Bronchial smooth muscle remodelling impacts the exacerbation frequency of severe preschool wheezers

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May 12, 2023

Abstract

INTRODUCTION: Exacerbations in preschool wheezers increase the risk of impaired lung function and asthma persistence at school age. Bronchial remodelling-based latent classes identify severe preschool wheezers at increased risk of frequent exacerbations (>3) but failed to distinguish those without exacerbation from those with low exacerbations rate (1-2 exacerbations) in the year following bronchoscopy. We thus aimed to identify further independent factors associated with no, low or high exacerbation rates. **METHODS:** In this *post-hoc* analysis, 80 severe preschool wheezers from the "P'tit Asthme" and "RESPIRE" studies were divided into 3 groups: No-Ex (0 exacerbation in the year following the bronchoscopy, n=20), Low-Ex (1-2 exacerbations, n=27) and High-Ex ([?]3 exacerbations, n=33). Associations between variables and groups were assessed using multinomial logistic regressions. **RESULTS:** Atopic dermatitis, age at the first wheezing episode, *Haemophilus* and *Streptococcus* genera in the bronchoalveolar lavage fluid (BALF), bronchial smooth muscle (BSM) area, reticular basement membrane (RBM) thickness and RBM-BSM distance were all significantly different between No-Ex and/or Low-Ex and/or High-Ex. However, only atopic dermatitis, age at first episode of wheezing, *Haemophilus* genus in the BALF, RBM-BSM distance and BSM area were significantly and independently associated with exacerbation frequency. Among them, the BSM area was the sole parameter independently associated with each group. **CONCLUSION:** While atopic dermatitis, age at the first episode of wheezing, *Haemophilus* in BALF, RBM-BSM distance and BSM area appeared to be relevant independent parameters associated with exacerbation susceptibility in severe preschool wheezers, only the increased BSM area discriminated between each of the three-exacerbation frequency-based groups.

Bronchial smooth muscle remodelling impacts the exacerbation frequency of severe preschool wheezers

Brief title: Exacerbation risk in preschool wheezers

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Manuscript word count: 3264/3500 words

Number of figures: 1; Number of tables: 4

ACKNOWLEDGEMENTS

The authors thank the staff from the clinical investigation center (CIC), the pathology and microbiology departments of the university hospital of Bordeaux (CHU de Bordeaux).

This study has received a research grant from the French Ministry of Health n° B101222-30; 2010-A01134-35, and the "Fondation Bordeaux Université (Fonds FGLMR/AVAD pour les Maladies Chroniques)". Clinical Trial n°: NCT02806466 and NCT04558671.

ABSTRACT

INTRODUCTION: Exacerbations in preschool wheezers increase the risk of impaired lung function and asthma persistence at school age. Bronchial remodelling-based latent classes identify severe preschool wheezers at increased risk of frequent exacerbations (> 3) but failed to distinguish those without exacerbation from those with low exacerbations rate (1-2 exacerbations) in the year following bronchoscopy. We thus aimed to identify further independent factors associated with no, low or high exacerbation rates.

METHODS: In this *post-hoc* analysis, 80 severe preschool wheezers from the "P'tit Asthme" and "RESPIRE" studies were divided into 3 groups: No-Ex (0 exacerbation in the year following the bronchoscopy,

n=20), Low-Ex (1-2 exacerbations, n=27) and High-Ex ([?]3 exacerbations, n=33). Associations between variables and groups were assessed using multinomial logistic regressions.

RESULTS: Atopic dermatitis, age at the first wheezing episode,*Haemophilus* and *Streptococcus* genera in the bronchoalveolar lavage fluid (BALF), bronchial smooth muscle (BSM) area, reticular basement membrane (RBM) thickness and RBM-BSM distance were all significantly different between No-Ex and/or Low-Ex and/or High-Ex. However, only atopic dermatitis, age at first episode of wheezing,*Haemophilus* genus in the BALF, RBM-BSM distance and BSM area were significantly and independently associated with exacerbation frequency. Among them, the BSM area was the sole parameter independently associated with each group.

CONCLUSION: While atopic dermatitis, age at the first episode of wheezing, *Haemophilus* in BALF, RBM-BSM distance and BSM area appeared to be relevant independent parameters associated with exacerbation susceptibility in severe preschool wheezers, only the increased BSM area discriminated between each of the three-exacerbation frequency-based groups.

Words: 250/250

Keywords: Asthma; Child; Atopic dermatitis; Haemophilus; bronchial remodelling, airway

INTRODUCTION

Wheezing during preschool age is a very common symptom which occurs at least once in about one third of children under 3 years of age and about a half under 6 years of age (1). Among these patients about 25% have recurrent preschool wheezing which increases the risk of asthma persistence at school age and altered lung function throughout life (2–4). Several risk factors for persistent recurrent wheezing have been identified, such as tobacco smoke exposure, the early onset of atopic dermatitis, allergic sensitization and/or the first episode of wheezing. However, the mechanisms are yet not fully understood (5).

Belgrave *et al.* reported that severe exacerbations within the first three years of life was predictive of persistent low lung function throughout life (3). In addition, Deliu *et al* . described an early-onset frequent exacerbation trajectory characterized by a persistent wheezing phenotype, an increased prevalence of atopic dermatitis at 1 and 3 years of age, decreased lung function at school age and an increased prevalence of asthma persistence at 16 years of age compared to preschool wheezers without exacerbations or those with infrequent exacerbation trajectories (6).

Exacerbations are frequently related to viral and/or bacterial infections (7), which involve most frequently respiratory syncytial viruses, rhinoviruses, *Moraxella catarrhalis* , *Haemophilus influenzae* or *Streptococcus pneumoniae* (8) in preschool wheezers. Moreover, bronchial dysbiosis which contributes to recurrent wheeze heterogeneity and severity independently of allergic sensitization (9,10), increases the risk of subsequent exacerbations in recurrent wheezing children (11). Indeed, a longitudinal study demonstrated that appropriate antibiotics course administered when bronchoalveolar lavage fluid (BALF) bacterial culture was positive decreased wheeze-related frequency for at least 6 months (11).

Previous studies also demonstrated that the risk of subsequent exacerbations in preschool wheezers was increased in patients previously hospitalized for severe wheezing exacerbation (12,13). Using bronchial remodelling-based latent class analysis, we recently identified two classes of severe preschool wheezers, one being characterized by a decreased reticular basement membrane (RBM) to bronchial smooth muscle (BSM) distance, submucosal fibrosis area, mucus gland area, and an increased BSM area, density of blood vessels, and RBM thickness compared to the other (14). This class was at increased risk of frequent subsequent exacerbations (*i.e* ., 3 or more in the year following fibreoptic bronchoscopy) despite not being significantly different from the second class in terms of clinical, biological or BALF parameters at baseline (14). However, even if our bronchial remodelling-based latent classes identified patients at increased risk of subsequent exacerbations, it failed to distinguish patients without exacerbations or those with infrequent exacerbations (*i.e.* , one or two) in the year following fibreoptic bronchoscopy (14).

We thus hypothesized that several additional parameters to those of bronchial remodelling, may be inde-

pendently associated with short-term exacerbation frequency. The objective was then to identify clinical, biological and bronchial remodelling parameters independently associated with exacerbation frequency group membership in the year following fibreoptic bronchoscopy.

METHODS

Study design

This post-hoc study pooled the eighty severe preschool children (1-5 years old) with analysable bronchial biopsies from the "P'tit Asthme" and the "RESPIRE" studies (Ndeg NCT02806466 and NCT04558671 at*ClinicalTrials.gov*) (14). The design, inclusion criteria, and procedures for these studies have been previously described (14). Briefly, for all patients, clinical (sex, age, body mass index, birth history, personal history (age at first episode of wheezing, birth weight and gestational age), tobacco exposure, atopic dermatitis, allergic sensitization, gastroesophageal reflux, parental history of atopy and treatment), biological (total blood immunoglobin E & eosinophils) and fibreoptic bronchoscopy parameters (BALF cytology, BALF microbiology (positive bacterial cultures and viral multiplex PCR identification), bronchial remodelling parameters (RBM-BSM distance, submucosal fibrosis area, mucus gland area, epithelial integrity, density of blood vessels, BSM area and RBM thickness) and density of inflammatory cells (neutrophils, eosinophils, lymphocytes, mast cells and macrophages) in bronchial biopsies were assessed. Patients were then followed-up for one year and the number of exacerbations (acute symptoms requiring oral corticosteroids burst), hospitalisations, emergency visits and treatments were collected (14). Patients were then divided into three groups according to the number of exacerbations in the year following fibreoptic bronchoscopy: "No-Ex" (no exacerbation), "Low-Ex" (one or two exacerbations) and "High-Ex" (three or more exacerbations).

Statistical Analyses

Results are expressed as absolute numbers with percentages for categorical variables and medians with interquartile ranges (IQR) (median [IQR_{25}; IQR_{75}]) for continuous variables. For categorical variables comparisons were performed using Chi-square tests between the three groups and in the case of a significant difference, the groups were compared two by two using Fisher's exact tests. For quantitative variables comparisons were performed using the Kruskal-Wallis test with Dunn's post-test. Correlations between parameters were performed using the Spearman test. Univariate multinomial logistic regression was used to identify the association between parameters and exacerbation frequency and the Odds ratio with its 95% confidence interval was determined. Then, parameters significantly associated with exacerbation frequency were included in a stepwise multiple multinomial logistic regression analysis to identify parameters independently and significantly associated with exacerbation frequency (15,16). Analyses were performed using SPSS software 29.0 (IBM Corp., Armonk, NY). A $p\text{-}value < 0.05$ was considered significant.

RESULTS

Patients' characteristics

During the year following the fibreoptic bronchoscopy, 20/80 (25.0%) preschool children had no exacerbation (No-Ex), 27/80 (33.7%) experienced 1 or 2 exacerbations (Low-Ex) and 33/80 (41.3%) experienced 3 or more exacerbations (High-Ex). The prevalence of atopic dermatitis was higher in the "High-Ex" than in the "Low-Ex" groups (Figure 1A) and the age at the first episode of wheezing was lower in the "High-Ex" than in both the "No-Ex" and "Low-Ex" groups (Figure 1B). Conversely, there was no significant difference between groups with respect to the other clinical and biological characteristics (Table 1).

Regarding the BALF parameters (Table E1), "No-Ex" had a lower prevalence of positive bacterial culture than both "Low-Ex" and "High-Ex" (Figure 1C). The genera identified in the BALF were in descending order of frequency *Haemophilus* , *Streptococcus* ,*Neisseria* , *Moraxella* , *Corynebacterium* ,*Staphylococcus* and *Pseudomonas* (Table E1). The prevalence of *Haemophilus sp* . was significantly more elevated in "Low-Ex" than in "High-Ex" than in "No-Ex" (Figure 1D) and the prevalence of *Streptococcus sp.* was significantly higher in both "Low-Ex" and "High-Ex" than in "No-Ex" (Figure 1E). At the species level, the most frequently identified bacteria were in descending order of frequency *Haεμοπηλυς ινφλυενζαε, Στρεπτοςοσσυς*

α-ηεμολιτισυς, Ηαεμοπηλυς παραινφλυενζα, Μοραξελλα ςαταρρηαλις, Στρεπτοςοςςυς πνευμονιαε, Στρεπτοςοςςυς οραλις/μιτις (Table E1). Among these bacteria species, only the prevalence of *Streptococcus pneumoniae* was significantly higher in "Low-Ex" than in "No-Ex" (Table E1). There was no significant difference between groups according to the BALF cytology (Table E1).

Regarding bronchial biopsies, RBM-BSM distance was significantly decreased (Figure 1F) whereas both the BSM area (Figure 1G) and the RBM thickness (Figure 1H) were significantly increased in High-Ex compared to the two other groups (Table E1). There was no significant difference between groups according to all other remodelling parameters or inflammatory cell densities on bronchial biopsies (Table E1).

Patients' follow-up within twelve months after fibreoptic bronchoscopy

Following fibreoptic bronchoscopy, patients from both the "Low-Ex" and "High-Ex" groups were more frequently treated by antibiotics than those from the "No-Ex" group without any difference between "Low-Ex" and "High-Ex" (Table 2). However, the percentages of patients with pathogenic bacteria in the BALF treated by antibiotics after fibreoptic bronchoscopy were not significantly different between the three groups (Table 2).

During the twelve months after fibreoptic bronchoscopy, the proportion of patients admitted at least once in the emergency department was increased in both "Low-Ex" and "High-Ex" vs. "No-Ex" and the proportion of patients requiring at least one hospitalization for wheeze were significantly increased in descending order of frequency in "High-Ex" compared to "Low-Ex" compared to "No-Ex" (Table 2). In addition, the proportion of patients requiring an increased daily dose of inhaled corticosteroids (ICS) was higher in "High-Ex" compared to the two other groups whereas the proportion of patients with unchanged daily dose of ICS was not significantly different between the three groups (Table 2).

During the follow-up period, there were no significant differences between groups according to the number of patients treated with ICS, leukotriene receptor antagonists, long-acting beta2-agonists, antihistamine agents (Table 2). By contrast, there were higher daily doses of ICS in "High-Ex" (1600µg [1000; 2000]) than in both "Low-Ex" (1000µg [800; 2000]) and "No-Ex" patients (1000 [425; 1000], $p<0.001$).

Univariate associations with exacerbation frequency groups during the year following fibreoptic bronchoscopy

Using univariate multinomial logistic regression, we identified the different parameters significantly associated with the different groups of patients. An early age at the first episode of wheezing, a decreased RBM-BSM distance, an increased BSM area and an increased RBM thickness were predictors of "High-Ex" (Table 3). The presence of atopic dermatitis (past history or current) and a low density of lymphocytes in the BALF were also predictors of "High-Ex" compared with "Low-Ex" and "No-Ex", respectively (Table 3). BAL neutrophilia (decreased percentage of macrophages and increased percentage of polymorphonuclear cells (PMN)) and the presence of the *Haemophilus* genus in the BALF were predictors of "Low-Ex". The absence of bacteria in the BALF and the absence of *Haemophilus* genus in the BALF were predictors of "No-Ex" membership (Table 3). Furthermore, the absence of hospitalizations within the year prior the bronchoscopy, the absence of antibiotic treatment after fibreoptic bronchoscopy, the absence of*Haemophilus influenzae* and *parainfluenza* ,*Streptococcus* genus or *Streptococcus pneumoniae* in the BALF were also predictors of "No-Ex" membership compared to "Low-Ex" only (Table 3). All other parameters were not significantly associated with any group membership (Table E2).

Multinomial logistic regression models associated with exacerbation frequency groups during the year following fibreoptic bronchoscopy

To identify parameters that were independently associated with exacerbation frequency during the year following the fibreoptic bronchoscopy, a stepwise multinomial regression model analysis was performed (Table E3). All parameters significantly associated with at least one of the three groups described in Table 3 were initially selected and the following parameters were included in the multinomial regression model

analysis: presence of atopic dermatitis (history or current), age at the first episode of wheezing, number of hospitalisations within the year, treatment with LABA at inclusion, the presence of *Haemophilus* genus in the BALF, the presence of*Streptococcus* genus in the BALF, RBM-BSM distance, sub-mucosal fibrosis area, BSM area, and RBM thickness (Table E3). However, parameters with more than 10% missing data (*i.e.* , Macrophages and PMN in the BALF as well as and density of lymphocytes in bronchial biopsies) were excluded, as previously proposed (15,16). Moreover, when two parameters were strongly associated with each other, only the most discriminating parameter between the groups was included in the analysis. The presence of bacteria, *Haemophilus influenzae* ,*Haemophilus parainfluenza* , *Streptococcus pneumonia* , all in the BALF and post fibreoptic antibiotic treatments were discarded from the model because already represent by *Haemophilus* or*Streptococcus* genera in the BALF. Indeed, 97% patients with positive bacteria isolated in the BALF and 95% of those who received a post-fiberoptic antibiotic treatment had at least either a*Haemophilus* or a *Streptococcus* genera bacteria. Macrophages/PMN count in the BALF as well as density of lymphocytes in bronchial biopsies were excluded from the model due to excessive missing values (19% and 35%, respectively).

The best model identified atopic dermatitis, age at first episode of wheezing, the presence of*Haemophilus* genera in the BALF, the RBM-BSM distance and the BSM area as factors significantly and independently associated with the exacerbation frequency in the year following the bronchoscopy (Table 4 & E3). This model was able to accurately identify the exacerbation frequency group membership in 94.3% of patients (Table E3). In addition, the risk factors independently associated with "Low-Ex" compared to "No-Ex" were the absence of atopic dermatitis, the presence of *Haemophilus* genus in the BALF and an increased in both the RBM-BSM distance and BSM area (Table 4). The risk factors independently associated with "High-Ex" compared to "No-Ex" were an early age at first episode of wheezing and an increased BSM area (Table 4). The risk factors independently associated with "High-Ex" compared to "Low-Ex" were the presence of atopic dermatitis, an early age at first episode of wheezing, the absence of *Haemophilus* genus in the BALF, a decrease in the RBM-BSM distance and an increased BSM area (Table 4). This analysis thus identified BSM area as the sole parameter independently and significantly associated with each exacerbation frequency group: the greater the BSM area, the higher the frequency of exacerbations (Table 4).

DISCUSSION

Taking together, these results identified independent factors associated with subsequent exacerbation frequency rates within the year following fibreoptic bronchoscopy in severe preschool wheezers. These factors include atopic dermatitis, age at first episode of wheezing, the presence of *Haemophilus* in the BALF, the RBM-BSM distance and the BSM area. The BSM area was the most important factor and was associated with each exacerbation frequency group.

The risk of subsequent exacerbations has been previously associated with a range of various parameters such as markers of uncontrolled disease (*i.e.* , uncontrolled symptoms of wheezing, at least one severe exacerbation in the previous year) in preschool wheezers (12,17). Thus, it was not surprising to note that three quarters of the patients in our study that included severe preschool children who experienced at least one exacerbation or one hospitalization for wheeze within a year prior to inclusion, continued to experience exacerbations during the follow-up year. Moreover, using univariate multinominal regression analysis, we identified a significant association between the number of hospitalizations within a year prior to inclusion and the exacerbation frequency in the follow-up year in agreement with previous reports (12,17).

The risk of exacerbations in preschool wheezers has also been associated with type-2 inflammation, highlighted by the presence of atopic dermatitis or allergic sensitizations, increased levels of blood eosinophils and/or exhaled NO levels (17–21). On the one hand, we do confirm, in the present study, that atopic dermatitis was an independent risk factor associated with the exacerbation frequency in severe preschool wheezers and that its prevalence was increased in the high exacerbation group in agreement with previous studies (17–19,21). However, the presence of atopic dermatitis was also associated with the no-exacerbation group. Therefore, this result suggest that isolated atopic dermatitis was not a risk factor of subsequent exacerbations but when combined with other exacerbation risk factors, its presence increased this risk. On

the other hand, previous studies demonstrated an increased risk of exacerbations in atopic and/or smoking mothers' infants with elevated exhaled NO levels after birth (20) and in preschool children with single (17) or early multiple allergic sensitizations (18,21) or increased blood eosinophils (19). However, we did not find any difference in exacerbation rate according to allergic sensitization or blood eosinophils levels. The association between exacerbation risk and blood eosinophil levels or allergic sensitization has previously been reported in populations including patients treated or not with ICS (17–19,21). In our study, all patients were treated at inclusion with high doses of ICS with a second controller which may have decreased the impact of both blood eosinophils level and allergic sensitization on the risk of exacerbations (22). Indeed, Fitzpatrick *et al* . showed that the risk of exacerbations was significantly decreased by ICS and abolished the statistical significance between the sensitized or blood eosinophils based groups (18,19).

Non-type 2 inflammation related parameters (*i.e.* , neutrophilic inflammation, isolation of bacteria on BALF culture) have also been associated with an increased risk of subsequent exacerbations (9,11). Despite no significant differences between exacerbation frequency-groups according to BALF and bronchial inflammation, the univariate analysis showed that a decreased percentage of macrophages and an increased percentage of PMN were significantly associated with infrequent exacerbations whereas a low density of lymphocytes in bronchial biopsies was a significant risk factor for frequent exacerbations. Unfortunately, these parameters were not included in the multivariate analysis due to the great number of missing values. We also demonstrated that bacteria in the BALF represent an independent risk factor for subsequent exacerbations but was mostly associated with a low annual exacerbation rate. This was consistent with studies reporting the efficacy of antibiotic or bacterial lysate therapies to decrease wheezing attacks rates and the use of oral corticosteroids compared to placebo (11,23,24).

In the present study, the risk of subsequent exacerbations was associated with several bronchial remodelling parameters (*i.e* ., submucosal fibrosis area, BSM area, RBM thickness, RBM-BSM distance) as previously reported (25–28). However, only the BSM area and the RBM-BSM distance appeared as independent risk factors of subsequent exacerbations in severe preschool wheezers using multivariate analysis. Moreover, it is interesting to note that the group with the highest exacerbation frequency is characterized by the greatest BSM area, the lowest RBM-BSM distance and the earliest age at first episode of wheezing. Thus, it is tempting to speculate that early-life respiratory tract infections promote BSM remodelling which in turn increased the risk of virus- and/or bacteria-induced exacerbation creating a vicious circle. Indeed, the first year of life appeared as a period of high susceptibility of BSM remodelling (29) characterized by an increased mitochondria-dependent BSM cell proliferation (30), which has been involved in BSM remodelling of both preschool wheezers (31) and adult asthma (32). Moreover, in adult asthma, we recently demonstrated an increased viral replication within the bronchial epithelium induced by the BSM which can increased exacerbation susceptibility (33) and in turn induced a specific BSM cell migration toward bronchial epithelium decreasing RBM-BSM distance (34). However, further mechanistic studies would be necessary to assess both the underlying mechanisms of increased BSM mass and the impact of BSM remodelling on viral replication in the specific population of preschool wheezers.

All these findings reinforce the usefulness of fibreoptic bronchoscopy in the specific population of severe preschool wheezers. Indeed, while it can be argued that fibreoptic bronchoscopy with bronchial biopsies is an invasive procedure requiring anaesthesia and of limited interest especially in such young children. Our study has, on the contrary, demonstrated its usefulness to provide information on bronchial inflammation, dysbiosis and remodelling and then on the risk of subsequent exacerbations. This well-tolerated examination allows for better characterization of the disease and helps the clinician to optimize the therapeutic strategy with a favourable benefit-risk ratio in severe preschool wheezing (14).

Several limits of the present study must be pointed out: (*i*) The relatively small number of patients limited the size of each group may have increased the risk of type 2 error in the group comparisons and the 95% confidences interval in the logistic regression analysis. In addition, the small size of our population has limited the maximal number of parameters included in the multinomial logistic regression model number. Indeed, some of the significant parameters in univariate analyses (*i.e.* , Macrophages and PMN in the BALF

as well as density of lymphocytes) had too many missing values in bronchial biopsies, and were assessed only in the P'tit Asthme" cohort (14). However, this pooled cohort represent the largest cohort describing both clinical and fibreoptic bronchoscopy parameters in severe preschool wheezers. (*ii*) As highlighted recently (35), our data did not include long term follow-up data (*i.e* ., more than one year) allowing for the assessment of the stability of exacerbation frequency over time and the consequences of bronchial remodelling on lung function and asthma persistence at school age. Additional, long term longitudinal studies are thus necessary.

CONCLUSION

This study highlights the importance of BSM remodelling in the susceptibility of severe preschool wheezers to present subsequent exacerbations. The combination of the level of BSM remodelling with atopic dermatitis, age at first wheeze and the presence of*Haemophilus* in the BALF allows to differentiate preschool wheezers from the three groups of exacerbation frequency (*i.e* ., No-Ex, Low-Ex and High-Ex). Mechanistic studies are required to better understand the pathophysiological mechanisms leading to BSM remodelling in this specific population as well as longitudinal studies to assess the long-term stability of such exacerbation groups and its consequences over time.

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TABLES

Table 1. Baseline patient characteristics according to the number of exacerbations during the year following flexible bronchoscopy

Variables
n
Male sex
Age (years)
Body mass index (Z-score)
Gestational age (weeks)
Birth weight (grams)
Post-natal cigarette smoke exposure
Parental history of atopy
History or current atopic dermatitis
Aeroallergen allergy/sensitization
Food allergy/sensitization
Gastroesophageal reflux
Multi-trigger wheezing
Disease duration (mo)
Age at 1st wheezing episode (mo)
Within a year prior to inclusion
No. of OCS bursts
3 OCS bursts
Hospitalized
No. of hospitalizations
Total IgE (kIU/L)

Blood eosinophils (/mm^3)
Blood eosinophils (%)
Drug History
Inhaled steroids
Daily dose (µg, eq. beclomethasone)
Montelukast
Long-acting beta-agonists
Antihistamine agents

Mo = month; No.= Number of; OCS= oral corticosteroids; IgE= Immunoglobulin E. Patients were divided according to the

Table 2. Follow-up patient characteristics according to the number of exacerbations during the post-fibreoptic bronchoscopy year

Variables
n
Within days following fibreoptic bronchoscopy
Patients treated by ATB
Pathogenic bacteria treated by ATB
During the 12 months following fibreoptic bronchoscopy
1 Hospitalization for wheeze
1 ED visit for wheeze
Increased daily dose of ICS
Unchanged daily dose of ICS
Decreased daily dose of ICS
12 months after fibreoptic bronchoscopy, number of patients treated with
ICS
Leukotriene receptor antagonists
Long-acting beta-agonists
Antihistamine agents

ATB= antibiotics; ICS= inhaled corticosteroids; eq. = equivalent, ED = Emergency department. Patients were divided acc

Table 3. Univariate analysis according to the number of exacerbations during the year following fibreoptic bronchoscopy

	No-Ex vs. Low-Ex OR (95% CI)	**No-Ex vs. Low-Ex** *p***1**	**No-Ex vs. High-Ex OR (95% CI)**	**No-Ex vs. High-Ex** *p***2**	**Low-Ex vs. High-Ex OR (95% CI)**	**Low-Ex vs. High-Ex** *p***3**	**p for overall model**
Atopic dermatitis (yes *vs* no)	0.001 (0.001; 0.207)	**0.011**	24.367 (0.113; 5268.368)	0.244	25420.421 (8.282; 78020.150)	**0.013**	**<0.001**
Age at 1st wheeze (per month increased)	1.115 (0.971; 1.281)	1.222	0.390 (0.174; 0.874)	**0.022**	0.350 (0.151; 0.811)	**0.014**	**<0.001**
Haemophilus genus in the BALF (yes *vs* no)	121.758 (4.836; 3065.791)	**0.004**	0.069 (0.001; 9.416)	0.287	0.001 (0.001; 0.231)	**0.015**	**0.020**
RBM-BSM distance (per 50µm increased)	6.895 (1.568; 30.321)	**0.011**	0.002 (0.000; 2.303)	0.085	0.001 (0.001; 0.479)	**0.031**	**<0.001**
Norm. BSM area (per 5% increased)	4.606 (1.398; 15.173)	**0.012**	91.105 (3.767; 2203.273)	**0.006**	19.778 (1.485; 263.461)	**0.024**	**<0.001**

BALF = Bronchoalveolar lavage fluid; PMN = Polymorphonuclear cells; RBM= Reticular basement membrane; BSM = Bronchial smooth muscle. Patients were divided according to the number of exacerbations in the year following the fibreoptic bronchoscopy in No-Ex (0 exacerbation, n=20), Low-Ex (1 or 2 exacerbations, n=27) and High-Ex ([?]3 exacerbations, n=33). Parameters associated with the number of exacerbations in the year following the fibreoptic bronchoscopy were determined using

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FIGURE LEGENDS

FIGURE 1. Clinical and bronchial characteristics according to the number of exacerbations in the year following fibreoptic bronchoscopy.

Patients were divided according to the number of exacerbations in the year following the fibreoptic bronchoscopy in No-Ex (0 exacerbation, n=20), Low-Ex (1 or 2 exacerbations, n=27) and High-Ex ([?]3 exacerbations, n=33). Comparison were performed according to the presence of atopy (history or current) (A), age at first wheezing episode (B), presence of bacteria in the BALF (C), *Haemophilus*genus in the BALF (D), *Streptococcus* genus in the BALF (E), reticular basement membrane (RBM) to bronchial smooth muscle (BSM) distance (F), normalized (norm.) BSM area (G) and RBM thickness (H). For categorical variables (A, C-E), results were presented as percentage of patients with (grey bars) or without (white bars) the tested parameter and comparison between groups were performed using Chi square tests. For quantitative variables (B, F-H), results were presented as absolute values with the median (line) and comparisons between groups were performed using Kruskal-Wallis tests with Dunn's post-tests. *$p<0.05$.