

Bronchial Smooth Muscle Remodeling in Nonsevere Asthma

Pierre-Olivier Girodet^{1,2,3}, Benoit Allard^{1,2}, Matthieu Thumerel^{1,2,3}, Hugues Begueret³, Isabelle Dupin^{1,2}, Olga Ousova^{1,2}, Régis Lassalle^{1,2}, Elise Maurat^{1,2}, Annaig Ozier^{1,2,3}, Thomas Trian^{1,2}, Roger Marthan^{1,2,3}, and Patrick Berger^{1,2,3}

¹University Bordeaux, Centre de Recherche Cardio-thoracique de Bordeaux, U1045, Département de Pharmacologie, CIC1401, Bordeaux, France; ²INSERM, Centre de Recherche Cardio-thoracique de Bordeaux, U1045, CIC1401, Bordeaux, France; and ³CHU de Bordeaux, Service d'Exploration Fonctionnelle Respiratoire, CIC1401, Service d'Anatomopathologie, Pessac, France

Abstract

Rationale: Increased bronchial smooth muscle (BSM) mass is a key feature of airway remodeling that classically distinguishes severe from nonsevere asthma. Proliferation of BSM cells involves a specific mitochondria-dependent pathway in individuals with severe asthma. However, BSM remodeling and mitochondrial biogenesis have not been examined in nonsevere asthma.

Objectives: We aimed to assess whether an increase in BSM mass was also implicated in nonsevere asthma and its relationship with mitochondria and clinical outcomes.

Methods: We enrolled 34 never-smoker subjects with nonsevere asthma. In addition, we recruited 56 subjects with nonsevere asthma and 19 subjects with severe asthma as comparative groups (COBRA cohort [Cohorte Obstruction Bronchique et Asthme; Bronchial Obstruction and Asthma Cohort; sponsored by the French National Institute of Health and Medical Research, INSERM]). A phenotypic characterization was performed using questionnaires, atopy and pulmonary function testing, exhaled nitric oxide measurement, and blood collection. Bronchial biopsy specimens were processed for immunohistochemistry and electron microscopy analysis. After BSM remodeling assessment, subjects were monitored over a 12-month period.

Measurements and Main Results: We identified characteristic features of remodeling (BSM area >26.6%) and increased mitochondrial number within BSM in a subgroup of subjects with nonsevere asthma. The number of BSM mitochondria was positively correlated with BSM

area ($r = 0.78$; $P < 0.001$). Follow-up analysis showed that subjects with asthma with high BSM had worse asthma control and a higher rate of exacerbations per year compared with subjects with low BSM.

Conclusions: This study reveals that BSM remodeling and mitochondrial biogenesis may play a critical role in the natural history of nonsevere asthma (Mitasthme study).

Clinical trial registered with www.clinicaltrials.gov (NCT00808730).

Keywords: asthma; exacerbation; mitochondria; remodeling; smooth muscle

At a Glance Commentary

Scientific Knowledge on the Subject: Bronchial smooth muscle remodeling and mitochondrial biogenesis are implicated in severe asthma pathogenesis. However, these processes have not yet been explored in nonsevere asthma.

What This Study Adds to the Field: Bronchial smooth muscle remodeling and enhanced mitochondrial biogenesis are present in some individuals with nonsevere asthma. Increased bronchial smooth muscle mass in patients with nonsevere asthma is related to a higher rate of exacerbations and worse asthma control. Eosinophilia and early onset of nonsevere asthma are not associated with increased exacerbation frequency and uncontrolled asthma.

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Correspondence and requests for reprints should be addressed to Patrick Berger, M.D., Ph.D., Centre de Recherche Cardio-thoracique de Bordeaux, INSERM, U1045, Université de Bordeaux, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France. E-mail: patrick.berger@u-bordeaux.fr

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Asthma is a heterogeneous inflammatory disease characterized by variable respiratory symptoms associated with the presence of bronchial hyperresponsiveness and bronchial remodeling (1). Bronchial remodeling is a complex process related to various pathological pathways, the onset of which can occur very early in the asthmatic life, concomitantly with the inflammatory process (2). It consists in an abnormal increased thickening of the bronchial wall due to various structural alterations, including abnormal epithelium, subepithelial membrane thickening, enhanced deposition of the extracellular matrix, neoangiogenesis, mucus gland hypertrophy, and increased bronchial smooth muscle (BSM) mass (1). Among these various alterations, marked increased BSM mass is the key structural change that distinguishes severe asthma from moderate asthma (3). The amount of BSM mass is correlated with a decrease in lung function (4). Moreover, in individuals with severe asthma, the increased BSM mass has been associated with chronic airflow obstruction (5). Among various potential mechanisms, the increased BSM mass has been associated with an increased proliferation of asthmatic BSM cells both *in vitro* (6–8) and *in vivo* (4). *In vitro*, the increased proliferation of asthmatic BSM cells has been related to an increased mitochondrial biogenesis, in severe asthmatic BSM cells only (8). *In vivo*, the BSM of individuals with severe asthma is also characterized by an increased number of mitochondria per surface area and by an increased density of mitochondria, which reflects mitochondrial activity (8). However, the presence of BSM remodeling in individuals with nonsevere asthma is probably underestimated, and the relationships between BSM mass and various clinical, functional, and inflammatory outcomes remain largely unknown in this population with asthma. Along the same line, the number of mitochondria in the BSM of individuals with nonsevere asthma has not been evaluated.

We hypothesized that the natural history of nonsevere asthma could be influenced not only by the inflammatory process but also by this key feature of bronchial remodeling (i.e., increased BSM mass). To test this hypothesis, we have quantified BSM mass in a population with nonsevere asthma that includes subjects with variable intensity of bronchial

remodeling. The aim of this study was thus to assess the potential role of BSM remodeling in nonsevere asthma.

Methods

Patients and Samples

A total of 34 patients with nonsevere asthma were enrolled in the Mitasthme trial from the Centre Hospitalier Universitaire, Bordeaux, France (see Figure E1 in the online supplement). The study received approval from the local ethics committee and has been registered under the No. NCT00808730 at ClinicalTrials.gov. All patients had bronchial hyperresponsiveness (9) and were graded as having nonsevere asthma according to GINA (Global Initiative for Asthma) (i.e., GINA steps 1, 2, or 3) (10). Three visits were scheduled (see Table E1): visit 1 (inclusion), visit 2 (bronchoscopy), and visit 3 (12 months $\pm$ 7 days after visit 2).

Bronchial specimens from 30 subjects were obtained by fiber-optic bronchoscopy, as described previously (8). For all patients, we ascertained age at onset of asthma, Juniper Asthma Control Questionnaire (ACQ) score (11), spirometry, fractional exhaled nitric oxide (F_{ENO}), serum total IgE, and blood eosinophils assessed within the week before the fiber-optic bronchoscopy.

In addition, 56 subjects with nonsevere asthma were recruited from the COBRA cohort (Cohorte Obstruction Bronchique et Asthme; Bronchial Obstruction and Asthma Cohort; sponsored by the French National Institute of Health and Medical Research, INSERM), as a second comparative group, using the same inclusion criteria. Patients from the Mitasthme study were also finally compared with a third group of 19 subjects with severe asthma (12), who were also recruited from the COBRA cohort and who underwent fiber-optic bronchoscopy. Details of inclusion and exclusion criteria are provided in the online supplement.

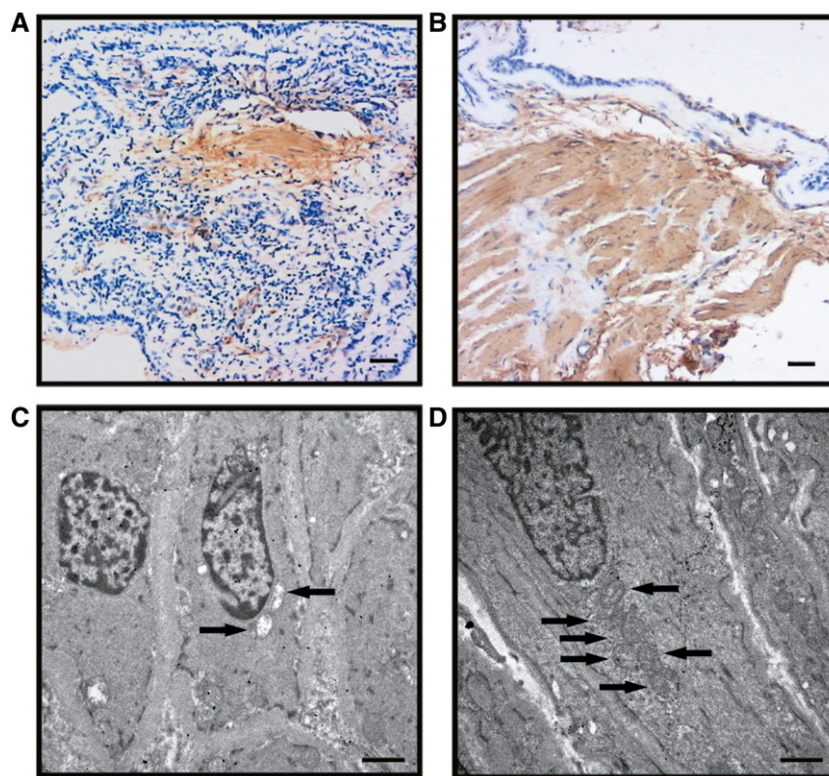


Figure 1. Bronchial smooth muscle (BSM) remodeling and mitochondrial biogenesis in nonsevere asthma. (A and B) Representative optic microscopic images from bronchial sections stained with antibody anti-actin and (C and D) representative electronic microscopic images were obtained from two patients with nonsevere asthma with low BSM (A and C) or high BSM mass (B and D). Some smooth muscle mitochondria were visualized (arrows). Bronchial wall from a subject with low BSM shows a dense inflammatory infiltrate (A). (A and B) Magnification, $\times 100$; scale bars, 30 μm . (C and D) Magnification, $\times 4,200$; scale bars, 1 μm .

Twelve months after the inclusion visit, the number of asthma exacerbations, as-needed bronchodilator use (short-acting β_2 -agonist [SABA]), ACQ score, respiratory function, and airway inflammation were assessed. Asthma exacerbations were defined as events of worsening asthma (10) that required a short course of oral or parenteral corticosteroids for 5 to 10 days. We documented the occurrence of exacerbation using medical records. Subject verbal reports were not acceptable.

Optical Microscopy and Immunohistochemistry

Bronchial specimens were fixed in 10% buffered formalin and processed in standard fashion for paraffin embedding as described previously (8, 13). The total area of the BSM layer was assessed manually in a blinded fashion using Quancoul software (QuantImage, Bordeaux, France) at a

magnification of $\times 100$ as described previously (8, 14–16). This BSM area was normalized to the whole area of the corresponding tissues and presented as percentages of the whole area, as described previously (17). Three biopsies were collected for each patient, and the BSM was analyzed in six to eight sections in each biopsy.

Electron Microscopy

Specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon as described previously (18).

Statistical Analysis

Comparisons between the two groups were performed using two-sided independent *t* tests for variables with a parametric distribution, Fisher's exact test for comparison of proportions, or the Mann-Whitney *U* test for comparison of

nonparametric variables. Exacerbation frequency was calculated and compared among the study groups using a negative binomial model, as previously described (17). A Pearson correlation matrix was built. Stepwise multiple linear regressions were performed to determine the parameters that significantly influenced BSM area. Comparisons between data obtained in the Mitasthme trial and those from the COBRA cohort are considered to be *post hoc* analyses. Values are presented as mean $\pm$ SEM. Results were considered significant at a *P* value of less than 0.05.

Results

Detection of BSM Remodeling in Patients with Nonsevere Asthma

Of the 30 bronchoscopies performed in the patients with nonsevere asthma in the

Table 1. Demographic and Baseline Characteristics of the Patients

	Low BSM Group	High BSM Group	<i>P</i> Values
N	14	14	
Age, yr	33.8 $\pm$ 4.0	33.0 $\pm$ 3.4	0.88
Sex, M/F	6/8	5/9	1.00
Score on Juniper Asthma Control Questionnaire	0.5 $\pm$ 0.1	0.8 $\pm$ 0.2	0.06
Age at onset of symptoms			
Early/late	9/5	9/5	1.00
Years	10.2 $\pm$ 2.7	13.1 $\pm$ 3.9	0.87
Asthma duration, yr	24.3 $\pm$ 3.7	20.5 $\pm$ 3.1	0.63
Atopic status, Y/N	10/4	13/1	0.33
Total IgE, U/ml	499 $\pm$ 234	868 $\pm$ 592	0.45
High eosinophil status, Y/N	6/8	8/6	0.71
Blood eosinophil count, $\times 10^9/L$	0.21 $\pm$ 0.04	0.29 $\pm$ 0.06	0.36
FE_{NO} , ppb	36.5 $\pm$ 10.0	37.0 $\pm$ 6.3	0.29
Chronic airflow obstruction, Y/N	0/14	0/14	1.00
Prebronchodilator FEV_1 , % of predicted value	91.9 $\pm$ 3.4	94.2 $\pm$ 3.7	0.66
$FEV_1:FVC$ ratio, %	75.7 $\pm$ 1.6	78.5 $\pm$ 2.1	0.29
PC_{20} for methacholine, mg	0.9 $\pm$ 0.3	0.9 $\pm$ 0.4	0.74
Obesity, Y/N	0/14	2/12	0.48
Body mass index, kg/m^2	23.3 $\pm$ 0.9	25.4 $\pm$ 2.3	0.80
Smoking status			
Current smoker, Y/N	0/14	0/14	1.00
Ex-smoker, Y/N	0/14	0/14	1.00
Pack-years	0	0	
Frequent exacerbations > 2/yr, Y/N	0/14	0/14	1.00
Severe exacerbations per subject in previous year, n	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.29
Treatment			
Dose of ICS, beclomethasone equivalent, $\mu g/d$	732 $\pm$ 182	786 $\pm$ 167	0.83
Use of long-acting β -agonists, Y/N	8/6	8/6	1.00
Regular use of oral prednisolone, Y/N	0/14	0/14	1.00
Use of montelukast, Y/N	3/11	3/11	1.00
Use of omalizumab, Y/N	0/14	0/14	1.00

Definition of abbreviations: BSM = bronchial smooth muscle; FE_{NO} = fraction of nitric oxide in exhaled air, ICS = inhaled corticosteroid; N = no; PC_{20} = provocative concentration of inhaled methacholine required to lower the FEV_1 by 20%; Y = yes.

Plus/minus values are means $\pm$ SEM.

Early onset of asthma: <12 years; late onset of asthma: ≥ 12 years. High eosinophil status: $FE_{NO} > 50$ ppb or blood eosinophils $> 0.3 \times 10^9/L$. Chronic airflow obstruction: FEV_1 measures persistently lower than 80% of predicted value. *P* values were calculated using a two-sided independent *t* test for variables with a parametric distribution, Fisher's exact test for comparison of proportions, and the Mann-Whitney *U* test for comparison of nonparametric variables.

Mitasthme study, BSM was assessable in 28 patients. The median BSM normalized area was 26.6%. Patients were thus divided into high BSM ($>26.6\%$; $n = 14$) and low BSM ($<26.6\%$; $n = 14$) groups (Figures 1A and 1B). Between and within-biopsies variabilities in normalized BSM area were similar in high BSM versus low BSM groups (coefficients of variation were 0.32 vs. 0.34; $P = 0.45$). The clinical and functional characteristics of these two BSM groups are presented in Table 1. Patients in the both low and high BSM groups were similar for all initially tested parameters, and in particular, age at onset of symptoms, asthma duration, eosinophils status, respiratory function, and asthma treatment.

Increased Mitochondrial Number and Density in Nonsevere Asthma

The number and the density of BSM mitochondria (Figures 1C and 1D) were increased in the high BSM group compared with the low BSM group (Figures 2A and 2C). In addition, the number and the density of BSM mitochondria were both significantly and positively correlated with the BSM area within the whole population with nonsevere asthma (Figures 2B and 2D). This relationship between BSM mass and mitochondrial density is in agreement with results obtained in individuals with severe asthma. Both BSM area and the number of mitochondria within BSM cells were significantly increased in severe

asthma compared with patients from the Mitasthme trial (see Table E2).

BSM Remodeling and Progression of Nonsevere Asthma

We next evaluated whether the presence of high BSM could define specific subsequent clinical outcomes. The number of exacerbations for the 12 following months (Figures 3A and 3B) was increased 12 months after the fiber-optic bronchoscopy in the high BSM group compared with the low BSM group (relative risk, 5.50; 95% confidence interval, 1.551–19.503; $P = 0.008$) (Figure 3B). Interestingly, the BSM area was significantly correlated with the number of exacerbations, which subsequently occurred within the following 12 months after the fiber-optic bronchoscopy (Figure 3C). The ACQ score (Figure 3D) and the mean SABA use per week (Figure 3E) were also increased in the high BSM group, but there was no difference in terms of FE_{NO} (Figure 3F) or FEV_1 (data not shown).

Key Role of BSM Cell Mitochondria in Nonsevere Asthma

To further explore the role of BSM mass variability in subjects with nonsevere asthma, several clinical and/or functional parameters were analyzed in a correlation matrix (Table 2). Then, these variables were entered into a stepwise multiple regression analysis to determine both the best model and the strength of each individual parameter to predict BSM area (Table 3). We first removed the independent variables with significant cross correlations from the models. We found that the best model associated the number of mitochondria and the initial ACQ score, which explained 69% ($R^2 = 0.69$) of the variation in this measurement (Table 3). However, of these variables, the number of mitochondria explained 55% of the variation alone, whereas the initial ACQ score only explained 14%.

Clinical Asthma Phenotypes and Progression of Nonsevere Asthma

When differentiating patients with nonsevere asthma from the Mitasthme trial in terms of early versus late onset asthma, no prospective outcome was significantly different at year 1 after the inclusion visit (see Figure E2). Moreover, when differentiating patients with asthma in terms of eosinophilic versus

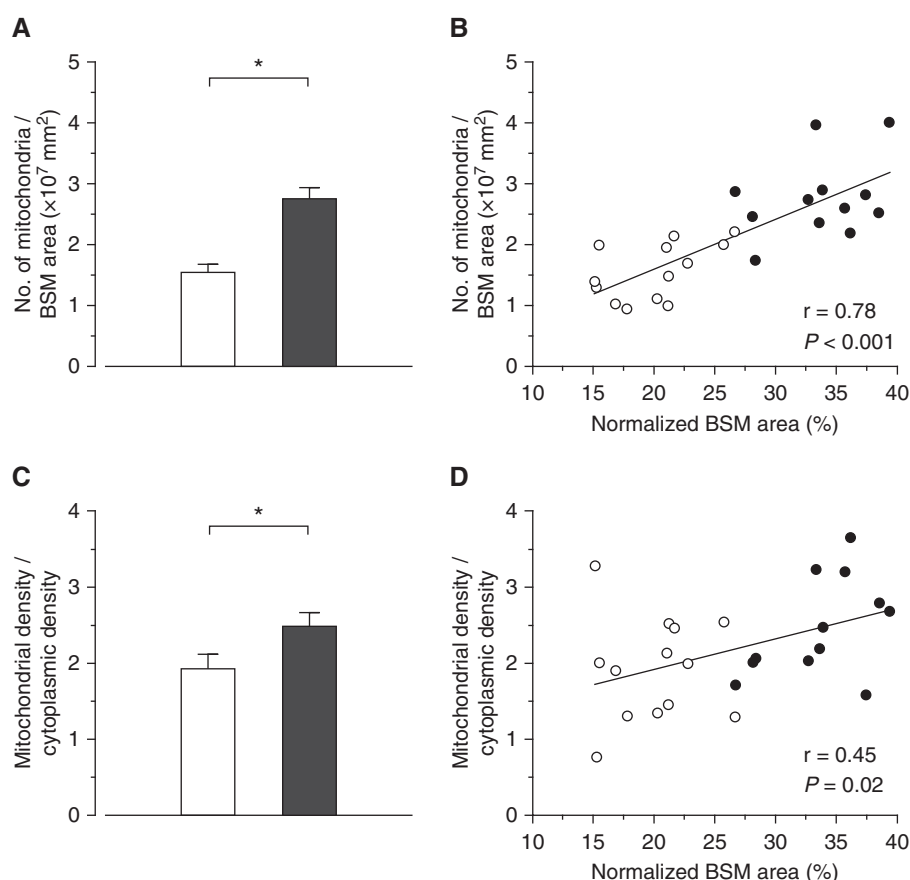


Figure 2. Bronchial smooth muscle (BSM) remodeling is associated with an increased mitochondrial number and density in patients with nonsevere asthma. Bronchial specimens were obtained from patients with nonsevere asthma with low BSM (BSM area $<26.6\%$; $n = 14$; open bars) or high BSM mass (BSM area $>26.6\%$; $n = 14$; solid bars). (A) The number and (C) the density of mitochondria were assessed from electronic microscopic images. Data are the mean $\pm$ SEM. $*P < 0.05$ using Student's t tests. (B and D) Scatterplots show correlation between normalized BSM area and mitochondrial biogenesis parameters. The normalized area of BSM layer was assessed using Quancoul software on bronchial sections at a magnification of $\times 100$. The BSM area was normalized by the whole area of the corresponding tissues and presented as percentages of whole area. Open circles: low-BSM group; solid circles: high-BSM group. r = Pearson correlation coefficient.

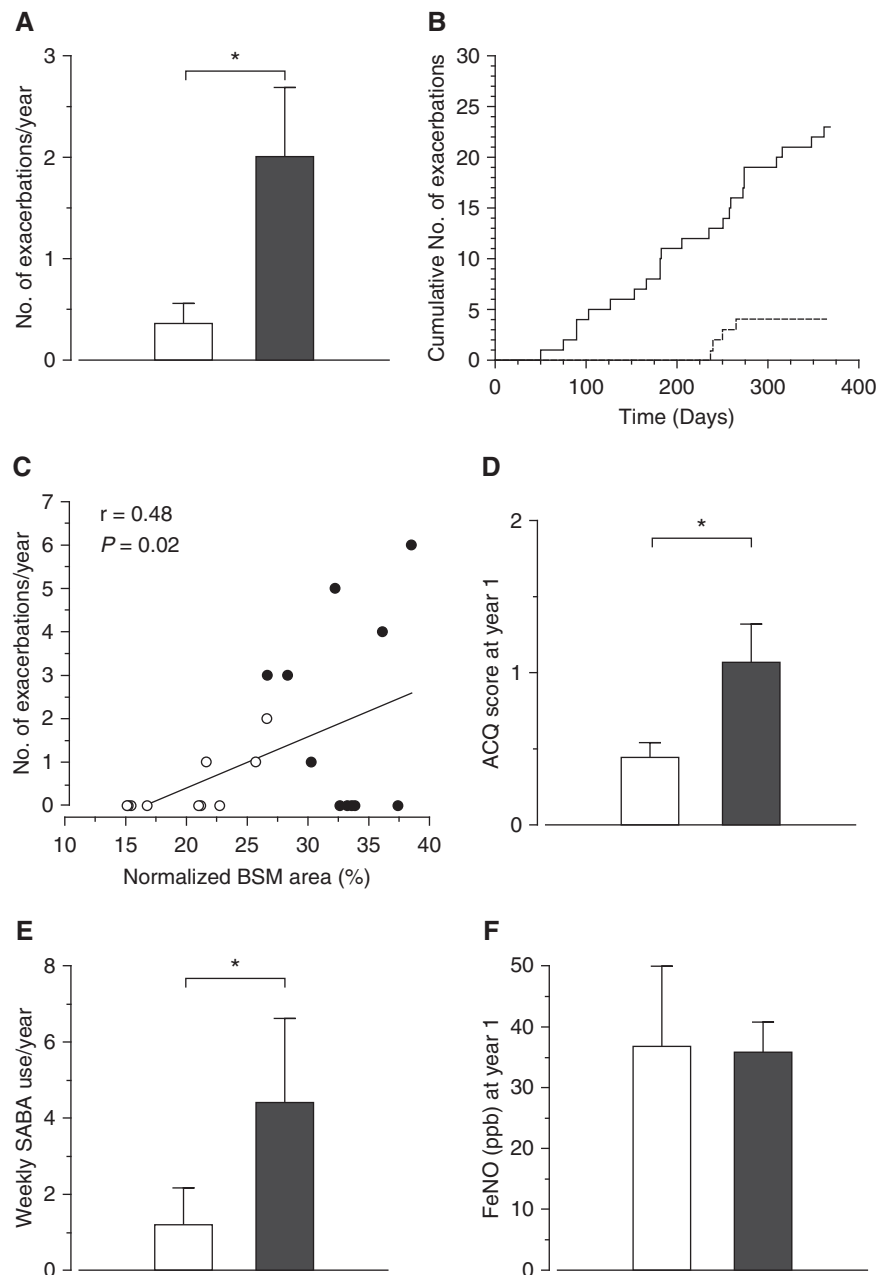


Figure 3. Impact of bronchial smooth muscle (BSM) remodeling on exacerbation frequency and control in nonsevere asthma. (A and B) At year 1 after the fiber-optic bronchoscopy, patients with nonsevere asthma with high BSM mass ($n = 12$) had higher numbers of exacerbations compared with subjects with low BSM mass ($n = 12$). (C) Scatterplot shows correlation between normalized BSM area and number of exacerbations per 12 months. (D–F) Patients with asthma with high BSM had lower asthma control (D and E) and similar levels of airway inflammation (F) compared with patients with low BSM. Low BSM group: BSM area $<26.6\%$ (open bars, dashed line, and open circles); high BSM group: BSM area $>26.6\%$ (solid bars, solid line, and solid circles). Short-acting β_2 -agonist (SABA) use is expressed as the number of puffs per week for 12 months. Data are mean $\pm$ SEM. * $P < 0.05$ using Mann-Whitney test. ACQ = Juniper Asthma Control Questionnaire; $FeNO$ = fractional exhaled nitric oxide; r = Pearson correlation coefficient.

noneosinophilic asthma, only $FeNO$ remained elevated in eosinophilic asthma (see Figure E3). Similar results were obtained in a larger sample of patients with

nonsevere asthma ($n = 56$) (see Table E3) from the COBRA cohort (see Figures E2 and E3). Thus, in contrast to BSM mass increase, eosinophilia was not associated

with higher exacerbation frequency and poorer asthma control in nonsevere asthma. Patients from the Mitasthme trial with total serum IgE level greater than 214 IU/mL (19) had lower asthma control at year 1 compared with patients with low total IgE levels (≤ 214 IU/mL), but this result was not reproduced in the COBRA cohort (see Figure E4).

Discussion

For the first time, this study demonstrates that a subgroup of patients with nonsevere asthma with BSM remodeling present with increased exacerbation frequency and worse asthma control compared with patients without BSM remodeling. Both BSM area and number of mitochondria from the patients with nonsevere asthma were lower than in those with severe asthma. Moreover, both the number of BSM mitochondria and the initial ACQ score could predict the variation of BSM mass.

The issue of the heterogeneous distribution of BSM mass within the bronchial tree must be first addressed. Jones and colleagues recently demonstrated that BSM area was different in large, medium, or small airways in patients with asthma, and that the coefficient of variation of BSM area between sections reached 9.1% in the large airways of subjects with asthma (20). In the present study, we paid special attention to averaging the BSM area from a variety of sections from each biopsy and from a variety of biopsies per patient. These biopsies were obtained by fiber-optic bronchoscopy from the same large airways in each patient. Bronchoscopy performed in patients with asthma was safe in our experience (8, 13, 15–17, 21). The use of bronchoscopy should thus be limited to expert centers, because measuring BSM area requires large forceps and dedicated pathologists. However, it remains invasive, and alternative biomarkers of BSM area are needed. Although there is no validated BSM biomarker, the circulating levels of YKL-40 (22) have been correlated with other aspects of bronchial remodeling (e.g., subepithelial membrane thickness). Periostin also contributes to subepithelial thickening in asthmatic airways (23), and its serum levels are related to greater decline in FEV_1 in patients with asthma on inhaled corticosteroid (ICS) treatment (24).

Table 2. Correlation between Bronchial Smooth Muscle Area and Multiple Variables

Parameter	BSM Area (P Value)
Number of mitochondria/BSM area, $\times 10^7/\text{mm}^2$	0.72 (0.0001)*
Score on Juniper Asthma Control Questionnaire	0.50 (0.007)*
Number of exacerbations per 12 mo	0.47 (0.03)*
SABA use, puffs/wk	0.45 (0.04)*

Definition of abbreviations: BSM = bronchial smooth muscle; SABA = short-acting β_2 -agonist. Data are Pearson correlation coefficient between BSM area and clinical variables or mitochondrial biogenesis (n = 28 patients with nonsevere asthma).

*P < 0.05.

One of the main interests of this study is to define a new nonsevere asthma group based on BSM mass (i.e., the key structural change in bronchial remodeling). Pathophysiological processes leading to increased BSM mass are complex and are still not completely understood (1). The involvement of mitochondria in the increased mass of BSM was first evidenced in our laboratory (8). Here, we show in the high BSM group of subjects with nonsevere asthma that both the number and the density of mitochondria were increased. It should be noted that the number of mitochondria was normalized to the BSM area, and that the density of BSM mitochondria is known to be related to mitochondrial activity (8). These results can thus be related to the increased number and density of BSM mitochondria that we previously reported in the BSM of patients with severe asthma (8). In such patients, the increased mitochondrial biogenesis was a key event leading to the increased BSM cell proliferation and BSM remodeling (8). Thus, our results suggest that this is also the case in patients with

nonsevere asthma. The number of mitochondria was correlated with the BSM area, and using multiple regression analysis, the number of mitochondria alone explained the majority of the variation in BSM area in nonsevere asthma. However, further *in vitro* studies are required to confirm that similar pathways are involved in nonsevere asthmatic BSM remodeling.

The high BSM group appears to be related to some specific clinical outcomes, such as the rate of future exacerbations. We assessed the number of asthma exacerbations per patient per year at two time points, before and 1 year after the bronchoscopy, similarly. Despite the lack of validated criteria, the definition of asthma exacerbations in our study included a physician-documented deterioration in symptoms, such as shortness of breath, cough, wheezing, or chest tightness (10). This definition also included use of systemic corticosteroids for 5 to 10 days to be consistent with American Thoracic Society/European Respiratory Society Task Force recommendations on retrospective assessment of asthma exacerbations (25).

The T-helper cell type 2 high eosinophilic asthma, which is one of the most studied endotypes (26), represents half of the population with asthma (27). It has been previously shown that, after ICS reduction, sputum eosinophil numbers increase before the onset of exacerbations (28). Moreover, management strategies based on controlling eosinophilic inflammation are associated with a reduction in the frequency of exacerbations (29, 30). In the present study, the rates of both previous and future exacerbations were similar in eosinophilic compared with noneosinophilic asthma in the Mitasthme trial. This result was confirmed in a second larger group of patients with nonsevere asthma (from the COBRA cohort), who were also prospectively followed for 1 year to confirm this absence of difference. Our focus has been on patients with nonsevere asthma, both in the Mitasthme trial and the COBRA cohort, whereas previous studies (29, 30) recruited patients with asthma of variable severity, a high proportion of whom had severe asthma (29). Moreover, the pathophysiology of asthma exacerbations is complex and probably involves multiple pro-inflammatory cells. Further studies are required to dissect the mechanisms that lead to exacerbations in individuals with nonsevere asthma, notably the role of eosinophils and its potential interaction with BSM cells.

Focusing on the amount of BSM mass raises the issue of bronchial thermoplasty, which is supposed to target BSM for the treatment of asthma (31). Although such a process has been shown to significantly reduce the rate of exacerbations and improve asthma control (31), the target population with asthma remains to be defined. From the present study, we demonstrate that defining a high BSM group of patients has clinical implications, even in nonsevere asthma. However, we did not demonstrate a clear causal relationship between BSM mass and the occurrence of future exacerbations. Further larger-scale clinical trials should be performed to determine which patients might preferentially benefit from bronchial thermoplasty or other processes that are able to decrease BSM size (17).

In conclusion, we have related the presence of BSM remodeling in patients with nonsevere asthma with an increased mitochondrial number. Patients with high BSM mass appear to experience specific

Table 3. Stepwise Multiple Linear Regression Analysis of Bronchial Smooth Muscle Area for Asthma Control and Mitochondrial Biogenesis

	BSM Area			
	r^2	β	SE	P Value
Number of mitochondria/BSM area ($\times 10^7/\text{mm}^2$)	0.55	7.17	1.15	0.0001
Score on Juniper ACQ at V1	0.14	0.60	0.27	0.04
Full model*	0.69	—	—	0.0001

Definition of abbreviations: ACQ = Asthma Control Questionnaire; BSM = bronchial smooth muscle; V1 = visit 1.

Dependent variable is BSM area. Independent variables are the number of mitochondria and the initial ACQ score assessed at V1 (n = 28 patients with nonsevere asthma). β is the regression coefficient. SE is the standard error of β .

*For the full model, the F ratio is 24.0, and the square root of the mean square error is 517.8 for BSM area. This model has been found to be the best in terms of r^2 , square root of the mean square error, and the F ratio.

important clinical outcomes, such as increased rate of future exacerbations. Noninvasive techniques for bronchial remodeling measurement are needed to distinguish individuals with low BSM from

individuals with high BSM, especially in nonsevere asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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References

- Girodet PO, Ozier A, Bara I, Tunon de Lara JM, Marthan R, Berger P. Airway remodeling in asthma: new mechanisms and potential for pharmacological intervention. *Pharmacol Ther* 2011;130:325–337.
- Turato G, Barbato A, Baraldo S, Zanin ME, Bazzan E, Lokar-Oliani K, Calabrese F, Panizzolo C, Snijders D, Maestrelli P, et al. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. *Am J Respir Crit Care Med* 2008;178:476–482.
- Pepe C, Foley S, Shannon J, Lemiere C, Olivenstein R, Ernst P, Ludwig MS, Martin JG, Hamid Q. Differences in airway remodeling between subjects with severe and moderate asthma. *J Allergy Clin Immunol* 2005;116:544–549.
- Ramos-Barbón D, Fraga-Iriso R, Brienza NS, Montero-Martínez C, Vereza-Hernando H, Olivenstein R, Lemiere C, Ernst P, Hamid QA, Martin JG. T cells localize with proliferating smooth muscle alpha-actin+ cell compartments in asthma. *Am J Respir Crit Care Med* 2010;182:317–324.
- Kaminska M, Foley S, Maghni K, Storness-Bliss C, Coxson H, Ghezzi H, Lemiere C, Olivenstein R, Ernst P, Hamid Q, et al. Airway remodeling in subjects with severe asthma with or without chronic persistent airflow obstruction. *J Allergy Clin Immunol* 2009;124:45–51.e1–e4.
- Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, Burgess JK, Black JL. Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001;164:474–477.
- Roth M, Johnson PR, Borger P, Bihl MP, Rüdiger JJ, King GG, Ge Q, Hostettler K, Burgess JK, Black JL, et al. Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. *N Engl J Med* 2004;351:560–574.
- Trian T, Benard G, Begueret H, Rossignol R, Girodet PO, Ghosh D, Ousova O, Vernejoux JM, Marthan R, Tunon-de-Lara JM, et al. Bronchial smooth muscle remodeling involves calcium-dependent enhanced mitochondrial biogenesis in asthma. *J Exp Med* 2007;204:3173–3181.
- Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, et al. Guidelines for methacholine and exercise challenge testing—1999. *Am J Respir Crit Care Med* 2000;161:309–329.
- Global Initiative for Asthma. Global strategy for asthma management and prevention; 2012 [accessed 2015 Sep 30]. Available from: <http://www.ginasthma.org>
- Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14:902–907.
- American Thoracic Society. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am J Respir Crit Care Med* 2000;162:2341–2351.
- Bara I, Ozier A, Girodet PO, Carvalho G, Cattiaux J, Begueret H, Thumerel M, Ousova O, Kolbeck R, Coyle AJ, et al. Role of YKL-40 in bronchial smooth muscle remodeling in asthma. *Am J Respir Crit Care Med* 2012;185:715–722.
- Berger P, Compton SJ, Molimard M, Walls AF, N'Guyen C, Marthan R, Tunon-De-Lara JM. Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. *Clin Exp Allergy* 1999;29:804–812.
- Berger P, Girodet PO, Begueret H, Ousova O, Perng DW, Marthan R, Walls AF, Tunon de Lara JM. Tryptase-stimulated human airway smooth muscle cells induce cytokine synthesis and mast cell chemotaxis. *FASEB J* 2003;17:2139–2141.
- El-Shazly A, Berger P, Girodet PO, Ousova O, Fayon M, Vernejoux JM, Marthan R, Tunon-de-Lara JM. Fraktalkine produced by airway smooth muscle cells contributes to mast cell recruitment in asthma. *J Immunol* 2006;176:1860–1868.
- Girodet PO, Dournes G, Thumerel M, Begueret H, Dos Santos P, Ozier A, Dupin I, Trian T, Montaudon M, Laurent F, et al. Calcium channel blocker reduces airway remodeling in severe asthma. A proof-of-concept study. *Am J Respir Crit Care Med* 2015;191:876–883.
- Begueret H, Berger P, Vernejoux JM, Dubuisson L, Marthan R, Tunon-de-Lara JM. Inflammation of bronchial smooth muscle in allergic asthma. *Thorax* 2007;62:8–15.
- Martins TB, Bandhauer ME, Bunker AM, Roberts WL, Hill HR. New childhood and adult reference intervals for total IgE. *J Allergy Clin Immunol* 2014;133:589–591.
- Jones RL, Elliot JG, James AL. Estimating airway smooth muscle cell volume and number in airway sections. Sources of variability. *Am J Respir Cell Mol Biol* 2014;50:246–252.
- Girodet PO, Ozier A, Trian T, Begueret H, Ousova O, Vernejoux JM, Chanez P, Marthan R, Berger P, Tunon de Lara JM. Mast cell adhesion to bronchial smooth muscle in asthma specifically depends on CD51 and CD44 variant 6. *Allergy* 2010;65:1004–1012.
- Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007;357:2016–2027.
- Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, Muller SJ, Fahy JV. Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci USA* 2010;107:14170–14175.
- Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, Kuwabara K, Tomii K, Otsuka K, Fujimura M, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;132:305–312.e3.
- Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, et al.; American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;180:59–99.
- Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;18:716–725.
- Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, Koth LL, Arron JR, Fahy JV. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388–395.
- Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. *Am J Respir Crit Care Med* 2000;161:64–72.
- Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715–1721.
- Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemièrre C, Pizzichini E, Cartier A, Hussack P, Goldsmith CH, Laviolette M, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006;27:483–494.
- Cox G, Thomson NC, Rubin AS, Niven RM, Corris PA, Siersted HC, Olivenstein R, Pavord ID, McCormack D, Chaudhuri R, et al.; AIR Trial Study Group. Asthma control during the year after bronchial thermoplasty. *N Engl J Med* 2007;356:1327–1337.