Non-invasive biomarkers in paediatric asthma

IMPACT PARAGRAPH

1. Research goals of the thesis

Asthma is the most common chronic respiratory disease in adults and children, affecting more than 350 million people globally. Asthma is a well-defined disease, characterised by respiratory symptoms due to an underlying airway inflammation and airway obstruction. Diagnosis is made by combining asthma-specific symptoms with lung function abnormalities. Unfortunately, asthma is a heterogeneous disease, which could make an adequate and timely diagnosis difficult. This is particularly true for young children. Wheezing, a hallmark symptom of asthma, is one of the most common respiratory symptoms in this age group. In the majority of children < 6 years of age, wheezing episodes will be transient. On the other hand, approximately one third of these children will have persistent symptoms and develop asthma. Currently, we do not have reliable tests to predict which wheezing children will develop asthma. Objective lung function tests, needed for a reliable asthma diagnosis, are not feasible in this age group. Furthermore, prediction rules based on clinical parameters alone, are not reliable enough and show inadequate performance in individual cases. As a result, an accurate and timely asthma diagnosis is very difficult at young age. This potentially leads to an underdiagnosis of true asthma, or an overdiagnosis of asthma in children with transient wheezing symptoms. Underdiagnosis and consequently undertreatment could affect quality of life, disease progression, and parental work absenteeism resulting in an increased economic burden on society. On the other hand, overdiagnosis and overtreatment could lead to unnecessary pharmacological side effects, and unnecessary high health care costs. Beside the lack of an accurate asthma prediction, we also do not have reliable tests to predict which wheezing children will benefit from (anti-inflammatory) asthma treatment. Again, this potentially leads to overtreatment or undertreatment with the aforementioned potential consequences. Hence, we need both reliable biomarkers for an accurate asthma diagnosis in wheezing preschool children, and biomarkers to better predict a treatment response. Ideally, these biomarkers should be non-invasive and impose the least burden on this young group of children.

This thesis focussed on the usefulness of non-invasive biomarkers, including exhaled breath analysis and the gut microbiome, in the prediction of asthma in wheezing preschool children. Furthermore, we assessed the diagnostic value of exhaled breath analysis for diagnosing asthma in children above 6 years of age. Finally, we assessed the usefulness of exhaled breath analysis in the prediction of a clinical ICS treatment response in wheezing preschool children.

2. Relevance of the research in this thesis: non-invasive biomarkers in preschool wheeze and paediatric asthma

2.1 Exhaled breath analysis to predict asthma in wheezing preschool children

As many markers of airway inflammation are invasive, e.g. bronchoscopy, or semi-invasive, e.g. blood sampling, we focussed on non-invasive biomarkers in exhaled breath. Exhaled breath analysis can be divided in different domains, including exhaled breath condensate (EBC) and exhaled volatile organic compounds (VOCs).

EBC is obtained by cooling of exhaled breath. The resulting condensate contains a small fraction of volatile and non-volatile molecules, including various markers of airway inflammation.

The contribution of the research in this thesis to scientific knowledge was that biomarkers in EBC with the current methodology are not accurate and reliable enough for an early prediction of asthma in wheezing preschool children. Moreover, EBC is far from implementation in daily clinical practice due to an important lack of methodological standardisation.

Exhaled VOCs are carbon based chemicals in exhaled breath. VOCs can both originate from internal body processes (e.g. airway inflammation), and from external sources (e.g. environment, medication). As a result, thousands of VOCs are identified in exhaled breath. For the analysis of exhaled breath, two methods are currently mostly applied: gas chromatography mass spectrometry (GC-MS) to identify specific VOCs, and an electronic nose (eNose) sensor technology based on a pattern recognition technique.

The contribution of the research in this thesis on exhaled VOCs to scientific knowledge was that exhaled VOCs has shown high potential for prediction of asthma in wheezing preschool children. However, literature on this topic is sparse and these findings need further confirmation and replication.

The societal impact of this finding is that application of exhaled breath analysis in a large group of children with wheezing symptoms might prevent underdiagnosis and undertreatment of true asthmatic children, and prevent overtreatment of preschool children with transient wheeze. This probably has a large beneficial influence on symptom control, lung function, and quality of life in these children. Moreover, this will also reassure parents of young children with respiratory symptoms, improve their quality of life and might potentially even decrease work and school absenteeism. All together this will result in a better cost-effectiveness and lower economic costs in this group of patients.

In the recently initiated ADEM2 study at the Maastricht University Medical Centre^{+}, the health gain and increase in cost-effectiveness of treatment in preschool children with wheeze will be established.

The production and implementation of a breath test in the Netherlands in this age group is potentially an interesting business model for various companies and for the Dutch society.

2.2 Gut microbiome to predict asthma in wheezing preschool children

Analysis of the gut microbiome is another interesting non-invasive technique as stool is easily collected, even in young children. Moreover, there is evidence of a so-called gut-lung axis, which means that changes in gut microbial composition are linked with altered immune responses and homeostasis in the airways. In this thesis we investigated the gut microbiome in wheezing preschool children, and found that a high relative abundance of the genus *Escherichia* was associated with subsequent asthma development. In contrast, overall gut microbial diversity and overall gut microbial community structure at age 2-4 years was not associated with preschool wheezing or future asthma development. Earlier studies on this topic showed that gut microbial dysbiosis, particularly in the first year of life, is associated with asthma development. The exact mechanisms are incompletely understood, although diet (rich in fibres and probiotics) might beneficially influence the gut microbiome, whereas medication (antacids and antibiotics) negatively influence the gut microbiome. Our findings stimulate future research to investigate how gut microbial dysbiosis at preschool age could be prevented in order to prevent future asthma development.

The societal impact of these findings might be that innovative dietary and probiotic interventions for the prevention of asthma in young children can be developed.

2.3 Exhaled breath analysis to diagnose asthma in children

In this thesis we explored the usefulness of a newly developed eNose, the Aeonose (The eNose Company, Zutphen, The Netherlands), to diagnose asthma in children. First, we found a high feasibility of this newly developed eNose: it was an easy-to-use, patient friendly eNose, capable of direct sampling. Next, we found a modest to good diagnostic accuracy to differentiate children with asthma from healthy controls. Previous studies using GC-MS technology for this purpose showed higher diagnostic accuracies. However, GC-MS cannot be used for real-time measurement, and it is an expensive, time-consuming technique. An eNose might therefore be an easy-to-use and cheaper alternative. An additional advantage of the Aeonose eNose, compared to other eNoses, is its aspect of direct sampling, which brings exhaled breath analysis closer to daily clinical practice.

The contribution to scientific knowledge is that the Aeonose eNose has good overall feasibility in children but too little diagnostic accuracy in childhood asthma to support broad clinical implementation. As multiple eNose sensor systems exist, of which the diagnostic performance in asthma or preschool wheezing is currently insufficiently

known, future studies should focus on the development and validation of asthma-specific eNoses in children.

2.4 Exhaled breath analysis to predict a treatment response in wheezing preschool children

Finally, we investigated whether exhaled VOCs, as assessed by GC-MS, could predict a clinical ICS treatment response. In our study we showed that exhaled breath profiles (VOCs) were significantly different between children who responded to ICS and children who did not respond to ICS. This effect was dependent on the future diagnosis of true asthma or transient wheeze at age 6 years, suggesting that wheezing children who eventually developed asthma had a higher chance of being ICS responsive. The treatment of wheezing preschool children, in particular the decision whether or not to start with ICS, has been part of debate for years. For this purpose other biomarkers, such as exhaled nitric oxide (FeNO), blood eosinophilia, and atopic sensitisation, have been studied with varying results. As both preschool wheezing and (paediatric) asthma are too heterogeneous, probably no single biomarker is capable of adequately predicting a clinical ICS response.

Our findings show a novel and innovative potential application of exhaled breath analysis in guiding treatment decisions in children with respiratory symptoms. Moreover, our results could ultimately help in personalising treatment in wheezing preschool children in order to improve cost-effectiveness and reduce side effects.

3. Relevance to patient groups: non-invasive biomarkers in preschool wheeze and paediatric asthma, how to proceed?

Despite our increased knowledge and understanding of asthma, we are still facing the following issues in children, particularly at young age:

- Which wheezing preschool children will eventually develop asthma?
- Which wheezing children will benefit from therapy (ICS)?
- How can we reliably diagnose asthma if routine lung function tests are not feasible?
- How can we better phenotype wheezing or asthmatic children without performing invasive tests?

The common denominator to the above questions is that current available techniques and tests do not fulfil our requirements in (young) children. We need simpler tests, feasible at young age, and preferably non-invasive. Moreover, these tests must be accurate and reliable.

Exhaled breath analysis has the potential to fill this gap. However, it should be noted that important steps have to be undertaken to move towards true clinical application.

These predominantly relate to standardisation of the sampling procedure of exhaled breath, measurement of VOCs, and consecutive statistical analysis.
In this thesis, the pearls and pitfalls of exhaled VOCs and EBC are summarised. Moreover, the feasibility and accuracy of a new handheld device is tested, and the potential confounding role of ICS is investigated. To further progress in this research field, more collaboration between research groups should be sought to address current knowledge gaps.
The gut microbiome should probably not be seen as a future biomarker to diagnose or predict asthma in wheezing preschool children. However, the findings from this thesis add to the growing knowledge of the effects of early life gut microbial dysbiosis on future disease. This might eventually lead to novel dietary interventions with hopefully positive effects on the prevention of asthma.
It is unrealistic to believe that the diagnosis of a complex and heterogeneous disease like asthma could be based on one single test or biomarker. The advent of newly developed biomarkers or techniques, such as exhaled VOCs, could aid by improving diagnostic algorithms, better monitoring of disease, and by guiding treatment decisions. This might ultimately lead to an earlier diagnosis of asthma in wheezing preschool children, and a reduction in the burden of asthma, with an increased quality of life for our patients.

4. Dissemination and implementation of the findings in this thesis

Methods used for knowledge transfer to relevant stakeholders include presentations on annual conferences, publications in journals, media attention, and accessible articles on websites of e.g. the Dutch Lung Foundation, and the Netherlands Respiratory Society.
The possibilities to implement exhaled breath analysis in primary care and specialist care are good: there is a high clinical need, the test is easy to perform, takes little time, and fits within the work-flow of general practitioners and paediatricians.
Recently we started the ADEM2 project in order to further demonstrate the health gain and cost-effectiveness of care by application of exhaled breath analysis in wheezing preschool children. During the project, an implementation task force with representatives of relevant stakeholders in the Netherlands are involved: patients (also children) and parents, paediatric pulmonologists, general practitioners, health insurance companies, and the Dutch Lung Foundation.
Hopefully the ADEM2 project will provide further evidence for health gain and diagnostic value of exhaled breath analysis in order to reduce the significant disease burden in preschool children with wheezing symptoms and their parents.