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Mobile instrumentation platform to distinguish airway disorders

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Abstract

Asthma and chronic obstructive pulmonary disease (COPD) are distinct but clinically overlapping airway disorders which often create diagnostic and therapeutic dilemmas. Current strategies to discriminate these diseases are limited by insensitivity and poor performance due to biologic variability. We tested the hypothesis that a gas chromatograph / differential mobility spectrometer (GC/DMS) sensor could distinguish between clinically well-defined groups with airway disorders based on the volatile organic compounds (VOCs) obtained from exhaled breath. After comparing VOC profiles obtained from 13 asthma, 5 COPD, and 13 healthy control subjects, we found that VOC profiles distinguished asthma from healthy controls and also a subgroup of asthmatics taking the drug omalizumab from healthy controls. The VOC profiles could not distinguish between COPD and any of the other groups. Our results show a potential application of the GC/DMS for non-invasive and bedside diagnostics of asthma and asthma therapy monitoring. Future studies will focus on larger sample sizes and patient cohorts.

Keywords

high-field asymmetric waveform ion mobility spectrometry; differential mobility spectroscopy; asthma; chronic obstructive pulmonary disease COPD; exhaled breath condensate; breath analysis; biomarkers; non-invasive diagnostics; volatile organic compounds (VOCs)

1. Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are common and important respiratory disorders that lead to substantial morbidity and lung dysfunction. While the overt manifestation of these disorders is breathlessness, the underlying physiologic mechanisms and treatment paradigms are different. For example, asthma is often characterized by increased levels of allergen-mediated immunoglobulin E (IgE) production, which leads to mast cell release of bronchoconstrictive and pro-inflammatory mediators. For a subset of difficult-to-control asthmatics, treatment with an anti-IgE monoclonal antibody, omalizumab, may improve symptoms and reduce asthma flares over time (Rodrigo et al., 2011). In contrast, COPD is characterized by progressive breathlessness with exertion often due to dynamic hyperinflation and fixed airflow obstruction. Therefore, treatment with long-acting bronchodilators is a front-line therapy in COPD (Karner and Cates, 2012, Decramer et al., 2009, Qaseem et al., 2011). However, the reality is that approximately 50% of patient

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who present to primary care physicians and specialists have clinical features suggestive of both asthma and COPD (Zeki et al., 2011).

Current strategies to differentiate asthma from COPD include measurements of lung function, a prolonged smoking history in COPD, the presence of daily sputum production in COPD, and exhaled biomarkers including nitric oxide (eNO), which is classically elevated in asthma and normal in COPD (Barnes et al., 2010, Dweik et al., 2011). However, eNO may not correctly classify patients as evidenced by normal levels of eNO in nonallergic asthma (Dweik et al., 2010) and variable eNO levels in COPD (Gelb et al., 2012).

Similarities in clinical presentation, limited discriminatory power of conventional diagnostic tools, and a limited armamentarium of validated biomarkers create both diagnostic and therapeutic dilemmas. This confusion can result in inappropriate therapies such as monotherapy with long-acting bronchodilators in asthma leading to an excess of asthma-related deaths (Nelson et al., 2006) or monotherapy with inhaled steroids in COPD leading to an excess of pneumonias (Yang et al., 2012).

Complimentary diagnostic modalities to properly characterize asthma and COPD include the assessment of volatile organic compounds (VOCs) from exhaled breath. Several clinical studies have assessed VOCs in asthma and COPD, however, with certain limitations. Some clinical studies analyzing VOCs have used chemical sensors designed to detect but not identify VOCs (Fens et al., 2011, Fens et al., 2009, Montuschi et al., 2010, Dragonieri et al., 2007). The results led to acceptable disease classification, but no further analysis including the specific VOC identification and eventual biomarker discovery was possible. Alternative studies used gas chromatography / mass spectrometry (GC/MS) which is considered the gold standard for VOC identification but does not provide an instrumental platform for bedside or office use (Caldeira et al., 2012, Ibrahim et al., 2011, Van Berkel et al., 2010).

Additional studies used portable diagnostic platforms including ion mobility spectrometry methods with promising results. Westhoff et al. were able to differentiate 97 cases of COPD from 35 healthy control subjects using ion mobility spectrometry (IMS) coupled with a multi-capillary column (MCC) analysis of 10 mL exhaled breath (Westhoff et al., 2010, Westhoff et al., 2011). In this study the investigators identified a single compound, cyclohexanone, which classified COPD from healthy controls with 91% specificity and 95% predictive value. A similar study using IMS looked at 13 COPD patients and 33 healthy controls (Bessa et al., 2011). The investigators found 1 out of 98 peaks which significantly separated the clinical groups. Though they did not overtly identify this peak, the authors suggest the peak identification was benzofuran, phenol, 4-methylanisol or 1,2,4-trimethylbenzol. A third study looked at COPD patients with and without alpha-1-antitrypsin (A1AT), a protective enzyme found in the blood and lungs (Koczulla et al., 2011). This study identified 5 of 22 peaks which separated 17 A1AT-deficient COPD subjects from 8 non-A1AT-deficient COPD subjects.

Basanta et al. used differential mobility spectrometry (DMS) to analyze exhaled breath from 20 COPD subjects and 6 healthy controls who smoke cigarettes (Basanta et al., 2010). Using a validated exhaled breath collection method, the authors were able to separate the clinical groups based on partial least squares-discriminant analysis of the datasets with good reproducibility, a 19% Type I error rate (false-positive), and 12.4% Type II error rate (false-negative). One characteristic not addressed in this study was that all 6 healthy controls were active smokers while only 2 of the 20 COPD subjects were active smokers. This fact could have introduced confounders.

Given the difficulties in accurate diagnosis and the potential for harmful therapy, there remains an urgent need for improved diagnostic tools to classify asthma and COPD. Despite

a number of illuminating studies, there is not a comprehensive understanding of the correlation of VOCs between asthma and COPD. Therefore, it is important to pursue further research efforts in these very common lung diseases with the ultimate goal of developing a protocol using a suitable analytical method that can be used for bed side diagnostics

As in the Basanta study, our lab has experience with DMS which is a highly miniaturized (i.e. micromachined), portable sensor for VOC detection. DMS (also known as high field asymmetric waveform ion mobility spectrometry [FAIMS]) (Shvartsburg et al., 2006, Guevremont, 2004, Eiceman et al., 2002, Borsdorf and Eiceman, 2006) is commonly applied for a wide variety of analytic purposes, including but not limited to: explosives detection (Perr et al., 2005, Guerra-Diaz et al., 2010, Buxton and Harrington Pde, 2003), drug detection (Gryniewicz et al., 2009, Dunn et al., 2011, Jafari et al., 2009), bacteria detection (Shnayderman et al., 2005), and VOC detection in human samples (Molina et al., 2008, Krebs et al., 2006).

DMS may be used as an ion-filtering device in combination with mass spectrometry (MS) or liquid chromatography / mass spectrometry (LC/MS), or it can function as a stand-alone sensor. The latter renders DMS a suitable technology for VOC detection in breath diagnostics as it is small and portable, highly sensitive, and consumes little power. The analytical signal in DMS is generated from the differences in ion mobility at low and high electric field conditions. Each chemical species is characterized by a unique dependence of its mobility within an electric field; therefore, the differences in ion mobility may be used to identify and differentiate VOCs. An additional direct current (DC) voltage, called a “compensation voltage” (CV), is applied to compensate for ion drift at differential field conditions and to enable a particular chemical species to propagate through the device. The value of the CV is related to ion’s structure and, therefore, is specific for each ion species. In combination with gas chromatography (GC), DMS is highly suitable to profile complex VOC distributions.

In the present study, we performed proof-of-concept experiments to explore the feasibility of the gas chromatography / differential mobility spectrometry (GC/DMS)-based chemical sensing to discriminate between VOC profiles from subjects with asthma, COPD, and healthy controls using exhaled breath condensate. The DMS device provides orthogonal information to drift-time (DT) IMS methods in that in DMS the ion mobility-related parameter (CV) only weakly correlates with ion size (Aksenov et al., 2012), unlike in DT IMS where ion drift time is essentially linear with the ion cross-section (Borsdorf and Eiceman, 2006, Eiceman and Karpas, 2005). By this, DMS could differentiate two ions which are structurally different but have the same or similar mass and charge such as isomers and isobars. Though DT IMS is an excellent portable diagnostic instrument, we chose to use DMS because of our experience and for future studies where precise ion species identification is paramount. In addition to the differential mobility parameter, compensation voltage (CV), an additional dimension of separation can be achieved through a scan of radiofrequency waveform amplitude (RF) (Basanta et al., 2007). This may enable further expansion of information achievable in DMS measurements. The ultimate goal of this work is to identify VOC combinations which will improve diagnostic accuracy and differentiation of asthma and COPD, in addition to existing diagnostic tools. It is further anticipated that advanced VOC analysis may lead to specific identification of exhaled biomarkers of disease and inform future mechanistic studies.

2. Materials and Methods

2.1 Subject Selection

We performed a cross-sectional observational study in subjects with asthma and COPD, and in healthy control subjects. All subjects provided informed consent prior to enrollment. The UC Davis institutional review board approved all study activities including subject recruitment, methods, and informed consent documents. We used standard American Thoracic Society and Global Initiative for Chronic Obstructive Lung Diseases (GOLD) definitions to define asthma and COPD (1987, Rabe et al., 2007, Miller et al., 2005). The inclusion criteria for asthma were a subject having a clinical diagnosis of asthma by a lung specialist, compatible symptoms by history (e.g. episodic chest tightness, wheezing, and shortness of breath), a <10 pack-year smoking history, and partially or completely reversible airflow obstruction on spirometry or a positive response to a bronchoprovocation challenge (a reduction in the forced expiratory volume at 1 second [FEV₁] by >20% after inhaling <8 mg/dL of methocholine). COPD subjects were defined as having a clinical diagnosis of COPD by a lung specialist, compatible symptoms by history (chronic shortness of breath with or without intermittent exacerbations), a smoking history of >10 pack-years, and predominantly fixed airflow obstruction on spirometry with an FEV₁ to forced vital capacity ratio (FEV₁/FVC) of <0.7. Healthy control subjects could not smoke, could not have any pulmonary disease, and required normal spirometry.

Medication use was not restricted, but subjects needed to be free of an asthma or COPD exacerbation for the preceding 28 days. All subjects were adults >18 years. Exclusion criteria were pregnancy, active asthma or COPD exacerbations, and inability to provide consent. Additionally, all subjects were asked to refrain from eating or drinking at least 4 hours prior to the study to avoid confounding factors.

2.2 Study Measurements

Anthropomorphic measurements were obtained as well as a brief history and physical exam. Subjects provided spirometry (if not done in the preceding 6 months) via an SDI Spirolab II spirometer (Easton, MA) and two exhaled nitric oxide (eNO) samples. The eNO samples were obtained by blowing into mylar bags per ATS standards (American Thoracic and European Respiratory, 2005). These were then analyzed on a Sievers 280i NO Analyzer (Boulder, CO).

2.3 Exhaled Breath Condensate Collection

Subjects rinsed their mouths with tap water and placed on noseclips to minimize oral and nasopharyngeal contamination. Exhaled breath condensate (EBC) was provided by breathing into a commercially available RTube™ EBC collector (Respiratory Research, Charlottesville, VA). We chose this modality at the time of study because of the RTube's™ portability and ease of use. A sleeve cooled to between −40°C to −50 °C by dry ice was placed over the RTube™ prior to collection. Subjects breathed between 10–15 minutes of tidal volume into the RTube™. The EBC was collected by sterile pipette and placed into an Eppendorf tube for storage at −80 °C.

2.4 GC/DMS Analysis

A 500 microliter to 1 mL aliquot of EBC was placed into a borosilicate vial capped with a silicon septum. The vial was placed in a water bath, heated to 90 °C, and the headspace over the EBC was sampled using a polyacrylate (PA) solid phase microextraction fiber (SPME, Supelco, Inc., Bellefonte, PA) to promote extraction of polar compounds from the sample head space. For extraction, the SPME fibre was inserted through septum and kept above the sample for 1 hour without EBC sample agitation. This was performed to optimize VOC

collection on the SPME fibre according to previously published work in this area (Snow, 2000a, Davis et al., 2010). The sorbed chemicals were then analysed using a GC/DMS method described below.

The operational principles and design specifications the DMS sensor are described elsewhere (Kolakowski and Mester 2007) (Anderson, Markoski et al. 2008). A schematic representation of the system is shown in Fig. 1. For this study we utilized the microDMX sensor, manufactured by Sionex (Waltham, MA). The detailed design specifications and performance descriptions are given elsewhere (Petinarides et al., 2005, Coy et al., 2008, Miller et al., 2006) Specifically, the DMS device is comprised of two parallel electrodes with 0.5 mm gap. The GC column eluent in helium at 1 mL / min flow rate was mixed with ultra high purity nitrogen carrier gas (Airgas, Sacramento, CA) and passed at the flow rate of 300 mL / min through the DMS device. The analyte was ionized with ^{63}Ni ionization source prior to entering the DMS device. The water content in the carrier gas was below 1 ppm, as reported by the manufacturer. The DMS unit was operated under the following conditions: asymmetric waveform amplitude 1000 V (in the gap of 0.5 mm, this corresponds to electric field of 20V/cm, ~80 Td); RF frequency 1.196 MHz with the asymmetric waveform about 34% high field and 66% low field; and CV scan range -43 to +15 V. The CV range of 60 V was scanned with 0.6 V step at 10 millisecond per step, corresponding to 100 steps and 1 second total CV scan time. The wave function generator employed in this study can produce waveform with maximum amplitude of 1500 V. However, due to the presence of helium in the carrier gas from the GC column eluent, we chose to operate at the lower dispersion voltage of 1000 V to avoid possible electrical breakdown..

2.5 Statistical Analysis

All demographic patient characteristics were compared between clinical groups using the Student's *t*-test or Wilcoxon Rank Sum test where appropriate in STATISTICA (StatSoft, Tulsa, OK). DMS data analysis performed using MatLab R2011a (Mathworks, Torrence, CA) and PLS Toolbox 6.7.1 (Eigenvector Research Inc, Wenatchee, WA).

3. Results

3.1 Subjects

A total 31 subject samples were screened. The clinical groups differed significantly in their baseline characteristics including age, body mass index, pre-bronchodilator spirometry, and eNO levels (see Table 1). These differences were expected as patients with asthma tend to be younger, have better lung function, and a higher BMI than patients with COPD. There was an unanticipated similarity between eNO levels in the asthma and COPD groups, however, our subjects reflect patients often encountered in practice rather than an artificially-selected cohort.

3.2 Algorithm for sample selection for analysis

Fig 2 delineates the algorithm for EBC VOC data sample selection used during multivariate data analysis.

To select the DMS datasets which display high-quality information, the data was subject to several evaluation techniques. Initially, all datasets were preprocessed with baseline correction and smoothing (Savitzky and Golay, 1964) to facilitate data comparison. Principle component analysis (PCA) was used to reduce dimensionality (Wold et al., 1987). This enables composite data analysis, outlier detection, and selection of data fitting within an acceptable range for further analysis. Following the selection of datasets from PCA, we used multiway partial least square (N-PLS) regression to find the relation between the *X*

(cube of data) and Y (labels) data spaces in order to model the covariance structures (Bro, 1996).

An N-PLS model attempts to determine the multidimensional direction in the X space that explains the maximum multidimensional variance direction in the Y space. A representative sample dataset from the asthma group is displayed in Figure 2a and 2b.

3.3 Group separation based on GC/DMS data

After processing the DMS datasets to select appropriate sets to analyze, we performed a validation based on previously published methods (Westerhuis et al., 2008). Briefly, the data cube was divided into a “test set” containing 10% of data and a model set containing 90% of data. The test set was then introduced into the model as quasi-unknown data resulting in a classification output. This output was compared to the *a priori* known classification of the datapoints (i.e. asthma, control or COPD) resulting in a correct classification (true positive, TP, or true negative, TN) or an incorrect classification (false positive, FP, or false negative, FN). This process was repeated several times in iterations called “loops” in order to identify the performance of the established model.

Figures 3a and 3b represent the confusion matrices produced from such multiple loops. The best levels of classification resulted from the asthma versus control groups and from the subjects taking omalizumab versus healthy patients not on this medication. The results show the mean percent classification for TP and FP for each group from all performed loops (20 for asthma vs. control, 40 for omalizumab vs. none), with each mean assigned a standard error.

4. Discussion

4.1 Interpretation of the results

In the present study we demonstrated that clinically-relevant groups may in part be classified and identified using GC/DMS analysis of the VOCs from EBC and using appropriate multivariate data analysis strategies. After executing 20 classification optimization loops on the asthma-control groups, we were able to correctly classify asthma subjects 75% of the time. While this number is certainly lower than desired for a diagnostic test, the potential of the proposed analytical technique is readily demonstrated. With improvements in our small sample size, the classification may be further enhanced. Similarly, we were able to correctly discriminate subjects taking omalizumab from subjects not taking this medication 70% of the time after executing 40 loops.

Our study differs from previous efforts in the field of mobile high-dimensional breath diagnostics in several key ways. First, no study using DMS technology to discriminate between asthma and COPD populations has been conducted to date. Second, our study used EBC rather than single-breath capture. EBC theoretically contains a higher abundance of VOCs and non-volatile compounds, is easier to pre-concentrate, and may be easier to standardize, though data on this is lacking. Ultimate breath diagnostic methods will ideally use single breath capture, however, in our study we aimed to maximize the quantity of VOCs. Last, our study design included a mixed cohort of patients reflective of those commonly encountered in clinical practice. The intent was to present a potential real-world application of the DMS technology, though our groups may have been more similar biochemically than different (see Limitations below). Future studies of this nature will need to utilize highly-selected groups (i.e. COPD with advanced fixed airflow obstruction and radiographic emphysema).

The ability to classify asthma from non-asthmatic patients is of high clinical relevance. For example, a condition called vocal cord dysfunction (VCD) may mimic the symptoms of asthma, yet it is entirely different in clinical course and therapy (Benninger et al., 2011). Hence, correctly classifying a patient as not having asthma if confusing symptoms are present may prompt an appropriate evaluation and, consequently, effective therapy. Though the model established herein does not have sufficient discriminatory power for a diagnostic test to correctly differentiate asthma from non-asthma, the results are promising and future studies with larger numbers of subjects and more samples per case should provide significantly improved classification models.

The determination of significant differences in VOC profiles between subjects taking omalizumab and healthy controls not taking the medication is interesting. Asthmatics who are taking omalizumab often have moderate to severe disease, and they may represent a distinct biological group from those patients with mild asthma. Asthma severity may engender more robust VOC metabolites and, thus, severe asthmatics may be a good patient cohort to study with respect to noninvasive diagnosis. An alternative hypothesis is that omalizumab itself may yield a set of distinct VOCs after being metabolized, and this may contribute to the observed differences. Finally, omalizumab produces a host of pharmacological effects, which help bring down the inflammatory status in the omalizumab-treated patients, which, in turn, may ultimately manifest in alteration of VOC profile. While to date there is little knowledge on the secondary effects of medication on VOC production and presence in exhaled breath, a noteworthy contribution may be suspected and will be subject to future studies.

While the initial aim of this study was differentiating asthma from COPD as the clinically most interesting goal, the limitations of the currently available dataset did not support this classification. This is most likely a result of the limited sample size (see below); yet, the quality of the obtained data clearly indicates that with a larger group of subjects a separation between asthma and COPD is very likely. Also, in the course of the study it turned out that the asthma and COPD groups may have been biologically more related rather than different, which reflects in the elevated eNO levels of both groups when they should have been significantly lower in the COPD group. Elevated eNO levels in the COPD cohort suggest a significant amount of allergic disease, and future studies will need to identify and exclude such overlapping populations (Zeki et al., 2011) when trying to validate novel diagnostics. However, the DMS system was able to distinguish between the control and asthma groups, which had significantly different eNO levels after removing outliers.

4.2 Limitations

While this feasibility study clearly demonstrates the utility of GC/DMS for exhaled breath VOC analysis to discriminate clinically well-defined patient groups, several limitations of the current experimental design and analysis are recognized.

Most evident is that the small number of subjects within the clinical groups is insufficient for establishing robust predictive models, as reflected in the standard errors. In the investigated patient cohort, several of the COPD subjects were of the emphysema subtype, and thus, produced only small volumes of EBC. This resulted in inadequate EBC samples for analysis from many subjects and further limited the available sample size. In addition, the GC/DMS analysis and sampling methodologies could be further optimized. At present, there is little *a priori* knowledge of defined chemical constituents that are distinctive within the exhaled breath of asthma or COPD populations. Once such information is available, sorbent matrices may specifically be selected to enhance the recovery of specific VOCs of interest from the EBC headspace while simultaneously minimizing the matrix background. Finally, given the fact that this was a feasibility study, the recruitment numbers were

expectedly low. Yet, demonstrating the potential of the method should significantly increase the number of subjects available to future extended studies. Nevertheless, it is evident that the between-group differences determined herein would likely be significantly more pronounced if the sample sizes per case were larger, thus advancing the discriminatory power of the developed predictive models.

During the present study, the RTube™ was used to collect EBC. Alternative breath collection devices may yield different quantities and qualities of EBC, although reliable data on this matter is disparate (Huttmann et al., 2011, Davidsson and Schmekel, 2010, Koczulla et al., 2009). Based on recent studies of our laboratory and others (Loyola et al., 2008, Czebe et al., 2008, Horvath et al., 2005, Liu et al., 2007), it is anticipated that conducting a similar clinical trial using continuously cooled EBC collection devices may yield more robust VOC data and further enhance the between-group differences.

The SPME headspace sample extraction procedure employed in the present work, though guided by the previous studies (Davis et al., 2010, Snow, 2000b) and directly utilizing protocols suggested by the SPME manufacturers, may not be specifically optimized for the cohort of subjects included in this study. We have conducted the extraction from the sample heated to 90 °C as suggested by the manufacturer as a possible mean to promote SPME headspace extraction (<http://www.sigmaaldrich.com/analytical-chromatography/sample-preparation/spme/faq.html>, 2012). This temperature is at the upper portion of the recommended temperature range, and we recognize that some VOC loss may occur into the septum using this protocol.

During future studies, several adaptations may further enhance the discriminatory power with the most substantial impact possibly achieved via concerted mass spectrometry studies for reliably establishing the chemical identities of asthma and COPD VOC biomarkers. Apart from the optimization of sampling methods, DMS measurements may also be improved. The ionization of analyte occurs according to the basicity distributions in proton transfer reactions or according to dissociation energies in charge transfer reactions. Thus, they differ in efficiency for each individual chemical moiety. Therefore, it is possible that the analytes of interest are suppressed by matrix interferences or are not efficiently ionized. Both scenarios lead to suppressed useful analytical signals, and thus, to impaired diagnostic results. For example, if the analytes of interest include aliphatic hydrocarbons, the ⁶³Ni source used in the present study would not efficiently ionizes these species. However, it has been reported that alkanes are highly relevant biomarkers indicative of oxidative stress associated with inflammation processes (Phillips et al., 2010). Further advancements may be achieved by modification of the carrier gas. In the present study, predominantly (dry) nitrogen was used. Addition of up to 50% of He would lead to a significant increase of DMS separation space (McCooye et al., 2001), and may thus enable better resolving signals from biomarkers species that are clinically important. While the DMS device used herein was interfaced to a conventional bench-top GC system, the potential of GC/DMS technology for bed-side monitoring could be fully unlocked upon further miniaturization of the GC component.

Last, an analyte diagnostic library does not currently exist for the DMS system in spite of the parameters which could be used for species identification. In this study, comparison of the GC/DMS data to GC/MS data would have greatly helped with species identification. Future studies may need to obtain both GC/DMS and GC/MS data from the same subjects until a suitable DMS library exists.

Despite these shortcomings, this proof-of-principle study shows the potential of DMS sensing in combination with gas chromatography providing potentially valuable diagnostic

information on VOC biomarker panels in exhaled breath for non-invasive disease diagnostics and disease discrimination.

5. Conclusion

Asthma and COPD represent two airway disorders which are difficult to accurately diagnose and treat. Consequently, improved diagnostic strategies are needed which are sensitive, portable, and complementary to available diagnostic tools. DMS is such a platform, and this study demonstrates that analysis of DMS VOC spectra can recognize differences between clinically well-defined groups. Though this study discriminated asthma from healthy controls as well as the subgroup of asthmatics taking omalizumab from healthy controls, the DMS technology can be applied to many other respiratory disorders. DMS has implications for both office diagnostics and as a research tool to ultimately identify specific VOCs which may function in the pathobiology of respiratory diseases. Further clinical studies should focus on larger subject recruitment numbers, improved EBC collection, and improved DMS (or other FAIMS technology) analyte measurements.

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Figure 1.

Schematic representation of the GC/DMS instrument setup. The volatile compounds collected from headspace of EBC are introduced into gas chromatograph (GC) column for chromatographic separation. The sample eluting from GC column is ionized with ^{63}Ni ion source prior to entering the DMS sensor. The filtered ions are detected at their corresponding compensation voltage values. E_l : low-field electric field strength, t_l : duration of the low-field portion of the waveform ; E_h : high-field electric field strength; t_h : duration of the high-field portion of the waveform Rf, radiofrequency waveform; Vc, compensation voltage

Figure 2.

Algorithm for DMS dataset selection. The raw data is assessed for analytic suitability by applying principle component analysis (PCA); the prediction model is built after Partial Least Square analysis (N-PLS); the data is validated; and a confusion matrix is created

Figure 3.

Figure 3a & 3b. Representations of quality DMS plots from our asthma data. Fig. 2a (top) shows a 3-dimensional plot with compensation voltage (CV) on the x-axis, retention time on the y-axis, and ion count (IC) on the z-axis. Each VOC has a unique RT & CV which can be used to ultimately identify the VOC. Fig 2b (bottom) shows a typical 2-dimensional output view of the 3-dimensional graph in 2a.

4a.				4b.			
		Mean (%)	SEM (%)			Mean (%)	SEM (%)
Control (n=13)	TP	57.5	2.3	No med (n=13)	TP	70	3.6
	FP	25	2.3		FP	30	3.6
Asthma (n=13)	TP	75	2.3	Omalizumab (n=9)	TP	70	5.1
	FP	42.5	2.3		FP	30	5.1
20 Loops				40 Loops			

Figure 4.

Figure 4a & 4b. Confusion matrices describing the performance of the models to classify asthma from controls (Fig. 3a, left) and subjects taking omalizumab from those not taking this medication (Fig 3b, right). The left graph shows the mean results of 20 executed loops of the dataset. Right, the results of 40 loops are shown.

Figure 5.

Box-whisker plot of exhaled nitric oxide (eNO) levels by group. Though there were no statistical eNO differences between the asthma and COPD groups, there is a clear separation between the control and asthma groups after appropriate removal of 3 outliers from each group ($p < 0.001$, unpaired Student's t -test). This shows that the selected control group was biologically different than the asthma group, as detected by the DMS system. The similar eNO levels between the asthma and COPD groups indicates a biologic similarity between these groups.

Table 1

Subject baseline characteristics.

	Healthy	Asthma	COPD
n	13	13	5
Age in yrs (range) ^{*,†,§}	33 (23–66)	50 (18–69)	70.5 (48–89)
Sex, M/F (n/n)	11/12	7/14	13/5
BMI (range) ^{*,§}	24.2 (18.2–30.9)	30.9 (20–53.4)	25.2 (18.6–34.5)
Spirometry ^{*,†,§}			
FEV1 ±SD, prebronchodilator (L)	3.53 ± 0.68	2.13 ± 0.93	1.21 ± 0.57
FEV1/FVC ±SD (% predicted)	82 ± 8.2	69 ± 13	49 ± 14
NO ±SD (ppb) [†]	16 ± 11	24 ± 22	25 ± 12
Omalizumab use	0	9	0

*
p<0.05;**
p<0.001 Normal-Asthma†
p<0.05;‡
p<0.001 Normal-COPD§
p<0.01 Asthma-COPD