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Benefits and risks of bronchoalveolar lavage in severe asthma in children

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LIST OF ABBREVIATIONS

ACT	Asthma Control Test
BAL	Bronchoalveolar lavage
CFU	Colony Forming Unit
GINA	Global Initiative for Asthma
IgE	Immunoglobulin E
SD	Standard deviation

Take home message

Bronchoalveolar lavage can help characterizing severe asthma in children. However, it can be poorly tolerated and in most cases its impact on the patient's management remains limited.

ABSTRACT

Background: Although bronchoscopy can be part of the exploration of severe asthma in children, the benefit of bronchoalveolar lavage (BAL) is unknown. The present study aims at deciphering if systematic BAL during a flexible bronchoscopy procedure could better specify the characteristics of severe asthma and improve asthma management.

Material and Methods: The study took place in two departments of a university hospital in Paris. Children who underwent flexible bronchoscopy for the exploration of severe asthma between April 2017 and September 2019 were retrospectively included.

Results: In total, 203 children were included, among whom 107 had a BAL. BAL cell count was normal in most cases, with an increasing number of eosinophils with age, independently from the atopic status of the patients. Compared with bronchial aspiration only, BAL increased the rate of identified bacterial infection by 1.5. Nonatopic patients had more bacterial infections ($p < 0.001$). BAL induced a therapeutic modification only for azithromycin and omalizumab prescriptions. The practice of a BAL decreased bronchoscopy tolerance ($p = 0.037$), especially in the presence of tracheobronchial malacia ($p < 0.01$) and when performed in a symptomatic patient ($p = 0.019$).

Discussion and conclusion: Although BAL may provide interesting information in characterizing severe asthma, in most cases its impact on the patient's management remains limited. Moreover, BAL can be poorly tolerated and should be avoided in the case of tracheobronchial malacia or current asthma symptoms.

Key words: Asthma; Childhood; Bronchoalveolar lavage; Bronchoscopy

INTRODUCTION

Asthma is the most frequent chronic disease in childhood, with 8% to 11% prevalence in school- and preschool-aged children, respectively. The disease is poorly controlled in more than a third of the cases (1,2). In severe and poorly controlled asthma, bronchoscopy can guide therapeutic management and optimize asthma control: bronchoscopy may estimate the magnitude of inflammation of the lower airway respiratory tract and allow microbiological analyses of bronchial aspirations. Bronchoscopy can be complemented by bronchoalveolar lavage (BAL). A BAL fluid analysis includes cell count, specific staining, and distal airway microbiologic analyses. Cell count allows a precise description of the type of predominant cells, *i.e.* eosinophils or neutrophils, to better describe the asthma phenotype (3,4). However, even when a bronchoscopy is done, BAL is not systematically performed in asthma exploration and its usefulness and safety remains to be ascertained (5). In our specialized pediatric hospital, two departments deal with severe asthma but with different habits regarding BAL. Whereas bronchoscopy is performed in both departments when necessary, a systematic BAL is performed in one of them but not the other. Based on these heterogeneous practices, the current study aimed to evaluate the benefit of a systematic BAL during a flexible bronchoscopy procedure in comparable populations of children with pediatric asthma. The main objective was to determine if a BAL fluid analysis improved asthma evaluation. The secondary objective was to evaluate its impact on flexible bronchoscopy's morbidity.

MATERIAL AND METHODS

The study took place in two departments (pediatric pulmonology and pediatric allergology) at the University Armand Trousseau Hospital in Paris. The patient (when possible) and his parents received an information and gave their consent to the study. The study was approved by the Institutional Review Board of the French Society for Respiratory Medicine (Société de Pneumologie de Langue Française, # CEPRO_2020-005) and by the local ethics committee of our institution (MR004-2216637).

Patients

Asthmatic patients older than 3 months of age who underwent flexible bronchoscopy between April 2017 and September 2019 were included in two departments of a single pediatric hospital. Asthma diagnosis and severity were assessed following the Global Initiative for Asthma (GINA). We also considered as severe asthma the patients treated with high doses corticosteroids or medium dose corticosteroids plus another treatment and an incomplete asthma control. The local usual procedure for flexible bronchoscopy is conscious sedation. To avoid any overinterpretation of the neutrophil cell count and of the procedure morbidity, patients who had bronchoscopy under general anesthesia were excluded (6). Other exclusion criteria were patients with another underlying disease, such as hemopathy, immune deficiency, congenital cardiopathy, neuromuscular disease, or respiratory disease other than asthma (cystic fibrosis, primary ciliary dyskinesia, etc.).

The following data were collected: age at asthma onset (defined as the age at the first wheezing episode), the treatments for asthma prescribed two months before bronchoscopy (oral and/or inhaled corticosteroid; long- and short-acting beta agonist, anticholinergic, montelukast, azithromycin, biologic therapy and antibiotics). Atopic asthma was defined when one or more commonly inhaled allergens had been identified by one of the following test: prick test, multiallergic blood test (Phadiatop, Phadia, Thermo Fisher Scientific, Uppsala, Sweden), or specific immunoglobulin (Ig)E dosage (Phadia, Thermo Fisher Scientific, Uppsala, Sweden) (7). Asthma severity and control were carried out before the bronchoscopy and during the following visit, one to five months after the bronchoscopy using an asthma control questionnaire (Supplemental Figure 1) before the age of 4 years-old, and the Asthma Control Test (ACT) in patients over 4 years-old. Absence or presence of respiratory symptoms beyond 24 hours was noted. Severe asthma was defined as uncontrolled asthma despite a well-conducted strong therapy (high-dose inhaled corticosteroid therapy in children under 6 years of age, in combination with another treatment in the elderly).

The bronchoscopy was performed under conscious sedation using atropine and midazolam premedication (**Supplemental Table 1**). After a local anesthesia of the nostril and the pharynx with

lidocaine, a flexible fiberscope was introduced in the right nostril (or in the mouth in case of nostril obstruction). A macroscopic evaluation of the tracheobronchial anatomy, kinesis (absence or presence of a significant malacia (>70%) and inflammation (absent, mild, moderate, severe) was first realized, followed by a bilateral bronchial aspiration for microbiologic analysis. Inflammation was assessed using the following criteria, as described by Thompson et al. erythema, edema, friability of the mucosa, and presence of secretions (8).

BAL was usually performed in a segmental bronchus of the middle lobe. A total volume of 10% of the functional respiratory capacity (FRC) of saline solution was distributed in 6 syringes (plus 2 ml per syringe corresponding to the fiberscope channel volume). Each syringe's fluid was instilled in the same distal bronchus and sucked. The 2 first ml were retrieved whereas the following fluid of each suction were pooled for cytology, pathology and microbiology analyses. BAL fluid cytology was considered normal when the total cell count was below 500,000 cells/mL with 80% to 95% macrophages, 10% to 15% lymphocytes, 1% to 5% neutrophils, and less than 0.2% (or 500/mm3) eosinophils (9). BAL fluid was also analyzed for microbiology. A lower airway bacterial infection was defined by the identification of a bacterial charge over 10^4 colony-forming unit (CFU)/mL (10). Bronchoscopy complications such as bronchospasm, fever, oxygen, or hospitalization requirements were collected.

Statistical analyses

Patients with and without BAL were compared. Quantitative variables were expressed as mean ± standard deviation (SD). Chi2 or exact Fisher tests were applied when the expected values were below 5. The grouped quantitative variables were compared with student's or Mann-Whitney tests. Univariate and multivariate logistic regressions were carried out for the qualitative variables. The Spearman correlation coefficient was used to measure the relationship between the quantitative variables. Excel and R software were used for the statistical analyses. A p-value (p) below 0.05 was considered significant.

RESULTS

Patients' characteristics

Among the 515 patients who underwent a bronchoscopy for the exploration of severe asthma during the 29-month period of inclusion, 203 were included: 96 without BAL (non-BAL group) and 107 with BAL (BAL group) (**Figure 1**). The clinical characteristics of the patients and their current treatments are provided in **Table 1** and **Table 2** respectively. At the time of the bronchoscopy, compared with the

non-BAL group, the patients in the BAL group were older ($p<0.001$), had a later asthma onset ($p<0.01$), and were more often atopic ($p<0.001$). Both groups displayed similar proportions of severe asthma: 60 (63%) patients in the non-BAL group versus 78 (75%) in the BAL group ($p = 0.07$). Asthma control was comparable in both groups in the different age classes (<3 years, 3–6 years, >6 years).

Macroscopic bronchoscopy findings

Compared with the BAL group, the non-BAL group had less bronchial inflammation (50% vs. 93%, respectively, $p<0.001$) and more frequent bronchial anatomic disorders, such as bronchial atresia or unusual bronchial segmentation (53% vs. 28%, respectively, $p<0.001$) (**Supplemental Table 2**).

BAL cytology analysis

The mean BAL fluid cell count was inversely correlated with the child's age, with a Spearman correlation coefficient between age and a total cell count of -0.44 ($p<0.001$). Lymphocyte cell count was higher in the 3–6-year-old patients, whereas eosinophil cell count was higher in the over 6-year-old patients (**Table 3**). Blood eosinophil count was correlated to BAL eosinophil count in number and percentage ($p<0.01$), with a respective correlation coefficient of 0.266 ($p = 0.032$) and 0.248 ($p = 0.047$). In atopic patients, the mean eosinophil cell count was positively correlated with age: 0.3% (±1.12) in the 3–6-year-old patients versus 2.08% (±5.38) in the over 6-year-old patients ($p = 0.048$). After adjusting for age, atopy, bacterial and viral infection, a higher total cell count remained associated with the young age (less than 3 years) (**Supplemental Table 3**).

Microbiologic analyses

Viral analyses were performed in bronchial aspiration in the non-BAL group and BAL fluid in the BAL group (**Figure 2**). The most frequently identified virus was *Rhinovirus*, independently of age (**Table 4 and Supplemental Table 4**). *Rhinovirus* presence was associated with a higher lymphocyte count in the youngest (<3 years old: 11.9% (±5.71) vs. 9.32 (±6.24), $p = 0.042$). *Adenovirus* was more often found in bronchial aspirations than in BAL and in patients under 6 years old ($p<0.05$).

Bacterial analyses were performed in bronchial aspiration in both groups, and also in BAL fluid in the BAL group (**Figure 2**). Both the non-BAL and BAL groups presented a similar rate of bacterial infections (29% vs. 24%, respectively, $p = 0.47$), regardless of the patient's age (**Figure 2**). *Haemophilus influenza* was the most frequently identified bacteria in both groups (15.7%), but was never found in the six patients treated with long-term azithromycin. The other identified bacteria were mainly *Branhamella catarrhalis* (9.2%) followed by *Streptococcus pneumoniae* (4.3%),

Staphylococcus aureus (1.1%) and *Mycoplasma pneumonia* (0.5%). Among the 19 patients for whom bacterial analyses were performed in both bronchial aspiration and BAL fluid, six (31.6%) had positive bacterial cultures in BAL only, increasing the rate of bacterial identification by 1.5 (15.5% to 22.6%). The rate of bacterial infections was not related to the age of the patients in the non-BAL vs. BAL groups, respectively: 22 (33%) vs. 17 (44%) in the patients under 3-year-old; 3 (17%) vs. 2 (9.1%) in the patients between 3–6-year-old; and 1 (17%) vs. 4 (12%) in the patients over 6-year-old. Interestingly, the atopic patients presented with fewer bacterial infections than nonatopic patients (20% vs. 47%, respectively, $p<0.001$). A bacterial and viral coinfection was more often identified in the non-BAL group (n = 12, 14%) than in the BAL group (n = 1, 1.1%), $p<0.001$. None had a positive PCR for *Pneumocystis Jirovecii* .

Bronchoscopy and BAL adverse events

Only 2 children received hydroxyzine as a premedication. All the other children had been premedicated only with midazolam and atropine; and complications included peri-endoscopic and post-bronchoscopy adverse events (**Table 5**).

The length of sedation and the peri-endoscopic tolerance were similar between the groups (**Table 5**). However, it appeared that when the bronchoscopy was performed in a patient with current asthma symptoms, the overall tolerance of bronchoscopy (at least one complication of the procedure among increased length of sedation, poor per-bronchoscopy tolerance (hypoxia, important cough, commotion related to midazolam side effect), post-bronchoscopy complication including fever, bronchospasm, oxygen requirement, hospitalization) was poorer ($p = 0.019$) and the length of the sedation was increased ($p<0.01$) in the BAL group compared to the non-BAL group (**Supplemental Table 5**). Moreover, the observation during the bronchoscopy of a tracheobronchial malacia (reduction of more than 70% of the size of airways on exhalation) was associated with a poorer global tolerance (one or more complications) of bronchoscopy ($p = 0.016$).

After the bronchoscopy, a total of 27 (13.3%) patients required additional oxygen therapy, and this was more often observed in the non-BAL group ($p = 0.03$). Consequently, more patients in the non-BAL group required hospitalization during the following night ($p = 0.038$) (**Table 5**). These hospitalized patients were younger than the ones who could be discharged home on the day of bronchoscopy (2.21 ±2.81 years vs. 4.45 ±3.83 years respectively, $p<0.01$).

Postbronchoscopy management of asthma

A treatment modification was documented in 135 patients after bronchoscopy, with no difference between the non-BAL and BAL groups (71% vs. 63% respectively, p = 0.22). The only significant change was the addition of a short-term antibiotic treatment in 31 (32%) patients in the non-BAL group and 48 (45%) patients in the BAL group, with, however no difference between groups. A few therapeutic modifications were different between the non-BAL and BAL groups, such as initiation of long-term azithromycin (4.3% vs. 25%, respectively, p<0.001) and omalizumab (0% vs. 5.7%, respectively, p = 0.03) (**Supplemental Table 6**).

Improvement of asthma control could be assessed for 156 patients and ACT only in a quarter of the patients. An improvement in asthma control after bronchoscopy was more often observed in the non-BAL group than in the BAL group (n = 54, 75% vs. n = 45 (54%), respectively, p < 0.01).

DISCUSSION

In the current study, we documented the benefits and risks of performing a BAL during bronchoscopy when exploring severe asthma in children. Using two groups with a fairly symmetric distribution of patients who did and did not have BAL, we observed that (i) BAL improves the identification of bacterial infection compared with bronchial aspiration; (ii) BAL cytology alone could not differentiate non-atopic from atopic asthma; and (iii) a BAL analysis has a limited impact on therapeutic management. Moreover, BAL was associated with a poorer tolerance of bronchoscopy in the presence of a tracheobronchial malacia, or when the bronchoscopy was performed in a symptomatic patient.

BAL cytology and asthma phenotype

The interest in BAL fluid cytology analysis in defining the asthma phenotype is controversial (11). As found herein, in asthmatic children, the total cell count is usually normal or slightly increased compared with control individuals (12,13). As shown by Just *et al.* in a previous study population, we evidenced an inverse correlation between BAL cell count and age, which could be explained by the fact that the youngest patients present with viral asthma more frequently, whereas the oldest present more frequently with atopic asthma (14,15). Conversely, some other authors did not find any correlations between BAL fluid total cell count and age in asthmatic pediatric patients of all ages (16–18). Thus, this parameter hardly seems to help depict the asthma phenotype in a single patient.

In a large study including patients aged 6-17 year, a correlation between the cell profile based on neutrophils and eosinophil repartition and clinical characteristics was suggest (19). Another study failed to find a correlation between neutrophil cell count and lung function, but suggested a link between an increased intraepithelial airway neutrophilia and a better lung function (20). Our study population was younger but we couldn't find a correlation between the eosinophil count or the neutrophil count and the clinical characteristics of the patients, nor with their lung function tests. An increased neutrophil count was noticed in patients under 3 years of age, which may be related to an increased rate of lower airway infections in the youngest, promoting neutrophil recruitment and, therefore, asthma development (4,11,16,17). Eosinophil count was increased in patients older than 6 years old. This has been previously documented by other authors, especially in polyallergic severe asthma (4,15,17). Interestingly, our study and other research showed no difference in eosinophil counts between atopic and nonatopic patients (12). The link between eosinophil rates in BAL fluid and the risk of developing persistent asthma remains controversial, arguing for the need for further convincing studies (13,18,21,22) .

Microbiology

BAL allows for a culture of distal airway samples along with bronchial aspiration analyses. With a total of 26% of documented bacterial infections, the present study is below others that report up to 40% of infections using similar thresholds ($>10^4$ CFU/ml), despite a low rate of antibiotic treatment prior to bronchoscopy (a total of 20 (9.8%) patients, including four (1.9%) on long-term antibiotics and seven (3.4%) long-term azithromycin treatments) (18,21). This could be related to an older study population than in other studies (21,22). Among the 19 patients who benefited from bacterial analyses in both bronchial aspiration and BAL, six bacterial infections were documented exclusively in the BAL fluid, increasing the rate of bacterial detection by 1.5. Even though an association between viral asthma and bacterial infections could be expected (23), surprisingly, atopic patients also displayed elevated rates of bacterial infections. This result encourages the practice of BAL for bacteriologic purposes in the case of uncontrolled asthma in children, whatever the atopic status may be.

Therapeutic modifications

BAL did not seem to be associated with significant changes in asthma management. Indeed, only azithromycin and omalizumab introductions were significantly more common in the BAL group. However, it is important to question the true impact of BAL in the decision of biologic therapy.

prescription in these children, for whom the treatment's indication could be based on the lack of asthma control associated with an elevated total IgE level.

Complications

The overall tolerance of the sedated-conscious bronchoscopy without or with an additional practice of BAL was good. BAL was associated with a poorer tolerance of bronchoscopy when performed in a symptomatic patient (increased length of sedation and increased rate of complications) and when tracheobronchial malacia was diagnosed. These results suggest two recommendations: postpone bronchoscopy as much as possible when asthma symptoms are present, and re-evaluate the benefit of performing BAL when a tracheobronchial malacia is observed during bronchoscopy.

Conversely, the need of additional oxygen therapy was more often observed in the non-BAL group, probably because a premedication with nebulized salbutamol was much less frequent ($p < 0.001$) in this group, as well as a long-term controller treatment with anticholinergics (which effect lasts for up to 6 hours). Moreover, the younger age and more frequent tracheobronchial malacia in the non-BAL group may be another explanation (24,25).

Strengths and limits

The major strength of the current study is that all of the patients were included in a single center, allowing a high comparability of the procedures and comparable cytological and microbiological analyses. Furthermore, this study draws from a large cohort of children with a fairly symmetric distribution of those who did and did not have BAL. Another strength is the differential analysis of bronchoscopy and BAL complications in the case of concomitant asthma symptoms. Finally, the study of the cellularity of the BAL fluid in subgroups according to age and the presence or lack of an atopy is an original and informative approach. However, even if the BAL were mainly performed in stable state (96.3%), the treatment effect may be confounding the cytologic evaluation and also safety assessment, especially for corticosteroids (26% of the patients in the month before the BAL) that could impact eosinophil and neutrophil count (19). Cytokine profile could also have been an interesting was to phenotype the BAL and could be discussed in future studies as part of the systematic BAL analysis (26). Another limitation of the study is mostly regarding it being retrospective, which resulted in data loss, especially in the evaluation of asthma control (ACT tests documented only for a quarter of the patients).

Conclusion

The present study has highlighted the limited benefit of performing BAL during bronchoscopy for the exploration of severe asthma in children. BAL seems to improve the detection of bacterial infections and this study encourages the practice of BAL for bacteriologic purposes in the case of uncontrolled asthma in children, whatever the atopic status may be. Moreover, BAL led to limited therapeutic modifications. In clinical practice, it seems cautious to avoid BAL when a tracheobronchial malacia is known or suspected or in a patient with current asthma symptoms, two conditions associated with a poor tolerance of the BAL. Finally, the impact of cytology and inflammatory marker analyses of BAL fluid on predicting the asthma phenotype remains to be evaluated.

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AUTHOR CONTRIBUTION

RBT, JJ, and NN conceived of the study. RBT and NN wrote the manuscript. JJ, HC, LB, BP, and JT provided their expertise and reviewed the manuscript. NN is the guarantor of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article.

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FIGURE LEGENDS

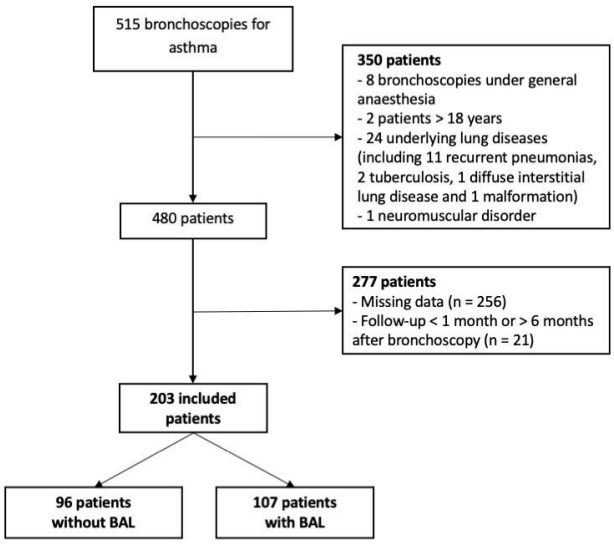

Figure 1: Flow-chart of the study

Between April 15, 2017, and September 30, 2019, 480 flexible bronchoscopies under conscious sedation were performed in Armand Trousseau Hospital for uncontrolled asthma in children. A total of 203 patients could be included in the study.

Figure 2

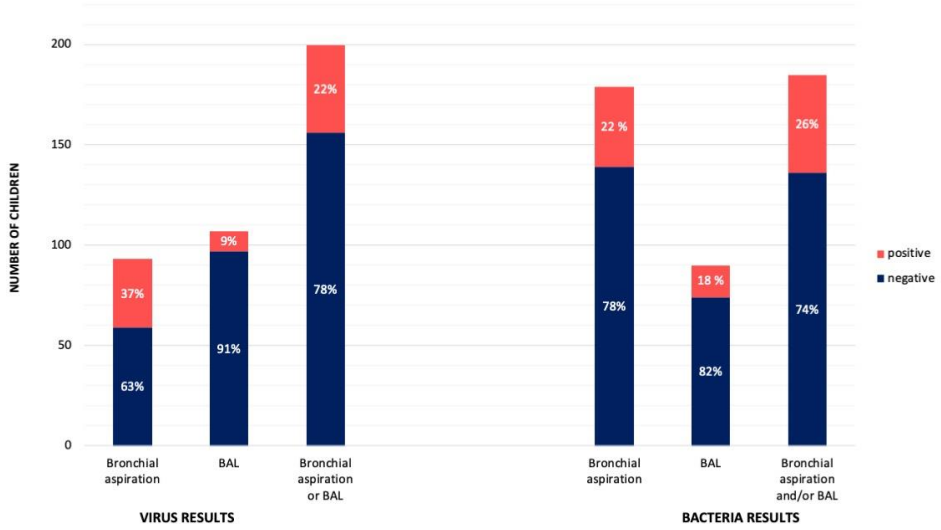

Figure 2: Distribution of virus and bacteria findings by sampling method

Results of the microbiological culture/detection were assessed for viruses in bronchial aspiration, bronchoalveolar lavage, or both and for bacteria in bronchial aspiration bronchoalveolar lavage or both.

TABLES

Table 1: Clinical characteristics of the included patients

		Non-BAL group (n = 96)	BAL group (n = 107)	n	p-value
Male, n (%)		63 (66%)	61 (57%)	124	0.21
Prematurity <35 WG, n (%)		16 (17%)	18 (17%)	34	0.98
Age, years	**Mean (± SD)**	2.24 (±2.12)	5.53 (±4.13)	203	**< 0.001**
	< 3 years, n (%)	72 (75%)	42 (39%)	114	**< 0.001**
	3–6 years, n (%)	18 (19%)	26 (24%)	44	0.34
	> 6 years, n (%)	6 (6.2%)	39 (36%)	45	**< 0.001**
Age at onset, months, mean (± SD)		6.15 (±8.98)	12.5 (±21.6)	200	**< 0.01**
Atopy	**Patient*, n (%)**	34 (47%)	83 (84%)	117	**< 0.001**
	Family*, n (%)	70 (80%)	90 (87%)	160	0.14
Passive smoking*, n (%)		29 (35%)	37 (35%)	66	0.99
Hospitalization*, n of patients (%)		79 (84%)	72 (68%)	151	**< 0.01**
Hospitalization, mean n (± SD)		2.42 (±1.41)	2.86 (±1.87)	151	0.11
ICU hospitalization*, n (%)		22 (24%)	16 (15%)	38	0.12
Current asthma symptoms, n (%)		29 (30%)	4 (3.7%)	33	**< 0.001**
High dose inhaled corticosteroids associated with another controller therapy*, n (%)		24 (25%)	70 (68%)	94	**< 0.001**
Uncontrolled or partially controlled asthma		72 (75%)	85 (79%)	157	0.45
Systematized alveolar opacities on chest radiography*, n (%)		7 (8.1%)	5 (5.4%)	12	0.46
Elevated eosinophils > 500/mm3*, n (%)		9 (13%)	19 (19%)	28	0.27
Lung function tests, n (%)		3 (3.1%)	36 (33.6%)	39	**< 0.001**
	Normal	3 (100%)	26 (72%)	29	0.56

Abbreviations: BAL, bronchoalveolar lavage; ICU, intensive care unit; WG, weeks of gestation

High dose inhaled corticosteroids: according to GINA, inhaled fluticasone > 200 µg/day for children under 6 years old and > 500 µg/jour over 6 years of age, inhaled budesonide > 400 µg/day under 12 years old and > 800 µg/day over 12 years old; nebulized budesonide > 1000 µg/day for all children

* based on 171 to 200 patients

Table 2: Basal treatment of the included patients

	Non-BAL group (n = 96)	BAL group (n = 107)	Total (n = 203)	p
Controller steroid treatment				
No corticosteroids	4 (4.2%)	1 (0.97%)	0 (0%)	0.19
Low dose inhaled corticosteroids	3 (3.2%)	6 (5.8%)	4 (3.9%)	0.5
Medium dose inhaled corticosteroids	15 (16%)	14 (14%)	15 (15%)	0.66
High dose inhaled corticosteroids	73 (77%)	82 (80%)	84 (82%)	0.64
Oral corticosteroids	37 (39%)	17 (16%)	54 (26%)	**<0.001**
Bronchodilators				
Long-acting beta agonist (LABA)	5 (5.2%)	17 (16%)	18 (17%)	**0.015**
Short-acting beta agonist (SABA)	47 (49%)	83 (78%)	87 (81%)	**<0.001**
Anticholinergic	13 (14%)	75 (70%)	76 (71%)	**<0.001**
Other				
Montelukast	15 (16%)	22 (21%)	16 (15%)	0.36
Omalizumab	0 (0%)	2 (1.9%)	8 (7.5%)	0.5
Antibiotics				
Azithromycin	2 (2.1%)	5 (4.7%)	31 (29%)	0.45
Long-term antibiotics	1 (1%)	3 (2.8%)	14 (13%)	0.62
Short-term antibiotics	8 (8.3%)	1 (0.93%)	48 (45%)	**0.014**

Table 3: BAL cell count according to age

	Total population	< 3 years (n = 41)	3-6 years (n = 25)	> 6 years (n = 37)	n	p
Total cells (10^3/ml)	255 (175)	341 (±219)	244 (±109)	166 (±95.8)	103	**<0.001**
Macrophage (%)	83.6 (13.9)	82.4 (±16.3)	83.4 (±10.8)	85.1 (±13.2)	105	0.43
Lymphocytes (%)	10.5 (6.47)	10.2 (±6.12)	13.7 (±7.80)	8.57 (±4.96)	105	**<0.01**
Neutrophiles (%)	4.48 (12.3)	7.44 (±16.4)	2.19 (±2.75)	2.73 (±10.4)	105	0.0503
Eosinophiles (%)	1.00 (3.83)	0.131 (±0.314)	0.269 (±0.992)	2.50 (±6.16)	105	**<0.01**

Results were available for 103 patients.

Table 4: Viral infections in non-BAL and BAL groups

	All patients (n = 200)	**Non-BAL group (bronchial aspiration) (n = 93)**	**BAL group (n = 107)**	**p**
≥ 1 infection	91 (45.5%)	50 (52%)	41 (38%)	**0.029**
Adenovirus	21 (10.5%)	18 (19%)	3 (2.8%)	**<0.001**
Enterovirus	14 (7%)	9 (9.7%)	5 (4.7%)	0.17
Parainfluenza virus	7 (3.3%)	5 (5.4%)	2 (1.9%)	0.25
Metapneumovirus	4 (2%)	4 (4.3%)	0 (0%)	**0.045**
Influenza virus*	8 (4%)	6 (8.1%)	2 (1.9%)	0.067
Respiratory syncitial virus*	8 (4%)	6 (8.1%)	2 (1.9%)	0.067
Rhinovirus **	37 (18.5%)	12 (52%)	25 (24%)	**<0.01**
Bocavirus **	10 (5%)	4 (17%)	6 (5.7%)	0.079
Coronavirus **	10 (5%)	2 (8.7%)	8 (7.6%)	1

*Researched respectively in 74 patients in the non-BAL group and 105 patients in the BAL group

** Researched respectively in 23 patients in the non-LBA group and in 105 patients in the BAL group

Table 5: Adverse events of bronchoscopy and BAL

	Non- BAL group (n = 96)	**BAL group (n = 107)**	n	p
Midazolam dose (mg / kg), mean (± SD)	0.397 (±0.231)	0.264 (±0.0912)	191	**< 0.001**
During bronchoscopy				
Length of sedation, mean (± SD), minutes	10.8 (±3.61)	11.4 (±5.27)	203	0.41
Poor bronchoscopy tolerance*, n (%)	7 (7.7%)	15 (15%)	22	0.1
After bronchoscopy				
Fever, n (%)	19 (20%)	13 (12%)	32	0.14
Bronchospasm, n (%)	13 (14%)	8 (7.5%)	21	0.16
Oxygen requirement, n (%)	18 (19%)	9 (8.4%)	27	**0.03**
≥ 1 night hospitalization, n (%)	15 (16%)	7 (6.5%)	22	**0.038**

* During the bronchoscopy (hypoxia, important cough, commotion related to midazolam adverse effect)

Supplemental Material for

Benefits and risks of bronchoalveolar lavage in severe asthma in children

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e-Table 1: Sedation doses per age and weight

	Midazolam	Atropine
< 30 kgs and/or < 5 years	0.3 mg/kg IR	0.02 mg/kg IR
> 30 kgs and/or > 5 years	0.1 mg/kg (max 2.5 mg per dose), max 3 doses	0.02 mg/kg IV

IR, intrarectal; IV, intravenous

e-Table 2: Bronchoscopy macroscopic findings

	Non-BAL group (n = 96)	**BAL group (n = 107)**	n	**p**
Normal	23 (24%)	5 (4,7%)	28	<0.001
Inflammation	48 (50%)	99 (93%)	147	<0.001
Secretions				<0.001
Presence	75 (78%)	103 (96%)	178	
Fluid and light	64 (67%)	93 (87%)	157	
Thick or purulent	11 (11%)	10 (9,3%)	21	
Anatomical abnormality/variation	51 (53%)	30 (28%)	81	<0.001
Tracheal or bronchial malacia	42 (44%)	29 (27%)	71	0.013
Bronchial partial atresia*	5 (5%)	1 (1%)	6	NA
Unusual bronchial segmentation	3 (3%)	0	3	NA
Subglottic nodule	1 (1%)	0	1	NA

e-Table 3: Factors that may influence total cellularity

	Coefficients	**p**
< 3 years	94.1 [9.48; 179]	**0.03**
> 6 years	-69.9 [-148; 7.83]	0.077
Viral infection	51.1 [-14.1; 116]	0.12
Bacterial infection	-74.1 [-163; 14.9]	0.1
Atopy	-59.2 [-162; 43.7]	0.25

e-Table 4: Viral infections according to age and group

	< 3 years			3-6 years			> 6 years		
	Non-BAL group (bronchial aspiration) (n = 72)	**BAL group (n = 42)**	**p**	**Non-BAL group (bronchial aspiration) (n = 16)**	**BAL group (n = 26)**	**p**	**Non-BAL group (bronchial aspiration) (n = 5)**	**BAL group (n = 39)**	**p**
≥ 1 infection	41 (57%)	23 (55%)	0.82	7 (44%)	8 (31%)	0.39	2 (40%)	10 (26%)	0.6
Adenovirus	15 (21%)	2 (4.8%)	**0.02**	3 (19%)	0 (0%)	**0.049**	0 (0%)	1 (2.6%)	1
Enterovirus	8 (11%)	4 (9.5%)	1	0 (0%)	1 (3.8%)	1	1 (20%)	0 (0%)	0.11
Parainfluenza virus	4 (5.6%)	1 (2.4%)	0.65	1 (6.2%)	0 (0%)	0.38	0 (0%)	1 (2.6%)	1
Metapneumovirus	4 (5.6%)	0 (0%)	0.29	0 (0%)	0 (0%)	1	0 (0%)	0 (0%)	1
Influenza virus*	6 (10%)	0 (0%)	0.079	0 (0%)	0 (0%)	1	0 (0%)	2 (5.3%)	1
Respiratory syncitial virus*	5 (8.3%)	1 (2.4%)	0.4	1 (8.3%)	0 (0%)	0.32	0 (0%)	1 (2.6%)	1
Rhinovirus*	9 (53%)	14 (34%)	0.18	2 (50%)	7 (27%)	0.56	1 (50%)	4 (11%)	0.24
Bocavirus*	1 (5.9%)	6 (15%)	0.66	3 (75%)	0 (0%)	**<0.001**	0 (0%)	0 (0%)	1
Coronavirus*	2 (12%)	5 (12%)	1	0 (0%)	0 (0%)	1	0 (0%)	3 (7.9%)	1

e-Table 5: Adverse events of bronchoscopy and BAL by age

	< 6 years				≥ 6 years			
	Non- BAL group (n = 90)	**BAL group (n = 68)**	**n**	**p**	**Non- BAL group (n = 6)**	**BAL group (n = 39)**	**n**	**p**
Midazolam dose, mean ± SD, mg/kg	0.382 (±0.0399)	0.304 (±0.0513)		**< 0.001**	0.729 (±1.18)	0.181 (±0.0998)		0.9
During bronchoscopy								
Length of sedation, mean ± SD (minutes)	10.9 (±3.63)	10.7 (±3.81)		0.69	9.17 (±3.19)	12.5 (±7.06)		**0.048**
Poor bronchoscopy tolerance*, n (%)	6 (7.1%)	10 (16%)	16	0.095	1 (17%)	5 (15%)	6	1
After bronchoscopy								
Fever, n (%)	19 (21%)	11 (16%)	30	0.43	0 (0%)	2 (5.1%)	2	1
Bronchospasm, n (%)	13 (14%)	5 (7.4%)	18	0.16	0 (0%)	3 (7.7%)	3	1
Oxygen requirement, n (%)	18 (20%)	7 (10%)	25	0.098	0 (0%)	2 (5.1%)	2	1
≥ 1 night hospitalization, n (%)	14 (16%)	6 (8.8%)	20	0.21	1 (17%)	1 (2.6%)	2	**0.25**

* During the bronchoscopy (hypoxia, important cough, commotion related to midazolam side effect)

e-Table 6: Pre-post bronchoscopy therapeutic changes

	Non-BAL group			**BAL group**			**Non- BAL group *versus* non-BAL group**	
	Pre (n = 96)	**Post (n = 96)**	**p**	**Pre (n = 107)**	**Post (n = 107)**	**p**	**Pre (p)**	**Post (p)**
Low dose inhaled corticosteroids	3 (3.2%)	1 (1.1%)	0.62	6 (5.8%)	4 (3.9%)	0.52	0.5	0.37
Medium dose inhaled corticosteroids	15 (16%)	11 (12%)	0.4	14 (14%)	15 (15%)	0.84	0.66	0.53
High dose inhaled corticosteroids	73 (77%)	83 (87%)	0.058	82 (80%)	84 (82%)	0.72	0.64	0.26
No corticosteroids	4 (4.2%)	0 (0%)	0.12	1 (0.97%)	0 (0%)	1	0.19	
Oral corticosteroids	37 (39%)			17 (16%)			**<0.001**	
Long-acting beta agonist (LABA)	5 (5.2%)	8 (8.3%)	0.39	17 (16%)	18 (17%)	0.85	**0.015**	0.071
Short-acting beta agonist (SABA)	47 (49%)	50 (52%)	0.66	83 (78%)	87 (81%)	0.5	**<0.001**	**<0.001**
Anticholinergic	13 (14%)	16 (17%)	0.55	75 (70%)	76 (71%)	0.88	**<0.001**	**<0.001**
Montelukast	15 (16%)	15 (16%)	1	22 (21%)	16 (15%)	0.28	0.36	0.89
Omalizumab	0 (0%)	0 (0%)		2 (1.9%)	8 (7.5%)	0.052	0.5	**<0.01**
Azithromycin	2 (2.1%)	5 (5.2%)	0.44	5 (4.7%)	31 (29%)	**<0.001**	0.45	**<0.001**
Long-term antibiotics	1 (1%)	6 (6.2%)	0.12	3 (2.8%)	14 (13%)	**<0.01**	0.62	0.1
Short-term antibiotics	8 (8.3%)	31 (32%)	**<0.001**	1 (0.93%)	48 (45%)	**<0.001**	**0.014**	0.059