies to *Aspergillus fumigatus*. There is currently no reference range for *A. fumigatus* IgG antibodies in healthy individuals in South Africa. The aim of this study was to develop a reference range in South African adults. Sera were collected from healthy blood donors who had no history of hypersensitivity to *A. fumigatus*. Specific IgG against *A. fumigatus* was determined using the ImmunoCAP system (Phadia, Uppsala, Sweden (now Thermo Fisher Scientific)). The distribution for specific IgG antibodies to *A. fumigatus* did not have a Gaussian distribution. The log-transformed data showed excellent agreement with normality. The reference range of the log-transformed data was delimited by the 2.5th centile (mean less 1.96 standard deviations) and the 97.5th centile (mean plus 1.96 standard deviations). The (antilogged) 2.5th centile [with 90% confidence intervals] was 2.79 [2.27-3.43] mgA/l. Similarly the 97.5th centile was 66.45 [54.01-81.77] mgA/l. Thus the reference range for specific IgG antibodies to *A. fumigatus* is 2.79 mgA/l to 66.45 mgA/l.

INTRODUCTION

Fungal spores are inhaled daily and the level of airborne fungal spores can be 1 000 times higher than pollen levels.¹ It is therefore counterintuitive that diseases caused by these fungi are of such low prevalence.¹ Although many different species of fungi are inhaled, *Aspergillus fumigatus*, a widely distributed fungus, is responsible for many diseases including aspergillosis, aspergilloma of lung and maxillary sinus and chronic pulmonary aspergillosis.¹ Allergic bronchopulmonary

aspergillosis (ABPA) occurs after colonisation of the bronchial tree with *A. fumigatus* in predisposed patients.¹ ABPA is a serious complication causing significant respiratory morbidity in patients with cystic fibrosis (CF) and asthma as a result of a hypersensitivity reaction to bronchial colonisation by *A. fumigatus*.^{2,3} If left untreated, ABPA can result in serious and irreversible lung damage leading to significant morbidity and mortality.^{2,3} Patients with aspergillosis and ABPA usually present with high levels of specific IgG antibodies to *A. fumigatus*.^{4,5} As there is currently no reference range for *A. fumigatus* IgG antibodies in healthy individuals in South Africa, the aim of this study was to develop a reference range for specific IgG antibodies to *A. fumigatus* in the South African adult population. The reference range will provide clinicians with guidance regarding the diagnosis of ABPA.

MATERIALS AND METHODS

Study population

One hundred and twenty-two blood samples were collected from healthy, HIV-negative blood donors from the Western Province Blood Transfusion Services. The donors ranged in age from 17 to 68 years and had no history of hypersensitivity to *A. fumigatus* and no prior exposure to birds (to exclude patients who might be suffering from extrinsic allergic alveolitis as a result of 'bird breeder's disease').⁶ Sera samples were collected and stored at -20°C until required. These donors included the various ethnic groups from the Western Cape region of South Africa. Ethical approval for this study was obtained from the University of Cape Town Research Ethics Committee.

Anti-*Aspergillus fumigatus* IgG detection in sera

Specific IgG against *A. fumigatus* was determined using the ImmunoCAP system (Phadia, Uppsala, Sweden (now Thermo Fisher Scientific))^{4,5} according to the manufacturer's instructions. Results were expressed as milligrams of *A. fumigatus*-specific IgG antibodies per litre (mgA/l). All reagents were from the same lot numbers and internal quality control samples were included in each run. The coefficient of variation between runs for the quality control samples was <10% (as per manufacturer's instructions should be ≤12%).⁷

RESULTS

The final sample size was 120, as two results were below the limit of quantitation (2.00 mgA/l) and were excluded from the parametric analysis provided here. The inclusion of the two results that were below the limit of quantitation did not affect the reference range in the non-parametric analysis (not presented) but did increase the confidence limits. The median age was 42 years and the interquartile range (IQR) 33 years. Specific IgG antibody results ranged from 2.10 to 99.20 mgA/l, with a median result of 14.50 mgA/l and IQR of 7.29 mgA/l.

STATISTICS

Statistical analysis of data

The statistics program, R (v 2.7.0 for Windows, The Comprehensive R Archive Network) was used for statistical analysis. The data were investigated for normality by visual inspection, construction of Q-Q-plots and the Shapiro-Wilk test. The data were not normally distributed. Log transformation of the data produced a Gaussian distribution. The reference range was established using a parametric method on the log-transformed data. The range (of the log-transformed data) was delimited by the 2.5th centile (mean less 1.96 standard deviations) and the 97.5th centile (mean plus 1.96 standard deviations).⁸ The 2.5th centile was 3.09 [2.10-3.62] mgA/l. The 90% confidence interval is in square brackets. The 97.5th centile was 58.16 [50.10-99.20] mgA/l.

Statistical results

The frequency distribution for specific IgG antibodies to *A. fumigatus* did not have a Gaussian distribution (Fig. 1A) and both Q-Q-plots (Figure 1B) and the Shapiro-Wilk test rejected the normality ($p=1.272e-12$). The log-transformed data showed excellent agreement with normality (Fig. 2A). The Q-Q-plot for the log-transformed data indicates that the data approximate a Gaussian distribution very well (Fig. 2B). Finally the Shapiro-Wilk test could not detect a statistically significant difference from normality ($p=0.8294$). For the logged data, the mean was 1.13 with a standard deviation of 0.35. The 2.5th centile was 0.45 [0.36-0.54] where the quantity in the square brackets is the 90% confidence interval of the centile. Similarly, the 97.5th centile is 1.82 [1.73-1.91]. The antilogged results were 2.5th centile 2.79 [2.27-3.43] mgA/l with 97.5th centile 66.45 [54.01-81.77] mgA/l.

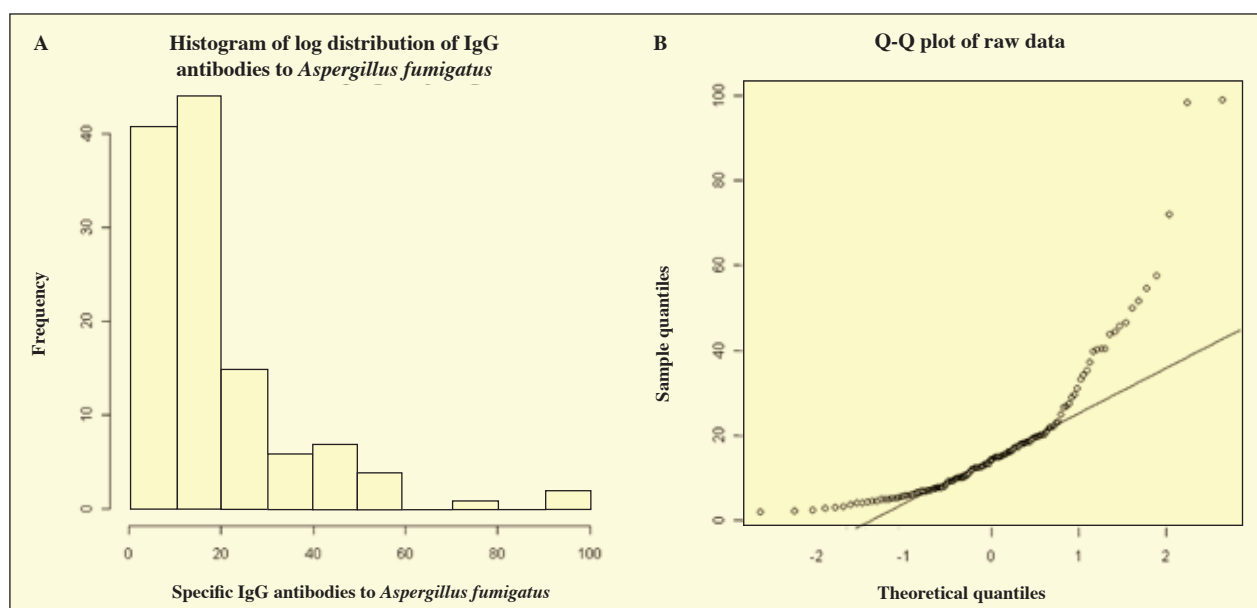


Fig. 1. The frequency distribution for the specific IgG antibodies to *Aspergillus fumigatus* data is indicated and is clearly not normally distributed (A). The deviation from Gaussian distribution is confirmed by the Q-Q-plot for the raw specific IgG antibodies to *A. fumigatus* data (B).

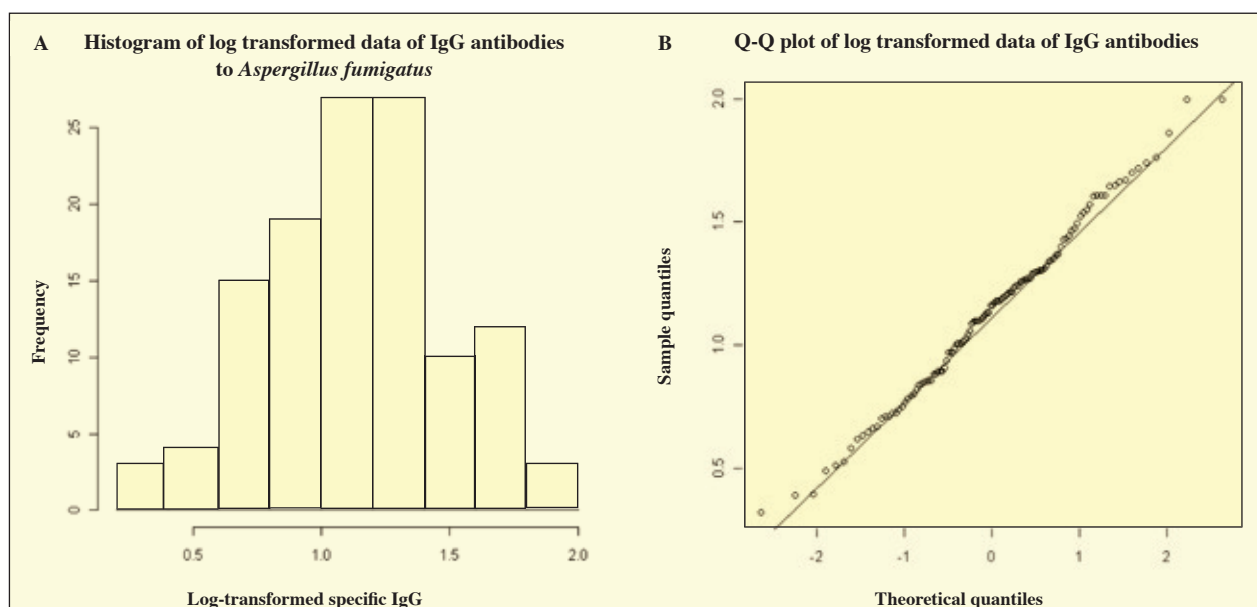


Fig. 2. The histogram for the log-transformed data of specific IgG antibodies to *Aspergillus fumigatus* (A). Log-transformed IgG antibodies to *A. fumigatus* Q-Q-plot for normality (B).

DISCUSSION

The reference range has been determined parametrically on the log-transformed data. The rejection of the parametric approximation on the raw data was based on the prominent deviation from normality. The decision to use the parametric method on the log-transformed data was both due to the excellent agreement of the log-transformed data with normality and the efficiency of parametric analysis.⁹ As a consequence the confidence intervals are small.

A comparison was made between the results obtained in this study and published reference ranges for IgG antibodies to *A. fumigatus* in healthy individuals. Research undertaken on healthy children in Finland, using an enzyme-linked immunosorbent assay (ELISA) to measure IgG antibodies to *A. fumigatus* reported that antibody levels increased until 6-7 years of age, after which the values remained constant.¹⁰ Median antibody levels in 7-9-year-olds were reported as 45.0 mg/l and 95th centile 67.8, which is similar to results in this study.¹⁰ In addition, Van Hoeyveld and co-workers relate median levels of 13.75 mgA/l and 97.5th centile of 70.10 mgA/l of these specific antibodies in healthy adult subjects, using the ImmunoCAP system.⁴ The published results in the adult population are comparable to the levels measured in the healthy South African adult population. In contrast, increased levels of *A. fumigatus* antibodies in ABPA patients were reported, with a median of 70.10 mgA/l and 75th centile of 113.45 mgA/l in a study by Van Hoeyveld and co-workers⁴ and a mean of 132.5 mgA/l reported by Barton and co-workers.⁵ Aspergillosis patients also demonstrated high IgG *A. fumigatus* antibodies, with a median of 103 mgA/l and 75th centile of 147 mgA/l.⁴

CONCLUSION

The reference range for specific IgG antibodies to *A. fumigatus* is 2.79-66.45 mgA/l. The confidence intervals are relatively small because of the efficiency of parametric analysis. The establishment of this reference range and the accuracy thereof is due to the fortuitous normality of the log-transformed data. The reference range will assist clinicians regarding the diagnosis of patients with ABPA.

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Declaration of conflict of interest

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