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ORIGINAL ARTICLE **Asthma and Rhinitis**

# Prediction of asthma exacerbations in children: results of a one-year prospective study

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**Summary**

*Background* Underdiagnosis and low levels of asthma control are frequent occurring problems in patients with asthma.

*Objective* The study aim was to evaluate the ability of non-invasive inflammatory markers in exhaled breath to predict exacerbations of childhood asthma, and to assess the time course of changes in these exhaled markers before, during and after exacerbations.

*Methods* The design was a prospective one-year longitudinal study. Regular two-month visits at the outpatient clinic were performed. Forty children with asthma (aged 6–16 years) participated. The primary outcome measure was the occurrence of an exacerbation. Assessment was made of the presence and severity of pulmonary symptoms, use of medication, and measurements of forced expiratory volume in 1 s using home monitor. The following independent parameters were assessed during outpatient visits: (1) exhaled nitric oxide, (2) inflammatory markers in exhaled breath condensate: acidity, nitrite, hydrogen peroxide, interleukin-1$\alpha$, -5, -13, interferon-$\gamma$, (3) lung function, (4) asthma control score.

*Results* Thirty-eight of 40 children completed the study. Sixteen children developed exacerbations, of which ten were moderate and six severe. Univariate Cox regression analysis revealed that condensate acidity, interleukin-5 and asthma control score were significant predictors of an asthma exacerbation ($P < 0.05$). In the multivariate Cox regression analysis, exacerbations were best predicted by the asthma control score and by the level of interleukin-5 in exhaled breath condensate (Wald scores of 7.19 and 4.44, $P = 0.007$ and $P = 0.035$ respectively). The predicted survival curve of this multivariate model showed a two times reduced risk on exacerbations in the category of children with the 10% most optimal values of IL-5 and asthma control score.

*Conclusions and Clinical Relevance* Both exhaled breath condensate interleukin-5 level and asthma control score were significant predictors of asthma exacerbations. These findings open up the possibility of assessing the potential of such parameters to titrate asthma treatment in future studies.

**Keywords** asthma control score, exhaled breath condensate, monitoring, paediatric asthma
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## Introduction

Asthma is the most common chronic disease in children, affecting 300 million people globally [1]. In the past 30 years, the prevalence of childhood asthma has increased substantially in many countries worldwide although a stabilization has been described recently [2, 3]. Underdiagnosis of childhood asthma in general practice is still present [4]. Several cross-sectional surveys worldwide have indicated that the degree of

asthma control falls short of goals for long-term management set by international guidelines both in general practice and specialist care [5–7]. Still many patients suffer from asthma exacerbations with an increase in symptoms, loss in lung function, school absence, work absence of the parents, and impaired quality of life. One probable explanation is that although asthma is characterized by chronic airway inflammation, monitoring of the disease is currently performed by assessments of symptoms and lung function, but not by measurements of airway inflammation [8, 9]. However, correlation between parameters of airway inflammation on one hand, and symptoms and lung function on the other, is generally low [8, 9]. Moreover, many children with asthma have poor perception of dyspnoea, which results in underreporting of symptoms and even underdiagnosis of asthma in childhood [4, 7].

Standard techniques to assess airway inflammation such as bronchoscopy with bronchoalveolar lavage or bronchial biopsy are far too invasive for routine use [9, 10]. There is a need for specific non-invasive tests. Previous studies have shown that non-invasive inflammatory markers in exhaled breath like fractional exhaled nitric oxide (FeNO) and inflammatory markers (IM) in exhaled breath condensate (EBC) considered together may be useful in the diagnosis of asthma, and in the identification of asthma control and severity [11]. It has been shown that non-invasive tests based on FeNO and biomarkers in EBC, such as Th1/Th2 cytokines, nitrate, nitrite, and hydrogen peroxide could discriminate between healthy and diseased children [12–15]. These findings have shown that EBC may be a promising tool for diagnostic and monitoring purposes. However, previous studies were conducted with a cross-sectional design and longitudinal data are lacking.

Therefore, a prospective longitudinal study over a period of 1 year was performed on children with asthma, in which regular non-invasive inflammatory markers in exhaled breath (condensate) were carried out (FLAME study: Inflammation Asthma Monitoring study). The specific aims were:

1 To assess the ability of exhaled inflammatory markers (FeNO, and EBC pH, nitrite, hydrogen peroxide, IL-1$\alpha$, IL-5, IL-13, IFN-$\gamma$), symptoms and lung function to predict asthma exacerbation.
2 To study the time course of changes in exhaled inflammatory markers, symptoms and lung function during asthma exacerbations.

## Methods

### *Patients*

Children aged 6–16 years with doctor-diagnosed asthma were recruited. They were selected from the outpatient clinic of the Department of Paediatric Pulmonology, Maastricht University Medical Centre, and had been known to have had an asthma diagnosis at our clinic for at least 6 months. Asthma was defined as a chronic inflammatory disorder with paroxysmal wheezing, breathlessness, chest tightness, or coughing with a variable but reversible airway obstruction and airway hyperresponsiveness [15]. Both allergic and non-allergic asthmatic children were selected. Allergic children had a positive Phadiatop or a positive Radio Allergo Sorbent Tests (RAST) (at least two RAST classes $\geq$ class 2). Exclusion criteria were: (1) diseases that may interfere with the results of the study (e.g. heart disease, anatomical abnormalities of the airway, other chronic inflammatory diseases, such as Crohn's disease or rheumatoid arthritis), (2) mental retardation, (3) inability to perform measurements properly, or (4) active smoking. All parents or children gave informed consent. This study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre, identification code MEC 05-114.1. The international study number was NCT00404859.

### *Study design*

The design was a prospective longitudinal study during a one-year period. Every 2 months, routine visits took place at the outpatient clinic. In addition to these fixed visits, patients were asked to visit the outpatient clinic an additional four times during an exacerbation. Extra measurements were planned at days one, three, and five of the exacerbation and after stabilization.

### *Primary outcome measure: asthma exacerbation*

The occurrence of an asthma exacerbation was the primary outcome measure of this study. A moderate exacerbation was defined as an increase in asthma symptoms (dyspnoea, chough, wheezing) and/or use of short acting $\beta_2$-agonists for not more than 2 days [16]. In this case, the fall in $FEV_1$ was not below the 80% personal maximum value. In case of a severe exacerbation, one or more of the following items were present: (1) The $FEV_1$% of maximum personal value fell below 80% of the person maximal value for at least two consecutive days, (2) need for treatment with oral corticosteroids, and/or (3) need for hospital admission [16].

To recognize an exacerbation in an early stage, AM1 home monitors (Viasys, Hoechberg, Germany) were used. All patients received a personal home monitor and a corresponding modem (HC1, Viasys, Hoechberg, Germany). Using this monitor, patients could measure $FEV_1$ at home. In addition, use of rescue medication, overall well-being, and the presence and severity of pulmonary symptoms were recorded. The intensity

of symptoms was scored on a scale of zero to three. Patients were asked to use the AM1 once daily, at a fixed time of the day. All patients were asked to perform the manoeuvres three times within 10 min, and the highest $FEV_1$ [L] was stored in the memory. Data were sent to a computer at the Maastricht University Medical Centre by means of a modem once per week. In case of deterioration of $FEV_1$ values, and/or an increase in presence and severity of pulmonary symptoms, patients were called into the hospital for additional measurements and a consultation with the paediatric pulmonologist responsible.

*Measurements of independent predictors*

At each clinical visit, parameters were assessed in the following order: (1) FeNO, (2) IM in EBC, (3) lung function indices, (4) asthma control score, and (5) asthma severity (criteria used as published previously) [11]. All measurements were performed within one hour.

*NO in exhaled air*. First, FeNO was measured online using a NIOX® chemiluminescence analyser (Aerocrine, Solna, Sweden). Measurements were carried out according to the American Thoracic Society/European Respiratory Society (ATS/ERS) criteria for children [17].

*Exhaled breath condensate collection and analysis*. EBC was collected by means of an optimized borosilicate glass tube, cooled by counter-current circulating ice water, as described in previous research [18]. Children breathed tidally for 10 min, while wearing a nose-clip, through a mouthpiece connected to a two-way non-re breathing valve (series 1420; Hans Rudolph Inc, Kansas City, MO, USA). Acidity of EBC was measured immediately after collection (Radiometer, type PHM201; Radiometer Nederland BV, Zoetermeer, NL, USA) without de-aeration. Subsequently, EBC samples were frozen using dry ice and stored at −80°C. Levels of nitrite (fluoremetric) and hydrogen peroxide ($H_2O_2$, spectrometric) were assessed as described in an earlier study [11]. Concentrations of IL-1α, IL-5, IL-13, and IFN-γ in 100 μL of EBC were determined using the multiplex immunoassay Luminex® 100 analyser (Luminex Corporation, Austin, TX, USA) as described earlier [19, 20]. All EBC samples of a specific patient were analysed simultaneously to minimize inter-assay variation.

*Lung function tests*. Dynamic spirometry was performed by means of the Flowscreen®, according to ATS/ERS standards (Viasys, Hoechberg, Germany) [21]. Highest values of three correctly performed manoeuvres were used for analysis. Recorded indices were forced expiratory volume in one-second ($FEV_1$), forced vital capacity (FVC), and maximal expiratory flow at 50% of FVC ($MEF_{50}$), all expressed as a percentage of the predicted normal value [21]. Reversibility to a beta-2-agonist was determined 15 min after inhalation of 400 μg salbutamol. Significant reversibility was defined as at least 9% increase in $FEV_1$. was defined as the provocative concentration of histamine to produce a 20% fall in FEV1% predicted value of < 8 mg/mL.

*Asthma control score*. The asthma control score was assessed two-monthly using a validated questionnaire, as in previous studies [6, 7]. The questionnaire contained questions about chronic airway symptoms, sleep disturbance, limitation of daily activity, asthmatic attacks, emergency or urgent care visits, and need for short acting beta-2-agonists [7].

*Treatment*

During the one-year study period, children were treated by their own paediatric pulmonologist according to GINA guidelines [22].

*Statistic analysis*

The statistical tests to compare and correlate normally distributed parameters between individuals and populations were Student's *t*- or paired *t*-test and the Pearson correlation coefficient test respectively. Non-normally distributed parameters were compared and correlated using the Mann–Whitney U or Friedman tests and Spearman's rank test respectively. The influence of independent predictors (non-invasive IM, lung function, asthma control) on the time until an exacerbation was analysed by univariate and multivariate Cox regression analyses. Wald score are used to analyse the effect (level of inflammatory marker) onto a response (asthma exacerbation). Wald scores cannot be compared to each other. In addition, it is not possible to calculate the sensitivity, specificity, positive and negative predictive value of parameters since the values were continuous in time.

Samples with IM levels below the detection limit were given an arbitrary value between zero and the lower limit of detection. Two-sided *P*-values $< 0.05$ were taken as lower limit of statistical significance.

*Power analysis*

To find clinically meaningful correlations between independent predictors and exacerbation rate of 0.6, $N = 35$ children are necessary to assess such a relationship with a two-sided alpha of 0.05 and a power of 98%. During the 12 month follow-up period, a drop-out rate of 10% was assumed. Therefore, 40 children with asthma were included in this study.

*Role of the funding source*

AstraZeneca had no role in the study design, in writing the manuscript or in the decision to submit this paper.

## Results

### *Population characteristics*

Lung function indices showed no obstructive or restrictive abnormalities (Table 1). The majority of the group was allergic: 29/40 (73%). With one exception, all patients received maintenance treatment with inhaled corticosteroids.

Thirty-eight of the 40 children completed the study while two dropped out. Of these two, one patient moved out of the region. Before he had to withdraw, he experienced one exacerbation. The other child lost interest in future participation. She had stable asthma.

### *Exacerbations*

During the one-year study period, 16 children developed an exacerbation of which 10 were moderate and six severe. Only three patients developed a second exacerbation.

### *Cox regression analyses*

The results of Cox regression analysis are shown in Table 2. In the univariate analysis, asthma control score, EBC acidity, and IL-5 were significantly related to the occurrence of an exacerbation (Table 2a, $P < 0.05$). FeNO did not appear to be a significant predictor ($P = 0.60$). When analysed using a multivariate model, only EBC IL-5 and asthma control score remained significant (Table 2b).

The predicted survival curve of this multivariate model showed that children with the 10% most optimal values of IL-5 and the asthma control score had a more than two times lower risk of exacerbations after 1 year than the children with the 10% least optimal values (Fig. 1).

### *Independent predictors before and during exacerbations*

Significant changes were found in nitrite, IL-13, and IFN-γ (Friedman test, $P < 0.05$). In Figs 2a and b, the course of $FEV_1$, IL-5, and FeNO are shown respectively. A rise in IL-5 was observed before the onset of an exacerbation with a gradual decrease during the exacerbation. The drop in lung function ($FEV_1$% predicted value) and the top in FeNO occurred at the start of the exacerbation. FeNO dropped rapidly in the course of the exacerbation during treatment.

## Discussion

Our study supports the hypothesis that non-invasive measurements of airway inflammation can be helpful during the monitoring of a chronic inflammatory lung

**Table 1.** Clinical characteristics of asthmatic children at commencement of study

| | |
|---|---|
| Age (years) | 10.7 ± 0.4 |
| Weight (kg) | 38.8 ± 2.0 |
| Height (cm) | 142.3 ± 2.4 |
| Male/Female | 29/11 |
| Asthma control score (minimum–maximum) | 27.6 ± 2.3 (9–64) |
| Lung function indices | |
| Reversibility (increase in $FEV_1$% predicted) | 5.9 ± 1.1 |
| $FEV_1$% predicted | 99.6 ± 2.2 |
| $FEV_1$/VC% | 83.9 ± 1.5 |
| VC% predicted | 99.2 ± 2.4 |
| $MEF_{50}$% predicted | 82.6 ± 3.8 |
| ATOPY (yes/no) | 29/11 |
| Total IgE (kU/L) | 493.3 ± 120.7 |
| Active eczema | 7 (18%) |
| Allergic rhinitis | 4 (10%) |
| Treatment | |
| Dose of inhaled budesonide or equivalent (μg) | 587 ± 53 |
| Long-acting beta-2-agonist | 21 (53%) |
| Leukotriene receptor antagonist | 8 (20%) |

$FEV_1$, forced expiratory volume in 1 s; VC, vital capacity; $MEF_{50}$%, mean expiratory flow at 50%.
Data were given as mean ± SEM except where indicated otherwise.

**Table 2.** (a) Univariate Cox regression analysis of the time until an exacerbation. (b) Multivariate Cox regression analysis of the time until an exacerbation

| Parameter | β ± SE | Wald score Chi-square (df = 1) | *P*-value |
|---|---|---|---|
| $FEV_1$% predicted | −0.02 ± 0.03 | 0.64 | 0.43 |
| Asthma control score | **0.04 ± 0.02** | **4.82** | **0.03** |
| FeNO (p.p.b.) | 0.01 ± 0.01 | 0.27 | 0.60 |
| EBC pH | **1.18 ± 0.52** | **5.10** | **0.03** |
| Nitrite (μM) | −0.005 ± 0.03 | 0.03 | 0.87 |
| $H_2O_2$ (μM) | −0.11 ± 0.09 | 1.28 | 0.26 |
| IL-1α | −0.0004 ± 0.0006 | 0.45 | 0.50 |
| IL-5 | 0.0004 ± 0.0002 | 3.76 | 0.05 |
| IL-13 | 0.0001 ± 0.001 | 0.01 | 0.92 |
| IFN-γ | −0.0002 ± 0.002 | 0.01 | 0.92 |

| Parameter | β ± SE | Wald score Chisquare (df = 1) | *P*-value |
|---|---|---|---|
| Asthma control score | 0.04 ± 0.02 | 5.7 | 0.007 |
| EBC pH | 0.59 ± 0.56 | 1.1 | 0.29 |
| IL-5 | 0.0004 ± 0.0002 | 3.2 | 0.035 |

β, regression coefficient; SE, standard error; df, degrees of freedom.

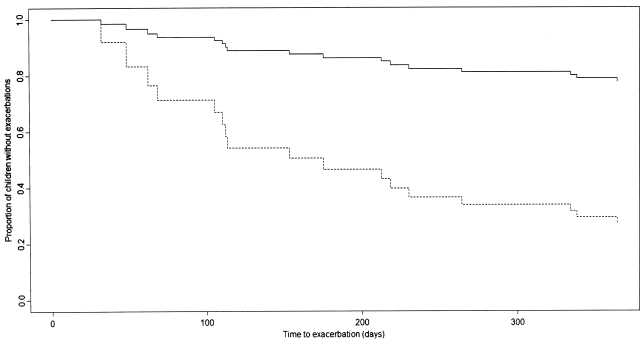


Fig. 1. The predicted survival curve of a patient of which the IL-5 value and the asthma control score were equal to the 90% percentile of all values of IL-5 and asthma control score in the dataset, and in addition, in case the values were equal to the 10% percentile. These curves show examples of the course of the risk of no exacerbation during the next 12 months when IL-5 and asthma control score values do not change.

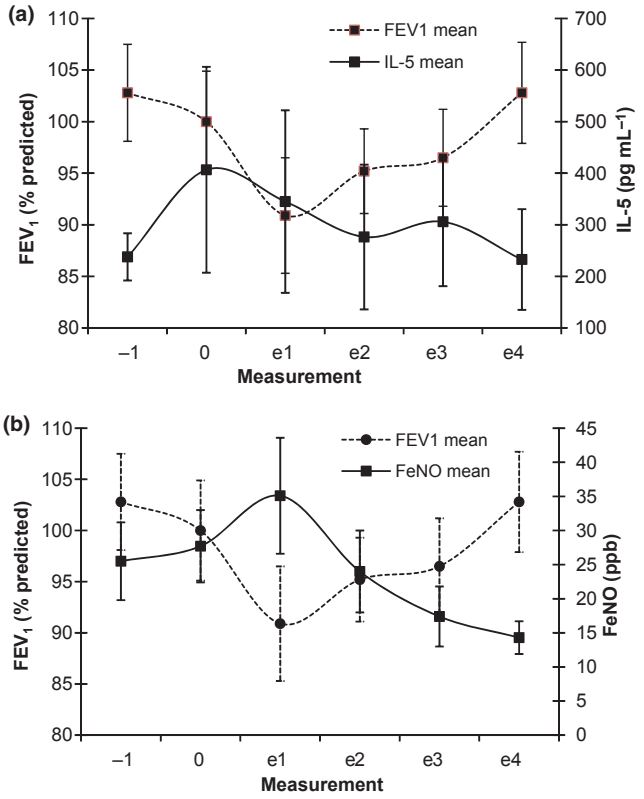


Fig. 2. (a) The course of $FEV_1$ and IL-5 before and during an asthma exacerbation. Measurements −1 and 0 represent on average 3 and 1 months before the exacerbation. Measurements e1, e2, e3, and e4 represent the measurements at day 1, 3, 5, and at the end of exacerbation. (b) The course of $FEV_1$ and fractional exhaled nitric oxide before and during an asthma exacerbation. Measurements −1 and 0 represent on average 3 and 1 months before the exacerbation. Measurements e1, e2, e3, and e4 represent the measurements at day 1, 3, 5, and at the end of exacerbation. $FEV_1$, forced expiratory volume in 1 s.

disorder like asthma. Exacerbations of childhood asthma were significantly predicted by regular assessments of EBC IL-5 levels, EBC acidity, and the asthma control score. In the multivariate analysis, EBC acidity was no longer a significant predictor of the exacerbation rate. The predicted survival curve showed an increase in the exacerbation rate from 0.3 to 0.8 per year in the category of patients with the 10% least optimal values of IL-5 and asthma control score compared to children with the 10% most optimal values. Strikingly, lung function and FeNO could not predict exacerbations.

The assessment of inflammatory markers in EBC has been suggested as a simple, rapid, safe, and non-invasive technique, suitable for assessment of airway inflammation in humans of all ages [10, 23]. Potentially, non-invasive biomarkers in EBC may be of help in the discrimination between different inflammatory phenotypes, the assessment of the severity of airway inflammation, and to monitor treatment and disease control [23].

The strengths of the present study are the prospective one-year study period, the use of various non-invasive inflammatory markers and lung function and the asthma control score as independent predictors, and the use of an optimized glass condenser system [18]. Moreover, the study population was well defined and already known to have had asthma for a significant period of time.

### *How can we explain the predicting value of EBC IL-5?*

IL-5 is an interdigitating homodimeric glycoprotein. It is produced by Th-2 cells and mast cells and induces immunoglobulin synthesis and activation of eosinophils in asthma. The importance of IL-5 in the pathophysiology of asthma has been demonstrated by a recent study showing that a monoclonal antibody against IL-5, Mepolizumab, was able to diminish exacerbations of asthma [24]. To the authors' current knowledge, the study described in this paper is the first research showing that IL-5 in exhaled breath condensate is a significant predictor of asthma exacerbations. However, the relationship between IL-5 and exacerbations of asthma has been demonstrated in other studies. Norzila and colleagues studied the characteristics of airway inflammation in exacerbations of childhood asthma using sputum cell counts and fluid-phase measurements at presentation to the emergency department and 2 weeks later at recovery. During exacerbation, the total cell count, sputum myeloperoxidase, IL-8, and IL-5 were increased [25]. Kocak and colleagues found an increase in serum IL-5, eosinophilic cationic protein, and CD4+/CD25+ lymphocytes on day one and five of an exacerbation of childhood asthma compared to control subjects, which normalized after glucocorticoid treatment [26]. Corrigan and co-workers assessed expression of

Th-1 and Th-2 cytokine mRNA and spontaneous secretion of IL-3, IL-5, and GM-CSF by peripheral blood CD4 and CD8 T-lymphocytes from asthmatics before and after oral glucocorticoid therapy, and in healthy controls. In asthmatics, the percentages of CD4 T-lymphocytes expressing IL-5 mRNA correlated with disease severity and the numbers of peripheral blood eosinophils. After oral glucocorticoid therapy, lung function improved and the number of CD4 lymphocytes expressing mRNA encoding IL-5 were reduced [27].

In the univariate analysis, the acidity of EBC was a significant predictor. However, in the multivariate analysis this influence disappeared. A lower pH of EBC seems to result from both eosinophilic and neutrophilic airway inflammation and apart from asthma also occurs in other diseases such as cystic fibrosis or COPD [28]. On the basis of the results of our study, it is possible that EBC IL-5 gives more specific information about airway inflammation in (exacerbations of) asthma than EBC pH. Although nitrite, IL-13, and IFN-gamma increased significantly during exacerbations, these biomarkers had no significant predictive value for exacerbations in the Cox regression models. EBC IL-5 levels were already elevated before the onset of an exacerbation which explains its predictive value in the Cox models. The multivariate analysis shows that the asthma control score and IL-5 considered together have most predictive value. Therefore, our study shows that asthma exacerbations in these children are best predicted by the combination of a simple clinical tool like asthma control and the EBC IL-5 levels. Potential of FeNO or lung function for the prediction of exacerbations of childhood asthma was not found in this study. This coincides with the result of a recent Cochrane in which on average no benefit was observed for titration of asthma treatment based on FeNO and clinical symptoms compared to symptoms only in patients with asthma [29]. Both FeNO and EBC IL-5 are markers of the eosinophilic inflammatory process, however, this study showed that only IL-5 was a significant predictor of an asthma exacerbation. The present study was designed to study the predictive value of several biomarkers in exhaled breath for an asthma exacerbation, and not the ability of these parameters to titrate asthma treatment.

Zacharasiewicz and co-workers performed a comparable study in which weaning of inhaled corticosteroids was studied in 40 asthmatic children. Two-monthly assessments of FeNO and sputum eosinophils were significantly related to failed steroid reduction but lung function and nitrate/nitrite in EBC were not [30]. This coincides with the results of the authors' study, in which EBC nitrite and lung function were not related to exacerbations. In contrast, two-monthly assessment of the asthma control score was found to be a significant predictor of the exacerbation rate. This indicates that asthma exacerbations are often preceded by a period with less asthma control, and also demonstrates the relevance of regular assessments of this validated questionnaire, for instance during visits to the outpatient clinic.

The results of this study should be reproduced in a new study with preferably a larger patient sample. In the future it is relevant to study whether titration of anti-inflammatory treatment on the basis of non-invasive markers such as IL-5 in EBC is of help in the prevention of exacerbations of asthma.

In this one-year longitudinal study, both exhaled breath condensate IL-5 levels and the asthma control score were significant predictors of an exacerbation of childhood asthma. This opens up the possibility of assessing the potential of these parameters to titrate asthma treatment in future studies.

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CMHHT Robroeks was main researcher. She was involved in the study design, management of the study, data analysis and writing of the manuscript. D van Vliet was partly responsible for measurements of the patients. Q Jöbsis was involved in the study design and in the evaluation of the study and manuscript. R Braekers was responsible for analysis of the longitudinal data. GT Rijkers was responsible for cytokine analysis in exhaled breath condensate. WKWH Wodzig was responsible for 8-isoprostane measurements in exhaled breath condensate. A Bast and GJM den Hartog were responsible for hydrogen peroxide measuments in exhaled breath condensate. LJI Zimmermann was involved in the study design and in the evaluation of the study and manuscript. E Dompeling was head supervisor of the study. All authors state that they have no conflict of interests to declare.

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