Repeated breath profiling by eNoses identifies asthma exacerbations and airway eosinophilia

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Rationale
- Symptoms and lung function are only moderately related to airway inflammation.
- Asthma exacerbations / loss of control are likely due to increased airway inflammation as induced by virus infections or other environmental exposures.
- Sputum inflammatory markers in asthma and COPD are related to exhaled molecular profiles (breathprints) obtained by eNose and GC-MS (Ibrahim et al Thorax 2011, Fens et al ERJ 2011).

Hypothesis: Breathprints obtained by an eNose platform are associated with exacerbations / loss of asthma control after inhaled steroid withdrawal.

Aim
To assess the relationship between profiles of exhaled biomarkers (eNose platform breathprints) and exacerbations / loss of asthma control after inhaled steroid withdrawal.

Methods
Subjects: Moderate-severe asthma
- 23 asthmatics based on GINA-criteria.
- (partly) Controlled asthma.
- At least 1 exacerbation in the past 2 years.
- At least 500 mcg fluticasone daily or equivalent.
- Non-smoker or ex-smoker (<5 PY).

Design: Longitudinal study with complete stop of ICS at T1

<table>
<thead>
<tr>
<th>Screening</th>
<th>Baseline</th>
<th>Exhaled breath</th>
<th>Loss of control</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T3</td>
</tr>
<tr>
<td>2 weeks</td>
<td>0-8 weeks</td>
<td>4 weeks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Loss of control / exacerbation defined as:
1. 2 out of 3 on 2 consecutive days
2. Awakening due to asthma
3. ≥8 puffs short-acting beta-agonist

Measurements:
- Spirometry, sputum induction, FeNO, ACQ
- eNose measurement:
  - Expiratory vital capacity volume collected by standardized method in Tedlar bag, transferred to Tenax tubes (Fens, AJRCM 2009).
  - Breathprints obtained by eNose platform: 158 sensors based on quartz microbalance, polymers, metal oxide, IMS (Wagener, PATS 2013).

Statistical analysis (SPSS):
- Sensor values were normalized, followed by principal component (PC) analysis.
- PC with Eigenvalue >1 were analysed by mixed models followed by post-hoc paired t-tests.

Results

Table 1. Patient characteristics at all timepoints

<table>
<thead>
<tr>
<th>Asthma (n=23)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)*</td>
<td>28 (19-50)</td>
<td>29 (21-3.9)</td>
<td>0.5 (0-2.1)</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>6/17</td>
<td>5/15</td>
<td>102 (14)</td>
</tr>
<tr>
<td>Atopy Y/N</td>
<td>21/2</td>
<td>102 (12)</td>
<td>108 (14)</td>
</tr>
<tr>
<td>LABA Y/N</td>
<td>17/6</td>
<td>102 (12)</td>
<td>108 (14)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0 (4.3)</td>
<td>16.5 (5-147.5)</td>
<td>17.5 (7-95)</td>
</tr>
<tr>
<td>ACo*</td>
<td>1.0 (0-3.0)</td>
<td>2.9 (21-3.9)</td>
<td>0.5 (0-2.1)</td>
</tr>
<tr>
<td>Pre-FEV, (% predicted)*</td>
<td>101 (12)</td>
<td>89 (15)</td>
<td>102 (14)</td>
</tr>
<tr>
<td>Post-FEV, (% predicted)*</td>
<td>107 (12)</td>
<td>102 (12)</td>
<td>108 (14)</td>
</tr>
<tr>
<td>FeNO (ppb)*</td>
<td>16.5 (5-147.5)</td>
<td>32.5 (7.15-75)</td>
<td>17.5 (7-95)</td>
</tr>
<tr>
<td>PC20*</td>
<td>1.96 (0.03-6.93)</td>
<td>0.4 (0.01-12)</td>
<td>3.5 (0.41)</td>
</tr>
</tbody>
</table>

* Median (range) † Mean (Standard Deviation)

Conclusions
- Using breathprints obtained by an eNose platform it is possible to discriminate between stable periods and periods with (induced) loss of control / exacerbation in asthma.
- At recovery, breathprints returned to baseline values.
- eNose platform breathprints show a correlation with sputum eosinophils.

Implications
eNose platform breathprints may be used for monitoring asthma control.

Figure 3 eNose breathprints PC8 at T1 vs T2, T1 vs T3

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References
- Ibrahim et al Thorax 2011
- Fens et al ERJ 2011
- Sputum inflammatory markers in asthma and COPD are related to airway inflammation as induced by virus infections or other environmental exposures.
- Mixed model analysis showed significant changes in PC8 (fig. 3) and PC9, FeNO and sputum eosinophils comparing all three timepoints.
- Post-hoc paired t-tests showed that significant changes were explained by differences between T1 and T2.
- PC8 showed a strong correlation with sputum eosinophils at T1 but not at T2 and T3 (p<0.008; R<0.60; see fig. 4).