Peripheral blood neutrophils and upregulated surface expression of interleukin-17 and CD177 in patients with bronchial asthma: an association with fungal allergy

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Received: 12 September 2019
Accepted: 6 January 2020
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:934–942

Background
Asthma is a prevalent debilitating airway disease, with a tremendous effect worldwide. Fungi and their spores are identified as major culprits in allergic asthma (AA) etiology. Peripheral blood neutrophils and interleukin 17 (IL-17), which are considered crucial players in both bronchial asthma and host defense mechanisms against fungi, increase concomitantly; moreover, certain subsets of neutrophils express and even release IL-17.
Objectives
In this study, we sought to determine the peripheral frequency of certain neutrophil subpopulations, expressing both CD177 and IL-17, among AA patients, particularly those with fungal allergy.
Methods
This observational study comprised 40 patients with AA (age range 19–60 years) and 20 age-matched and sex-matched healthy controls (age range 20–55 years). All patients had positive allergy skin prick test results, and accordingly, they were further subdivided into two groups (18 reactive to fungal allergens and 22 to other aeroallergens). The frequency of IL-17^{+}CD177^{+} neutrophils in the peripheral blood was assessed by flow cytometry in all studied participants.
Results
The authors observed significantly increased frequency of circulating IL-17^{+}CD177^{+} neutrophils among AA patients (especially mild to moderate cases) compared with healthy controls (43.3±13.9 vs 15.3±4.8). On the contrary, and surprisingly, patients with fungal allergy and those without did not show any difference with respect to this neutrophil subpopulation (44.6±14.4 vs 42.2±13.7).
Conclusion
Despite limited sample size, we reported elevated IL-17+CD177+ neutrophil proportion in all AA, regardless of fungal aeroallergenicity. This observation points to a role played by this neutrophil subpopulation in asthma pathophysiology, especially the allergic phenotype, and hopefully offer a new therapeutic approach in asthma management.

Keywords:
allergic asthma, CD177^{+}neutrophils, fungal allergy, interleukin &minus, 17

Egypt J Intern Med 31:934–942

1110-7782

Introduction

Bronchial asthma is a chronic inflammatory disease of the airways, with increasing prevalence worldwide. It is characterized by bronchial hyper-responsiveness and variable airflow obstruction. Asthma manifests clinically as recurrent episodes of wheezing, breathlessness, chest tightness, and cough [1]. One of the strongest identifiable risk factor for the development of asthma is atopy; the link between asthma and atopy is unquestioned, where various allergens are known to be major triggers of asthma [2–4]. Fungi and their spores are regarded as the principal aeroallergens inducing asthma, as they exist ubiquitously indoors and outdoors [5]. Among atopic individuals, those who have respiratory allergy to molds are ~20–30% [6]. The close relationship between asthma and fungi is well recognized, and the correlation between visible domestic mold growth and wheezing attacks in children is evident [7,8]. Being allergic to fungi significantly affects the development, persistence, and severity of allergic asthma (AA) [9,10].

It is now well established from a variety of studies that AA is more heterogeneous and complex than mere T-helper 2 (Th2) mechanisms. These studies revealed

that non-Th2 factors such as interleukin (IL)-17 and neutrophils are frequently found in the lungs of patients with asthma, particularly those with severe asthma [11]. IL-17 expression is increased in the lung, sputum, and bronchoalveolar lavage fluid in patients with asthma [12]. Besides $CD4^+IL\text{-}17A^+$cells, serum IL-17 levels were found to be high as well in persistent asthmatic patients rather than intermittent asthmatics [13]. IL-17 is also critically important in host defense against fungal pathogens at mucosal barrier sites, especially in the lung [14]. Likewise, neutrophils are considered one of the essential components of the innate immune system involved in binding and killing of various fungi [15].

IL-17 is produced by multiple cells, including activated $CD4^+$ T cells [16], γδ T cells [17], Th17 [18], and neutrophils. IL-17 release from neutrophils has been detected in several mouse models of infectious and autoimmune inflammation [19,20], in human psoriatic lesions [21], and in patients with corneal ulcers caused by filamentous fungi [22]. Furthermore, recently, Ramirez-Velazquez *et al.* [23] reported that $CD177^+$ neutrophils were able to express IL-17 in asthmatic patients. CD177 is a glycoprotein expressed in blood specifically by neutrophils, neutrophilic metamyelocytes, and myelocytes [24]. CD177 plays an important role in neutrophil transmigration through the endothelium [25], and the circulating levels of $CD177^+$ neutrophils are augmented in severe inflammatory conditions [26,27], which facilitates increased neutrophil tissue infiltration in these conditions [28].

Taken together, these observations make it reasonable to suppose that neutrophils may be activated in the circulation of AA with increased expression of surface molecules CD177 and IL-17. Hence, the main objective of this work was to determine if the fraction of $IL\text{-}17^+CD177^+$ neutrophils might be increased in AA patients, especially those with fungal allergy.

Materials and methods

Participants and recruitment

We enrolled 40 asthmatic participants fulfilling Global initiative for Asthma (GINA) criteria for asthma diagnosis [29]. Only skin prick test (SPT)-positive patients were selected, with an age range of 19–60 years. We further subdivided our patients into two groups according to SPT results: 18 (45%) patients had positive results for common fungal aeroallergens such as Candida, *Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., and *Penicillium* spp., whereas 22 (55%) had negative results for fungi but positive for other aeroallergens. Patients were recruited from the allergy outpatient clinic at Ain Shams University Hospital during the period from November 2014 to December 2015.

We excluded individuals with severe dermatographism, smokers, ex-smokers, and those who gave history of respiratory tract infection in the past month before the study. Patients taking corticosteroids, long-acting β2-agonists, antileukotriene, or antihistamines a month before the study were excluded. Another 20 healthy controls were enrolled; they were age and sex matched and of the same social class as far as possible. Controls had no history of allergy or symptoms suggestive of bronchial asthma, with negative allergen SPT result (except only one individual) and with the same exclusion criteria as patients. The Ethics Committee of Ain Shams University approved the study, and each participant gave written informed consent. All procedures performed in this study were in accordance with the ethical standards of Ain Shams University Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Study design

All the patients were subjected to detailed history and clinical examination. Spirometric measurements were performed as described in other studies [30,31], and asthma grading was done according to GINA criteria [29]. We also performed SPTs and collected peripheral blood for complete blood picture, serum total immunoglobulin (Ig)E levels, and flow cytometry for $IL\text{-}17^+CD177^+$ neutrophils %.

Skin prick test

All participants underwent SPT using a panel of the commonest fungal aeroallergens, such as Candida, *Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., and *Penicillium* spp., and other aeroallergens, such as mites, pollens, cat hair, dog hair, horse hair, and cockroach. Histamine and saline were positive and negative controls, respectively. A mean wheal 3 mm in diameter larger than the negative control was considered to be positive. Atopy was defined as a positive SPT to at least one of the allergens in the panel.

Total serum immunoglobulin E and eosinophil count

Serum total IgE Ab level in serum was measured by enzyme-linked immunosorbent assay (ELISA; Euroimmun AG, Lübeck, Germany). Values were interpreted as the following: negative (<100 kU/l), low positive (100–500 kU/l), and high positive

(>500 kU/l). Count of eosinophils was done using Fuchs-Rosenthal counting chamber (Hausser Scientific, PA, USA).

Isolation of neutrophils from peripheral blood mononuclear cells

Isolation of neutrophils was done from whole blood sample which is heparinized, by sedimentation of red blood cell with dextran 1%. This is followed by Ficoll-Hypaque (Lonza, Greenwood, SC, USA) density gradient centrifugation and hypotonic lysis of erythrocyte as previously described [32]. Cell viability exceeded 98% as detected by trypan blue dye exclusion test. By Wright-Giemsa staining, neutrophil percentage was greater than 95% in the suspension.

Flow cytometry for detection of interleukin-17 and CD177 expression on neutrophils

Using 1 ml lysing solution, cell populations from each respective sample were lysed followed by PBS wash, and then centrifugation was performed for 3 min at 1500 g (3200 rpm). Staining the cell pellet was done using combinations of the following antibodies (5 µl each): fluorescein isothiocyanate-labeled antihuman CD177 (BioLegend, San Diego, CA, USA) and phycoerythrin-labeled antihuman IL-17 (MiltenyiBiotec, Bergisch Gladbach, Germany). Then incubation of the cells for thirty minutes was done in the dark at room temperature after light vortex, followed by a single wash with PBS. Data acquisition and analyses were done on EPICS XL flow cytometer (Coulter Electronics, Hialeah, FL, USA) utilizing SYSTEM II version 3 software with a standard three-color filter configuration. Gating of neutrophils was determined according to their forward and side scatter properties, and CD177-positive neutrophils were gated out of total granulocytes. IL-17 expression was specified as a percentage of gated CD177-positive neutrophils.

Statistical analysis

The collected data were revised, coded, tabulated, and introduced to the computer using Statistical package for Social Science (SPSS 15.0.1 for windows, 2001; SPSS Inc., Chicago, Illinois, USA). The demographic and laboratory data were shown as mean±SD or ratio and compared among groups by the unpaired Student's *t*-test. χ^2-test was used for relationship between two qualitative variables. Analysis of variance test was applied to assess the statistical significance of the difference between more than two study group means. Post-hoc test was used for comparisons of all possible pairs of group means. Logistic regression procedure was performed to determine whether the likelihood of atopic asthma could be predicted from the independent variables, including IL-17$^+$CD177$^+$ neutrophil frequency.

Table 1 Demographic data and skin test reactivity to fungal aeroallergens in the study population

Variables	Asthmatic patients	Healthy controls	*P* value
Number	40	20	
Age (years)	42.3±12.0	41.9±10.8	0.894¶
Sex (male/female)	24/16	13/7	0.707§
Fungal allergy (+/–)	18/22	1/19	0.002§

Data were expressed as mean±SD or ratio. ¶Unpaired test. §χ^2-tests.

Results

A total of 60 participants were enrolled in this study, of whom 40 were patients with allergic bronchial asthma (24 males and 16 females; mean age: 42.3±12.0 years) and 20 were healthy controls (13 males and seven females; mean age: 41.9±10.8 years). Demographic characteristics and SPT reactivity to fungal and other aeroallergens in both groups are presented in Table 1.

IL-17+CD177$^+$ neutrophil frequency in patients with allergic bronchial asthma vs healthy controls

The frequency of CD177$^+$ neutrophils expressing the IL-17 was significantly higher in asthmatic participants (n=40) compared with controls (*n*=20) ($P<0.05$), and as predicted, significant differences in mean serum total IgE and mean forced expiratory volume in first second % between patients and controls were observed as well. However, no differences were seen in the percentage of serum neutrophils or eosinophils between participants with asthma and controls, as shown in Table 2.

IL-17$^+$CD177$^+$ neutrophil frequency among asthmatic patients with different asthma severity grades

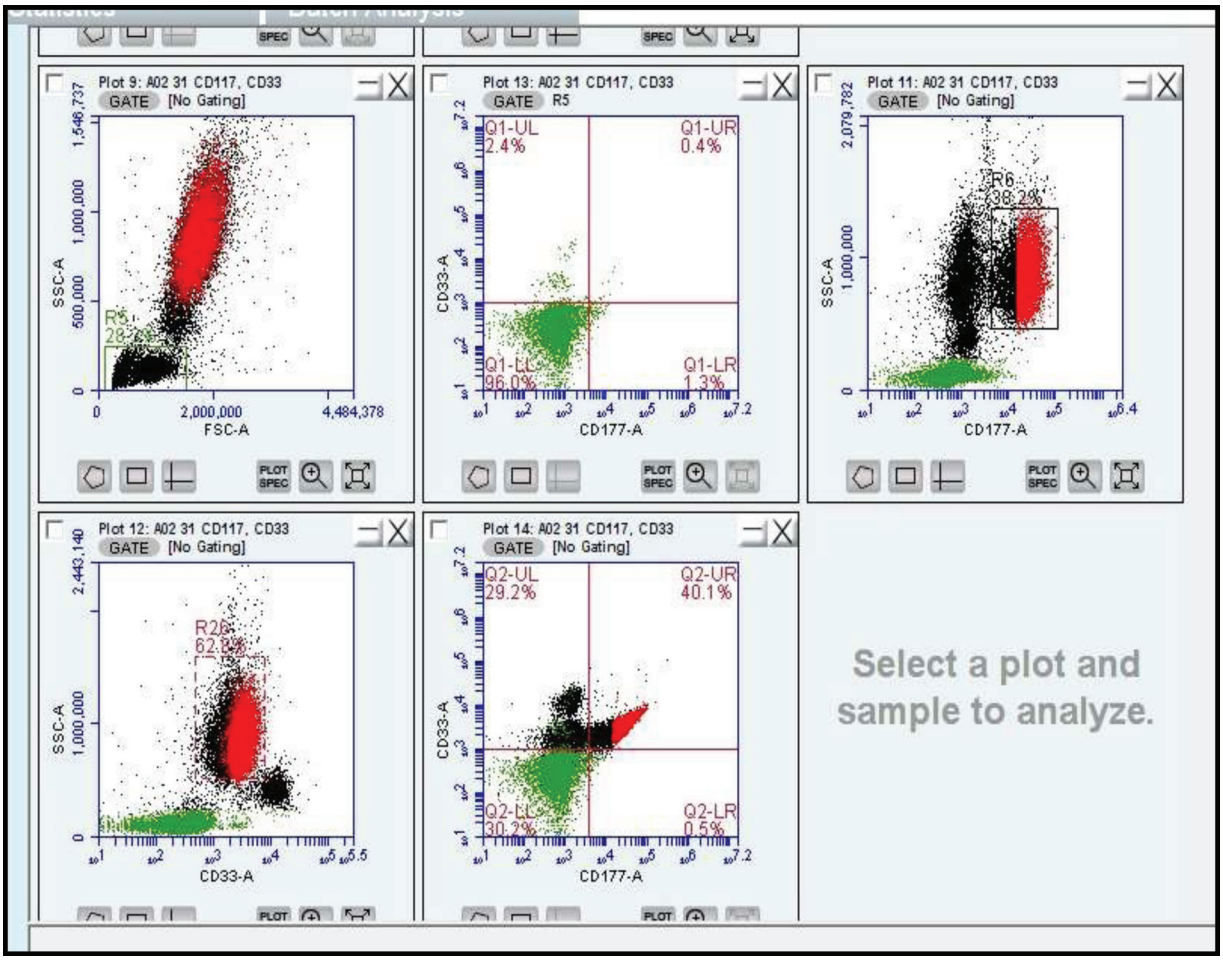
We categorized asthma severity in our patients according to GINA criteria: 14 (35%) patients had mild persistent asthma (mean age: 46.8±9.9 years), 17

Table 2 Laboratory data of asthmatic patients and healthy controls

Variables	Asthmatic patients	Healthy controls	*P* value
Serum neutrophils%	59.7±12.9	55.2±13.3	0.203¶
IL17$^+$CD177$^+$ neutrophils%	43.3±13.9	15.3±4.8	0.0001¶
Serum total IgE	728.3±603.8	194.8±108.4	0.0001¶
Serum eosinophils%	2.9±2.2	2.2±0.9	0.146¶
FEV_1%	70.8±12.3	102.6±5.2	0.0001¶

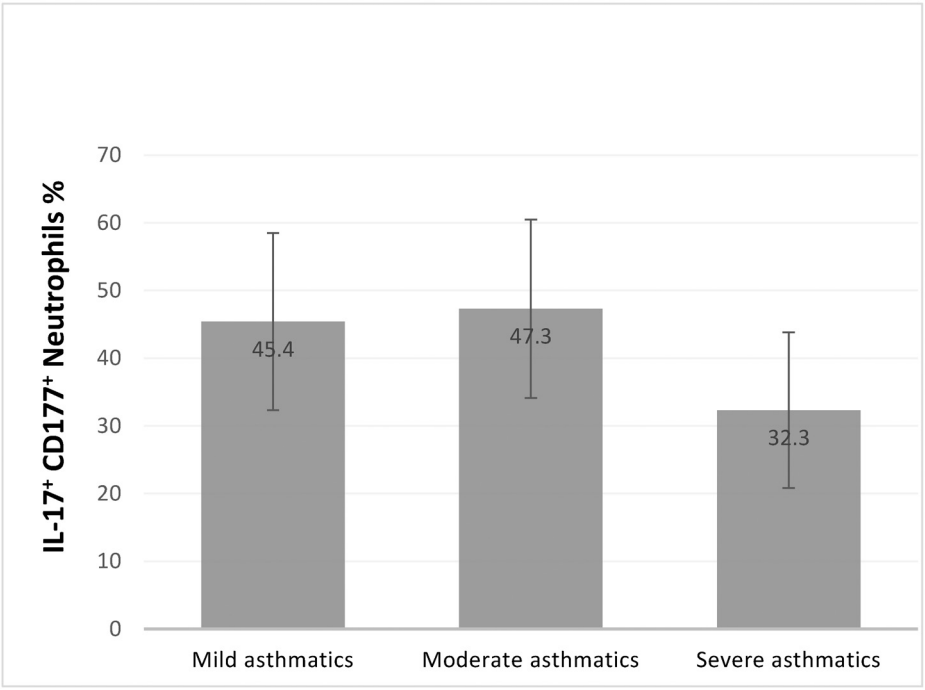
Data were expressed as mean±SD. FEV, forced expiratory volume in first second; Ig, immunoglobulin; IL, interleukin. ¶Unpaired test.

Figure 1



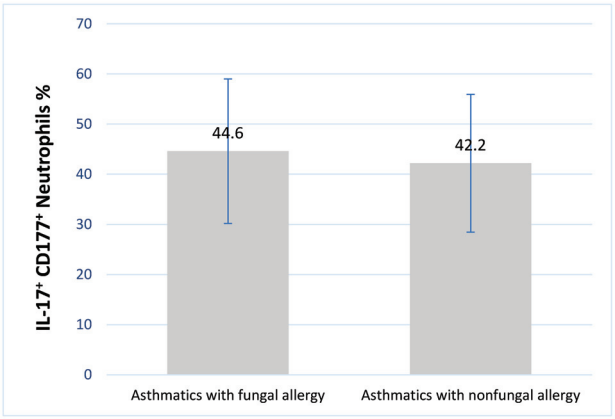
IL-17^{+}CD177^{+} neutrophils were recognized within granulocytes by size (forward scatter), complexity (side scatter), and IL-17 and CD177 expression. IL, interleukin.

Figure 2



IL-17^{+}CD177^{+} neutrophil frequency among asthmatic patients with different asthma severity grades. IL, interleukin.

Figure 3



IL-17$^+$CD177$^+$ neutrophil frequency in asthmatic patients classified according to fungal aeroallergen sensitivity. IL, interleukin.

(42.5%) had moderate persistent asthma (mean age 38.1±12.7 years), and nine (22.5%) had severe persistent asthma (mean age 43.4±12.1 years). IL-17$^+$CD177$^+$ neutrophil frequency was significantly higher in patients with mild to moderate asthma compared with severe asthmatics (Figs. 1 and 2).

IL-17$^+$CD177$^+$ neutrophil frequency in asthmatic patients classified according to fungal aeroallergen sensitivity

To determine if the presence of fungal allergy preferentially upregulates this distinct neutrophil subpopulation, we further subdivided our AA patients into two groups according to SPT reactivity to fungal aeroallergens (18 with positive fungal allergy vs 22 without). We assessed the frequency of CD177$^+$ neutrophils expressing the IL-17 in both groups, and surprisingly mean percentage of these cells was comparable between both groups (44.6±14.4 vs 42.2 ±13.7), with no statistically significant difference ($P>0.05$) (Fig. 3).

IL-17$^+$CD177$^+$ neutrophils as a predictor of allergic asthma

Lastly, we performed a logistic regression analysis which showed that IL-17$^+$CD177$^+$ neutrophils is an independent predictor of allergic asthma. The results from this analysis are shown in Table 3. Results are expressed as log odds with 95% confidence interval (CI). As for IL17$^+$CD177$^+$ neutrophils%, odds ratio was 1.405, with 95% CI, 1.036–1.905 ($P<0.05$).

Table 3 Logistic regression analysis using interleukin 17$^+$CD177$^+$ neutrophils% and other parameters as candidate predictors for allergic asthma

Predictors	Odds ratio	*P*	95% CI for odds ratio	
Positive fungal allergy	2.246	0.706	0.034	150.234
Serum neutrophils%	1.023	0.752	0.889	1.177
IL17$^+$CD177$^+$ neutrophils%	1.405	0.029	1036	1.905
Serum total IgE	1.009	0.149	0.997	1.022
Serum eosinophils%	5.934	0.154	0.513	68.639

CI, confidence interval; Ig, immunoglobulin; IL, interleukin.

Discussion

Many types of cells are involved in the pathophysiology of asthma predominantly eosinophils, mast cells, CD4$^+$ lymphocytes, and neutrophils [33]. In the current study, we demonstrated a significantly higher percentage of IL17$^+$CD177$^+$ neutrophils in the peripheral blood of patients with AA compared with healthy controls. Neutrophil has long been regarded as a mere phagocytic cell, yet it is now known to be an important participant in allergic process in general and in bronchial asthma in particular. It releases multiple mediators with subsequent potent effect on asthmatic airways, resulting in nonresolving chronic inflammation [34–36]. In asthmatics, IL-17 is upregulated, as evidenced by elevated serum levels of IL-17 and pulmonary IL-17 mRNA and protein [37,38]. It triggers airway responsiveness [39,40] and has an indirect role in bronchoconstriction, boosting the proliferation and survival of human airway smooth muscle cells *in vitro* [41]. IL-17 and neutrophils are closely linked. Several human and animal studies have declared neutrophils as a source of IL-17 [20,23,42–44], besides recent evidence showing IL-17-cytokine expression by CD177$^+$neutrophils as well [23].

An initial objective of the current study was to assess peripheral frequency of IL17$^+$CD177$^+$ neutrophils in asthmatic patients, and it is worth mentioning that all our patients were atopic with significantly high mean serum total IgE. The studied patient group indeed showed high frequency of CD177$^+$ neutrophils expressing IL-17. This association is corroborated by the findings of previous work published by Manise *et al.* [45], who noted that sputum IL-17 levels were higher in asthmatics with high IgE rather than asthmatics with low IgE. Moreover, another study showed that in response to Dermatophagoides farinae extract, IL-17 production by T cells was significantly induced in atopic asthmatics when compared with nonatopic asthmatics and normal controls [38]. Together, these results suggest that IL-17 may play a role in AA. IL-17 stimulates the activation and proliferation of B cells, thus promoting IgE production [46,47]. However, this finding appeared not to be the case in two other contradictory studies. Raedler *et al.* [48] in contrast to our results, demonstrated highly increased IL-17 levels, in conjunction with neutrophil generation and

recruitment, in children with nonallergic asthma (NAA) vs controls, and slightly in those with nonallergic vs AA, denoting that cellular environment in the airway of NAA children is predominantly neutrophilic. Fattahi *et al.* [49] as well found higher IL-17 expression, mainly by neutrophils, in bronchial specimens obtained from nonatopic asthmatics compared with atopic asthmatics. This inconsistency could be understood by the well-described involvement of IL-17 in the pathogenesis of nonatopic (neutrophil-dominant) rather than atopic (eosinophil-dominant) asthma.

A study from Australia used a slight different approach than ours, but its data are in accordance with our findings. In murine models of allergic airways disease, Essilfie *et al*. [50] have demonstrated increased count and proportions of pulmonary neutrophils releasing excessive IL-17 in *Haemophilus influenzae*-infected allergic mice vs uninfected allergic controls. Lung neutrophils isolated on day 1 also had increased levels of IL-17 mRNA transcripts. This raises the question of whether is it infection alone or the synergistic effect of infection and allergy that is responsible for the creation of this neutrophilic asthma phenotype. *H. influenzae* infection alone induced airway neutrophilia, IL-17 mRNA expression in the lung, and increased percentages of neutrophils producing IL-17 1 day after infection. These changes are normal responses to infection; however, after the primary insult, a persistent and long-lasting airway inflammatory environment may result, with subsequent development of neutrophilic asthma upon allergen challenge. Moreover, a previous research study by Li *et al.* [51] seems to be consistent with our data. They observed significantly increased percentage of IL-17RB$^+$ granulocytes that were of the type 2 myeloid (T2M) cells subtype (IL-17Rb$^+$CD177$^+$CD11b$^+$CD16$^+$) in asthma patients vs controls. However, the authors studied T2M cells, which represents a separate subpopulation of granulocytes, and their finding are in line with what we detected, as T2M cells appear to be a steroid-resistant cell population, similar to neutrophils. In the same study, they found no correlation between forced expiratory volume in first second and percentage of T2M cells, similar to what we detected in our study (data not shown). Furthermore, our results match those published by Ramirez-Velazquez *et al.* [23] who detected increased IL-17-producing peripheral blood CD177$^+$ neutrophils in AA patients compared with healthy controls. In addition, they reported that the percentage of CD177$^+$IL-17$^+$ neutrophils increased in mild, moderate, and acute asthma in comparison with healthy controls. The CD177$^+$IL-17$^+$ neutrophil percentage was highest in patients diagnosed with mild asthma, when compared with those with moderate or acute asthma. Notably, we also detected significantly high IL17$^+$CDl77$^+$ neutrophils% in the peripheral blood of mild to moderate cases compared with severe cases. The reason for this is not clear, but it may have something to do with the vital role played by this cell population in early phases of the disease. Or an alternative explanation for this could be preferential migration of these cells to the pulmonary tissues in severe asthmatics. This hypothesis was supported by Al-Ramli *et al.* [52] who detected higher number of IL-17A$^+$-positive cells in the bronchial submucosa of adults with severe asthma compared with mild asthma or controls. In spite of this, Andersson *et al.* [53] found no difference in the number of IL-17A$^+$-positive cells in the bronchial adventitia between children with severe therapy resistant asthma and controls, attributing this to time of biopsy collection, being taken during stable disease rather than following challenge or during disease exacerbation. Moreover, Doe *et al.* [54] observed no increase in IL-17A expression in the bronchial submucosa of severe asthmatics, and referred this to high dose inhaled and/or oral corticosteroids given to participants with severe asthma, which may have attenuated the IL-17A expression in this group.

Fungi have been incriminated in allergic responses. Airborne fungi and their products may contribute to the evolution and aggravation of allergic airway diseases [55,56]. A considerable amount of literature has been published on the role of neutrophils as the most important innate immune effector cells controlling fungal infections, as well as IL-17 implication in antifungal immunity [57–60]. This study was set out to evaluate IL-17$^+$CD177$^+$ neutrophil frequency in AA patients particularly those with fungal allergy. Surprisingly, we did not observe any significant difference between asthmatics those with fungal allergy and those without. Our finding does not support the previous research done by Ramirez-Velazquez *et al.* [23] who revealed higher percentage of peripheral CD177$^+$IL-17$^+$ neutrophils in asthmatic patients allergic to fungi (four out of 17) compared with AA reactive to other allergens. Reviewing the literature, no other data were found on this association between allergic fungal asthma and CD177$^+$IL-17$^+$ neutrophils except by the same authors who afterward detected elevated percentage of CD177$^+$ CRTAM$^+$ neutrophils in peripheral blood of AA patients reactive to fungal and nonfungal aeroallergens [61]. However, Ghosh *et al.* [62],

using an Aspergillus fumigatus (Af) murine inhalation model to mimic human fungal AA, found significantly elevated IL-17A in JH-/- mice airways after fungal challenge compared with BALB/c controls, pointing to IL-17A involvement in fungal AA. Notably, IL-17 was not produced by T cells in this model, and although its cellular source in JH-/- mice during Af infection was not recognized, others cells including macrophages, neutrophils, natural killer T cells, and mast cells could produce IL-17 in AA [63,64]. In support of this, CD11b Ly-6G neutrophils have been identified by Werner *et al.* [65] as one cell type capable of producing IL-17A in a Dectin-1-dependent manner, during invasive lung fungal infection with Af in mouse. IL-17 production was impaired in dectin-1-deficient mice, and they were unable to get rid of Af. This could suggest that fungal allergens and their derivatives might stimulate neutrophils through the dectin-1 receptor, provoking IL-17 production and contributing to bronchial inflammation during AA. Likewise, Inoue *et al.* [66,67] reported that exposing the lungs of mice to β-glucan isolated from Candida albicans induced neutrophilic airway inflammation, concomitant with augmented lung expression of IL-17A.As mentioned before and contrary to the expectation, in the present study, the percentage of IL-17$^+$CD177$^+$ neutrophils did not differ between patients with positive fungal allergy and those with nonfungal allergy. This finding does not rule out the well-accepted theory of proactive role played by neutrophils and IL-17 in asthma and against infections with molds. However, it is difficult to explain this result, but it could be related to a small sample size (since slightly higher percentage was shown with fungal allergy); besides, this difference has been shown in only one previous Mexican study [23], therefore warranting future large-scale studies. Whatever the case is, we undoubtedly demonstrated significantly increased IL-17$^+$CD177$^+$ neutrophils in patients with AA particularly those with mild to moderate disease, and to note, this increase was not augmented in those with fungal allergy. Finally, we found IL-17$^+$CD177$^+$ neutrophil to be an independent predictor of future AA development, and to the best of our knowledge, this is the first time to be demonstrated. This result was obtained by logistic regression analysis (odds ratio=1.405, 95% CI: 1.036–1.905); consequently, this cell count might be considered when evaluating patients at high risk of developing AA.

Conclusion

In this study, we reported significantly elevated percentage of circulating IL-17$^+$CD177$^+$ neutrophils in patients with AA; this finding highlights the potential role played by this novel cell subset in the pathophysiology of AA. Despite these promising results, a question remains unanswered, whether fungal allergy in asthmatics is associated with expanded IL-17$^+$CD177$^+$ neutrophils or not. Accordingly, future studies on this topic are recommended.

Limitations of the current study

The major limitation of this study is being a single-center observational one. Besides, it is unfortunate that the study is based on a small sample of participants and did not include a group of NAA patients to compare their data with allergic group. In spite of its limitations, the study certainly adds to our understanding of asthma pathogenesis and offers some new insight into therapeutic interventions.

Acknowledgements

All authors contributed to the work presented in this study; Sabry M.K. and Ahmed E.E. conceived the ideas; Abd Elbadie H.E. implemented study design and collected the data; and Melek N.A. was involved in proper patient selection and writing and revising the manuscript.

Financial support and sponsorship

Nil.

Conflict of interest

There are no conflicts of interest.

References

1 Agarwal R, Dhooria S, Aggarwal AN, Maturu VN, Sehgal IS, Muthu V, *et al.* Guidelines for diagnosis and management of bronchial asthma: joint ICS/NCCP (I) recommendations. Lung India 2015; 32(Suppl 1):S3–S42.

2 National Asthma Education and Prevention Program: Expert panel report II. 1997. Guidelines for the diagnosis and management of asthma. National Heart, Lung, and Blood Institute (NIH publication no. 97-4051), Bethesda, MD

3 Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, *et al.* A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003; 349:1414–1422.

4 Vonk JM, Boezen HM. Predicting adult asthma in childhood. Curr Opin Pulm Med 2006; 12:42–47.

5 Tanaka A, Fujiwara A, Uchida Y, Yamaguchi M, Ohta S, Homma T, *et al.* Evaluation of the association between sensitization to common inhalant fungi and poor asthma control. Ann Allergy Asthma Immunol 2016; 117:163–168.

6 Wuethrich B. Epidemiology of allergic diseases: are they really on the increase. Int Arch Allergy Appl Immunol 1989; 90:3–10.

7 Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, *et al.* Fungal allergy in asthma-state of the art and research needs. Clin Transl Allergy 2014; 4:14.

8 Karvonen AM, Hyvärinen A, Roponen M, Hoffmann M, Korppi M, Remes S, *et al.* Confirmed moisture damage at home, respiratory symptoms and atopy in early life: a birth-cohort study. Pediatrics 2009; 124: e329–e338.

9 Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J 2006; 27:615–626.

10 Salo PM, Arbes SJ jr, Sever M, Jaramillo R, Cohn RD, London SJ, Zeldin DC. Exposure to alternaria alternata in US homes is associated with asthma symptoms. J Allergy Clin Immunol 2006; 118:892–898.

11 Lee YT, Wu CT, Sun HL, Ko JL, Lue KH. Fungal immunomodulatory protein-fve could modulate airway remodel through by affect IL17 cytokine. J Microbiol Immunol Infect 2018; 51:598–607.

12 Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, *et al.* Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. J Allergy Clin Immunol 2003; 111:1293–1298.

13 Chien JW, Lin CY, Yang KD, Lin CH, Kao JK, Tsai YG. Increased IL-17A secreting $CD4^+$ T cells, serum IL-17 levels and exhaled nitric oxide are correlated with childhood asthma severity. Clin Exp Allergy 2013; 43:1018–1026.

14 Rudner XL, Happel KI, Young EA, Shellito JE. Interleukin-23 (IL-23)-IL-17 cytokine axis in murine *Pneumocystis carinii* infection. Infect Immun 2007; 75:3055–3061.

15 Borregaard N. Neutrophils, from marrow to microbes. Immunity 2010; 33:657–670.

16 Infante-Duarte C, Horton HF, Byrne MC, Kamradt T. Microbial lipopeptides induce the production of IL-17 in Th cells. J Immunol 2000; 165:6107–6115.

17 Lockhart E, Green AM, Flynn JL. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. J Immunol 2006; 177:4662–4669.

18 Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005; 6:1123–1132.

19 Hoshino A, Nagao T, Nagi-Miura N, Ohno N, Yasuhara M, Yamamoto K, *et al.* MPO-ANCA induces IL-17 production by activated neutrophils in vitro via classical complement pathway-dependent manner. J Autoimmun 2008; 31:79–89.

20 Li L, Huang L, Vergis AL, Ye H, Bajwa A, Narayan V, *et al.* IL-17 produced by neutrophils regulates IFN-gamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J Clin Invest 2010; 120:331–342.

21 Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, *et al.* Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. J Immunol 2011; 187:490–500.

22 Karthikeyan RS, Leal Jr, Prajna SM, Dharmalingam NV, Geiser DM, Pearlman E, Lalitha P. Expression of innate and adaptive immune mediators in human corneal tissue infected with Aspergillus or Fusarium. J Infect Dis 2011; 204:942–950.

23 Ramirez-Velazquez C, Castillo EC, Guido-Bayardo L, Ortiz-Navarrete V. IL-17-producing peripheral blood CD177+ neutrophils increase in allergic asthmatic subjects. Allergy Asthma Clin Immunol 2013; 9:23.

24 Stroncek DF. Neutrophil-specific antigen HNA-2a, NB1 glycoprotein, and CD177. Curr Opin Hematol 2007; 14:688–693.

25 Bauer S, Abdgawad M, Gunnarsson L, Segelmark M, Tapper H, Hellmark T. Proteinase 3 and CD177 are expressed on the plasma membrane of the same subset of neutrophils. J Leukocyte Biol 2007; 81:458–464.

26 Stroncek DF, Shankar RA, Noren PA, Herr GP, Clement LT. Analysis of the expression of NB1 antigen using two monoclonal antibodies. Transfusion 1996; 36:168–174.

27 Zhou G, Yu L, Fang L, Yang W, Yu T, Miao Y, *et al.* CD177+neutrophils as functionally activated neutrophils negatively regulate IBD. Gut 2018; 67:1052–1063.

28 Hu N, Westra J, Huitema M, Bijl M, Brouwer E, Stegeman CA, *et al.* Coexpression of CD177 and membrane proteinase 3 on neutrophils in antineutrophil cytoplasmic autoantibody–associated systemic vasculitis: anti–proteinase 3–mediated neutrophil activation is independent of the role of CD177-expressing neutrophils. Arthritis Rheumatol 2009; 60:1548–1557.

29 Global initiative for Asthma (GINA). Global strategy for asthma management and prevention. GINA; 20014; 2014. 2014.

30 Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al.* ATS/ERS TaskForce. Standardisation of spirometry. Eur Respir J 2005; 26:319–338.

31 Ranu H, Wilde M, Madden B. Pulmonary function tests. Ulster Med J 2011; 80:84–90.

32 Schreiber A, Busjahn A, Luft FC, Kettritz R. Membrane expression of proteinase 3 is genetically determined. J Am Soc Nephrol 2003; 14:68–75.

33 Hamid Q, Tulic' MK, Liu MC, Moqbel R. Inflammatory cells in asthma: mechanisms and implications for therapy. J Allergy Clin Immunol 2003; 111 (Suppl 1):S5–S17.

34 Monteseirín J. Neutrophils and asthma. J Investig Allergol Clin Immunol 2009; 19:340–354.

35 Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH, *et al.* Mucosal inflammation in asthma. Am Rev Respir Dis 1990; 142:434–457.

36 Davies RJ, Devalia JL. Asthma: epithelial cells. Br Med Bull 1992; 48:85–96.

37 Honda K, Wada H, Nakamura M, Nakamoto K, Inui T, Sada M, *et al.* IL-17A synergistically stimulates TNF-α-induced IL-8 production in human airway epithelial cells: a potential role in amplifying airway inflammation. Exp Lung Res 2016; 42:205–216.

38 Hashimoto T, Akiyama K, Kobayashi N, Mori A. Comparison of IL-17 production by helper T cells among atopic and nonatopic asthmatics and control subjects. Int Arch Allergy Immunol 2005; 137(Suppl 1):51–54.

39 Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, *et al.* Antigen-specific T cell sensitization is impaired in IL-17A-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 2002; 17:375–387.

40 Kudo M, Melton AC, Chen C, Engler MB, Huang KE, Ren X, *et al.* IL-17A produced by αβ T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. Nat Med 2012; 18:547–554.

41 Chang Y, Al-Alwan L, Risse PA, Halayko AJ, Martin JG, Baglole CJ, *et al.* Th17-associated cytokines promote human airway smooth muscle cell proliferation. FASEB J 2012; 26:5152–5160.

42 Taylor PR, Roy S, Leal SM, Sun Y, Howell SJ, Cobb BA, *et al.* Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, ROR gammat and dectin-2. Nat Immunol 2014; 15:143–151.

43 Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A. IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. J Immunol 2003; 170:2106–2112.

44 Katayama M, Ohmura K, Yukawa N, Terao C, Hashimoto M, Yoshifuji H, *et al.* Neutrophils are essential as a source of IL-17 in the effector phase of arthritis. PLoS One 2013; 8:e62231.

45 Li L, Huang L, Vergis AL, Ye H, Bajwa A, Narayan V, *et al.* IL-17 produced by neutrophils regulates IFNgamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J Clin Invest 2010; 120:331–342.

46 Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R. Sputum IgE and cytokines in asthma: relationship with sputum cellular profile. PloS One 2013; 8:e58388.

47 Liu Y, Zeng M, Liu Z. Th17 response and its regulation in inflammatory upper airway diseases. Clin Exp Allergy 2015; 45:602–612.

48 Newcomb DC, Peebles RS. Th17-mediated inflammation in asthma. Curr Opin Immuno 2013; 25:755–760.

49 Raedler D, Ballenberger N, Klucker E, Böck A, Otto R, Prazeres da Costa O, *et al.* Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. J Allergy Clin Immunol 2015; 135:81–91.

50 Fattahi F, Brandsma CA, Lodewijk M, Reinders-Luinge M, Postma DS, Timens W, *et al.* Atopy and inhaled corticosteroid use associate with fewer IL-17+ cells in asthmatic airways. PLoS One 2016; 11:e0161433.

51 Essilfie AT, Simpson JL, Horvat JC, Preston JA, Dunkley ML, Foster PS, *et al. Haemophilus influenzae*infection drives IL-17-mediated neutrophilic allergic airways disease. PLoS Pathog 2011; 7:e1002244.

52 Li L, Lukacs NW, Schaller MA, Petersen B, Baptist AP. IL-17RB +granulocytes are associated with airflow obstruction in asthma. Ann Allergy Asthma Immunol 2016; 117:674–679.

53 Al-Ramli W, Prefontaine D, Chouiali F, Martin JG, Olivenstein R, Lemière C, Hamid Q. T(H)17-associated cytokines (IL-17A and IL-17F) in severeasthma. J Allergy Clin Immunol 2009; 123:1185–1187.

54 Andersson C, Adams A, Nagakumar P, Bossley C, Gupta A De Vries D, *et al.* Intraepithelial neutrophils in pediatric severe asthma are associated with better lung function. J Allergy Clin Immunol 2017; 139:1819–1829.

55 Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, *et al.* Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. Chest 2010; 138:1140–1147.

56 Inoue Y, Matsuwaki Y, Shin SH, Ponikau JU, Kita H. Nonpathogenic, environmental fungi induce activation and degranulation of human eosinophils. J Immunol 2005; 175:5439–5447.

57 Bush RK, Prochnau JJ. Alternaria-induced asthma. J Allergy Clin Immunol 2004; 113:227–234.

58 Gazendam RP, van de Geer A, Roos D, van den Berg TK, Kuijpers TW. How neutrophils kill fungi. Immunol Rev 2016; 273:299–311.

59 Gazendam RP, van Hamme JL, Tool AT, Hoogenboezem M, van den Berg JM, Prins JM, *et al.* Human neutrophils use different mechanisms to kill aspergillus fumigatus conidia and hyphae: evidence from phagocyte defects. J Immunol 2016; 196:1272–1283.

60 Conti HR, Gaffen SL. IL-17-mediated immunity to the opportunistic fungal pathogen *Candida albicans*. J Immunol 2015; 195:780–788.

61 Sparber F, Leibund Gut-Landmann S. Interleukin 17-mediated host defense against *Candida albicans*. Pathogens 2015; 4:606–619.

62 Ramirez-Velazquez C, Beristain-Covarrubias N, Guido-Bayardo L, Ortiz-Navarrete V. Peripheral blood T cells and neutrophils from asthma patients express class-I MHC-restricted T cell-associated molecule. Allergy Asthma Clin Immunol 2014; 10:46.

63 Ghosh S, Hoselton SA, Asbach SV, Steffan BN, Wanjara SB, Dorsam GP, Schuh JM. B lymphocytes regulate airway granulocytic inflammation and cytokine production in a murine model of fungal allergic asthma. Cell Mol Immunol 2015; 12:202–212.

64 Nembrini C, Marsland BJ, Kopf M. IL-17-producing T cells in lung immunity and inflammation. J Allergy Clin Immunol 2009; 123:986–994.

65 Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol 2010; 10:479–489.

66 Werner JL, Gessner MA, Lilly LM, Nelson MP, Metz AE, Horn D, *et al.* Neutrophils produce interleukin 17A (IL-17A) in a dectin-1- and IL-23-dependent manner during invasive fungal infection. Infect Immun 2011; 79:3966–3977.

67 Inoue K, Takano H, Koike E, Yanagisawa R, Ichinose T, Yoshikawa T. Candida soluble cell wall beta-glucan facilitates ovalbumin-induced allergic airway inflammation in mice: possible role of antigen-presenting cells. Respir Res 2009; 10:68.