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Abbreviation list

BHR= bronchial hyperresponsiveness

BPT= bronchoprovocation test

COPD= chronic obstructive pulmonary disease

ICS= inhaled corticosteroids

NAEB = nonasthmatic eosinophilic bronchitis

SPT = skin prick test

SAD=small airway dysfunction

Will nonasthmatic eosinophilic bronchitis develop chronic airway obstruction? A prospective, observational study

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Running head: prognosis of nonasthmatic eosinophilic bronchitis

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【 Abstract 】 Objective: The long-term prognosis of nonasthmatic eosinophilic bronchitis (NAEB) is still unclear. This study aims to observe the frequency of relapse

among NAEB patients and the likelihood of developing into chronic airflow obstruction over time.

Methods: The eligible patients with NAEB from 2003 to 2013 were followed up for at least 1 year. During the follow-up period, we collected the patients' clinical symptoms, sputum eosinophil count, spirometry and bronchial hyperresponsiveness for evaluation. Linear mixed model was adopted to determine the relationship between time and lung function.

Results: A total of 234 patients with NAEB were identified, of whom 141 were followed up for over one year with the median period of 4.1 years. Up to 59.6% patients had relapse after treatments. Both allergic rhinitis (OR 4.37, 95%CI 1.049~18.203, $P=0.043$) and sputum eosinophilia after 4-week treatment with inhaled corticosteroids (OR 9.493, 95%CI 2.381~37.850, $P=0.001$) were risk factors for relapse. Among the 141 patients, eight of them (5.7%) developed mild asthma. During the follow-up period, no progressive decline in FVC, FEV_1 , FEV_1/FVC were observed ($P>0.05$). Although the proportion of small airway dysfunction ($MMEF<65\%$) significantly increased at the last visit in all groups (all $P<0.05$), only relapse group showed MMEF decline at the end of follow-up ($P<0.05$) in linear mixed model.

Conclusion: Over 50% patients with NAEB have repeated episodes associated with persistent sputum eosinophilia after treatment and allergic rhinitis. In our cohort, chronic airway obstruction does not develop despite small airway dysfunction increases over time.

Introduction

Non-asthmatic eosinophilic bronchitis (NAEB) was originally described by Gibson in

1989¹, and has been reported as one of common causes of chronic cough in 10-22% of the patients²⁻⁶. NAEB shares a similarity with asthma in terms of airway eosinophilic inflammation and response to corticosteroids⁷⁻⁸, but lack of airflow obstruction and bronchial hyperresponsiveness (BHR).

Approximately 30-49% patients with chronic obstructive pulmonary disease (COPD) also showed sputum eosinophilia, with good response to corticosteroids⁹⁻¹⁰. A case report showed one patient developed irreversible airflow obstruction eventually¹¹. It is important to identify whether NAEB is a chronic airway inflammation disease and develop chronic airway disease eventually.

Only a few prospective studies with relatively small sample size have conducted to prognosis of NAEB and committed inconsistent results. Hancox et al¹² reported that seven out of eight NAEB patients were in complete remission of cough and airway inflammation during 5-10 years follow-up. Among them, one patient showed asthmatic symptoms, but not confirmed by spirometry. Pouljoki and his colleagues¹³ found that 16% of patients with chronic cough developed into asthma after 4.4 years follow-up. The diagnosis of NAEB was not confirmed in those patients, as induced sputum test was not adopted in this study. An observational study conducted by Berry and his coworkers¹⁴ showed that only one out of 32 (3%) patients had complete relief in cough and normal sputum eosinophil count. Twenty three (72%) patients showed persistent cough and sputum eosinophilia, among which three developed asthma and five were irreversible airway obstruction. In Park's study¹⁵, five (21%) patients contracted with a recurrent episode. Among them, two had experience in progressive reduction in FEV₁ (>20%), and the others (around 76%) did not show any recurrence of cough. The observational results of these studies remain inconsistent and the risk factor of relapse is still unknown.

This study aims to investigate the frequency and risk factors of NAEB relapse in patients with NAEB and attempts to determine whether chronic airflow obstruction will develop in NAEB patients with a relatively large sample and long follow-up period.

Methods

Subjects

All Patients referred to our hospital and diagnosed with NAEB were recruited. The

medical history of the patients was recorded using a standardized questionnaire, applied in our previous study⁶. In the first visit, patients were examined through allergen skin prick test, serum total IgE and specific IgE, chest radiograph, induced sputum test, spirometry and bronchial challenge test. NAEB was diagnosed regarding criteria: (1) chronic cough lasting for more than 8 weeks, (2) no abnormality in chest radiograph, (3) $FEV_1 > 80\%$ pred, $FVC > 80\%$ pred, $FEV_1/FVC > 80\%$, maximum within-day PEF variability over 2 weeks $< 20\%$, $PC_{20}FEV_1$ (methacholine) > 16 mg/ml, (4) sputum eosinophilia ($\geq 2.5\%$).

All patients were treated with inhaled corticosteroids (budesonide 400 μ g/d) for at least 4 weeks and oral prednisone with a dose of 10-15mg/d in the initial 3 days according to Chinese cough guideline¹⁶. The Ethic Committee of The First Affiliated Hospital of Guangzhou Medical University approved this study, the IRB approval number is 2002-02. The informed written consent was obtained from each subject before inclusion.

Follow-up visits were conducted annually and whenever necessary for example, the reoccurrence of cough, or the emergence of new symptoms, including wheeze and chest tightness. Telephone interviews were also conducted when patients had no such symptoms, unable, or unwilling to do the site visit. In addition, we collected questionnaire (supplement 1) through either the site visit or telephone interview to guarantee the accuracy of our patients' data. Induced sputum test, spirometry, bronchial challenge test were performed in each visit. An additional induced sputum test was conducted in the first month of follow-up.

Measurement

Induced Sputum Test

Sputum was induced with 3% hypertonic saline inhaled in consecutive 10min via ultrasonic nebulizer, processed as described by Pizzichini¹⁷ et al. Sputum was mixed with 4 times its volume of 0.1% dithiothreitol, filtered through a 48 μ m mesh gauze and centrifuged at 3000 rpm for 10min. The cell pellet was resuspended in PBS. The cell smear was prepared, and stained with haematoxylin-eosin stain. Differential cell count was obtained by counting 400 nonsquamous cells^{16,17}.

Bronchial Challenge Test

Spirometry and bronchial challenge test were performed as recommended by the American Thoracic Society (ATS)¹⁸. The forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC) and FEV₁/FVC ratio were recorded. Bronchial hyperresponsiveness was defined as a $\geq 20\%$ decrease in the FEV₁ at a methacholine dose of 12.8 μmol or less. Small airway dysfunction was defined as MMEF% less than 65% predicted value.

The relapse of NAEB was defined as repeated episodes of cough with sputum eosinophilia ($\geq 2.5\%$), normal lung function, and absence of BHR, responses well to inhaled corticosteroids. Asthma was defined as presenting asthmatic symptoms such as wheeze, chest tightness, and objective evidence of BHR or bronchial reversibility at any time point during the follow-up period. Fixed airflow obstruction was defined as a persistent FEV₁/FVC value less than 70% following inhalation of salbutamol (400 μg) throughout the subsequent observation period.

Statistic Analysis

Statistical analysis was performed with SPSS 19.0. The clinical characteristics and physiologic data are presented as mean $\pm$ SD or median (minimum, maximum). The differences in the two different groups were analyzed by using two independent-samples t-test or chi-square test. The differences between first and last visit within the same group were analyzed using paired-samples t-test or chi-square test. Non-normal distribution or heterogeneity of variances was calculated using Mann-Whitney U test. Logistic regression analysis was applied to identify the risk factors associated with the recurrence of NAEB. The confidence intervals were set up at the level of 95%. Linear mixed model was used to determine whether the time had an influence on lung function decline. Time (in years) was chosen for fixed effect.

Results

1. Clinical characteristics of subjects

A total of 234 subjects were diagnosed with NAEB in our study. Among them, 141 have been followed up for at least one year and the mean duration of following-up was 4.1 years (range, 1 to 10 years). The clinical characteristics of these subjects were

summarized in Table 1.

Table 1. The clinical characteristics of patients with NAEB

2. Clinical course during the follow-up period

As demonstrated in Table 1, after treatment, 57 (40.4%) patients did not cough any more, and 84 (59.6%) patients showed recurrent episode of NAEB, 8 (5.67%) patients, who contracted with one or more recurrences of cough. Nearly 90% of the relapse events occurred at the first year.

3. Risk factors associated with EB relapse

The proportion of small airways dysfunction, allergic rhinitis (AR), and sputum eosinophil count in relapse group were higher than that in the non-relapse group at the very beginning (All $P<0.05$). Higher sputum eosinophil count as well as the proportion of patients with sputum eosinophilia was noticed in relapse group after 4 weeks treatment ($P<0.01$). The multiple regression analysis indicated that allergic rhinitis and persistent sputum eosinophilia after treatment were risk factors of relapse. (Table 2)

Table 2. Multiple logistic regression analysis for risk factors for relapse

4. Characteristics of EB patients developing asthma

Among eight patients with NAEB (5.7%) developing asthma. There were five patients showing typical asthmatic symptoms and six with nocturnal cough. Most patients had history of atopy or exposure to noxious substance in asthmatic group (Table 3). The average length of time in developing asthma was 27.5 ± 15.80 months and the mean sputum eosinophil count was $11.42\pm8.47\%$, when the diagnosis of asthma was confirmed.

Table 3. The clinical characteristics of asthmatic patients and non-asthmatic patients

5. Changes of lung function during the follow-up

At last visit, a normal but significant decreased FEV₁/FVC in relapse group was observed ($P=0.007$). FEV₁%pred was comparable between all groups (Table 4). A significant lower MMEF% pred at last visit was observed in relapse group ($P=0.003$). No significant difference was shown between the last visit and the initial visit regarding to spirometry parameters in non-relapse group (All $P>0.05$).

Both relapse group and non-lapse group revealed a higher rate of small airway dysfunction at last visit as compared with initial visit ($P=0.012$, $P=0.036$, respectively). The proportion of small airway dysfunction in relapse group was higher than that in non-relapse group at initial visit ($P=0.043$), however, there was no insignificant difference between two groups at the last visit ($P>0.05$) (Table 4).

Table 4. Change of Lung function in patients with NAEB during follow-up

Lung function parameters, including FVC, FEV₁/FVC, FEV₁ and MMEF, were stable in follow-up group and non-relapse group over the follow-up period in our linear mixed model (all $P>0.05$). FEV₁, FVC, FEV₁/FVC in relapse group were stable over time(all $P>0.05$), but the MMEF slightly decreased between year 8 and 10 of the follow-up period after being level in the first 7 years, and then dropped significantly at the last year ($P<0.05$)(Figure1).

Figure 1. Change of lung function during follow-up period in linear mixed model.

Discussion

The mean age was over 50 and female was predominant, as reported in previous studies among population in western countries^{2,14,15,19}. In our study, the patients were younger, with an average age of 41. The number of patients was similar in both genders, which was consistent with our previous studies^{5,6}. A very low proportion of cigarette smoking in patients with NAEB was observed, indicating that cigarette smoking might not be related to the onset of NAEB. One third of patients with NAEB had either allergic

rhinitis or atopy, implying an association between allergic rhinitis and NAEB.

A few studies focused on the prognosis of NAEB, but the results were not consistent^{12,14,15}. To our knowledge, our study is of merit due to its largest sampling size of NAEB patients and the longest following-up period. In this study, up to 60% patients had experienced with at least one relapse, which was higher than those in Park's study (21%)¹⁵, albeit being slightly lower than that of Berry's study (97%)¹⁴. The variation of relapse is still unclear. Short follow-up period might be associated with the low percentage of relapse in Park's¹⁵ study. High proportion of smokers, persistent airway eosinophilic inflammation, and elderly patients might contribute to the high frequency of relapse and persistence of symptoms in Berry's study¹⁴. We were the first to find that allergic rhinitis and prolonged sputum eosinophilia were risk factors of the relapse of NAEB.

Compared with patients without eosinophilic inflammation after treatment, patients with persistent sputum eosinophilia experienced more relapses. The persistent sputum eosinophilia following 4-week treatment with ICS indicated that those patients might need a longer course of treatment to reduce relapse. A comprehensive assessment of symptoms and airway inflammation is suggested as an approach to guide treatment. Although patients who experienced relapse showed a higher percentage of sputum eosinophils, no relationship between baseline sputum eosinophil and relapse was observed in our study. Coincidentally, Newby and colleagues found that lung function decline in adult asthmatics with eosinophilic inflammation was related to variability in the inflammation over time rather than eosinophilic inflammatory burden *per se*²⁰.

The underlying relationship between NAEB and rhinitis might be similar to that of asthma and rhinitis. Coincidentally, around 40-90% asthmatics have the comorbidity of AR, which was considered as an independent risk factor of asthma^{21,22}. Meanwhile, inflammation in lower airways could be induced after nasal allergen provocation²³. Similar to asthma, many allergens, such as mites, mushroom spore, flour, cyanoacrylate and methacrylate and latex were also involved in the onset of NAEB²⁴⁻²⁶. Patients with AR may be more likely to suffer from lower airway inflammation. Therefore, the relapse of NAEB was more common in patients with co-morbidity of AR.

Similar to asthma, NAEB is involved in many types of inflammatory cells and cytokines²⁷⁻³¹. Although the relapse rate was high, the percentage of developing to asthma remained quite low, which was similar to the prevalence of asthma (6.9%) in the

general population of Guangzhou³². This result indicated that NAEB should be a distinct entity, rather than the early stage of asthma.

Unlike the previous studies, no progressive FEV₁, FVC or FEV₁/FVC reduction was observed in all patients in our study, which indicated that relapse was unlikely to affect FEV₁, FVC and FEV₁/FVC in EB patients. Berry and coworkers¹⁴ found that fixed airflow obstruction developed in 16% of patients with NAEB. The percentage of smokers was quite low in this study, whereas the higher proportion of smokers (19%) in Berry's study may explain the high prevalence of fixed airflow obstruction. In the study by Park and associates¹⁵, NAEB reemerged in 5 non-smokers, and three of them were found to have a progressive FEV₁ reduction >20%, the mechanism of developing COPD was not clear. However, none of our patients developed COPD during the follow-up period.

Small airway dysfunction, one of pathophysiological characteristics in patients with asthma and COPD, was identified in a small proportion of patients with NAEB. This figure increased over time among relapse patients in particular. However, the declined MMEF%pred value was only observed in relapse group at the last year, but not in follow-up group and non-relapse group. These results indicated that EB relapse might accelerate the decrease of MMEF in some patients. Many studies have recently showed that small airway dysfunction is highly related to severe asthma³² or poor controlled asthma³³. Although small airway dysfunction is common in COPD and asthma in association with chronic airflow obstruction, it has been reported to less likely to be associated with asthma symptoms³⁴. The small airway dysfunction in NAEB could be different from asthma and COPD. Some methods are available to detect small airway abnormality, including spirometry, fractional exhaled nitric oxide, inflammatory cells³⁵. Further study is needed to explore the difference of small airway dysfunction between asthma and NAEB. Although it could not be completely excluded that airflow obstruction will develop once a sufficient proportion of small airways is affected, it is thought that persistent small airway dysfunction alone is likely to exist in certain conditions such as NAEB, and might not subsequently develop airflow obstruction. NAEB is unlikely to be an early stage of COPD or asthma.

NAEB shares a similar airway inflammation with asthma, but the underlying mechanism of the lack of BHR and airflow obstruction in patients with NAEB is still unclear. Compared with asthmatic subjects, less mast cell in the bundles of airway

smooth muscle, lower level of IL-13 and elevated PGE2 in sputum were identified in patients with NAEB³⁶⁻³⁹. These differences may result in the absence of airway obstruction in NAEB.

There are some limitations in our study. Firstly, we were not able to trace the natural courses of NAEB without the interference by ICS treatment regarding ethical requirement. Secondly, the prevalence of relapse might be underestimated because some patients failed to keep the site visit each year. For example, some asymptomatic patients only accepted telephone interview. Thirdly, we evaluated small airway function with spirometry only in patients with NAEB. Finally, the significant decrease of MMEF in relapse group appeared at the end of our observation, it was uncertain that whether this decrease was transient or not, thus a longer time, larger sample, controlled, observational study is need to clarify this issue.

In conclusion, relapse is common in EB patients even after treatment. For the first time, we have demonstrated that allergic rhinitis and persistent sputum eosinophilia after treatment are the risk factors of relapse. During follow-up, only a few patients with NAEB develop mild asthma. In our cohort, there is no significant progressive decline in FVC, FEV₁, FEV₁/FVC despite the increase in small airway dysfunction in some patients over time. NAEB is highly likely to be a distinct entity, rather than an early stage of asthma or COPD.

Author Contributions

Dr. Kefang Lai contributed to conceptualization of the study, all aspects of the study, and manuscript preparation, revising of the submitted manuscript, read and approved the final manuscript.

Dr. Baojuan Liu: contributed to data collection, data analysis and interpretation, revising of the submitted manuscript, read and approved the final manuscript.

Dr. Danyuan Xu: contributed to data collection, data analysis and interpretation, and drafting the manuscript, and read and approved the final manuscript.

Dr. Lina Han: contributed to data collection, data analysis and interpretation, and drafting the manuscript, and read and approved the final manuscript.

Dr. Ling Lin: contributed to data collection, and read and approved the final

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Dr. Ying Xi: was responsible for patient recruiting and follow-up, and read and approved the final manuscript.

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Dr. Wei Luo: was responsible for sputum induction and differential cell count, and read and approved the final manuscript.

Dr. Qiaoli Chen: was responsible for sputum induction and differential cell count, and read and approved the final manuscript.

Dr. Nanshan Zhong: contributed to study design and critical review of the manuscript and read and approved the final manuscript.

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Reference

1. Gibson PG, Dolovich J, Denburg J, et al. Chronic cough: eosinophilic bronchitis without asthma. *Lancet*.1989;1(8651):1346-1348.
2. Brightling CE, Ward R, Goh KL, et al. Eosinophilic bronchitis is an important cause of chronic cough. *Am J Respir Crit Care Med*.1999;160(2):406-410.
3. Melvin R. Overview of common chronic cough ACCP evidence-based clinical practice guideline. *Chest*.2006;129(Suppl):59-62.
4. Ayik SO, Basoglu OK, Erdinc M, et al. Eosinophilic bronchitis as a cause of chronic

cough. *Respir Med*.2003;97(6):695-701.

5. Lai K, Chen R, Liu C, et al. Etiology and a diagnostic protocol for patients with chronic cough. *Chin J Tuberc Respir Dis*.2006;29(2):96-99.[Article In Chinese]

6. Lai K, Chen R, Lin J, et al. A prospective, multicenter survey on causes of chronic cough in China. *Chest*.2013;143(3):613-620.

7. Brightling CE, Ward R, Wardlaw AJ, et al. Airway inflammation, airway responsiveness and cough before and after inhaled budesonide in patients with eosinophilic bronchitis. *Eur Respir J*.2000;15(4):682-686.

8. Kelly MM, Leigh RJ, Lata G, et al. Eosinophilic bronchitis in asthma: A model for establishing dose-response and relative potency of inhaled corticosteroids. *J Allergy Clin Immunol*.2006;117(5):989-994.

9. Brightling CE, McKenna S, Hargadon B, et al. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. *Thorax*.2005;60(3):193-198.

10. Fujimoto K, Kubo K, Yamamoto H. Eosinophilic inflammation in the airway is related to glucocorticoid reversibility in patients with pulmonary emphysema. *Chest*.1999;115(3):697-702.

11. Brightling CE, Woltmann G, Wardlaw AJ. Development of irreversible airflow obstruction in a patient with eosinophilic bronchitis without asthma. *Eur Respir J*. 1999; 14(5):1228-1230

12. Hancox RJ, Leigh R, Kelly MM, et al. Eosinophilic bronchitis. *Lancet*.2001;358(9287):1104-1104.

13. Puolijoki H, Lahdensuo A. Chronic cough as a risk indicator of broncho-pulmonary disease. *Eur J Respir Dis*.1987;71(2):77-85.

14. Berry MA, Hargadon B, McKenna S, et al. Observational study of the natural history of eosinophilic bronchitis. *Clin Exp Allergy*.2005;35(5):598-601.

15. Park SW, Lee YM, Jang AS, et al. Development of chronic airway obstruction in patients with eosinophilic bronchitis: a prospective follow-up study. *Chest*.2004;125(6):1998-2004.

16. Asthma Workgroup, Chinese Society of Respiratory Diseases (CSRD), Chinese

Medical Association. The Chinese national guidelines on diagnosis and management of cough (December 2010). *Chin Med J (Engl)*.2011;124(20):3207-3219.

17. Pizzichini E, Pizzichini MM, Efthimiadis A. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med*.1996;154(2Pt1):308-317.

18. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing; 1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med*.2000;161(1):309-329.

19. Birring SS, Parker D, Brightling CE, et al. Induced sputum inflammatory mediator concentrations in chronic cough. *Am J Respir Crit Care Med*.2004;169(1):15-19.

20. Newby C, Agbetile J, Hargadon B, et al. Lung function decline and variable airway inflammatory pattern:longitudinal analysis of severe asthma, *J Allergy Clin Immunol*.2014;134(2):287-294.

21. Celine B, Qutayba H. Relationship between asthma and rhinitis:epidemiologic, pathophysiologic, and therapeutic aspects. *Allergy, Asthma, and Clin Immunol*.2005;1(2):81-87.

22. Guerra S, Sherrill DL, Martinez FD, et al. Rhinitis as an independent risk factor for adult onset asthma. *J Allergy Clin Immunol*.2002;109(3):419-425.

23. Braunstahl GJ, Overbeek SE, Kleinjan A. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. *J Allergy Clin Immunol*.2001;107(3):469-476.

24 Tanaka H, Saikai T, Sugawara H, et al. Workplace-related chronic cough on a mushroom farm. *Chest*.2002;122(3):1080-1085.

25. Lemiere C, Efthimiadis A, Hargreave FE, et al. Occupational eosinophilic bronchitis without asthma: an unknown occupational airway disease. *J Allergy Clin Immunol*.1997;100(6Pt1):852-853.

26. Quirce S, Fermandea-Nieto M, Miguel JD, et al. Chronic cough due to latex-induced eosinophilic bronchitis. *J Allergy Clin Immunol*.2001;108(1):143.

27. Luo W, Lai K, Chen R, et al. Characteristics of airway inflammatory cells and

mediators in eosinophilic bronchitis patients. *Chin J Tuberc Respir Dis.*2005;28(9):626-629.[Article In Chinese]

28. Brightling CE, Symon FA, Bradding P, et al. Th2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. *J Allergy Clin Immunol.*2002;110(6):899-905.

29. Matsuoka T, Hirata M, Tanaka H, et al. Prostaglandin D2 as a mediator of allergic asthma. *Science.*2000;287(5460):2013-2017.

30. Brightling CE, Ward R, Woltmann G, et al. Induced sputum inflammatory mediator concentrations in eosinophilic bronchitis and asthma. *Am J Respir Crit Care Med.*2000;162(3Pt1):878-882.

31. Luo W, Lai K, Chen R, et al. Pathological features of airway inflammation in eosinophilic bronchitis. *Chin J Pathophysio.* 2006;22(5):943-947.[Article In Chinese]

32. In't Veen JC, Beekman AJ, Bel EH, et al. Recurrent exacerbations in severe asthma are associated with enhanced airway closure during stable episodes. *Am J Respir Crit Care Med.*2000;161(6):1902-1906.

33. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. *Lancet Respir Med.*2014;2(6):497-506.

34. van der Wiel E, Postma DS, van der Molen T, et al. Effects of small airway dysfunction on the clinical expression of asthma: a focus on asthma symptoms and bronchial hyper-responsiveness. *Allergy.*2014;69(12):1681-1688.

35. Contoli M, Santus P, Papi A. Small airway disease in asthma: pathophysiological and diagnostic considerations. *Curr Opin Pulm Med.* 2015;21(1):68-73.

36. Li J, Wang H, Chen Y. House dust mite sensitization is the main risk factor for the increase in prevalence of wheeze in 13- to 14-year-old schoolchildren in Guangzhou city, China. *Clin Exp Allergy.*2013; 43(10):1171-1179.

37. Brightling CE, Bradding P, Symon FA, et al. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med.*2002;346(22):1699-1705.

38. Berry MA, Parker D, Neale N, et al. Sputum and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. *J Allergic Clin Immunol.*2004;114(5):1106-1109.

39. Sastre B, Fernández-Nieto M, Mollá R, et al. Increased prostaglandin E2 levels in the airway of patients with eosinophilic bronchitis. *Allergy*.2008;63(1):58-66.

Table 1. The clinical characteristics of patients with NAEB

	Total	Follow-up group	Relapse group	Non-relapse group
Subjects, n	234	141	84	57
Male/Female, n	102/132	72/69	46/38	26/31
Age (yrs)	41.88±13.14	41.11±11.56	42.03±11.83	38.85±10.49
Cough duration (M)	12(1, 480)	12(1, 396)	12(1, 396)	6 (1, 156)
Daily cough	145(61.96)	86(60.99)	51(60.71)	35(61.40)
Nocturnal cough	3(1.28)	0(0.00)	0(0.00)	0(0.00)
Daily and nocturnal cough	86(36.75)	55(39.00)	33(39.28)	22(38.60)
Dry cough	198(84.62)	118(83.69)	71(84.52)	47(82.46)
Sensitive to fumes, odor	111(47.44)	68(48.22)	43(51.19)	25(43.86)
VAS score	5 (2, 10)	6 (3, 10)	6 (3,10)	5 (3,10)
Atopy	84(35.90)	58(41.13)	42(50.00)	16(28.07)**
AR	82(35.04)	50(35.46)	37(44.2)	13(22.70)*
Smoker	19(8.12)	8(5.67)	4(4.76)	4(7.02)
Current Smoker	11(4.70)	6(4.26)	3(3.57)	3(5.26)
Ex-smoker	8(3.41)	2(1.42)	1(1.19)	1(1.75)
Sputum Eos (%) at baseline	7.75 (2.50, 84.50)	8.00 (2.50,84.50)	11.00 (2.50,78.00) *	6.50 (2.50,84.50)
Sputum Eos (%) After 4w ICS treatment	1.75 (0.00, 63.00)	3.50 (0.00,63.00)	5.00 (0.25, 63)	1.00 (0.00, 25.50)**
Sputum eosinophilia after 4w ICS treatment	101(43.16)	78(55.32)	65(77.38)	13(22.80)**
FEV1 %pred	99.64±14.19	99.86±15.44	98.92±17.57	101.49±11.94
FEV1/FVC%	84.47±8.56	82.77±11.16	83.32±7.86	86.45±9.15
MMEF % pred	86.48±25.53	83.84±22.18	83.32±22.40	84.57±21.83

Note: Data are presented as median (minimum, maximum), n (%) or mean $\pm$ SD; AR: allergic rhinitis, ICS: Inhaled corticosteroids, FEV1: forced expiratory volume, FVC: forced vital capacity, MMEF: maximum mid expiratory flow ;Relapse group vs non-relapse group, *: $P < 0.05$, **: $P < 0.01$.

Table 2. Multiple logistic regression analysis for risk factors for relapse

	β value	SE	Wald value	P value	OR	95%CI for OR
AR	1.475	0.728	4.104	0.043	4.370	1.049~18.203
Sputum eosinophilia after 4w therapy	2.251	0.706	10.171	0.001	9.493	2.381~37.850
Constant	-2.695	0.722	13.948	0.000	0.068	

Note: AR: allergic rhinitis

Table 3. The clinical characteristics of asthmatic patients and non-asthmatic patients

	Asthmatic group	Non-asthmatic group
Subjects, n	8	76
Gender(female)	6 .00 (75.00%)	32.00 (42.11%)
Age (yrs)	36.25 $\pm$ 7.29	42.83 $\pm$ 12.17
Cough duration (month)	5.00 (1.5-72)	18.00(1.00-396)
History of exposure to noxious materials	5(62.50)	25(32.89)
AR	3(37.50)	27(35.53)
Atopy	7(87.50)	49(64.47)
Sputum eosinophil %		
Baseline	12.25 (7.50-64.00)	9.00 (2.50-78.00)
Sputum eosinophil % after 4-week ICS treatment	10.88 (0.00-63.5)	4.75 (0.25-63.00)

FEV1 pred%	96.69±12.10	99.17±18.39
FEV1/FVC%	79.40±2.93*	83.54±8.30

Note: Data are presented as median (minimum, maximum), n (%) or mean±SD; AR: allergic rhinitis,ICS: Inhaled corticosteroids, FEV1: forced expiratory volume, FVC: forced vital capacity,*:P<0.05.

Table 4.Change of Lung function in the patients with NAEB during follow-up

	Follow-up group		Non-relapse group		Relapse group	
	baseline	last visit	baseline	last visit	baseline	last visit
FEV1%	99.64±14.19	100.68±11.15	101.49±11.94	100.94±8.58	98.92±17.57	97.64±11.77
FVC%	102.38±14.06	104.01±13.27	102.87±14.14	102.73±8.79	103.50±15.00	103.18±13.82
FEV1/FVC %	89.10±12.01	92.05±12.09	86.45±9.15	83.05±6.44	83.32±7.78	79.15±5.98 [#]
MMEF%	86.48±25.53	73.38±28.65	84.57±21.83	82.12±21.21	83.32±22.40	68.31±20.73 ^{&}
SAD	23 (16.33)	53 (37.59)*	3 (5.26)	14 (24.56)*	16 (19.05) ^{\$}	37 (44.07) *

Note: Data are presented as mean±SD or n (%); FEV1: forced expiratory volume, FVC: forced vital capacity, MMEF: maximum mid expiratory flow, SAD: small airway dysfunction; comparison between baseline and last visit in the same group, * P<0.05, [#]: P=0.01; compared with non-relapse group at baseline, ^{\$}: P<0.05; compared with non-relapse group at last visit, [&]: P<0.05.

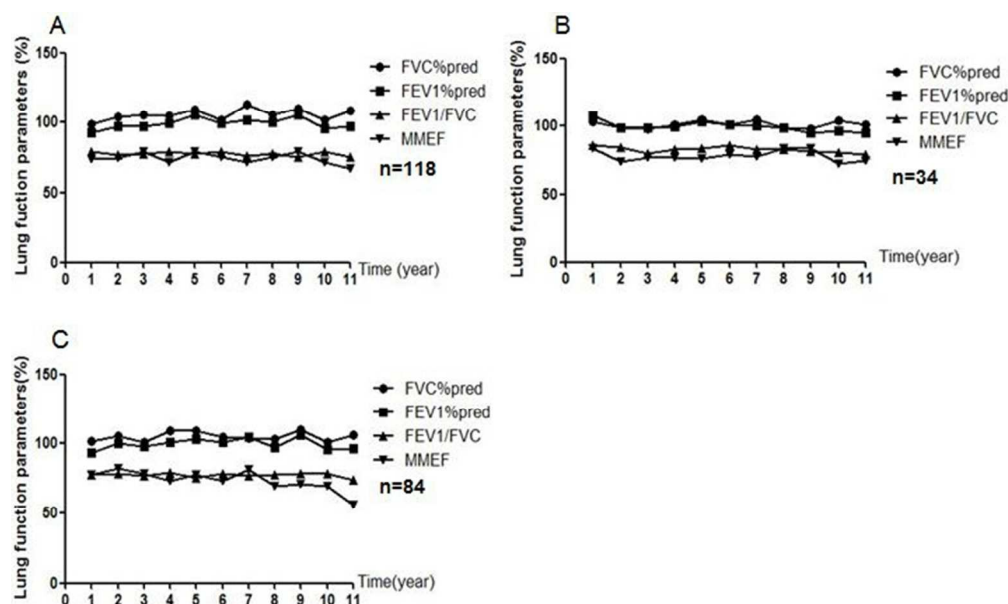


Figure 1. Change of lung function during follow-up period in linear mixed model. The number on X axis represented the year in a cis-order, namely, 1 represented 2003, and 11 represented 2013. A: all lung function parameters were stable in follow-up group ; Year main effect for FVC%pred: $F=0.748$, $P=0.683$; Year main effect for FEV1%pred: $F=0.709$, $P=0.708$; Year main effect for FEV1/FVC: $F=1.669$, $P=0.239$; Year main effect for MMEF: $F=3.973$, $P=0.110$; B: all lung function parameters were stable in non-relapse group; Year main effect for FVC: $F=0.479$, $P=0.874$; Year main effect for FEV1%pred: $F=1.012$, $P=0.477$; Year main effect for FEV1/FVC: $F=1.657$, $P=0.195$; Year main effect for MMEF: $F=1.561$, $P=0.318$; C: FEV1%pred, FVC%pred, FEV1/FVC were stable in relapse group ; Year main effect for FVC%pred: $F=1.631$, $P=0.157$; Year main effect for FEV1%pred: $F=2.095$, $P=0.075$; Year main effect for FEV1/FVC: $F=0.709$, $P=0.709$; Year main effect for MMEF: $F=2.299$, $P=0.144$, but MMEF significantly decreased at the last year of follow-up period ($P<0.05$). FEV1: forced expiratory volume, FVC: forced vital capacity, MMEF: maximum mid expiratory flow.

189x112mm (96 x 96 DPI)

Guangzhou Institute of Respiratory Disease

Cough Questionnaire for EB patients follow up

Date: Y M D

Name		Gender		Age		Occupation	
Address				Contact		ID	

1. Did you experience cough in the past 12 months?

☐ No ☐ Yes: If yes, please answer the following questions

1.1. Duration of Cough Month Week

1.2 Trigger of the onset of cough: _____

1.3 Cough timing ☐ Daytime ☐ Bedtime ☐ Nocturnal ☐ Morning
 ☐ Persistent ☐ Intermittent (cough free time month week)

1.4 Seasonal cough ☐ Yes (☐ Spring ☐ Summer ☐ Autumn ☐ Winter)
 ☐ No

1.5 Cough profile ☐ Dry cough ☐ Productive cough
 ☐ Single cough ☐ Serial of spouts ☐ Wheezing

1.6 Sputum property Color _____ Quantity _____

1.7 Daytime cough score _____ **Nocturnal cough score** _____

Cough symptom score

Daytime cough symptom score 0: No cough at all 1: 1 or 2 short coughs 2: Cough more than 3 times 3 : Frequent cough , without daily activity impairment 4 : Frequent cough , with daily activity impairment 5: Frequent cough, with severe daily activity impairment	Nocturnal cough symptom score 0: No cough at all 1: Cough when awakening or before sleep 2: Awakening during sleep or get up earlier because of cough 3: Frequent awakening during sleep because of cough 4: Cough at almost the whole night 5: Can not sleep at all because of cough
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1.8. V A S: 0 1 2 3 4 5 6 7 8 9 10

2. Did you feel pain in abdomen muscle when you cough? ☐ Yes ☐ No

3. Did you feel incontinence when you cough? ☐ Yes ☐ No

4. Did the cough related to diet? ☐ No ☐ Yes ☐ Before meal

☐ After meal ☐ Having meal

5.Triggers of cough ☐ No ☐ Yes (☐Dust ☐Cooking fumes ☐ Cold air

☐ Common cold ☐ Lie on the back

☐ Cigarette smoking ☐ Exercise ☐ Talking

☐ Itching throat ☐ Drinking (If there is
any other triggers ,please specify _____)

6. Noxious substance exposure ☐ NO ☐ Yes, please specify _____

7. Smoking history

☐ No ☐ Yes (pack/day , year, quit for year)

8. Cough related symptoms: ☐ Itching pharynx ☐ Itching below-pharynx

☐ Foreign body sensation in throat ☐ Regurgitation ☐ Nausea ☐ Belching

☐ .Heartburn ☐ Abdomen pain ☐ Sneezing ☐ Itching nose ☐ Rhinorrhoea

☐ Clearing throat ☐ Post nasal drip ☐ Chest tightness ☐ Breathless

☐ Nasal obstruction ☐ Mucus adhesion sensation in throat

Diagnosis: ☐ EB relapse ☐ Others (please specify_____) ☐ Normal