Airway inflammation in children and adolescents with bronchiolitis obliterans

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ABSTRACT

Background: Airway inflammation plays a major role in the progression of chronic lung diseases. The features of airway inflammation are not well defined among patients with cases of bronchiolitis obliterans (BO) that began in childhood.

Objectives: To investigate the sputum cell and cytokine profiles of stable cases of BO regarding lung function and the involvement of small airway disease (SAD).

Methods: Twenty patients with BO (median age = 14.5, range = 7–23 years) and 22 healthy controls (median age = 16.5 years, range = 7–24 years) were investigated. Lung function parameters and bronchial reversibility testing as well as sputum cell and cytokine profiles (IL-1α, IL-6, IL-8, TNF-α, IL-5, IFN-γ, and NFκB regulation) were analysed using quantitative RT-PCR and cytometric bead assay (CBA) in induced sputum.

Results: Patients with BO had significantly lower lung function values, including FVC, forced expiratory volume (FEV1), the Tiffeneau index (FEV1/VC), and MEF25, but increased functional residual capacity (RV/TLC) values. Bronchial reversibility was found in five patients (25%). Moreover, airway inflammation (as indicated by total cells, neutrophils, IL-1β, IL-6, IL-8, TNF-α, and NFκB) was significantly increased among patients with BO compared with controls.

Conclusions: BO is predominantly a neutrophilic disease of the small bronchioles featuring elevated levels of pro-inflamatory cytokines leading to tissue remodelling and fibrosis of the small airways. Future therapies for patients with BO should more efficiently target the small airways.

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1. Introduction

Bronchiolitis obliterans (BO) is a rare, chronic, inflammatory and fibrosing lung disease. In children, it typically begins with an injury of the bronchiolar epithelium followed by an inflammatory reaction that progresses towards airway obliteration as the repair process of restoring the epithelium and microvasculature to its previous state, is severely altered [1]. Inflammatory cells and mesenchymal structures can be found in the lumen, the wall and around the bronchiale sparing other parenchymal structures [2].

This severe injury to the lower respiratory tract can be caused by either common pathogens (adenovirus, influenza, measles, respiratory syncytial virus, or Mycoplasma pneumonia) or by lung, and bone marrow transplantation. Rare causes of bronchiolitis include drugs, collagen vascular disease, graft versus host disease, and chronic occult aspiration [3–5]. The aetiology is used for clinical classification schemes and is important to guide the investigator to the diagnosis when tachypnoea, wheezing, and hypoxaemia persist for at least 2 months after a causative event [1]. Still the gold standard for diagnosing and classification of BO is the histopathology, as it shows an improved correlation with radiological manifestations, the natural history of the disease and the response to therapy [5]. BO can be classified histopathologically i.e. as cellular BO, bronchiolitis with intraluminal polyps, constrictive bronchiolitis and peribronchiolar fibrosis. Due to its invasive nature this procedure cannot be performed routinely. Thus, many experts define BO using clinical course, lung function (hyperinflation and obstruction) [6,7], and characteristic CT findings (mosaic attenuation pattern with ground glass opacities and central bronchiectasis) [6,8] especially in patients with post-infectious BO as these patients often show a mild clinical course.

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The pathologic processes of BO are poorly understood. BO might be caused by a progressive inflammatory response with elements of tissue remodelling, fibrosis of the small airways, airway obstruction, and reduced expiratory flow rates that can eventually lead to death from respiratory failure. Although the importance of inflammation in the pathophysiology of BO is intriguing, few studies have measured airway inflammation among patients with cases of BO that began in childhood [9,10]. A study of bronchoalveolar lavage (BAL) samples found increased levels of pulmonary neutrophils, IL-8, and CD8+T lymphocytes, all of which might play a role in the pathogenesis of post-infectious BO [9]. These findings resemble BO after lung transplantation, where several BAL parameters, including neutrophil count and IL-8 levels, were associated with small airway disease (SAD) [11]

Early closure of the small airways due to neutrophilic inflammation is reflected by lung function tests that reveal peripheral airway obstruction, raised RV/TLC, or both in diseases such as cystic fibrosis and chronic obstructive bronchitis [12,13]. CT scans and lung biopsies have confirmed the central role of small airways in BO [8,14]. Thus, whether we can more precisely define SAD among patients with stable BO is relevant and should be investigated. To our knowledge, the inflammatory pattern present in induced sputum specimens from patients with BO originating from childhood has not been elucidated. The aim of this study was to investigate sputum cell and cytokine profiles in stable BO in relation to lung function and involvement of small airway disease (SAD).

2. Materials and methods

2.1. Patients

Patients with BO were recruited from the outpatient clinic of the paediatric pneumology of the Goethe-University, Frankfurt, Germany. Initially, an electronic chart review of 2006–2011 was performed. A diagnosis of BO in children older than 6 years was found in 36 of the 6,924 records. In 24 of these 36 patients, the diagnosis was confirmed using patient history, lung function data, and CT scans. In 6 patients the diagnosis was confirmed by histopathology. All 24 patients with BO were invited to participate; four declined, but the remaining 20 were enrolled in the study.

The following lower respiratory tract pathologies/events were initially diagnosed: bone marrow respectively stem cell transplantation (4), viral infections (3), Mycoplasma pneumoniae (3), prematurity (2), gastroesophageal reflux (1), immunodeficiency (1) and 6 of unknown origin.

All patients suffered from BO for more than 2 years (median = 6 years, range = 2.5–18 years). Control participants were recruited using public announcements. After the informed consent process, all patients performed lung function tests, and induced sputum was collected during the first visit. The collected sputum was processed and stored until certified laboratory personnel blind to the clinical findings analysed it. During the second visit, reversibility testing was performed as described below.

Exclusion criteria were acute lung infection during the 4 weeks before enrolment, therapy with systemic corticosteroids, regular treatment with inhaled corticosteroids, and the inability to perform lung function tests.

The Medical Ethics Committee of the University Hospital, Frankfurt/Main approved this study, and it was registered at clinicaltrial.gov (NCT01327248).

2.2. Lung function tests

Baseline lung function was evaluated using a body plethysmograph (VIASYS Healthcare GmbH; Hoechberg, Germany). The following parameters were recorded: VC, FEV1, FEV1/VC, maximum expiratory flow 25% (MEF 25%), RV, total lung capacity (TLC), and RV/TLC. Bronchodilator reversibility was tested after 400 μg of Salbutamol. Reversibility was defined as yes/no using a ≥12% and 200-ml post-bronchodilator change.

2.3. Exhaled NO (eNO)

eNO was measured using the NIOX® system (Aerocrine Inc., Solna, Sweden) according to ATS guidelines [15]. This chemiluminescence gas analyser is sensitive enough to measure NO at concentrations between 1.5 and 200 ppb, with a standard deviation of ±2.5 ppb <50 ppb or ±5% of >50 ppb. We controlled for intra-participant variability using the mean of three consecutive measurements.

2.4. Sputum collection and processing

Participants first inhaled salbutamol and consecutively nebulised hypertonic saline at increasing concentrations of 3%, 4%, and 5% NaCl every 7 min. During this procedure, participants rinsed and cleaned their noses to reduce squamous epithelium cells in the samples. Sputum was processed within 1 h of collection. The selected sputum plugs (which contained as little saliva as possible) were placed in a weighed Eppendorf tube and processed with 4× weight/volume of 0.1% dithiothreitol (DTT). Afterward, 2× weight/volume of phosphate-buffered saline (PBS) was added. Samples were filtered through a 48-μm mesh and centrifuged for 10 min at 390g to remove the cells. Supernatants were stored at −80 °C for additional analysis with a protein assay.

2.5. Sputum cells

Specimens in which fewer than 10% of the cells were squamous epithelial cells were considered adequate. At least 400 inflammatory cells were counted for each specimen. Neutrophils, lymphocytes, eosinophils, and macrophages were expressed as cells per mL and as proportions of the total cell count.

2.6. RNA extraction

Total RNA from induced sputum (IS) was extracted using the innuPrep RNA Mini Kit (Analytic Jena, Jena, Germany) according to the manufacturer’s instructions. All sputum plugs (at least 80 mg) were processed with 0.1% DTT and PBS/BSA according to the manufacturer’s instructions. Then, 5 μL of RNA was diluted with RNase-free water in a 1:5 ratio, and the absorbance was measured to determine the amount of RNA. In addition, the RNA quality was checked using the BioRad Experion (BioRad, Hercules, CA, USA) according to MIQE guidelines [16]. The RQI values were between 8.9 and 9.7. Before reverse transcription, a DNase treatment was performed using DNase I (Qiagen, Hilden, Germany).

2.7. mRNA reverse transcription

This preparation was supplemented with 9 μL of a master mix of 1-μL iScript Reverse Transcriptase (BioRad, Hercules, CA, USA), a random hexamer, oligo dT mix, 4 μL of 10× iScript RT buffer, and 4 μL of nuclease-free water and then incubated in a thermocycler at 25 °C for 5 min, followed by 42 °C for 30 min and 85 °C for 5 min.

2.8. Real-time-PCR

Transcripts were quantified using a two-step real-time (RT)-PCR with an Eppendorf Mastercycler realplex S detection system (Eppendorf, Hamburg-Eppendorf, Germany) in Greiner 25-mL
96-well reaction plates (Greiner, Germany). The housekeeping gene glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was used as a control in each plate; Qiagen designed specific QuantiTect primer assays for IL-1ß, IL-6, IL-8, TNF-α, IL-5, and IFN-γ. PCR reactions were performed according to the manufacturer’s instructions in a final volume of 25 μl using QuantiTect SYBR Green Master Mix (Qiagen). A fluorescent product was detected at the last step of each cycle. A melting curve analysis was conducted immediately after amplification in accordance with the manufacturer’s instructions. Data from the genes involved in immune cells and inflammation markers were analysed as previously described [17].

2.9. mRNA quantitative analysis

Quantitative analyses were performed based on the threshold CT values for each well, which were calculated using the realplex S Database tool. Expression levels of amplicons were quantified using the 2(−ΔΔCT) method [18].

2.10. Cytometric bead array

The concentrations of six cytokines were determined in sputum samples using the BD™ CBA Flex Set System (BD Bioscience-PharMingen, San Diego, CA, USA) to measure the cytokines in the samples using the BD™ CBA Flex Set System (BD Bioscience-PharMingen, San Diego, CA, USA). The BD™ CBA Flex Set contained a one-bead population with distinct fluorescence intensity and the appropriate phycoerythrin (PE) detection reagent and standard. The tests were performed according to the manufacturer’s instructions, and samples were tested in duplicate. To analyse the cytokines, we added the same concentration of DTT (0.025%) as in the sputum supernatant to the standard curve and manufacturer’s instructions, and samples were tested in duplicate. The lower detection limits of the cytokines were IL-1ß, 1.0 pg/ml; IL-6, 1.6 pg/ml; IL-8, 1.2 pg/ml; TNF-α, 0.7 pg/ml; IL-5 1.1 pg/ml; and IFN-γ, 1.8 pg/ml.

2.11. Data analyses

Data were analysed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) and Microsoft Excel. Group differences between BO controls were analysed using a Kruskal–Wallis test (GraphPad Software Inc., La Jolla, CA, USA) and Microsoft Excel. Group differences were analysed using a Mann–Whitney test depending on the normality and homogeneity of variance assumptions. A probability of p < 0.05 was considered significant.

3. Results

3.1. Study population

The median age of the 22 control participants was 16.5 (range = 7.8–24.5 years); they were well matched for age with the patients with BO (median age = 14.5, range = 7–23 years). Further clinical characteristics of the two study populations are summarised in Table 1.

3.2. Lung function testing

Patients and controls were compared in means of lung function and bronchodilator reversibility. As shown in Fig. 1, FVC, FEV1, and MEF25 were significantly lower but RV/TLC was higher among patients with BO compared with the control group, as expected. In our group of mild to moderate cases of BO some of the patients with histologically diagnosed BO still had normal lung function values at the time of the study visit.

3.2.1. Bronchodilator reversibility

The FEV1 of patients with BO increased by 6.63% after 400 μg of Salbutamol (mean FEV1, 63.62 ± 20.2% and 70.25 ± 22.05% before and after inhaling Salbutamol, respectively). Five of the 20 patients (25%) had post-bronchodilator changes consistent with the standard reversibility criteria based on the unchanged FEV1. RV and RV/TLC after Salbutamol inhalation (mean RV, 172.2 ± 65.9% and 165.4 ± 55.2% before and after Salbutamol inhalation, respectively; mean RV/TLC 166.2 ± 42.3% and 158.6 ± 40.1% before and after Salbutamol inhalation, respectively).

3.3. Measurement of bronchial inflammation

3.3.1. Results of eNO

The control group had significantly higher eNO levels than patients with BO (Table 1). All patients with BO showed levels below 30 ppb indicating the absence of eosinophilic inflammation in the airways.

3.3.2. Analysis of inflammatory cells in induced sputum

We analysed the number and proportion of inflammatory cells (neutrophils, lymphocytes) in both cohorts. Acceptable sputum samples were obtained from 18 patients with BO and from 22 controls. Patients with BO had significantly higher counts of total cells

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Table 1

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<th>Characteristics of controls and patients with BO.</th>
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FVC (% pred.), forced vital capacity in% predicted; FEV1 (% pred.), forced expiratory volume in 1 s; MEF25 (% pred.), maximum expiratory flow; RV (%), residual volume; RV/TLC, functional residual volume; eNO, exhaled nitric oxide.

Data are shown as medians (range). The non-parametric Mann-Whitney Test was used to compare controls with patients with BO.
and of neutrophils in induced sputum compared with the control group (see Table 2). As displayed in Fig. 2, significantly more total cells and neutrophils were observed in all patients with BO.

3.4. Measurement of inflammation by qRT-PCR from sputum cells

We measured six cytokines (IL-1β, IL-6, IL-8, TNF-α, IFN-γ, and IL-5) and NFκB in the sputum of patients with BO and controls. All seven mediators were detectable using qRT-PCR in approximately all sputa obtained from patients with BO. As shown in Table 3, all pro-inflammatory neutrophilic biomarkers (IL-1β, IL-6, and IL-8) except TNF-α and NFκB were significantly greater in patients with BO compared to the non-affected control-group. No between-group differences were observed with regard to the TH1 and TH2 cytokines IFN-γ and IL-5. The individual levels of all RT-PCR analyses with regard to IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-5, and NFκB are displayed in the colour blot (Fig. 3).

3.4.1. Protein levels of cytokines via CBA

The protein levels of all cytokines were detected in the sputum supernatants of patients with BO and controls. As shown in Fig. 4, all levels of the pro-inflammatory neutrophilic biomarkers (IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-5, and NFκB) were significantly different between patients with BO and controls.

**Table 2**

| Sputum cell counts in induced sputum of BO patients and healthy controls. |
|-----------------------------|-----------------------------|
| Controls (n = 23) | BO (n = 20) | p-Value |
| Total cells × 10⁴/ml | 22.5 (6.6–153.0) | 115.0 (20.0–818.0) | <0.0001 |
| Macrophages × 10⁴/ml | 384.0 (191.0–400.0) | 91.0 (29.0–256.0) | <0.0001 |
| Macrophages (%) | 97 (63–100) | 28.3 (7–64) | <0.0001 |
| Neutrophils × 10⁴/ml | 5.0 (0.0 – 128.0) | 275.5 (47.0–371.0) | <0.0001 |
| Neutrophils (%) | 2 (0–12) | 68.9 (30–93) | <0.0001 |
| Lymphocytes × 10⁴/ml | 3.0 (0.0–36.0) | 7.0 (0.0–28.0) | 0.01 |
| Lymphocytes (%) | 1 (0–9) | 2 (0–7) | 0.01 |
| Eosinophils × 10⁴/ml | 0.0 (0.0–42.0) | 0.0 (0.0–4.0) | n.s. |
| Eosinophils (%) | 0 (0–8) | 0 (0–1) | n.s. |

Data are shown as medians (range). The non-parametric Mann–Whitney Test was used to compare controls with patients with BO.

**Table 3**

| Cytokines measured by CBA in the induced sputum supernatant of controls and patients with BO. |
|-----------------------------|-----------------------------|
| Cytokines | Controls (n = 23) | BO (n = 20) | p-Value |
| IL-1β pg/ml | 147.5 (56–3511) | 923.7 (82–99,313) | <0.001 |
| IL-6 pg/ml | 0 (0–604) | 324.7 (0–3541) | <0.001 |
| IL-8 pg/ml | 1742 (529–49,658) | 21110 (579–49,658) | <0.001 |
| TNF-α pg/ml | 0 (0–2314) | 0.0 (0–99) | <0.001 |
| IL-5 pg/ml | 0 (0–99) | 0.0 (0–122) | n.s. |
| IFN-γ pg/ml | 116 (0–174) | 26.5 (0–325) | n.s. |

Data are shown as medians (range). The non-parametric Mann–Whitney Test was used to compare controls with patients with BO.

Fig. 1. Lung function parameters of controls and patients with BO Data are shown as medians (range). The non-parametric Mann–Whitney Test was used to compare controls with patients with BO. Post-transplant patients are depicted in red triangles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Total cells and Neutrophils in controls and patients with BO Data are shown as medians (range). The non-parametric Mann–Whitney Test was used to compare controls with patients with BO. Post-transplant patients are depicted in red triangles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
IL-6, IL-8, and TNF-α were significantly higher among patients with BO compared with controls. Again, IFN-γ and IL-5 levels did not differ between the study groups. Table 3 shows the median levels and ranges of the patients with BO and controls.

### 3.5. Correlation to lung function

We correlated the clinical lung function data indicative of SAD (MEF25 and RV/TLC) to the biomarkers in the sputum levels of patients with BO. Sputum levels of neutrophils, IL-1β, IL-6, IL-8, and TNF-α were negatively correlated with MEF25 and RV/TLC (Table 4).

### 4. Discussion

Distinct inflammatory profiles have emerged as an important area of investigation that advances the understanding of on-going inflammation and remodelling in airway diseases such as BO.
as cystic fibrosis (CF) and asthma [20,21]. Airway inflammation in asthma is usually characterised as an eosinophilic, TH-2 driven inflammation [17,22]. CF is associated with an exuberant pro-inflammatory state primarily driven by IL-8 and neutrophils, which leads to a decline in lung function, tissue destruction, and bronchiectasis [19,20]. In contrast, less is known about the nature and mechanisms of airway inflammation in cases of BO that begin in childhood.

BAL is usually the primary method of studying inflammatory processes in adults with BO; however, because this procedure is invasive, it has been used rarely in children and adolescents [11]. The non-invasive sampling of induced sputum is an alternative to BAL for patients with BO and control participants; this method allows for the characterisation of cells and the measurement of biomarkers in sputum.

This study showed that total cells and neutrophils were significantly elevated in the induced sputum of patients with BO. The total cell load greatly exceeded the level of neutrophilic inflammation that we recently observed among patients with mild CF (data not shown). Notably, all pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) were significantly increased in the induced sputa of patients with BO when measured using either RT-PCR or proteins. These potent chemo-attractants for neutrophils might account for the excess of neutrophils observed even among patients with mild lung impairments. In addition, neutrophils and their reactive oxygen species (ROS) are able to perpetuate inflammation and remodel airways [23,24]. In fact, it was recently shown that lipid and protein oxidation was significantly elevated in BAL specimens from children with post-infectious BO [25].

Increased pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α as well as and neutrophilic influx are prominent features of inflammation in BAL specimens from adults with BO after bone marrow and lung transplantation [26–28]. A recent review summarised the strength of the associations between BAL parameters and BO. The analysis indicates that neutrophil count, IL-8, alpha defensins, and matrix metalloproteinases were highly and reproducibly associated with BO [11]. The effects of inflammatory cytokines might not be limited to their actions on neutrophils: IL-8 might act on airway smooth muscle and contribute to airway remodelling through the promotion of smooth muscle cell migration within the airway wall [29]. Moreover, high levels of IL-8 might be attributed to significantly up-regulated NFκB expression [30]. Interestingly, immunologically relevant genetic variations in the IκBα promoter (the major negative regulator of NF-κB) are associated with differential susceptibility to severe bronchiolitis following infection from respiratory syncytial virus [31]. Moreover, mutations of the NOD2 receptor, a pattern recognition molecule, have been associated with BO. The cumulative incidence of post-transplant BO increased from 1.3% in donor-recipient pairs without this mutation to 18.7% in pairs with donor or recipient NOD2 variants [32]. The functional implications of these mutations are not well understood, but evidence shows that NOD2 acts via the NF-κB signalling of proinflammatory cytokine induction [33]. In addition, NOD2 mutations lead to an impaired control of the immune response and a reduction of antimicrobial peptides [34]. These data highlight the importance of negative regulators of innate immunity cells in chronic inflammatory lung disease and provide insight into important determinants of genetic predisposition to BO.

Next, we analysed the potential role of inflammatory cytokines with regard to disease activity/progression. We found significant correlations between sputum biomarker levels and the presence of SAD as indicated by MEF 25 and RV/TLC (Table 4). Prior studies correlating single sputum biomarkers (e.g., IL-8 and airway neutrophils with lung function parameters) have reported results comparable to our findings [11,27,28]. Devouassoux et al. found that the number of neutrophils was correlated to advanced BO stages [28]. The early closure of the smallest airways due to neutrophilic inflammation can cause air trapping and peripheral airway obstruction. As expected, RV and RV/TLC functional residual capacity (which have been used to characterise air trapping and, hence, small airway function) differed significantly between patients with BO and controls in our study.

Sputum is seldom analysed in clinical practice because this procedure requires highly trained personnel and is time consuming. A more convenient approach might be to measure exhaled NO via simple hand-held devices as a marker of airway inflammation. NO is clinically useful, notably for diagnosing asthma, but more importantly for identifying steroid responsive airway inflammation [35]. The findings regarding the role of NO in patients with BO are inconsistent. Neurohr et al. reported that NO measurements can be used prognostically among transplanted patients [36]. These authors found that NO was higher in patients with BO compared with non-patients with BO (23.8 ± 1.2 vs. 17.8 ± 0.4 ppb, p < 0.01). However, others who did not find difference in the NO levels of transplanted patients with or without BO could not confirm these data [37]. In contrast, our data showed significantly lower NO levels among patients with BO, which is difficult to explain. Currently, definite guidelines do not exist with regard to “normal” values of NO. Low NO levels have been reported in patients with CF, pulmonary hypertension, or bronchiectasis [35]. Our finding of reduced NO levels in patients with BO might indicate that, in addition to on-going airway inflammation, the innate airway defence is altered in patients with BO [35].

Because the small airways play an important role in the pathophysiology of BO, patients can be characterised phenotypically according to the presence or absence of airway reversibility due to bronchodilator therapy using lung function tests. In the present study, five of the 20 patients showed post-bronchodilator changes consistent with the standard criteria of reversibility based on FEV1 only (increments ≥ 12% and 200 ml). This bronchodilator response is consistent with previous reports showing that approximately 25% of patients with BO showed airway reversibility [37,38]. In addition, Tiotropium decreased airway obstruction and air trapping up to 24 h in a sample of children with BO [39]. Nevertheless, discussion continues regarding which lung function parameter should be used to detect airway reversibility. Simple spirometry is not sufficiently sensitive to changes in the diameter of the small airways. Moreover, body plethysmography, a specific measurement of lung hyperinflation using RV and RV/TLC, is of greater value with regard to detecting SAD. A significant decrease in RV was recently found in response to bronchodilator use in approximately one-half of patients with BO after stem cell transplantation [38]. However, our data did not confirm these findings. Only two of 20 patients showed a significant decrease in RV, RV/TLC, or both. Next, we addressed the question of whether patients with a positive bronchodilator response had a distinct clinical or inflammatory phenotype. However, we did not find this result most likely because the sample was too small to detect significant differences.

Controlling excessive neutrophilic airway inflammation is an important goal of the treatment of BO. Treatments with azithromycin have been proven to be effective with regard to diffuse panbronchiolitis, CF, and BO after lung or bone marrow transplantation [40]. With regard to BO, azithromycin has been proven to reduce airway neutrophilia and IL-8 levels. Although not previously studied in BO diagnosed in childhood, a therapeutic trial with azithromycin is warranted with regard to this inflammatory mediated disease.

In conclusion, our study demonstrated that patients with BO had predominantly neutrophilic inflammation with elevated pro-inflammatory cytokines leading to tissue remodelling and
fibrosis of the small airways. Future therapies for patients with BO should more efficiently target the small airways.

Acknowledgement

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