

Role of Small Airways in Asthma



Lindsay K. Finkas, MD^{a,*}, Richard Martin, MD^b

KEYWORDS

• Small airways • Extrafine particle inhalers • Asthma • Nocturnal asthma

KEY POINTS

- The small airways are distal airways that measure less than 2 mm in luminal diameter.
- Inflammation and increased peripheral airway resistance have been demonstrated in the small airways of patients with asthma.
- The development of extrafine particle inhalers has improved drug delivery to the small airways.

INTRODUCTION

Asthma is a common chronic condition characterized by reversible airflow limitation. Prevalence has increased in the United States, affecting approximately 8% of the population and can be associated with high morbidity and health care costs.¹ Asthma is a heterogeneous disease affecting both small and large airways. In the past, much of the focus in asthma has been on the larger airways given the difficulty associated with measuring the small airways. The small airways, as a result, are frequently ignored, though remain an important aspect of asthma. Recently, there has been increased work in elucidating the contribution of the peripheral airways in asthma, more specifically severe asthma.²

The distal (small) airways are defined as those distal to the seventh or eighth division of the tracheobronchial tree and measure less than 2 mm in luminal diameter.³ The distal airways represent most of the total surface area of the airways and play an important role in the pathophysiology of asthma.⁴ The alveolar tissue area, in addition to the airways, also contributes to distal airway inflammation.⁵

Studies have been undertaken to evaluate inflammation and the physiology of the distal airway as well as develop clinical parameters that may be used to measure the small airways. New inhalers and drug formulations with improved corticosteroids

^a Division of Allergy and Clinical Immunology, Department of Medicine, National Jewish Health, 1400 Jackson Street, Denver, CO 80206, USA; ^b Department of Medicine, National Jewish Health, 1400 Jackson Street, Denver, CO 80206, USA

* Corresponding author.

E-mail address: FinkasL@NJHealth.org

deposition are able to reach the small airways that are an area that larger-particle corticosteroid inhalers have been unable to target in significant concentration.⁶

Nocturnal asthma (NA) can be used as a model for studying the small airways and is used in several studies. NA is common in individuals with asthma and affects approximately 30% to 75% of individuals with asthma.⁷ NA is a risk factor for mortality (70% association) and respiratory arrests (80% association).⁸ In asthma, the peak lung function achieved is seen at 4:00 PM with a nadir at 4:00 AM.⁹ For NA, this circadian change is markedly increased. In addition, bronchial hyperreactivity increases at night as well. These changes are felt to be related to circadian changes that occur in multiple factors, including cortisol levels.¹⁰

INFLAMMATION

Histologic evaluation of autopsy specimens from patients with asthma has allowed airway inflammation to be evaluated in both large and small airways. Carrol and colleagues¹¹ demonstrated that lymphocytes and eosinophils were present in both the proximal and distal airway in patients with asthma. Further studies have demonstrated that regional variations among the small airways and large airways exist as well. Haley and colleagues¹² found that the proximal airways had a significantly greater density of CD45-positive lymphocytes in the subbasement membrane and airway smooth muscle than the same areas in small airways. The small airways were noted to have CD45-positive lymphocytes and eosinophils in the area between airway smooth muscle and alveolar attachments.

Subjects with cystic fibrosis were also studied and they showed a similar pattern of proximal airway inflammation as subjects with asthma; however, the inflammation pattern in the small airways was not present. This suggests that small airway inflammation is not a characteristic of all obstructive airway disease and may be a specific finding seen in asthma.

A study in stable patients with chronic asthma in 1996 by Kraft and colleagues¹³ evaluated 21 subjects with NA and 10 subjects with non-nocturnal asthma (NNA). Subjects underwent transbronchial and endobronchial biopsy at 4:00 PM and 4:00 AM. There were significant differences in the number of eosinophils in the alveolar tissue area from the 4:00 AM sample with increased eosinophils seen in the NA group. These findings were not seen in the biopsies from the proximal airway. In comparison of the 4:00 PM and 4:00 AM samples in the NA group, there were a significantly greater number of eosinophils at 4:00 AM. Similar findings were present for macrophages as well with increased values seen at 4:00 AM in the NA group. Circadian differences were not seen in the large airways.¹³ An example of the inflammation in the small airways is shown in [Fig. 1](#).¹⁴

CD4 T lymphocytes produce inflammatory cytokines including tumor necrosis factor, interleukin (IL)-4, IL-5, and IL-13 that are important mediators in eosinophil migration. Kraft and colleagues¹⁵ sought to evaluate whether T lymphocytes were responsible for eosinophil migration in the distal airways in patients with NA. They found the number of CD4+ T lymphocytes in alveolar tissue at 4:00 AM was greater than that seen in subjects with NNA. In addition, they found that the number of alveolar CD4+ lymphocytes in NA positively correlated with the number of EG2+ eosinophils and inversely with FEV1 at 4:00 AM. Interestingly, proximal tissue inflammation of the larger airways did not have a significant correlation.¹⁵

Mediator release was also studied by Schulman and colleagues.¹⁶ Human lung parenchyma tissue mediator release was measured and compared with airway tissue in a ragweed-challenged *in vitro* study. The model allowed investigators to compare if bronchus or parenchyma had greater mediator release following allergen challenge.

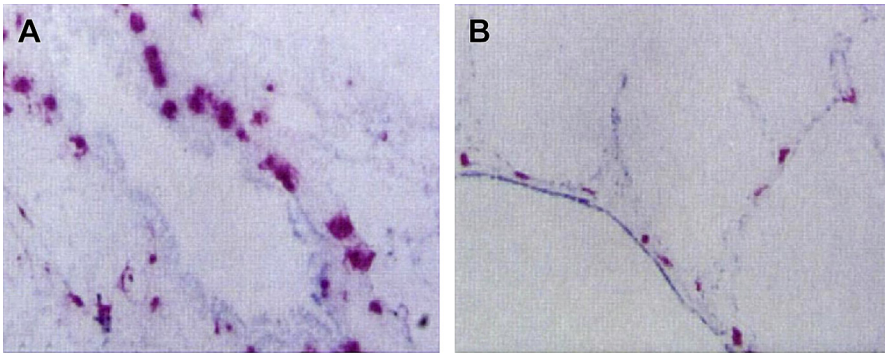


Fig. 1. Representative distal airway inflammation. (A) Small airway less than 2 mm in an subject with asthma with EG2-positive cells (activated eosinophils). (B) Major basic protein-stained lung parenchyma from a subject with asthma. (From Hamid Q, Song Y, Kotsimbos TC, et al. Inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997;100(1):46; with permission.)

They found twofold to threefold greater concentrations of prostaglandins and thromboxane B₂ in the lung parenchyma compared with the airway. Histamine was also measured and there was a fivefold greater concentration in the lung parenchyma than in the larger airways. These findings support differences in inflammatory responses in different airway tissue.

Conventional computed tomography (CT) and chest radiographic imaging have been unable to measure the distal airways. With the development of high-resolution CT (HRCT), there is now the ability to measure the small airway with diameter of 1.5 to 2.0 mm as well as wall thickness as small as 0.25 mm.¹⁷ Okazawa and colleagues¹⁸ used HRCT to evaluate airway wall thickness as well as the site and magnitude of narrowing of the airway in response to methacholine in subjects with asthma and healthy controls. In the subjects with asthma, the small airways were thickened compared with healthy controls. The small airways also showed a significantly decreased luminal area following methacholine inhalation. Goldin and colleagues¹⁹ also used HRCT, and found that methacholine induced bronchoconstriction in up to 95% of airways with diameter between 1.6 and 2.5 mm.

PHYSIOLOGY

Wagner and colleagues²⁰ improved our understanding of physiology of the small airways by using a peripheral airway resistance technique. With this technique, a bronchoscope is wedged into the airway and airflow rate is progressively increased via one port and pressure change is measured in the second port. Peripheral airway resistance (R_p) is then calculated dividing the change in pressure by the change in flow ($\Delta\text{Pressure}/\Delta\text{flow}$). They used this method to compare healthy controls with individuals with mild asthma who had similar lung function. They found significantly increased peripheral airway resistance in the subjects with asthma (Fig. 2).²⁰

A study by Kraft and colleagues²¹ subsequently evaluated peripheral airway resistance in NA, NNA, and healthy controls. In addition, they assessed peripheral function to B-2 agonist by administering subcutaneous terbutaline in all groups. All subjects underwent bronchoscopy with R_p at 4:00 PM and 4:00 AM separated by 1 week. Peripheral airway resistance was measured via wedge bronchoscope as discussed previously. The peripheral airway resistance was highest in the NA group at both 4:00 PM and 4:00 AM compared with both NNA and control groups; however, there

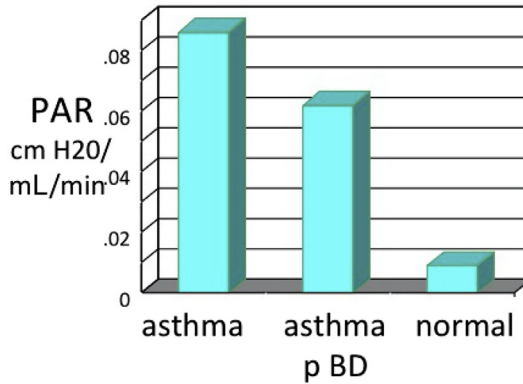


Fig. 2. Measurement of peripheral airways resistance: wedged bronchoscopic technique. pBD, post bronchodilator; PAR, peripheral airway resistance. (Data from Wagner EM, Liu MC, Weinmann GG, et al. Peripheral lung resistance in normal and asthmatic subjects. *Am Rev Respir Dis* 1990;141(3):584–8.)

was no significant difference found in R_p within the NA group at both times. Plateau pressures were noted to significantly increase from 4:00 PM to 4:00 AM in the NA group only. Closing pressures were found to be decreased in the NA group compared with NNA and control groups at both times. Following beta agonist treatment, R_p decreased in both asthma groups, although was significant only at 4:00 AM. The increase in plateau pressure in the NA group from 4:00 AM to 4:00 PM is suggestive of a loss of collateral channels and this may be due to smooth muscle contraction, inflammation, and/or edema.²¹

As direct measurement of peripheral airway resistance is invasive, surrogate markers to measure the small airways is desirable and multiple modalities and testing have been investigated. Spirometry is the most common test used to evaluate and monitor asthma. It is a simple, noninvasive method. Reproducibility on testing is important, as measurements such as the forced vital capacity (FVC) are highly effort dependent.²² The forced expiratory volume in 1 second (FEV1) and FVC are reflective of the proximal airways, whereas the forced expiratory flow 25% to 75% of the forced vital capacity (FEF25%–75%) has long been purposed as a measurement of the small airways. It is highly dependent on the validity of the FVC measurement and the level of expiratory effort.²³

A pediatric study by Rao and colleagues²⁴ found a low FEF 25% to 75% in the setting of normal FEV1 was associated with increased asthma severity. The FEF 25% to 75%, however, has shown poor reproducibility and comparability if not adjusted for lung volumes. The FEF 25% to 75% is also often normal when the FEV1/FVC ratio is greater than 75%.²⁵

Closing volume (CV) is defined as the volume of gas remaining in the vital capacity (VC) at the intersection of phases III and IV. It is typically expressed as a percentage of the VC (CV/VC%). Higher CV has been attributed to earlier closing of the distal airways. In a pediatric study by In't Veen and colleagues,²⁶ CV was found to be elevated in the severe asthma group with recurrent exacerbations. This suggests that recurrent exacerbations may be related to the distal airway.

A study by Sutherland and Martin²⁷ looked at the relationship between physiology and distal lung inflammation by comparing transbronchial biopsy, spirometry, and plethysmography in patients with asthma. Distal lung tissue inflammation at

4:00 AM poorly correlated with previously suggested measurements of small airways FEF 25% to 75% and maximal to partial VC maneuver (M:P ratio). The distal lung tissue eosinophils at 4:00 AM, however, did correlate with both the thoracic gas volume and total lung capacity. This study suggests that FEF 25% to 75% and M:P ratio may not be reflective of distal airway inflammation and may not be the best reliable measurements of the inflammation in distal airways. Thoracic gas volume and total lung capacity may be better measurements of distal airway inflammation, as they correlate with distal airway eosinophil count.²⁷

Impulse oscillometry (IOS) is another noninvasive objective measurement of lung function and particular values are associated with the small airways. IOS is a tool that is of benefit, as it is independent of effort and requires minimal patient cooperation. The R5 is a measurement that includes both small and large airways, whereas the R20 reflects the large airways only. Therefore, the difference between the R5 and R20 ($R5 - R20$) is reflective of the small airways only. Reactance area (AX) may also be used as a measurement of small airways and is the reactance at all frequencies between 5 Hz and the frequency at which the reactance is 0. In a study in children with asthma as well as controls, there was a significant difference in the $R5 - R20$, as was reactance in subjects with poorly controlled asthma versus those with controlled asthma and healthy controls.²⁸ Additionally, a study by Takeda and colleagues²⁹ used IOS in subjects to assess associations with health status, dyspnea, and asthma control. They found the R20 as well the $R5 - R20$ to be independently associated with lower health status as well as dyspnea. The AX was associated with loss of asthma control.²⁹

OTHER MODALITIES

Exhaled nitric oxide (NO) is a newer, safe, noninvasive measurement of airway inflammation that is standardized. As NO is produced throughout the airway, it is recommended to obtain the measurement during exhalation against positive pressure to close the velum, and therefore exclude the upper airway contributions.³⁰ NO is produced by the reaction catalyzed by NO synthase. The inducible form of the enzyme is increased by proinflammatory mediators.²² Fraction of exhaled nitric oxide measurements are flow dependent and require a constant expiratory flow and measurements obtained at a single flow rate are primarily reflective of the proximal airway.³¹

Two compartment models of pulmonary NO productions can be used to calculate the alveolar production of exhaled NO concentration.³² Alveolar NO was calculated using a nonlinear model. Elevated alveolar NO can be seen in other conditions involving distal airway inflammation, such as pulmonary fibrosis and chronic obstructive pulmonary disease. A study from the United Kingdom compared subjects with refractory asthma, mild-to-moderate asthma, and healthy controls. They found that alveolar NO concentration was reproducible in subjects with refractory asthma. The study also showed there was also a positive correlation between alveolar NO concentration and bronchoalveolar lavage eosinophil count, but a similar association was not seen in bronchial wash or sputum eosinophil count. In addition, alveolar NO was found to be higher in subjects with refractory asthma as compared with mild-to-moderate asthma and healthy controls and values decreased following treatment with prednisolone. Alveolar NO, however, was not decreased following a doubling dose of inhaled steroids. This suggests that alveolar NO is a measurement of the distal lung and that refractory asthma is associated with increased distal airway inflammation.³³

Lehtimäki and colleagues³⁴ found that subjects with asthma with nocturnal symptoms had higher levels of alveolar NO compared with both patients with asthma

without nocturnal symptoms and healthy controls. This again suggests the role of the small airways in NA.

Nitrogen breath washout has also been proposed as a noninvasive measurement of the peripheral airways. By using a slope III analysis of multiple washout versus single washout, the ability to distinguish between the conductive airways (proximal airways) from acinar (distal airways) is achieved.³⁵

THERAPEUTICS

Inhaled corticosteroids (ICS) are the mainstay of treatment for persistent asthma in both pediatric and adult populations. Inhaled drug delivery to the distal lung is dependent on numerous factors, with the most important being drug particle size. Formulation and preparation of the drug, patient technique, as well as the device in which the drug is delivered can also play a role in deposition in the distal airway. Studies have shown that the ideal particle size for drug delivery to the distal airway is a mass median aerodynamic diameter between 1 μm and 2 μm. Particle size less than 1 μm tends to be inhaled and exhaled without deposition. Particle size between 2 and 5 μm is deposited mainly in the medium-sized and large-sized airways. Particle size greater than 5 μm is associated with deposition in the oropharynx as well as in the larger, central airways.³⁶

Particle size and drug efficacy have been well studied in inhaled bronchodilators. Improvements in FEF 25% to 75% have been shown with smaller particle size inhaled isoproterenol 2.5 μm compared with 5.0 μm. Studies looking at varying particle size with inhaled ipratropium bromide demonstrated peak bronchodilation was achieved with smaller particle size. This improvement is felt to be due to improved deposition into the small airways.³⁷

In 1987, the Montreal Protocol mandated elimination of substances that deplete the ozone layer and included chlorofluorocarbons (CFCs) that were used as propellants in pressurized metered-dose inhalers (MDIs).³⁸ This led to the development of alternative forms of propellants, including hydrofluoroalkane (HFAs) that are currently used. With the development of HFAs, there were also changes in drug formulation. For beclomethasone dipropionate (BDP), this meant reformulation from a suspension to a solution. This change in formulation resulted in a change in particle size as well as a decrease in speed in which the drug leaves the MDI, both positively effecting drug delivery.³⁹ In addition to HFAs, breath-actuated dry powder inhalers (DPIs) have also been developed and are increasing in use. Particle sizes in DPIs are in some cases larger than previously used in MDIs with CFCs.⁴⁰ See Table 1 for selected ICS and particle size.

Table 1 Particle size of select inhaled corticosteroids	
Drug	Particle Size (MMAD), μm
Beclomethasone-HFA ^a	1.1
Beclomethasone-CFC ^b	3.5
Ciclesonide-HFA ^a	1.1
Flunisolide-HFA ^a	1.2
Flunisolide-CFC ^b	3.8
Fluticasone-DPI	5.4
Fluticasone-CFC ^b	2.4

Abbreviations: CFC, chlorofluorocarbon; DPI, dry powder inhaler; HFA, hydrofluoroalkane; MMAD, mass median aerodynamic diameter.
^a Indicates extrafine particle inhalers.
^b No longer available.

The smaller the particle size of the ICS, the greater the distal airway deposition and possibly, improved efficacy. CFC and DPI inhalers typically have larger particle sizes and, as a result, only approximately 5% to 30% of the drug is delivered to the lungs. The remainder of the drug is deposited in the oropharynx. The change in BDP to solution form with HFA resulted in a reduced mass median aerodynamic diameter to 1.1 μm from 3.5 μm .⁴¹

To evaluate drug delivery and deposition in the airway and relation to particle size, Leach and colleagues⁴² used technetium-99m-radiolabeled BDP. Beclomethasone was then delivered via CFC suspension or with HFA solution. There was a significant difference between airway deposition between the 2 products, with a significantly increased airway deposition associated with the BDP-HFA. In healthy subjects, BDP-HFA delivered 55% to 60% of the drug to the airways with 29% to 30% deposited in the oropharynx compared with the CFC, in which 4% to 7% of the drug was delivered to the lung with the remaining 90% to 94% having oropharyngeal deposition. Similar findings were also seen in the subjects with asthma. The pattern of delivery also differed between the 2 formulations. CFC-BDP had more central distribution as opposed to the HFA-BDP that had more diffuse delivery, in which the investigators concluded as improved delivery to the distal airways.

A randomized, placebo-controlled trial in 1999 by Gross and colleagues⁴³ compared HFA-BDP 400 $\mu\text{g}/\text{d}$, CFC-BDP 800 $\mu\text{g}/\text{d}$ and placebo for 12 weeks. Peak expiratory flow rates were used as an outcome and HFA-BDP was shown to be equivalent to CFC-BDP. The investigators concluded that there was similar efficacy at half the dose with the HFA-BDP due to peripheral lung deposition.

Flunisolide has also been studied and compared in HFA versus CFC formulations. Corren and colleagues⁴⁴ studied varying dosing of HFA flunisolide and CFC flunisolide in asthmatic subjects. FEV1 was used as the primary endpoint and similar improvement in FEV1 was achieved in flunisolide HFA at one-third the dose compared with the CFC flunisolide-treated group.

Particle size has also been studied radiographically by following air trapping on HRCT. Goldin and colleagues⁴⁵ randomized subjects to HFA-BDP and CFC-DBP. Pre-HRCT and post-HRCT performed at residual volume before and after methacholine challenge. Following 4 weeks of treatment, subjects in the HFA-BDP group had greater improvement in regional airttrapping than those in the CFC-BDP group.

Increased dose of small-particle ICS as well as standard-size ICS as compared with the addition of long-acting beta agonists (LABA) has also been studied retrospectively by Israel and colleagues.⁴⁶ Increased dose of small and standard particle ICS was as effective as adding LABA as evaluated by asthma control and rates of severe exacerbations over 1 year.

In a retrospective matched cohort study, the cost-effectiveness of extrafine particle ICS as compared with standard-size particle ICS for both the United States and the United Kingdom in patients older than 12 demonstrated that the odds of overall control were greater in the extrafine ICS cohorts in both countries. Cost related to asthma was also significantly lower with the extrafine ICS cohorts in both countries.⁴⁷

SUMMARY

Asthma is an obstructive airway disease that involves both large and small airways. Studies evaluating the small airways have shown increased inflammation as well as increased peripheral airway resistance in individuals with asthma. Additional studies are needed to correlate airway inflammation and resistance with surrogate markers that may readily provide information about the distal airways and assist in the

evaluation of patients with asthma. With the development of new inhalers and drug formulations, there is an increased ability to deliver drugs to the distal airway that may improve asthma control and reduce exacerbations.

REFERENCES

1. Akinbami LJ, Bailey C, Zahran HS, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. *NCHS Data Brief* 2012;(94):1–8.
2. van der Wiel E, ten Hacken NH, Postma DS, et al. Small-airways dysfunction associates with respiratory symptoms and clinical features of asthma: a systematic review. *J Allergy Clin Immunol* 2013;131(3):646–57.
3. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. *N Engl J Med* 1968;278(25):1355–60.
4. Tulic MK, Christodoulouopoulos P, Hamid Q. Small airway inflammation in asthma. *Respir Res* 2001;2(6):333–9.
5. Martin RJ. Exploring the distal lung: new direction in asthma. *Isr Med Assoc J* 2008;10(12):846–9.
6. Trikha AM, Martin RJ. Small airways in asthma. *Curr Respir Care Rep* 2013;2:226–32.
7. Turner-Warwick M. Epidemiology of nocturnal asthma. *Am J Med* 1988;85(1B):6–8.
8. Hetzel MR, Clark TJ, Branthwaite MA. Asthma: analysis of sudden deaths and ventilatory arrests in hospital. *Br Med J* 1977;1(6064):808–11.
9. Hetzel MR, Clark TJ. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. *Thorax* 1980;35(10):732–8.
10. Martin RJ. Small airway and alveolar tissue changes in nocturnal asthma. *Am J Respir Crit Care Med* 1998;157(5 Pt 2):S188–90.
11. Carroll N, Elliot J, Morton A, et al. The structure of large and small airways in nonfatal and fatal asthma. *Am Rev Respir Dis* 1993;147(2):405–10.
12. Haley KJ, Sunday ME, Wiggs BR, et al. Inflammatory cell distribution within and along asthmatic airways. *Am J Respir Crit Care Med* 1998;158(2):565–72.
13. Kraft M, Djukanovic R, Wilson S, et al. Alveolar tissue inflammation in asthma. *Am J Respir Crit Care Med* 1996;154(5):1505–10.
14. Hamid Q, Song Y, Kotsimbos TC, et al. Inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997;100(1):44–51.
15. Kraft M, Martin RJ, Wilson S, et al. Lymphocyte and eosinophil influx into alveolar tissue in nocturnal asthma. *Am J Respir Crit Care Med* 1999;159(1):228–34.
16. Schulman ES, Adkinson NF Jr, Newball HH. Cyclooxygenase metabolites in human lung anaphylaxis: airway vs. parenchyma. *J Appl Physiol Respir Environ Exerc Physiol* 1982;53(3):589–95.
17. King GG, Muller NL, Pare PD. Evaluation of airways in obstructive pulmonary disease using high-resolution computed tomography. *Am J Respir Crit Care Med* 1999;159(3):992–1004.
18. Okazawa M, Muller N, McNamara AE, et al. Human airway narrowing measured using high resolution computed tomography. *Am J Respir Crit Care Med* 1996;154(5):1557–62.
19. Goldin JG, McNitt-Gray MF, Sorenson SM, et al. Airway hyperreactivity: assessment with helical thin-section CT. *Radiology* 1998;208(2):321–9.
20. Wagner EM, Liu MC, Weinmann GG, et al. Peripheral lung resistance in normal and asthmatic subjects. *Am Rev Respir Dis* 1990;141(3):584–8.
21. Kraft M, Pak J, Martin RJ, et al. Distal lung dysfunction at night in nocturnal asthma. *Am J Respir Crit Care Med* 2001;163(7):1551–6.

22. Liang BM, Lam DC, Feng YL. Clinical applications of lung function tests: a revisit. *Respirology* 2012;17(4):611–9.
23. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005;26(2):319–38.
24. Rao DR, Gaffin JM, Baxi SN, et al. The utility of forced expiratory flow between 25% and 75% of vital capacity in predicting childhood asthma morbidity and severity. *J Asthma* 2012;49(6):586–92.
25. van den Berge M, ten Hacken NH, Cohen J, et al. Small airway disease in asthma and COPD: clinical implications. *Chest* 2011;139(2):412–23.
26. 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):1902–6.
27. Sutherland ER, Martin RJ. Distal lung inflammation in asthma. *Ann Allergy Asthma Immunol* 2002;89(2):119–24 [quiz: 124–5, 211].
28. Shi Y, Aledia AS, Tatavoosian AV, et al. Relating small airways to asthma control by using impulse oscillometry in children. *J Allergy Clin Immunol* 2012;129(3):671–8.
29. Takeda T, Oga T, Niimi A, et al. Relationship between small airway function and health status, dyspnea and disease control in asthma. *Respiration* 2010;80(2):120–6.
30. Silkoff PE, McClean PA, Slutsky AS, et al. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. *Am J Respir Crit Care Med* 1997;155(1):260–7.
31. Linkosalo L, Lehtimäki L, Holm K, et al. Relation of bronchial and alveolar nitric oxide to exercise-induced bronchoconstriction in atopic children and adolescents. *Pediatr Allergy Immunol* 2012;23(4):360–6.
32. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* (1985) 1998;85(2):653–66.
33. Berry M, Hargadon B, Morgan A, et al. Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. *Eur Respir J* 2005;25(6):986–91.
34. Lehtimäki L, Kankaanranta H, Saarelainen S, et al. Increased alveolar nitric oxide concentration in asthmatic patients with nocturnal symptoms. *Eur Respir J* 2002;20(4):841–5.
35. Verbanck S, Schuermans D, Paiva M, et al. Nonreversible conductive airway ventilation heterogeneity in mild asthma. *J Appl Physiol* (1985) 2003;94(4):1380–6.
36. Sutherland ER, Martin RJ. Targeting the distal lung in asthma: do inhaled corticosteroids treat all areas of inflammation? *Treat Respir Med* 2005;4(4):223–9.
37. Zanen P, Go LT, Lammers JW. Optimal particle size for beta 2 agonist and anticholinergic aerosols in patients with severe airflow obstruction. *Thorax* 1996;51(10):977–80.
38. UNEP Environmental Effects Panel, United Nations Environment Programme. Environmental effects of ozone depletion: 1994 assessment: pursuant to article 6 of the Montreal Protocol on substances that deplete the ozone layer. Nairobi (Kenya): UNEP; 1994.
39. Anderson PJ. Delivery options and devices for aerosolized therapeutics. *Chest* 2001;120(Suppl 3):89S–93S.
40. Martin RJ. Therapeutic significance of distal airway inflammation in asthma. *J Allergy Clin Immunol* 2002;109(Suppl 2):S447–60.
41. Vanden Burgt JA, Busse WW, Martin RJ, et al. Efficacy and safety overview of a new inhaled corticosteroid, QVAR (hydrofluoroalkane-beclomethasone extrafine inhalation aerosol), in asthma. *J Allergy Clin Immunol* 2000;106(6):1209–26.

42. Leach CL, Davidson PJ, Boudreau RJ. Improved airway targeting with the CFC-free HFA-beclomethasone metered-dose inhaler compared with CFC-beclomethasone. *Eur Respir J* 1998;12(6):1346–53.
43. Gross G, Thompson PJ, Chervinsky P, et al. Hydrofluoroalkane-134a beclomethasone dipropionate, 400 microg, is as effective as chlorofluorocarbon beclomethasone dipropionate, 800 microg, for the treatment of moderate asthma. *Chest* 1999;115(2):343–51.
44. Corren J, Nelson H, Greos LS, et al. Effective control of asthma with hydrofluoroalkane flunisolide delivered as an extrafine aerosol in asthma patients. *Ann Allergy Asthma Immunol* 2001;87(5):405–11.
45. Goldin JG, Tashkin DP, Kleerup EC, et al. Comparative effects of hydrofluoroalkane and chlorofluorocarbon beclomethasone dipropionate inhalation on small airways: assessment with functional helical thin-section computed tomography. *J Allergy Clin Immunol* 1999;104(6):S258–67.
46. Israel E, Roche N, Martin RJ, et al. Increased dose of inhaled corticosteroid versus add-on long-acting beta-agonist for step-up therapy in asthma. *Ann Am Thorac Soc* 2015;12(6):798–806.
47. Martin RJ, Price D, Roche N, et al. Cost-effectiveness of initiating extrafine- or standard size-particle inhaled corticosteroid for asthma in two health-care systems: a retrospective matched cohort study. *NPJ Prim Care Respir Med* 2014; 24:14081.