

High-resolution computed tomography scan and airway remodeling in children with severe asthma

Jacques de Blic, MD,^a Isabelle Tillie-Leblond, MD, PhD,^b Sophie Emond, MD,^c Bruno Mahut, MD,^d Thanh Lan Dang Duy, MD,^a and Pierre Scheinmann, MD^a
Paris and Lille, France

Background: Children with severe asthma have a significantly higher bronchial wall thickness (BWT) on high-resolution computed tomography scan than control children.

Objective: We sought to determine whether a BWT score correlates with markers of airway remodeling and inflammation.

Methods: In 37 children with severe asthma, we determined reticular basement membrane thickness; number of intra-epithelial neutrophils and eosinophils on bronchial biopsy; IFN- γ , IL-4, IL-5, and eosinophil cationic protein levels and IFN- γ /IL-4 ratio on bronchoalveolar lavage specimen; and alveolar nitric oxide (NO) concentration and the maximum airway wall NO flux.

Results: The BWT score significantly correlated with reticular basement membrane thickening ($r = 0.34$; $P = .04$) and NO production by the airway wall ($r = 0.45$; $P = .02$). The correlation with the eosinophil cationic protein level was just significant ($r = 0.40$; $P = .05$), whereas there was no correlation with IFN- γ /IL-4 ratio ($r = -0.31$; $P = .08$). The BWT score did not correlate with FEV₁ or forced expiratory flow at 25% to 75% of forced vital capacity.

Conclusion: High-resolution computed tomography scan is a noninvasive technique that might be valuable for quantifying airway remodeling in children with severe asthma. The new generations of multislice computed tomography scanners will allow higher definition and lower radiation exposure and probably give a better assessment of airway remodeling and efficacy of treatment in children with asthma. (*J Allergy Clin Immunol* 2005;116:750-4.)

Key words: Severe asthma, nitrous oxide, HRCT scan, bronchial wall thickening, reticular basement membrane, remodeling, airway inflammation, ECP, IFN- γ , IL-4

Abbreviations used

ATS:	American Thoracic Society
BWT:	Bronchial wall thickening
Calv _{NO} :	Alveolar nitric oxide concentration
ECP:	Eosinophil cationic protein
eNO:	Exhaled nitric oxide
HRCT:	High-resolution computed tomography
J'aw _{NO} :	Maximum airway wall nitric oxide flux
NO:	Nitric oxide
RBM:	Reticular basement membrane

Airway inflammation and remodeling are 2 important mechanisms in the pathophysiology of asthma.¹ Evaluation of these mechanisms requires invasive techniques, in particular bronchoscopy with bronchoalveolar lavage and bronchial biopsy, although noninvasive techniques, including induced sputum, exhaled breathe condensate, and exhaled NO (eNO) measurement, have been developed. We recently reported that eNO was associated with both inflammation and remodeling variables, indicating that in some conditions, eNO reflects the 2 major features of the disease.² Moreover, evaluations of high-resolution computed tomography (HRCT) in adults have shown that abnormalities of the airways, and particularly the extent of bronchial wall thickening (BWT), correlate with lung functions, reticular basement membrane (RBM) thickness,³ and metalloproteinase 9/tissue inhibitor of metalloproteinase 1 production imbalance.⁴

Children with severe asthma represent a subgroup at high risk of developing an irreversible airflow obstruction. We recently showed that bronchial wall thickening (as a score) was significantly higher in children with severe asthma than in control children.⁵

Here we investigated whether the BWT correlates with markers of bronchial inflammation and remodeling in a group of children with severe asthma. These children belong to a cohort study in which patients are followed prospectively. Some of the data for this cohort have been published.^{2,6} We determined RBM thickness; number of intraepithelial neutrophils and eosinophils on bronchial biopsy; INF- γ , IL-4, IL-5, and eosinophil cationic protein (ECP) levels and IFN- γ /IL4 ratio on bronchoalveolar lavage specimen; and alveolar nitric oxide (NO) concentration (Calv_{NO}) and the maximum airway wall NO flux (J'aw_{NO}).

From ^aService de pneumologie et d'allergologie Pédiatriques, Hôpital Necker-Enfants Malades, Université René Descartes, Paris; ^bInstitut National de la Santé et de la Recherche Médicale U 416, Institut Pasteur de Lille; ^cService de Radiologie, Hôpital Necker-Enfants Malades, Paris; and ^dLaboratoire d'Exploration Fonctionnelle Respiratoire—Radio Isotope, Hôpital Européen Georges Pompidou, Paris.

Supported by a grant from GlaxoSmithKline, Paris.

Received for publication April 21, 2005; revised July 1, 2005; accepted for publication July 13, 2005.

Available online September 3, 2005.

Reprint requests: Professor Jacques de Blic, Service de Pneumologie et d'allergologie Pédiatriques, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75015 Paris, France. E-mail: j.deblic@nck.aphp.fr.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.07.009

METHODS

Thirty-seven children were prospectively included between July 2000 and January 2002. These children attended the clinic every 2 to 3 months for at least 2 years, and symptoms, bronchodilator use, exacerbations, and pulmonary function test results were regularly recorded. Asthma was diagnosed according to American Thoracic Society (ATS) criteria.⁷ All children had severe asthma in accordance with the ATS workshop recently reviewed by Wenzel.⁸ Severe asthma was defined as the presence of 1 major criterion—need for high-dose inhaled corticosteroids—and at least 2 of the following 5 minor criteria: (1) requirement for daily long-acting β -agonist or leukotriene antagonist in addition to inhaled corticosteroids, (2) asthma symptoms requiring short-acting β -agonist use on a daily or nearly daily basis, (3) persistent airway obstruction ($FEV_1 < 80\%$ predicted), (4) 1 or more emergency care visits for asthma per year, and (5) 3 or more oral steroid bursts per year. Eleven children had no airway obstruction but persistent symptoms (defined before the study as the use of more than 3 bronchodilator rescue treatments per week) and/or exacerbations defined as asthma attacks requiring systemic corticosteroids and/or hospitalization (more than 3 exacerbations in the past year). Twenty-six children had persistent airway obstruction with $FEV_1 < 80\%$ of predicted value whatever their clinical symptoms. All had a trial course of corticosteroids (2 mg/kg/d prednisolone for 14 days) that proved negative, defined as an increase in FEV_1 of no more than 15%.

The study was approved by the local ethical committee of Necker Enfants Malades Hospital. Written consent for participation was obtained from the parents and verbal assent from the children.

Bronchoscopy and endobronchial biopsies

All flexible bronchoscopy procedures were performed with a 4.9-mm external diameter bronchoscope (Olympus Winter IBE GMBH, Hamburg, Germany) under deep sedation. Bronchial biopsy samples were taken from segmental or subsegmental bronchi of the right or left lower lobes by using cupped forceps. Biopsy samples were fixed in 10% formaldehyde and embedded in paraffin blocks. They were stained with hematoxylin and eosin staining and May-Grünwald-Giemsa by standard methods. The numbers of inflammatory cells in the epithelium were assessed as previously described.⁶ Results are expressed as the number of cells per millimeter length of basement membrane.⁹ RBM thickness was determined as the average of 20 measurements in sections where the epithelium was oriented in such a way that measurement artifacts did not occur.¹⁰

Bronchoalveolar lavage was performed in the right middle lobe by using $3 \times 1 \text{ mL} \cdot \text{kg}^{-1}$ aliquots of normal saline.¹¹ Return fluid was collected into sterile polypropylene vials (Falcon 50 mL; Becton Dickinson, Rutherford, NJ). We determined the concentrations of IL-4, IFN- γ (Diaclone, Besançon, France), and IL-5 (Coulter-Immunotech, Villepinte, France) by ELISAs according to the manufacturers' protocols. ECP levels were evaluated by means of a competitive radioimmunoassay (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Results are expressed in pg/mL for IL-4, IL-5, and IFN- γ ; the sensitivity of these assays was 0.3, 1, and 3 pg/mL, respectively. For ECP, the results are expressed in $\mu\text{g/L}$, and the limit of detection was 1 $\mu\text{g/L}$.

Spirometry

Spirometry was performed and flow volume curves obtained according to the ATS guidelines (Gould 9000; Sensormedics; Dayton, Ohio).¹²

NO measurements

A multiple constant exhalation flow approach was used to assess alveolar NO concentration and $J'aw_{NO}$. The theoretical basis, tech-

nical aspects, and feasibility in children have been reported previously.^{2,13} Briefly, 4 to 6 FE_{NO} (fractional exhaled NO) values were obtained at several expiratory flow rates between 50 and 200 mL/s. FE_{NO} was measured by using a chemiluminescent NO analyzer (EVA4000; Seres, Aix en Provence, France). Each NO measurement was performed as recommended by ATS guidelines.¹³ At each expiratory flow (V), the NO output was calculated as $Q_{NO} = FE_{NO} \times V \times 0.06$, where Q_{NO} is expressed in nL/min, FE_{NO} in parts per billion, and V in mL/s (0.06 is a unit-correcting factor).

The slope of this regression line between NO output and expiratory flow rates is $Calv_{NO}$, and the intercept to 0 flow is $J'aw_{NO}$.¹⁴

HRCT scan

All patients underwent HRCT of the chest on a helical scanner (ProSpeed; General Electric Medical Systems, Milwaukee, Wis). HRCT scans were obtained at full inspiration. The following parameters were used: 1-mm sections at 8-mm intervals, 120 kV, 100 mAs, and a 512×512 matrix. Lung window settings were photographed at a width of 1600 H and a level of -600 H. BWT was assessed as previously described by Marchac et al.⁵ Two BWT scores were obtained, in the right and the left lung at 5 levels each, and a simplified score at 3 levels in the right lung. The 3-level score proved to be as satisfactory as the 5-level score and therefore was used for this study. This score was based on the number of visible sections of bronchi (all circular and longitudinal bronchi except hilar bronchi) at 3 levels in the right lung on HRCT: 1 cm below the carina, 2.5 to 3 cm below the top of the right pulmonary vein, and above the right side of the diaphragm. All computed tomography scans were analyzed by 2 physicians blinded to the characteristics of the asthma.

Statistics

Data are expressed as median and 25th to 75th percentiles. Differences between groups were assessed by using the Mann-Whitney U test for continuous variables and Fisher exact test for qualitative variables. Correlations were evaluated by using a non-parametric test: the Spearman rank correlation coefficient.

Probability values less than .05 were considered statistically significant.

RESULTS

The characteristics of the patients are described in Table I, and results of tests for non-HRCT variables are given in Table II.

BWT score

Agreement between the 2 observers was good. The interclass correlation coefficient was 0.997.

The median BWT score was 14 (10-19). There was no difference between children with and without persistent obstruction and between symptomatic children and those with few symptoms (Table III). The BWT score did not correlate with FEV_1 , FEV_1 /forced vital capacity, or forced expiratory flow at 25% to 75% of forced vital capacity.

Correlation between BWT score and bronchial biopsy variables

There was a weak but significant relationship ($r = 0.34$; $P = .04$) between BWT score and RBM thickness (Fig 1). There was no correlation between the BWT score and intraepithelial polynuclear neutrophil or eosinophil infiltration (Table IV).

TABLE I. Characteristics of the 37 children with severe asthma*

Age (y)	10.4 (9-13)
Sex ratio (M/F)	21/16
Duration of asthma (y)	8.8 (7.2-11)
Positive skin prick test result or positive specific IgE (n)	25
Children with obstructive disease	26
Children with nonobstructive symptomatic disease	11
FEV ₁ (% predicted)	77.5 (70.5-87)
Forced expiratory flow at 25% to 75% of forced vital capacity (% predicted)	50 (41-70.5)
FEV ₁ /forced vital capacity (%)	75 (67-83)
Inhaled corticosteroids ($\mu\text{g}\cdot\text{d}^{-1}$)	1000 (800-1000)

*Data are expressed as medians and 25th and 75th centiles or absolute results (n).

TABLE II. Non-HRCT variables in the 37 children with severe asthma*

Median 27 th -75 th centiles	n	
RBM (μm)	36	6.7 (5.37-7.7)
Intraepithelial neutrophils (per mm length of RBM)	32	6.5 (5.0-11.2)
Intraepithelial eosinophils (per mm length of RBM)	32	2.5 (0.0-5.0)
IL-4 (pg/mL)	30	1.4 (0.8-1.6)
IL-5 (pg/mL)	30	2 (2.0-4.8)
IFN- γ (pg/mL)	30	8.53 (2.0-15.7)
IFN- γ /IL-4	30	5.1 (2.0-9.7)
ECP ($\mu\text{g/L}$)	24	4.2 (1-29)
Calv _{NO} (ppb)	27	5.2 (3.0-7.9)
J'aw _{NO} (nL/min)	27	51 (29.7-125.6)

*Data are expressed as medians and 25th and 75th centiles.

Correlation between BWT score and bronchoalveolar lavage variables

Bronchial wall thickening score did not correlate with IFN- γ , IL-4, or IL-5. The correlation with IFN- γ /IL-4 ($r = -0.31$; $P = .08$) did not reach significance, whereas that with the ECP level was just significant ($r = 0.40$; $P = .05$; Table IV).

Correlation between BWT score and NO variables

The BWT score correlated with J'aw_{NO} ($r = 0.45$; $P = .02$), but not with Calv_{NO} (Table IV; Fig 2).

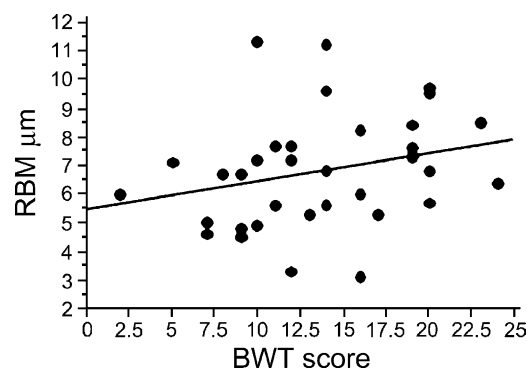
DISCUSSION

In children with severe asthma, bronchial thickening as assessed by the BWT score on HRCT was correlated with RBM thickening and NO production by the airway wall. Although these correlations were not strongly significant and did not remain significant when correcting for multiple comparisons using the Bonferroni procedure, to our

TABLE III. Bronchial wall thickening score in the 37 children with severe asthma*

	n	Median score	25th-75th Centiles
All children	37	14	10-19
Children with persistent airway obstruction	26	14	11-19
Children with persistent symptoms	25	12	9.75-19
Children without airway obstruction but persistent symptoms	11	12	9.2-16
Children with persistent airway obstruction but few symptoms	12	14	11-17

*Data are expressed as medians and 25th and 75th centiles.

**FIG 1.** Correlation between BWT score and RBM thickness ($r = 0.34$; $P = .04$).

knowledge, this is the first pediatric study suggesting a concordance between radiological findings and both a classic parameter of remodeling (RBM thickness) and a relatively new marker (eNO).

The corresponding coefficient of determination of the change in W3 score explained by a change in RBM thickening was 11%. The relative weakness of our study appears mainly related to a lack of power because of our limited sample size, because a timed complete data set was not available in all children. Further studies are needed to confirm the values of these radiological measurements.

Until now, remodeling of the airway could be demonstrated only by using endobronchial biopsies. Thickening of the lamina reticularis is diagnostic of asthma¹⁵ and appears to reflect thickening of the entire airway wall, including the airway smooth muscle.¹⁶ However, bronchoscopy is an invasive procedure that is admitted in children with severe asthma but cannot be repeated.

Technical improvements have increased the spatial resolution of HRCT sufficiently to quantify the extent of airway remodeling.^{17,18} In adults, qualitative or semi-quantitative analyses measured airway wall dimensions, particularly thickness or area of the bronchial wall, and bronchial area.^{3,4,19-25} The main result is the finding of a correlation between the degree of thickening and severity of the disease^{21,23,26} and airflow obstruction,^{3,21} but the relationship is not strong.⁸ Some studies found

TABLE IV. Correlations between BWT score and bronchial biopsy, bronchoalveolar lavage, and NO variables in the 37 children with severe asthma

	Correlation	P
RBM thickness	0.35	.04
Intraepithelial PNN	−0.08	.6
Intraepithelial PNE	0.03	.87
ECP	0.40	.05
IFN- γ	−0.25	.17
IL-4	0.13	.5
IFN- γ /IL-4	−0.31	.08
IL-5	−0.09	.6
J'aw _{NO}	0.45	.02
Calv _{NO}	0.11	.56

correlations between HRCT variables and markers of inflammation/remodeling,^{3,4} and others not.²³ In agreement with previous pediatric studies,^{5,27} we did not find correlations with airway obstruction. This may be because other factors such as distension (measured by residual volume) are also important.²⁸

The relative contributions of airway remodeling and persistent airway inflammation to the bronchial alterations detected by HRCT are unknown. Children with persistent airway obstruction and few symptoms had less intraepithelial inflammatory cell infiltration but comparable RBM thickening.⁶ Here, the BWT score in the group of children with few symptoms was not different from that for symptomatic children. Only 1 study correlated the metalloproteinase 9/tissue inhibitor of metalloproteinase 1 ratio with the magnitude of HRCT scan abnormalities,⁴ but these mediators are also responsible for an increase in bronchial permeability.²⁹

Measurement of eNO is an alternative approach to airway inflammation and structural changes. Airway inflammation is sustained by the relationships between eosinophilic infiltration and eNO.³⁰ Structural changes seem to be a consequence of the multiple intracellular and extracellular roles of NO.³¹ In our study, J'aw_{NO} correlated with the BWT. Correlations between BWT score and RBM thickness might be secondary to correlations between J'aw_{NO} and RBM thickness.² Nevertheless, although weak, the BWT score was more closely correlated with J'aw_{NO} than with RBM thickness, an observation that could be explained by 2 mechanisms. First, HRCT score depends not only on RBM thickness but also on other remodeling factors—for example, hyperplasia of bronchial smooth muscle and myofibroblastic hypertrophy. Increased exhaled NO has both relaxation³¹ and antiproliferative^{32,33} effects on airway smooth muscle and may reflect an adaptive response to the airway remodeling. Second, possible persistent airway inflammation, poorly detected in our biopsies, may influence both HRCT score and NO production by the airways.^{6,25} In fact, inflammation and remodeling may act in parallel in chronic asthma but interact each other.¹ ECP, which is a classic marker of eosinophil-driven airway inflammation, is also involved in remodeling.^{34,35} The higher levels of ECP in children with

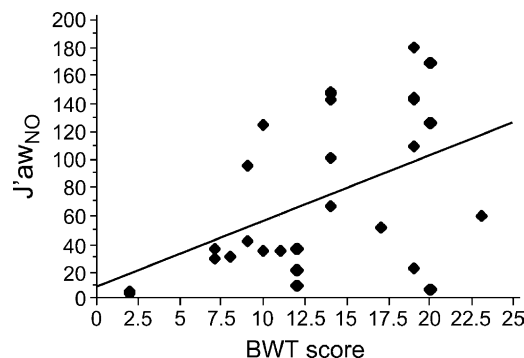


FIG 2. Correlation between BWT score and J'aw_{NO} ($r = 0.45$; $P = .02$).

higher BWT scores may be related to persistent inflammation and remodeling.

In conclusion, this pilot study shows that a simple BWT score on HRCT correlates with RBM thickness on endobronchial biopsy and with eNO measurement. Further prospective studies are necessary to confirm whether HRCT scanning will prove to be valuable. Until now, grading severity of asthma was based on clinical score, pulmonary function, and perhaps eNO testing, and the major contribution of HRCT scanning was to eliminate alternative diagnosis. Our results were obtained in a selected population of children with severe asthma and cannot necessarily be generalized to children with less severe asthma. However, the longitudinal prospective study of our cohort of children with severe asthma will help to determine whether a high BWT score is associated with acceleration in the decline of FEV₁ and to determine the respective role of other parameters of remodeling implicated in BWT such as airway smooth muscle hypertrophy.³⁶ Finally, the new generations of multislice computed tomography scanners will allow higher definition and lower radiation exposure and probably give a better assessment of airway remodeling and efficacy of treatment.¹⁸

REFERENCES

- Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. *J Allergy Clin Immunol* 2003;111:215-25.
- Mahut B, Delclaux C, Tillie-Leblond I, Gosset P, Delacourt C, Zerach-Lancner F, et al. Both inflammation and remodeling influence nitric oxide output in children with refractory asthma. *J Allergy Clin Immunol* 2004;113:252-6.
- Kasahara K, Shiba K, Ozawa T, Okuda K, Adachi M. Correlation between the bronchial subepithelial layer and whole airway wall thickness in patients with asthma. *Thorax* 2002;57:242-6.
- Vignola AM, Paganin F, Capieu L, Scichilone N, Bellia M, Maakel L, et al. Airway remodelling assessed by sputum and high-resolution computed tomography in asthma and COPD. *Eur Respir J* 2004;24:910-7.
- Marchac V, Emond S, Mamou-Mani T, Le Bihan-Benjamin C, Le Bourgeois M, De Blic J, et al. Thoracic CT in pediatric patients with difficult-to-treat asthma. *AJR Am J Roentgenol* 2002;179:1245-52.
- de Blic J, Tillie-Leblond I, Tonnel AB, Jaubert F, Scheinmann P, Gosset P. Difficult asthma in children: an analysis of airway inflammation. *J Allergy Clin Immunol* 2004;113:94-100.

7. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
8. Wenzel S. Severe asthma in adults. *Am J Respir Crit Care Med* 2005;175:149-60.
9. Saetta M, Jeffery PK, Maestrelli P, Timens W. Biopsies: processing and assessment. *Eur Respir J Suppl* 1998;26:20S-5S.
10. Vignola AM, Chanez P, Campbell AM, Souques F, Lebel B, Enander I, et al. Airway inflammation in mild intermittent and in persistent asthma. *Am J Respir Crit Care Med* 1998;157:403-9.
11. de Blic J, Midulla F, Barbato A, Clement A, Dab I, Eber E, et al. Bronchoalveolar lavage in children. ERS Task Force on bronchoalveolar lavage in children. European Respiratory Society. *Eur Respir J* 2000;15:217-31.
12. Standardization of spirometry, 1994 update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152:1107-36.
13. Mahut B, Delacourt C, Zerah-Lancner F, De Blic J, Harf A, Delclaux C. Increase in alveolar nitric oxide in the presence of symptoms in childhood asthma. *Chest* 2004;125:1012-8.
14. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* 1998;85:653-66.
15. Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520-4.
16. James AL, Maxwell PS, Pearce-Pinto G, Elliot JG, Carroll NG. The relationship of reticular basement membrane thickness to airway wall remodeling in asthma. *Am J Respir Crit Care Med* 2002;166:1590-5.
17. Nakano Y, Muller NL, King GG, Niimi A, Kalloger SE, Mishima M, et al. Quantitative assessment of airway remodeling using high-resolution CT. *Chest* 2002;122:271S-5S.
18. Mitsunobu F, Tanizaki Y. The use of computed tomography to assess asthma severity. *Curr Opin Allergy Clin Immunol* 2005;5:85-90.
19. Paganin F, Trussard V, Seneterre E, Chanez P, Giron J, Godard P, et al. Chest radiography and high resolution computed tomography of the lungs in asthma. *Am Rev Respir Dis* 1992;146:1084-7.
20. Park JW, Hong YK, Kim CW, Kim DK, Choe KO, Hong CS. High-resolution computed tomography in patients with bronchial asthma: correlation with clinical features, pulmonary functions and bronchial hyperresponsiveness. *J Investig Allergol Clin Immunol* 1997;7:186-92.
21. Niimi A, Matsumoto H, Amitani R, Nakano Y, Mishima M, Minakuchi M, et al. Airway wall thickness in asthma assessed by computed tomography: relation to clinical indices. *Am J Respir Crit Care Med* 2000;162:1518-23.
22. Harmanci E, Kebapci M, Metintas M, Ozkan R. High-resolution computed tomography findings are correlated with disease severity in asthma. *Respiration* 2002;69:420-6.
23. Little SA, Sproule MW, Cowan MD, Macleod KJ, Robertson M, Love JG, et al. High resolution computed tomographic assessment of airway wall thickness in chronic asthma: reproducibility and relationship with lung function and severity. *Thorax* 2002;57:247-53.
24. Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M. Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. *Am J Respir Crit Care Med* 2003;168:983-8.
25. Bumbacea D, Campbell D, Nguyen L, Carr D, Barnes PJ, Robinson D, et al. Parameters associated with persistent airflow obstruction in chronic severe asthma. *Eur Respir J* 2004;24:122-8.
26. Awadh N, Muller NL, Park CS, Abboud RT, FitzGerald JM. Airway wall thickness in patients with near fatal asthma and control groups: assessment with high resolution computed tomographic scanning. *Thorax* 1998;53:248-53.
27. Ketai L, Coutias C, Williamson S, Coutias V. Thin-section CT evidence of bronchial thickening in children with stable asthma: bronchoconstriction or airway remodeling? *Acad Radiol* 2001;8:257-64.
28. Pifferi M, Caramella D, Ragazzo V, Pietrobelli A, Boner AL. Low-density areas on high-resolution computed tomograms in chronic pediatric asthma. *J Pediatr* 2002;141:104-8.
29. Lemjabbar H, Gosset P, Lamblin C, Tillie I, Hartmann D, Wallaert B, et al. Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. *Am J Respir Crit Care Med* 1999;159:1298-307.
30. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001;164:1376-81.
31. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;84:731-65.
32. Hamad AM, Johnson SR, Knox AJ. Antiproliferative effects of NO and ANP in cultured human airway smooth muscle. *Am J Physiol* 1999;277:L910-8.
33. Patel HJ, Belvisi MG, Donnelly LE, Yacoub MH, Chung KF, Mitchell JA. Constitutive expression of type I NOS in human airway smooth muscle cells: evidence for an antiproliferative role. *FASEB* 1999;13:1810-6.
34. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004;305:1776-9.
35. Zagai U, Skold CM, Trulsson A, Venge P, Lundahl J. The effect of eosinophils on collagen gel contraction and implications for tissue remodelling. *Clin Exp Immunol* 2004;135:427-33.
36. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003;167:1360-8.