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Running Title: Immunology and Microbiology Assessment in Asthma

Utility of Immunology, Microbiology and Helminth Investigations in Clinical Assessment of Severe Asthma

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Abstract

Objective: Systematic assessment of patients with potential severe asthma is key to identification of treatable traits and optimal management. Assessment of anti-microbial immune function is part of that assessment at many centres although there is little evidence-base on its added value in clinical assessment of this patient group. As part of reviewing our local pathway we have retrospectively reviewed these tests in 327 consecutive referrals to our severe asthma service, in an evaluation to describe the utility of these tests and allow refinement of the local guideline for patient assessment.

Methods and Results: Serum immunoglobulin concentrations were in the normal range in most patients though 12 patients had serum IgG < 5.5 g/L and many had suboptimal anti-*Haemophilus* (127 of 249 patients tested) and anti-Pneumococcal (111 of 239) immune responses. As expected many patients had evidence of sensitisation to *Aspergillus* although specific IgG was not confined to those with evidence of allergic sensitisation/allergic bronchopulmonary aspergillosis (ABPA). Eighteen of 277 patients tested had serological evidence of *Strongyloides* infection. Bacteria and/or yeast were cultured from the sputum in 76 out of 110 patients productive of sputum, and the most common microbes cultured were *Candida sp.* (44 patients), *Staphylococcus aureus* (21 patients), *Haemophilus influenzae* (18 patients).

Conclusions: Many patients had evidence of infection, colonisation or sensitisation to potential pathogens relevant to asthma. *Strongyloides* infection was evident in several patients, which may be a major issue when considering the risk of hyper-infection following immunosuppression and supports our local screening strategy.

Keywords: Strongyloides, Candida, Hypogammaglobulinaemia, Corticosteroids, Immunoglobulins

Introduction

Difficult-to-treat asthma is a heterogeneous condition in which individual patient disease is characterised by combinations of treatable traits (1, 2). Systematic assessment of patients, using assessment protocols and investigation bundles, improves detection and diagnosis of these treatable traits but is resource intensive (3–5). Addressing comorbidities and considering disease phenotypes through systematic assessment underpins the international European Respiratory Society (ERS) and American Thoracic Society (ATS) joint guidelines for diagnosis of severe asthma and its management (6).

Licensing of anti-eosinophil monoclonal antibody therapies has greatly increased the number of severe asthma patients who are candidates for treatment with these high-cost biologic therapies (7). In England, UK, the National Health Service for England (NHSE) mandates these high-cost

therapies are only prescribed by specialist centres capable of conducting appropriate systematic assessments. The large increase in patients referred to our centre, in common with other national centres, has increased the volume of assessments to be conducted with resulting resource implications necessitating evaluation of which aspects of systematic assessment are most beneficial to patient management.

Assessment of anti-microbial immune function is part of severe asthma assessment at many centres. Th2-biased immune responses themselves (8, 9) and corticosteroids used to treat Th2 inflammation (10–12) are both known to compromise anti-microbial immune responses. Furthermore there is an overlap between asthma and bronchiectasis (13), and immune deficiency states such as antibody deficiencies can present with similar symptoms to severe asthma (14, 15). The importance of the airway microbiome in asthma, and the pathological role of airway infections in asthma are both emerging areas of importance in asthma management (16).

Another concern is asymptomatic chronic *Strongyloides* infection. *Strongyloides stercoralis* is a parasite endemic to resource-poor tropical and sub-tropical regions, with an estimated global prevalence of 100 million cases (17). *Strongyloides* is almost unique amongst parasites in that it can complete its entire life-cycle within the human host and as a result chronic, often asymptomatic, infection can persist for decades after primary infection (17). Immune-mediated control of helminth infection is thought to rely on an intact Th2 immune response (18). With immune suppression, particularly with corticosteroids (19), there is an increased risk of developing hyper-infection, which carries a high mortality. This increased risk also hypothetically applies to anti-Th2 monoclonal antibody therapies. Concerns around the risk of hyper-infection, especially given the high prevalence of *Strongyloides* sero-positivity within the population that the hospital serves (20), led to introduction of screening patients for asymptomatic *Strongyloides* infection with an increasing volume of tests as the service grew.

The evidence-base on which laboratory tests to request, and their utility in severe asthma assessment, is poor to-date. Given local resource implications we therefore undertook a service evaluation to ascertain which of these tests were influencing patient management, and to allow informed discussion and refinement of the local guideline for systematic assessment investigations. Here we present data from the service evaluation, describing clinical immunology and microbiology assays in consecutive patients referred for systematic severe asthma assessment.

Methods

Patients

Patients referred to the North Central and East London Severe Asthma Service for confirmation of severe asthma diagnosis as per ERS/ATS criteria (6) and consideration for biologic therapy undergo a systematic assessment as per NHSE specification. The internal guideline for this systematic assessment includes as standard of care the following investigations in all new referrals: measurement of serum immunoglobulins; serological assays of antibodies to *Haemophilus*, *Pneumococcus*, *Aspergillus* and *Strongyloides*; and sputum culture in patients spontaneously productive of sputum.

The results of these clinical immunology and microbiology tests from consecutive adult patients undergoing severe asthma assessments between September 2016 and October 2018 were collated and analysed by members of the clinical team as part of this local service evaluation (Barts Health NHS Trust Clinical Effectiveness Unit references 9269 and 7643).

Serum Immunoglobulins

Serum IgA, IgG and IgM levels were measured using a Roche c 702 module (Roche Diagnostics) according to the manufacturer's instructions. Serum total IgE was measured by Phadia ImmunoCAP (Thermo Fisher Scientific) on a Phadia 250 instrument according to the manufacturer's instructions.

Anti-microbial Serological Assays

Anti-Haemophilus influenzae serotype B IgG antibodies were measured by ELISA (Binding Site) according to the manufacturer's instructions. The minimum protective level for IgG antibody for Haemophilus is >0.15mg/L, the optimal protective level is >1.00mg/L. Serotype-specific Pneumococcal IgG antibodies were measured by a multiplex bead assay previously described (21, 22). The assay measures 13 of the commonest pneumococcal serotype antibody levels. For each serotype measured a level >0.35IU/ml is considered protective for invasive pneumococcal disease. There is only partial consensus in the UK as to what constitutes a satisfactory response (23, 24) but regionally when 7 or more out of the 13 pneumococcal serotypes are above the protective level this is considered an adequate response (22). Aspergillus fumigatus IgE and IgG were measured by Phadia ImmunoCAP (Thermo Fisher Scientific) on a Phadia 250 instrument, according to the manufacturer's instructions. Strongyloides infection was examined by measuring anti-Strongyloides IgG (Bordier Affinity Products, Crissier, Switzerland) as previously described (25).

Routine Sputum Culture Methodology

Upon receipt of sputum samples a liquefying agent (Dithiothreitol and phosphate buffer, pH 7.0, (Mycolyse), Pro-Lab Diagnostics) was added to a dilution of 1:2, and the sample vortexed for up to 20 minutes. The end-point was complete liquefaction. 10µl of liquefied sputum was inoculated for colony enumeration onto two agar plates and incubated as follows:

- Blood agar (Thermo Fisher Oxoid) with an optochin disc, incubated in CO₂ at 37°C for 48 hours.
- Bacitracin Chocolate agar (Thermo Fisher Oxoid), incubated in CO₂ at 37°C for 48 hours.

Quadrant streak culture methodology was used to determine sputum culture growth in a semi-quantitative manner. To allow comparison to quantitative analysis of extended sputum cultures, dependent on the number of serial quadrant streak dilutions in which organisms were grown, microbial growth was recorded as colony forming units (cfu) as follows. Growth only in sputum inoculum as 100 cfu/ml; and growth in serial quadrant dilutions as 1 000 cfu/ml, 10 000 cfu/ml and 100 000 cfu/ml.

Extended Sputum Culture Methodology

Upon receipt of sputum samples a liquefying agent (Dithiothreitol and phosphate buffer, pH 7.0, (Mycolyse), Pro-Lab Diagnostics) was added to a dilution of 1:2, and the sample vortexed for up to 20 minutes. The end-point was complete liquefaction. 20µl of liquefied sputum was inoculated for colony enumeration onto an array of six different agar plates and incubated as follows:

- Blood agar (Thermo Fisher Oxoid) (plus optochin disc), incubated in CO₂ at 37°C for 48 hours.
- MacConkey agar (Thermo Fisher Oxoid), incubated in CO₂ at 37°C for 48 hours.
- Chromogenic *Staphylococcus aureus* agar (E&O Laboratories Ltd), incubated aerobically at 37°C for 48 hours.
- *Pseudomonas* selective agar (Thermo Fisher Oxoid), incubated in CO₂ at 37°C for 3 days.

- *Burkholderia cepacia* selective agar (Thermo Fisher Oxoid), incubated in CO₂ at 37°C for 3 days.
- Sabouraud agar (Thermo Fisher Oxoid), incubated in CO₂ at 37°C for 5 days.

In addition, a 200µl sample of liquefied sputum was further diluted in 0.9% saline, to a final dilution of 1:10,000 and 20µl of this dilute sample was inoculated onto a Bacitracin Chocolate agar (Thermo Fisher Oxoid) plate.

Colonies of organisms isolated were counted, identified by MALDI-TOF, and the growth quantity of each organism reported in colony forming units/ml of direct sputum.

Statistics

Results were analysed in R (version 3.5.1; www.r-project.org) using the following additional packages: eepTools, gplots, factoextra and cluster. Parametric distributed data is presented as mean with standard deviation, and non-parametric data as median with interquartile range. Associations were studied using linear regression models (lm function) and Pearson's correlation coefficient (cor.test function with pearson model). Graphs were produced with R and GraphPad Prism 6.0 (GraphPad Software, USA). Heat map produced in R using heatmap.2 function with matrix shading intensity proportionate to log (1 plus cfu from sputum culture).

Results

During the evaluation period 327 patients underwent systematic assessment with protocolled investigations (Table 1). Of these 270 patients were diagnosed with severe asthma as per ERS/ATS criteria after multi-disciplinary review and initial investigations. The remaining patients either did not have severe asthma or needed further assessment to clarify diagnosis.

Serum Immunoglobulins

Serum immunoglobulin concentrations were measured in 285 of the patients undergoing assessment (not measured in 42). Of those 285 patients, 238 were subsequently diagnosed with severe asthma after multidisciplinary assessment. The mean (SD) serum Immunoglobulin A (IgA) concentration for the patients referred for assessment was 2.40 g/L (1.17) and for the subgroup with confirmed severe asthma 2.37 g/L (1.09). For IgG the mean (SD) concentrations were 10.1 g/L (3.36) and 10.1 g/L (3.33) respectively, and for IgM 1.14 g/L (1.03) and 1.13 g/L (1.08) respectively (Figure 1A). Few patients were found to have clinically low serum immunoglobulin concentrations with 8 patients having serum IgA < 0.8 g/L, 12 patients with serum IgG < 5.5 g/L including 1 patient with serum IgG < 3.5 g/L, and 30 patients with serum IgM < 0.4 g/L.

We next examined using linear regression models whether there were any associations between serum immunoglobulin concentrations and patient reported courses of oral antibiotics taken over the preceding year. There were no significant associations between either serum IgA or serum IgM and courses antibiotics, but a significant association between serum IgG and courses of antibiotics was evident with lower serum IgG in patients receiving more courses of antibiotics (p=0.0237; Figure 1B). However visual examination of the data was suggestive of a possible confounding effect of courses of oral corticosteroids (prednisolone) over the preceding year with a significant correlation between courses of oral corticosteroids and oral antibiotics (Pearson's Coefficient, r=0.7594, p<0.001; Figure 1C). Therefore a further multiple regression was undertaken to examine whether there was any association of serum IgG with courses of antibiotics, short courses of oral corticosteroids for exacerbations and/or maintenance oral corticosteroid dose. There was a significant association between serum IgG and maintenance oral Prednisolone dose (p=0.022) with lower serum

IgG in patients on higher doses of maintenance oral Prednisolone, but not between serum IgG and either courses of antibiotics or short courses of Prednisolone.

Serum Antibodies to Microbes

Serum antibodies to *Haemophilus* and *Pneumococcus* were measured in patients undergoing assessment to examine immunological responses to different types of antigens (Figure 2A-B). The median (IQR) average anti-*Haemophilus* antibody titre was 0.89 mg/L (1.54) (i.e. above the minimum protective level of 0.15 mg/L but below the optimal protective level of 1.00 mg/L) and median (IQR) number of *Pneumococcal* serotypes to which patients had protective antibody response (≥ 0.35 IU/ml) was 7 (5) serotypes out of 13 tested. The proportion of patients with anti-*Haemophilus* antibody titre < 0.1 mg/L was 28/249 and proportion with titre < 1.0 mg/L was 127/249. The proportion of patients with protective responses to less than 7 *Pneumococcal* serotypes was 111/239.

Patients with suboptimal antibody responses to *Pneumococcus* (i.e. those who had < 7 out of 13 serotypes above the protective level of 0.35 IU/ml) and *Haemophilus* (< 1 mg/L) were asked to attend local primary care services for Pneumovax and Menitorix vaccinations respectively, and then serum antibodies re-measured after an appropriate period (at least 4 weeks or later after immunisation). Forty-nine of 111 patients have to-date had antibodies to *Pneumococcus* re-measured, and 33 of 127 patients to *Haemophilus* re-measured. Thirty five of the 49 had protective responses to at least 7 *Pneumococcal* serotypes on re-testing, and 20 of the 33 reporting immunisation with Menitorix had a protective anti-*Haemophilus* antibody titre > 1.0 mg/L on re-testing.

Mean (SD) serum concentration of IgG to *Aspergillus fumigatus* was 26.8 mg/L (26.8) and in 35 of 302 patients the serum *Aspergillus* IgG was abnormally elevated (above 50 mg/L) (Figure 3A). In many of the patients with raised serum IgG to *Aspergillus fumigatus*, serum IgE to *Aspergillus fumigatus* was not raised (Figure 3B) and serum total IgE concentrations were not raised sufficiently for a diagnosis of ABPA (Figure 3C).

Eighteen of 277 (7%) patients undergoing assessment (16 of 237 with confirmed severe asthma) tested had positive *Strongyloides* serology (Figure 3D). Data for ethnicity and travel history were incomplete, but in two patients with complete data no relevant risk factors (e.g. residence in or travel to an endemic country) were identified.

Sputum Culture

110 patients undergoing assessment were productive of sputum and gave a sample for microbiological cultures. 95 of these samples underwent extended spectrum cultures whilst the remainder underwent routine culture. Bacteria or yeast were cultured in 76 of the 110 samples. The most frequent microbe isolated was yeast, which when further identified in 44 individuals were *Candida* sp. *Staphylococcus aureus* was cultured from the sputum in 21 individuals and *Haemophilus influenzae* in 18 individuals. There was no clear pattern of growth of different microbes being associated with blood eosinophil count (Figure 4). In many cases these organisms were present in low abundance.

Discussion

In this evaluation of immunology, microbiology and helminth investigations in clinical assessment of severe asthma we found relatively few patients to have lower than normal serum immunoglobulin concentrations but many to have evidence of infection, colonisation or sensitisation to potential pathogens. In particular *Strongyloides* infection was evident in some patients, which may be a major

issue when considering consequences of immunosuppression. Due to the characteristics of *Strongyloides* that are distinct amongst parasites, namely its capacity to complete its life-cycle within the human body, it has the capacity to cause chronic asymptomatic infection, and to cause hyper-infection in the setting of immune-suppression.

Our finding that a proportion of patients with severe asthma have low serum immunoglobulin concentrations is consistent with previous reports (26, 27). In particular the association between lower serum IgG concentrations and treatment with oral corticosteroids is consistent with the previous findings of Hamilos *et al.* (26) and of Kawano *et al.* who found an association with daily dose of maintenance prednisolone in asthma, but not duration of prednisolone therapy (28). Although immunoglobulin therapy could be considered for corticosteroid-associated hypogammaglobulinaemia, our own experience shows little evidence of long term benefits from such therapy and certainly this is not a treatment for severe asthma as previous randomised clinical trials have shown (29, 30). The optimal management is to reduce systemic corticosteroids and this is now possible with the arrival of a host of biologic therapies in severe asthma with proven corticosteroid weaning action. Indeed the PROSE study in allergic asthma has shown Omalizumab to enhance anti-viral host defence (31).

A proportion of patients had suboptimal serological responses to *Pneumococcus* and/or *Haemophilus* at baseline. In the majority the serological responses incremented to protective levels after the patients were advised to undergo appropriate vaccination(s). Patient reports of vaccinations were not verified and might not be accurate, which may explain serological responses failing to increment in some patients. However, the fact that several patients continued to use systemic corticosteroids is a likely factor contributing to some patients not showing protective serological responses at follow-up after vaccination. As discussed below, the real-world nature of this service evaluation is a limitation to assessing why some patients did not have protective serological responses at follow-up. Failure to develop protective serological responses after vaccination, together with low serum immunoglobulin concentrations, is an indicator for further immunodeficiency testing and for immunoglobulin replacement therapy after multi-disciplinary agreement. It should be noted that in addition to the diagnostic value of testing the capacity of the immune system to respond to these vaccinations, there is also clinical value to ensuring through vaccination that patients have protective immune responses (23).

In addition to those patients diagnosed with impaired immune function secondary to long-term corticosteroids, to date at our centre two patients undergoing severe asthma assessment have been diagnosed with other immunodeficiencies. In both cases other features of the clinical assessment were atypical for severe asthma. In one case a non-tuberculous mycobacterial pulmonary disease (NTM-PD) was evident and in the other pulmonary fibrosis with bone marrow failure. It may therefore be possible to select out only a subset of patients for detailed immunological assessment based on clinical features identified at assessment. However a history of frequent chest infections is not useful to guide patient selection for further immunological assessment in this cohort.

Our local population has large ethnic diversity, including a proportion of first generation immigrants from South East Asia, with high rates of *Strongyloides* infection (20). We therefore undertake screening for *Strongyloides* due to concern in these individuals that chronic asymptomatic infection may progress to life-threatening hyper-infection after suppression of the Th2 immune system. Oral corticosteroids are associated with development of disseminated *Strongyloides*/hyper-infection (19). There is no data we are aware of examining risk of *Strongyloides* hyper-infection in patients on anti-eosinophil biologic therapy but there is good evidence that eosinophils may have a major protective role in immune defence against *Strongyloides* and other helminths (32). That some of our South East Asian patients had detectable anti-*Strongyloides* antibodies and therefore potential quiescent infection, was not surprising. However, unexpectedly, several patients with no reported history of travel to regions where *Strongyloides* is endemic, or other reported risk factors, had positive

Strongyloides serology. *Strongyloides* is not endemic in the UK. The index exposure may have been decades previously and so may not have been gleaned through a 'routine' travel history, which typically only covers travel in the preceding five years and relies on patient recall. Although positive serology does not necessarily mean current active infection, this result is in keeping with the fact that *Strongyloides* infection can persist for years following travel to an endemic area and potentially disseminate many years later. We treat severe asthma patients with positive *Strongyloides* serology with ivermectin and then consider commencing biologic therapy 3 months later. We have not seen any consistent impact on asthma control following ivermectin treatment and therefore the primary reason to treat *Strongyloides* infection is to prevent development of life-threatening hyper-infection upon immunosuppression with biologics or further systemic corticosteroids. The risk of re-activation of latent tuberculosis was not at first appreciated in the early days of anti-TNF α treatments and there was a delay before widespread screening was introduced (33). There is a danger of a similar delay in recognising the possibility of *Strongyloides* hyper-infection in patients started on anti-Th2 biologics or receiving frequent systemic corticosteroids.

Raised serum IgG to *Aspergillus* was evident in a proportion of patients but in many of these there was no serological evidence of allergic broncho-pulmonary aspergillosis (ABPA). This finding is consistent with that of Woolnough and colleagues who did however find an association between serum IgG to *Aspergillus* and radiological evidence of airway damage (34). Raised *Aspergillus* IgG may reflect past airways infection with *Aspergillus*. Interestingly *Aspergillus* can also stimulate Th2 immune responses through non-allergenic (non-IgE) mechanisms such as fungal proteases (35), and it is possible that past aspergillus infection of the airway may contribute to the development of severe asthma. Overall routine testing for serum *Aspergillus* IgG did not contribute to our patient management.

The most common microbe cultured from our patients' sputum was *Candida* sp. The high prevalence of *Candida* sp. likely reflects a consequence of corticosteroid therapy, and consequent increase in oropharyngeal candidiasis (36, 37). Often historically thought relatively benign, there is increasing evidence for a potential role in pathological immunological responses (38–41). Furthermore case-series evidence suggests patients may benefit from anti-fungal treatment for chronic *Candidal* bronchitis (42). The second commonest microbe was *Staphylococcus aureus*, though typically at low abundance. This is often thought to represent commensal colonisation but similarly to *Candida* sp. there is increasing evidence of the capacity of *Staphylococcus aureus* to induce Th2-type inflammation through multiple different mechanisms (35, 43).

The major limitation of these findings is that the data collection was from a service evaluation analysing which immunological and microbiologic investigations were being undertaken in our service, which were beneficial to assessment in our local severe asthma systematic assessment, and which were not contributing to clinical assessment. Although the local guidance for systematic assessment states all the above investigations are to be requested for all new patients, in some cases not all tests were conducted, for example with serum immunoglobulins not tested in 42 undergoing systematic assessment (32 subsequently diagnosed with severe asthma). Additionally, dedicated research tests were not conducted as part of the evaluation. For example 16S RNA sequencing of sputum is a better method than culture for analysing sputum microbiome but not part of our routine clinical assessment of patients. Furthermore oropharyngeal contamination of sputum samples cannot be excluded. This may explain the lack of any clear association between sputum cultures and blood eosinophil counts in contrast to research studies such as Taylor *et al.* that have suggested *Haemophilus* to be more abundant in non-eosinophilic asthmatics (16).

As a result of this service evaluation we have revised our standard panel of immunology and microbiology tests undertaken in all new referrals. Whilst local decisions will always be dependent on local resources, we would recommend testing *Strongyloides* serology in all new referrals. Similarly, we would recommend sputum cultures as beneficial to patient assessment in those patients

who are productive of sputum. Analysis of immunoglobulins and ‘vaccine’ responses can be helpful although in most cases poor responses are indicative of chronic steroid exposure, and such testing could be limited only to those patients where there are specific concerns. A reported history of antibiotics for chest infections does not help identify patients for further immunology assessment given the strong association between courses of antibiotics and courses of oral corticosteroids received by patients, and difficulty patients have in identifying between infective and non-infective exacerbations. We would not recommend routine testing of IgG to *Aspergillus*.

Conclusions

In summary our major findings are that a proportion of severe asthma patients in our regional severe asthma service do have lower serum immunoglobulin concentrations, most likely as a consequence of systemic corticosteroid therapy rather than as a result of a primary immunodeficiency. *Candida* sp. and *Staphylococcus aureus* were the most frequently present microbes on sputum culture, which is of interest given growing evidence of the pathological actions of both. More unexpectedly ‘latent’ *Strongyloides* infection was evident in both individuals of high-risk and low-risk background, supporting our current strategy of screening all patients being considered for biological therapy.

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Declaration of Interests

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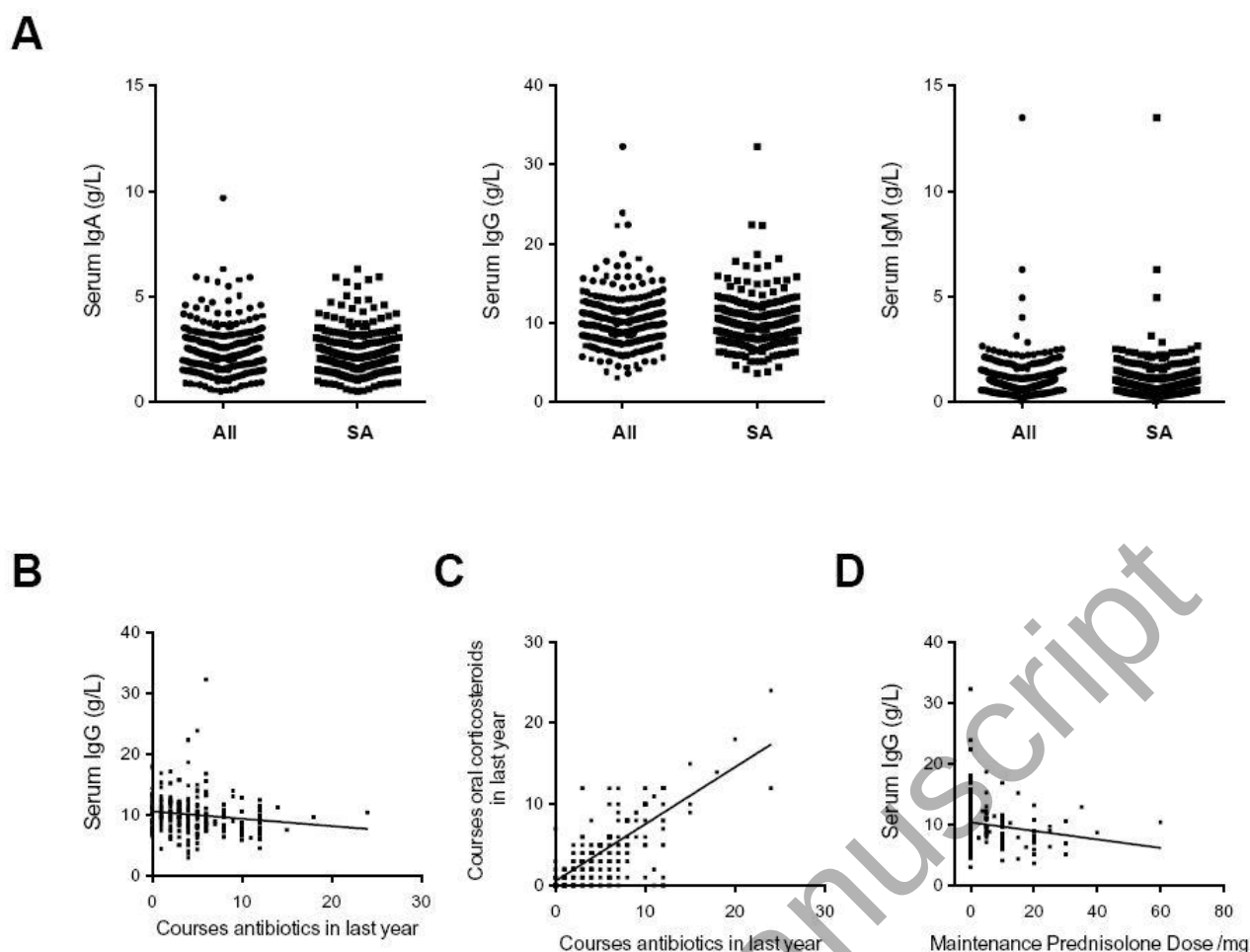


Figure 1: Serum immunoglobulin concentrations and associations with clinical characteristics.

(A) Serum immunoglobulin concentrations in **All**, all patients referred for severe asthma assessment; and **SA**, the subgroup of patients with confirmed severe asthma. (B) Comparison of serum immunoglobulin G (IgG) to courses of antibiotics for chest infections over preceding year. (C) Comparison between patient reported courses of oral corticosteroids for asthma exacerbations and courses of antibiotics for chest infections over preceding year. (D) Comparison of serum immunoglobulin G (IgG) to maintenance Prednisolone dose at time of assessment.

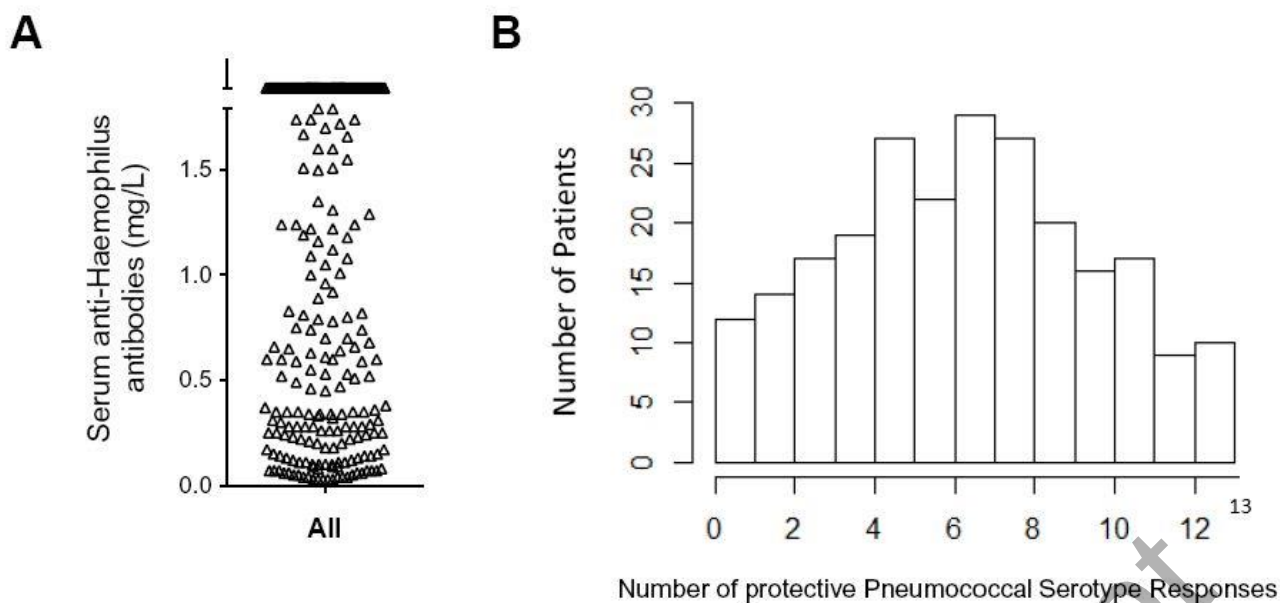


Figure 2: Serological responses to Haemophilus and Pneumococcal antigens.

(A) Serum concentrations of antibodies to Haemophilus in patients undergoing severe asthma assessment (mg/L). Top of range of detection, 1.8 mg/L. (B) Frequency histogram showing the number of Pneumococcal serotypes to which patients had protective responses.

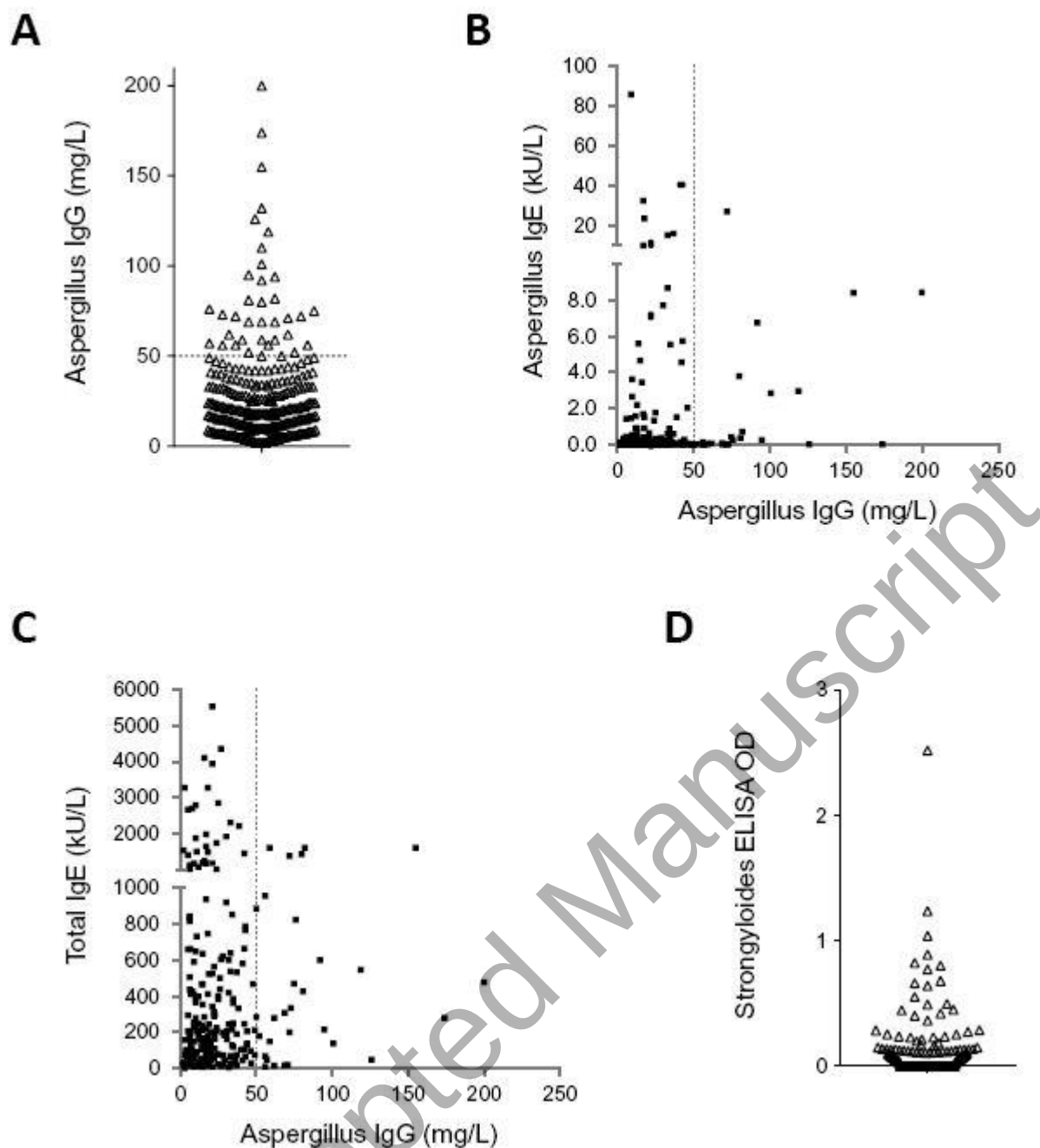


Figure 3: Serological responses to Aspergillus and Strongyloides antigens.

(A) Serum concentrations of IgG antibodies to Aspergillus in patients undergoing severe asthma assessment. (B-C) Comparison of patient serum IgG antibodies to Aspergillus to serum specific IgE antibodies and total serum IgE. (D) ELISA optical density (OD) for serum IgG antibodies to Strongyloides in patients undergoing severe asthma assessment. Axis intercept for Aspergillus IgG axis at upper limit of normal.

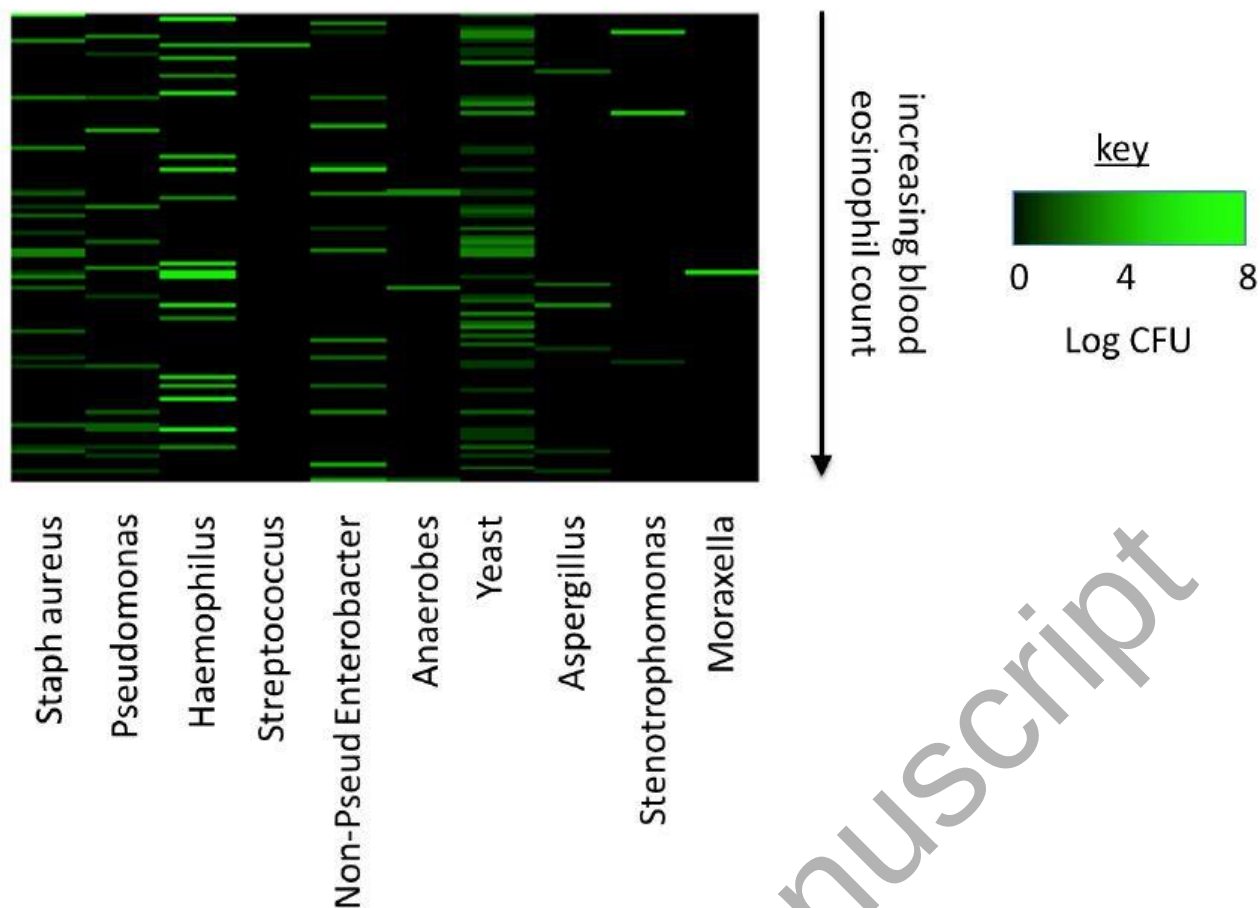


Figure 4: Heat-map of sputum culture microbial growths.

Heat-map showing growth of different microbes from individual patient sputum samples as colony forming units (cfu) on a logarithmic scale. Patients ordered on the vertical axis by blood eosinophil count from lowest (top) to highest (bottom).

Table 1: Demographics of patients undergoing systematic assessment.

	Patients undergoing systematic assessment
	(n = 327)
current age (years)	
mean (SD)	48.9 (14.8)
age onset asthma (years)	
mean (SD)	24.4 (18.3)
gender	
female	225 (69%)
male	102 (31%)
ethnicity	
Caucasian	103 (31%)
Asian	55 (17%)
Black	31 (9%)
other	22 (7%)
information declined	116 (35%)
inhaled corticosteroid dose (microg)	
mean BDP equivalent (SD)	1760 (650)
ACQ6 score	
mean (SD)	3.2 (1.5)

Presented as mean and standard deviation (SD) unless otherwise stated. BDP; beclometasone dipropionate. ACQ; Asthma Control Questionnaire.