

Original article

Specificity of basement membrane thickening in severe asthma

Arnaud Bourdin, MD,^a Dorine Neveu, PhD,^b Isabelle Vachier, PhD,^a Fabrice Paganin, MD, PhD,^a Philippe Godard, MD,^a and Pascal Chanez, MD, PhD^a Montpellier, France

Background: Reticular basement membrane (RBM) thickness is considered a hallmark for airway remodeling in airway diseases such as asthma. It is still unclear whether this measurement could be associated with disease severity or apply to chronic obstructive pulmonary disease (COPD). A wide range of results, at baseline or after therapeutic intervention, have been reported using different measurement methods. **Objective:** To determine whether increased RBM thickness could be associated specifically with severe asthma and in COPD in large samples.

Methods: We blindly measured RBM thickness in endobronchial biopsies from 50 patients with severe asthma (mean age, 53 years; FEV₁ 66% predicted, inhaled steroids ≥ 1500 μ g and 20 mg daily dose of oral corticosteroids, lifelong nonsmokers), 50 untreated patients with mild asthma (mean age, 33 years; FEV₁ 93% pred, lifelong nonsmokers), 50 patients with COPD (mean age, 57 years; FEV₁ 53% pred, all current smokers), and 18 control subjects using 2 different validated quantitative and computer-assisted methods (repeated multiple point-to-point vs area by length ratio). **Results:** Reticular basement membrane thickness was higher in severe asthma compared with mild asthma and COPD ($P = .0053$). On the basis of receiver operating characteristic curves, RBM thickness was effective in differentiating severe asthma from other groups (sensitivity and specificity, 98% and 95%, respectively, above a threshold of 5 μ m vs control, 70% and 75% at 7 μ m vs mild, 83% and 68% at 6 μ m vs COPD). **Conclusion:** Increased RBM thickness was specifically associated with severe asthma, whereas surprisingly, COPD and mild asthma had similar remodeling features. **Clinical implications:** Reticular basement membrane thickness can be considered a hallmark of severe asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, COPD, remodeling, basement membrane, ROC

Asthma is a chronic inflammatory disorder of the airways that leads to recurrent episodes of airway obstruction. Some structural changes can occur in association

Abbreviations used

AUC: Area under curve
COPD: Chronic obstructive pulmonary disease
EBB: Endobronchial biopsy
RBM: Reticular basement membrane
ROC: Receiver operating characteristic
TEM: Transmission electron microscopy

with inflammation, such as mucous gland hypertrophy, enlargement of bronchial smooth muscle, and collagen matrix deposition. This remodeling underlies chronic airflow obstruction. Thickening of the reticular basement membrane (RBM) has been attributed to excessive collagen deposition beneath the true basement membrane, especially involving subepithelial myofibroblasts and T-cell activation.^{1,2} James et al³ demonstrated in autopsy samples that RBM thickness correlated with the whole bronchial thickness, suggesting that a change in RBM thickness is a hallmark of the remodeling process. Some relationships with functional parameters that are thought to be linked with severity, such as FEV₁ or, more often, PC₂₀ methacholine, have sometimes been reported.⁴ The specificity of this phenomenon is still unknown, especially because it has also been reported in chronic obstructive pulmonary disease (COPD).⁵

Reticular basement membrane thickness is widely used in clinical and therapeutic studies as a marker of airway remodeling. A broad range of RBM thickness values, from 3 to 25 μ m,⁶⁻⁹ has been reported in controls and patients with asthma. Various techniques have been used, from transmission electron microscopy (TEM) to specific immunoassays of RBM components.⁸ Sullivan et al⁹ reported that multiple point-to-point repeated measurements at regular 20- μ m intervals were correct when 40 measurements per slide were performed. On the other hand, Wilson and Li⁸ described satisfactory results in hematoxylin-eosin-stained biopsies with RBM assessment on the basis of the area by length ratio, compared with using TEM measurements as a gold standard. In most of these studies, the number of cases investigated was quite low and the clinical relevance questionable.

Reticular basement membrane thickness may be difficult to assess because of fixation and embedding artefacts, as well as technical bias caused by the reorientation

From ^aService des Maladies Respiratoires and ^bDépartement d'Informatique Médicale, Hôpital Arnaud de Villeneuve, Centre Hospitalo Universitaire de Montpellier.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication May 17, 2006; revised January 19, 2007; accepted for publication January 24, 2007.

Reprint requests: Pascal Chanez, MD, PhD, Hôpital Arnaud de Villeneuve, 34295, Montpellier Cedex 5, France. E-mail: chanez@montp.inserm.fr. 0091-6749/\$32.00

© 2007 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2007.01.055

requirements, and irregularities.^{8,10} Stereologic approaches reduce such bias,¹⁰ but they are time-consuming and limit the potential of studies involving large patient populations.

We hypothesized that increased RBM thickness could be specific enough to discriminate between severe asthma and other chronic airway diseases, including milder forms of the disease. To test this hypothesis, we used 2 different validated methods involving light microscopy to obtain precise data with large patient populations.

METHODS

Patients

From 1996 to 2000, endobronchial biopsies (EBBs) were performed in 6 different clinical studies, approved by ethics committees, involving more than 300 patients. We selected the first 50 well characterized patients with mild-to-moderate untreated asthma, 50 current smokers with untreated COPD, and 50 patients with severe oral steroid-treated asthma. The diagnoses were made according to current guidelines.^{11,12} The first 2 groups of patients were naive for anti-inflammatory treatment at the time of sampling. Flow-volume curves were assessed at baseline and after inhaling 400 µg salbutamol via a spacer. Atopic status was assessed by skin prick tests for allergens from the Montpellier area. Smoking status was checked through patient interviews. All patients with asthma had a clear history of recurrent wheezing or breathlessness episodes that reversed spontaneously or after treatment, a familial history of asthma, and at least 15% reversibility after bronchodilator inhalation¹¹ or a positive methacholine provocation test with a PC₂₀ <4 mg/mL. Active smokers or exsmokers of more than 5 pack-years were excluded. Asthma severity was based on medication requirements (high daily dose of inhaled corticosteroids >1500 µg/d, long-acting β₂ agonists, and in some cases, oral corticosteroids at required doses) and permanent air-flow impairment in patients followed up for at least 1 year, as previously defined.^{13,14} Disease onset was recorded on the basis of patient interviews or medical files when available. Patients with COPD had a clear history of smoking, chronic productive cough or sputum, no history of asthma even in their family, no reported allergic diseases, and no significant reversibility after bronchodilator treatment. All patients with COPD had a high-resolution computed tomography scan in their files confirming the presence of centrilobular emphysema at the parenchymal level. All patients were stable for at least 1 month before the investigation. Eighteen slides of tissues from normal volunteer subjects stored in our tissue databank were used as controls to compare the results.

EBB sampling and selection

Between 3 and 6 EBBs were performed on 1 occasion, with flexible bronchoscopy under local anesthesia, in the left lower lobe with crocodile forceps according to recommendations.¹⁵ After fixation in neutral 4% formaldehyde buffer and embedding in paraffin, each biopsy was sliced and then stained with hematoxylin-eosin. Four to 6 sections were fixed on a slide for subsequent analysis.

Randomization

Each slide was twice randomly assigned a number according to 2 independent centralized lists. First, a single observer assessed the RBM thickness of all endobronchial biopsies by multiple point-to-point repeated measurements at regular intervals of 20 µm according to Sullivan et al.⁹ Then the observer assessed the RBM thickness by the area by length ratio according to Wilson and Li⁸ (Fig 1).

Measurements were performed over 1 mm while taking only the adequately preserved and oriented area into account. Metaplastic as well as fully shed areas were considered inadequate for measurement.

The intraobserver and intramethod reproducibilities were assessed on 40 randomly chosen slides. All measurements were made with a computerized image analyser (AnalySIS 3.0 for Windows, Olympus Soft Imaging Solutions, Muenster, Germany) linked to a CCD camera (Sony CCD DXC950P, Sony, Tokyo, Japan) connected to a light microscope at ×400 magnification (Olympus BHS, Olympus Optical, Tokyo, Japan). The pixel resolution was approximately 0.02 µm.

Assessment

Reticular basement membrane thickness was expressed as the geometric mean of repeated measurements by using the Sullivan method or as the average area to length ratio by using the Wilson method, as previously recommended.^{8,9}

Statistical analysis

Categorical variables were summarized as percentages and continuous variables as means and SDs for Gaussian variables and medians with interquartile ranges for non-Gaussian variables.

The *t* test and ANOVA or the Wilcoxon and Kruskal-Wallis tests were used for comparing continuous variables between groups, according to whether their distribution was Gaussian.

Correlations between functional or clinical continuous variables were estimated with the Spearman rank ρ index.

Intramethod agreement was evaluated with the Bland and Altman¹⁶ method and the intraclass correlation coefficient.

Receiver operating characteristic (ROC) curves were used to evaluate the validity of basement membrane thickening measurements for discriminating between airway diseases. Their areas under curve (AUCs) with 95% CIs were computed.¹⁷

In the current study, equal weight was given to sensitivity and specificity. Cut-points that optimize the differentiating ability of the basement membrane thickening were estimated with the Youden¹⁸ index, which is the maximum vertical distance between the ROC curve and the diagonal or chance line. Sensitivity and specificity were computed according to these optimal cut-points. *A posteriori* power calculations were performed with an α risk at 5% and a power of 80% and 90%.

The statistical significance was set at $P < .05$. As sequential tests were performed, we corrected the *P* value with the Bonferroni correction to maintain the overall 2-sided type 1 error rate at 5%.

The computations were performed with the SAS software package (version 8.2; SAS Institute Inc, Cary, NC).

RESULTS

Patient characteristics

Patients with mild and severe asthma and COPD presented markedly different phenotypes, as reported in Table I. Seventy percent of patients with mild asthma and 60% with severe asthma were atopic, and 3 of them were exsmokers (<5 pack-years, ceased for at least 1 year; Table I). Patients with COPD had a lower reversibility than patients with asthma ($P = .0001$). They were all current heavy smokers. Severe asthma and COPD groups presented with similar lung function at rest but significantly different reversibility after bronchodilator inhalation. No differences between groups were noted in terms of disease duration, whereas only patients with severe asthma patients were receiving high doses of inhaled corticosteroids.

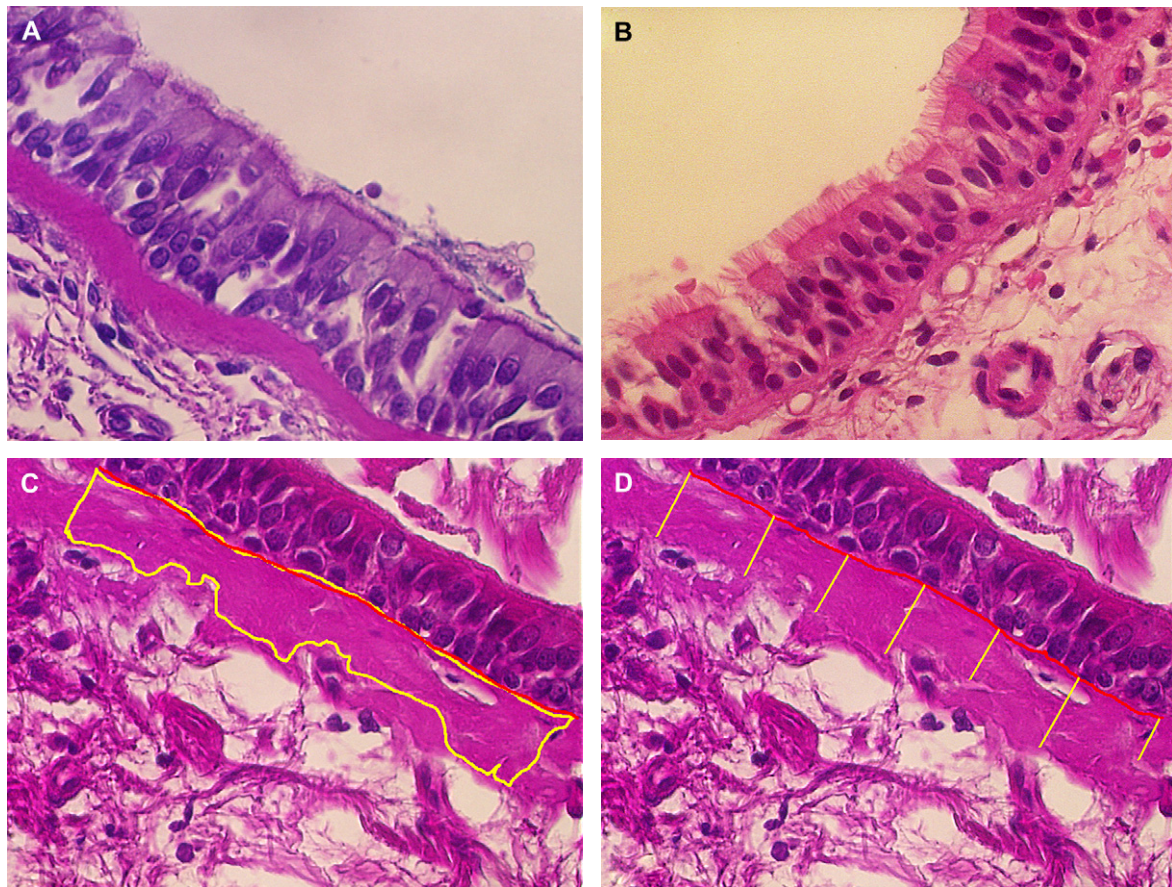


FIG 1. Different examples obtained in this study. A patient with mild asthma (A) and COPD (B). RBM thickness measurements in a patient with severe asthma according to Wilson (C) and Sullivan (D).

TABLE I. Patient characteristics*

	Mild (n = 50)	Severe (n = 50)	COPD (n = 50)	Controls (n = 18)	P value
Male (%)	64	57	88	50	NS
Age (y)	33 (26-49.75)	53 (39-62)	60 (51.5-66.75)	26 (22-34)	.003
Postbronchodilator FEV ₁ (%pred)	94.5 (79.25-100)	66 (51.50-74)	59 (45.25-67.75)	100 (97.25-104)	.02
Reversibility (%)	13 (11.5-18)	17 (14-22)	4 (0-9)	2 (0-3)	.0001
Smoking (pack-years)	0 (0-0.75)	0 (0-1)	40 (40-50)	0 (0-1.25)	.00004
Atopy (%)	70	62	0	0	.003
Disease duration (y)	8 (2-12)	8.4 (3-15)	9 (2-18)	NA	NS
Inhaled corticosteroids (μg/d)	0	2000 (1500-3000)	0	NA	.002

NA, Not applicable; NS, not significant.

*Comparisons were made using the Kruskal-Wallis test with adequate posttest corrections. Values are means with 95% CIs for Gaussian variables, and median with 25 and 75 interquartiles for others.

Assessment of RBM thickness

Reticular basement membrane thickness presented a normal distribution in all groups and homoscedasticity among the severe asthma, mild asthma, and COPD groups. Mean RBM thickness was statistically lower ($P < .001$) in the control group than in the 3 others (Table II). Slides not adequately reversed or oriented for

accurate measurements were excluded (3, 4, 6, and 2 slides per group, respectively).

With both methods, RBM was statistically thicker in severe asthma (mean, 7.23 μm according to Sullivan; 6.25 μm according to Wilson) than in mild asthma (mean, 6.85 μm according to Sullivan, $P = .0002$; 5.63 μm according to Wilson, $P < .0001$), in COPD biopsies (mean,

TABLE II. RBM thickness in asthma and COPD, according to both techniques

	Wilson		Sullivan	
	Mean (μm)	SD	Mean (μm)	SD
Controls (n = 18)	4.16	0.66	4.95	0.47
Mild asthma (n = 50)	5.63*	2.03	6.85*	1.99
COPD (n = 50)	4.80*	2.12	5.68*	1.90
Severe asthma (n = 50)	6.25*†	2.10	7.23*†	1.97

* $P < .0001$ vs controls.

† $P < .0001$ vs mild asthma and COPD (Kruskal-Wallis with the adequate Bonferroni and Dunn posttest corrections).

5.68 μm according to Sullivan, $P < .0004$; 4.80 μm according to Wilson, $P < .0001$, or in control biopsies (mean, 4.95 μm according to Sullivan, $P = .0004$; 4.16 μm according to Wilson, $P < .0001$; Table II).

No significant differences were noted between mild asthma and COPD, irrespective of the method used (Fig 2). A *posteriori* power calculations revealed that 150 to 300 subjects per group would be necessary to detect a statistically significant difference between mild asthma and COPD.

Reticular basement membrane was also thicker in the mild asthma group than in the control group (mean, 4.83 μm with Sullivan, $P = .0004$; 4.23 μm with Wilson, $P = .016$). Thickening was also encountered in the COPD group compared with the control with the Sullivan method ($P = .0004$). A trend toward RBM thickening was also noted when the Wilson method was used ($P = .06$).

The results were always higher with the Sullivan method compared with the Wilson method (paired *t* test, $P < .0001$ for the 4 groups).

We did not find any correlation with any clinical or functional parameters in mild and severe asthma, or in COPD (Spearman rank ρ index for FEV₁ and RBM thickness in the severe asthma group, $\rho = 0.224$; $P = .343$). A significant correlation between FEV₁ and RBM thickness was observed ($\rho = -0.56$, $P < .0001$ according to Wilson; $\rho = -0.51$, $P < .0001$ according to Sullivan; Fig 3) when considering mild and severe asthma together (Fig 3). Duration of the disease and RBM thickness correlate neither within nor among groups.

Reproducibility

Both methods were highly reproducible according to the Bland and Altman¹⁶ visual analysis (Fig 4). The intraclass correlation coefficient was 0.84 (95% CI, 0.66-0.94) for RBM measurement according to Wilson and 0.81 according to Sullivan (95% CI, 0.67-0.89).

ROC curves

We quantified the ability of RBM thickness to discriminate between diseases with AUC computed from ROC curves. AUC differentiated severe asthma from mild asthma, COPD, and control biopsies (Table III). The RBM thickness differentiated severe asthma from any

group among the 3 others: the AUC was 0.790 (95% CI, 0.698-0.883) for severe versus mild asthma, 0.838 (95% CI, 0.754-0.922) for severe asthma versus COPD, and 0.963 (95% CI, 0.92-1) for severe asthma versus controls (Fig 5).

The detection cutoff for severe asthma versus mild asthma (or COPD), which maximized the Youden index, was 7 μm and 6 μm , respectively. When these cutoffs were used, the sensitivity and specificity were 70% and 75%, respectively, when comparing severe and mild asthma, and 83% and 68% when comparing severe asthma and COPD (Table IV).

Because the cost of false-negatives and false-positives may be considered as similar in these diseases, we determined the cutoffs for pathology detection by maximizing the Youden index. The results are presented in Table IV. The cutoff values shifted with the technique. When comparing severe asthma to mild asthma, COPD, and controls, these cutoffs were associated with high sensitivity and specificity of RBM thickening—that is, 98% and 95%, respectively—at a threshold of about 5 μm versus controls, 70% and 75% at 7 μm versus mild asthma, and 83% and 68% at 6 μm versus COPD, according to Wilson's method (Table IV).

DISCUSSION

This study demonstrated that RBM thickness could differentiate patients with chronic airway disease when measured by 2 different validated methods. Remodeling in severe asthma was clearly different from remodeling in mild asthma and COPD, both of which differed from the situation observed in control subjects.

We chose the methods for RBM assessment by Wilson and Li⁸ and Sullivan et al⁹ because of their wide use and validation compared with methods as effective as TEM.⁸ Stereologic approaches have demonstrated their superiority in this respect, but they are time-consuming and difficult to use with large patient populations.¹⁰ Moreover, our biopsies were not processed for it. We decided to use 2 different methods to check our approach. The results gave satisfactory results with similar differences among groups. Comparing methods was not the aim of this study. Clearly, these 2 methods are not identical, nor do they measure the same structure, because the absolute results were different. The area-to-length ratio method enables more observer bias to delineate the possible nonlinear upper or lower RBM limits, whereas the repeated measurements of Sullivan's method provide a more accurate indication on possible variations in RBM thickness. The presence of capillary or inflammatory cells infiltrated within the basement membrane can be subtracted with Wilson's method, whereas this cannot be done with Sullivan's method because of the regular intervals required. The reproducibility of the methods assessed by the Bland and Altman¹⁶ graphical presentation and the intraclass coefficient of correlation assessment was as high as in other studies.

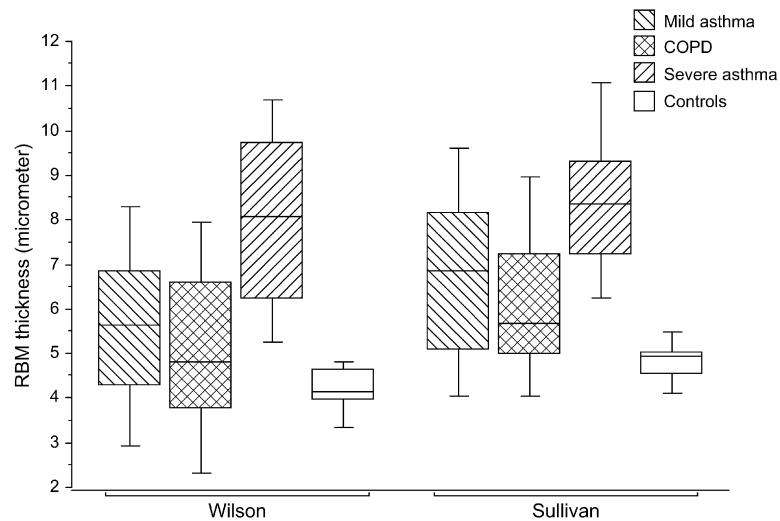


FIG 2. Box-plot distribution according to the 2 techniques.

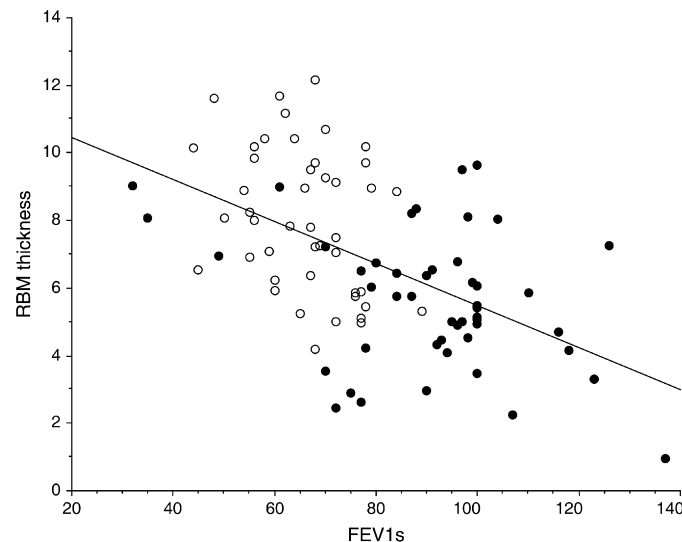


FIG 3. Spearman correlation between RBM thickness (μm) and post-bronchodilator FEV₁ (% of predicted value) in mild (solid circles) and severe (open circles) asthma. $\rho = -0.56$; $P < .0001$.

We ensured that the patients included in our study were clearly representative of well-described phenotypes to avoid overlapping forms of these diseases. Borderline conditions were thus avoided as much as possible. The patient populations studied here were much larger than those investigated in all other studies on the same topic. Nonetheless, the patients with the most severe asthma could not be safely tested with a methacholine provocative test, as could be done with patients with severe COPD. Otherwise, the tobacco smoking status was based on patient interviews.

Other confounding risk factors for increased RBM thickness such as diabetes mellitus¹⁹ were controlled before inclusion. The absence of discrimination between mild asthma and COPD, instead of being different from controls, is likely to induce many comments, because

the patients' phenotypes were at the ends of the clinical spectrum. Age differences could not account sufficiently for RBM thickness differences.¹⁹ First, it could be argued that the remodeling process may also occur at the proximal level in COPD, even though the results differed between techniques, with different mechanisms involved. This has been recently suggested by others.²⁰ Otherwise, increased RBM thickness in posttransplantation bronchiolitis or interstitial pulmonary fibrosis has already been reported.²¹ The excessive ECM deposition that occurs under the true RBM could be interpreted as nonspecific visual evidence of healing processes in response to different deleterious phenomena. Very few studies have been conducted on COPD endobronchial biopsies focused on RBM thickness. New studies focused on this aspect are required to investigate remodeling more precisely on

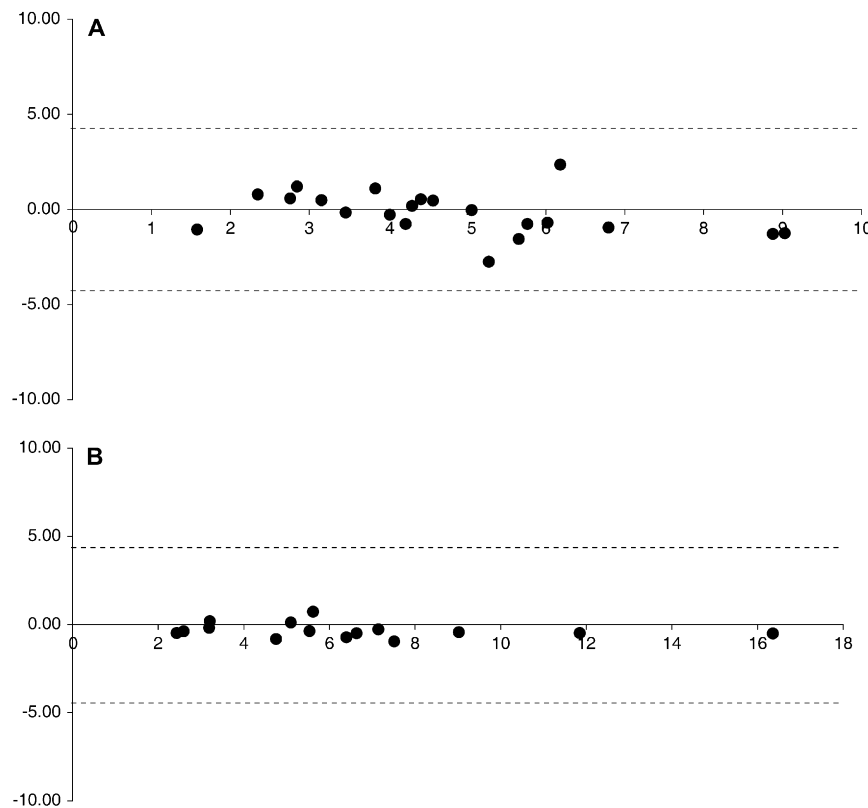


FIG 4. Bland and Altman graphs for reproducibility in Wilson's (A) and Sullivan's (B) techniques (intraobserver).

TABLE III. Values of the area under a ROC with 95% CI and *P* values¹⁴

	Wilson		Sullivan	
	AUC	<i>P</i> value	AUC	<i>P</i> value
Mild vs control	0.751 (0.630-0.872)	.002	0.798 (0.685-0.911)	.0003
COPD vs control	0.655 (0.511-0.801)	.058 NS	0.756 (0.629-0.884)	.002
COPD vs mild	0.574 (0.455-0.694)	.226 NS	0.596 (0.470-0.723)	0.143 NS
Severe vs control	0.978 (0.945-1)	10 ⁻⁹	0.964 (0.922-1)	<10 ⁻⁹
Severe vs mild	0.790 (0.690-0.882)	10 ⁻⁶	0.735 (0.632-0.840)	.0002
Severe vs COPD	0.838 (0.754-0.922)	10 ⁻⁸	0.815 (0.724-0.906)	10 ⁻⁷

proximal airways in COPD. The nature and amounts of extracellular matrix products involved could differ from those observed in asthma.²⁰ Increased collagen content and small airway fibrosis have been reported in COPD, while the molecular nature of extracellular matrix deposition and cellular sources were not clearly identified.^{12,20}

Severe asthma is more likely to induce such a pathological process, thus supporting the idea that remodeling increases with the disease severity. No correlations could be clearly observed within our groups, but phenotypic heterogeneity is a feature of severe asthma.¹³ Patients with permanent airflow obstruction represent only 1 phenotype of severe asthma. For instance, a positive relationship between RBM thickness and PC₂₀ methacholine was

controversially reported in the past.^{4,22,23} Moreover, the patients with severe asthma involved in this study were all receiving heavy treatments, including inhaled and oral steroids, that might affect RBM thickness to largely unknown extents. Recent clinical studies that used RBM thickness as a marker of therapeutic intervention against excessive airway remodeling reported that inhaled steroids tended to decrease RBM thickness.^{6,24,25} On the other hand, some *in vitro* studies suggested that steroids could increase extracellular matrix deposition.²⁶ Nonetheless, whereas the patients with the most severe asthma had the highest RBM thicknesses, in the current study we noted, like Minshall et al²⁷ in a previous study, a negative correlation between RBM thickness and FEV₁ when considering the 2 asthma groups. In a variable

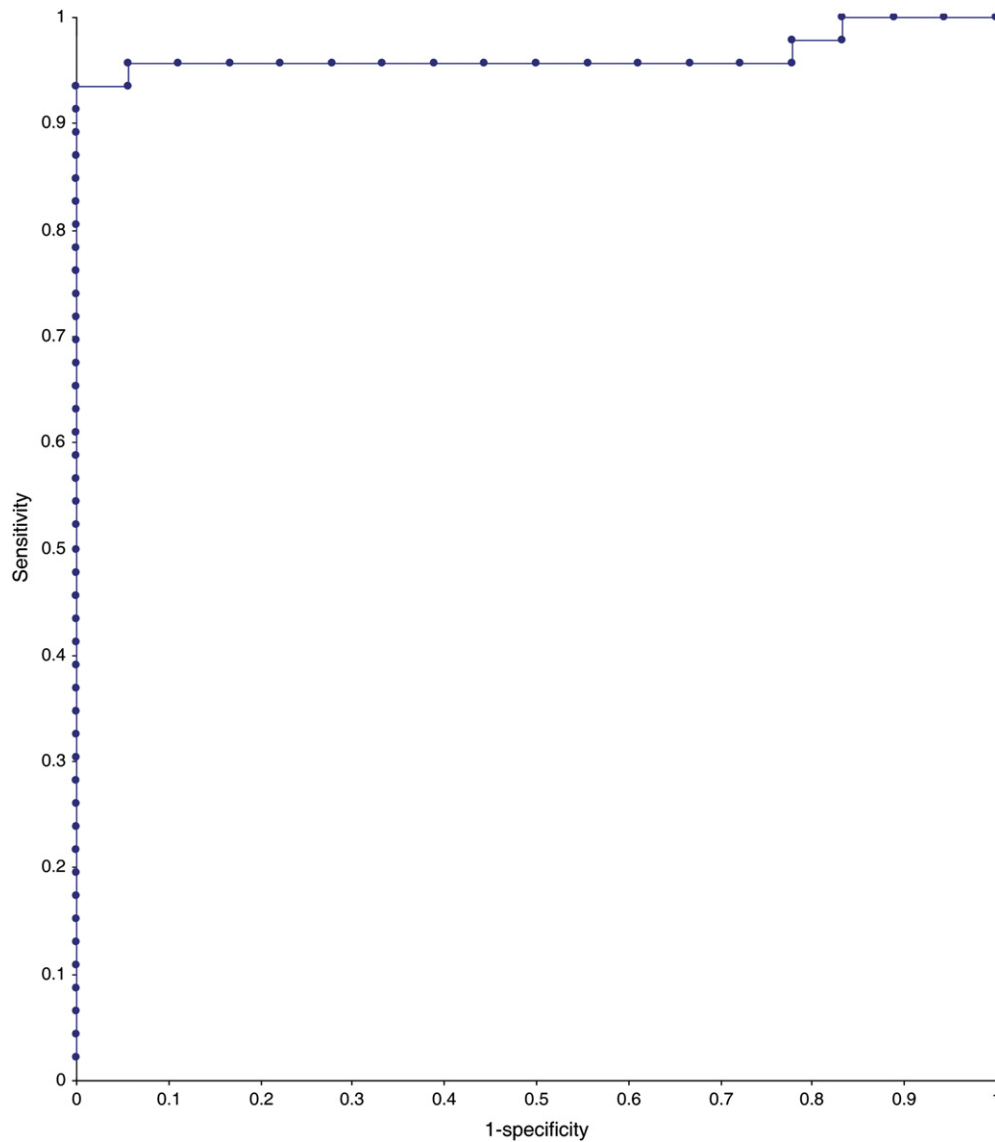


FIG 5. ROC graph illustrating the ability of RBM measurement to discriminate between controls and severe asthma according to the Wilson technique (sensitivity, 98%; specificity, 94.4% at a threshold of 4.96 μm).

TABLE IV. Sensitivity and specificity of increased RBM thickness for discriminating between groups, provided by the ROCs

Condition	Wilson			Sullivan		
	Threshold (μm)	Sensitivity (%)	Specificity (%)	Threshold (μm)	Sensitivity (%)	Specificity (%)
Mild vs control	4.92	66.6	94.4	5.88	71	100
COPD vs control*	5.05	49	94.4	5.38	67.5	88.8
Mild vs COPD*	4.8	51	66.6	6.72	70.3	53.7
Severe vs control	4.96	98	94.4	5.94	93.5	100
Mild vs severe	6.91	69.5	75	7.04	82.6	61
COPD vs severe	5.86	82.6	68	6.65	89	67.6

*Results not statistically significant and reported for informational purpose only.

and heterogeneous condition such as severe asthma, a single parameter such as FEV₁ did not account for the complexity of the disease. Jeffery²⁰ demonstrated that the true basement membrane was not affected by the remodeling process. The organization of the fibers in the thickened RBM in asthma, contrary to what is expected in fibrosis, does not resemble that of interstitial collagen,²⁸ suggesting a qualitative more than a quantitative pathway. The more severe disease then leads to more severe remodeling because duration of the disease judged by patient's interview was not different.

Irrespective of these potential biases, because our ROC curves sustained our results, such RBM thickening could thus be considered as a real feature associated with severe asthma. The exact potential role of this remodeling process—that is, protective against excessive airway narrowing²⁹ or responsible for chronic airflow impairment^{20,30}—should be investigated in more in-depth studies. Patients with severe asthma and COPD presented the same airflow impairment but different RBM thicknesses, as demonstrated before,³¹ suggesting a disease specific process rather than a simple association with a nonspecific airway remodeling mechanism.

Safety issues can be considered as potential limits but observing recommended cautions permit researchers to optimal results.³² In this study, we did not encounter any safety issues, because all patients requiring bronchoscopy had been previously investigated and eventually excluded according to international guidelines.¹⁵ This is a potential limiting factor with respect to investigating the patients with the most severe disease.

By comparison with the current study, the exhaled NO level has been studied as a diagnostic tool for asthma, and the results obtained in these studies were not as good as those obtained with methacholine provocation—that is, around 75% sensitivity and specificity.³³ Although the CI was higher than in controls, a RBM thickness assessed visually in a quasi-routine setting could be used as a diagnostic criterion for severe asthma, with positive predictive values that are higher than exhaled NO.³³

In conclusion, this study is the first to demonstrate the potential discriminative diagnostic benefits of routine RBM thickness assessment, at least in patients with severe asthma. These findings suggest that increased RBM thickness is related to the disease and its severity rather than nonreversible chronic airflow obstruction. Important questions still to be addressed concern differences in the remodeling process in severe asthma and COPD, and the functional and biological consequences of increased RBM thickening.

REFERENCES

1. Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520-4.
2. Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990;3:507-11.
3. 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.
4. Chu HW, Halliday JL, Martin RJ, Leung DY, Szefer SJ, Wenzel SE. Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. *Am J Respir Crit Care Med* 1998;158:1936-44.
5. 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.
6. Hoshino M, Takahashi M, Takai Y, Sim J. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol* 1999;104:356-63.
7. Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol* 1998;102:783-8.
8. Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. *Clin Exp Allergy* 1997;27:363-71.
9. Sullivan P, Stephens D, Ansari T, Costello J, Jeffery P. Variation in the measurements of basement membrane thickness and inflammatory cell number in bronchial biopsies. *Eur Respir J* 1998;12:811-5.
10. Ferrando RE, Nyengaard JR, Hays SR, Fahy JV, Woodruff PG. Applying stereology to measure thickness of the basement membrane zone in bronchial biopsy specimens. *J Allergy Clin Immunol* 2003;112:1243-5.
11. NHLBI. National Asthma Education and Prevention Program Expert Panel Report 2: guidelines for the diagnosis and management of asthma. In: NHLBI/WHO Workshop. Bethesda MN:NIH, editor. Bethesda (MD): National Institutes of Health; 2002.
12. NHLBI. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease, NHLBI/WHO Workshop. Bethesda (MD): National Institutes of Health; 2001.
13. Bel EH. Clinical phenotypes of asthma. *Curr Opin Pulm Med* 2004;10:44-50.
14. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med* 2000;162:2341-51.
15. Jeffery P, Holgate S, Wenzel S. Methods for the assessment of endobronchial biopsies in clinical research: application to studies of pathogenesis and the effects of treatment. *Am J Respir Crit Care Med* 2003;168:S1-17.
16. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307-10.
17. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29-36.
18. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950;3:32-5.
19. Kida K, Utsuyama M, Takizawa T, Thurlbeck WM. Changes in lung morphologic features and elasticity caused by streptozotocin-induced diabetes mellitus in growing rats. *Am Rev Respir Dis* 1983;128:125-31.
20. Jeffery PK. Remodeling and inflammation of bronchi in asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2004;1:176-83.
21. Ward C, De Soyza A, Fisher AJ, Pritchard G, Forrest I, Corris P. A descriptive study of small airway reticular basement membrane thickening in clinically stable lung transplant recipients. *J Heart Lung Transplant* 2005;24:533-7.
22. Boulet L, Belanger M, Carrier G. Airway responsiveness and bronchial-wall thickness in asthma with or without fixed airflow obstruction. *Am J Respir Crit Care Med* 1995;152:865-71.
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. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. *Am J Respir Crit Care Med* 1999;159:1043-51.
25. Chetta A, Zanini A, Foresi A, Del Donno M, Castagnaro A, D'Ipollito R, et al. Vascular component of airway remodeling in asthma is reduced

- by high dose of fluticasone. *Am J Respir Crit Care Med* 2003;167:751-7.
26. Fouty B, Moss T, Solodushko V, Kraft M. Dexamethasone can stimulate G1-S phase transition in human airway fibroblasts in asthma. *Eur Respir J* 2006;27:1160-7.
 27. Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17:326-33.
 28. Saglani S, Molyneux C, Gong H, Rogers A, Malmstrom K, Pelkonen A, et al. Ultrastructure of the reticular basement membrane in asthmatic adults, children and infants. *Eur Respir J* 2006;28:505-12.
 29. Milanese M, Crimi E, Scordamaglia A, Riccio A, Pellegrino R, Canonica GW, et al. On the functional consequences of bronchial basement membrane thickening. *J Appl Physiol* 2001;91:1035-40.
 30. Corsico A, Milanese M, Baraldo S, Casoni GL, Papi A, Riccio AM, et al. Small airway morphology and lung function in the transition from normality to chronic airway obstruction. *J Appl Physiol* 2003;95:441-7; discussion 35.
 31. Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, et al. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003;167:418-24.
 32. Elston WJ, Whittaker AJ, Khan LN, Flood-Page P, Ramsay C, Jeffery PK, et al. Safety of research bronchoscopy, biopsy and bronchoalveolar lavage in asthma. *Eur Respir J* 2004;24:375-7.
 33. Deykin A, Massaro AF, Drazen JM, Israel E. Exhaled nitric oxide as a diagnostic test for asthma: online versus offline techniques and effect of flow rate. *Am J Respir Crit Care Med* 2002;165:1597-601.