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Malacia, Inflammation and BAL culture in children with persistent respiratory symptoms

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Abstract
In children with persistent respiratory symptoms, despite regular anti-asthma inhalation treatment, diagnostic investigations to exclude underlying disease are warranted.
124 children were prospectively enrolled, 24-hour oesophageal pH measurement and fiberoptic bronchoscopy (FOB) with bronchoalveolar lavage (BAL) were performed. BAL fluid (BALF) was processed for neutrophil counting and bacterial culture. Inflammation of the respiratory mucosa was registered.
A structural abnormality of the central airways was found in 47% (40% females). In 19% neither anatomical anomalies nor inflamed respiratory mucosa were observed, whereas in 64% definite macroscopic mucosal inflammation was registered. Inflammation of the respiratory mucosa was associated with a significantly higher percentage of neutrophils in the BALF, 48% (IQR 14 – 82) compared to 7% (IQR 0 – 16) ($p< 0,025$). A positive BALF culture was found in 62% of the infants with mucosal inflammation compared to 25% in the group without inflammation ($p< 0.016$). Fifty six per cent of the BALF samples were positive for bacterial culture.
In children with persistent respiratory symptoms, nearly half have anatomical anomalies of the central airways. In 62% of the children with mucosal inflammation a positive BAL culture and a significantly higher percentage of BAL fluid neutrophils were detected.

Introduction

Respiratory symptoms such as productive cough, bronchorroea and wheezing, are common problems in infants and young children. Prevalence up to 33% are reported(1). A diagnosis of asthma at that age is difficult and relies on the clinical and familial history . Assessment of young children with recurrent wheeze or cough is difficult in the clinical setting due to a relative lack of objective physiological measures and diagnostic tools. Consequently, it is common practice to initiate empirical trials of anti-asthma medications, and use symptom responses as a diagnostic tool. The European Respiratory Society (2,3) recommends selectively referring young children who do not respond to such medical interventions for flexible bronchoscopy with bronchoalveolar lavage (BAL) to evaluate other aetiologies of chronic/recurrent wheeze and cough. In a retrospective study Schellhase et al. (4) reported airway anomalies in 57% of 30 children, 0 to 18 months of age, with recurrent episodes of wheezing that were clinically refractory or poorly responsive to inhaled albuterol. Saito et al. (5) reported a high percentage of positive BAL fluid (BALF) cultures in children with recurrent cough and/or wheeze who had failed to respond to empirical treatment for asthma and gastro-oesophageal reflux.

We initiated a prospective study, incorporating fiberoptic bronchoscopy (FOB) and broncho alveolar lavage (BAL) besides a sweat test and a 24-hour oesophageal pH measurement in children with treatment-resistant respiratory symptoms.

Methods

Patients were recruited in the Pediatric Pulmonary Departments of the Ghent University Hospital and the Universitair Ziekenhuis Brussel in Belgium between January 2006 and August 2008. Infants and young children aged 0 to 2 years who had had persistent respiratory symptoms, productive cough, bronchorroea and wheezing for at least 3 months and had been sent by their pediatrician for advice were evaluated. They received inhalation treatment by spacer: Fluticason dipropionate (100 µg twice a day) or aerosol (250 µg budesonide twice a day) combined with a beta-2-agonist salbutamol 100 µg (twice a day) during the first week, for at least one month. Patients unresponsive to adequate asthma treatment, with symptoms defined as ongoing or recurrent, and who had not undergone antibiotic treatment in the previous month, were enrolled. Treatment compliance was inquired about but not verified.

Children born prematurely or showing failure to thrive, as well as children with a positive sweat test, with prolonged endotracheal intubation or tracheotomy, dysmorphic children and children with congenital cardiopathies, frequently associated with primary tracheomalacia, and children with neurologic disorders were excluded (6).

Children with localized consolidations on a chest X-ray were excluded and treated with an antibiotic. In the course of a two-and-a-half-year-period, 124 children, representing 2,4% of all children between 0 and 2 years evaluated for sustained respiratory disorders during this period, were enrolled. Family history on allergy and asthma in first degree relatives was positive in 39% and 17% of the study patients respectively. Fourteen percent of the children had been chronically exposed to cigarette smoke.

A 24-hour oesophageal pH-measurement was performed in all patients (DL70 Eccemedical®) . A positive test was defined relying on gastro-enterological criteria: oesophageal pH value below 4 for more than 4 per cent of the measuring time; more than 25 reflux moments and at least 1 reflux period exceeding 5 minutes (7).

All recruited patients underwent FOB (Olympus BF-3C20). FOB was performed by nasal approach under sedation and local anaesthesia in a spontaneously breathing child. The BAL was performed in the right lower lobe by instillation and aspiration of 3 aliquots of 1 ml/kg body weight normal saline. BALF recovered after each instillation was gathered in different sterile recipients. The third BALF sample was used for bacterial culture and white blood cell differentiation. Cell pellets were made by cytospin. A smear of the cytospin was stained with Giemsa for white blood cell differentiation.

The slides were examined using light microscopy by 3 independent observers, blinded to the clinical status and bronchoscopic findings. The mean percentage of the three observations was reported.
In order to isolate Gram positive bacteria, Gram negative bacteria and Staphylococcus aureus BALF was cultured on blood agar, Mc Conkey agar and mannitol salt agar respectively. Different looking colonies were picked up for further identification. Every potential respiratory pathogen was reported, commensal flora was disregarded.

Laryngomalacia was defined as a dynamic anomaly of the supraglottis region causing stridor by narrowing of the laryngeal entry, tracheomalacia and bronchomalacia as an abnormal collapse (> 50%) of the trachea or the main bronchi, as estimated by the bronchoscopist, due to localized or generalized weakness of the airway wall, leading to respiratory obstruction (6).
Inflammation of the respiratory mucosa was defined as mucosal oedema and/or hyperaemia , and/or hypertrophic submucosal glands (cobble stone pattern) and/or longitudinal mucosal folds.
In children with localized pulsatile tracheomalacia an angio-MRI was done in order to detect vascular anomalies.
The study was approved by the Ethical Committees of both participating hospitals. Written informed consent was obtained from both parents.

Statistics

Statistical significance of differences between subgroups for prevalence of mucosal inflammation, allergy and asthma in first degree relatives, as well as passive smoking, were calculated using the χ^2 test. The non-parametrical Wilcoxon test was used for calculation of statistical significance of differences in granulocyte count between patients with and without respiratory mucosal inflammation. A P-value less than 0.05 was considered significant.

Results

We selected 124 children (57 females, median age 10 months, IQR 7-14) with treatment-resistant respiratory symptoms for additional diagnostic work up. A 24-hour oesophageal pH measurement was positive in 29% of the children. In 20 children the 24-hour oesophageal pH measurement was not conclusive, because of digital storage problems. A structural abnormality of the central airways was found in 46% (58/124, 40% females 23/58): 6% (7/124) laryngomalacia, 19% (24/124) tracheomalacia, 10% (12/124) combined laryngotracheomalacia, 9% (11/124) bronchomalacia and 3% (4/124) localised mid-tracheal pulsatile compression (Fig 1). In these 4 infants vascular MRI was conclusive for the innominate arteria syndrome.

In 24 children, neither anatomical anomalies nor inflammation of the respiratory mucosa were observed, whereas in 79 definite mucosal inflammation was reported. Mucosal inflammation was found in 64% (30/47) of the children with tracheomalacia and/or bronchomalacia. In children with inflammation of the respiratory mucosa a significantly higher percentage of polymorphonuclear leucocytes in the BALF was found: 48% (IQR 14 – 82) compared to 7% (IQR 0 – 16) ($p< 0,025$) in the non inflammation group (Fig 2).

In 62% (49/79) of the children with mucosal inflammation a positive BALF culture was obtained compared to 25% (6/24) in the non inflammation group ($p< 0.016$) (Fig 3). Fifty six per cent of the BALF samples was positive for bacterial culture: 51% *M. catharralis*, 28% *H. influenzae*, 13% *S. pneumoniae*, 10% *S. aureus*, 4% *E. coli*, 1% *K. ocytocica*, 1% *P. aeruginosa*. In patients with a positive BALF culture a higher median value of polymorphonuclear cells was found. Because of the large range, however, this finding is not significant. Regarding gastro-oesophageal reflux no significant difference was found in positive bacterial cultures between children with a positive or a negative test: 65% (19/29) compared to 55% (41/75).

Compared to the group with normal anatomy of the conducting airways, patients with tracheo- and bronchomalacia showed a significantly lower percentage of positive familial history of asthma in first relatives (15% versus 24%, $p< 0.016$) and of passive smoking (0% versus 18%, $p< 0.012$),. No significant difference in allergy in first degree relatives was observed (38% versus 43%, NS).

Discussion

We report high prevalence of anatomical anomalies of the central airways in children with treatment- resistant respiratory symptoms: laryngomalacia, tracheomalacia, laryngo-tracheomalacia and bronchomalacia were observed in 46%.

In a retrospective study Schellhase et al. (4) reported central airway anomalies in 57% of 30 children, 0 to 18 months of age, with recurrent episodes of wheezing that were clinically refractory or poorly responsive to inhaled albuterol. Central airway abnormalities were found in 17 (57%) and tended to be more common in the 0 to 6-month age group. They found comparable prevalence of total anomalies (57%) and laryngomalacia (6%) but a higher frequency of segmental tracheomalacia (40%), in 33% caused by vascular anomalies. This high prevalence of vascular compression may be due to the bias of the retrospective design of the study. Saglani et al. (8) studied the clinical benefit of further investigations in preschool

children with severe recurrent wheeze. A noisy breathing with or without wheezing periods was the main inclusion criterium. All patients had already failed to respond to bronchodilator or to (inhaled or oral steroid),therapy. The minimum duration of the inhaled steroid trial was 2 months. In a subgroup of 19 young children (< 18 months), structural anomalies of the central airways were reported in 53%. No further information on specific anomalies was given and children with enlarged tonsils and adenoids were also included.
Saito et al. (5) studied 19 infants with recurrent wheeze and cough and revealed tracheomalacia in 63% of them.
Despite subtle differences in inclusion criteria: recurrent wheezing poorly responsive to albuterol (3), noisy breathing with or without wheezing poorly responsive to beta-agonists and inhaled steroids (8), recurrent wheeze and cough (5), our prospective study of a large group of children revealed comparative prevalence of central airway abnormalities in children with treatment-resistant respiratory symptoms of productive cough, bronchorroea and wheezing. This finding corroborates the high prevalence of central airway anomalies in young children with treatment-resistant cough and/or noisy breathing with or without wheezing and justifies the referral for bronchoscopy.
Primary or congenital tracheo-bronchomalacia is seen in normal infants, prematurely born infants, in congenital anomalies of the cartilage and in congenital syndromes (6). According to Bogaard et al. [9] the estimated prevalence of primary tracheo- and or bronchomalacia amounted to 1 in 2100 children aged 0 to 17 years. In infants Callahan et al. (10) estimated the prevalence of congenital tracheomalacia at 1 in 1445. Secondary or acquired tracheo- and bronchomalacia is caused by prolonged intubation, tracheotomy, severe tracheobronchitis or compression. No data are available concerning the prevalence of acquired tracheo-bronchomalacia. In our study population tracheo- bronchomalacia was highly associated with mucosal inflammation. Although, an association doesn't prove a causal origin and our study was not designed to answer the question of what came first, one could speculate that malacia in this kind of patient is sustained and could even be induced by ongoing mucosal inflammation (6).
In 64% of the study group definite macroscopic inflammation of the respiratory mucosa was observed. In this subgroup of children a significant higher percentage of polymorphonuclear cells was found, corroborating the finding of inflammation of the respiratory mucosa. Sixty four percent of the children with trachea- bronchomalacia also displayed signs of mucosal inflammation. Saito et al. [5] reported visible inflammation of the respiratory mucosa in 42% of their study group and an increase of neutrophils in the BAL fluid (>10%) in 47%..
In the children with mucosal inflammation we found a significant higher percentage of positive bacterial BALF cultures., Moreover, in these children, as a characteristic of infection, a significant higher percentage of polymorphonuclear cells was counted. These findings are suggestive of protracted bacterial infection as a possible etiologic or at least a concomitant cause of persistent respiratory symptoms. Schellhase et al. (4) reported in the 27 infants in whom BAL was performed, 3 (11%) with a positive bacterial culture, *H. influenzae* and *M. catharralis*. In a retrospective study Donnely et al. (11) reported the possibility of persistent bacterial bronchitis in children with chronic wet cough diagnosed as asthma. Prolonged and sometimes recurrent treatment with antibiotics led to recovery in over half of these patients. They suggested that this treatment might prevent an evolution to bronchiectasis. In 56% of our study group a positive bacterial culture was obtained, mimicking the bacterial flora found in sputum of children with bronchiectasis.
Saglani et al. (8) identified bacterial bronchitis in 43% of children with persistent wheeze, despite adequate treatment for asthma (24% were receiving oral steroids).
Saito et al. (5) reported positive bacterial cultures of the BALF in 61% of his study group.

The latter stated that aspiration is likely to be an important predisposing condition in early childhood leading to disruption of the normal epithelium and colonisation by organisms such as *H. influenzae*. Strengthening this hypothesis, we found in a recent study aspiration to be a major problem in 48 % of children with laryngo-tracheomalacia and persistent respiratory symptoms (12).

In view of the widely accepted ‘‘vicious circle’’ hypothesis that the development of bronchiectasis, as evident on HRCT, is preceded by a period of chronic inflammation driven by persistent bacterial infection of the conducting airways, the high percentage of positive BAL cultures in our study group is important and worrying (13, 14). Double blind placebo controlled trials are warranted to study the immediate and delayed effects of antibiotic treatment in this small subgroup of patients.

Our study is the first to enrol prospectively a large group of infants with treatment-resistant respiratory symptoms of productive cough, bronchorroea and wheeze. The high prevalence of positive BALF cultures corroborated the results of smaller retrospective studies.

These observations emphasize the need for FOB and BAL in children with ongoing respiratory symptoms, productive cough, bronchorrhoea and wheezing, despite an adequate anti-asthma inhalation treatment (2,3). A diagnosis of malacia could explain to the parents the persistent character of the respiratory problems and point to additive treatment options such as physiotherapeutic “positive end expiratory pressure” techniques. In case of mucosal inflammation and a positive bacterial BALF culture a prolonged therapeutic trial with an antibiotic could help to control the respiratory symptoms and probably prevent an evolution to the development of bronchiectasis (11).

The present study has some weaknesses: mucosal inflammation was not proved by biopsy and BALF cultures were not performed in a quantitative way. The significant higher percentage of neutrophils in the BALF of patients with macroscopic mucosal inflammation could preclude this criticism. In order to preclude upper airway induced positive BAL cultures, samples were taken out of the third instillation. Moreover significantly more positive cultures were seen in the patient group with mucosal inflammation.

High prevalence of central airway anomalies, mucosal inflammation, high percentages of neutrophils and positive cultures of BALF are observed in young children with treatment-resistant respiratory symptoms. Our study emphasizes the need for further investigations to elucidate the role of each of these findings in the pathogenesis of treatment-resistant respiratory symptoms in young children.

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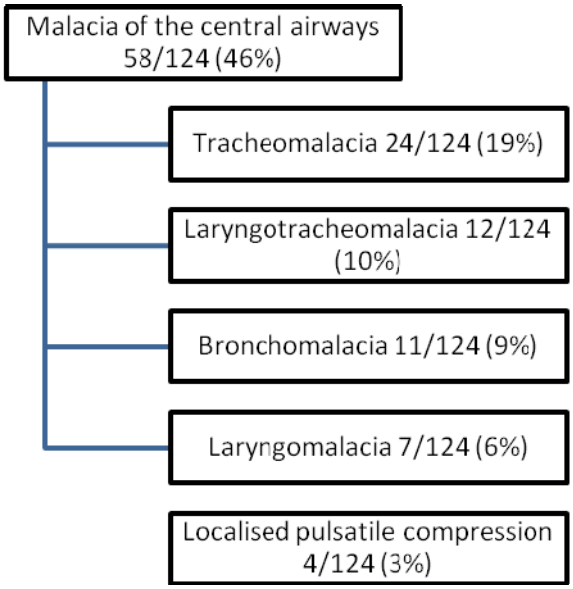

Figure 1: relative prevalence of different types of airway malacia

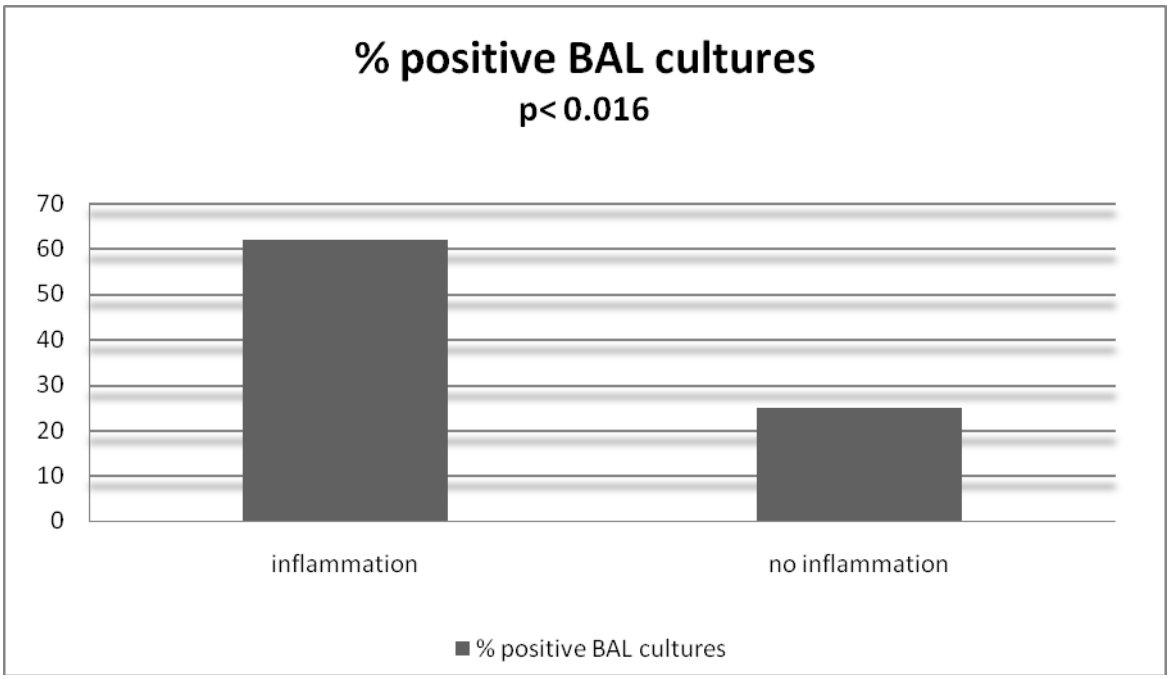

Figure 2: percentage of positive BAL cultures in infants with and without chronic inflammation of the respiratory mucosa.

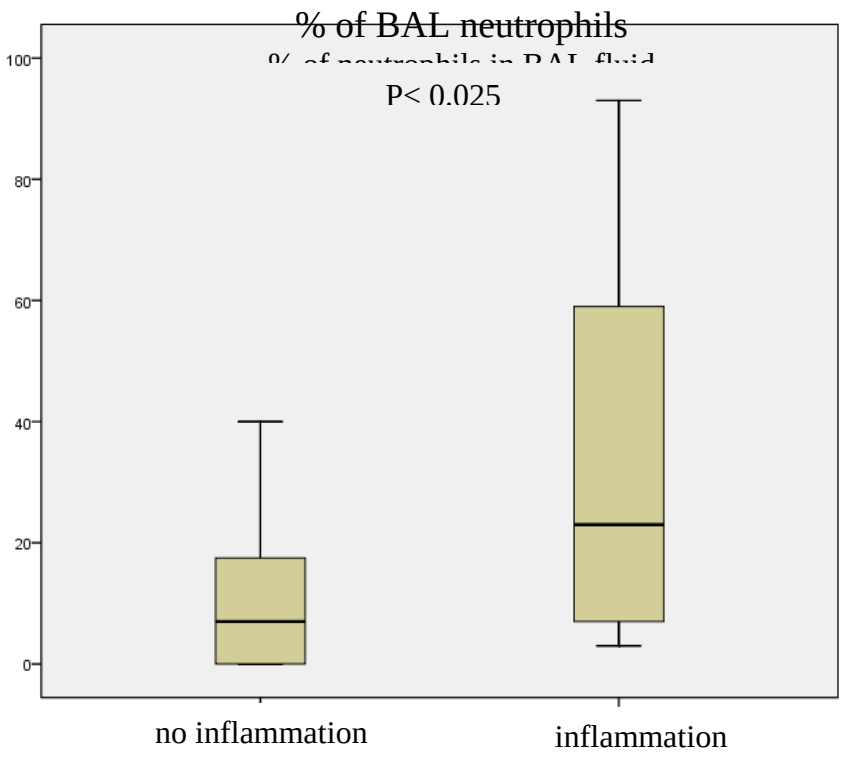

Figure 3: Percentage of neutrophils in BAL fluid of infants without and with inflammation of the respiratory mucosa.