Immune checkpoint inhibitors in mesothelioma

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Chapter 7

eNose in malignant mesothelioma – prediction of response to immune checkpoint inhibitor treatment

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Eur J Cancer. 2021 Jul;152:60-67

Abstract

Introduction

Recent clinical trials with immune checkpoint inhibitors (ICI) have shown that a subgroup of patients with malignant pleural mesothelioma (MPM) could benefit from these agents. However, there are no accurate biomarkers to predict who will respond. The aim of this study was to assess the accuracy of exhaled breath analysis using electronic technology (eNose) for discriminating between responders to ICI and non-responders.

Methods

This proof of concept prospective observational study was part of an intervention study (INITIATE) in patients with recurrent MPM who were treated with nivolumab (anti-PD-1) plus ipilimumab (anti-CTLA-4). At baseline and after six weeks of treatment breath profiles were collected by an eNose. Modified Response Evaluation Criteria in Solid Tumors (RECIST) were used to assess efficacy at six months follow up. For data processing and statistics, we used independent t-test analyses followed by linear discriminant and receiver operating characteristic (ROC) analysis.

Results

Exhaled breath data of 31 MPM patients who received nivolumab plus ipilimumab were available at baseline. There were 16 with and 15 without a response after six months of treatment. At baseline breath profiles significantly differed between responders and non-responders, with a cross validation value of 71%. The ROC-AUC after internal cross-validation was 0.90 (CI: 0.80-1.00)

Conclusion

An eNose is able to discriminate at baseline between responders and non-responders to nivolumab plus ipilimumab in MPM, thereby potentially identifying a subgroup of patients that will benefit from ICI treatment.

Introduction

Malignant pleural mesothelioma (MPM) is a rare disease, mainly caused by exposure to asbestos, with a latency time of 30 to 50 years (1). Since 2004, the first-line treatment consists of a platinum compound plus pemetrexed with a median overall survival (OS) of 12-16 months. The addition of bevacizumab is reported to increase the OS to 18 months in a selected group of patients (2,3).

Immune checkpoint inhibitors (ICI), both as single agent and combination therapy, have shown promising anticancer activity against mesothelioma in single arm phase II clinical trials. For single agent anti-programmed cell death 1 antibody (anti-PD-1) ICI treatment, the overall response (ORR) is about 20% and progression free survival (PFS) between 2.5 and 6 months (4-7). For combination treatment with anti-PD-1 plus anti-CTLA-4, the ORR is around 27% and mPFS 6 months (7-9). The phase III PROMISE-meso trial, comparing the efficacy of pembrolizumab, an anti-PD-1 antibody, versus chemotherapy in recurrent mesothelioma shows that the ORR, is nearly four times higher with pembrolizumab (22% vs 6%). Unfortunately, median PFS, OS and duration of response (DOR) are similar for both treatment arms. However, long-term responders to pembrolizumab are observed.(10) Results from the recently presented phase III Checkmate 743 study show a significant OS benefit for first-line nivolumab (anti-PD-1 ICI) plus ipilimumab (anti-CTLA-4 ICI) compared to platinum plus pemetrexed chemotherapy (18.1 versus 14.1 months, HR 0.74 (95% CI 0.61–0.89; P=0.002). (11) These results are expected to change practice guidelines for mesothelioma.

As in other cancers, not all mesothelioma patients will benefit from ICI treatment.(12) Upfront identification of the subgroup that will benefit (or will not) could ultimately lead to improved outcomes. Unfortunately, relevant biomarkers have not been identified yet (13,14).

Over the last decades, exhaled breath analysis has shown potential as a non-invasive and easy-to-use technology for diagnosis and phenotyping of a wide range of diseases including mesothelioma and lung cancer (15-19). Exhaled breath consists of up to thousands of volatile organic compounds (VOCs) that are produced by both physiological and pathophysiological processes in the body and respiratory tract (20). Among the different available techniques, electronic nose (eNose) technology can be applied for pattern recognition of the complete mixture of VOCs using multiple cross-reactive sensors. Combined sensor signals produce a characteristic "breath profile" that is unique for each person (21,22). Recently de Vries *et al.* have shown that eNose technology allows for upfront discrimination between responders and non-responders to pembrolizumab or nivolumab in patients with advanced non-small cell lung cancer (NSCLC) with an accuracy as high as 90% (23). The results were confirmed in a separate validation set of patients, suggesting that this technology can be used upfront to predict the efficacy or failure of ICI therapy in these patients (23).

Therefore, in the current study, we aimed to assess as proof of concept whether the eNose was able to discriminate at baseline between mesothelioma patients with and without clinical response to anti-PD-1 plus anti-CTLA-4 therapy. Next, we explored the changes in breath profiles of responders and non-responders from baseline after 6 weeks of treatment with ICI.

Methods

Study design and population

This is a prospective observational study linked to a prospective single-center, single arm, phase II trial (the INITIATE trial) in patients with recurrent MPM who were eligible for treatment with nivolumab (anti-PD-1) plus ipilimumab (anti-CTLA-4). Details of the INITIATE trial have been published elsewhere.(8) In short, patients were treated with nivolumab 240mg every two weeks plus ipilimumab 1mg/kg every 6 weeks for a maximum of 4 times. In the INITIATE trial pulmonary function tests were performed at baseline and after six weeks of treatment. All patients provided written informed consent before enrolment in the INITIATE trial. Exclusion criteria for participating in the present study were the recent (<12hours) intake of alcohol (which affects eNose signals) or if patients were not willing or able to participate. In order to increase the applicability in clinical practice, there were no further restrictions. Patients completed a short survey about factors relevant for exhaled breath analysis, such as smoking history and food intake in the last two hours.

Definition of Treatment Response

Response to therapy was monitored by computed tomography (CT) scans performed every 6 weeks, using the modified Response Evaluation Criteria in Solid Tumors (mRECIST) for mesothelioma (24,25). The outcome of complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) was recorded. Since our aim was to differentiate between responders and non-responders, we grouped patients with CR, PR and SD for more than 6 months as responders and patients with PD at 6 months as non-responders.

Measurements

Exhaled breath analysis was performed at baseline and after six weeks of treatment using a cloud-connected eNose, the so-called SpiroNose (23). This SpiroNose is an integration between eNose technology and routine spirometry and has been technically and clinically validated (23,26). It has 7 different cross-reactive metal-oxide semiconductor sensors. These sensors are present in duplicate on both the inside (to measure VOCs in exhaled breath) and on the outside of the SpiroNose (to measure VOCs in ambient air). During the measurement, patients were instructed to perform five tidal breaths followed by a single inspiratory capacity maneuver up to total lung capacity, a five second breath-hold and slow

(<0.4 L/s) maximal expiration towards residual volume. The exhaled breath measurement was performed in duplicate for each patient. The sensor signals were uploaded in real-time to the online analysis platform, BreathBase, for signal processing and analysis. From each sensor two variables were determined, 1) the highest sensor peak, normalized to the most stable sensor (sensor 2), to minimize inter-array differences; and 2) the ratio between the sensor peak and the breath hold (BH) point. A detailed description of the SpiroNose and the processing of data is available in the supplementary material.

Signal processing

The processing of the SpiroNose sensor signals included filtering, detrending, ambient correction and peak detection as was previously published (21,22). The signal processing resulted in a .csv file containing the selected parameters (sensor peak- and peak/BH ratios) serving as the source document for statistical analysis.

Statistical analysis

SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) and MatLab (2019B, MathWorks, Natick, MA) were used for data analysis. Descriptive statistics were expressed as mean ±SD if data were normally distributed and as median (interquartile range) for non-normally distributed data. Between-group comparisons were carried out using Mann–Whitney U tests, two-sample unpaired t-tests or chi-squared tests.

Exhaled Breath Analysis

The normalized sensor peaks and peak/BH ratios were compared between groups using independent sample t-tests. The variables that discriminated ($P<0.05$) between responders and non-responders were selected for further analysis. Independent t-tests were internally validated by 1000 iterations of bootstrap. Subsequently, linear discriminant analysis was carried out using the selected variables. A discriminant function was calculated that best distinguished between the two groups. The accuracy of this model was defined as the percentage correctly classified patients. Cross-validation using the leave-one-out method was used to calculate the cross-validated accuracy value (CVV, %). The discriminant scores were used to construct receiver operating characteristics (ROC) curves. Finally, mean baseline and follow-up sensor values were compared using independent sample t-tests.

Results

Response to ICI treatment

In the INITIATE trial, 35 patients with MPM were included (8). ENose data were available for 31 (89%) patients and they were included in this observational study. From the other

4 patients we only have measurements after start of treatment (1; 3%), was no response evaluation available (1; 3%) or were not scheduled for measurements at all (2; 5%). Baseline characteristics of these patients are shown in table 1. As in most mesothelioma trials, the mean age was 65 years, most patients were male (74%) and the majority had epithelioid subtype (88%). After 6 months of treatment, 16 patients (52%) had a response (PR 39% plus SD 13%) and 15 patients (48%) were non-responders.

There were no significant differences between responders and non-responders regarding their demographic data and baseline characteristics.

Table 1: Baseline characteristics and radiological response data

	All	Responder	Non-responder	P value
N (%)	31	16 (52)	15 (48)	
Age, years (range)	65 (37-79)	67	63	0.25
Gender, n (%)				0.35
Male	23 (74)	13 (81)	10 (67)	
Female	8 (26)	3 (19)	5 (33)	
Ethnic background, n (%)				0.32
White	30 (97)	15 (94)	15 (100)	
Black	1 (3)	1 (6)		
WHO PS, n (%)				0.81
0	10 (32)	6 (37)	5 (33)	
1	21 (68)	10 (63)	10 (67)	
Smoking, n(%)				0.096
current smoker	5 (16)	1 (6)	4 (27)	
ex-smoker	14 (45)	10 (63)	4 (27)	
never smoker	12 (39)	5 (31)	7 (46)	
BMI ($kg.m^{-2}$) *	25.5	25.2	25.8	0.69
FEV1 (L) *	2.20	2.20	2.20	0.96
FEV1 (% predicted) *	71	74	67	0.34
Histologic subtype, n(%)				0.37
Epithelioid	27 (88)	13 (81)	14 (93)	
Sarcomatoid	2 (6)	2 (13)	0	
Mixed	2 (6)	1 (6)	1 (7)	
Line of treatment, n(%)				0.68
2	26 (84)	13 (81)	13 (87)	
>2	5 (16)	3 (19)	2 (13)	
Radiological response, n (%)				0.000
Complete response	0	0	0	
Partial response	12 (39)	12 (75)	0	
Stable disease	4 (13)	4 (25)	0	
Progressive disease	15 (48)	0	15 (100)	

WHO, world health organization; PS, performance status; BMI, body mass index; FEV1, forced expiratory volume in one second.
*data of one non-responder missing.

Exhaled breath analysis at baseline

Results of the independent t-test analysis showed that at baseline, sensor 3 (p=0.034), sensor 5 (p=0.04) and sensor 6_BH (p=0.017) were significantly different between patients with (n=16) and without response (n=15). Sensor 3 and 5 indicate the normalized sensor peak and sensor 6_BH the ratio between the highest sensor peak and the breath hold point. Linear discriminant analysis showed a cross-validated value of 71%. The ROC-Area Under the Curve (AUC) after internal cross-validation was 0.90 (95%CI: 0.80-1.00) (Figure 1).

A: **B:**

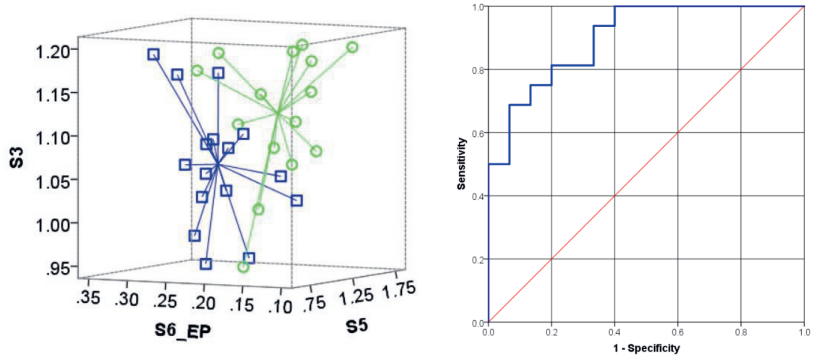

Figure 1. A: Three-dimensional scatter plot showing discrimination of exhaled breath profiles between responders (blue) and non-responders (green) along discriminative variables. The x and y axes represent normalized sensor values. B. ROC-curve showing sensitivity and specificity for the identification of non-responders (ROC-AUC: 0.90 (CI: 0.80-1.00))

Exhaled breath analysis after six weeks of treatment

In 25 patients (81%) of this cohort, follow-up exhaled breath measurements were performed after 6 weeks of treatment. In patients with a partial response (n=11), normalized sensor peak of sensor 3 and sensor 5 at follow-up were significantly (p<0.01) different from baseline measurements (Figure 2). In patients with progressive disease (n=10), a significant difference (p<0.01) between follow-up and baseline parameters was seen from sensor 3 and sensor 5 (Figure 3). In patients with a partial response, an increase in normalized sensor peak values was noted while in patients with progressive disease, a decrease in parameters was found (Figure 2 & 3). In patients with long-term stable disease, no significant changes in sensor values were seen during treatment. However, follow up measurements were only available for 4 patients (data not shown).

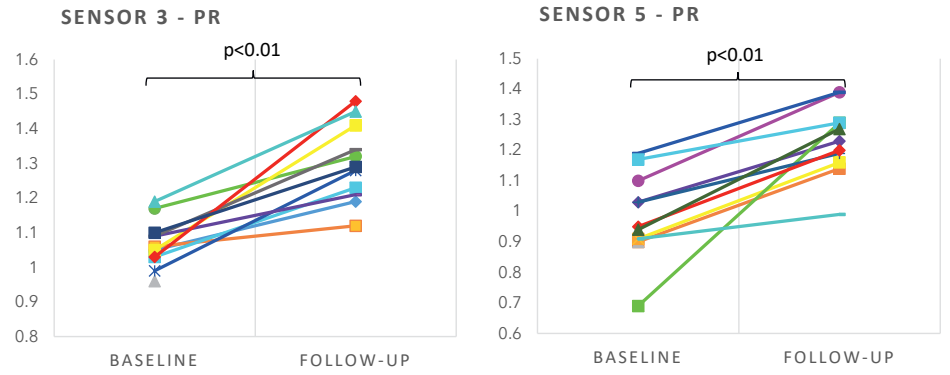

Figure 2. Change between baseline and follow up at week six in two significantly different sensors (sensor 3 and sensor 5) in patients with a partial response (PR). Y-axis in both figures correspond to the highest sensor peak normalized to the most stable sensor (sensor 2).

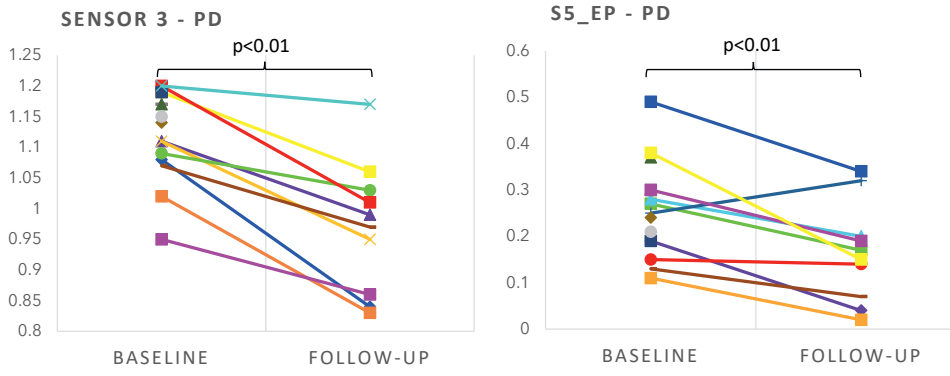

Figure 3. Change between baseline and follow up at week six in two significant different sensors (sensor 3 and sensor 5_BH) in non-responding patients. Left, Y-axis from sensor 3 corresponds to the highest sensor peak normalized to the most stable sensor (sensor 2). Right, Y-axis from sensor 5_BH corresponds to ratio of breath hold point and highest sensor peak.

Discussion

In this study, we showed that exhaled breath analysis by eNose at baseline allows for discrimination between mesothelioma patients with and without clinical response to nivolumab plus ipilimumab. The eNose could become a tool for prediction of response.

We also assessed within-patient changes in breath profiles during 6 weeks of treatment with ICI. We observed a significant change in sensor values from baseline both in those with partial response and progressive disease, though in opposite directions. Although some questions have to be addressed concerning the effect of changes in tumor size, and thereby tumor metabolites and/or changes in inflammatory response on VOCs,(27) this however suggests that the eNose may also be suitable as a monitoring tool to assess prognosis or effect of therapy of MPM. Especially since radiological response measurements are difficult and often inaccurate in MPM due to the unique nonradial pleural rind, eNose could discriminate between responders and non-responding patients.

Results from several clinical trials suggest that there is a subgroup of MPM patients that benefit from ICI therapy (7,8,10). Identifying those has been difficult, however, this is of utmost importance. Particularly, since in the near feature, many patients with MPM will be treated with nivolumab plus ipilimumab in first line (11). In NSCLC similar results for eNose analysis were reported in a cross-sectional study of 143 patients (training: 92, validation: 51) who received ICI therapy. De Vries *et al.* demonstrated that the eNose was able to prevent ineffective anti-PD-1 therapy in 24% of patients with NSCLC, without withholding anyone effective treatment. The study also showed that the eNose outperformed the currently used biomarker PD-L1 in NSCLC (90% vs 66% accuracy) (23). In the INITIATE study, PD-L1 expression at baseline on both tumor and immune cells correlated with response, but both proved insufficient for prediction of response (8).

To the best of our knowledge, this is the first trial to study the use of exhaled breath analysis by eNose to assess clinical responsiveness to anti-PD-1 plus anti-CTLA-4 therapy among patients with MPM. Most patients in the INITIATE trial were measured, and the patients included in this trial adequately represent the normal mesothelioma population since inclusion criteria and baseline characteristics are comparable to those in other MPM trials. Another strength is the eNose data are comparable to NSCLC. However, since nivolumab plus ipilimumab is not (yet) standard therapy for MPM, we could not include a higher number of patients, or validate our results in a separate cohort. Therefore, despite these encouraging results, the main limitation of the study is the lack of external validation. Since nivolumab plus ipilimumab will soon be standard of care in first line, these results can then be validated in an independent set of patients.

Both characteristics of the host and characteristics of the tumor microenvironment such as infiltration of lymphocytes, extracellular matrix, cytokine expression and tumor mutation burden, are known to have a significant influence on response to immunotherapy (28,29). Evidence shows high inter-patient and intra-tumor heterogeneity in the mesothelioma microenvironment, which can further complicate the prediction of response to anti-PD-1 therapy (30,31). Considering the complex and dynamic nature of the tumor

microenvironment, it is not surprising that a single marker such as PD-L1 is not able to provide sufficient information to predict response.

ENoses contain an array of cross-reactive sensors, each interacting with overlapping groups of VOCs without the identification of individual compounds (20). The technology applies pattern recognition algorithms and artificial intelligence for the discovery of multi-dimensional and composite biomarkers that are considered to be more informative than single markers (32). Thus, the high accuracies in these studies are not unexpected. Whether the associations between VOCs and treatment response are a direct effect of metabolite production by the tumor cells, or the immunological or inflammatory host responses remains to be determined (33,34). However, this does not influence the clinical utility of a breath test for the prediction of response to anti-PD-1 therapy.

Interestingly, in both MPM and NSCLC (23) normalized sensor peaks of sensors 3 and 5 were significantly different between responders and non-responders. This suggests that VOC compositions that differentiate between responders and non-responders may be similar in both diseases. This could reflect overlapping mechanisms within the tumor microenvironment or host that influence responsiveness to anti-PD-1 therapy (35). Sensor 3 has the highest sensitivity to hydrocarbons such as natural gas and methane. Di Gilio *et al.* have recently shown that 10 VOCs including hydrocarbons, ketones and alkanes can discriminate between MPM patients and healthy controls (36). Similarly, *in-vitro* studies also report hydrocarbons to differentiate between lung cancer and normal lung cell lines (33,37). Studies with analytical chemistry technologies such as Gas Chromatography-Mass Spectrometry (GC-MS) can provide more insight into individual VOCs involved in these processes (22). In order to unravel the underlying mechanisms, other high-throughput technologies like (epi)genomics, proteomics and transcriptomics may be more appropriate (38).

In conclusion, eNose technology has the potential to become a novel tool for predicting response to nivolumab plus ipilimumab among patients with MPM. In first line, many patients with mesothelioma will be treated with nivolumab plus ipilimumab in the near future (11), but this will not be effective for all patients. Therefore, eNose might be of importance to identify those patients who are at risk of failure or those who are candidates for continuation of treatment with ICI's when the CT scan is indiscriminative. Further validation of the results in a larger prospective multi-center study may lead to the use of eNose technology as a rapid and non-invasive tool at the point-of-care.

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Supplementary data

Exhaled breath analysis

SpiroNose measurement setup

The eNose measurement setup used in this study included a mouthpiece, nose clamp, viral/bacterial filter (Lemon Medical GmbH) attached to a Masterscreen™ pulmonary function testing system (Masterscreen, Jaeger, CareFusion) and the SpiroNose (Figure S1, left) (26,39). The SpiroNose consists of 8 separate sensor arrays, 4 reference sensor arrays to monitor environmental air and 4 sensor arrays used to monitor the VOCs in exhaled breath (Figure S1, right). The SpiroNose contains 7 different metal oxide semiconductor sensors (Table S1) and each sensor is present in duplicate in both the reference and breath-monitoring sensor arrays (in total 28 sensors). The sensor stability was verified, as previously described, using the standard test gas for pulmonary diffusion capacity measurements as quality control gas every morning before patient measurements (26,39).

Patients were not allowed to have used alcohol in the 12 hours before the breath test. All patients rinsed their mouth thoroughly 3 times with water. Patients were instructed to perform five tidal breaths followed by a single inspiratory capacity manoeuvre up to total lung capacity, a five second breath hold and slow (<0.4 L/S) maximal expiration towards residual volume (26,39). Exhaled breath was real-time measured (<1 minute) by the SpiroNose, which is connected to an Ethernet cable for immediate secured data transmission to an online server for further automated analysis. From each sensor two variables are determined, first the highest sensor peak, normalized to the most stable sensor (sensor 2), to minimize inter-array differences; and second the ratio between the sensor peak and the breath hold (BH) point (Figure S2).

The normalized sensor peaks and ratios are compared between groups by independent sample t-tests. The variables that discriminated ($p<0.05$) between responders and non-responders to ICI treatment were selected for further analysis. The t-tests were internally validated by 1000 iterations of bootstrap. Linear discriminant analysis was performed using the selected variables. A discriminant function was calculated that distinguished between patients with and without clinical benefit. This was used to construct receiver operating characteristics (ROC) curves.

Table S1 Sensors of the SpiroNose. ppm: parts per million, VOCs: volatile organic compounds

	Type	Highest sensitivity for:	Range (ppm)
Sensor 1	TGS 2602	VOCs (e.g. toluene) and odorous gases (e.g. ammonia and hydrogen sulphide)	1 - 30
Sensor 2	TGS 2610	butane and propane	500 - 10.000
Sensor 3	TGS 2611-COO	methane and natural gas	500 - 10.000
Sensor 4	TGS 2600	air contaminants (e.g. hydrogen, carbon monoxide and ethanol)	1 - 30
Sensor 5	TGS 2603	air contaminants (e.g. trimethylamine, methyl mercaptan)	1 - 30
Sensor 6	TGS 2620	alcohol and solvent vapors	50 - 5.000
Sensor 7	TGS 2612	methane, propane and iso-butane	500 - 10.000

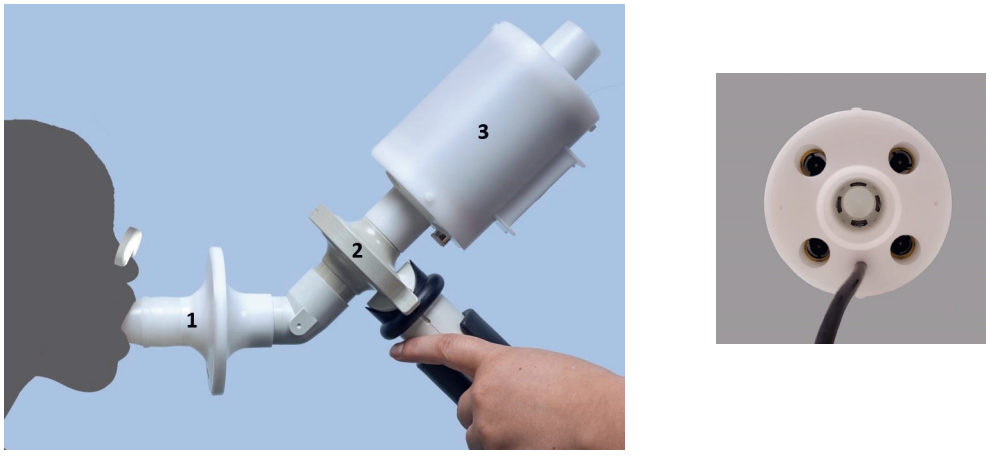

Figure S1. Left: SpiroNose measurement setup: (1) Mouthpiece, nose clamp and bacteria filter, (2) Spirometer, (3) SpiroNose. Right: Front view of the SpiroNose and the positioning of the sensor arrays. Yellow arrow: four sensor arrays monitoring exhaled breath. Red arrow: four reference sensor arrays monitoring ambient VOCs.

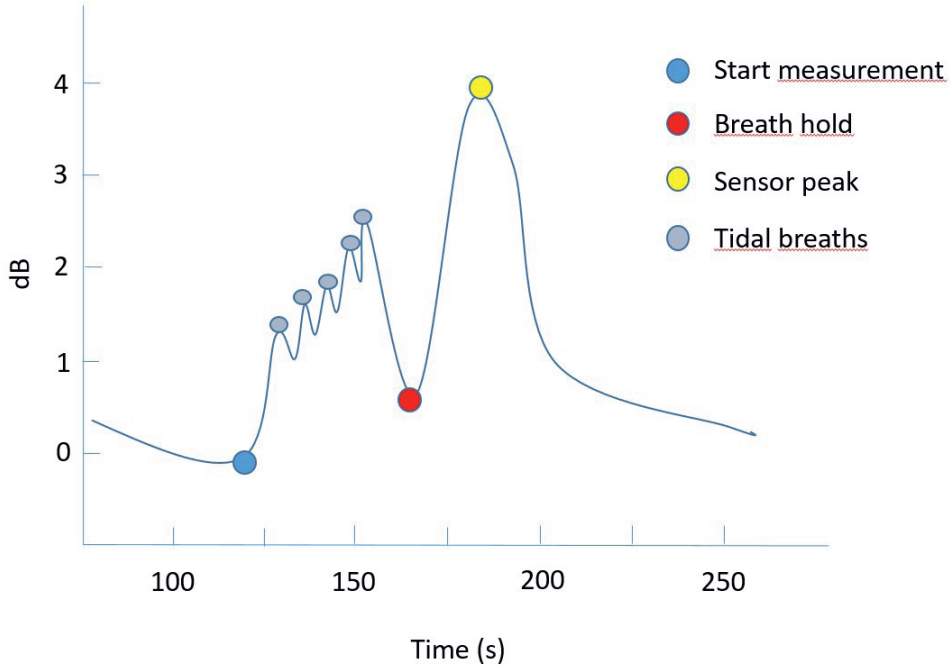

Figure S2. Data analysis SpiroNose.