Bronchoscropy and biopsy study in children with asthma

Research Clerkship 2011/2012
Fardou Heida
S1804782

Supervisor: B.L. Rottier, M.D., Pediatric Pulmonology
Location: University Medical Center Groningen
PREFACE & ACKNOWLEDGMENTS

Not earlier I had to perform a complete scientific research project during my medical training on this level. With the start of this project I started with a learning process as well. This learning process and the results of the research would not been as successful without the help of my supervisor and experts in the field of research. I would like to say thanks to:
Dr. B.L. Rottier, M.D. (supervisor), Dr. M. Pijnenburg, M.D. PhD (pediatrician), prof. dr. W. Timens (pathologist), M. Luinge (pathologist), L. Vanlaeken (medical student).

I - ABSTRACT

INTRODUCTION:
Bronchoscopy and endobronchial biopsies are diagnostic tools in the evaluation of difficult asthma and other paediatric airway diseases. The yield of these procedures in clinical practice is uncertain. Therefore our aim was to evaluate if bronchoscopy and endobronchial biopsies change management.

METHODS:
Retrospectively we collected data on diagnosis and treatment before and after bronchoscopy in all children undergoing bronchoscopy with endobronchial biopsies between 0 and 18 years. Difficult asthma was defined as uncontrolled asthma despite high dose ICS dose and LABA. In endobronchial biopsies reticular basement membrane (RBM) thickness was measured.

RESULTS:
Of the 74 children (37 male, mean age 7.8 years), 26 had asthma and 13 had difficult asthma. The diagnosis of children undergoing bronchoscopy changed in 31.3 % of the cases: 10 children got a second diagnosis, which could explain the persistent symptoms, 8 children were misdiagnosed. In these 18 children airwaymalacia (n=10) and anatomic abnormalities (n=7) were the most common. In 9.5 % of the cases biopsies changed the diagnosis; in 3 of them primary ciliary dyskinesia was suspected. In 25 % medication was changed after bronchoscopy.

Children without asthma had a RBM of 5.6 μm (5.73 [3.07-7.03]); in children with asthma RBM thickness was 5.11 μm (4.85 [3,25 – 6,64]) and in those with difficult asthma 6.0 μm (6.2 [3,93 – 7,31]) (p=0.49).

CONCLUSION:
Bronchoscopy showed an important role to reveal underlying causes of persistent symptoms such as airway malacia and anatomic abnormalities. In 25% of patients bronchoscopy led to a change in treatment. We found no significant difference in RBM thickness between non-asthmatic, asthmatic children and children with difficult asthma.
III - BACKGROUND

3.1 General
Asthma is a multifactorial, chronic inflammatory disease of the airways. The main features of asthma include a variable degree of air-flow obstruction, bronchial hyper-responsiveness and chronic airways inflammation (1). These features lead to coughing, wheezing and shortness of breath, but these symptoms are not limited to an asthma diagnosis. (RW.ERROR - Unable to find reference:5).

3.2 Etiology & Pathophysiology
Asthma is a disease that results from airway inflammation, hyper-responsiveness, and structural changes of the airway, called remodeling. Many cells are involved in the pathology of asthma, like mast cells, mediators, eosinophil granulocytes, macrophages and the T-lymphocytes. Interactions between environmental, such as environmental tobacco smoke, viral infections, cold air, exercise, and genetic factors, result in acute and chronic inflammatory processes. In the classical form of asthma, the eosinophil granulocyte plays the most important role and the eosinophils are increased in the asthmatic airways. The inflammation reaction is persistent, even when the symptoms are episodic, and the presentation is individual dependent. Besides an increase of eosinophils or neutrophils, other changes as reticular basement membrane (RBM) thickening due to airway remodeling, increased extracellular matrix protein deposition, increased smooth muscle mass, new vessel formation, and mucus gland hyperplasia occur in the asthmatic airways (RW.ERROR - Unable to find reference:12).

3.3 Epidemiology of asthma
Around 115,000 children have asthma in the Netherlands, this is around the 4.5 percent of the Dutch children till an age of 14 years. Boys suffer almost one-and-a-half times more from asthma than girls during their childhood (2).

3.4 Diagnosis
Asthma is still a clinical diagnosis. Clinical features that increase or decrease the probability of asthma are stated in the BTS guidelines (table 1) (3):

<table>
<thead>
<tr>
<th>Clinical features that increase the probability of asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>* More than one of the following symptoms – wheeze, cough, difficulty breathing, chest tightness – particularly if these are frequent and recurrent; are worse at night and in the early morning; occur in response to, or worse after, exercise or other triggers, such as exposure to pets; cold or damp air, or with emotions or laughter; or occur apart from colds.</td>
</tr>
<tr>
<td>* Personal history of atopic disorder. Family history of atopic disorder and/or asthma. Widespread wheeze heard on auscultation. History of improvement in symptoms or</td>
</tr>
</tbody>
</table>
lung function in response to adequate therapy.

### Clinical features that lower the probability of asthma

* Symptoms with colds only, with no interval symptoms. Isolated cough in the absence of wheeze or difficulty breathing. History of moist cough

* Repeatedly normal physical examination of chest when symptomatic. Normal peak expiratory flow (PEF) or spirometry when symptomatic. No response to a trial of asthma therapy. Clinical features pointing to alternative diagnosis

* Prominent dizziness, light-headedness, peripheral tingling.

There is not one main symptom of asthma that is sufficiently specific and sensitive to serve as a diagnostic. However, wheezing is considered a core symptom. Besides the clinical criteria increasing or decreasing the probability of asthma as stated in the GINA guideline, The GINA guideline recommends additional testing to confirm the asthma diagnosis:

- Measurement of non-invasive markers of airway inflammation (FeNO).
- Measurement of allergic status. (4).

### 3.5 Treatment

#### 3.5.1 General

The goal of asthma treatment is to achieve and maintain clinical control (4). With the increased knowledge of asthma's chronicity and the role of inflammation, a greater focus has been placed on early diagnosis and daily, maintenance strategies of children with asthma, in parallel with confirming adherence to prescribed therapies (1). Current guidelines for asthma treatment are based on a combination of medicaments and non-medicamentous therapy. Inhaled Corticosteroids (ICS) for maintenance therapy and short acting Beta-agonist for symptom control play an essential role in asthma treatment (figure 1).
3.5.3 Non-medicamentous therapy

Non-medicamentous therapy aims at the avoidance of potentially modifiable factors such as exposure to environmental tobacco smoke or ongoing allergen exposure, as cats, dogs, and house dust mites.
3.5.4 Difficult asthma

The term difficult asthma generally refers to a clinical situation where a prior diagnosis of asthma exists, and asthma-like symptoms and exacerbations persist, despite prescription of high-dose asthma therapy. Previous consensus studies have suggested failure to achieve symptom control despite prescribed high-dose inhaled steroid as a minimum requirement, whilst more recent consensus work has stipulated a treatment level equivalent to at least step 4 (5).

 Probably 5 percent of the children with asthma are diagnosed with difficult asthma (6). Instead of changing directly the treatment of children when asthma treatment fails, the primary goal is to first search for an explanation for the failure (7).

Table 2 shows the difficult asthma protocol with the purpose to answer the fundamental question as to what has made this particular child’s asthma is so difficult to treat. The protocol is a compromise between what is scientifically ideal and ethical acceptability (6). A systemic approach to the child with difficult asthma comprises two steps. The first step in the management of a child with difficult asthma involves detailed diagnostic assessment to exclude an alternative diagnosis (“not asthma at all”) (RW.ERROR - Unable to find reference:1), see table 3. Recognition of the associated conditions will allow target treatment, rather than an escalation in asthma therapy. The second step, the assessment of asthma severity and the degree control should be identified if no either mimic or other condition coexists (6).

Only a small group with a truly diagnosed difficult asthma should be investigated according to a strict protocol, aimed at the specific characteristics of the individual child. After two weeks of systemic steroids the asthma is further phenotyped by means of lung function measurements, bronchoscopy, bronchoalveolar lavage and mucosal biopsies of the bronchus. Further treatment is then based on the outcomes of this phenotyping (RW.ERROR - Unable to find reference:2). Bronchoscopy, bronchoalveolar lavage fluid and bronchial biopsy will only be performed when there are no other diagnostic options available.

**Table 2: Difficult asthma protocol**

<table>
<thead>
<tr>
<th>Fase 1:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Confirm the diagnosis</td>
</tr>
<tr>
<td>2. Criticize the treatment and the reaction on the treatment</td>
</tr>
<tr>
<td>3. Assess asthma severity and the degree control</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fase 2: investment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Complementary diagnostics like HRCT thorax, pH, bronchoscopy, BAL and mucosal biopsies</td>
</tr>
<tr>
<td>2. Phenotyping</td>
</tr>
</tbody>
</table>
Table 3: Diagnosis that may or mimic or coexist with asthma

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroesophageal reflux</td>
<td>pH study, isotope milk scan</td>
</tr>
<tr>
<td>Vascular ring</td>
<td>Chest X-ray, spirometry, barium swallow, flexible bronchoscopy</td>
</tr>
<tr>
<td>Vocal cord dysfunction</td>
<td>Siprometry, laryngoscopy</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Sweat test, DNA analysis</td>
</tr>
<tr>
<td>Inhaled foreign body</td>
<td>Rigid bronchoscopy</td>
</tr>
<tr>
<td>Obliterative bronchiolitis</td>
<td>CT scan, respiratory viral titers in serum</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>CT scan</td>
</tr>
<tr>
<td>Primary ciliary dyskinesia</td>
<td>Ciliary brushing, nasal nitric oxide</td>
</tr>
<tr>
<td>Tracheobronchomalacia</td>
<td>Flexible bronchoscopy, bronchography</td>
</tr>
<tr>
<td>Recurrent aspiration</td>
<td>Bronchoalveolar lavage for lipid-laden macrophages, chest X-ray, CT scan, videofluoroscopy</td>
</tr>
<tr>
<td>Immune deficiency</td>
<td>Immune function testing</td>
</tr>
</tbody>
</table>

Together with the information gathered from the asthma protocol new treatment options should be overlooked for children with difficult asthma.

3.6 Bronchoscopy

3.6.1 Bronchoscopy

The use of bronchoscopy and endobronchial biopsies is mentioned in the 3rd step of the difficult asthma protocol. Bronchoscopy can be used for inspection of superficial airway structures in children with airway pathology, like difficult asthma, recurrent infections, congenital tracheomalacia, and to obtain cultures like in cystic fibrosis. Mucosal biopsies are found to be safe in the assessment of children with difficult asthma, there is effectively no risk of a pneumothorax and bleeding is minimal (8). However, the value of bronchoscopy and endobronchial biopsies in the management of children with difficult asthma is uncertain, and therefore should be studied.

3.6.2 Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) is usually performed when a child needs bronchoscopic examination. Bronchoalveolar lavage fluid (BALF) is examined for cell differentiation, bacterial cultures and lipid-laden macrophages. Lipid laden macrophages are a diagnostic clue for aspiration.

3.6.3 Biopsy

The best site for the biopsies is the subcarina of a segmental or sub-segmental bronchus. (8). In the biopsy, RBM thickness can be measured.
IV - INTRODUCTION TO THE STUDY

Asthma is a prevalent, inflammatory disease as we described above. Most children with asthma are easy to manage. In children with difficult to manage asthma both bronchoscopy and bronchial biopsies may be valuable in the diagnostic workup. It is surprising to find only a small number of hits on PubMed on this topic. A medline search in 2011 combining [asthma and biopsy (and endobronchial)] yielded 237 references. The same search combined with ‘pediatric’ resulted in only nine relevant hits. Biopsies are a tool to investigate asthma in children, but whether they actually change diagnosis or treatment has not been evaluated. Therefore it is relevant to investigate if there are correlations between the outcome of bronchoscopies and biopsies and whether these change patient management. In the case of a positive correlation, the regular management for children with difficult asthma can be optimized with the outcomes of this research. (RW.ERROR - Unable to find reference:3).

Endobronchial biopsies have been taken during bronchoscopies in children in the Beatrix Children’s Hospital in Groningen. The diagnostic yield of the biopsies has not been analyzed on a group level; but the results were always used to tailor individual patient care. The aim of this study is to analyze the yield from bronchoscopies and the results of the endobronchial biopsies and whether these change patient management.
V - OBJECTIVES

Primary objective:
To determine if bronchoscopy and the results of endobronchial biopsies change the management in children who had a bronchoscopy performed as a work up for their persisting respiratory symptoms.

Secondary objectives:
- What is the success rate of performing endobronchial biopsies in children?
- Are there differences in RBM thickness between children with and without asthma.
- Are there differences in RBM thickness between the subgroups of asthma.
- Is there a difference between the ‘10 point measurement method’ or the ‘area measurement method’ for the measurement of RBM thickness in children?
VI - MATERIAL & METHODS

6.1 Study procedure
This descriptive, retrospective study was completed in the Beatrix Children’s Hospital in Groningen, the Netherlands. All children who underwent bronchoscopy and bronchial biopsies of the muscosa for clinical reasons between January 2004 and December 2011 were registered in a bronchoscopy-biopsy database. The data included age, gender, lung function, fraction of exhaled nitric oxide, medication before and after the bronchoscopy, diagnosis before and after the bronchoscopy, number of biopsies, number of evaluable biopsies, inflammation infiltrate in BAL fluid and RBM thickness.

The bronchial biopsies were digitalized by the scanner ‘Hamatsu’ (Hamamatsu, Herts), and RBM was measured using ImageScope viewing software.

Asthma was defined as a doctor’s diagnosis and the use of ICS and/or bronchodilators and/or Leukotriene receptor antagonists.

Difficult asthma was defined as a condition where asthma control was not gained despite the use of ICS of twice daily 200 micrograms of fluticasone or equivalent AND the use of long acting beta2 agonists (LABA) or leukotriene receptor antagonists.

6.2 Flexible bronchus bronchoscopy procedure
All bronchoscopic procedures were performed using Olympus bronchoscopes under general anesthesia. Bronchial biopsy samples were taken from third- and fourth-order bronchial divisions from either the left or right side, using a cupped forceps. All children tolerated the procedure well with no complications.

6.3 Staining and immunohistology
Tissues for biopsy were fixed in 10 percent formaldehyde and embedded in paraffin blocks prior to sectioning. Small sections, with different sizes, were cut and stained with haematoxylin and eosin (H.E). These sections were scanned and re-evaluated with Aperio ImageScope Viewing Software (magnification 400x) in random order by the observer.

6.4 Patients
All children undergoing a bronchoscopy with endobronchial biopsies from January 2004 till December 2011 in the Beatrix Children’s Hospital in Groningen were eligible for inclusion. The data were collected retrospectively from the UMCG electronic patient files.

6.5 Characteristics
6.5.1 General
As outcome measures, the data from the respiratory symptoms were collected from the electronic patient files. As also lung functions, fraction of exhaled nitric oxide,
medication use pre- and post bronchoscopy, BAL outcomes, biopsy outcomes and diagnosis pre- and post bronchoscopy.

6.5.2 Lung function
Children underwent spirometry for the outcomes of FEV1, FEF75 and FVC.

6.5.3 Allergy
Total and specific IgE using the RAST test, provocation tests, or skinpricktests were recorded if available

6.5.4 Fraction Exhaled Nitric Oxide
The quantity of exhaled Nitric Oxide (FeNO) as measured with NIOX (Aerocrine, Sweden)

6.5.5 BAL outcomes
The BAL fluid was analyzed by the pathologist for cell differentiation and lipid-laden macrophages. The cell differentiation was documented in the categories none, few and many of granulocytes, macrophages and lymphocytes. The results from standard bacterial cultures were recorded.

6.5.6 RBM thickness
RBM thickness was measured with two methods
1) the 10-point measurement: the RBM thickness was measured on 10 random places, then the average RBM thickness was calculated.
2),the RBM thickness was assessed by the ‘area method’ the length and the area starting in the lower left angle of the coupe were measured and the viewer software calculated average RBM thickness.
Measurements were only performed on undamaged, uninterrupted RBM, and tangential sections were avoided.
A RBM thickness of > [4.16 μm +/- SD 0.66] was considered as thickened (9).
To avoid influence on the results by a learning effect, the first ten biopsies were scored again in random order with both methods at the end of the study

6.6 Main study parameters/endpoints:
The percentage of bronchoscopies that changed diagnosis or management
The percentage of biopsies resulted in treatment changes.

6.7 Ethic approval
Bronchoscopy and endobronchial biopsies were performed as part of the clinical assessment of children with respiratory symptoms. Therefore, the primary aim of the intervention was to examine the airway pathology of the child instead of guidance for the individual management of asthma patients.
Approval was obtained by the ethics committee (METc) of the UMCG. The study was found not-WMO obligatory.

6.8 Analysis
Inter-observer reproducibility was expressed as single-occasion coefficient of variance for repeat 10 percent of the measurements of RBM thickness.

6.9 statistics
All statistic analyses were performed using SPSS version 18.0. Intra-observer repeatability and between-biopsy variability were analyzed with interclass correlation coefficients and by the method of Bland and Altman. Within biopsy variability was expressed as coefficient of variance (%CV) for the RBM thickness. Correlations between RBM measurement methods and RBM and other parameters were assessed with Spearman Correlation Coefficients. Differences between asthmatic, difficult to treat asthmatic and non-asthmatic children were assessed with both parametric and non-parametric tests.
VII. RESULTS

7.1 General results

74 children were included (37 male, mean age 7.8 years). Their diagnosis before bronchoscopy was mild asthma in 26/74 children and 13/74 had difficult asthma. The control group underwent bronchoscopy for other clinical indications. These are shown in figure 3. Only children with incomplete data of bronchoscopy were excluded from this study.

Figure 3: gender and pre-bronchoscopy diagnosis of included children

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Mild asthma</th>
<th>Difficult to treat asthma</th>
<th>No asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>26</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>Mean Age (year)</td>
<td>7.0 [1.1 – 16.9]</td>
<td>9.3 [0.7 – 16.8]</td>
<td>7.1 [0.1 – 18.0]</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Man</td>
<td>17 (65.4 %)</td>
<td>6 (46.2 %)</td>
<td>14 (40 %)</td>
</tr>
<tr>
<td>- Female</td>
<td>9 (34.6 %)</td>
<td>7 (53.8 %)</td>
<td>21 (60 %)</td>
</tr>
<tr>
<td>Atopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eczema</td>
<td>5 (19.2 %)</td>
<td>3 (23.1 %)</td>
<td>3 (8.6 %)</td>
</tr>
<tr>
<td>High rate</td>
<td>1 (3.8 %)</td>
<td>5 (38.5 %)</td>
<td>8 (22.9 %)</td>
</tr>
<tr>
<td>Granulocytes &gt; 0.50 * 10^9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes &gt;10,0 *10^9</td>
<td>3 (11.5 %)</td>
<td>5 (38.5 %)</td>
<td>3 (8.6 %)</td>
</tr>
<tr>
<td>Lipid laden Macropahges</td>
<td>5 (19.2 %)</td>
<td>2 (15.4 %)</td>
<td>8 (22.9 %)</td>
</tr>
<tr>
<td>Culture Found</td>
<td>No culture found</td>
<td>H. Influenza</td>
<td>H. Parainfluenza</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------</td>
<td>--------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Number</td>
<td>19 (73 %)</td>
<td>8 (30,7 %)</td>
<td>-</td>
</tr>
<tr>
<td>Percentage</td>
<td>73 %</td>
<td>30,7 %</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>26 (73 %)</td>
<td>8 (30,7 %)</td>
<td>-</td>
</tr>
<tr>
<td>Medication before:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No medication</td>
<td>2 (7,7 %)</td>
<td>10 (38,5 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>ICS</td>
<td>-</td>
<td>-</td>
<td>2 (5,7 %)</td>
</tr>
<tr>
<td>LABA</td>
<td>9 (34,6 %)</td>
<td>0 (0 %)</td>
<td>1 (3,9 %)</td>
</tr>
<tr>
<td>ICS+ LABA</td>
<td>6 (46,2 %)</td>
<td>13 (100 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>11 (314 %)</td>
<td>3 (8,6 %)</td>
<td>5 (19,2 %)</td>
</tr>
<tr>
<td>Singular</td>
<td>3 (8,6 %)</td>
<td>0 (0 %)</td>
<td>100 (100 %)</td>
</tr>
<tr>
<td>Triamcinolon</td>
<td>11 (314 %)</td>
<td>1 (2,9 %)</td>
<td>32 (100 %)</td>
</tr>
<tr>
<td>other</td>
<td>3 (8,6 %)</td>
<td>0 (0 %)</td>
<td>1 (2,9 %)</td>
</tr>
<tr>
<td>Mean FEV1 (%)</td>
<td>100,2 [77,0 – 226,0]</td>
<td>84,3 [49,0 – 109,0]</td>
<td>91,0 [32,0 – 116,0]</td>
</tr>
<tr>
<td>Mean FeNO (%)</td>
<td>15,3 [5,6 – 33,5]</td>
<td>25,3 [5,0 – 65,5]</td>
<td>9,7 [3,9 – 21,2]</td>
</tr>
</tbody>
</table>

### 7.2 Management

74 children underwent bronchoscopy including mucosal biopsies and 42 of them had assessable endobronchial biopsies. Only 15% of the children had a minor bleeding treated with instilled xylometazaline. None of these complications had a negative influence for the accomplishment of the procedure.

The diagnosis of the children after the bronchoscopy changed in 31,3% of the cases, while the diagnosis after biopsies changed in 9,5% of the cases (figure 4). Most of them had an extra anatomical explanation for the prolonged symptoms. Only in 8 children were diagnosed differently after bronchoscopy (table 5), and in 2 children after evaluation of biopsies. In 24,7% of the children the medication was adjusted after the investigations, for coexisting comorbidity or for symptomatic treatment.

**Table 5: Change of management/diagnosis after bronchoscopy or biopsy**

<table>
<thead>
<tr>
<th></th>
<th>misdiagnosed</th>
<th>Extra diagnosis to explain the symptoms</th>
<th>Confirmation of diagnosis</th>
<th>The main diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>After bronchoscopy</td>
<td>8 children</td>
<td>10 children</td>
<td>5 children</td>
<td>- Airwaymalacia - Anatomic disorders - Chronic cough</td>
</tr>
<tr>
<td>After biopsies</td>
<td>2 children</td>
<td>3 children</td>
<td>2 children</td>
<td>- PCD</td>
</tr>
</tbody>
</table>
7.3 Biopsy success rate of endobronchial biopsy
From the 74 included children in the study, 173 coupes were received from biopsy for evaluation (2 to 3 biopsies per child). From 59/74 patients had evaluable coupes. The others had a specific staining that could not be used for measuring RBM thickness, macroscopically empty coupes (coupes were not able to see with the naked eye before microscopy, or to low quality). In the coupes that were not assessable because of low quality the RBM thickness could not be measured with either method.

Table 5: Biopsy success rate

<table>
<thead>
<tr>
<th></th>
<th>Number of biopsies</th>
<th>Success rate in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Biopsies</td>
<td>173</td>
<td>100</td>
</tr>
<tr>
<td>Total assessable biopsies per child</td>
<td>59</td>
<td>34,1</td>
</tr>
<tr>
<td>Total Individual HE-staining</td>
<td>53</td>
<td>71,8</td>
</tr>
<tr>
<td>Success rate of obtaining evaluable biopsies</td>
<td>42</td>
<td>58,3</td>
</tr>
<tr>
<td>- total</td>
<td>42</td>
<td>31,3</td>
</tr>
<tr>
<td>- mild asthma</td>
<td>11</td>
<td>30,8</td>
</tr>
<tr>
<td>- difficult asthma</td>
<td>9</td>
<td>21,4</td>
</tr>
<tr>
<td>- no asthma</td>
<td>20</td>
<td>53,5</td>
</tr>
</tbody>
</table>
In this study there was a success rate of 58.3%. This means that 32 children were excluded for RBM thickness measurements. 14.9% of these children had no assessable coupes and in 28% another staining than H.E. staining was used. In comparison to other studies, which show a success rate of 78.2% the success rate is low (10). In the clinical practice the use of immuno-staining and electro microscopy is highly used for evaluation of the biopsies.

7.4 RBM method measurement in comparison

The procedure of measuring RBM thickness according to both methods is shown in figure 5 and the results in table 4.

**Figure 5: Area measurement and 10-point measurement**

![Figure 5](image)

**Table 4: RBM Measurements**

<table>
<thead>
<tr>
<th></th>
<th>10-point measurement (µm)</th>
<th>Area measurement (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole population</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5.36</td>
<td>5.63</td>
</tr>
<tr>
<td>Range</td>
<td>2.96 – 8.43</td>
<td>3.07 – 8.86</td>
</tr>
<tr>
<td><strong>Non-Asthmatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5.61</td>
<td>5.73</td>
</tr>
<tr>
<td>Range</td>
<td>2.96 – 8.43</td>
<td>3.07 – 7.03</td>
</tr>
<tr>
<td><strong>Mild Asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5.11</td>
<td>4.85</td>
</tr>
<tr>
<td>Range</td>
<td>3.33 – 7.13</td>
<td>3.25 – 6.64</td>
</tr>
<tr>
<td><strong>Difficult Asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.06</td>
<td>6.2</td>
</tr>
<tr>
<td>Range</td>
<td>4.15 – 8.26</td>
<td>3.93 – 7.31</td>
</tr>
</tbody>
</table>
The correlation [Correlation coefficient: 0.87] between the both methods of RBM measurement meaning that there is positive correlation between the methods, and thus show similarity.

7.5 RBM subgroups comparison
While the group without asthma [area measurement: 5.73 μm (3.07 - 7.03), 10 point measurement: 5.61 μm (2.96 – 8.43)] showed a visual thicker RBM thickness than group with mild asthma [area measurement: 4.85 μm (3.25 – 6.64), 10 point measurement: 5.11 μm (3.33 – 7.13)], the group with difficult asthma showed the thickened RBM [area measurement: 6.2 μm (3.93 – 7.31), 10 point measurement: 6.06 μm (4.15 – 8.26)]. We observed a trend towards increased RBM thickness between the subgroups, however this was not significant [p=0.49].

7.6 RBM in comparison to the other parameters
The exploration of the relationship of RBM to the other parameters in the dataset was done by creating several graphs and descriptive statics. Some of the graphs gave the visual impression of an association. We found a significant correlation between the granulocytes in BAL and the subgroups in asthma [p= 0.013]. There were no significant correlations between RBM thickness and sex [r= 0.014 p= 0.931], lung function [Spearman Correlation Coefficient FeNO: r=0.074 p= 0.779 FEV1: r = 0.332 p= 0.091 FVC: r = 0.013 p= 0.948], atopy [Spearman Correlation Coefficient: r=0.006 p=0.970] and BAL outcomes [Spearman Correlation Coefficient gran: r= 0.062 p=0.695, macr: r= 0.026 p= 0.872, leuco: r= 0.127 p= 0.423]. Also, there were no significant correlations found between the asthma subgroups and FeNO [p= 0.134]. At last, there was no significance found between the RBM thickness and the change trough biopsy [Spearman Correlation Coefficient: r=0.277 p=0.076].
7.7 Inter-observer

The first 10 children in our data with assessable coupes were reevaluated for the RBM thickness with both methods, to avoid influence of the learning curve during the study (table 5). The inter-observer had an Interclass Correlation Coefficient (ICC) of 0.95. Figure 7 shows this strong correlation between both outcomes, which means that there were little variables that influenced the measurements of the RBM thickness.

Table 5: Inter-observer

<table>
<thead>
<tr>
<th></th>
<th>10 point method (μm)</th>
<th>Area method (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>5.52 +/- SD 1.30</td>
<td>5.43 +/- SD 1.36</td>
</tr>
<tr>
<td>Inter-observer</td>
<td>5.06 +/- SD 0.98</td>
<td>5.69 +/- SD 1.03</td>
</tr>
</tbody>
</table>

Figure 7: Inter-Observer measurement RBM thickness
VIII – DISCUSSION

This study shows that the diagnostic yield from bronchoscopy itself is low, especially for the management of asthmatic children, but important to exclude trachea- or bronchomalacia and anatomic abnormalities. The diagnostic yield from biopsies is less. Only children with an indication for Primary Ciliary Dyskinesia show true benefits of the biopsies. Then, our study shows that although RBM is thicker in children with asthma.

We can also conclude that bronchoscopy with BAL and endobronchial biopsy can be performed safely in children with asthma and difficult asthma under general anesthesia, and that the procedure is acceptable to parents and children. This is in agreement with existing literature (RW.ERROR - Unable to find reference:3). However, the procedure of bronchoscopy and endobronchial biopsies will be useful, if it is subsequently shown to contribute to patient management ((RW.ERROR - Unable to find reference:3). Research shows that bronchoscopy and biopsies are able to indicate airway remodeling and airway inflammation in children with asthma. The outcomes of bronchoscopy and biopsies are assessed for clinical management on an individual level.

Still we should doubt the outcomes of bronchoscopy and biopsies, due to the fact that in the pathogenesis of asthma, i.e. eosinophils, mast cells and T-lymphocytes, do not follow a normal frequency distribution (11). On this manner the outcomes could be misinterpret. As children can only be broncoscoped with small caliber bronchoscopes, only small forceps can be used to obtain biopsies. The resulting tissue samples are small and might decrease the diagnostic yield compared to larger samples as can be obtained from adults.

This study shows on a group level that the procedure does not lead to a significant different approach to treatment. While Payne states that the different phenotypes in the subgroups of asthma require a different approach to treatment (6), the clinical approach stays behind. Also Ranganathan et al, concluded earlier that despite an extensive search, for the majority of children in his study they were unable to offer an alternative diagnosis to difficult to threat asthma after bronchoscopy and endobronchial biopsies (12). Still it is important that if a child with asthma is not responding to treatment, one should first ask what it is about this child’s asthma that makes it therapy resistant or difficult to manage (6). Our study as well as existing research confirms that the results of bronchoscopy may result in a change of diagnosis, most often PCD, airway malacia and anatomical abnormalities. This means, that the procedure could be very important on the individual level.

A RBM thickness of > [4,16 μm +/- SD 0,66] was considered as thickened based on the study of Bourdin. This means that in our study the mean of the population showed a thickened RBM thickness compared to children with healthy airways (9). We found no significant difference between the RBM thickness in children with asthma and non-asthmatic children. Even though there is a visual impression of a difference between the
subgroups in asthma children, this turned out not to be significant. These outcomes differ from other studies, of Payne and Bush, stating that RBM is thickened in asthmatic children compared to a controls. Another explanation for equal RBM thickness in with and without asthma is that the non-asthmatic children might have an increased RBM thickness because of chronic airway infections while the use of corticosteroids by asthmatic children might lower the RBM thickness. In children with difficult to treat asthma, the children often show a poor response on the use of corticosteroids, which explains the thicker, albeit non-significant average mean RBM thickness compared to children with mild asthma.

How could the yield of bronchoscopies and endobronchial biopsies be increased? The answer might be technology enabling larger biopsies in children without increased risks or to search for a different method to assess airway pathology. Bronchoscopy might still be needed to validate the use of non-invasive techniques (13). There is a successful development of non-invasive methods to assess airway pathology Non invasive imaging techniques include computed tomography (CT), magnetic resonance imaging (MRI) and optical coherence tomography. Of these methods, CT is the best characterized (13). This method can be used for qualitative description of various changes to lung structure, in particular airway wall thickening, not to be confused with RBM thickening in asthmatic patients (13).

Sullivan et al. state that there is no agreement as to how many measurements of the RBM should be made to obtain a reliable estimate of its thickness (11). In this study we demonstrated that there is no significant difference between the 10-point RBM measurement and the area RBM measurement. While the area RBM measurement is a more objective measurement for studies because of the same location of the measurement in every included coupe, it did not make a difference in outcome compared to the 10-point measurement. For further study we recommend to include the interval RBM-measurement in the comparison, a frequent used measurement, the interval method, 40 points over 20 um intervals are measured in this method. Only on this way we conclude fully that there is no aim for a specific measurement.

We could indeed confirm that there is no significant preferable method for the measurement of the RBM in children. However, there are three commonly used ways of measurements and in this study we only included two methods. The third way to measure RBM thickness includes 40 measure points over 100 μm RBM. Besides that, we should be aware of the fact that the methods are very subjective. While the area-measurement is less subjective because the measuring is performed every time in the same angle of the coupe, still both measurements are influenced by human interpretations and visual limitations. The 10-point measurement might be more sensitive for subjectivity, due to own influence about which part of the RBM will be measured, although in our study no difference between the 2 methods was found. The area measurement is constantly measured on the same angle of the biopsy, in the left under angle of the coupes in this study, and is therefore less subjective.
Study limitations
Potential limitations of our study are that the quality of the biopsies could be low, due to poor techniques, poor staining or too little tissue for evaluation. Likewise, the biopsies in children are smaller due to the small airways of children wherefore a smaller endoscope is used in the procedure. This means that biopsies are smaller and may be correspondingly more difficult to interpret (6) Therefore the interpretation of the biopsies in children with asthma could be difficult.

Other limitations of the study were, the population size and the use of H.E. stained coupes. 74 children were included in the study, while 59 children had evaluable coupes. If more children were included in the study, the study could have found significant differences. This could also explain the controversy with the existing literature. Further a control group should include healthy children but these children will not undergo diagnostic bronchoscopy. Therefore, no healthy endobronchial tissue could serve for acquisition of control data. This may contribute to the fact no significance was found between the non-asthmatic and asthmatic group.

We acknowledge that this study is retrospective and for clinical data we are dependent on adequate registration of diagnosis in the electronic patient files, which may have influenced our results.

The use of H.E. stained coupes had no limitations for measurements of the RBM thickness. Nevertheless, it was a limitation for the eosinophil counts in the coupes. Besides that, we used in this study light microscopy, while better outcomes would be predicted when we used electronic microscopy to analyze the coupes in more detail. At last, this study was performed as a retrospective, which is totally based on registration in the electronic patient files.

IX - CONCLUSION
Taken together, that the value of bronchoscopy is mainly to explore important other explanations for persistent respiratory symptoms. Evaluable biopsies can be obtained in a majority of children. In most of those biopsies, RBM measuring is feasible. We found no difference in RBM thickness between children with asthma, difficult to treat asthma or children with another diagnosis. There is no difference between the two methods of measuring RBM thickness we used. The measurement of RBM thickness did not result in treatment change.

X - NEDERLANDSE SAMENVATTING
INTRODUCTIE:
Bronchoscopie en endobronchiale biopsieën zijn diagnostische instrumenten bij de evaluatie van moeilijk behandelbaar astma en andere kinderlongziekten. De diagnostische winst van deze procedures is niet bekend. Daarom was het doel van deze studie om de uitkomsten van bronchoscopieën en endobronchiale biopsieën te reevalueren om te zien of ze van invloed waren op de behandeling van kinderen met astma.

METHODE:
De studie was opgezet als een descriptieve en retrospectieve studie en is uitgevoerd in het Beatrix Kinderziekenhuis in Groningen. Endobronchiale biopsieën zijn genomen tijdens klinisch geindiceerde bronchoscopieën. De coupes werden ingescand met de ‘Hamatsu’ scanner en geëvalueerd op reticulaire basaal membraan (RBM) dikte met Aperio ImageScope software.

RESULTATEN:
Van de 74 kinderen (37 man, gemiddelde leeftijd 7.8 jaar), hadden 26 astma en 13 moeilijk behandelbaar astma. De diagnose veranderde in 31.3 % van de gevallen na bronchoscopie: 10 van de kinderen kregen een extra diagnose die de persisterende symptomen kon verklaren, bij 8 kinderen werd een andere diagnose gesteld: luchtwegmalacie (n=10) en anatomische afwijkingen (n=7) werden gevonden. Bij 9,5 % van de gevallen veranderde de diagnose na de uitkomsten van de biopsie, 3 van hen hadden een mogelijk PCD.. In 25 % van de gevallen werd de medicatie aangepast na bronchoscopie.
Kinderen zonder astma hadden een RBM dikte van 5,6 μm (5,73 [3,07-7,03]); bij kinderen met astma was dit 5,11 μm (4,85 [3,25 – 6,64]) en in de kinderen met moeilijk behandelbaar astma was dit 6,0 μm (6.2 [3,93 – 7,31]) (p=0.49).

CONCLUSIE;
De waarde van bronchoscopie is voornamelijk het opsporen van belangrijke alternatieve verklaringen voor persistente luchtwegsymptomen. Metingen van basaal membraan dikte is uitvoerbaar in de praktijk en er is geen verschil aantoonbaar voor de tweede methoden. Echter, de uitkomsten van de biopsieën, inclusief de dikte van de basaalmembraan leidde niet tot verandering in de behandeling van de meeste kinderen.

XI - REFERENCES


(40) Vanlaeken L. Endobronchial biopsies in children: feasibility and results.:16.
