Detection of obstructive sleep apnoea by an electronic nose

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Abstract

**Rationale:** Diagnosis of obstructive sleep apnoea syndrome (OSAS) is technically demanding, cost-intensive and time-consuming. The measurement of volatile organic compounds by an electronic nose is an innovative method that determines distinct molecular patterns of exhaled breath in different patient groups. We addressed the following questions: What is the diagnostic accuracy of an electronic nose in the detection of OSAS and the ability to detect effects of standard therapy in patients with OSAS? Are these results related to changes in distinct markers of airway inflammation and extracellular remodelling?

**Study Participants/Methods:** We included 40 OSAS patients and 20 healthy controls. Exhaled breath of all participants was analysed using the Cyranose 320™. Pharyngeal washings were performed to sample the upper airway compartment. For statistical analysis linear discriminant analysis was employed.

**Results:** We identified a Linear Discriminant function separating OSAS from control (p<0.0001). The corresponding area under the Receiver Operating Curve was 0.85 (95%CI 0.75-0.96; sensitivity 0.93; specificity 0.7). In pharyngeal washing fluids of OSAS patients we observed higher levels of alpha-1-antitrypsin and markers of extracellular remodelling compared to controls.

**Conclusion:** The electronic nose can distinguish between OSAS patients and controls with high accuracy.
Keywords

Alpha-1-antitrypsin, continuous positive airway pressure, matrix metalloproteases, pharyngeal washing fluid, tissue inhibitor of matrix metalloproteases (TIMP), volatile organic compounds
Introduction

Obstructive sleep apnoea syndrome (OSAS) is a common disease [1] associated with an increased risk for cardiovascular disorders [2;3]. The current gold standard to confirm OSAS is multi-channel polysomnography (PSG) [4]. This is technically demanding, time-consuming, and labour- and cost-intensive with limited availability.

Different screening tools have been developed to reduce the number of patients requiring PSG but most of them lack sensitivity and/or specificity. Clinical parameters like Epworth Sleepiness Scale (ESS), neck circumference and a composite clinical score show a large overlap between healthy controls and OSAS patients [5-7].

Technical tools sensing a change of body position as a surrogate for respiratory movements [8], sound analysis [9] and complex computerized analyses of electrocardiogram (ECG) recordings [10] have been evaluated with different success rates. A novel screening tool predicting OSAS with acceptable accuracy and without overnight measurements could improve OSAS screening algorithm.

Several studies revealed that OSAS is associated with increased oxidative stress [11] as well as systemic and local inflammation [12], as indicated by increased concentrations of pro-inflammatory cytokines and other markers in the exhaled breath condensate (EBC) and serum [11]. The serum level of alpha-1-antitrypsin (AAT), an acute phase reactant playing a major role in the control of inflammation [13], also seems to be elevated [14]. Other compounds such as matrix metalloproteases (MMP) and tissue inhibitor of matrix metalloproteases (TIMP) might also be involved in the disease process as mediators of the ongoing airway remodelling [15].
Exhaled breath (EB) contains hundreds of volatile organic compounds (VOCs), as demonstrated by mass spectrometry [16], and its analysis can provide information about systemic or local inflammation. Instead of identifying single compounds, the assessment of EB can also be performed by devices enabling the recognition of patterns of VOCs [17]. Indeed, such devices can distinguish between a number of diseases via their VOC profiles [18-20]. A recent review article summarising potential medical applications has been published elsewhere [21]. To our knowledge VOC profiles have not been assessed in patients with OSAS.

Our hypothesis was that a pattern-recognizing electronic nose is capable of distinguishing between OSAS patients and healthy controls (HC). In addition, we assumed that therapy with continuous positive airway pressure (CPAP) has a detectable effect on airway inflammation and consequently the VOC profile. To substantiate the data by direct measurement of biochemical compounds, we assessed whether markers of inflammation and airway remodelling differ between HC and OSAS before/after CPAP treatment. For this purpose we selected pH and conductivity of EBC, and MMP-9, TIMP-1, and AAT in pharyngeal washing fluids.

Methods

Subjects and study design

Twenty healthy volunteers were recruited from the hospital staff and 40 OSAS patients from a sleep apnoea outpatient clinic before receiving CPAP therapy. Inclusion criteria for HC were the ability and the willing to participate. Volunteers with any known chronic disease or any acute disease in the last four weeks before study entry or any medication taken on a regular basis were excluded. OSAS was defined
as an AHI > 5 in combination with clinical signs of obstructive sleep apnoea. We excluded patients with any other chronic and/or acute respiratory disease of the upper and/or lower airways (e.g. asthma, COPD). These diseases were assessed by a questionnaire (patient-reported). In any case of doubt lung function testing was performed. The following co-morbid conditions were documented systematically: coronary heart disease, diabetes, arterial hypertension. Disease specific medical therapy was allowed and left unaltered in the course of the study. The study was approved by the local ethics committee (Marburg Ethics Committee AZ 59/06 Amendment 3) and written informed consent was obtained from each subject.

All participants had sleep studies and answered a questionnaire regarding symptoms, smoking habits, health status, medication and medical history. In addition, the following examinations were performed: collection of pharyngeal washing fluid, collection of EBC, measurement of EB with the electronic nose Cyranose 320™. The first 20 of the 40 OSAS patients were examined again after 3 months of CPAP therapy.

**Polysomnography/Polygraphy**

Patients with suspected OSAS underwent an overnight polysomnography (Embla N7000, TNI® Medical AG, Germany). Electroencephalogram (EEG), electrooculogram (EOG) and electromyogram (EMG) were measured using established procedures. Further, thoracic and abdominal respiratory excursions, breath sounds, nasal airflow, electrocardiogram (ECG) and oxygen saturation were recorded. In HC home polygraphy (SOMNOcheck2 R&K, Weinmann, Hamburg, Germany) was used to exclude OSAS. An apnoea-hypopnoea index (AHI) smaller than 5 events/h was defined as the absence of OSAS.
Pharyngeal washing fluid

All participants had to be fasting for at least two hours, including chewing gum or any kind of candy and tobacco smoking. They washed out their mouth with water before rinsing the throat with 25 ml water by gargling. The fluid was stored at -80 °C for ELISA-based analysis of TIMP-1 and MMP-9 (R&D Systems, Minneapolis, USA) which were conducted according to the manufacturer’s suggested routine procedure. AAT was measured by ELISA as described before [22]. The sensitivity of the ELISAs for TIMP-1, MMP-9, and AAT was 30 pg/ml, 30 pg/ml, and 40 pg/ml, respectively.

Exhaled breath condensate

EBC was collected by tidal breathing over 15 min using the ECoScreen Turbo (VIASYS, CareFusion Germany 234 GmbH, Höchberg, Germany) as described [23].

Electronic nose

The EB was assessed with the Cyranose 320 electronic nose (Smiths Detection Group Ltd., Watford, UK). The participants breathed medicinal air (Aer medicinalis Linde, Linde Gas Therapeutics GmbH, Unterschleißheim, Germany) and exhaled for 10 s at a flow rate of 100 ml/s to 200 ml/s into a disposable collection bag, which then was assessed within 60 s. This medicinal air was also used as reference air for the 60-s baseline, followed by a 60-s sample draw from the collection bag, and completed by a 60-s purging of the electronic nose. These measurements were performed in triplicate [20].

Data analysis

The three data sets obtained by the electronic nose were averaged by taking their arithmetic mean for each individual. On these data principal component analysis was performed. The resulting transformed data were fed into a linear discriminant
analysis (LDA). The LDA results were then used for further analyses, including nonparametric statistical significance tests. The Mahalanobis distance (MD) between the groups was determined and a leave-one-out cross-validation of the data was performed to calculate the cross-validation value (CVV) like described before [24]. Additionally a receiver operating characteristic (ROC) curve using the LD as discriminative variable was constructed to determine the area under the curve (AUC). The values for sensitivity and specificity were derived from LDA “self-prediction” [25], meaning that the complete data set was used to calculate the values. To support this analysis we additionally performed a split-half analysis using the first 20 patients as training set and the second half as the test set (and vice versa). Sensitivity and specificity were reported at the specific cut-off level, where the sum of the sensitivity and the specificity (Youden-Score) was highest [26].

Prior to the statistical comparisons the data were checked for normal distribution by the Kolmogorov-Smirnov test. For data not normally distributed non-parametric tests were used (Mann-Whitney U test for unpaired, Wilcoxon matched-pairs signed-rank test for paired data), otherwise a Student’s t-test (unpaired or paired). To determine the correlation between the LDA and parameters of inflammation, the Spearman's rank correlation coefficient was calculated. Data are presented as mean ± standard deviation (SD) unless stated otherwise. The software GraphPad Prism 5.00 (GraphPad Inc., California, USA) and SPSS Version 20 (IBM Germany, Ehingen, Germany) was used for all statistical analyses.
**Results**

**Patients versus controls**

Baseline characteristics of the 40 OSAS patients and 20 healthy volunteers are shown in table 1.

<table>
<thead>
<tr>
<th></th>
<th>OSAS patients</th>
<th>Healthy controls</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number ♂ / ♀</strong></td>
<td>3 / 37</td>
<td>8 / 12</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td><strong>Age [yr]</strong></td>
<td>55 (10)</td>
<td>40 (8)</td>
<td>p &lt; 0.001†</td>
</tr>
<tr>
<td><strong>Height [cm]</strong></td>
<td>175 (7)</td>
<td>175 (8)</td>
<td>p = 0.888</td>
</tr>
<tr>
<td><strong>Weight [kg]</strong></td>
<td>99 (15)</td>
<td>78 (14)</td>
<td>p &lt; 0.001†</td>
</tr>
<tr>
<td><strong>BMI [kg/m²]</strong></td>
<td>32.00 (4.5)</td>
<td>25.4 (4.1)</td>
<td>p &lt; 0.001†</td>
</tr>
<tr>
<td><strong>AHI [events/h]</strong></td>
<td>33.65 (22.00)</td>
<td>2.7 (1.7)</td>
<td>p &lt; 0.001†</td>
</tr>
<tr>
<td><strong>AI [events/h]</strong></td>
<td>11.9 (16.7)</td>
<td>0.6 (1.1)</td>
<td>p &lt; 0.001‡</td>
</tr>
<tr>
<td><strong>HI [events/h]</strong></td>
<td>22.6 (19.5)</td>
<td>2.1 (1.5)</td>
<td>p &lt; 0.001‡</td>
</tr>
<tr>
<td><strong>SO₂ [%]</strong></td>
<td>92.8 (2.7)</td>
<td>95.5 (1.1)</td>
<td>p = 0.01†</td>
</tr>
</tbody>
</table>

**Table 1: Baseline characteristics of OSAS patients and healthy controls**

OSAS = obstructive sleep apnoea syndrome, BMI = body mass index, AHI = apnoea-hypopnoea index, AI = apnoea index, HI = hypopnoea index, SO₂ = oxygen saturation.

*† student’s t-test
* Fishers’s exact test
‡ Mann–Whitney U-test
The LDA scores of OSAS patients and healthy controls differed statistically significantly from each other \( (p < 0.0001; \text{Mann-Whitney-U test; figure 1a}) \). The MD between the two groups was 1.88 and the CVV 79.5 \%. When using a split-half analysis, 80 \% of the “second half” were predicted to have OSAS correctly. Vice versa, when predicting the “first half” after having used the second half as training set, 85 \% were predicted to suffer from OSAS correctly. The corresponding area under the ROC curve was 0.85 (95 \% confidence interval (95\%CI) 0.745 to 0.960; figure 1b), indicating a sensitivity of 0.93 and a specificity of 0.70. Furthermore, the LDA was significantly correlated with the apnoea hypopnoea index (Spearman’s \( r = 0.58, p < 0.001 \)), indicating a “dose-response” relationship. There was no significant correlation between the LDA and the other measured markers (EBC pH, EBC conductivity, inflammatory markers in pharyngeal washings).

EBC pH values (8.16 ± 0.47 in OSAS vs. 8.09 ± 0.40 in HC; \( p = 0.27; \text{t-test} \)) and EBC conductivity (175.5 µS/cm ± 292.9 µS/cm in OSAS vs. 93 µS ± 33.36 µS/cm in HC; \( p = 0.17; \text{t-test} \)) of both groups did not differ significantly (figure 2).

AAT concentrations in pharyngeal washing fluids were significantly higher in OSAS patients compared to HC (60.6 µg/ml ± 52.0 µg/ml vs. 25.3 µg/ml ± 21.7 µg/ml; \( p = 0.007; \text{t-test; figure 3a} \)). In contrast, the difference in concentrations for MMP-9 (OSAS 5077.3 pg/ml ± 9104.5 pg/ml vs. HC 1008.6 pg/ml ± 872.9 pg/ml; \( p = 0.06; \text{t-test} \)) and TIMP-1 (OSAS 7918.9 pg/ml ± 7075.7 pg/ml vs. HC 6961.6 pg/ml ± 11412 pg/ml; \( p = 0.16; \text{Mann–Whitney U-test} \)) did not reach statistical significance. However, the MMP-9/TIMP-1 ratio showed a significant difference between groups (OSAS 0.69 ± 1.11 vs. HC 0.24 ± 0.25; \( p=0.02; \text{Mann–Whitney U-test; figure 3b} \)). Compared to the VOC analysis, any other marker (EBC pH, EBC conductivity, any marker in pharyngeal washing fluid) was inferior in predicting OSAS (AUC of the
ROC curves ranged from 0.59 to 0.71; data not shown). However, the combination of inflammatory markers in pharyngeal washings and EBC pH/conductivity with the LDA increased the diagnostic accuracy to 100 % (AUC of the ROC 1; 95% CI 0.94 to 1.00).

**Patients before and after 3 months of CPAP therapy**

The characteristics of the first 20 OSAS patients measured before and after initiation of CPAP therapy are listed in table 2.

<table>
<thead>
<tr>
<th>OSAS patients</th>
<th>Pre-initiation</th>
<th>After 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number♀ / ♂</td>
<td>1 / 19</td>
<td></td>
</tr>
<tr>
<td>Age [yr]</td>
<td>57 (9)</td>
<td></td>
</tr>
<tr>
<td>Height [cm]</td>
<td>175 (7)</td>
<td></td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>101 (15)</td>
<td></td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>32.9 (4.4)</td>
<td></td>
</tr>
<tr>
<td>AHI [events/h]</td>
<td>32.0 (22.8)</td>
<td>2.9 (3.4)</td>
</tr>
<tr>
<td>AI [events/h]</td>
<td>9.4 (17.4)</td>
<td>1.3 (2.8)</td>
</tr>
<tr>
<td>HI [events/h]</td>
<td>22.6 (21.7)</td>
<td>1.7 (2.1)</td>
</tr>
<tr>
<td>SO₂ [%]</td>
<td>92.5 (2.5)</td>
<td>94.4 (1.5)</td>
</tr>
</tbody>
</table>
Table 2: Characteristics of the subgroup of OSAS patients with measurements at baseline and after 3 month of CPAP therapy

OSAS = obstructive sleep apnoea syndrome, BMI = body mass index, AHI = apnoea-hypopnoea index, AI = apnoea index, HI = hypopnoea index, SO2 = oxygen saturation.

The LDA values of before and after initiation of standard CPAP therapy differed significantly (p = 0.0003; Wilcoxon test; figure 4a). The MD between the two groups was 1.83, the CVV 63.1 %, the corresponding area under the ROC curve 0.82 (95%CI 0.6825 to 0.9475; figure 4b), with a sensitivity of 0.80 and a specificity of 0.65.

EBC pH values of both visits were similar (pre 8.08 ± 0.52 vs. post 8.05 ± 0.85; p = 0.63; Wilcoxon test; figure 5a), however conductivity differed (pre 186.7 ± 303.6 µS/cm vs. post 97.9 ± 59.35 µS/cm; p < 0.05; Wilcoxon test; figure 5b).

Moreover, the AAT concentration decreased significantly after CPAP treatment (pre 66.3 µg/ml ± 48.4 µg/ml vs. post 42.8 µg/ml ± 36.3 µg/ml; p = 0.017; paired t-test; Figure 6a. For MMP-9 (pre 7785.6 pg/ml ± 10500 pg/ml vs. post 9185.8 pg/ml ± 11003 pg/ml; p = 0.421; Wilcoxon test) and TIMP-1 (pre 8344.7 pg/ml ± 7201.1 pg/ml vs. post 13201 pg/ml ± 13151 pg/ml; p = 0.314; Wilcoxon test) there was no significant difference. The same applied to the MMP-9/TIMP-1 ratio (pre 0.87 ± 1.2 vs. post 0.98 ± 0.97; p=0.37; Wilcoxon test; figure 6b.

Discussion

This study shows that the electronic nose Cyranose 320™ can distinguish the pattern of VOCs present in the EB of patients with OSAS from that of healthy subjects. A significant correlation between the linear discriminant and the AHI could be observed.
Furthermore, the electronic nose could discriminate the state before and after treatment with CPAP. This indicates that specific VOC patterns in EB are associated with untreated OSAS.

EB analysis for VOCs is a relatively novel option to obtain information about diseases. Gas chromatography and mass spectrometry (GC-MS) have been used in the past [16]. The simpler pattern-recognizing electronic noses allow the rapid recognition of VOCs mixtures in terms of “smellprints” [27], but not the identification of individual molecular components [28].

Electronic noses of various sophistications have been tested in a variety of respiratory diseases. In principle, the diagnosis of ear, nose and throat infections [29] or pneumonia [30] is possible. Moreover, patients with lung cancer [31], asthma [32], chronic obstructive pulmonary disease (COPD) [18] or alpha-1 antitrypsin deficiency [33] could be recognized when compared with healthy controls or individuals with other respiratory diseases. In our study we aimed to test the hypothesis that OSAS could be recognized by its VOC profile and that the profile would change after CPAP treatment. To our knowledge this is the first study in which the smellprints of OSAS patients have been compared to healthy subjects.

Our results might be of interest, as nearly all screening tools for OSAS require overnight measurements. Although the available devices are easy to handle, part of the recordings exhibit poor quality limiting their diagnostic value. Furthermore, some patients are unwilling to sleep “connected to an electronic device”. Conversely, overnight polygraphy with a limited number of channels does not require expensive medical staff but two patient visits at the clinic. Thus a diagnosis from other sources, e.g. “within a breath”, would be desirable.
One might wish that single substances could be described to predict differences in the exhaled breath profile of different patient groups. While gas chromatography and/or mass spectrometry could achieve that, due to tremendous costs and time-consuming data analysis these methods would not be feasible in a clinical setting. The idea to describe (on a computational level) patterns without knowing the disease-specific substance is a promising new attempt with a clinically-directed on-site approach.

It could be argued that an AUC of 0.85 is not sufficient for a diagnostic tool. However, diagnostic tests are used in specific clinical situations. The eNose could be useful in two occasions: First, to rule out the disease in a low prevalence population, e.g. in a general practitioner’s office where the prevalence of OSAS is about 2 % to 4 % [1]. A negative result would have a negative predictive value of 99.6 % (95 % CI 90.3 – 100 %), thus provide a high degree of certainty. Second, the device could be a decision aid on whom to conduct overnight polysomnography. In a population with a high prevalence of OSAS (as high as 35 % in obese, snoring subjects), a positive result would have a positive predictive value of 62.4 % (95 % CI 43.3 - 79.1 %) and would pave the way towards overnight polysomnography. On the other hand, a negative result would have a negative predictive value of 94.5 % (95 % CI 78.9 - 99.6 %), thus drawing the attention to other diagnoses than OSAS. Furthermore, a value of 0.85 for an AUC is in the range of other diagnostic tests that are used in daily clinical practice like troponin for the diagnosis of myocardial infarction (AUC of 0.87 [34]).

To reveal whether there would be specific chemical or physicochemical alterations in OSAS patients, we also analysed EBC, with focus on easy accessible markers such as pH and electrical conductivity, which we recently found to be robust and reproducible markers unaltered by respiratory manoeuvres [24]. EBC pH values were
in the range of 8 which is comparable to what has been reported before [35-37]. However we did not find major differences in these measures which might be too unspecific for the disease.

In contrast, AAT in pharyngeal washing fluid as a marker of inflammation showed elevated values in OSAS patients and these were reduced after only 3 months of CPAP treatment. This could reflect the response to the intermittent hypoxia and/or cyclic shear forces that are alleviated by CPAP. Similar results have been shown for local and systemic markers of inflammation and/or oxidative stress [38;39]. As further markers we used MMP-9, TIMP-1 and their ratio MMP-9/TIMP-1. At least, the ratio showed elevated levels in OSAS. To our knowledge this is the first time that MMP-9/TIMP-1 ratio has been examined in a local compartment in OSAS. On a systemic level, increased MMP-9 concentration and activity have already been described in OSAS and these alterations could be reduced by CPAP therapy [40]. This leads us to believe that the increased ratio of MMP-9/TIMP-1 is an indicator of upper airway remodelling following chronic intermittent hypoxia and sheer forces.

Quite naturally, this study has a number of limitations. The groups were not matched for age and body mass index (BMI), both of which are known as potential confounders in EB analysis. To investigate if age and BMI were confounders for the altered VOC mixture we performed a logistic regression analysis with AHI as the dependent variable. The electronic nose derived LDA was the best and solely significant predictor of AHI (odds ratio OR 3.03, 95%CI 1.41 - 6.51) compared to BMI (OR 1.17, 95%CI 0.99 - 1.37) and age (OR 1.05, 95%CI 0.98 - 1.12). This was also reflected in a significant correlation between AHI and LDA (Spearman’s r = 0.58, p < 0.001). Moreover, the significant change of the VOC profile before vs. after CPAP treatment suggests that the differences between groups in the VOC profile were
mainly due to the presence of OSAS. This represents a major advantage compared to previous publications where the VOC profile was assessed only once and significant correlations to other biological markers were not demonstrated.

Because of the high prevalence of co-morbid conditions in OSAS patients it could be argued, that the altered VOC mixture was mainly due to comorbidities and/or associated medication. However, looking at the LDA graph (figure 1a) and marking patients with coronary heart disease (n=3), diabetes (n=5), arterial hypertension (n=27) there was no trend in the distribution of patients with comorbidities (supplemental data).

The data on inflammatory markers in pharyngeal washing are limited by missing standardized procedures to assess the absolute values of specific markers. Herr et al. described defensin measurements in smokers [41], pharyngeal washings have been used for the detection of potential respiratory pathogens [42]. Irrespective of this, the data for AAT strengthen the assumption that AAT is upregulated in untreated OSAS and pharyngeal washings are helpful to investigate this further.

Additionally, a sham CPAP and follow-up data would have strengthened the study and would have added data about the repeatability of the breath print over time. However, the focus of this proof-of-concept study was on the diagnostic approach with the goal of demonstrating the general possibility of the diagnostic potential of exhaled breath analysis regarding OSAS. We used the limited before/after comparison of exhaled breath to show that standard therapy changes the breath print and to underline the assumption that the observed EB profile differences of OSAS vs. HC were mainly due to the presence/absence of obstructive episodes.
Most importantly, the results have to be validated in a separate cohort, possibly in an independent centre, in line with the STARD statement for the validation of diagnostic tests [43].

We conclude that the electronic nose Cyranose 320™ is capable of distinguishing the exhaled breath of OSAS patients and control subjects with high accuracy.
Acknowledgement and Support

Silke Mronga und Angelique Holland replaced the first and senior authors in their clinical duties in 2010 and 2011. Otherwise the work would not have been possible. The study was supported by the German Research Foundation (DFG), SFB/TR22 A19 and by a grant of the Behring-Roentgen Foundation (56-0036).


(38) Carpagnano GE, Kharitonov SA, Resta O, Foschino-Barbaro MP, Gramiccioni E, Barnes PJ. 8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy. Chest 2003 October;124(4):1386-92.


Figure legends

Figure 1:

(A): The values of the linear discriminant analysis (LDA) of sleep apnoea patients and healthy controls differ statistically significantly. OSAS = obstructive sleep apnoea syndrome, HC = healthy control, **** indicates p < 0.0001 (Mann–Whitney U test).

(B): The area under the receiver operator characteristics (ROC) curve equals 0.85 resulting in a sensitivity of 0.93 and a specificity of 0.70.

Figure 2:

The EBC values of pH (panel A) and conductivity (panel B) do not differ statistically significantly (p = 0.27 and p = 0.17, respectively; student’s t-test). EBC = Exhaled breath condensate, OSAS = obstructive sleep apnoea syndrome. HC = Healthy controls.
Figure 3:

AAT concentrations (panel A) and MMP-9/TIMP-1 ratio (panel B) in pharyngeal washings were significantly higher in OSAS patients compared to healthy controls ($p = 0.02$, Mann–Whitney $U$-test and $p = 0.007$; student’s $t$-test, respectively).
**Figure 4:**

(A) The (paired) values of the linear discriminant analysis (LDA) of sleep apnoea patients before (pre-init) and three months after (post-init) initiation of CPAP therapy differ statistically significantly (** indicates $p < 0.001$, Wilcoxon signed-rank test).

(B): The area under the receiver operator characteristics (ROC) curve equals 0.82 resulting in a sensitivity of 0.80 and a specificity of 0.65.

![Figure 4](image)

**Figure 5:**

(A): The pH values of the EBC of both visits do not differ statistically significantly ($p = 0.63$; Wilcoxon signed-rank test).

(B): The conductivity values of the EBC of both visits differ statistically significantly (*) indicates $p < 0.05$, Wilcoxon signed-rank test).
Figure 6:

(A): The AAT concentration in pharyngeal washing fluids decreased significantly after three months of CPAP treatment ($p = 0.017$; student’s $t$-test for paired samples).

(B): MMP-9/TIMP-1 ratio did not change significantly in OSAS patients before and after treatment ($p = 0.37$, Wilcoxon signed-rank test).

Supplemental Figure 1:

To demonstrate that the comorbidities diabetes (A), arterial hypertension (B), and coronary artery disease (C) did not cause a confounding bias in the interpretation of the LDA, we marked these patients separately.