

ORIGINAL ARTICLE

Can exhaled inflammatory markers predict a steroid response in wheezing preschool children?

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Summary

Background The efficacy of inhaled corticosteroids (ICS) varies among wheezing preschool children. Currently, it is not possible to predict which fraction of wheezing children will benefit from an ICS treatment.

Objective We explored whether fractional exhaled nitric oxide (FeNO) and inflammatory markers in exhaled breath condensate (EBC) can predict an ICS response in preschool wheezers.

Methods An 8-week ICS study (registered at Clinicaltrials.gov: NCT 00422747; 200 µg; beclomethasone extra-fine daily) was performed in 93 wheezing children (age range 2.0–4.4 years). At baseline, FeNO was determined off-line. EBC was collected using a closed glass-condenser. The acidity of EBC was determined and other EBC markers [interleukin (IL)-1α, IL-2, IL-4, IL-5, IL-10, soluble intercellular adhesion molecule, interferon-γ, eotaxin] were measured using a multiplex immunoassay. The change in airway resistance (Rint) and symptom score following ICS treatment was related to atopy (positive Phadiatop Infant test), FeNO and EBC markers.

Results Airway resistance and symptoms mildly improved after ICS treatment [median (IQR): 1.4 (1.2–1.7) to 1.3 (1.1–1.5) kPa s/L, symptom score: 26 (23–28) to 28 (24–29), $P < 0.01$, respectively]. Only IL-10 and atopy had limited predictive value regarding a change in symptoms [β (SE) = –0.13 (0.07), $P = 0.08$, β (SE) = 2.05 (1.17), $P = 0.08$, respectively].

Conclusions and Clinical Relevance We did not find convincing evidence that FeNO and EBC markers could predict an ICS response in preschool wheezers. Recommendations for future studies on this topic are given.

Keywords airway inflammation, chemokines, cytokines, EBC, infants, inhaled corticosteroids, nitric oxide, wheeze

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Introduction

Wheeze is a common symptom in preschool children. About one third of children will experience intermittent wheeze during preschool life [1]. The underlying cause of childhood wheeze can differ substantially. Various phenotypes of childhood wheeze have been defined [2]. These phenotypes differ in pathophysiology and show a differential response to inhaled corticosteroids (ICS) [3–5]. ICS are effective in preschool children who wheeze due to

asthma [3]. In contrast, ICS are not very effective in preschool children who wheeze due to a viral respiratory tract infection, so-called viral or transient wheezers [4, 5]. Because a reliable distinction between asthmatic children and viral wheezers cannot be made before the age of 6 years, it is currently not possible to predict which fraction of the wheezing children will benefit from an ICS treatment. An early, reliable prediction of an ICS response can optimize treatment in preschool children with respiratory symptoms.

Eosinophilic airway inflammation is a central feature in asthma. In contrast, in transient wheezers, neutrophilic inflammation is believed to play a central role [6]. The assessment of (different forms of) airway inflammation in preschool wheezers might therefore improve the prediction as to which children will respond to ICS treatment.

The currently available techniques to measure airway inflammation, such as biopsy and bronchoalveolar lavage, are too invasive for routine use in children. Consequently, there is an increasing interest in the non-invasive assessment of exhaled inflammatory markers, such as fractional exhaled nitric oxide (FeNO) and solutes in exhaled breath condensate (EBC). We previously showed that the analysis of inflammatory markers in EBC is a feasible and non-invasive method to assess airway inflammation in preschool children, and demonstrated an increased inflammatory response in preschool children with recurrent wheeze [7]. Whether these markers are clinically useful to predict a response to anti-inflammatory treatment has not been studied as yet. Our objective was to investigate whether exhaled inflammatory markers are able to predict an ICS response in wheezing preschool children.

Methods

Study population

We included children from the ADEM study (Asthma DEtection and Monitoring study: registered at Clinical-trial.gov: NCT 00422747), which started in 2006 in the Netherlands. In the ADEM study, children who experienced at least two wheezing episodes during life (assessed by the ISAAC questionnaire [8]) and control subjects who

never wheezed are included at 2–4 years of age and are studied prospectively until 6 years of age. The primary objective of the ADEM study is to develop a non-invasive instrument for an early asthma diagnosis using exhaled inflammatory biomarkers and early lung function measurements. The study protocol has been described previously [9].

Figure 1 presents the flow chart of the current study. We included children who experienced respiratory symptoms (wheeze, cough or dyspnoea) during the preceding month, as assessed by a questionnaire [10]. If applicable, the administration of prescribed ICS was stopped 4 weeks before the initial visit. Children were excluded if ICS could not be stopped. Ethical approval was obtained from the Dutch National Medical Ethical Committee (CCMO). All parents gave written informed consent.

Study design

During an initial visit, EBC was collected and FeNO was measured. In addition, symptoms were assessed and lung function measurements were performed (details are described below). Measurements were postponed in case of an airway infection. Solid foods and physical exercise were not allowed for 1 hour before the measurements. The presence of atopy was determined using a Phadiatop Infant test[®] (Phadia, Uppsala, Sweden). Subsequently, children received ICS for 8 weeks (Beclomethasone extra-fine, two times 100 µg daily, via the Aerochamber[®], Teva Pharma NL, Haarlem, the Netherlands). Parents were allowed to give salbutamol (Airomir[®]) to their child for symptom relief. No other asthma medication was allowed. Compliance to the study medication was measured by weighing the ICS canisters before and after therapy.

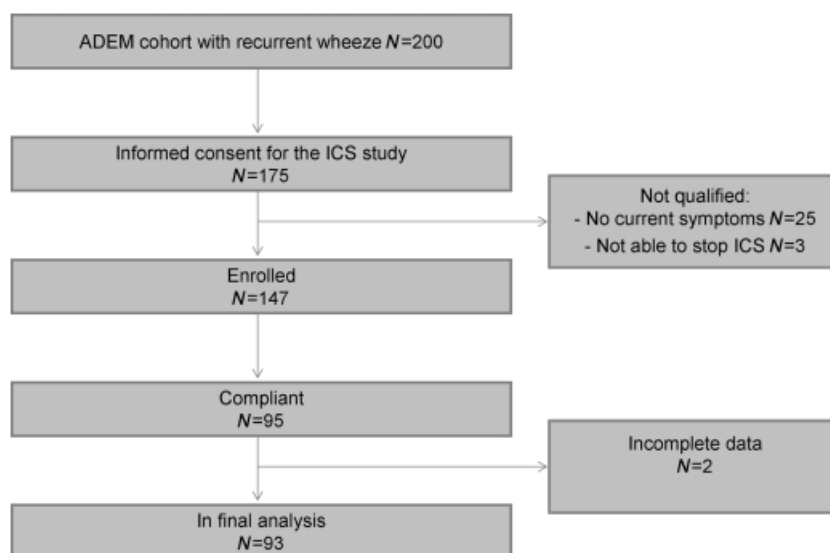


Fig. 1. Study flow chart. Current symptoms were defined as experiencing respiratory symptoms during the preceding month. Compliance to the study medication was defined when children used at least 80% of the prescribed study medication.

Children were excluded when less than 80% of the prescribed study medication was used. After 8 weeks of ICS treatment, all measurements were repeated. We did not include a control group refrained from ICS because this was not approved by the Dutch National Medical Ethical Committee.

Airway resistance and symptoms

The outcome variable was defined as change in airway resistance and symptoms before and after ICS treatment.

Airway resistance. Lung function was assessed using airway resistance measurements (MicroRint[®], Micro Medical Ltd., Rochester, UK). Children breathed tidally through a facemask while sitting in an upright position. Airflow interruptions were made on the expiratory peak flow. The median value of at least five successful interruptions before and after treatment was used for analysis.

Symptoms. Parents completed a questionnaire to record patterns of wheeze and other respiratory symptoms [10]. Parents scored three items (cough, wheeze, dyspnoea) on day-time symptoms and on night-time symptoms. Each item had five possible, graded responses (symptoms experienced: every day, 1; most days, 2; some days, 3; a few days, 4; not at all, 5). A total symptom score (ranging from six points for children with the most severe symptoms to thirty points for those with no symptoms) before and after therapy was calculated.

Cut-off point for illustrative purposes. From a clinical perspective, a cut-off point can be helpful to determine whether a child should be treated with ICS or not. A child was considered to be a responder when there was a decrease in respiratory symptoms of $\geq 30\%$ and/or an improvement in airway resistance of $\geq 10\%$, based on ICS responses reported in similar populations [5, 11].

Fractional exhaled nitric oxide assessment

FeNO was sampled before EBC collection and lung function assessments. FeNO was collected off-line in a 500 mL inert balloon during tidal breathing and analysed using a nitric oxide monitoring system, as described previously (NIOX[®], Aerocrine, Solna, Sweden) [9].

Exhaled breath condensate collection

To collect EBC, a closed glass condenser system with a breath recirculation unit was used, which was developed at our institute [9, 12]. This efficient closed condenser system enables sufficient EBC collection in less collection time (10 min), as described previously [9, 12]. Directly after collection, the acidity (pH) of EBC was analysed (Radiometer, Zoetermeer, the Netherlands). Inflammatory markers

in EBC [interleukin (IL)-1 α , IL-2, IL-4, IL-5, IL-10, soluble intercellular adhesion molecule (sICAM), interferon- γ (IFN- γ) and eotaxin (CCL-11)] were assayed using the multiplex immunoassay (Luminex Corporation, Austin, TX, USA) [13].

Statistical analysis

Data were analysed using SPSS 16.1 (SPSS Inc., Chicago, IL, USA). Because the airway resistance values, symptoms scores, FeNO levels and EBC markers were skewed to the right, they were expressed as medians and inter quartile ranges (IQR). Samples with levels of EBC markers below the detection limit received a randomly generated value taken from the uniform distribution between zero and the detection limit [7].

Differences in the baseline characteristics between responders and non-responders (secondary analysis) were evaluated using independent *t*-tests for parametric variables, Mann-Whitney tests for non-parametric variables and the χ^2 test for categorical variables. Wilcoxon' signed-rank tests were used to test for differences in parameters before and after ICS treatment. Differences were considered statistically significant when the *P*-value was < 0.05 .

The effect of each individual inflammatory marker on the ICS outcome was tested via separate linear regression models adjusted for possible confounders. The influence of possible confounders (gender, atopy, eczema, history of ICS use, season of measurements, parental asthma, parental smoking and number of wheezing episodes from birth until present) was studied using linear regression models. Parameters were considered to be confounders when they changed the association between EBC markers and ICS outcome by $> 10\%$ when included in the model. Only atopy altered the association between the majority of markers and change in symptoms with $> 10\%$ and was therefore included in the subsequent regression models. In addition, a multivariable model was fitted to study the combined effects of inflammatory markers. Because EBC markers were highly correlated with each other, a principal component analysis (PCA) with orthogonal rotation (varimax) was conducted, reducing the nine markers to fewer uncorrelated composite variables. These variables, instead of the original EBC markers, were, together with atopy and FeNO, used in a multivariable model, thereby avoiding multicollinearity. A backward procedure was used for variable selection with a significance level of 5%.

Power analysis

A sample size calculation for correlation between two quantitative variables was performed (formula: $N = 6 + [(Z_{1-\alpha/2} + Z_{1-\beta})^2(1 - \rho^2/\rho^2)]$) [14]. Previous research in asthmatic adults (paediatric data are lacking) showed correlation coefficients ranging from 0.3 to 0.6 between exhaled markers and improvement in lung function after ICS [15–17]. When using a correlation of 0.3, at least 85 children were required for the

analysis, with an α of 0.05 and a power of 80%. When considering a non-compliance rate of 30% [18], at least 111 children had to be enrolled.

Results

A total number of 147 children participated in the study. For the analysis, 93 children [mean age (range); 3.3 (2.0–4.4)] were included; see Table 1 for the baseline characteristics. The rest of the children were excluded because of non-compliance ($N=52$) or incomplete data ($N=2$). Figure 1 presents the study flow chart. The reasons for non-compliance were withdrawn consent of parents ($N=6$), refusal of the child to take ICS ($N=9$), personal constraints ($N=5$), forgot to give ICS daily ($N=20$) and unknown reasons ($N=12$). Children who were not compliant did not differ in symptoms, airway resistance, atopy, FeNO and markers in EBC at the baseline compared with children who were compliant.

Inhaled corticosteroid treatment

In general, both airway resistance and symptoms improved significantly after 8 weeks of ICS treatment. Airway resistance decreased from 1.4 (1.2–1.7) to 1.3 (1.1–1.5) kPa s/L (median (IQR), $P<0.01$, Table 2). Symptom

scores improved from a total score of 26 (23–28) to 28 (24–29) [median (IQR), $P<0.01$, Table 2]. Changes in airway resistance and symptoms scores after ICS treatment were significantly different from zero ($P<0.05$). None of the inflammatory marker concentrations changed significantly after ICS treatment (Table 2). When we divided children into responders and non-responders for a secondary analysis ($\geq 30\%$ decrease in symptoms and/or $\geq 10\%$ decrease in airway resistance), ICS responders had higher airway resistance at baseline than ICS non-responders (Table 1, $P<0.01$). There were no other baseline differences between both groups (Table 1).

Confounders

Of all possible confounders tested (see statistical analysis), only atopy was modestly associated with an ICS response regarding symptoms [$\beta = 2.05$, β (SE) = 1.17, $P = 0.08$], and altered the association between most markers and change in symptoms with $> 10\%$. Therefore, atopy was included in the regression models with inflammatory markers.

Exhaled inflammatory markers

We could successfully measure FeNO and collect EBC in all children. The mean EBC volume was 90 μ L (range:

Table 1. Characteristics at baseline of responders and non-responders

	Responders	Non-responders	Total group
Study population (N)	47	46	93
Mean age (range) (years)	3.2 (2.0–4.3)	3.4 (2.1–4.4)	3.3 (2.0–4.4)
Gender: male/female (n)	21/26	26/20	47/46
Atopy, n/N (%) [*]	12/46 (26)	9/44 (20)	21/90 (23)
Eczema, n/N (%)	15/45 (33)	13/46 (28)	28/91 (31)
Wheezing episodes, n (IQR) [†]	8 (3–8)	8 (3–8)	8 (3–8)
Use of ICS, n/N (%)	12/47 (26)	9/46 (20)	21/93 (23)
Use of short-acting B ₂ agonist, n/N (%)	23/47 (49)	21/46 (46)	44/93 (47)
Use of long-acting B ₂ agonist, n/N (%)	1/47 (2)	0/46 (0)	1/93 (1)
Total symptom score	25 (20–27)	26 (24–28)	26 (23–28)
Airway resistance (kPa s/L) ^{***}	1.5 (1.3–1.9)	1.3 (1.1–1.5)	1.4 (1.2–1.7)
IL-1 α (pg/mL)	40.2 (11.4–125.1)	51.5 (21.6–79.1)	41.9 (19.2–90.8)
IL-2 (pg/mL)	57.0 (29.0–103.0)	55.8 (42.7–77.4)	56.8 (38.9–78.5)
IL-4 (pg/mL)	9.7 (6.8–18.5)	9.8 (5.6–15.5)	9.7 (6.7–15.2)
IL-5 (pg/mL)	28.4 (18.0–91.7)	38.7 (19.7–66.5)	33.4 (18.9–66.5)
IL-10 (pg/mL)	5.1 (2.6–8.6)	5.1 (3.1–7.8)	5.1 (3.1–8.4)
sICAM (pg/mL)	204.2 (86.6–614.7)	238.4 (117.0–393.1)	220.0 (95.8–423.0)
IFN- γ (pg/mL)	28.5 (19.4–57.6)	29.1 (18.9–45.9)	28.6 (19.4–46.9)
CCL-11 (pg/mL)	8.6 (5.9–18.3)	9.1 (6.0–13.2)	8.6 (6.0–15.0)
pH	6.0 (5.7–6.4)	5.9 (5.6–6.3)	5.9 (5.7–6.3)
FeNO (p.p.b.)	8.4 (3.9–14.5)	6.9 (3.2–12.7)	7.8 (3.9–13.4)

^{*}Atopy is defined as a positive Phadiatop infant test; numbers may not add up to 47, 46 or 93 in, respectively, responders, non-responders and total group due to missing values.

[†]Numbers of wheezing episodes from birth until present.

Symptom scores, airway resistance and inflammatory markers are expressed as medians and IQR.

^{***} $P<0.01$ between responders and non-responders (MW test).

CCL-11, eotaxin; FeNO, fractional exhaled nitric oxide; ICS, inhaled corticosteroids; IFN- γ , interferon- γ ; IL, interleukin; IQR, interquartile range; sICAM, soluble intercellular adhesion molecule.

Table 2. Parameters before and after steroid treatment

Parameter	Steroid therapy		P-value
	Before	After	
Symptom score	26 (23–28)	28 (24–29)	<0.01
Airway resistance, (kPa s/L)	1.4 (1.2–1.7)	1.3 (1.1–1.5)	<0.01
IL-1 α (pg/mL)	41.9 (19.2–90.8)	59.4 (15.7–55.8)	0.09
IL-2 (pg/mL)	56.8 (38.9–78.5)	57.8 (42.4–104.1)	0.22
IL-4 (pg/mL)	9.7 (6.7–15.2)	9.8 (7.2–17.6)	0.62
IL-5 (pg/mL)	33.4 (18.9–66.5)	37.0 (20.0–120.8)	0.07
IL-10 (pg/mL)	5.1 (3.1–8.4)	5.5 (3.3–9.1)	0.36
sICAM (pg/mL)	220.0 (95.8–423.0)	260.4 (106.6–754.9)	0.14
IFN- γ (pg/mL)	28.6 (19.4–46.9)	30.6 (16.9–60.8)	0.36
CCL-11 (pg/mL)	8.6 (6.0–15.0)	9.8 (5.6–19.6)	0.09
pH	5.9 (5.7–6.3)	5.8 (5.6–6.2)	0.27
FeNO (p.p.b.)	7.8 (3.9–13.4)	9.1 (4.1–15.8)	0.51

For abbreviations see Table 1. Wilcoxon' signed-rank tests. Data are presented in median (inter quartile range).

Table 3. Factor loadings of the Principal Components

	Component 1	Component 2	Component 3
IL-1 α	0.90	0.20	0.36
IL-2	0.69	0.29	0.65
IL-4	0.44	0.84	0.29
IL-5	0.88	0.36	0.24
IL-10	0.54	0.41	0.72
sICAM	0.88	0.29	0.27
IFN- γ	0.78	0.44	0.38
CCL-11	0.78	0.42	0.32
pH	–0.03	0.01	0.00

For abbreviations see Table 1. Rotation method: Varimax with Kaiser' normalization.

60–145 μ L). EBC markers could be detected in 97–100% of the samples.

The analysis of separate parameters demonstrated that none of the inflammatory markers significantly predicted an improvement in lung function after ICS treatment ($P > 0.05$). In addition, none of the markers were significant predictors for an improvement in symptoms, although IL-10 showed a negative trend [$\beta = -0.13$, β (SE) = 0.07, $P = 0.08$, $R^2 = 0.07$].

Before multivariable linear regression modelling, PCA was conducted on the nine markers in EBC. Markers could be summarized in three components that both had eigenvalues over Kaiser's criterion of 1 and in combination explained 93.6% of the variance. Table 3 shows the factor loadings after rotation. Component 1 had high loadings on various inflammatory markers including a pro-inflammatory cytokine (IL-1 α), a Th2 cytokine (IL-5), an adhesion molecule (sICAM), a Th1 cytokine (IFN- γ) and a chemokine (CCL-11). Component 2 had the highest loading on the Th2 cytokine IL-4, while component 3 had the highest loading on the anti-inflammatory cytokine IL-10.

Table 4. Multivariable regression for change in symptoms and lung function

Outcome Parameter	Δ Symptoms		Δ Lung function	
	B+SE	P-value	B+SE	P-value
EBC component 1	–0.04+0.56	0.94	–0.04+0.05	0.41
EBC component 2	–0.96+0.87	0.27	0.02+0.07	0.77
EBC component 3	–0.69+0.51	0.18	–0.04+0.04	0.40
FeNO	–0.00+0.06	0.96	0.00+0.05	0.86
Atopy	2.00+1.19	0.09	–0.10+0.10	0.35

R^2 model with symptoms = 0.08; R^2 model with lung function = 0.03.

Table represents the initial models; with backward procedure, only atopy remained in the final model for Δ symptoms ($B + SE = 2.05 + 1.17$, $P = 0.08$, $R^2 = 0.06$).

EBC, exhaled breath condensate; FeNO, fractional exhaled nitric oxide.

The three EBC components, FeNO and atopy were included in two multiple regression models, with change in symptoms and lung function as dependent variables. In both models, none of the parameters were significant in the initial model (Table 4). With the backward procedure, only the presence of atopy was mildly positively associated with an improvement in symptoms after ICS in the final model [$\beta = 2.05$, β (SE) = 1.17, $P = 0.08$, $R^2 = 0.06$]. As the PCA loading pattern did not change after log transformation, the results are reported in the original scale.

Discussion

At present, there are no validated biomarkers that can predict a treatment response to ICS in wheezing preschool children. ICS are believed to improve symptoms and lung function due to their anti-inflammatory effect. The incorporation of non-invasive markers of airway inflammation in the therapy strategy may facilitate the choice of proper treatment.

This is the first study in wheezing preschool children that has explored the value of exhaled inflammatory markers in directing therapy. We showed that symptoms mildly improved after an 8-week period of ICS treatment in preschool children with wheezing symptoms. In addition, lung function improved, although it can be argued whether the improvement in lung function is clinically relevant. Whether these improvements can be attributed to ICS is somewhat contentious, due to the lack of a control group. However, in contrast to compliant children, non-compliant children (who might be considered as controls because they hardly received ICS) did not show any improvement in both symptoms and lung function ($N = 52$, data not shown). This strengthens the finding that improvement in symptoms and lung function was ICS induced. From all the inflammatory biomarkers and potential confounders studied, only IL-10 and atopy were shown to be associated with an ICS response, although not in a multivariable approach.

From a clinical viewpoint, the negative predictive trend between IL-10 and improvement in symptoms is meaningful. IL-10 is an anti-inflammatory cytokine and is believed to play an important inflammatory down-regulating role in asthma [19, 20]. Possibly, wheezing preschool children with lower levels of IL-10 might have an enhanced inflammatory status and therefore might react more strongly on ICS. Although one should keep in mind that our population in general demonstrated only a mild clinical improvement after ICS. Equally plausible is the positive predictive trend regarding atopy and improvement in symptoms after ICS. In atopic wheezing children, mainly eosinophilic airway inflammation is present. Conversely, in viral-induced wheeze, neutrophilic inflammation is believed to play a central role [6]. ICS mainly target eosinophilic airway inflammation, which might explain why atopic wheezing children responded slightly better to ICS.

Whereas this is the first study in wheezing preschool children, more studies were performed in older children and adults with asthma to explore the value of exhaled inflammatory markers in directing therapy. The majority of studies conducted hitherto with FeNO support its predictive role for an ICS response either in adults or in children with asthma [15, 16, 21, 22]. In contrast, there are some studies that could not demonstrate a predictive role of FeNO either in asthmatic adults or in children [23, 24], in concordance with our findings. In our specific case, however, it is important to emphasize that we collected FeNO off-line during tidal breathing. One of the factors that can influence FeNO is the expiratory flow rate. A single breath online manoeuvre is recommended in older children but is not feasible in preschool children [25]. The tidal breathing method has been successfully used previously, but standardization is less developed than for the single-breath online method.

Regarding markers in EBC, only Matsunaga and colleagues investigated whether these markers were able to predict an ICS response in adults with asthma [26]. Although their sample size was small ($N=18$), they found that increased levels of IL-4 and RANTES and reduced levels of IFN- λ -inducible protein-10 were significantly correlated with a steroid-induced improvement in lung function. We showed previously the clinical potential of EBC markers in asthmatic children when using these markers in the evaluation of the anti-inflammatory effects of ICS [27]. EBC collection has huge diagnostic potential, as there is cumulative evidence that the analysis of exhaled inflammatory markers is a non-invasive technique to study airway inflammation in young children [7, 12, 28, 29]. Nevertheless, their clinical role in the guidance of ICS treatment in young children has not been studied previously. This is partly due to the fact that the collection of EBC is notoriously difficult in preschool children. In the present study, the high success rate of EBC collection is a

result of two factors. Firstly, the efficient system enables sufficient EBC collection in a short collection time. Secondly, EBC markers were measured using multiplexed liquid bead arrays (Luminex Corporation). This technology is highly sensitive and is able to measure different cytokines accurately in a small sample of EBC [13].

Given the body of evidence and the sound theoretical underpinning regarding the clinical role of EBC, the lack of their significant predictive value in our study should not be overinterpreted. Methodological issues concerning the composition of our study population may explain the non-significant results. For instance, the majority of our children had minor acute symptoms and therefore had little room for improvement after ICS (symptom scores were bounded upwards). Children with severe symptoms and high airway resistance, in contradistinction, would have been expected to improve more significantly. Moreover, we cannot fully discard the possibility that minor improvements in symptoms and lung function were related to habitual ICS use in the minority of our study population (although their ICS administration was stopped 4 weeks before the study).

To test these hypotheses, we performed two additional sub-analyses: (1) including children with severe symptoms (symptom score of ≤ 24 , $N=36$) and (2) including steroid-naïve children ($N=74$). In the former, the EBC markers remained non-significant (possibly due to loss of power – data not shown). In the latter, we found that next to IL-10, IL-4 also showed a negative trend with improvement in symptoms, and that the pH of EBC was negatively associated with improvement in lung function after ICS ($\beta=-0.06$, $P=0.07$, $R^2=0.10$; $\beta=-0.20$, $P=0.01$, $R^2=0.11$, respectively). Therefore, it is warranted to assess the predictive role of exhaled inflammatory markers regarding an ICS response in a large cohort of steroid-naïve children with more severe symptoms.

In order to analyse an ICS response, we included children who received at least 80% of the subscribed ICS. Because children who were not compliant did not differ in symptoms, airway resistance, atopy and exhaled markers at baseline compared with children who were compliant, we believe that excluding these children did not influence our results.

Next to exhaled biomarkers, inflammatory markers in induced sputum have been successfully used in asthmatics as clinical parameters to predict an ICS response [15, 17, 22]. However, sputum is a semi-invasive method that needs the co-operation of the child and technical expertise, leading to a lower success rate and more side effects than EBC collection. The collection of inflammatory markers in EBC is non-invasive and can be easily performed in non-sedated preschool children. Although an American/European Taskforce presented recommendations about the methodology of EBC collection, the technique needs further standardization and challenging

issues such as the influence of expiratory flow and dilution factor on EBC collection need to be addressed in further investigations [30].

Clinical relevance

At present, no clinical test is available to determine which young children with wheeze will benefit from an ICS treatment. We hypothesized that non-invasive markers of airway inflammation could be of added value to proper treatment decisions in wheezing preschool children. However, we did not find convincing evidence that exhaled inflammatory markers can predict an ICS response in preschool wheezers. Hence, the search for a reliable, non-invasive and feasible technique in young children needs to be continued. In asthmatic adults and older children, there is increasing evidence of the clinical value of

exhaled inflammatory markers regarding ICS treatment. The clinical use of these markers still needs to be established in young children. For this purpose, it is advisable to use large cohort studies of preschool children with (more severe) recurrent wheezing symptoms.

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