Journal of Infection and Public Health (2011) 4, 235—243

http://www.elsevier.com/locate/jiph

Clinical relevance of peripheral blood eosinophil count in allergic bronchopulmonary aspergillosis

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Received 21 February 2011; received in revised form 17 August 2011; accepted 21 August 2011

KEYWORDS
Allergic bronchopulmonary aspergillosis (ABPA); *Aspergillus fumigatus*; Eosinophils

Summary
Background and aims: Currently, there is not a uniform consensus regarding the number of criteria or specific cut-off values for the variety of tests that are used to diagnose allergic bronchopulmonary aspergillosis (ABPA). Traditionally, an eosinophil count >1000 cells/μl is considered an important criterion in the diagnosis of ABPA. The goal of this study was to delineate the significance of the peripheral blood eosinophil count in the diagnosis of ABPA, and the relationship between eosinophil counts and lung function and immunological and radiological parameters.
Methods: This study was a retrospective analysis of the data from ABPA patients who were managed in our chest clinic. Based on their eosinophil count, the patients were classified into the following three categories: <500, 500—1000 and >1000 cells/μl. The spirometric, immunological and radiological characteristics were also assessed.
Results: We studied 108 males and 101 females with a combined mean (±SD) age of 34.1 ± 12.5 years. The median (IQR) eosinophil count at diagnosis was 850 (510—1541) cells/μl, and 60% of the patients had an eosinophil count of <1000 cells/μl. We found no relationship between eosinophil count and lung function using spirometry and other immunological parameters. The median eosinophil count was higher in patients with an high resolution computed tomography (HRCT)

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1876-0341/$ — see front matter
doi:10.1016/j.jiph.2011.08.006

chest finding of bronchiectasis (986 vs. 620, $p < 0.001$) vs. those without and in patients with high-attenuation mucus (1200 vs. 800, $p < 0.001$) compared to those without high-attenuation mucus.
Conclusions: A peripheral blood eosinophil count has limited utility in the diagnosis of ABPA, and there is no relationship between eosinophil count and lung function or other immunological parameters. The higher eosinophil count that we observed in patients with central bronchiectasis or high-attenuation mucus suggests that eosinophils are primary mediators of inflammatory activity in ABPA.

Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is an immunological pulmonary disorder and occurs in response to antigens that are released by the ubiquitous fungus *Aspergillus fumigatus*, which colonizes the tracheobronchial tree [1]. The ABPA is a common serious complication in the natural disease course of patients with bronchial asthma and cystic fibrosis. Indeed, a recent systematic review estimated that the prevalence of ABPA in asthma and cystic fibrosis is approximately 13 and 8%, respectively [1,2]. ABPA was first described in a report by Hinson et al. in 1952 in the United Kingdom, in which they described three cases of bronchopulmonary aspergillosis [3]. The classic criteria that were proposed by Rosenberg and Patterson are commonly used in diagnosing ABPA [4,5]. However, there is currently no uniform consensus with regard to the number of criteria that are needed for diagnosis nor are there optimum disease-specific cut-off values for the various tests that are employed in establishing a diagnosis [6].

Depending upon the stage of the disease, the immune-mediated damage in ABPA is reflected by elevated levels of both total and *A. fumigatus*-specific IgE and eosinophils. Eosinophils are leukocytes that are derived from the bone marrow [7,8]. In most reviews, a peripheral blood eosinophil level above 1000 cells/μl has been used as a criterion for diagnosis of ABPA [1,9—11]. However, an eosinophil count may be of limited diagnostic utility, as many patients may not have an elevated eosinophil count [12]. However, there is a paucity of convincing published data that either support or refute these claims. We observed that many of the ABPA patients in our chest clinic had been screened using an eosinophil count by their general/family physicians. Many of these patients had counts that were lower than 500—1000 cells/μl, which precluded additional work-up of these patients. Using a multivariate analysis, we demonstrated previously that an eosinophil count does not reflect remission in ABPA [13,14].

However, the relevance of eosinophil counts — particularly, their role in the diagnosis of ABPA and its pathophysiological mechanisms — and their correlation with other immunological and radiological findings, remain poorly understood. We hypothesized that an elevated eosinophil count is not related to the others markers of severity in ABPA. In the present study, we further delineated the role of the eosinophil count in the diagnosis of ABPA and the relationship of eosinophil levels with other diagnostic markers in a heterogeneous group of patients with ABPA.

Materials and methods

The study was a retrospective analysis of previously published data and includes glucocorticoid-naïve patients with ABPA that were diagnosed in the Chest Clinic of Postgraduate Institute of Medical Education and Research, Chandigarh from January 2002 through June 2008. The clinical characteristics and outcomes of these patients have been described previously [13—16]. In our chest clinic, every patient with asthma is screened for *Aspergillus* sensitization using an intradermal skin test, and patients who exhibit immediate cutaneous hyperreactivity are evaluated further by examining their IgE levels (both total and *A. fumigatus*-specific), eosinophil count, *Aspergillus* precipitins, and by performing high-resolution computed tomography (HRCT) of the chest. Patients who met both of the following criteria were classified as ABPA cases: a total IgE level >1000 IU/ml and an *A. fumigatus*-specific IgE levels >0.35 kilounit of antibody (kUA)/l. Additionally, the ABPA patients had to have any two of the following four criteria: (a) the presence of serum precipitins against *A. fumigatus*; (b) radiographic pulmonary opacities (fixed/transient); (c) an absolute eosinophil count

>1000 cells/μl; and (d) central bronchiectasis that was visible on HRCT [13—15,17]. All procedures, including the CT scans, were performed prior to initiating the glucocorticoid therapy and were performed within a 2—3-day period. The Institutional Ethics Committee approved the study, and each patient provided informed consent.

Aspergillus skin test

This test was performed using an *A. fumigatus* antigen that was prepared in the Department of Medical Mycology [18]. The test forearm was injected intradermally with 0.2 ml of the *Aspergillus* antigen (100 PNU/ml), and 0.2 ml of phosphate-buffered saline was injected in the other forearm to serve as a negative control. Reactions were classified as type I if wheal and erythema occurred within a minute, peaked after 10—20 min and resolved within 1—2 h. The antigen reaction in the arm skin needed to be at least 8 mm larger than the reaction in the control arm. Any residual subcutaneous edema after 6 h was classified as a type III reaction.

Serum total IgE and *A. fumigatus*-specific IgE levels:

IgE levels were measured using commercially available kits that are based on the quantitative enzyme-linked immunosorbent assay (Demeditec diagnostics GmbH, Kiel, Germany) and fluorescent enzyme immunoassay (UniCap Systems; Pharmacia & Upjohn; Stockholm, Sweden).

High-resolution CT (HRCT) of the chest:

HCRT was performed using a 16-row, multiple-detector CT scanner (LightSpeed Plus; GE Medical Systems; Slough, UK) with a matrix size of 512 × 512. The scans were obtained from the lung apex to the lung base using a scan time of 3 s while the patient was in the supine position at full end-tidal inspiration. Image acquisition was contiguous, and the images (1.25 mm at 10-mm intervals) were reconstructed using a high-spatial-frequency algorithm. The presence and extent of bronchiectasis on the HRCT chest were categorized in accordance with the criteria that were described by Reiff et al. [19]. Individual bronchopulmonary segments were identified based on their relationship with the major and minor fissures and the appropriate lobar bronchi [20]. Bronchiectasis was classified as 'central' when it was confined to the medial half (the midway point between the hilum and the chest wall) of the lung [21]. High-attenuation mucus (HAM) was considered to be present if the mucus was visibly denser than the paraspinal skeletal muscle [13,16,22].

A. fumigatus precipitins

The *A. fumigatus* precipitins were detected using the Ouchterlony gel diffusion technique according to the method that was described by Longbottom and Pepys [23].

Eosinophil count

A 2-ml peripheral blood sample was collected into an EDTA tube to perform an eosinophil count. The number of white blood cells (WBCs) was measured using an automated blood cell analyzer with a 5-part differential (LH-750 or SF-3000). To measure the eosinophils, a blood slide was prepared using the Wright—Giemsa stain. An initial evaluation at 10× magnification (total magnification 100×, including the eyepiece) provided an assessment of the overall smear quality and the distribution of the cells. A differential leukocyte count was performed at 100× (total 1000×) magnification. This process involved counting and classifying 100 WBCs and recording the percentage of each WBC subtype. We then multiplied the percentage by the total leukocyte count to obtain the absolute eosinophil count.

Based on their absolute eosinophil counts, the patients with ABPA were stratified into the following three categories: <500, 500—1000, and >1000 cells/μl. The relationship between these counts and the spirometric, immunological, and radiological characteristics were assessed within each group.

Spirometry

Each subject performed spirometry on a dry rolling-seal spirometer (Spiroflow; PK Morgan Ltd.; Kent, UK). The values were measured according to the American Thoracic Society guidelines, and the highest measurements from among three technically acceptable and reproducible maneuvers were obtained at body temperature and pressure that was saturated with water vapor [24]. The spirometer was calibrated frequently to ensure consistent and reliable performance. Age, gender, height and spirometry data were recorded for each patient using our previously developed computer software [25].

Statistical methods

The data are presented as the mean (SD), median (IQR) or number (percentage). Differences with a *p*-value of lower than 0.05 were considered to be significant. The differences between the continuous variables were analyzed using the Mann—Whitney *U* test or the Kruskal—Wallis test (with Dwass—Steel—Critchlow—Fligner test for post hoc analysis) where appropriate. The categorical variables were compared using the Chi-square test. Chi-square tests for more than 2×2 rows or columns were performed by extracting the 2×2 table of interest from the original table and applying the Bonferroni adjustment for *p*-values. Linear regression was performed to ascertain the strength of the relationship between two quantitative variables. A scatter plot was initially constructed to assess how closely the eosinophil counts approximated the serum IgE levels. The points were then fitted to a linear regression curve using the ordinary least-squares method.

Results

This study comprised 108 males and 101 females with a combined mean (SD) age of 34.1 (12.5) years. The mean (SD) duration of asthma prior to the diagnosis of ABPA was 9.4 (8.7) years. Eighty-seven (41.6%) of the subjects had inappropriately received anti-tuberculous therapy in the past. The baseline characteristics and outcome of the patients were described previously [13—15]. One hundred and eighty-one (86.6%) of the subjects were positive for *Aspergillus* precipitins. Central bronchiectasis was present in 162 (77.5%) of the patients, and the remaining patients were classified as serological ABPA patients. HAM was present in 41 (19.6%) of the patients based on the chest HRCT.

Eosinophil counts at diagnosis

The median (IQR) eosinophil count was 850 (510—1541) cells/μl, and the distribution of the eosinophil counts is shown in Table 1. Approximately 60% of the patients had an eosinophil count of fewer than 1000 cells/μl.

Table 1 Distribution of the eosinophil counts in 209 patients with allergic bronchopulmonary aspergillosis.

Category of eosinophil count[a]	No. (%) of patients
Fewer than 500	50 (23.9)
500—1000	73 (34.9)
More than 1000	86 (41.1)
Quartile	**Range of eosinophil count**
1st quartile ($n = 52$)	80—509
2nd quartile ($n = 51$)	512—847
3rd quartile ($n = 54$)	850—1520
4th quartile ($n = 52$)	1562—14,124

[a] All eosinophil counts are given in cells/μl.

Relationship between the eosinophil counts and spirometry results

Forty-one (19.6%) of the patients exhibited normal lung function on spirometry at diagnosis. There was no significant relationship between lung function abnormalities on spirometry and the level of eosinophils ($p = 0.63$; Table 2). Bronchodilator reversibility was present in 95 (45.5%) of the patients and did not differ significantly ($p = 0.51$) between the groups (Table 2).

Relationship between the eosinophil counts and the immunological findings

The prevalence of *Aspergillus* precipitins was similar between the three categories of eosinophil counts (Table 2). However, in the post hoc analysis, the median IgE levels were significantly different between the categories ($p = 0.012$) with a trend towards significance in the patients whose counts exceeded 1000 cells/μl vs. those who had only 500—1000 cells/μl (Table 2). The eosinophil counts and total IgE levels were converted to their corresponding logarithmic values, and a linear regression was applied to ascertain the significance of the resulting relationship. The linear regression yielded an r^2 value of 0.018 (with a variance of 2%), which suggests that only 2% of the eosinophil counts can be explained by the IgE level. This was confirmed by analyzing a scatter plot, which revealed a wide distribution of the points along the intercept of the fitted line (Fig. 1). The *A. fumigatus*-specific IgE levels were also not significantly different between the three groups, and this was confirmed by linear regression analysis (Fig. 2).

Relationship between the eosinophil counts and the chest HRCT findings

The prevalence of central bronchiectasis and HAM were significantly different between the groups ($p = 0.003$ and $p = 0.004$, respectively), with both findings having a higher prevalence in the >1000 cells/μl group compared with the <500 cells/μl group (Table 2). The median (range) eosinophil counts in the groups of patients with

Table 2 Spirometric, immunological, and chest CT findings in the patients with different categories of eosinophil counts.

	Fewer than 500 cells/μl ($n = 50$)	500—1000 cells/μl ($n = 73$)	More than 1000 cells/μl ($n = 86$)	p value
Spirometry				
Normal	7 (14)	17 (23.3)	17 (19.8)	0.63
Mild obstruction	14 (28)	22 (30.1)	18 (20.9)	
Moderate obstruction	18 (36)	21 (28.8)	34 (39.5)	
Severe obstruction	11 (22)	13 (17.8)	17 (19.8)	
Bronchodilator reversibility	19 (38)	36 (49.3)	40 (46.5)	0.51
Immunological findings				
Aspergillus precipitins	43 (86)	66 (90.4)	72 (83.7)	0.46
IgE levels (total), IU/ml; median (IQR)	4345 (2475—7964)	4095 (2629.5—9193.5)	5769 (2926.3—10,965.3)[a]	0.012
A. fumigatus-specific IgE levels, kUA/l; median (IQR)	2.19 (1.3—9.88)	4.7 (1.94—11.53)	4.8 (1.07—14.85)	0.3
HRCT findings				
Central bronchiectasis	32 (64)	54 (74)	76 (88.4)[a]	0.003
No. of segments involved in central bronchiectasis; median (IQR)	6 (0—8)	5 (0—8)	6 (5—8)	0.46
High-attenuation mucus	3 (6)	13 (17.8)	25 (29.1)[a]	0.004

[a] The IgE levels, the occurrence of central bronchiectasis and the presence of high-attenuation mucus were significantly higher in patients with eosinophil counts >1000 cells/μl compared to those with <500 cells/μl. There was no significant difference between those with <500 cells/μl and those with 500—1000 cells/μl or between those with 500—1000 cells/μl and those with eosinophil counts >1000 cells/μl.

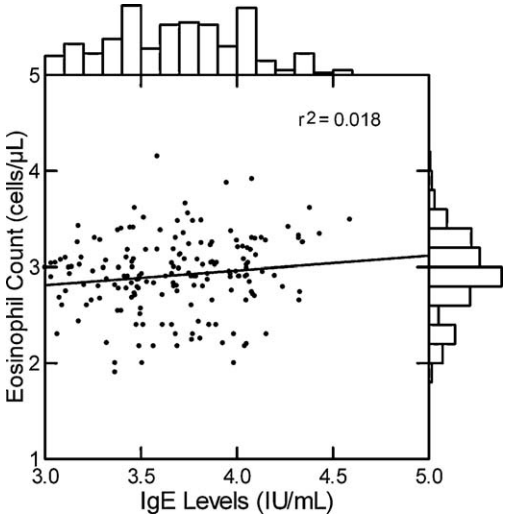

Figure 1 Scatter plot showing the strength of the relationship between the eosinophil counts and the total IgE levels. The individual eosinophil counts and IgE levels were converted to their corresponding logarithmic values, and the regression line was fit using the ordinary least-squares method. The r^2 value is 0.018 (with a variance of 2%), which suggests a weak relationship between the two variables.

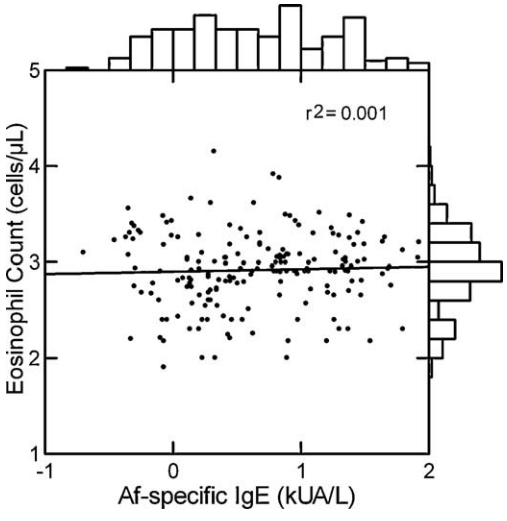

Figure 2 The relationship between the eosinophil counts and the *A. fumigatus*-specific IgE levels. The scatter plot presents the logarithmic transformations of the individual eosinophil counts and IgE levels. The regression line was fit using the ordinary least-squares method. The r^2 value is 0.001, which suggests a weak relationship between the two variables.

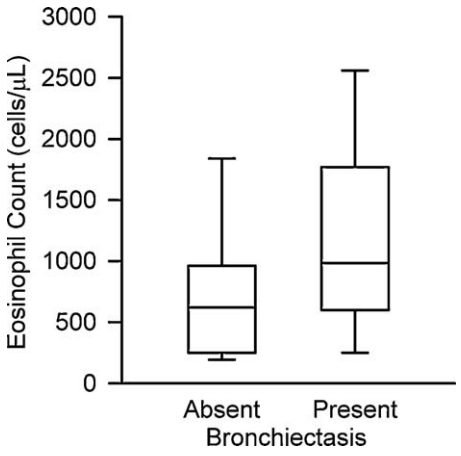

Figure 3 Box and whisker plot of the eosinophil counts for the patients with and without bronchiectasis. The box plot represents the 25th and 75th percentiles, the internal horizontal line indicates the median and the *T* bars indicate the 5th and 95th percentiles. The eosinophil counts were significantly higher in the patients with bronchiectasis ($p = 0.001$).

and without bronchiectasis were 620 (100—14,124) and 986 (80—8200) cells/μl, respectively, and the eosinophil counts were significantly higher in the patients with bronchiectasis ($p = 0.001$, Fig. 3). Similarly, the median eosinophil counts were higher in the patients with HAM than in the patients without HAM (800 vs. 1200 cells/μl, respectively, $p = 0.0001$; Fig. 4). However, the severity of bronchiectasis (as assessed by the number of involved bronchopulmonary segments) was similar among the three groups.

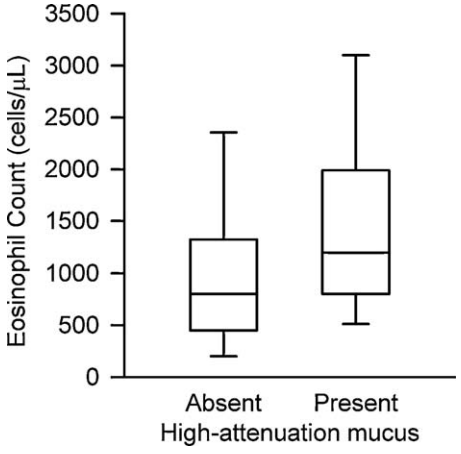

Figure 4 Box and whisker plot of the eosinophil counts for the patients with and without high-attenuation mucus. The box plot represents the 25th and 75th percentiles, the internal horizontal line indicates the median and the *T*-bars indicate the 5th and 95th percentiles. The eosinophil counts were significantly higher in the patients with high-attenuation mucus ($p = 0.0001$).

Discussion

The results of this study suggest that the eosinophil count has a limited role in the diagnosis of ABPA, as 60% of the patients in our cohort had an eosinophil count of fewer than 1000 cells/μl, which is the cutoff that is commonly used for diagnosis. There was no relationship between the eosinophil counts and lung function on spirometry, nor with the other immunological parameters. Although the prevalences of central bronchiectasis and high-attenuation mucus were higher in the patients with high eosinophil counts, the severity of bronchiectasis was not correlated with the degree of peripheral eosinophilia.

The Rosenberg—Patterson criteria are most commonly used criteria in the diagnosis of ABPA (Table 3) [4,5], and the presence of six of the eight primary criteria provides a near-definitive diagnosis. However, many of the criteria that were proposed by Rosenberg and Patterson can be considered redundant in the diagnosis of ABPA.

Table 3 Criteria that were used in the diagnosis of allergic bronchopulmonary aspergillosis complicating bronchial asthma.

Classic criteria [4,5]

Major criteria

- Immediate cutaneous hyper-reactivity to *A. fumigatus* antigen
- Elevated serum total IgE levels (>1000 IU/ml)
- Elevated serum levels of *A. fumigatus*-specific IgG and/or IgE
- Central bronchiectasis (CB) on HRCT of the chest
- Precipitating serum antibodies against *A. fumigatus*
- Fleeting pulmonary opacities on a chest radiograph
- Peripheral blood eosinophilia (>1000 cells/μl)

Minor criteria

- Presence of *Aspergillus* in sputum
- Expectoration of brownish-black mucus plugs
- Delayed skin reaction to *Aspergillus* antigen (type III reaction)

The presence of six out eight of the major criteria provides a near-definitive diagnosis. The disease is further classified as ABPA-S or ABPA-CB based on the absence or presence, respectively, of CB.

Minimal diagnostic criteria [26,27]

- Immediate cutaneous hyper-reactivity to the *A. fumigatus* antigen
- Elevated serum total IgE levels (>1000 IU/ml)
- Elevated *A. fumigatus*-specific IgG and/or IgE levels
- Central bronchiectasis on HRCT chest

The disease is further classified as ABPA-S or ABPA-CB based on the absence or presence, respectively, of CB.

Therefore, a set of minimal diagnostic criteria for ABPA has been proposed to facilitate an earlier diagnosis of ABPA while using fewer diagnostic tests (Table 3) [26,27]. For example, serum precipitins against *A. fumigatus* — although present in 69—90% of ABPA patients [13,23,28—30] — are also present in up to 10% of patients who have other pulmonary disorders, including asthma [23,31,32]. Therefore, this criterion represents supportive — but not diagnostic — evidence of ABPA. A peripheral blood eosinophil count that is higher than 1000 cells/μl is also regarded as a primary criterion in the diagnosis of ABPA. However, the results of the present study clearly suggest that the eosinophil count has limited utility in the diagnosis of ABPA and should not be used as a screening criterion. In our study, approximately 60% of the ABPA patients had an eosinophil count of lower than 1000 cells/μl, and approximately 25% of the patients did not even present with peripheral blood eosinophilia (i.e., higher than 500 cells/μl).

ABPA is characterized by an increased production of eosinophils in the bone marrow, followed by the accumulation of eosinophils in the tissues and blood [33—38]. The inhaled spores and the subsequently released particulate *A. fumigatus* antigens trigger a Th2 response in the lungs. This response leads to release of IL-5, which then stimulates progenitor cells in the bone marrow to release eosinophils that feed back onto the site of inflammation (i.e., the lung) [33,36,39]. Eosinophils are predominantly tissue-dwelling cells, and their numbers in the tissues far exceed what is typically found in the blood [7]. Similarly, in ABPA patients, the levels of eosinophils in the lung are far higher than in the peripheral blood, although there seems to be little correlation between these two factors [34,40]. This may explain the apparent lack of peripheral eosinophilia in ABPA patients despite studies that have demonstrated high concentrations of eosinophils in the pulmonary parenchyma. We also did not find any relationship between the eosinophil counts and other inflammatory markers, including the total and *A. fumigatus*-specific IgE levels. The elevated IgE levels in ABPA patients are due to the *A. fumigatus*-induced production of IL-4. IL-5, however, is responsible for inducing the differentiation and recruitment of eosinophils in the lung [41]. Thus, IgE and eosinophilia are regulated independently [41], albeit by a common antigenic source (i.e., *A. fumigatus*).

The presence of large numbers of eosinophils in the lungs of untreated ABPA patients suggests a primary proinflammatory role for eosinophils in ABPA. Eosinophil cationic protein (ECP), a constituent of eosinophil granules, and eosinophil peroxidase have been implicated in epithelial shedding and mast cell degranulation in patients with asthma [42]. In one study, eosinophils that were isolated from patients with ABPA were stimulated *in vitro* with *A. fumigatus* allergens and exhibited higher eosinophil peroxidase levels when compared with control cells [43]. Similarly, another study found that the inflammatory response in stable ABPA patients with bronchiectasis was characterized by an increase in the sputum eosinophil count and by evidence of marked eosinophil degranulation with elevated sputum ECP levels [40]. Furthermore, a significant relationship has been reported between sputum eosinophil numbers and the extent of the disease based on HRCTs of the chest [40]. In our study, we found an association between the eosinophil levels and occurrences of central bronchiectasis and HAM. This finding suggests that eosinophils are one of the principal determinants of inflammatory activity in ABPA. However, the extent of bronchiectasis did not correlate significantly with the eosinophil count, possibly because we used only the peripheral blood eosinophil count, which might not accurately reflect ongoing pulmonary inflammation [34,40].

Finally, our study has several limitations. Most importantly, we did not perform an eosinophil count on the sputum or bronchoalveolar lavage fluid. This might have been more definite, as eosinophils would have been recovered from the site of the ongoing inflammation. Another limitation is the retrospective nature of the study, especially given the limited amount of data. Finally, we used a manual method to count total eosinophils. Although manual microscopy is the current gold standard for WBC differential counts, it is time- and labor-intensive and can be associated with large coefficients of variation. Automated cell counters are the preferred method for differential counts in most laboratories, as they provide reliable clinical results in a shorter time and can be more reproducible than the manual approach [44]. Despite these limitations, one strength of this study — in addition to the large sample size — is the fact that all of the patients were glucocorticoid naïve. This provided us with an accurate estimation of the eosinophil count in ABPA patients at the time of their diagnosis, as eosinophil levels drop rapidly with corticosteroid therapy.

Conclusions

In conclusion, the results of this study suggest that an eosinophil count has limited utility for diagnosing ABPA, as we found no correlation between the eosinophil count and lung function or any other immunological parameters. The relationships

between the peripheral blood eosinophil count and central bronchiectasis and high-attenuation mucus suggest that eosinophils are one of the primary mediators of inflammation in ABPA.

Funding: No funding sources.

Competing interests: None declared.

Ethical approval: Approved by the Ethics Committee.

References

[1] Agarwal R. Allergic bronchopulmonary aspergillosis. Chest 2009;135:805—26.

[2] Agarwal R, Aggarwal AN, Gupta D, Jindal SK. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis. Int J Tuberc Lung Dis 2009;13:936—44.

[3] Hinson KFW, Moon AJ, Plummer NS. Broncho-pulmonary aspergillosis: a review and a report of eight new cases. Thorax 1952;7:317—33.

[4] Rosenberg M, Patterson R, Mintzer R, Cooper BJ, Roberts M, Harris KE. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. Ann Intern Med 1977;86:405—14.

[5] Patterson R, Greenberger PA, Halwig JM, Liotta JL, Roberts M. Allergic bronchopulmonary aspergillosis. Natural history and classification of early disease by serologic and roentgenographic studies. Arch Intern Med 1986;146:916—8.

[6] Agarwal R. Controversies in allergic bronchopulmonary aspergillosis. Int J Respir Care 2010;6:53—4, 56—63.

[7] Weller PF. The immunobiology of eosinophils. N Engl J Med 1991;324:1110—8.

[8] Nutman TB. Evaluation and differential diagnosis of marked, persistent eosinophilia. Immunol Allergy Clin North Am 2007;27:529—49.

[9] Greenberger PA. Diagnosis and management of allergic bronchopulmonary aspergillosis. Allergy Proc 1994;15:335—9.

[10] Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. Chest 2002;121:1988—99.

[11] Slavin RG, Hutcheson PS, Chauhan B, Bellone CJ. An overview of allergic bronchopulmonary aspergillosis with some new insights. Allergy Asthma Proc 2004;25:395—9.

[12] Tillie-Leblond I, Tonnel AB. Allergic bronchopulmonary aspergillosis. Allergy 2005;60:1004—13.

[13] Agarwal R, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A, Jindal SK. Clinical significance of hyperattenuating mucoid impaction in allergic bronchopulmonary aspergillosis: an analysis of 155 patients. Chest 2007;132: 1183—90.

[14] Agarwal R, Gupta D, Aggarwal AN, Saxena AK, Saikia B, Chakrabarti A, et al. Clinical significance of decline in serum IgE Levels in allergic bronchopulmonary aspergillosis. Respir Med 2010;104:204—10.

[15] Agarwal R, Gupta D, Aggarwal AN, Behera D, Jindal SK. Allergic bronchopulmonary aspergillosis: lessons from 126 patients attending a chest clinic in north India. Chest 2006;130:442—8.

[16] Agarwal R, Khan A, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A. An alternate method of classifying allergic bronchopulmonary aspergillosis based on high-attenuation mucus. PLoS One 2010;5:e15346.

[17] Agarwal R, Hazarika B, Gupta D, Aggarwal AN, Chakrabarti A, Jindal SK. *Aspergillus* hypersensitivity in patients with chronic obstructive pulmonary disease: COPD as a risk factor for ABPA? Med Mycol 2010;48:988—94.

[18] Agarwal R, Noel V, Aggarwal AN, Gupta D, Chakrabarti A. Clinical significance of Aspergillus sensitization in bronchial asthma. Mycoses 2011;54:e531—8.

[19] Reiff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: limited value in distinguishing between idiopathic and specific types. AJR Am J Roentgenol 1995;165:261—7.

[20] Osborne D, Vock P, Godwin JD, Silverman PM. CT identification of bronchopulmonary segments: 50 normal subjects. AJR Am J Roentgenol 1984;142:47—52.

[21] Hansell DM, Strickland B. High-resolution computed tomography in pulmonary cystic fibrosis. Br J Radiol 1989;62:1—5.

[22] Agarwal R, Aggarwal AN, Gupta D. High-attenuation mucus in allergic bronchopulmonary aspergillosis: another cause of diffuse high-attenuation pulmonary abnormality. AJR Am J Roentgenol 2006;186:904.

[23] Longbottom JL, Pepys J. Pulmonary aspergillosis: diagnostic and immunological significance of antigens and C-substance in *Aspergillus fumigatus*. J Pathol Bacteriol 1964;88:141—51.

[24] Standardization of spirometry, 1994 update. American Thoracic Society. Am J Respir Crit Care Med 1995;152: 1107—36.

[25] Aggarwal AN, Gupta D, Jindal SK. Development of a simple computer program for spirometry interpretation. J Assoc Physicians India 2002;50:567—70.

[26] Schwartz HJ, Greenberger PA. The prevalence of allergic bronchopulmonary aspergillosis in patients with asthma, determined by serologic and radiologic criteria in patients at risk. J Lab Clin Med 1991;117:138—42.

[27] Eaton T, Garrett J, Milne D, Frankel A, Wells AU. Allergic bronchopulmonary aspergillosis in the asthma clinic. A prospective evaluation of CT in the diagnostic algorithm. Chest 2000;118:66—72.

[28] Campbell MJ, Clayton YM. Bronchopulmonary aspergillosis. A correlation of the clinical and laboratory findings in 272 patients investigated for bronchopulmonary aspergillosis. Am Rev Respir Dis 1964;89:186—96.

[29] McCarthy DS, Pepys S. Allergic broncho-pulmonary aspergillosis. Clinical immunology: (1) clinical features. Clin Allergy 1971;1:261—86.

[30] McCarthy DS, Pepys S. Allergic broncho-pulmonary aspergillosis. Clinical immunology: (2) skin, nasal and bronchial tests. Clin Allergy 1971;1:415—32.

[31] Greenberger PA. Allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1984;74:645—53.

[32] Vlahakis NE, Aksamit TR. Diagnosis and treatment of allergic bronchopulmonary aspergillosis. Mayo Clin Proc 2001;76:930—8.

[33] Kurup VP, Mauze S, Choi H, Seymour BW, Coffman RL. A murine model of allergic bronchopulmonary aspergillosis with elevated eosinophils and IgE. J Immunol 1992;148:3783—8.

[34] Murali PS, Dai G, Kumar A, Fink JN, Kurup VP. *Aspergillus* antigen-induced eosinophil differentiation in a murine model. Infect Immun 1992;60:1952—6.

[35] Murali PS, Kumar A, Choi H, Banasal NK, Fink JN, Kurup VP. *Aspergillus fumigatus* antigen induced eosinophilia in mice is abrogated by anti-IL-5 antibody. J Leukoc Biol 1993;53:264—7.

[36] Murali PS, Kurup VP, Guo J, Fink JN. Development of bone marrow eosinophilia in mice induced by *Aspergillus fumigatus* antigens. Clin Immunol Immunopathol 1997;84:216—20.

[37] Kurup VP, Seymour BW, Choi H, Coffman RL. Particulate *Aspergillus fumigatus* antigens elicit a TH2 response in BALB/c mice. J Allergy Clin Immunol 1994;93:1013—20.
[38] Kurup VP, Choi H, Murali PS, Resnick A, Fink JN, Coffman RL. Role of particulate antigens of *Aspergillus* in murine eosinophilia. Int Arch Allergy Immunol 1997;112:270—8.
[39] Togias A. Systemic effects of local allergic disease. J Allergy Clin Immunol 2004;113:S8—14.
[40] Wark PA, Saltos N, Simpson J, Slater S, Hensley MJ, Gibson PG. Induced sputum eosinophils and neutrophils and bronchiectasis severity in allergic bronchopulmonary aspergillosis. Eur Respir J 2000;16:1095—101.
[41] Kurup VP, Choi H, Murali PS, Coffman RL. IgE and eosinophil regulation in a murine model of allergic aspergillosis. J Leukoc Biol 1994;56:593—8.
[42] Calhoun WJ, Sedgwick J, Busse WW. The role of eosinophils in the pathophysiology of asthma. Ann N Y Acad Sci 1991;629:62—72.
[43] Murali PS, Kurup VP, Bansal NK, Fink JN, Greenberger PA. IgE down regulation and cytokine induction by *Aspergillus* antigens in human allergic bronchopulmonary aspergillosis. J Lab Clin Med 1998;131:228—35.
[44] Simson E, Groner W. The state of the art for the automated WBC differential. Lab Hematol 1995;1:13—22.