Title Page

Title: Exhaled breath analysis in childhood rheumatic disorders – a longitudinal study

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Abstract

Objectives. To evaluate fraction of exhaled nitric oxide (F\textsubscript{E}NO\textsubscript{50}) and deaerated exhaled breath condensate pH (dEBCpH) as non-invasive markers of subclinical airway inflammation in pediatric patients with rheumatologic disorders.

Methods. We determined F\textsubscript{E}NO\textsubscript{50} and dEBCpH in a prospective study spanning at least 12 months, comprising 85 pediatric patients with rheumatologic disorders, including juvenile idiopathic arthritis (JIA, n=63), chronic recurrent multifocal osteomyelitis (CRMO, n=6), systemic lupus erythematosus (SLE, n=3), juvenile dermatomyositis (JDM, n=1) and other rheumatic disorders (n=12). dEBCpH was determined once in a group of children without evidence of rheumatologic or pulmonary disease (controls, n=90). Findings were correlated with results of pulmonary function tests. Atopic sensitization was assessed by RAST or skin prick test in 76 patients.

Results. Atopic sensitization was detected in 34% (26/76) of patients. Neither F\textsubscript{E}NO\textsubscript{50} nor dEBCpH correlated with disease activity, but intermediately (20-35 ppb) or highly elevated (>35 ppb) levels were observed at least once in 26 patients (31%), 19 of whom had atopic sensitization. Median dEBCpH did not differ between cases and controls (8.05 vs. 8.02; p=0.48). Median dEBCpH decreased slightly over the study period (p=0.02), whereas F\textsubscript{E}NO\textsubscript{50} values did not change significantly (p=0.89). There were several patients with significantly abnormal dEBCpH values that could not be readily explained by diagnosis, higher disease activity, medications, or atopic sensitization.

Conclusions. There were no consistent abnormalities in F\textsubscript{E}NO\textsubscript{50} or dEBCpH in this cohort of Caucasian patients with relatively stable rheumatologic disorders, but there were some patients with abnormal values of unknown significance.

Key words: exhaled breath analysis, F\textsubscript{E}NO\textsubscript{50}, dEBCpH, pulmonary function, juvenile idiopathic arthritis, chronic recurrent multifocal osteomyelitis, systemic lupus erythematosus.
Introduction

Most rheumatologic disorders of childhood can feature lung involvement in one way or another. However, clinically significant lung involvement is usually rare in the most common disease entity, juvenile idiopathic arthritis (JIA) [1], and the advent of potent disease-modifying antirheumatic drugs (DMARDs) and biologicals has reduced the burden of disease due to pulmonary involvement across the board in most childhood rheumatic disorders. On the other hand, new technologies have made it possible to noninvasively monitor relatively subtle changes in pulmonary inflammation, even in asymptomatic periods [2]. The fraction of exhaled nitric oxide ($F_{E\text{-NO}}$) and deaerated breath condensate pH (dEBCpH) constitute the most widely used of these methods, both of which have been studied extensively in the context of asthma and related pulmonary diseases [3-5]. $F_{E\text{-NO}}$ has been found useful as a non-invasive biomarker of disease activity in several non-pulmonary diseases [6]. It has been assessed in several rheumatologic disorders in adults, where they were found to be elevated in two conditions that often feature pulmonary involvement, namely systemic lupus erythematosus (SLE), Sjögren syndrome, and systemic sclerosis [6-8], whereas dEBCpH has not received much attention in this regard. In particular, neither $F_{E\text{-NO}}$ nor dEBCpH have been studied in pediatric patients with rheumatologic disorders. We have therefore used these methods to screen pediatric patients with rheumatologic disorders for evidence of pulmonary inflammation and correlated findings with indices of pulmonary function and, in a subpopulation, of rheumatologic disease activity. We do not find obvious differences in $F_{E\text{-NO}}$ or dEBCpH between patients and controls, although significantly abnormal values were detected in a small number of patients without evidence of atopic sensitization. However, these could not be explained by diagnosis, disease activity, or medications taken.

Participants, Materials and Methods

Patients were recruited between 2008 and 2011 from the Division of Rheumatology of the Department of Pediatrics, Medical Faculty “Carl Gustav Carus”, Technical University (TU) Dresden, in Dresden, Germany. Most participants were clinically stable patients who came in for follow-up appointments. The final study population (n=85) comprised patients with juvenile idiopathic arthritis (JIA, n=63, consisting of oligoarticular JIA [n=17], polyarticular JIA [n=5], enthesitis-related JIA [n=8], systemic onset JIA [n=6], psoriatic JIA [n=1], and unspecified JIA [n=26]), chronic recurrent multifocal osteomyelitis (CRMO, n=6), SLE (n=3), and miscellaneous rheumatologic disorders (n=13; consisting of 2 cases each of nonspecific periodic fever syndrome, Lyme arthritis, Wegener granulomatosis, Sjögren syndrome, and 1
case each of juvenile dermatomyositis, reactive arthritis, systemic sclerosis, and Behcet
disease [n=1 of each]). Measurements were taken in 3-monthly intervals during routinely
scheduled clinic visits. For dEBCpH measurements, control subjects were recruited among
inpatients of the same hospital who did not have evidence of rheumatologic, atopic, or
pulmonary disease and were fit enough to walk to the pulmonary function testing unit and to
cooperate with the tests (n=90). Controls were matched to patients by age and sex. $F_{eNO50}$
was measured using a commercially available, clinically validated chemiluminescence
analyzer (CLD 88 sp, Eco Medics, Duernten, Switzerland). $F_{eNO50}$ values were not measured
among controls, since commonly accepted pediatric reference values were available [9,10].
dEBCpH was measured as described in [11]. In brief, deaeration was performed with argon,
and a stable dEBCpH was achieved after 10-12 minutes at a flow rate of 350 mL/min. Disease
activity of the underlying rheumatologic disease was determined in a subset of patients with
the Childhood Health Assessment Questionnaire (CHAQ) [12,13]. Atopic sensitization was
determined by Radio-Allergo-Sorbent-Test (RAST) or skin prick test in order to assess its
ctribution to any abnormalities in $F_{eNO50}$ and dEBCpH. Atopic sensitization was defined as
a RAST class of greater than 2, a positive skin prick test response to at least 1 common
airborne allergen, or both. Skin prick tests (Allergopharma, Reinbek, Germany) were
performed against *Penicillium* species, *Cladosporium* species, *Aspergillus* species, *Mucor*
species, *Alternaria* species, mugwort, ribwort, grasses, alder, hazelnut, birch, rye,
*Dermatophagoides pteronyssinus*, and *Dermatophagoides farina*. Sensitization was defined as
a wheal at least 3 mm greater in diameter than that elicited by the negative control. Plasma
total and specific IgE concentrations to common airborne allergens (same panel as skin prick
test) were assessed with RAST, and total IgE and eosinophil cationic protein levels were
assessed with fluorescence immunoassay (all with UniCAP 205; Phadia, Freiburg, Germany).
Pulmonary function was measured by spirometry and body plethysmography using the
equipment available for clinical care in the hospital (MasterScreen Body, Jaeger™,
CareFusion, Hoechberg, Germany). The reference values to calculate the percent of predicted
values were chosen according to Zapletal et al. [14].

**Ethics approval**
The study was approved by the Ethics Committee of the Medical Faculty “Carl Gustav
Carus”, TU Dresden. Informed consent was obtained from all parents, and assent from all
participants.
Statistical analysis

Correlations between pulmonary function parameters and $F_{E}NO_{50}$ and dEBCpH values were examined by Spearman’s rank correlation analysis. Differences in dEBCpH between cases and controls were compared with the Mann-Whitney-U-Test, as data were not normally distributed. Changes in $F_{E}NO_{50}$ and dEBCpH values over the study period were tested with the Friedman test, a nonparametric two-way analysis of variance. Statistical analyses were carried out with IBM SPSS Statistics, version 20 (IBM Corporation, Armonk, NY, USA). Figures were made with the online functions available through the R Foundation for Statistical Computing, version 3.1.3.

Results

Table 1 summarizes demographic and clinical characteristics of the patients and controls. Consistent with the predominantly Caucasian ethnicity of the patients, most of the patients had JIA, whereas SLE was rare. Atopic sensitization could be assessed in 76 patients, 26 (34%) of whom were classified as atopic. Of the 85 patients, 37 (43.5%), 36 (42.4%), 14 (16.5%) and 12 (14.1%) received nonsteroidal anti-inflammatory drugs (NSAIDs), DMARDs (including methotrexate [MTX]), biologicals, and corticosteroid therapy, respectively (multiple choices were possible).
Table 1. Demographic and clinical characteristics of the study and control populations

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls, n (%)</th>
<th>All cases, n (%)</th>
<th>Case subgroups, n</th>
<th>CRMO (n=6)</th>
<th>SLE (n=3)</th>
<th>Miscellaneous (n=13)</th>
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<tr>
<td></td>
<td>(n=90)</td>
<td>(n=85)</td>
<td>JIA (n=63)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Oligoarticular</td>
<td>Polyarticular</td>
<td>Enthesitis-related</td>
<td>Systemic onset</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>33 (37%)</td>
<td>29 (34%)</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Girls</td>
<td>57 (63%)</td>
<td>56 (66%)</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Median age</td>
<td>13 (6-23)</td>
<td>13 (5-26)</td>
<td>12 (6-18)</td>
<td>12.5 (5-17)</td>
<td>15 (9-19)</td>
<td>9 (8-13)</td>
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<td>(range), years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CHAQ*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No activity</td>
<td>NA</td>
<td>17 (20%)</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mild activity</td>
<td>NA</td>
<td>7 (8.2%)</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>NA</td>
<td>5 (5.9%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>activity</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations used: CHAQ, Childhood Health Assessment Questionnaire; CRMO, chronic recurrent multifocal osteomyelitis; JIA, juvenile idiopathic arthritis; NA, not assessed; SLE, systemic lupus erythematosus.

* Assessed at baseline
Pulmonary function tests

Fig. 1 shows the results of those successively measured PFTs that were deemed to be most relevant to the study aims, i.e. forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV₁). Only a few patients had abnormal FVC and FEV₁ values: two patients had values lower than 80% and seven patients had values higher than 120%. There were no significant differences in FVC and FEV₁ values across the rheumatologic diagnoses (Fig. 1).

Figure 1. Major parameters of pulmonary function tests. Forced vital capacity (left) and forced expiratory volume in 1 sec (right).

The dotted lines delineate the range of normal values.

$F_{E}NO_{50}$ and dEBCpH

Fig. 2 (left panel) shows the spread of the $F_{E}NO_{50}$ values across all patients and also separated according to diagnosis. The median values of $F_{E}NO_{50}$ for JIA, CRMO and SLE were 10.9, 11.7 and 14.7, respectively. Fig. 2 (right panel) shows $F_{E}NO_{50}$ values at the four time points of the study; the median values did not change during the study period (p=0.89, Friedman test). There were a total of 26 (31%) children with any value >20 ppb (i.e. intermediately or highly elevated levels), 19 of whom were atopic. Specifically, intermediately elevated $F_{E}NO_{50}$ values (20-35 ppb) were observed in at least one visit in 14 (16%) children, 9 of whom were classified as atopic. The diagnoses of the 5 non-atopic patients in this group were JIA (n=4).
and SLE (n=1), and treatments were corticosteroids (n=2), NSAID (n=1), and sulfasalazine (n=1); one patient was without pharmacological treatment. Highly elevated $F_{E\text{NO}_{50}}$ values (>35 ppb) were observed in at least one visit in 11 (13%) children, 10 of whom were classified as atopic. The one non-atopic patient in this group had stable JIA, was treated with MTX, and had no clinical evidence of lung disease.

**Figure 2.** Values of the fraction of exhaled nitric oxide ($F_{E\text{NO}_{50}}$) by diagnosis (left, baseline values) and at four time points of the study (right).

![Graph showing $F_{E\text{NO}_{50}}$ values by diagnosis and time points](image)

The horizontal line represents the mean $F_{E\text{NO}_{50}}$ values of healthy individuals aged 13 years according to [10]. The dashed lines at 20 and 35 ppb correspond to intermediate and high risk of eosinophilic inflammation, respectively, according to [9]. The red dots on the right graph represent the median values. Changes in $F_{E\text{NO}_{50}}$ values over the study period were not significant ($p=0.89$, Friedman test, n=43). Measurements were taken in 3-monthly intervals during routinely scheduled clinic visits.

dEBCpH values are shown in Fig. 3. Since there are no age appropriate reference values for this parameter, values were compared to a matched control group of children and adolescents without atopic, pulmonary or rheumatologic disease. The median dEBCpH values in cases and controls were 8.05 and 8.02, respectively ($p=0.48$, Mann-Whitney-U-test, Fig. 3, left panel). The median dEBCpH values were similar across rheumatologic diagnoses. There were several outliers, only one of whom had atopic sensitization. The most severe outlier was a boy...
with anti-phospholipid antibody syndrome due to SLE, who consistently had values between pH 4 and 5, but did not have atopic sensitization. At 6 years follow-up (2015), he has not developed atopic disease or pulmonary involvement of the SLE. Longitudinal data show that dEBCpH values decreased slightly during the study period (T1: median of 8.06; T4: median of 7.79; p=0.02, Friedman test, Fig. 3, right panel).

**Figure 3.** Deaerated breath condensate pH (dEBCpH) values by diagnosis (left, baseline values) and changes over the study period (right).

Left panel; the difference in dEBCpH between controls and cases was not significant (p=0.48, Mann-Whitney-Test). Right panel; each dashed line represents one patient. The red line represents median values; the median dEBCpH values decreased slightly during the study period (p=0.02, Friedman test, n=31). Measurements were taken in 3-monthly intervals during routinely scheduled clinic visits.

**Correlations with the CHAQ score**

Results of the CHAQ were available from a subgroup of patients (n=29) because they were also enrolled in a related study in which CHAQ scores were assessed. We thus looked for correlations between the CHAQ score and F_{E}NO_{50} or dEBCpH values. There was no significant correlation (F_{E}NO_{50}: Spearman’s r=-0.05, p=0.80, n=26; dEBCpH: r=0.06, p=0.75, n=27).
Effects of medications

We finally addressed the question whether abnormal $F_{E\text{NO}_{50}}$ or dEBCpH values could be attributed to pharmacological interventions. However, when testing for any effects due to the major classes of medications used to treat rheumatologic disorders, there were no associations between elevated $F_{E\text{NO}_{50}}$ values and treatment with NSAIDs (Fisher’s exact test, $p=0.52$), corticosteroids ($p=0.21$), or DMARDs including MTX ($p=0.43$). There was a positive association between elevated $F_{E\text{NO}_{50}}$ values and treatment with biologicals ($p=0.007$). However, this effect disappeared after controlling for atopic sensitization as a confounding variable ($p=1.0$). Likewise, there was no association between abnormally low dEBCpH (here defined as pH $\leq 7.0$, as Fig. 3 [right panel] suggests a step-off around this value) and these medication classes (NSAIDs, $p=1.0$; corticosteroids, $p=0.76$; DMARDs including MTX, $p=0.27$; biologicals, $p=1.0$).

Discussion

We have analyzed $F_{E\text{NO}_{50}}$ and dEBCpH in a heterogeneous population of patients with rheumatologic disorders of childhood and found, overall, few abnormal values, most of which could be explained by a co-existing atopic sensitization. This suggests that subclinical airway inflammation due to pulmonary involvement in pediatric rheumatologic disorders is rare in populations such as ours, which are usually clinically stable due to effective treatments. Indeed, recent studies of pulmonary function in pediatric patients with rheumatologic disorders have shown mostly normal function and age-appropriate development (e.g., ref. [15]). $F_{E\text{NO}_{50}}$ has been assessed in adult patients with rheumatologic disorders. For instance, a study of adult SLE patients showed that $F_{E\text{NO}_{50}}$ values were about twice as high as in controls and correlated with disease activity [8], and a study of patients with Sjögren syndrome revealed $F_{E\text{NO}_{50}}$ values about 50% higher in patients than in controls [7]. dEBCpH has proven useful in the detection of subclinical airway inflammation in children at risk of asthma during asymptomatic intervals [11], suggesting that it is a sensitive method to detect clinically inapparent pulmonary inflammation. However, we detected abnormal values only in a small number of patients, and their clinical significance is uncertain.

What may be the reasons for the small number of clearly abnormal values that could not be explained by atopic sensitization? dEBCpH values were persistently lowest in a boy with anti-phospholipid antibody syndrome due to SLE. At the time of the measurements he had stable disease, was not atopic, and did not develop pulmonary involvement during 5 years follow-up. At the time of the measurements he was on treatment with mycophenylate mofetil and...
ramipril, both of which are known to cause pulmonary side effect. Thus, his persistent, remarkably low dEBCpH values may have been due to pharmacological interventions, although there are no data to support this hypothesis. Likewise, unexplained elevated $F_{E}NO_{50}$ levels were detected in 5 non-atopic patients with intermediated elevation (20-35 ppb), and in one non-atopic patient with severe elevation (>35 ppb). It is currently not possible to explain the observed abnormalities in this small subset of patients (n=6; i.e. 7%), but this phenomenon certainly deserves to be addressed in future studies.

Limitations of the presented study
This study is limited by the fact that the patients were clinically stable and that no newly-diagnosed, treatment-naïve patients were included. It is conceivable that in newly manifesting disease, in particular SLE, abnormal values (reflecting disease-associated lung inflammation, albeit subclinical) might have been detected. Likewise, due to the composition of the patient population at our center, we could not include patients with sarcoidosis and only 1 patient with systemic sclerosis, both constituting disorders that typically feature lung involvement. However, it is known that at least $F_{E}NO_{50}$ is not elevated in adult patients with sarcoidosis [16] or interstitial lung disease associated with connective tissue diseases [17]. Furthermore, analysis for concentrations of nitric oxide metabolites, e.g. nitrites and nitrates, would have been of great interest but was not available during the study period. This should be included in future studies. Moreover, comparing $F_{E}NO_{50}$ and dEBpH with established screening tools for interstitial lung involvement such as diffusion lung capacity and echocardiography (to assess pulmonary artery pressure) would be important. Lastly, CHAQ scores were available for only a subpopulation of participants. However, the correlations between the CHAQ score and $F_{E}NO_{50}$ or dEBCpH were so clearly not significant that it is highly unlikely that availability of CHAQ scores for a larger proportion of participants would reveal any significant associations.

Conclusions
In summary, in this clinically stable Caucasian population, $F_{E}NO_{50}$ and dEBCpH values were mostly normal, and most abnormal values could be explained by concomitant atopic sensitization. However, the small number of patients with abnormal values not explained by atopic sensitization suggests that $F_{E}NO_{50}$ and/or dEBCpH may have some value as biomarkers of abnormal pulmonary or systemic processes in selected cases. Further research will be necessary to identify such narrowly defined subpopulations or clinical scenarios in which
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Authors’ contributions
NH recruited the patients, performed the F\textsubscript{E}NO\textsubscript{50} and dEBCpH measurements, and collected the pulmonary data. MKA performed data analysis, made the figures and edited the manuscript. JH acquired the CHAQ scores. CV oversaw the pulmonary aspects of the study and edited the manuscript. FP conceived and oversaw the study, wrote the first draft of the manuscript, had access to all data and takes responsibility for their integrity.
References


