Rapid Detection of Sepsis in Rats through Volatile Organic Compounds in Breath

Ana V. Guamán[1,2], Alba Carreras[3,4], Daniel Calvo[2], Idoya Agudo[1], Daniel Navajas[1,3,4], Antonio Pardo[2], Santiago Marco[1,2], Ramon Farré[3,4]

[1] Institute of Bioengineering of Catalonia (IBEC), Baldiri i Rexach 4 08028-BCN, Spain.

[2]Intelligent Signal Processing, Departament d'Electrònica,, Universitat de Barcelona,
Martí i Franquès 1, 08028-BCN, Spain

[3]Unitat de Biofísica i Bioenginyeria, Facultat de Medicina, Universitat de Barcelona-Institut d'Investigacions
Biomèdiques August Pi i Sunyer, 08036 Barcelona, Spain.

[4]CIBER de Enfermedades Respiratorias, 07110 Bunyola, Spain.

Corresponding author: Ramon Farré, Unitat de Biofísica i Bioenginyeria, Facultat de Medicina, Casanova 143, 08036, Barcelona, Spain, Phone: (34) 93 402 4515, Fax: (34) 93 403 5278; email: rfarre@ub.edu

Keywords: Sepsis, Volatile organic compounds, Ion mobility spectrometer, Rat model, Bedside patient systems, Noninvasive detection.

Abbreviations used

IP: intraperitonealy
LPS: lipopolysaccharide
VOCs: volatile organic compounds
IMS: ion mobility spectrometry
SPME- GC/MS: Solid Phase MicroExtraction-gas chromatography/mass spectrometry
COPD: Chronic Obstructive Pulmonary Disease
CAR/PDMS: Carboxen/Polydimethylsiloxane
RIP: Reactant Ion Peak
MCR-LASSO: Multivariate curve resolution- Least Absolute Shrinkage and Selection Operator
SFFS: sequential floating forward selection
kNN: k nearest neighbours
PCA: principal component analysis
LDA: linear discriminant analysis
PCT: ProCalcitonin Test
PCR: Polymerase Chain Reaction
PFA: Perfluoralkox
IL: Interleukin
TNF: Tumor necrosis factor

Abstract

BACKGROUND: Sepsis is one of the main causes of death in adult intensive care units. The major drawbacks of the different methods used for its diagnosis and monitoring are that they are unable to provide fast responses, being unsuitable for bedside use. In this study, performed using a rat sepsis model, we evaluate breath analysis with Ion Mobility Spectrometry (IMS) as a fast, portable and non-invasive strategy.

METHODS: This study was carried out on 20 Sprague-Dawley rats. Ten rats were injected with lipopolysaccharide from Escherichia coli and ten rats were IP injected with regular saline. After a 24-h period, the rats were anaesthetized and their exhaled breaths were collected and measured with IMS and SPME-gas chromatography/mass spectrometry (SPME-GC/MS) and data were analyzed with multivariate data processing techniques.

RESULTS: The SPME-GC/MS dataset processing showed 92% accuracy in the discrimination between the two groups, with a confidence interval of between 90.9% and 92.9%. Percentages for sensitivity and specificity were 98% (97.5%-98.5%) and 85% (84.6%-87.6%), respectively. The IMS database processing generated an accuracy of 99.8% (99.7%-99.9%), a specificity of 99.6% (99.5%-99.7%) and a sensitivity of 99.9% (99.8%-100%).

CONCLUSIONS: IMS involving fast analysis times, minimum sample handling and portable instrumentation can be an alternative for continuous bedside monitoring. IMS spectra require data processing with proper statistical models for the technique to be used as an alternative to other methods. These animal model results suggest that exhaled breath can be used as a point-of-care tool for the diagnosis and monitoring of sepsis.

Introduction

Thanks to the pioneering work of Pauling L et al, it has been known since the 1970s that human breath is a complex mixture of hundreds of compounds (1). Gas Chromatography-Mass Spectrometry has made it possible to identify some of these compounds, revealing that exhaled breath included traces of many volatile organic compounds (VOC), small inorganic molecules and non-volatile substances such as isoprostanes, cytokines or leukotrienes (2, 3). Accordingly, there is now a consensus about the diagnostic potential of breath, and there is considerable evidence available to support the use of breath analysis as a diagnostic tool for pulmonary diseases, liver diseases, gastric diseases, diabetes and inflammatory diseases such as sepsis (3, 4-7). The analysis of exhaled breath has a number of advantages compared with traditional diagnostic techniques: it is a non-invasive, painless procedure that does not require skilled medical staff (8, 9).

Despite this undeniable interest, however, only a few breath tests, such as capnography and the urea breath test, are typically used in clinical routine. One reason for the continued reluctance to use breath analysis as a common diagnostic tool in clinical practice is lack of knowledge about the compounds' metabolic pathways of the compounds, although another factor is the lack of normalization and standardization methods (8, 10). Furthermore, bedside systems are not always compatible with the sophistication now required of analytical instruments. Gas chromatography-mass spectrometry (GC/MS), for example, is the most widely used instrument in breath analysis (11-13) but, although it offers very good sensitivity, precision and resolution, the sampling procedures and subsequent interpretation of data can be demanding and time-consuming and require qualified personnel.

Alternative chemical sensing techniques, such as solid-state sensor arrays (e-noses) and ion mobility spectrometers (IMS), offer new approaches to this problem. E-noses have been applied for many preliminary studies (14) but their limited sensitivity and selectivity hinder their clinical application when the analytes of interest are at sub-ppm levels. Alternatively, IMS is a simple, portable and sensitive instrumental analytical technique that it is gradually expanding its range of applications, from security to food and environmental and clinical applications. IMS provides a spectra response of less than a second to the trace levels of volatile organic compounds, based upon the mobility of gas phase ions in weak electric fields (15). The mobility K of an ion depends on the ion's mass, charge, shape and size, but also on the measurement conditions, particularly pressure and temperature. An ion's mobility is usually reported by means of reduced mobility K0, where the effects of measurement conditions have been harmonized to standard conditions. Moreover, an IMS can offer two different responses: the mobility spectra for positive ions and for negative ions.

Promising results of breath analysis with IMS have been reported and its potential for application as a diagnostic instrument is huge. Lung cancer has been the main focus of attention (16, 17) but also interesting findings have also emerged with respect to COPD, sarcoidosis and vaginitis (18-20). Other diseases such as sepsis have yet to be tested by IMS technology, however, even although the potential capability of breath test for the diagnosis of sepsis has been posited in some works (3, 7).

Sepsis is a clinical condition characterized by systemic inflammation, aberrant immune response, and microcirculation/coagulation disorders generated by the host in combating an infection due to bacterial toxins absorbed from infected wounds and passed into the bloodstream (21). It is a common cause of morbidity and mortality in elderly, immuno-compromised and critically ill patients, and it is the commonest cause of death in adult intensive care units (22).

Several rodent models have been used for sepsis studies in various works (23-25) and, although the correlation between animal and human models is not perfect, rat models can make a positive contribution in many areas, such as the reduction and standardization of experimental variability and the simplification of the experimental setup. One of the common sepsis models used in murine is the induction of sepsis through an exogenous bacteria infection. It is well known that mimicking bacterial infection by using lipopolysaccharide (LPS), which is a structural component of gram-negative bacteria, has been used as a model to activate the immune system, inducing fever, sepsis and multi-organ injury (26). The main cytokines acting as endogenous pyrogens in response to LPS are interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α). Moreover, pulmonary dysfunction, including edema, is a well-recognized dysfunction in sepsis (27).

The analysis of exhaled breath in rats has been reported in some studies (28-30) and the analysis of rodent's breath with an IMS instrument has been reported in a recent feasibility study by Vautz et al (31).

This work explores the viability of the IMS instrumentation and chemometric techniques to generate a VOC discriminatory pattern of sepsis through breath sampling. This work has been performed in a rat model as a first step towards a possible future application in humans. Furthermore, although IMS is undoubtedly capable of providing fast VOC analysis, it also needs to be compared with a gold standard technique in VOC analysis. In order to fill this gap, this study includes GC/MS measurements of the rat's breath as a reference technique, while also providing analyte identification capabilities using proper MS libraries.

Materials and Methods

Animals

This study was carried out on 20 Sprague-Dawley male rats from Charles River (250-300g) following an experimental protocol approved by the Ethical Committee of Animal Research at the University of Barcelona. One day before the experiment, 10 of these rats were intraperitoneal (IP) injected with LPS from Escherichia coli 055:B5 (Sigma Chemical Co., St. Louis, MO) at a concentration of 4mg/kg and the other 10 rats were IP injected with saline solution as a control group. All the animals were housed in light-dark cycle-regulated air conditioned (23ºC) and air humidity (60%) animal quarters for 24h. After this period, the animals were IP sedated and anaesthetized with a mixture solution containing Rompun (Bayer) in a concentration of 0.7mL/kg and Imalgene 1000 (Merial Laboratories, Spain) in a concentration of 1mL per kilogram of animal body weight. A tracheotomy was performed and one cannula (16GA BD Adsyte Pro, Becton Dickinson, Spain) was introduced into the trachea. The intratraqueal cannula was connected, by means of a T-piece, to the inspiratory and expiratory lines of a conventional mechanical ventilator (Harvard, USA). The rats were ventilated normally, with a tidal volume of 7ml per kg of body weight, at a rate of 80 breaths/min using room air. The ventilation period lasted for 20 min and at the end of this period the expiratory gas was sampled for further VOC analysis. Figure 1 shows the sampling method used in this work. With GC/MS the rat's breath was collected in a perfluoralkoxy (PFA) bag for subsequent analysis. With IMS, the breath sample is directly introduced into the instrument via the expiratory line and measured online.

[Figure 1 about here]

Assessment of rat status

After expiratory gas sampling, the rats were sacrificed by aortic exsanguination. The rats' septic status as a result of the bacterial-LPS injection was investigated by assessing lung edema and systemic inflammation. To assess lung edema, the lungs of all the rats were excised, quickly weighed after removing the main airways, weighed (wet weight=W), dried at 70ºC for 48h and weighed again (dry weight=D). The ratio W/D was computed as a conventional index of lung edema.

The systemic inflammatory status of the rats injected with LPS was determined by measuring the plasma concentrations of two representative inflammatory cytokines: IL1-β and TNF-α. To this end, the peripheral blood was processed to isolate the plasma (centrifugation at 3,000 g using a vasculant rotor for 15 min at 4 ºC). Enzyme-linked immunosorbent assays (ELISA) for IL1-β and TNF-α were performed (Quantikine, R&D Systems, Minneapolis, MN, USA).

Instrumentation and measurements:

The IMS used in this study was the GDA2 (Airsense Analytics, Germany) based on a 100MBeq Ni^{63} ionization source that works in both positive and negative modes. An electrostatic gate allows the ions to travel at atmospheric pressure into the drift tube where a constant electric field accelerates them (length 6cm). At the end of the drift tube ions become neutralized in the collector and an electric current is measured. In this manner, the time that the ions need to reach the collector is measured. The collector current is sampled at 33.3kHz. The IMS provides a different sample mobility spectrum 28ms in length every 3s. This spectrum corresponds to an average of 16 consecutive spectra for noise reduction. In this work, the measurements were made at 50% internal dilution of sampling, with a sampling flow of 200ml/min. All the samples were measured twice for up to 40s after 5min of stabilizing the system and IMS. Once these measurements with the IMS were finished, 1L of breath was collected in PFA bags

(Jensen Inert Products, USA) for subsequent SPME-GC-MS analysis.

The GC/MS used in this study was FOCUS GC-DSQII (Thermo Scientific, USA). Breath was collected in 1L PFA bags and analyzed with SPME/Gas Chromatography-Mass Spectrometry. A Carboxen/Polydimethylsiloxane (CAR/PDMS) 75-µm-thick fiber from Supelco was used for the pre-concentration of the analytes. The sorption conditions were 30min at room temperature. The desorption of volatiles from the fiber was undertaken at 250ºC for 5min at the GC/MS injection port. The GC/MS analyses were performed on a Focus GC-DSQ II with a split/splitless injector. A 60m x 0.32mm x 1.8µm capillary column DB-624 (Agilent Technologies) was chosen for the chromatographic separation. Helium was used as carrier gas, with a flow rate of 1ml/min. The MS analyses were carried out in a full scan (scan range 35-350amu) with ionization energy of 70eV. The oven program temperature was as follows: initially, 40ºC held for 5 min, then ramped 10 ºC min-1 to 180ºC; held for 1 min, then ramped 15ºC/min to 230ºC; and then held for 10min.

At the beginning and at the end of each session of measurements, the blanks of the sampling system and the air of the laboratory were measured with IMS in order to ensure the reproducibility of the measurements. In order to counteract the anesthetic drugs in the ion mobility spectra and in the chromatogram, these products were measured in a head-space mode by IMS and by SPME-GC-MS.

Signal Processing and statistical analysis

The signal processing strategies used in this work were based on multivariate signal processing and implemented in MATLAB 7.5 (Mathworks, USA) using the PLS Toolbox 5.8 (Eigenvector Research, USA). The signal processing applied to the IMS dataset was designed to find differences between healthy and diseased rats, and the signal processing applied to the GC/MS dataset focused on identifying compounds that could be potentially useful as sepsis biomarkers

The IMS dataset pre-processing includes a base line correction by fitting a 4th order polynomial to specific spectral intervals devoid of peaks and a cubic Savitzky-Golay filter (32) of length fifteen smoothing procedure. The drift time of the IMS Reactant Ion Peak (RIP) was used as a reference for the spectra alignment and an area normalization procedure was applied to each spectrum. The multivariate signal processing strategy involved the use of the iterative algorithm MCR-LASSO (33) to estimate the pure contributions to the spectra, the Sequential Floating Feature Selection (SFFS) (34) to select the best subset of pure contribution for maximum discrimination between classes, and a kNN classifier (34) in the reduced space to evaluate the classification results under a bootstrap validation (35) strategy.

As regards the GC/MS dataset, the compounds were identified by comparison with mass spectra from the NIST 2005 library database available in the Thermo Xcalibur data system. The basic multivariate strategy involved using a combination of principal component analysis (PCA) (34) and linear discriminant analysis (LDA) (34), with a selection based on rank products (36, 37). This strategy made it possible to reduce dimensionality and order the identified compounds by their p-value. A kNN, SFFS and bootstrap validation were also used in the same way as in the IMS dataset analysis.

Results

Pathophysiological rat status

As expected, pulmonary edema was found only in the LPS-treated rat group (W/D=6.88±0.58; mean±SEM) compared to control animals (W/D=5.40±0.28). Moreover, concentrations of circulating inflammatory markers in plasma were significantly increased in LPS-infected mice compared to controls. Whereas in the control animals the concentration of IL1-β and TNF-α were 1.51±1.01 pg/mL and 1.43±0.14 pg/mL, respectively, in the LPS-injected animals these concentrations rose to 313.45±81.80 pg/mL and 5.99±0.30 pg/mL, respectively. All the differences observed between the controls and the animals with the bacterial endotoxin were statistically significant (t-test or Mann–Whitney rank sum test, as required): $p=0.034$, $p=0.002$ and $p<0.001$ for lung edema, IL1-β and TNF-α, respectively.

Ion Mobility Spectrometry dataset

The IMS dataset featured 10 spectra from 40 breath samples (10 healthy rats + 10 LPS treated rats and an additional replicate of each one). MCR-LASSO was used to decompose IMS raw spectra into their pure contributions: pure spectra components, S, and their related concentration time evolution, C, were extracted. As a result, fourteen relevant pure components were obtained from negative and positive spectra. In Figure 2, the plots show the components of the measured rat's breath in positive and negative IMS mode.

[Figure 2 about here]

Undesirable contributions appeared at a drift time of 9.575ms in positive mode and at a drift time of 8.99ms in negative mode. Anesthesia (drift time=12.48ms in negative mode) as well as pure components related to the RIP peaks in positive mode (drift time=8.06ms and 9.03ms) and negative mode (drift time 8.363ms) were identified but were not considered for further evaluation. At the end of this process, eight pure components had been obtained.

As a result of the SFFS selection, the subset consisting of compounds with reduced mobility of $K0_1$=9.96cm^{2}V^{-1}s^{-1} (positive spectra), $K0_2$=8.75cm^{2}V^{-1}s^{-1} and $K0_3$=11.79cm^{2}V^{-1}s^{-1} (negative spectra) were selected. Fig 3 shows the distribution of rats in the space of the three selected compounds. For easier interpretation, two plots of $K0_1$ versus $K0_2$ and $K0_1$ versus $K0_3$ have been shown, as opposed to a three-dimensional plot. Bootstrap validation was applied to estimate the discrimination between healthy and LPS-treated rats and the final result was an accuracy of 99.8% (99.7%-99.9%), a specificity of 99.6% (99.5%-99.7%) and a sensitivity of 99.9% (99.8%-100%). The confidence limits were calculated at a 95% confidence level.

[Figure 3 about here]

GC/MS

Figure 4 shows one chromatogram obtained from a diseased rat and one obtained from a healthy rat. Note the abundance of peaks and slight differences between both chromatograms.

[Figure 4 about here]

Although not all the peaks of the samples can be identified, Table 1 lists nineteen compounds found and identified in breath samples from diseased and healthy rats. Three compounds were identified as related to a fever induced by LPS and one compound was identified as linked to the anesthesia. All of these were discarded for the subsequent data evaluation study. In the end, fifteen compounds were selected as possible compounds associated with sepsis and the area under the peak was calculated for each one using MzMine2 (38).

[Table 1 about here]

The results of the application of PCA-LDA with rank products are shown in Table 1.

Five compounds with a p-value less than 0.001 were chosen by the algorithm as possible compounds related with sepsis.

Figure 5 shows the plot resulting from the discriminant model. Bootstrap validation was implemented for a strict validation of the discrimination model. The final results obtained with bootstrap validation have an accuracy of 85% with a confidence interval between 84.6% and 85.9%. The results for sensitivity and specificity are 91% (89.7%-92.2%) and 80% (79.3%-80.7%), respectively. Again, the confidence limits were calculated to a 95%.

[Figure 5 about here]

Discussion

Despite the evolution of intensive care medicine and the broad range of clinical systems nowadays, sepsis is still the first cause of death in non-coronary critical care units. Traditionally, sepsis diagnostics use culturing techniques of blood, urine, cerebrospinal fluid and bronchial fluid, among others. The major drawback of culturing techniques is the time needed to develop the culture, usually between 24 and 48 h. Although other techniques such as ELISA, ProCalcitonin Test (PCT) assays and DNA detection by Polymerase Chain Reaction (PCR) are faster, they need between 2 and 6 h to obtain a response and they are incapable of following the dramatic changes occurring in sepsis (39). In the face of a lack of a real-time monitoring system for sepsis, breath analysis with IMS must be considered a promising and prospective alternative.

The potential capability of breath tests for the diagnosis of sepsis has been indicated in some works (3, 7) but, as far as we know, sepsis still remains untested by IMS technology. Other technologies such as GC/MS are also capable of offering a high performance in breath analysis but usually they cannot provide the portability and simplicity of the IMS measurements. IMS is more suited to the clinical trend of developing bedside patient systems but unfortunately it cannot identify easily unknown volatile compounds in a sample, so, in this respect, GC/MS measurements complement this lack of knowledge as a reference technique. This study includes, for the first time, the measurement with IMS technology of rats' breath infused with LPS from *E. coli* as a sepsis animal model. This represents a first step in the potential applicability of IMS for the diagnosis of sepsis in human patients.

Although is well known that the injection of live bacteria and the injection of only LPS in an animal has some different pathological effects, LPS is commonly used in sepsis models because the injected dose is completely controlled by the experimenter, while this is not the case when injecting live bacteria. Further, although it has been demonstrated that LPS-induced models of endotoxic shock in rodents do not exactly reproduce septic complications in humans (40), they have been used to investigate endotoxin-dependent mechanisms in vivo. Thus, the LPS from the *E. coli* model used in rats in our study is adequate to examine LPS-dependent aspects of septic shock.

GC/MS measurements provided a list of compounds in the rat’s breath. After the elimination of the compounds from the SPME-fiber and the anesthesia, fifteen compounds can be potentially used to separate healthy rats from treated rats. To obtain a subset of compounds related to sepsis, PCA-LDA and rank products were used as techniques that allow a maximum discrimination between classes and a ranking of compounds according to their discrimination importance. Moreover, this methodology allows us to obtain a significance level for selected compounds considered as a p-value (37). Thus, the p-value represents the probability of observing a compound at a certain rank, and compounds with the lowest rank are the most important in the separation. In this study we selected compounds with a p-value lower than 0.001. In the end, the first five compounds listed in Table 1 were selected as the most representative compounds in the discrimination between septic and healthy animals, and this could be considered a pattern correlated with sepsis. In this reduced space, a pattern recognition system provides promising rates of bootstrap validation: 85% of accuracy, 91% of specificity and 80% of sensitivity. These percentages must be understood in the light of the bootstrap validation procedure: they mean that, after 500 random selections of different sets of rats, overall 85% of the rats were well classified, and the same interpretation can be made for the specificity and sensitivity figures.

Despite the good figures achieved with GC/MS measurements, the time, cost and infrastructure needed for the sampling and measurement make it impossible to use of these instruments in a bedside setting. The IMS alternative, however, does allow for this possibility because the sampling and measurement time takes only few minutes. With respect to the IMS results, multivariate signal processing was able to detect the spectra of pure breath constituents. After a fine counteraction of external pollutants and anesthesia, and after applying pattern recognition procedures, a pattern of three components was found. Although it is not possible to identify these compounds, they can be separated into two classes, with good levels of accuracy (99.8%), specificity (99.6%) and sensitivity (99.9%) figures under bootstrap validation. It must be stressed that bootstrap validation is designed to avoid over-optimistic results. It is interesting to note that even better results are achieved by processing the full IMS spectra instead of selected molecules. In this respect, we believe that sepsis produces a general alteration in the breath pattern and not just the secretion of a single or few biomarkers. Lack of knowledge about the metabolic pathway is therefore not a major issue, since the levels of many different VOCs are probably altered. The outstanding results obtained are encouraging and open up the prospect of performing new experiments to validate the model developed for the diagnosis of sepsis and beginning carefully controlled studies with human patients.

In conclusion, breath analysis with IMS has been presented as an alternative for a rapid diagnosis of sepsis. The performance of this methodology in separating a healthy rat group from a diseased rat group is excellent and provides encouraging conceptual evidence at the experimental level. Therefore, the results obtained in the present animal study warrant further clinical studies in septic patients, in order to explore the routine capability of IMS as a non-invasive point-of-care diagnostic tool.

Acknowledgments

The authors wish to thank Ms. Rocío Nieto for her technical contribution. This work was supported in part by the Spanish Ministry of Science and Innovation (SAF2008-02991 and PI081908).

AV. Guamán, D. Calvo, I. Agudo, A. Pardo and S. Marco belong to the 2009-SGR-753 research group of the Generalitat de Catalunya.

References

1. Pauling L, Robinson AB, Teranishi R, Cary P. Quantitative analysis of urine vapour and breath by gas-liquid partition chromatography. Proc Natl Acad Sci USA 1971; 68: 2374-76.

2. Smith D, Turnes C, Spanel P. Volatile metabolites in the exhaled breath of healthy volunteers: their levels and distributions. J Breath Res 2007; 1: R1-R12.

3. Miekisch W, Schubert JK, Noeldge-Schomburg GFE. Diagnostic potential of breath analysis: focus on volatile organic compounds. Clin Chim Acta 2004; 347:25-39.

4. A, Manolis. The diagnostic potential of breath analysis. Clin Chem 1983; 29/1: 5-15.

5. Cao W, Duan Y. Breath analysis: potential for clinical diagnosis and exposure assessment. Clin Chem 2006; 52/5: 800-11.

6. Amann A, Schmid A, Scholl-Burgi S, Telser S, Hinterhuber H. Breath analysis for medical diagnosis and therapeutic monitoring. Spectrosc Eur 2005; 17:18-20.

7. Scholpp J, Schubert JK, Mleklsch W, Geiger K. Breath markers and soluble lipid peroxidation markers in critically ill patients. Clin Chem Lab Med 2002; 40:587–94.

8. Buszewski L, Kesy M, Ligor t., Amann A. Human exhaled air analytics: biomarkers of diseases. Biomed Chromatogr 2007; 21:553-66.

9. Spinhirne JP, Koziel JA, Chirase NK. A device for non-invasive on-site sampling of cattle breath with solid-phase microextraction. Biosyst Eng 2003; 84/2:239–46.

10. Miekisch W, Schubert JK. From highly sophisticated analytical techniques to life-saving diagnostics: Technical developments in breath analysis. TrAC 2006; 25/7:665-73.

11. Van Berkel J, Dallinga JW, Moller GM, Godschalk RWL, Moonen EJ, Wouters EFM, Van Schooten FJ. A profile of volatile organic compounds in breath discriminates COPD patients from controls. Resp Med 2010; 104:557-63.

12. Phillips M, Cataneo RN, Ditkoff BA, Fisher P, Greenberg J, Gunawardena R, et al. Volatile markers of breast cancer in the breath. Breast J 2003; 9:184-91.

13. Van den Velde S, Nevens F, Van Hee P, van Steenberghe D, Quirynen M. GC-MS analysis of breath odor compounds in liver patients. J Chromatogr B-Analytical Technologies in the Biomedical and Life Sciences 2008; 875:344-8.

14. Di Natale C, Macagnano A, Martinelli E, Paolesse R, D'Arcangelo G, Roscioni C, et al. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. 10, 2003, Biosens Bioelectron 2003; 18/10:1209-18.

15. Eiceman GA, Karpas Z. Ion Mobility Spectrometry. CRC Press: Boca Raton, 1994.

16. Westhoff M, Litterst P, Freitag L, Urfer W, Bader S, Baumbach JI. Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. Thorax 2009; 64/9:744-8.

17. Baumbach JI, Westhoff M. Ion mobility spectrometry to detect lung cancer and airway infections. Spectrosc Eur 2006; 18:22–7.

18. Westhoff, M, Litterst P, Freitag L, Baumbach JI. Ion mobility spectrometry in the diagnosis of Sarcoidosis results of a feasibility study. J Physiol Pharmacol 2007; 58:739–51.

19. Bessa V, Darwiche K, Teschler H, Sommerwerck U, Rabis T, Baumbach JI, Freitag L. Detection of volatile organic compounds (VOCs) in exhaled breath of patients with chronic obstructive pulmonary disease (COPD) by ion mobility spectrometry. Int J Ion Mob Spectrom 2011; 14/1:7-13.

20. Karpas Z, Chaim W, Gdalevsky R, Tilman B, Lorber A. Novel application for ion mobility spectrometry: diagnosing vaginal infections through measurement of biogenic amines. Anal Chim Acta 2002; 474:115-23.

21. Opal SM. The host response to endotoxin, antilipopolysaccharide strategies, and the management of severe sepsis. Int J Med Microbiol 2007; 297:365–77.

22. Brun-Buisson C, Doyon F, Carlet J, Dellamonica P, Gouin F, Lepoutre A, et al Incidence, risk factors and outcome severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU Group for Severe Sepsis. JAMA 1995; 274/12:968-74.

23. Parker SJ, Watkins PE. Experimental models of gram-negative sepsis. Brit J Surg 2001; 88/1:22–30.

24. Schultz MJ, van der Poll T. Animal and human models for sepsis. Ann Med; 2002, 34:573–81.

25. Tarkowski A, Collins LV, Gjertsson I, Hultgren OH, Jonsson IM, Sakiniene E, Verdrengh M. Model systems: modeling human staphylococcal arthritis and sepsis in the mouse. Trends Microbiol 2001; 9/7:321-26.

26. Soares DM, Figueiredo MJ, Manvailer J, Resende R, Sorgi C, Faciolli LH, Alves-Filho JC, Cunha FQ, Souza G. A crucial role for IL-6 in the CNS of rats during fever induced by the injection of live *E.coli*. Med Microbiol Immunol. 2011 (Published online).

27. Lava J, Rice CL, Moss GS, Levine H, Rosen A, Sehgal L, Gould SA. Pulmonary dysfunction in sepsis: is pulmonary edema the culprit? J Trauma. 1982; 22:280-4.

28. Sethi JM, Choi AMK, Calhoun WJ, Ameredes BT. Non-invasive measurements of exhaled NO and CO associated with methacholine responses in mice. Respir Res 2008;

9:45.

29. Weicker S, Karachi TA, Scott JA, McCormack DG, Mehta S. Noninvasive measurement of exhaled nitric oxide in a spontaneously breathing mouse. Am J Resp Crit Care 2001; 163: 1113-6.

30. Dercho RA, Nakatsu K, Wong RJ, Stevenson DK, Vreman HJ. Determination of in vivo carbon monoxide production in laboratory. J Pharmacol Toxicol 2006; 54/3:288-95.

31. Vautz W, Nolte J, Bufe A, Baumbach JI, Peters M. Analyses of mouse breath with ion mobility spectrometry: a feasibility study. J Appl Physiol 2010; 108:697-704.

32. Savitzky A, Golay MJE. Smoothing + Differentiation of Data by Simplified Least Squares Procedures. Anal Chem 1964; 36/8:1627-39.

33. Pomareda V, Calvo D, Pardo A, Marco S. Hard modeling Multivariate Curve Resolution using LASSO: Application to Ion Mobility Spectra. Chemometr Intell Lab 2010; 104:318-32.

34. Webb AR. Satatistical Pattern Recognition 2nd Edition. Jhon Wiley & Sons, 2002.

35. Efron B. Bootstrap Methods: Another Look at the Jackknife. Ann Stat 1979; 7:1-26.

36. Breitling R, Armengaud P, Amtmann A, Herzyk P. Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. FEBS Lett 2004; 573: 83-92.

37. Smit S, van Breemen MJ, Hoefsloot HCJ, Smilde AK, Aerts JMFG,de Koster CG. Assessing the statistical validity of proteomics based biomarkers. Anal Chim Acta 2007; 592:210-17.

38. Pluskal, T, Castillo, S, Villar-Briones A, Oresic M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatic 2010; 11:395.

39. Carrigan SD, Scott G, Tabrizian M. Toward Resolving the challenges of sepsis diagnosis. Clin Chem 2004; 50/8:1301–14.

40. Gordon CJ .Response of the thermoregulatory system to toxic insults. Front Biosci. 2010; 2:293–311 Review.

Table 1: Identification of compounds from GC dataset

Compounds	Identification	Rank Product (p-value)
1	Cyclohexane, methyl	0.000005
2	Acetone	0.000007
3	CO2	0.00001
4	Pentafluoropropionamide	0.00003
5	Dimethylether	0.0002
6	Retention Time (18.57) Mazas(42,48,56)	0.0010
7	o-Xylene	0.0191
8	Hexane, 2,3,4-trimethyl-	0.2676
9	Octane, 4-methyl-	0.5343
10	Decane	0.6611
11	2-Propanol, 1,3-dichloro-	0.8983
12	Toluene	0.9702
13	Acetic acid	1.6955
14	Propane, 2-ethoxy-2-methyl-	2.3828
15	Benzene	4.1241
FIBRE	Silanediol, dimethyl-	
	Cyclotrisiloxane, hexamethyl-	
	Cyclotetrasiloxane, octamethyl-	
ANESTHESIA	Ketanone	

Figure captions

Figure 1: Diagram of the experimental setup. The expired air from the ventilator was analyzed on-line by the IMS device by using an air buffer. For the collection of expired air for GC/MS, the exhaled air was obtained by directly connecting a collecting bag to the expiratory outlet of the ventilator.

Figure 2: Pure components from MCR-LASSO results for rat's breath. Every component P1, P2,…P14 has his Reduced Mobility K0 (cm^{2}V^{-1}s^{-}1).

Positive Spectra: P1K0=2.35, P2K0=2.11, P3K0=1.97, P4K0=2.04, P5K0=1.89, P6K0=1.84, P7K0=1.82, P8K0=1.79

Negative Spectra: P9K0=2.25, P10K0=2.11, P11K0=1.52, P122.16, P13K0=2.01, P14K0=1.60.

Figure 3: Score plots of the final IMS pure component selection.

Figure 4: Chromatograms of rats with sepsis and healthy rats.

Figure 5: Score plot of the final GC/MS discriminant vector.

	COMPOUND	Rank Product Probability
1	Cyclohexane, methyl-	0.000058
2	Acetona	0.00006
3	CO2	0.00007
4	Ui1	0.00009
5	o-Xylene	0.0001
6	Ethyl alcohol	0.0003
7	Decane	0.0008
8	Ui3	0.002
9	Pentafluoropropionamide	0.003
10	2-Propanol, 1,3-dichloro-	0.05
11	Acetic acid	0.06
12	Propane, 2-ethoxy-2-methyl-	0.065
13	Octane, 4-methyl-	0.07
14	Hexane, 2,3,4-trimethyl-	0.3
15	Toluene	0.4
16	Ui2	0.7
FIBRE	Silanediol, dimethyl	
	Cyclotrisiloxane, hexamethyl	
	Cyclotetrasiloxane, octamethyl	
ANESTHESIA	Ketanone	

Tabla 1 Volatile Organic Compounds detected in breath samples and rank product probability of each compound. The first seven compounds could be linked with sepsis. UI: Unidentified compound

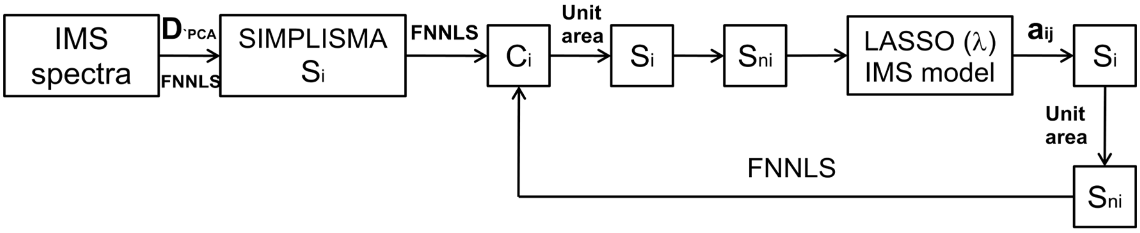
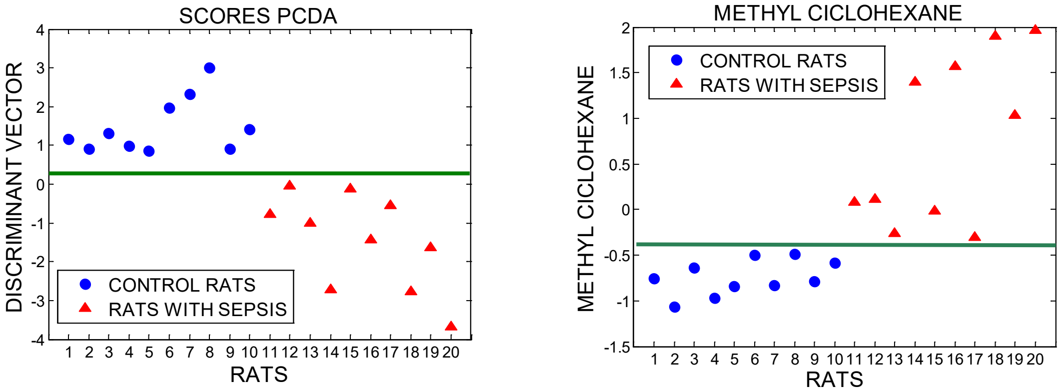
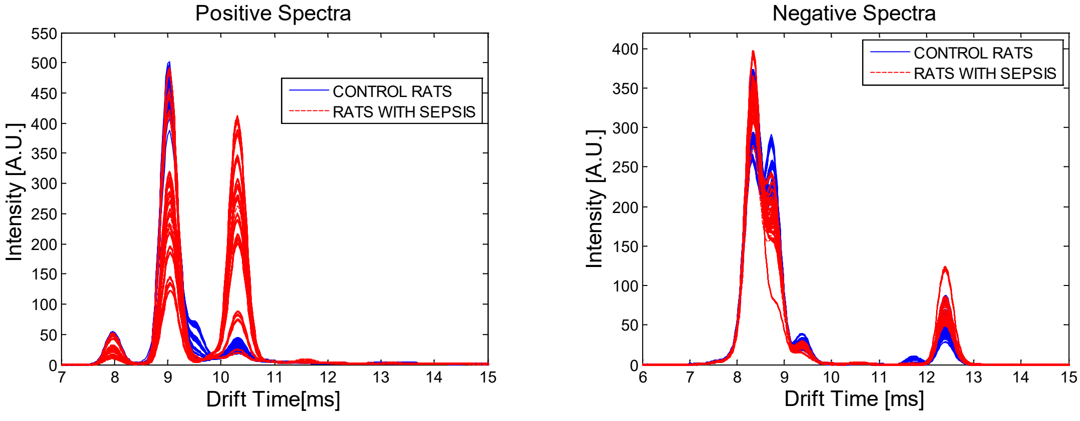


Fig. 1 MCR LASSO block diagram. (1)



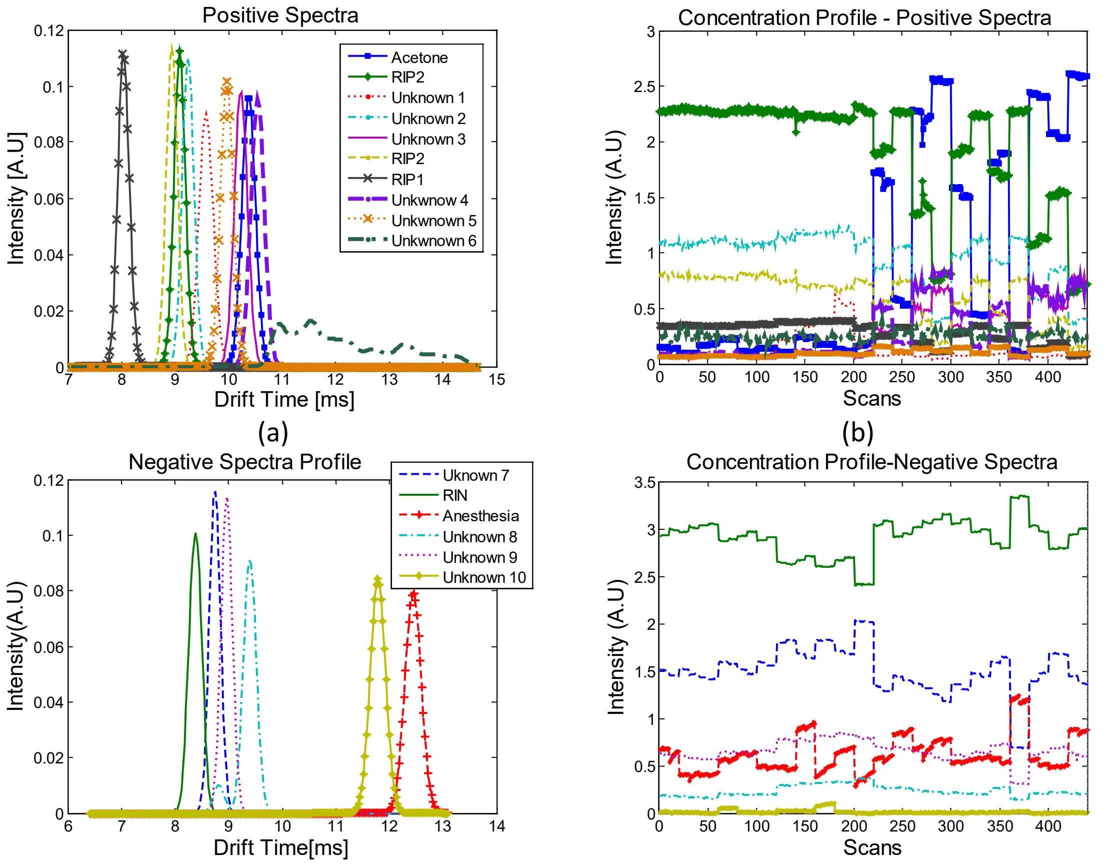
(a) (b)

Fig. 2 Analysis of chromatogram peak area of 10 control rats and 10 rats with sepsis a) Score Plot of PCDA model. b) Plot of Methyl Ciclohexane.



(a) (b)

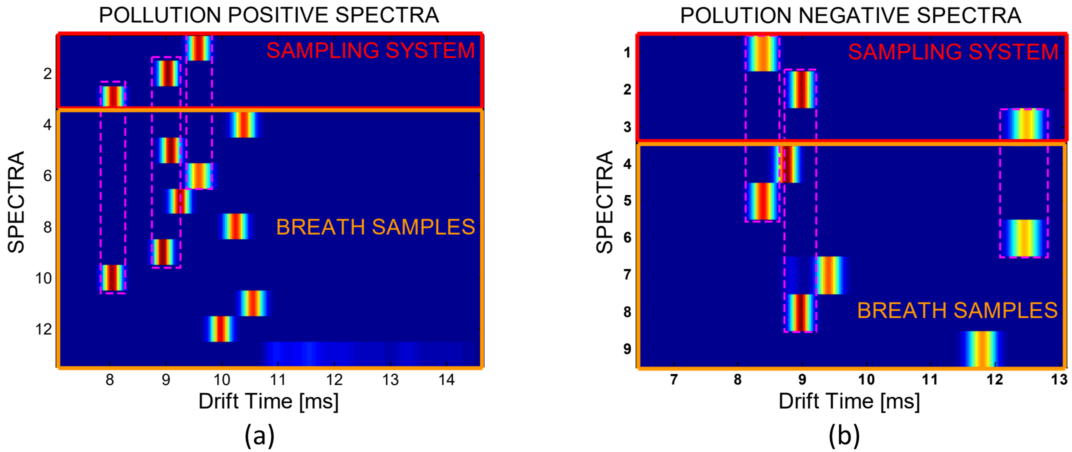
Fig. 3 IMS Spectra of control rats and rats with sepsis a) Positive Spectra b) Negative Spectra



(c) (d)

Fig. 4 Results of MCR-LASSO of IMS spectra of breath samples. a) Positive Spectra Profile. b) Conentratio Profile of Positive Spectra. c) Negative Spectra Profile. d) Concentration Profile of Negative Spectra

Fig. 5 Pollution study of the breath samples. a) Positive Spectra and b) Negative Spectra. The y-axis represents the spectra obtained using MCR-LASSO of breath samples and samples of the sampling system (on the top of the image). Dashed lines represent the spectra present both in the breath sample and the sampling system.



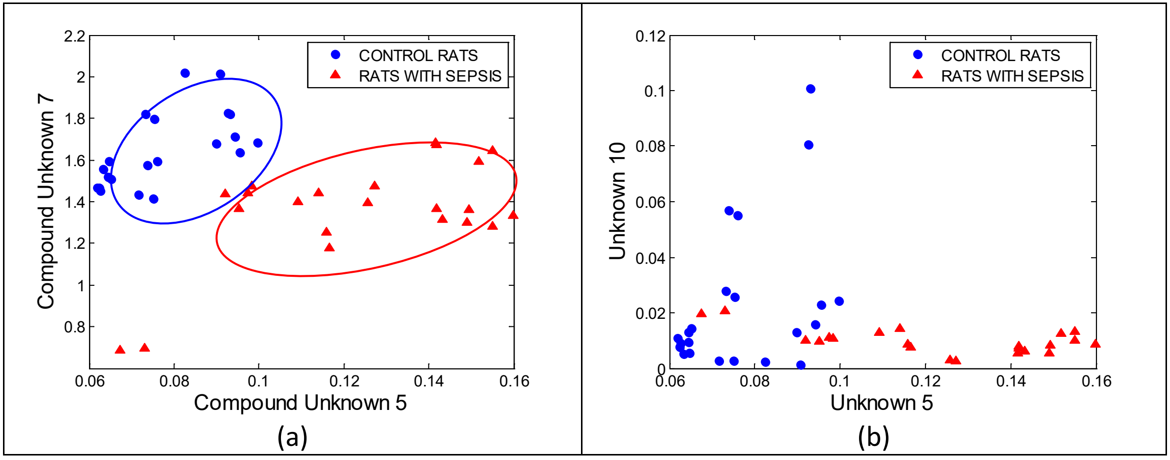


Fig. 6 a) Unknown 7 vs Unknown 5 b) Unknown 10 vs Unknown 5

DAY 1			DAY 2		
TYPE OF SAMPLE	GC/MS	IMS	TYPE OF SAMPLE	GC/MS	IMS
BLANK 1	☒	☒	**BLANK 1**	☒	☒
RAT HEALTHY 6	☒	☒	RAT SEPSIS 1	☒	☒
RAT HEALTHY 7	☒	☒	**BLANK 2**	▨	☒
RAT HEALTHY 8	☒	☒	RAT SEPSIS 2	☒	☒
RAT HEALTHY 9	☒	☒	RAT SEPSIS 3	☒	☒
BLANK 2	▨	☒	RAT SEPSIS 4	☒	☒
RAT HEALTHY 10	☒	☒	RAT SEPSIS 5	☒	☒
BLANK 3	▨	☒	**BLANK 3**	▨	☒
RAT SEPSIS 1	☒	☒	RAT SEPSIS 6	▨	☒
BLANK 4	▨	☒	**BLANK 4**	▨	☒
RAT SEPSIS 2	☒	☒	RAT HEALTHY 7	☒	☒
RAT SEPSIS 3	☒	☒	RAT HEALTHY 8	☒	☒
RAT SEPSIS 4	☒	☒	**BLANK 5**	▨	☒
RAT SEPSIS 6	☒	☒	RAT HEALTHY 9	☒	☒
BLANK 5	▨	☒	RAT HEALTHY 10	☒	☒
			RAT HEALTHY 11	☒	☒
			RAT HEALTHY 12	▨	☒
			BLANK 6	▨	☒

Tabla 2 Measurement in two different sessions of breath samples and blanks.

Reference

1. **Pomareda V, Calvo D, Pardo A, and Marco S**. Hard modeling Multivariate Curve Resolution using LASSO: Application to Ion Mobility Spectra. *Chemometrics and Intelligent Laboratory Systems* 104: 318-332, 2010.