Respiratory Medicine (2011) 105, 1284–1289

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Expiratory flows and airway inflammation in elderly asthmatic patients

Marie-Eve Ducharme [a], Philippe Prince [a], Nesreen Hassan [b], Parameswaran Nair [b], Louis-Philippe Boulet [a,*]

[a] Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, 2725, chemin Ste-Foy, Québec (Québec), Canada G1V 4G5

[b] Firestone Institute for Respiratory Health, McMaster University, 50 Charlton Avenue East, Hamilton (Ontario), Canada L8N 4A6

Received 23 December 2010; accepted 11 April 2011
Available online 4 May 2011

KEYWORDS
Airway inflammation;
Elderly asthma;
Induced sputum

Summary
Asthma in the elderly is often underrecognized and suboptimally treated, resulting in an increased morbidity and mortality. The characteristics of asthma-related bronchitis and its optimal treatment remain to be determined in this population. We aimed to compare lung function and airway inflammation in elderly and younger asthmatic subjects.

Data from two induced sputum databases were analyzed in three groups of asthmatic subjects (18–30 y, $n = 136$; 31–59 y, $n = 385$; 60–72 y, $n = 172$) and one group of healthy elderly subjects (60–89 y, $n = 16$). Expiratory flows and induced sputum cell counts were analyzed.

Airway obstruction was more marked in elderly asthmatics compared with healthy elderly or younger asthmatic subjects ($p < 0.01$). An increase in sputum neutrophils and a decrease in macrophages and lymphocytes were observed in elderly asthmatics ($p < 0.0001$). Neutrophil percentages significantly increased with asthma severity in the young and the middle-aged groups, while they remained similar in elderly asthmatics regardless of asthma severity ($p < 0.05$). Neutrophil percentages weakly correlated with the dose of ICS in all asthmatics ($r = 0.17$, $p < 0.0001$). Age and dose of ICS were independent predictors of neutrophil percentage in asthmatic subjects in a regression model ($R^2 = 0.12$). Asthma in the elderly is associated with a more marked airway obstruction and sputum neutrophilia. Both age and the dose of corticosteroids need to be considered in the interpretation of the clinical relevance of sputum neutrophil count.

* Corresponding author. Tel.: +1 418 656 4747; fax: +1 418 656 4762.
E-mail addresses: meve_ducharme@hotmail.com (M.-E. Ducharme), Philippe.Prince@criucpq.ulaval.ca (P. Prince), nesreena2000@yahoo.com (N. Hassan), parames@mcmaster.ca (P. Nair), lpboulet@med.ulaval.ca (L.-P. Boulet).

0954-6111/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2011.04.006

Introduction

Although most studies on asthma have been performed on young or middle-aged populations, 6–10% of elderly patients have asthma.[1] Asthma in the aged patient may have developed early in life, but it may also be of late-onset, beginning late in life, without past history of atopic or respiratory disease.[2] Elderly patients are responsible for a large proportion of hospitalizations due to asthma, associated with substantial health care costs.[3] Most guidelines do not specify if the management and treatment should be different in the elderly compared to the younger asthmatics, and current recommendations are based on studies conducted in younger patients with asthma.

Airway function of asthmatic patients has been shown to decline at a faster rate than in normal subjects.[4] Typical asthma symptoms, such as wheezing and chest tightness, may be absent in older asthmatic patients.[5] Bronchoprovocation studies have shown that increased airway resistance is poorly perceived by older people.[6] Moreover, distinguishing between asthma and chronic obstructive pulmonary disease (COPD) may be difficult in the elderly. Marsh et al. showed that 55% of COPD subjects also have asthma.[7] In older patients, the frequently associated comorbidities can induce symptoms considered falsely to be due to asthma, or can worsen the asthmatic condition. All these reasons, among others, may explain the underdiagnosis and undertreatment of asthma in older patients.[8]

However, there is also a possibility that asthma in the elderly presents a different phenotype, less responsive to current therapies. This is supported by previous observations of a lesser response to corticosteroids in this population,[9] although the mechanisms underlying such reduced response remain to be documented. To our knowledge, no study has yet compared respective physiological and inflammatory changes in elderly asthmatic patients compared to younger populations. In this study, we investigated the alterations in pulmonary function and changes in airway inflammatory features of elderly asthmatic patients.

Methods

A retrospective analysis of two large induced sputum databases from the Institut de cardiologie et de pneumologie de Québec and McMaster University was done. A total of 693 patients with stable asthma who had sputum cell counts and spirometry performed at our institutions between September 1999 and June 2008 were included in the analysis. Both centers were using the same procedures. Asthmatic subjects were divided in three different age groups: young (18–30 y, $n = 136$), middle-aged (31–59 y, $n = 385$), and elderly (60–72 y, $n = 172$). A control group of healthy elderly subjects (60–89 y; $n = 16$) was also included in the study. All subjects had no respiratory infection 4 weeks preceding their visit and were able to produce sputum. Asthma severity was determined according to the Canadian consensus guidelines, mostly based on medication needs to control asthma.[10] Current smokers and ex-smokers with a tobacco history of more than 10 pack-years were excluded. When that data was not available, subjects were excluded when "smokers' inclusions" were detected in sputum macrophages.[11] Sputum was induced and processed according to the methods described by Pizzichini et al.[12] Analyses of forced expiratory volume in 1 s (FEV_1) post bronchodilator (BD), forced vital capacity (FVC) post BD, and inflammatory cell counts (neutrophils, eosinophils, lymphocytes, and macrophages) were conducted.

We analyzed the different inflammatory phenotypes in each group of subjects. Based on the normal range values published by Belda et al.[13] and Spanevello et al.[14], we defined an eosinophilic phenotype as a sputum eosinophil percentage greater than 2.2%, a neutrophil phenotype as a percentage greater than 64.4%, a mixed phenotype when both eosinophils and neutrophils were above those limits, and a paucigranulocytic phenotype when cell percentages were in the normal range.

Other data collected at the time of sputum induction included the confirmation of a diagnosis of asthma by a respirologist and the dose of inhaled or oral asthma medication. The diagnosis of asthma was based on a compatible clinical history with evidences of reversible airflow limitation (bronchodilator reversibility of 12% or more or a provocative dose of methacholine inducing a 20% fall in FEV_1 ($PC_{20} < 8$ mg/ml)). The protocol was reviewed and approved by the local Institutional Ethics Committee.

Statistical analyzes

Data were expressed using mean ± SD/SEM or median [interquartile range]. The two-way ANOVA was used to analyse data. Two experimental factors, one associated to the comparison among age categories (18–30 years, 31–59 years, 60–72 years (asthmatic and control group)), factor fixed, and one associated to the comparison among the different asthma severities (mild, moderate, severe or healthy), factor fixed, with interaction terms between the fixed factors were defined. Variables expressed in percentage were analyzed using the $\sin^{-1}(\sqrt{\bullet})$ transformation. Cell counts data were analyzed using the log transformation to stabilize variances. In situations where the normality assumptions were unjustified after log transformation, the alternative procedure used was the rank transformation using the ordinary F test from the two-way ANOVA (non-parametric analyses). Reported p-values are based on these transformations. For variables using parametric analyses, the variance assumptions were verified using the Brown and Forsythe's variation of Levene's test statistic and the univariate normality assumptions were verified with the Shapiro–Wilk tests. Correlations between continuous variables were expressed using the Pearson's correlation coefficients. One-way ANOVA was used to compare asthma severity groups based on neutrophil values. The results were considered significant with p-values ≤ 0.05. All analyses were conducted using the statistical package SAS, version 9.1.3 (SAS Institute Inc, Cary, NC, USA.).

Results

Data from 693 asthmatic patients and 16 healthy elderly controls were analyzed. Characteristics of patients are shown in Table 1. FEV_1, FVC, and FEV_1/FVC ratio were significantly lower in the elderly asthmatic group compared

Table 1 Characteristics of study participants.

Groups	Young Asthmatics (18–30 y)	Middle-aged Asthmatics (31–59 y)	Elderly Asthmatics (60–72 y)	Healthy Elderly (60–89 y)
n	136	385	172	16
Mild asthmatics	84	169	59	–
Moderate asthmatics	33	125	70	–
Severe asthmatics	19	91	43	–
Age (years)	24 ± 4*	47 ± 8*	65 ± 4*	74 ± 9†
Sex (F/M)	90/46	210/175	90/82	9/7
FEV_1 (% predicted)	97 ± 20*	81 ± 24*	69 ± 23*	95 ± 12+
FVC (% predicted)	103 ± 18*	92 ± 19*	84 ± 20*	96 ± 15**
FEV_1/FVC (%)	80 ± 10*	71 ± 12*	65 ± 13*	76 ± 7**
ICS (μg)	423 ± 470*	565 ± 497	633 ± 471	N/A

Mean ± SD; FEV_1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; ICS: Inhaled corticosteroids (fluticasone equivalent, daily μg). *$p < 0.05$ vs the two other asthmatic groups. **$p < 0.01$ vs the elderly asthmatic group. †$p < 0.01$ vs the three asthmatic groups. +$p < 0.001$ vs middle-aged and elderly asthmatic groups.

with the control, middle-aged, and young asthmatic groups. Younger subjects were taking less ICS than middle-aged and elderly subjects.

Inflammatory cell counts are shown in Table 2. Sputum neutrophil percentages and absolute numbers were higher and macrophage and lymphocyte percentages were lower in the elderly asthmatics compared with the middle-aged and the young asthmatic groups. When analyzing all asthmatic subjects together, age and sputum neutrophil percentages were correlated ($r = 0.38$, $p < 0.001$). When analyzing data according to asthma severity, as determined by the Canadian guidelines,[10] neutrophil percentages significantly increased with asthma severity in the young and the middle-aged groups, while they remained similar in elderly asthmatics regardless of asthma severity (Fig. 1). Age and neutrophil percentages correlated in mild ($r = 0.44$, $p < 0.0001$) and moderate ($r = 0.28$, $p < 0.0001$), but not in severe asthmatics ($r = 0.16$, $p = 0.10$).

We also looked at the different inflammatory phenotypes in each group of subjects. We found that 62% of elderly subjects had a neutrophilic or mixed phenotype, compared with 37% in the middle-aged and 21% in the young group ($p < 0.0001$, Fig. 2). The majority of younger subjects (53%) had no airway inflammation, while it was the case for only 19% of elderly patients. The type of inflammation was not associated with asthma severity in elderly subjects ($p > 0.05$, Fig. 3).

A sub-analysis ($n = 554$) showed that daily ICS dose and neutrophil percentages were weakly correlated in all subjects ($r = 0.15$, $p = 0.0003$). Neutrophil percentages were weakly correlated with the dose of ICS in mild ($r = 0.15$, $p = 0.02$), but not in moderate ($r = 0.04$, $p > 0.05$) or severe asthmatics ($r = 0.11$, $p > 0.05$).

We then performed a regression analysis in all subjects with neutrophil percentage as the dependent variable and ICS dose and age together as predictors. Both age ($p < 0.0001$) and ICS dose ($p = 0.003$) remained significant in the model, suggesting that they are independent predictors of neutrophil percentage ($R^2 = 0.12$, Table 3). That relation was not present in the elderly group alone.

Discussion

In this study, we examined the lung function and sputum inflammatory cells in young, middle-aged, and elderly asthmatic subjects to determine whether age could affect

Table 2 Sputum cell counts according to age groups.

Groups	Young Asthmatics (18–30 y)	Middle-aged Asthmatics (31–59 y)	Elderly Asthmatics (60–72 y)	Healthy Elderly (60–89 y)
n	136	385	172	16
TCC ($\times 10^6$ cells/g)	3.05 [4.75]	3.80 [6.70]**	5.65 [9.73]	4.30 [4.30]
Neutrophils (%)	27.0 [40.6]*	52.5 [45.4]*	72.9 [34.3]*	65.6 [21.3]**
Eosinophils (%)	0.8 [3.8]	0.8 [4.3]	1.0 [4.6]	0.7 [1.8]
Lymphocytes (%)	1.0 [2.0]*	0.7 [1.7]*	0.5 [1.1]*	0.9 [1.3]
Macrophages (%)	63.9 [43.2]*	36.7 [39.9]*	19.5 [24.0]*	30.8 [25.5]**
Neutrophils ($\times 10^6$ cells/g)	0.74 [2.24]*	1.64 [5.00]*	3.94 [8.14]*	2.75 [3.46]
Eosinophils ($\times 10^6$ cells/g)	0.02 [0.12]	0.04 [0.21]	0.04 [0.37]*	0.02 [0.12]
Lymphocytes ($\times 10^6$ cells/g)	0.03 [0.07]	0.02 [0.08]	0.03 [0.08]	0.03 [0.13]
Macrophages ($\times 10^6$ cells/g)	1.40 [2.05]	1.35 [1.84]	1.11 [1.63]*	1.39 [1.29]

Median [interquartile range]; TCC: Total cell count; *$p < 0.01$ vs the two other asthmatic groups; **$p < 0.0001$ vs the young asthmatic group.

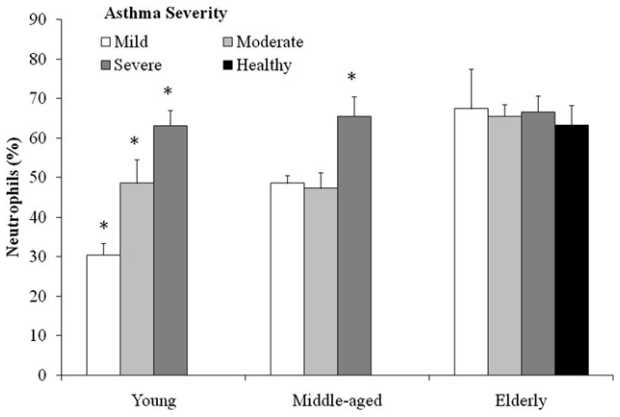

Figure 1 Neutrophil percentages according to asthma severity in young (18—30 y), middle-aged (31—59 y), and elderly (60—72 y) asthmatic and healthy (60—89 y) subjects. Mean ± SEM, $*p < 0.05$ vs the two other severity categories.

the asthma phenotype, particularly in regard to airway obstruction and inflammation. FEV_1, FVC, and FEV_1/FVC ratio were significantly lower in the elderly group compared with the middle-aged and the young asthmatic groups. An increase in neutrophils and a decrease in macrophages and lymphocytes were observed in the induced sputum of elderly asthmatics.

Our findings are consistent with previous studies showing a decline in FEV_1 with aging, in asthmatics. Lange et al.[4] published a 15-year follow-up study on lung function in adults with asthma and observed a faster deterioration of FEV_1 in asthmatic than in healthy subjects. Furthermore, Cassino et al.[15] showed that in non-smoking elderly asthmatics, FEV_1 was significantly lower in long-standing asthma compared with late-onset asthma, confirming a link between pulmonary function decline and disease duration, this decline in FEV_1 increasing with age. Such accelerated decline of respiratory function may be related to the possibility that asthma is diagnosed when the illness is already severe, due to the previously described reduction in symptoms perception in elderly asthmatic patients.[6] It may also be due to suboptimal treatment or management.[6] No data on asthma duration was available in our study so we could not assess this parameter.

It has been suggested that neutrophilic airway inflammation plays a role in the progression of persistent airflow limitation in adult asthma.[16] Neutrophils were significantly higher in the airways of the elderly group, and a neutrophilic phenotype was more frequently observed in these subjects than in the younger ones. These results suggest a change in the inflammatory cells phenotype in aging asthma. A recent report by Nyenhuis et al. on a small number of subjects also showed that neutrophil percentages were higher in the elderly compared with young subjects.[17] This study, however, did not include a healthy

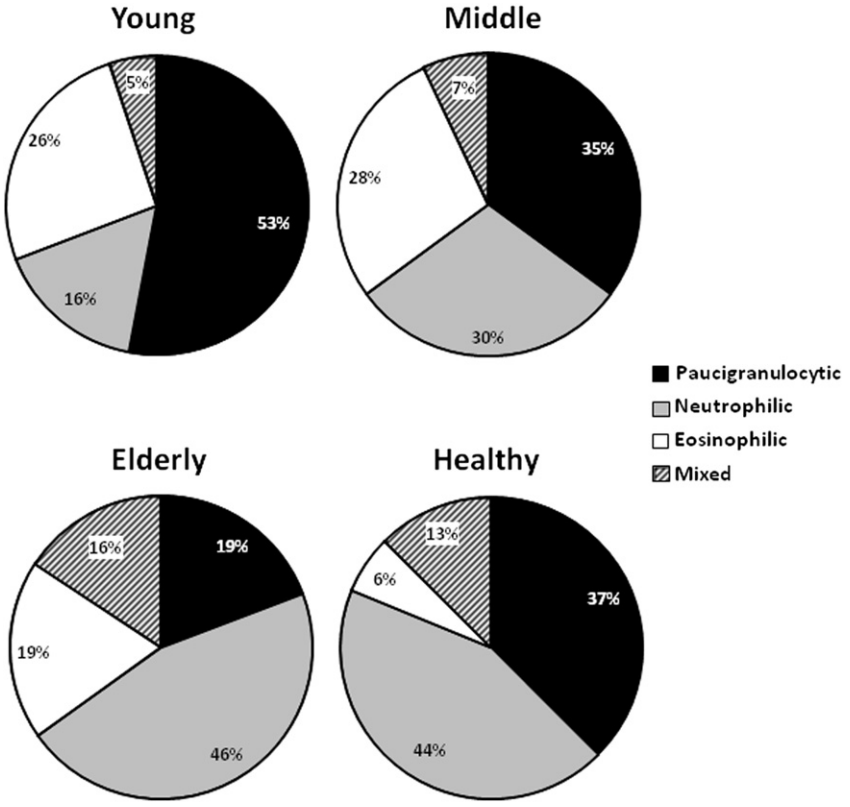

Figure 2 Inflammatory phenotypes of each group. Paucigranulocytic: Eosinophils < 2.2% and Neutrophils < 64.4%; Neutrophilic: Eosinophils < 2.2% and Neutrophils > 64.4%; Eosinophilic: Eosinophils > 2.2% and Neutrophils < 64.4%; Mixed: Eosinophils > 2.2% and Neutrophils > 64.4%; $p < 0.0001$.

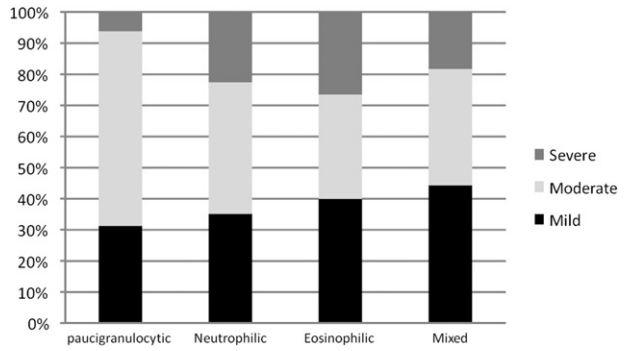

Figure 3 Inflammatory phenotypes according to asthma severity in the elderly group. $p > 0.05$.

control group. Vignola et al.[18] evaluated induced sputum samples from elderly asthmatic subjects and found that the number of neutrophils significantly correlated with the levels of both active and total elastase, which were both higher than in control non-asthmatic subjects. Neutrophils release oxygen free radicals, cytokines, and a wide variety of enzymes, including elastase, which may affect bronchial epithelium by degrading elastin and other extracellular matrix proteins.[19] This neutrophilic inflammation could cause airway damage and amplify the decrease of lung function in elderly asthmatics.

Thomas et al.[20] showed that sputum neutrophil counts increase with age in normal healthy adults. Our sub-analysis on 16 healthy elderly subjects showed an elevated percentage of neutrophils while pulmonary function remained normal. Neutrophil percentages and numbers were not statistically different than elderly asthmatic subjects. These findings suggest that high neutrophil levels may not be specific to asthmatic patients. The effect of age is therefore important for the interpretation of sputum differential cell counts in older subjects.

When considering the severity of asthma, the correlation between age and neutrophil counts was stronger for mild, lesser for moderate, and nonexistent in severe asthmatics. A regression analysis showed that both age and ICS were independent predictors of neutrophil percentage in all asthmatic subjects. This suggests that the neutrophilia observed can be influenced by age, but also by the ICS dose. This is in keeping with a recent study by Cowan and coworkers showing that ICS intake contributes to increased neutrophilia in asthmatic subjects.[21] Intake of inhaled corticosteroids may indeed have influenced the results and induced neutrophilia, particularly if taken at high doses, possibly by preventing neutrophil apoptosis.[22]

One of the key observations of this analysis is indeed the fact that neutrophilia increased with age and is similar in asthma vs controls in the elderly, while in younger patients, only severe asthmatics had elevated neutrophils. High doses of ICS may also induce neutrophilia. There was no relationship between severity of asthma and neutrophilia in the elderly, contrarily to younger asthmatic patients. Having said that, this does not preclude the possibility that neutrophilia does alter treatment response and clinical outcomes in elderly asthmatics. That feature has also been observed in the smoking population, where they are more resistant to treatment and have increased asthma-related morbidity. However, the results of this current study cannot provide answers in regard to the clinical outcomes of these patients. Another study would be needed to look at the possibility that such change in airway inflammation phenotype can influence diagnosis or change the clinical response to treatment and accelerate the decline in pulmonary function.

The response to asthma treatment is possibly reduced in elderly patients due to the alteration of the lung structure, including a more fixed component of airway obstruction and reduced lung elastic recoil.[23,24] It is also possible that it could be related to a different type of inflammation present in the airways of elderly patients. The presence of a neutrophilic inflammation may explain why patients are less responsive to corticosteroids. However, in our study, eosinophil percentages were similar between age groups, suggesting that elderly asthmatics also have a target on which ICS can act.

There are some intrinsic limitations that need to be acknowledged regarding the present study. Those include the retrospective analysis, the possibility (although unlikely) that some patients may have had a component of COPD, and the lack of data on complete smoking history for some patients. In that case, tobacco exposure was inferred from the presence of smoker's inclusions in macrophages, which can be considered reliable.[11] Further, the measurements represent a snapshot profile of bronchitis which can change over time in an individual patient.[25] Thus, a prospective longitudinal study is necessary to observe if persistent neutrophilic bronchitis can lead to progressive airflow limitation. Asthma and COPD are both components of obstructive airway diseases.[26] It is likely that persistent untreated neutrophilic bronchitis may have contributed to the chronic airflow limitation in elderly asthmatics.

Table 3 Regression analysis. Neutrophil percentages (dependant variable) vs age and ICS dose (independant variables) in all subjects.

Variable	Coefficient	Standard error	Standard coefficient	*t*-value	*p*-value
Intercept	22.495	4.020	22.495	5.595	<0.0001
Age	0.581	0.079	0.298	7.346	<0.0001
ICS dose	0.007	0.002	0.122	3.007	0.0028

ICS: Inhaled corticosteroids; $R^2 = 0.12$.

Conclusion

Our data suggest that elderly asthmatic subjects have a more severe airway obstruction and airway neutrophilia than younger patients. This neutrophilia could be attributed in part to aging and ICS intake, although the effect of age is more dominant than the dose of ICS in elderly subjects. More studies are needed to better characterize these patients in order to determine the best asthma management plan in this population.

Conflict of interest

None declared.

Acknowledgments

We are grateful to Serge Simard for the statistical analysis of data. M.E.D. holds a studentship from the FRSQ Respiratory Health Network. This work was supported by local funds.

Abbreviations

BD	bronchodilator
COPD	chronic obstructive pulmonary disease
FEV_1	forced expiratory volume in 1 s
FVC	forced vital capacity
ICS	inhaled corticosteroids

References

1. Gibson PG, McDonald VM, Marks GB. Asthma in older adults. *Lancet* 2010;**376**:803—13.
2. Braman SS. Asthma in the elderly. *Clin Geriatr Med* 2003;**19**: 57—75.
3. Krishman JA, Davis SQ, Charbeneau JT, Holguin F, Naureckas ET. Hospitalizations for asthma in the elderly in the U.S. in 2000 and 2005. *Am J Respir Crit Care Med* 2008;**177**: A667. Ref Type: Generic.
4. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;**339**:1194—200.
5. Marks GB, Yates DH, Sist M, Ceyhan B, De CM, Scott DM, Barnes PJ. Respiratory sensation during bronchial challenge testing with methacholine, sodium metabisulphite, and adenosine monophosphate. *Thorax* 1996;**51**:793—8.
6. Connolly MJ, Crowley JJ, Charan NB, Nielson CP, Vestal RE. Reduced subjective awareness of bronchoconstriction provoked by methacholine in elderly asthmatic and normal subjects as measured on a simple awareness scale. *Thorax* 1992;**47**:410—3.
7. Marsh SE, Travers J, Weatherall M, Williams MV, Aldington S, Shirtcliffe PM, Hansell AL, Nowitz MR, McNaughton AA, Soriano JB, Beasley RW. Proportional classifications of COPD phenotypes. *Thorax* 2008;**63**:761—7.
8. Parameswaran K, Hildreth AJ, Chadha D, Keaney NP, Taylor IK, Bansal SK. Asthma in the elderly: underperceived, underdiagnosed and undertreated; a community survey. *Respir Med* 1998;**92**:573—7.
9. Cho YJ, Lee KE. Decreased glucocorticoid binding affinity to glucocorticoid receptor is important in the poor response to steroid therapy of older-aged patients with severe bronchial asthma. *Allergy Asthma Proc* 2003;**24**:353—8.
10. Canadian Asthma Consensus Report Boulet LP, Becker A, Berube D, Beveridge R, Ernst P. Canadian asthma consensus group. *Can Med Assoc J* 1999;**161**:S1—61.
11. Brody AR, Craighead JE. Cytoplasmic inclusions in pulmonary macrophages of cigarette smokers. *Lab Invest* 1975;**32**:125—32.
12. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, Gleich GJ, Dolovich J, Hargreave FE. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 1996;**154**:308—17.
13. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000;**161**:475—8.
14. Spanevello A, Confalonieri M, Sulotto F, Romano F, Balzano G, Migliori GB, Bianchi A, Michetti G. Induced sputum cellularity. Reference values and distribution in normal volunteers. *Am J Respir Crit Care Med* 2000;**162**:1172—4.
15. Cassino C, Berger KI, Goldring RM, Norman RG, Kammerman S, Ciotoli C, Reibman J. Duration of asthma and physiologic outcomes in elderly nonsmokers. *Am J Respir Crit Care Med* 2000;**162**:1423—8.
16. Shaw DE, Berry MA, Hargadon B, McKenna S, Shelley MJ, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. *Chest* 2007;**132**:1871—5.
17. Nyenhuis SM, Schwantes EA, Evans MD, Mathur SK. Airway neutrophil inflammatory phenotype in older subjects with asthma. *J Allergy Clin Immunol*; 2010.
18. Vignola AM, Scichilone N, Bousquet J, Bonsignore G, Bellia V. Aging and asthma: pathophysiological mechanisms. *Allergy* 2003;**58**:165—75.
19. Lonky SA, McCarren J. Neutrophil enzymes in the lung: regulation of neutrophil elastase. *Am Rev Respir Dis* 1983;**127**: S9—15.
20. Thomas RA, Green RH, Brightling CE, Birring SS, Parker D, Wardlaw AJ, Pavord ID. The influence of age on induced sputum differential cell counts in normal subjects. *Chest* 2004; **126**:1811—4.
21. Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax* 2010;**65**:384—90.
22. Zhang X, Moilanen E, Kankaanranta H. Beclomethasone, budesonide and fluticasone propionate inhibit human neutrophil apoptosis. *Eur J Pharmacol* 2001;**431**:365—71.
23. Verbeken EK, Cauberghs M, Mertens I, Clement J, Lauweryns JM, van de Woestijne KP. The senile lung. Comparison with normal and emphysematous lungs. 2. Functional aspects. *Chest* 1992;**101**:800—9.
24. Verbeken EK, Cauberghs M, Mertens I, Clement J, Lauweryns JM, van de Woestijne KP. The senile lung. Comparison with normal and emphysematous lungs. 1. Structural aspects. *Chest* 1992;**101**:793—9.
25. Al-Samri MT, Benedetti A, Prefontaine D, Olivenstein R, Lemiere C, Nair P, Martin JG, Hamid Q. Variability of sputum inflammatory cells in asthmatic patients receiving corticosteroid therapy: a prospective study using multiple samples. *J Allergy Clin Immunol* 2010;**125**:1161—3.
26. Hargreave FE, Parameswaran K. Asthma, COPD and bronchitis are just components of airway disease. *Eur Respir J* 2006;**28**: 264—7.