Association of wheezing phenotypes with fractional exhaled nitric oxide in children

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Running title: Association of FeNO with wheezing phenotype

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Abstract

Asthma is a heterogeneous group of disorders characterized by airway inflammation, airway obstruction, and airway hyperreactivity (AHR). Airway inflammation is the key pathophysiology of asthma, which induces AHR and recurrence of asthma. Fractional exhaled nitric oxide (FeNO) is a noninvasive and reproducible measurement for eosinophilic airway inflammation, which is easy to perform in young children. As airway inflammation is present earlier than asthma attack and airway obstruction, high FeNO levels may be used as a predictive marker for recurrence of asthma.

Several wheezing phenotypes in early childhood have been studied in large-scale longitudinal studies. Wheezing phenotypes have been classified into three to six categories based on the onset and persistency of wheezing from birth to later childhood. Each phenotype showed characteristic features in atopic sensitization, lung function, AHR or FeNO. In one birth cohort study, children who have asthma and persistent wheezing at 7 years had higher FeNO levels at 4 years compared to those without wheezing, which suggests the possibility of FeNO as a predictive marker for later asthma development. In addition, preschool children with recurrent wheezing and stringent asthma predictive index also have higher FeNO levels in the first 4 years of life compared to wheezing children with loose index or children with no wheeze.

In conclusion, FeNO may serve as an additional parameter for predicting persistent wheezing in preschool children. More large scaled longitudinal studies are required to set up cut-off levels of FeNO as a risk factor for persistent asthma.

Key words: Atopy, Fractional exhaled nitric oxide, Lung function, Phenotype, Preschool children, Wheeze
Introduction

Asthma is a heterogeneous group of disorder, which is associated with recurrent episodes of wheezing, coughing, chest tightness, and shortness of breath. Although recurrent wheezing is the most common symptom suggesting asthma, not all the children with wheezing are asthmatic. Furthermore, only a small percentage of infants with wheezing will develop asthma in later childhood. Therefore, identification of asthma predictive marker in preschool children might help in early diagnosis and management of asthma. Large birth cohort studies suggested wheezing phenotypes which may help in predicting the development of asthma in later life.

Airway inflammation, airway hyperresponsiveness (AHR), and variable airway obstruction are key pathophysiology of asthma. Airway inflammation is present during asymptomatic period of asthma and may precede the onset of asthma. Airway inflammation also induces recurrent asthma attack and leads to airway remodeling. Therefore, early detection of airway inflammation is the cornerstone of asthma therapy. Although objective measurement for AHR or airway obstruction has made an advance in clinical practice, assessment of airway inflammation using bronchial biopsy or bronchoalveolar lavage is too invasive in children.

FeNO is a non-invasive, reproducible, easy, and simple biomarker of eosinophilic airway inflammation. In recent studies, FeNO has been shown to be useful in the diagnosis of asthma as well as monitoring steroid responsiveness in asthma. FeNO correlates well to AHR and atopy in children. Early intervention and treatment of asthma is important to improve the prognosis and decrease irreversible airway remodeling. Chronic airway inflammation is present even in children with mild asthma and leads to recurrent asthma attack.

Several longitudinal cohort studies tried to figure out clinical and laboratory characteristics
according to wheezing phenotypes to help distinguish children who will develop asthma from those who have transient symptoms only. FeNO can be assessed easily in preschool children with recurrent wheezing and may be used as a biomarker for persistence of asthma up to later childhood. Only a few studies have shown high FeNO levels were associated with persistent wheezing phenotypes compared to transient wheeze in preschool children. This review will focus on association of lung function test, atopic sensitization, and FeNO levels with wheezing phenotypes in early childhood and summarize the results of several large birth cohort studies in Table 1.

Wheezing phenotypes in early childhood

There are several large longitudinal cohort studies which classified wheezing phenotypes in preschool children according to the onset and persistency of wheezing. Based on the wheezing in the first 6 years of age, the Tucson Children’s Respiratory Study (TCRS) first classified wheezing into four phenotypes among 826 children from birth to 6 years: no wheezing (51.5%), transient early wheezing (19.9%), persistent wheezing (13.7%), and late-onset wheezing (15.0%). Early transient wheezing group has high prevalence of wheezing before age of 3 years and resolves at the age of 6 years, whilst persistent and late-onset wheezing groups have persistent wheezing at 6 years old. The prevalence of ever-diagnosed as asthma at 6 years was 46.0% in children with persistent wheeze compared to 22.5% in children with late onset wheeze.

On the other hand, the Avon Longitudinal Study of Parents and Children (ALSPAC) reclassified wheezing into six phenotypes in 6265 children from birth to 7 years using latent class analysis: no/infrequent wheezing (59%), transient early wheezing (16%), prolonged early wheezing (9%), intermediate onset wheezing (3%), late onset wheezing (16%), and
persistent wheezing (7%). Intermediate onset wheeze had a low prevalence of wheeze up to 18 months and high prevalence from 42 months of age, and prolonged early wheeze had a peak prevalence of wheeze at 30 months and declining prevalence from 69 months (Fig.1). They identified higher prevalence of asthma by 7 years in the intermediate onset wheeze compared to the late onset wheeze.

The Prevention of Infant Asthma and Mite Allergy (PIAMA) cohort identified five phenotypes of wheezing children after replicating the process with 2810 cases from birth up to 8 years of age\(^8\); never/infrequent (75.0%), transient early (16.7%), intermediate onset (3.1%), late onset (1.7%), and persistent wheeze (3.5%) (Fig.2). Transient early wheezing in PIAMA study was characterized by high prevalence of wheezing at 12 months and a decline thereafter to a low prevalence at 84 months. It represents a combination of prolonged early wheezing and transient early wheezing in ALSPAC study. In PIAMA model, persistent, late onset, and intermediate onset wheeze were strongly associated with asthma at 8 years of age\(^8\).

The Southampton Women’s Survey (SWS) successfully validated ALSPAC phenotypes using 6-year follow-up data of longitudinal lung function and atopic sensitization among 940 children from birth up to 6 years\(^9\). They performed infant lung function at 6 weeks, spirometry, FeNO, and methacholine challenge at 6 years, and skin prick test at 12 months, 3 and 6 years. The SWS cohort study assigned wheezing children into ALSPAC and Tucson phenotypes according to wheezing data at 6 and 12 months, and at 2, 3, and 6 years; never (40.3%), transient early (17.4%), prolonged early (27.6%), intermediate onset (6.2%), late onset (2.2%), and persistent (6.4%) in ALSPAC phenotypes and never (40.3%), transient early (45.0%), late onset (2.2%), and persistent (12.5%) in Tucson phenotypes.

Lung function and atopic sensitization in different phenotypes of wheezing
Tucson study identified transient early wheeze had diminished airway function at birth, which was still present at the age of 6 years, and was non-atopic, whilst persistent wheeze had normal lung function at birth, which diminished at 6 years of age, and had atopic sensitization\(^2\). Late onset wheeze had normal lung function both at birth and at 6 years and higher prevalence of atopic sensitization at 6 years compared to never wheeze. This was supported by the Manchester Asthma and Allergy Study (MAAS), a population-based birth cohort study. They measured specific airway resistance at age 3 and 5 years, and analyzed the association of lung function with wheezing phenotypes of Tucson. The MAAS study found persistent wheeze and transient early wheeze were associated with poor lung function at age 3 years and 5 years, while late onset wheeze had normal lung function in preschool age. In addition, persistent wheeze was associated with steeply increasing airway resistance at 5 years compared to at 3 years. However, the Copenhagen Prospective Studies on Asthma and Childhood (COPSAC) cohort study found children with asthma at 7 years of age had lung function deficit during neonatal period, which progressed during early childhood and also had AHR in the neonates\(^10\).

The ALSPAC study assessed atopic sensitization, spirometric lung function, and AHR at 7-9 years of age. They identified intermediate onset wheeze had the strongest associations with atopy, lung function deficit, and AHR at 7-9 years followed by late onset wheeze and persistent wheeze in order. Transient and prolonged early wheeze did not show associations with atopy and had weak associations with AHR\(^3\).

The PIAMA study showed airway resistance at 4 years measured with the interrupter technique was higher in persistent wheezer than in never and early wheezers\(^11\). In SWS study\(^9\), intermediate onset wheeze showed earlier atopic sensitization at 1 year, while persistent and late onset wheeze had atopic sensitization at 3 and 6 years. Intermediate
onset wheeze showed no lung function deficit at 6 weeks and 6 months, while persistent wheeze were associated with lower lung function at 6 weeks and 6 years. Prolonged early wheeze were non-atopic, but had deficit of lung function at infants and 6 years, while transient early wheeze showed no decreased lung function at 6 weeks and 6 years. Atopic sensitization at 4 years was suggested to be a predictive marker for asthma or wheezing at 8 years in the PIAMA study\textsuperscript{(12)}. A positive specific IgE to any aeroallergen was strongly associated with wheezing at 8 years. In most of cohort studies, wheezing at 6 to 8 years of age was strongly associated with atopic sensitization and AHR. Taken together, atopic sensitization in early childhood may be a very important factor for persistency of asthma in certain children with AHR.

FeNO levels in different phenotypes of wheezing

FeNO is a simple and easy objective marker for airway inflammation with excellent reproducibility. FeNO has been shown to be superior to lung function test by impulse oscillometry (IOS) in preschool children with a sensitivity of 86\% and specificity of 92\% to distinguish between children with probable asthma and healthy controls\textsuperscript{(13)}. The first FeNO measurement from preschool children participating in birth cohort study was reported in PIAMA study\textsuperscript{(14)}. At 4 years of age, they were divided into wheezing phenotypes of Tucson\textsuperscript{21} based on the wheeze episode before the first 3 years of life and in the 4\textsuperscript{th} year of life. Even though FeNO levels were higher in children with atopy or doctor’s diagnosed asthma, there was no association between FeNO levels and wheezing phenotypes\textsuperscript{(14)}. However, when they re-categorized children into 5 wheezing phenotypes of PIAMA\textsuperscript{8}) at 8 years of age and re-assessed the association of FeNO levels at 4 years and 8 years, FeNO levels at 4 years were higher in intermediate onset and persistent wheeze compared to never wheeze and
transient early wheeze\textsuperscript{15}). This is in line with our study showing higher FeNO levels in preschool children with persistent and late onset wheeze compared to no wheeze\textsuperscript{7}). Similarly, the SWS study identified higher FeNO levels at 6 years in children with intermediate onset, late onset, and persistent wheeze\textsuperscript{9}).

Atopy is an important factor for high FeNO levels in children with persistent asthma. In the PIAMA study, FeNO levels at 8 years showed a significant increase in children with intermediate onset, persistent, and late onset wheeze only who had atopic sensitization at 8 years compared to never/infrequent wheeze. Oh et al. also showed persistent wheezers with atopy and AHR had higher FeNO values compared to persistent wheezers without atopy or AHR\textsuperscript{7}). Interestingly, in this study, neither spirometry nor interrupted oscillometry (IOS) showed any difference between persistent wheezers and non-wheezers in preschool children\textsuperscript{7}).

This implies airway inflammation is present even during a period when there is no airway obstruction in preschool children with persistent asthma.

The possibility of FeNO as a risk factor for later asthma development has been shown in a cross-sectional study with preschool children aged from 3 to 47 months\textsuperscript{16}). They categorized subgroups using the asthma predictive index (API)\textsuperscript{17}) which is a clinical index to define the risk of asthma at 6 years in young children with recurrent wheeze. Preschool children with recurrent wheeze and stringent index had higher FeNO levels compared to both recurrent wheezing children with loose index and children with no wheeze\textsuperscript{16}). It suggests that preschool children with recurrent wheeze and a higher risk of asthma at 6 years have higher FeNO levels in the first 4 years of life. This was supported by another study from PIAMA\textsuperscript{12}). They showed a higher FeNO levels at 4 years was associated with doctor’s diagnosis of asthma at 7 years and more wheezing prevalence between 5 and 8 years of age. Atopic sensitization at 4 years was also associated with doctor’s diagnosis of asthma up to the age of 8 years. Recently,
new API including high FeNO as major criteria has been suggested in a cohort study with 391
high risk preschool children aged 3-47 months (18) (Table 2). In this study, median FeNO was
significantly increased in preschool children who developed asthma at school age compared
to those not developing asthma (10.5 ppb vs 7.4 ppb). They compared new API including
FeNO > 10 ppb as a major criterion to classical API including blood eosinophilia as a risk
factor for later asthma and the diagnostic performance was similar. New API does not require
blood sampling and identifies risk for later asthma development so that it can replace
classical API for the prediction of school-age asthma in clinically relevant preschool children.
There are several considerations of FeNO in clinical practice for young children. FeNO level
is influenced by factors such as height, age, atopic status, smoking, infection, medications,
measurement technique, exhalation flow rate, and the NO analyzer used. Furthermore, there
is several methods of FeNO measurement in children, which is categorized into on-line and
off-line methods with constant exhaled flow or tidal breathing (4,19). Measurements with
constant exhaled flow rate require patients’ cooperation and skills to breath in constant flow
of 50 ml/sec. Therefore, this method is difficult for young children less than 4 years old.
Methods collecting exhaled NO in balloons during spontaneous breathing were introduced
for young children less than 4 years of age (4,20-23). During quiet breathing, exhaled gas is
collected into balloons and FeNO concentration is measured with NO analyser. The ATS
guidelines recommend that age has to be considered as a factor influencing FeNO in children
younger than 12 years of age (24) and that FeNO levels >35 ppb is likely, and FeNO levels < 20
ppb is unlikely, to be used as cut-off level for eosinophilic inflammation in symptomatic
children (24). In healthy children aged from 1 year to 4 years, reference value of exhaled NO
using off-line tidal breathing methods was suggested as geometric mean 7.1 ppb (95% CI,
2.8-11.5 ppb) (22). FeNO is also highly affected by atopic status. It is required to determine cut-
off values of FeNO stratified for atopy and according to height and age, and validate the cut
points. However, recent study with healthy elementary school children showed no association
between age and FeNO values, while total IgE, blood eosinophil percent, and height was
positively associated with FeNO levels\textsuperscript{23}). They demonstrated FeNO reference equations by
multiple linear regression analysis, considering the variables of height, total IgE, and
eosinophil percent. Although FeNO measurement protocols were published by the ERS/ATS
in 2005, there can be different values depending on the detecting methods; electrochemical
sensor devices detects 4-10 ppb lower than chemiluminescent devices\textsuperscript{25}).

Conclusions

There are different wheezing phenotypes in children, but the predictive marker for
persistency of wheezing has yet to be clarified. Although AHR or lung function deficit
showed conflicting results among studies, atopic sensitization as well as high FeNO level has
been suggested as a risk factor for the persistency of wheezing in most studies. FeNO may
serve as an additional parameter for predicting persistent wheezing in preschool children.
More large scaled longitudinal studies are required to set up cut-off levels of FeNO as a risk
factor for persistent asthma.

Reference

1. Bacharier LB, Guilbert TW. Diagnosis and management of early asthma in preschool-aged children. J
   Allergy Clin Immunol 2012;130:287-96; quiz 97-8.
   phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-


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<tr>
<th>Wheezing phenotypes in each birth cohort study</th>
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The number of symbols (↑↓) represents the strength of association with each outcome in each wheezing phenotype and a negative symbol (-) represents absence of association. Blank represents no-check.

AHR, airway hyperresponsiveness; FeNO, fraction of exhaled nitric oxide
Table 2. Asthma predictive indices

Wheeze phenotypes

Early wheezer (EW): 1-2 attacks during last 12 months

Early frequent wheezer (EFW): ≥3 attacks during last 12 months

Asthma predictive criteria

Major

(A) Parental physician-diagnosed asthma

(B) Physician-diagnosed atopic dermatitis

(C) Fraction of exhaled nitric oxide >10 ppb

Minor

(a) Wheezing apart from colds

(b) Physician-diagnosed allergic rhinitis

(c) Eosinophils ≥4% of white blood cells

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<td>Loose index</td>
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<td>Stringent index</td>
<td>EFW + 1/2 major (A, B) or 2/3 minor (a-c)</td>
<td>EFW + 1/3 major (A-C) or 2 minor (a, b)</td>
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API, asthma predictive index

(Allergy 2013; 68: 531-538)
Fig. 1. Six wheezing phenotypes in ALSPAC study. Estimated prevalence of wheezing at each time point from birth to 81 months was identified by latent class analysis.

Fig. 2. Five wheezing phenotypes in PIAMA study. Estimated prevalence of wheezing at each time point from birth to 8 years was identified by latent class analysis.
(Thorax 2008;63:974-980)
(J Allergy Clin Immunol 2011;127:1505-12)