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Diagnostic potential of breath analysis – Focus on the dynamics of volatile organic compounds

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A B S T R A C T

Analysis of exhaled volatile organic compounds (VOCs) holds promise for the development of new and non-invasive procedures in many areas of clinical and home care applications. Recent research mainly focused onto cross-sectional pilot studies with single point measurements in order to detect specific VOC markers or marker sets for diseases such as cancer, infections or diabetes. Actual studies have shown the highly dynamic behavior of exhaled VOC profiles related to factors such as exposure, nutrition, exercise, medication but also to physiology and clinical interventions. Single point analyses cannot take into account neither the fundamentally dynamic nature of exhaled substance profiles nor can they sufficiently reflect the kinetics of exogenous contaminants and confounders and are, therefore, prone to erroneous interpretation, especially if single point analyses are applied for primary diagnostic purposes. These disadvantages cannot be overcome by means of any statistics as complex and sophisticated these algorithms may be.

While unique VOC markers for certain diseases could not be confirmed in independent studies, analytical techniques and tools to identify and monitor VOCs as well as the knowledge on exhaled VOCs have developed significantly during the last two decades of breath research.

As dynamic VOC profiling provides valuable information on kinetics of markers and confounders, it offers huge and so far unexplored potential for physiological, metabolic, therapeutic and environmental monitoring. Driven by new and innovative technologies such as real time mass spectrometry and highly specific sensor systems, future applications may range from home care to ICU monitoring.

1. Introduction

During the last decades breath VOC research was focused on cross-sectional pilot studies seeking to detect diseases such as cancers [1–3], cardio-respiratory disorders [4,5], infections [6–8] and diabetes [9,10]. Some studies recently succeeded in demonstrating the highly dynamic behavior of exhaled VOC profiles related to external factors such as exposure [11], nutrition [12,13], exercise [14–16], medication or clinical interventions. Concentrations of a single VOC and VOC profiles may change acutely in time windows of seconds but may also be related to long term dynamic effects such as excretion of (previously uptaken) contaminations over days and weeks or to diurnal rhythms in relation to regular physiology. In addition, a large number of potentially confounding factors onto VOC profiles have been identified – especially in the clinical environment. Without a deeper knowledge on marker origins, their distribution in the body, effecting vectors, previous intakes and physiological effects it is, therefore, nearly impossible to interpret exhaled VOC profiles in a proper way.

In parallel, analytical techniques for VOC profiling have improved dramatically in the last decades. Since Larson, Pauling and others first analyzed breath samples by means of gas chromatography [17,18], the advancement in analytical technology e.g., in the field of mass spectrometry and separation techniques – has immensely improved the ability to detect, identify and quantify volatile compounds by orders of magnitude. While combined separation techniques such as GC-MS and GCxGC-ToF still represent the gold standard for identification and quantitation of (unknown) volatile biomarkers [19], direct methods e.g., in the field of mass spectrometry have advanced significantly. Today, profiling of the whole spectrum of exhaled VOC in the ppbV/pptV (i.e., mmol/L – pmol/L) range is available with sub-sec time resolutions in real time by techniques such as proton transfer time of flight mass spectrometry (PTR-ToF) [20] and ultra-high resolution mass spectrometry such as secondary electrospray ionization (SESI) –Orbitrap [21].

Despite these developments most published clinical pilot studies aiming at potential diagnostic or medical applications still rely on cross-sectional setups with punctual measurements, often complicated by methodological bottlenecks such as bag sampling techniques at the hospital and later analysis in the laboratory. Due to the huge number of VOCs present in each breath sample, too comparably small patient groups in these setups and due to the highly dynamic behavior of most exhaled VOCs some concentration differences between patient groups

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list of abbreviation

COVID-19 - corona virus disease - 2019
ECC - extracorporeal circulation
ECMO - extra corporal membrane oxygenation
GCxGC-ToF - comprehensive (2D) gas chromatography – time of flight mass spectrometry
GC-MS - gas chromatography-mass spectrometry
HFO - high frequency oxygenation
ICU - intensive care unit
I:E - inspiration to expiration (ratio)
NTME - needle trap micro extraction
PEEP - positive end expiratory pressure
PTR-ToF-MS - proton transfer reaction - time of flight - mass spectrometry
SPE - solid phase extraction
SPME - solid phase micro extraction
SESI - secondary electrospray ionization
VOC(s) - volatile organic compound(s)

and healthy volunteers can always be found for mere reasons of statistical over fitting. When subsequently these differences are claimed as "specific markers" or "specific marker sets", it is not surprising, that not even one of these "highly specific and accurate tests" has ever made it into a clinically suited procedure or a diagnostic test.

Clinical interpretation of exhaled VOC profiles requires concrete and systematic understanding of origins, extrinsic and/or intrinsic influences and their actual effects onto current exhalation profiles. Validation of breath tests with respect to clinically applied procedures does not only require well adapted clinical setups and basic understanding of the described "biomarker" compounds and potential effectors onto the concentration profiles but also profound knowledge on the extremely dynamic behavior of these markers. Measurements of the dynamic behavior of VOC profiles in the exhaled breath are therefore, a key to understand and interpret exhaled biomarkers rationally.

2. Dynamic behavior of exhaled VOCs

Concentration changes in exhaled VOC profiles may mirror a broad range of processes related to different, partially contrasting effects such as normal physiology and metabolism, marker origins, intake of medications/supplements, lifestyle habits, (previous) exposure as well as to pathophysiological processes such as inflammation or infection (Fig. 1).

Due to the highly "volatile" nature of all these effects, VOC profiles are often not stable for longer periods of time but may change immediately (within seconds) and pronouncedly (by orders of magnitude) [22]. While some VOC concentration changes occur within seconds, minutes our hours other changes will take weeks, months or occur throughout an entire lifetime [12,23,24].

2.1. Effects of physiology and metabolism

Our physiological and metabolic states diversely affect exhaled VOC profiles [25]. On many occasions, such effects change exhaled end-tidal metabolite concentrations more pronouncedly than those reported as potential biomarker ranges in cross-sectional clinical studies. Normal physiological fluctuations associated with respiratory and hemodynamic parameters may immediately affect alveolar gas-exchange process and thereby, cause significant changes in VOC exhalations – both qualitatively and quantitatively.

In a series of physiological VOC profiling studies measured with an online PTR-ToF-MS with sub-second time resolution in healthy humans, we systematically demonstrated that any change in subjects' breathing patterns [26], postures [27], expiratory time or flow [28], breathing routes [29], upper-airway resistances [30] and/or respiratory rhythms [31] immediately influence his/her respiratory and/or hemodynamic parameters and thereby, significantly affect VOC concentrations. Fig. 2 demonstrates this behaviour for the most abundant human breath VOCs acetone and isoprene.

Besides normal physiological effects, we also reported that metabolic effects due to natural menstrual rhythms [32], and menopause in women as well as overall healthy aging [23] significantly alter breath VOC concentrations.

2.2. Importance of endogenous marker origins

The origins and human metabolic pathways of endogenous VOCs are largely unknown or putative. Even most abundant VOCs namely, acetone and isoprene have long-suggested major pathways based on *ex-vivo*/physiological models [33]. Such assumptions must be considered with great care as often more than one biochemical pathway may generate the same VOC or pathways suggested from animal models or cell cultures cannot directly be transferred to human in vivo conditions. Recently, the putative origin of exhaled isoprene from cholesterol

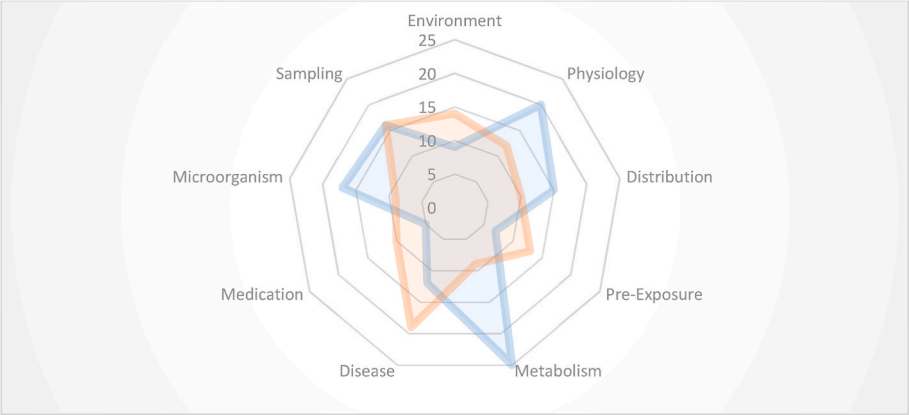

Fig. 1. Key intrinsic and extrinsic factors responsible for the dynamic nature of human exhaled breath VOC profiles. The colored area reflects the concentration of potential VOC biomarker at two different time points A (red) and B (blue). Note that the VOC concentration at point A is lower than at point B despite the fact that the "disease effect" is higher. Reprinted with permission from: W. Miekisch et al. in Volatile Biomarkers for Human Health - From Nature to Artificial Senses, ed. H. Haick, The Royal Society of Chemistry, 2022 [22]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

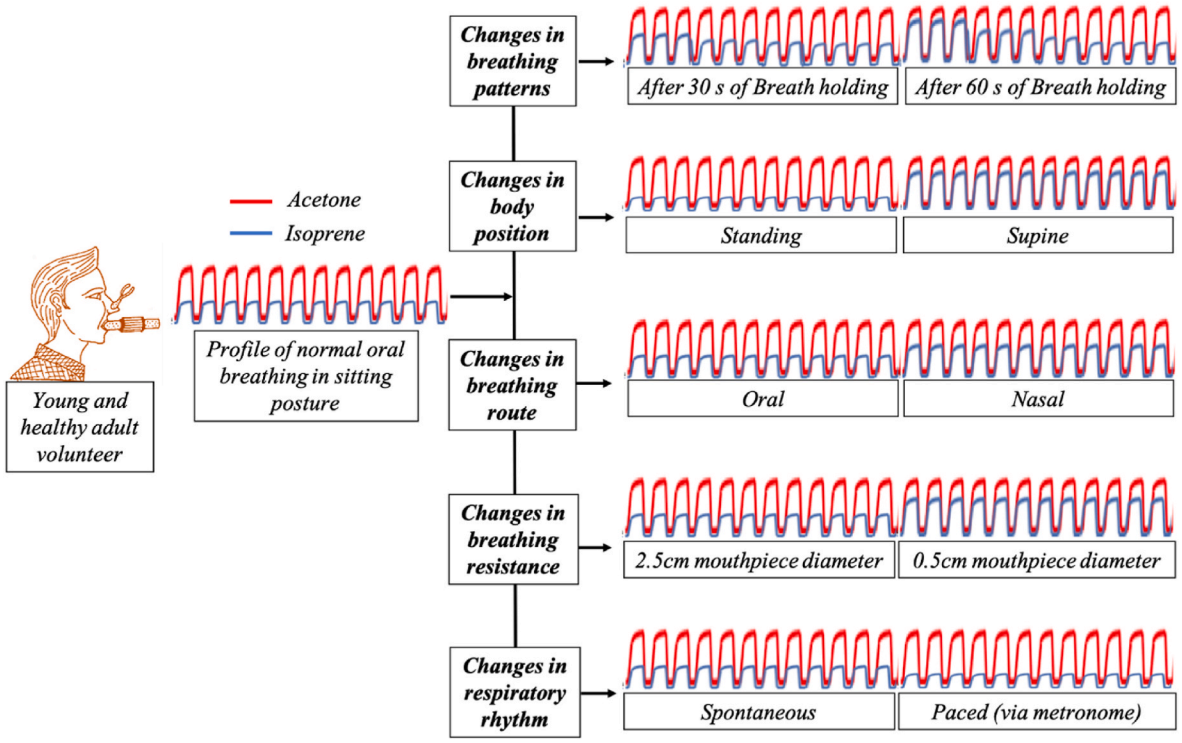

Fig. 2. Dynamic breath to breath changes in exhaled isoprene and acetone due to different physiological maneuvers (breath holding, changes of body position, changes of breathing route, changes in breathing resistance and changes in the respiratory rhythm). Each peak of the red (exhaled acetone) and blue (isoprene) curves represents exhalations (~3 s), each valley an inspiration (~1–2 s) Note that acetone concentrations do not change in this setups. Reprinted with permission from: W. Miekisch et al. in Volatile Biomarkers for Human Health - From Nature to Artificial Senses, ed. H. Haick, The Royal Society of Chemistry, 2022 [22]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

biosynthesis in the liver was disqualified [34]. A multi-omic investigation revealed that it originates from *IDI2* mediated lipolytic cholesterol metabolism in the peroxisomes of skeletal-myocytes [35]. Given the resulting dynamic range of exhaled isoprene (0–450 ppbV) the correct knowledge of metabolic origin is extremely important to understand the dynamic behavior of endogenous VOCs under any investigated condition of interest. The same holds true for many other VOCs described as biomarkers in the literature of the last 30 years. Without basic knowledge on marker origins, metabolism and distribution in the body, interpretation of breath tests may easily be biased or caused by random or external effects.

Further approaches suited to reveal metabolic pathways and origins of relevant VOCs could be the use of isotopically labeled substances and modelling of VOC elimination in adapted models. While a number of isotopically labeled tests are available for exhaled CO_2 [36], this field of research is still in its infancy for exhaled VOCs [37–39]. The development of multi-compartment models may further support the basic understanding of VOC uptake [40] and elimination [41,42] in the human body.

2.3. *Effects of medication, nutrition, and lifestyle habits*

Substance specific changes in exhaled VOCs have been observed In healthy pre-menopausal women during the menstrual cycle or due to the daily use of oral contraceptive pills (containing supplementary female sex hormones), within days [32] or even within seconds due to intravenous administration of drugs such as catecholamines and painkillers [43]. Changes in VOC profiles may also occur within hours or minutes due to metabolic adaptation [44,45]. Uptake of regular food such as high or low fat drinks [12] or diets [46,47] will change exhaled VOC profiles. Due to the effects of diseases or medication (such as insulin) or different genetic/enzymatic conditions concentration changes of metabolic relevant VOCs such as acetone differ between individuals (Fig. 3).

To avoid confounding effects of acute food intake, the test subjects should be advised to avoid eating >2 h before the breath measurements. In addition, special diets or medications (such as insulin) should be well controlled and reported for each volunteer/patient.

Smoking is another important confounder for breath VOC profiles [48,49]. Most inhaled smoking related VOCs such as acetonitrile, benzene or toluene and other hydrocarbons show a dynamic decay after consumption. Due to their highly lipophilic properties and the re-distribution through body compartments, some smoking related

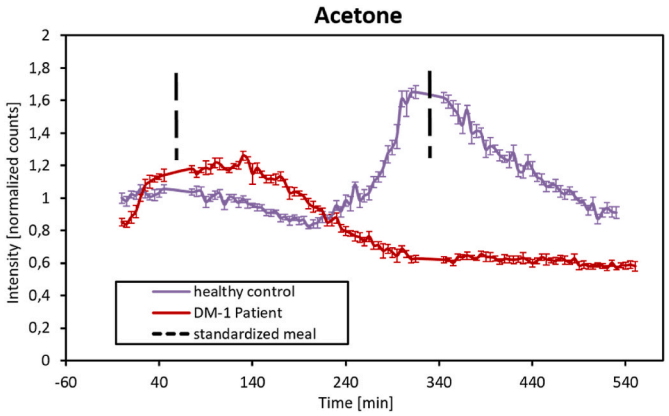

Fig. 3. Normalized one day profiles of acetone in a healthy subject and a DT1 patient; the black barks mark the time of uptake of a regular standardized meal. Reprinted with permission from: W. Miekisch et al. in Volatile Biomarkers for Human Health - From Nature to Artificial Senses, ed. H. Haick, The Royal Society of Chemistry, 2022 [22].

compounds are detectable even weeks after consumption and may bias any breath test if not taken properly into account [50].

2.4. *Effects of environmental uptake and confounding effects in the clinical environment*

A major part of the detectable VOCs in the exhaled breath is meant to come from external sources such as emissions from fuels, plastic materials, traffic or buildings [51]. Depending on their chemical nature and the intended use, plastic materials may emit a broad range of VOCs from (branched) hydrocarbons and aromatic compounds over oxygenated compounds such as alcohols, aldehydes, ketones, acids, esters, ethers to amines or sulfides [52]. The situation is further aggravated in the clinical environment as typical confounders such as disinfectants, plasticizers or volatile (breakdown products) from drugs such as anesthetics may occur in high amounts. Concentrations of those external VOCs may rise or change in very short time periods and by orders of magnitude (Figs. 4 and 5) [53]. These contaminations are then mirrored in the exhaled breath of the patients. Therefore, exhaled VOC profiling must take into account not only the endogenous dynamics of exhaled VOCs

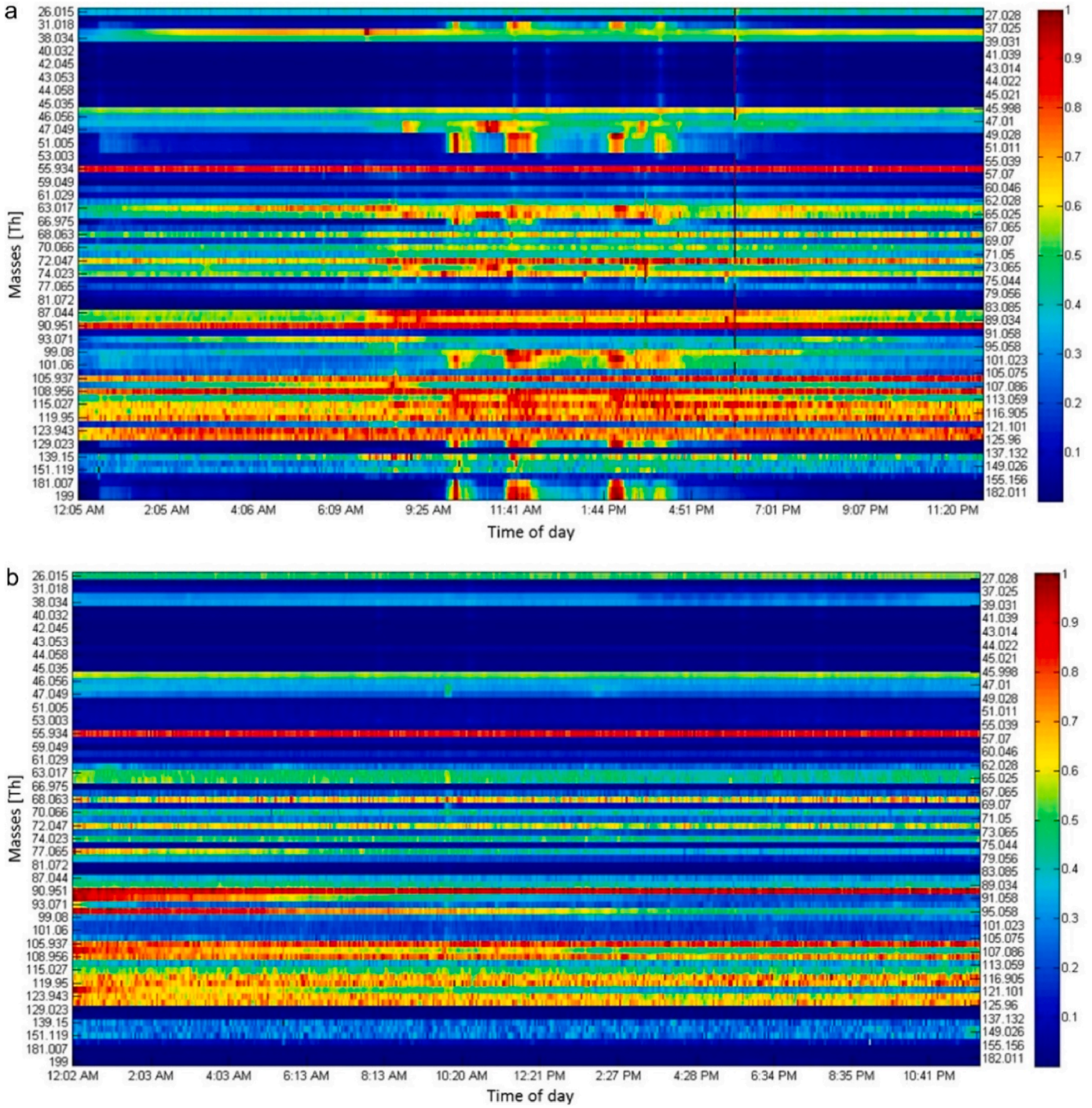

Fig. 4. 24 h VOC concentration changes in the room air of the PACU unit on a working day (a) and a Sunday (b) measured continuously by means of PTR-ToF-MS with a time resolution of 200 ms. Reprinted with permission from Trefz et al. Anal. Chem. 2013, 85, 21, 10321–10329. Copyright 2013 American Chemical Society [53].

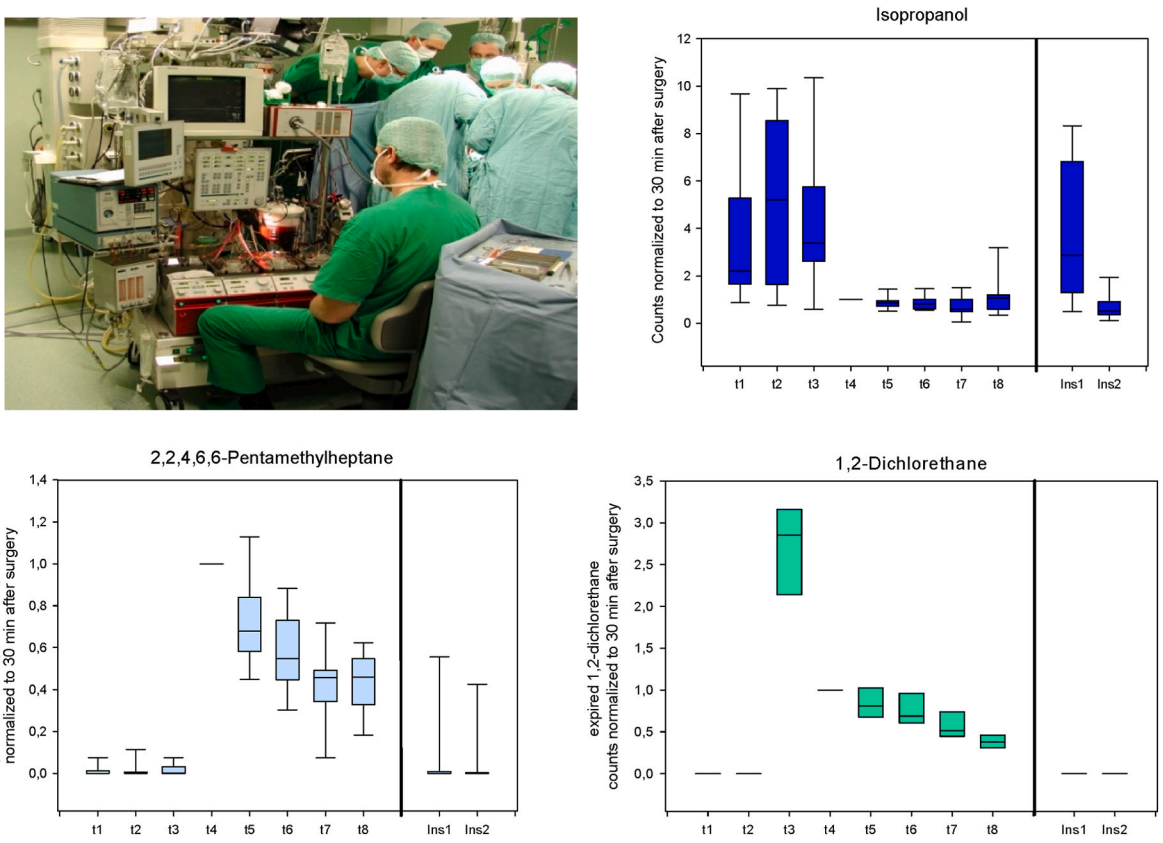

Fig. 5. Dynamic profiles of confounding VOCs in the exhaled breath of patients after cardiac surgery/extracorporeal circulation (ECC, n = 11); t1 = after induction of anaesthesia; t2 = after sternotomy; t3 = 5 min after end of ECC and t4 = 30 min, t5 = 60 min, t6 = 90 min, t7 = 120 min and t8 = 150 min after end of surgery; Ins1 = inspiration during surgery; Ins2 = inspiration in ICU. Adapted with permission from: Mieth et al. Anal. Chem. 2010, 82, 6, 2541–2551 [55]. Copyright 2010 American Chemical Society.

but also observe environmental effects during breath sampling and monitoring. Continuous or repeated monitoring of the dynamic VOC profiles in inhaled air is therefore mandatory for any clinical study. Inhaling filtered air for short time periods does not solve this problem as most substances are taken up via the blood stream and are distributed in different body compartments (tissue, muscle, fat) and will therefore be exhaled for prolonged time periods (hours – weeks) [54].

Even if the room air/inspiratory concentrations are low or no detectable, contaminations from clinical interventions such as administration of intravenous medication via plastic tubing, by protective filters [56], from sterilization of respiratory circuits may occur. Fig. 5 shows an example from measurements before and after heart surgery with extracorporeal circulation (ECC). While the increased concentrations of the disinfectant related isopropanol in the OR can be attributed to the environment by means of room air measurements, branched hydrocarbons (a typical by-products of plastic materials) or small halogenated hydrocarbons are rarely detectable in the inspired air and – therefore – may easily be misinterpreted as "diagnostic" biomarkers. The true origins in this setting were plastic residues from the tubing system for intravenous drug administration and disinfectant residues in the reused sterilized ventilation tubing, respectively, and this can only be revealed by means of dynamic measurements showing the typical washout after precedent exposure. High concentrations (in or above the ppmV range) of disinfectants such as isopropanol or ethanol in room air/inspired air may not only significantly affect exhaled VOC profiles as contaminations. In some cases, the adsorption efficacy of pre-concentration methods such as solid phase extraction (SPE), solid phase micro extraction (SPME) or needle trap micro extraction (NTME) may be reduced and response of sensor or e-nose systems my easily be biased. Even for hyphenated equipment such as real time mass spectrometry (e.g. PTR-MS and SIFT-MS) high concentrations of such volatile contaminants in room air or ventilation systems may lead to reduction or quenching of primary ions in the reaction chamber and, may, therefore, bias the analysis of endogenous exhaled compounds.

3. Analytical techniques suited for dynamic VOC measurements

Several analytical procedures may be applied for breath VOC measurements. Combined analytical techniques such as GC-MS are still regarded as a gold standard for identification of unknown compounds [19]. Techniques such as GC-MS or IMS may be applied for repeated monitoring of exhaled profiles but time resolution will be limited to the minute range and will require high amount of resource. Fast or continuous changes in exhaled VOC profiles can, however, only be monitored by techniques resolving in the sub-minute range. In addition, a continuous monitoring of blood borne VOCs requires either adapted, controlled sampling procedures to assess the tidal breath phase or a sub-second time resolution able to distinguish different breath phases during data processing.

3.1. Real time mass spectrometry

Direct MS analysis with soft ionization such as selected ion flow tube mass spectrometry (SIFT-MS) [57] and proton transfer reaction mass spectrometry (PTR-MS) [58–60] are the most often used technologies for the monitoring of exhaled breath profiles in experimental and clinical settings.

If time resolutions in the sub-second range are applied, no additional procedures for controlled sampling are required as tidal as well as inspiratory phases of the breath cycle can easily be identified during

data processing by adapted automated algorithms [61]. A major advantage of those techniques is the feasibility for continuous side stream monitoring without any further sample preparation or the need to affect the breathing pathway, even in ventilated patients [53]. While early studies of exhaled VOCs and quadruple based techniques had to define a small number of VOCs for real time (sub-second) monitoring [15,61,62], the introduction of ToF based detectors allows the profiling of the whole exhaled volatile VOC profile if the relevant compounds (molecular range from 20 to 500 Da) have a higher proton affinity than water [20]. Due to matrix effects, the quantitative amounts (based on reaction k-rates) of these techniques may have to re-adjusted if extreme conditions – such as high oxygen concentrations during clinical interventions – are applied [63].

Secondary electrospray ionization mass spectrometry in combination with high resolution mass spectrometry (SESI-HR-MS) is a relative new analytical platform with special characteristics such as low detection limits for compounds with relative higher molecular masses (150–3000 Da) [21,64]. In atmospheric ionization sources, the ionization efficiency of VOCs in the exhaled air is affected by the matrix. By introducing VOCs-free gas into the SESI source those effects are minimized. In contrast to PTR-MS and SIFT-MS the ionization probability increases with pressure [21]. The atmospheric pressure in the SESI interface allows the application with most atmospheric pressure ionization ultra-high resolution mass analysers (>100,000) such as Orbitrap [65]. This opens up new perspectives in breath analysis for both better identification of measured VOCs as well as the detection of (probably water or particle bound) larger molecules in the exhaled aerosols such as proteins. If quantitative correlations of this larger compounds to systemic levels can be established has still to be investigated. A current drawback of the SESI-HR-MS is the lack of an interface for direct continuous measurements in the side stream.

3.2. Sensor and sensor like technologies

Once potential VOC biomarkers are identified, sensor or sensor like technology based technologies can be developed to monitor single compounds. Target-specific detection in and real-time monitoring is often achieved with a combination of different coating materials or and the combination of different sensing techniques sensors including electrochemical, chemoresistive, and optical methods [66,67]. The combination of high selectivity, quick response times and VOC sensitivities in the ppbV range still represents a challenge for this kind of technology [68]. Also the selective detection of certain compounds out of complex VOC mixtures represents a challenge due to the sampling matrix (e.g. humidity, temperature) and dynamics of the VOCs contained [69]. Using multiple sensors as detection platform may improves the specificity through elimination of some interferences [70]. For dynamic monitoring e.g. combined photo-acoustic based sensor systems show promising results [71–73].

3.3. Methodological requirements for robust breath monitoring

Controlled sampling of the alveolar portion is mandatory if blood born VOC markers are to be accessed [74]. Breathing against resistance leads to altered VOC patterns in breath [30], so forced or restricted breathing, e.g., blowing through tubing of narrow diameter, or filling bags via a small orifice, must be avoided during sampling. Side-stream sampling can overcome this limitation. Furthermore, implementing custom respiratory rhythms, such as spontaneous breathing i.e., preceded by a minute of paced respiration (i.e., performing 10–12 breaths/min via visual or audible clues), does reduce ventilatory variations, significantly lowers the inter-breath variability [31] and, facilitate more accurate identification or extraction of breath phase being targeted. Besides the well-investigated contribution of ammonia from the oral cavity or nitric oxide from in the sinuses, comprehensive effects of respiratory routes on exhaled VOCs are studied recently via real-time mass-spectrometry [29]. In addition to exploring the potential origins and pH dependency of ammonia, concern about other VOCs that primarily originate in the oral or nasal cavity to influence (and confound) breath that is sampled via the corresponding breathing route. Substance generated by oral/nasal microbiota and physiological effects due to difference in upper-airway deadspace ventilation are important aspects in selecting the sampling route of interest [29]. Thus, while sampling breath from spontaneously breathing awake humans in cross-sectional and longitudinal studies, subject's posture, breathing patterns, kinetics and flows, respiratory route and rhythms must remain constant (wherever possible) as well as instrumental resistance against normal breathing must be set to minimal as possible.

The above recommendations differ in mechanically-ventilated subjects where, controlled inspiratory to experiatory (I:E) ratio and hemodynamic modes of intensive care and conduct must be determined case/study wise e.g., based on the presence/requirement of positive end expiratory pressure (PEEP), high frequency oxygenation (HFO) or extra corporal membrane oxygenation (ECMO) interventions etc.

Similar to physiological effects, normal metabolic state of a subject may cause marked differences in exhaled VOC concentrations. Metabolic adaptation and diurnal response depicted immediate effects on endogenous VOC exhalation in a longitudinal setup [44]. It was clearly observed that correlations of certain VOCs to blood parameters varies significantly based on the time of sampling. Thus, the sampling time of the day must be kept constant for both individual and cross-sectional metabolic comparisons. Studies incorporating female subjects should account for the menstrual cycle phase and presence of oral contraception [32]. Observed effects on VOC concentrations just due to the difference in age [24] have exceeded many limits/ranges that are proposed as biomarkers for various diseases. Therefore, subject's metabolic assessments must report in line with corresponding age effects.

In general the parallel control of inspired air/room air samples is mandatory to take into account effects of acute/previous exposure onto exhaled breath profiles (cf. section 2).

4. Potential applications for dynamic VOC profiling

Endogenous VOCs may be produced in different parts of the body, from the proximal airways to the interior organs including the gut microbiome. Most VOCs generated at the cellular level are transported to the lungs via the blood stream. In the alveoli, VOCs are then transferred into the gas-phase according to their solubility and volatility controlled by membrane diffusion capacities. These VOCs then pass through the lower and upper airways and are exhaled within a water saturated mixture of nitrogen (N_2), oxygen (O_2) and carbondioxid (CO_2). Exhalation takes place within seconds or minutes after substance generation and, therefore, enables monitoring of different processes in the whole body [75]. Endogenous VOC markers commonly proposed for diagnostic purposes are hydrocarbons such as ethane, pentane and isoprene, oxygen-containing compounds like acetone, acetaldehyde, methanol, ethanol and propanol, sulphur containing compounds such as dimethylsulfide, methyl and ethyl mercaptanes and carbon disulfide; and nitrogen containing substances like ammonia and dimethyl/-trimethylamine [76]. As they easily outnumber cells in the human body, bacteria in different compartments such as airways, the oral or nasal cavity, gut or lung may add major contributions to exhaled VOC profiles [77–80].

In order to assess physiological meanings and diagnostic potential of these substances, biochemical pathways of generation have to be known. Most pathways are, however, still only tentatively identified and rely on in vitro experiments. The unique dynamic nature of exhaled biomarkers opens up a wide field for potential applications.

4.1. Monitoring to track physiological processes

Based on the origins and physicochemical characters (e.g., solubility,

volatility and blood-gas partition-coefficients etc.) dynamic VOC profiles diversely mirror cardiac output and minute ventilation. Recent studies have demonstrated that physiological changes induced by different breathing patterns and/or body postures alter breath profiles. Exhaled concentrations of substances with low aqueous solubility and high volatility (e.g., isoprene) have changed immediately due to posture's influence on cardiac output and pulmonary distribution of ventilation, whereas compounds with high aqueous solubility (e.g., acetone) remained minimally affected [27]. Similarly, changes in ventilation patterns, such as hyper- or hypoventilation [81], or ventilation maneuvers, such as deep inhalations or forced exhalations [28], initiate an immediate change in breath VOC patterns. Breath holding, for example, can induce a two-to threefold increase in the concentrations of perfusion dependent volatiles, but does not affect other VOCs [26]. Dynamic breath profiles are able to track regular physiological changes such as increase or decrease of cardiac output or changes in the ventilation of the lung. Several studies describe the monitoring of VOC profiles under exercise [14,15] and conclude that hemodynamic and ventilatory changes may be mirrored by substances with high vapour pressure such as isoprene. In this context, even emotional processes such as sexual arousal [82] may also become traceable via breath monitoring.

4.2. Dynamic personalized home care and life style monitoring

The anaerobic threshold, currently determined by (blood based) lactate tests or respiratory coefficients may become accessible via dynamic breath monitoring [83]. Several studies report a maximum of exhaled acetone close to the anaerobic threshold in exhaustive exercise caused by the metabolic shift of energy consumption [14,16].

While looking at gender specific aspects, a recent study has demonstrated that endocrine changes during natural menstrual cycle and/or daily intake of oral contraceptive pills diversely affect VOC profiles [32]. Another study on healthy female aging has depicted pronounced differences in specific VOCs based on various stages of life and irreversible life events e.g., menopause [23]. Changes in bone density in elderly are reflected by changes in exhaled VOC profiles and probably related to changes in the gut microbiom [23]. A dynamic monitoring of female VOC profiles, therefore, bears the potential for assessing hormonal changes or menopausal events.

Identification of related marker compounds as well as the non-invasive nature of breath test give perspectives for a personalized m-health based monitoring at home. As longitudinal measurements rely on changes in exhaled concentrations rather than on absolute values, the general problem of broad inter-individual variations in breath biomarker levels can be overcome.

4.3. Monitoring to access exposure and drugs

For those compounds with a reasonable volatility/vapour pressure detection in the exhaled breath is possible and similar to the detection of breath alcohol intake of these compounds can be detected quantitatively [84]. Smoking and vaping generate own volatile emissions [42,85]. These emissions are taken up into the human body via the lung/blood interface. Some of them may be adsorbed or metabolized in the body [86], others act as direct confounders onto exhaled VOC profiles (see also section 2.3). Typical smoking related compounds such as acetonitrile or aromatic compounds are detectable for several days in exhaled breath profiles and may be used to prove active smoking or recent exposure to cigarette smoke [49,51,87]. Thermal decomposition and fragmentation products associated with vape exposure (first or second hand) are also detectable in exhaled VOC profiles [88]. Also the detection of d9-THC from previous use of marijuana is possible via breath analysis [89,90]. Dynamic monitoring of these VOCs typically shows a decay of related compounds reflecting washout after recent exposure. If pathways and involved biochemical systems are known, exogenous compounds could therefore even be used to assess parameters such as enzymatic activity in a dynamic setup.

Some other compounds, e.g. larger and more polar medical drugs, can only by detected qualitatively due their adherence to water droplets in the exhaled breath. As most of them are non-volatile, effects of these drugs are mainly due to the induced changes in hemodynamics or ventilation. If systemic/blood concentrations of these medical compounds are to be assessed, pharmacodynamic effects such as plasma binding or (re-) distribution have to be taken into account to avoid misinterpretations of breath levels [91]. Beside the detection of drugs of abuse [92,93], dynamic tracing of medical drugs and related volatile metabolites [94–96] may open interesting perspectives for pharmacodynamics investigations.

4.4. Monitoring of infections

Fig. 6 shows the in vivo profiles of seven VOCs during an influenza A infection in a controlled animal model [97]. Notably, the resulting peaks in the VOC profiles occur before clinical symptoms are detectable but only at a single time point in the course of the infection (day 4). The origin and increase of the relevant marker substances could be confirmed by related in vitro studies in infected cells [98].

In contrast to single point measurement repeated or continuous monitoring of exhaled VOC profiles would not only be able to indicate the infection status but also to observe potential therapeutic interventions.

VOC profiles during bacterial infections also show a highly dynamic behaviour. In large animal models mycobacterial infections yielded varying breath profiles reflecting the host response while emissions from faecal samples reflected the causing bacteria. Dynamic changes in these profiles could be observed over a period of several weeks [99].

Recent pilot studies in humans have reported specific marker sets for the (early) detection of CoVID-19 [70,100–102]. The proposed marker sets differ qualitatively and quantitatively and none of the proposed methods or marker sets could be confirmed in independent prospective validation studies under screening conditions. As the virus has not any metabolism of its own, VOCs potentially exhaled during infection mirror virus-host or virus-host-microbiome interactions. The host response depends on the individual and the time (relative to the start of infection), is highly dynamic and will differ between individual patients. Hence, the occurrence of stable and specific marker sets is highly improbable and a broad variance of VOC markers related to the host response and the dynamic behaviour of exhaled VOCs is to be expected. This behaviour of related VOC markers has been observed in measurements in a COVID-19 test centre with >700 test subjects under real screening conditions and a realistic disease pre-relevance [103]. As the host response is often not highly specific for a single causing pathogen, other respiratory infections may cause similar changes in breath profiles [104]. Therefore, monitoring of infection processes or effects of therapeutic actions can realistically be realized through dynamic analyses rather than identifying the causative agent by means of single point measurements.

4.5. Therapeutic monitoring

In contrast to single point measurements, adapted dynamic monitoring of exhaled VOC concentration changes may help to improve disease monitoring and to support therapeutic actions. At the intensive care unit (ICU) continuous monitoring of VOC profiles can be applied to mirror changes during lung recruitment maneuvers (Fig. 7) [105] but also to stage post-operative patients undergoing analgesic treatment [43].

While the technical options to analyze and monitor VOCs improved dramatically during the last decades (e.g. with the development of real-time mass spectrometry and highly specific sensors), basic knowledge on the origin of marker compounds and knowledge how (and how many) of these compounds end up in the breath is still in its infancy. (Continuous)

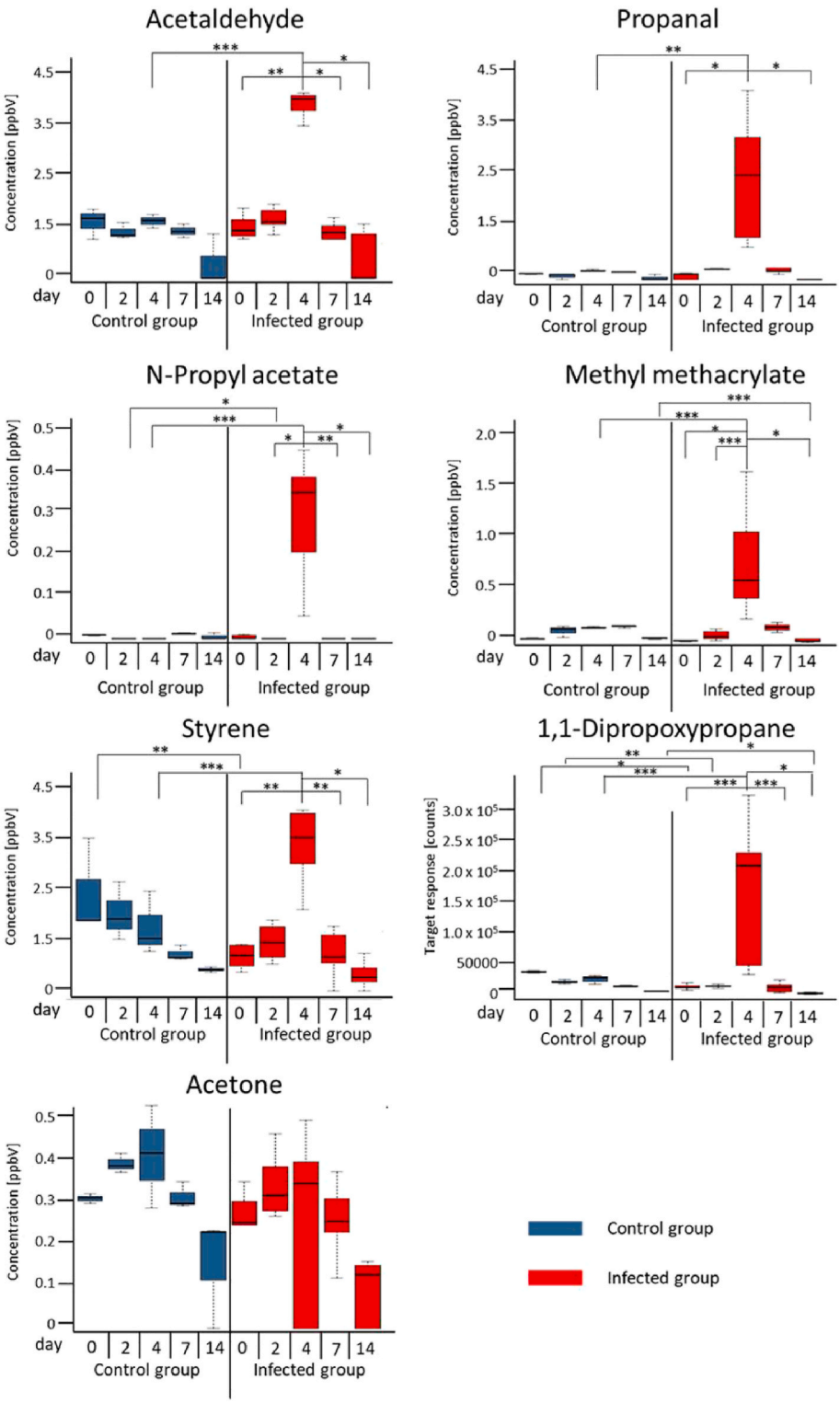

Fig. 6. In vivo changes in selected VOC concentrations during a viral H1N1 infection in a controlled animal model (pigs). Reprinted with permission from: Traxler et al. Sci Rep. 2018 5; 8(1):14857 [97].

dynamic monitoring of VOC profiles by state-of-the-art analytical techniques such as real time mass spectrometry does not only enable better understanding of exhaled biomarkers but may also open new paths to potential applications ranging from personalized metabolic and physiological monitoring to the improvement of monitoring of infections and therapeutic actions in clinical settings. In contrast to single point measurements, continuous or repeated monitoring has a huge potential to improve clinical tests and support therapeutic actions.

5. Conclusion

Breath analysis can provide insights into the human body through a noninvasive window. Physiological as well as pathological processes may be assessed in this way. However, results in terms of VOC or other

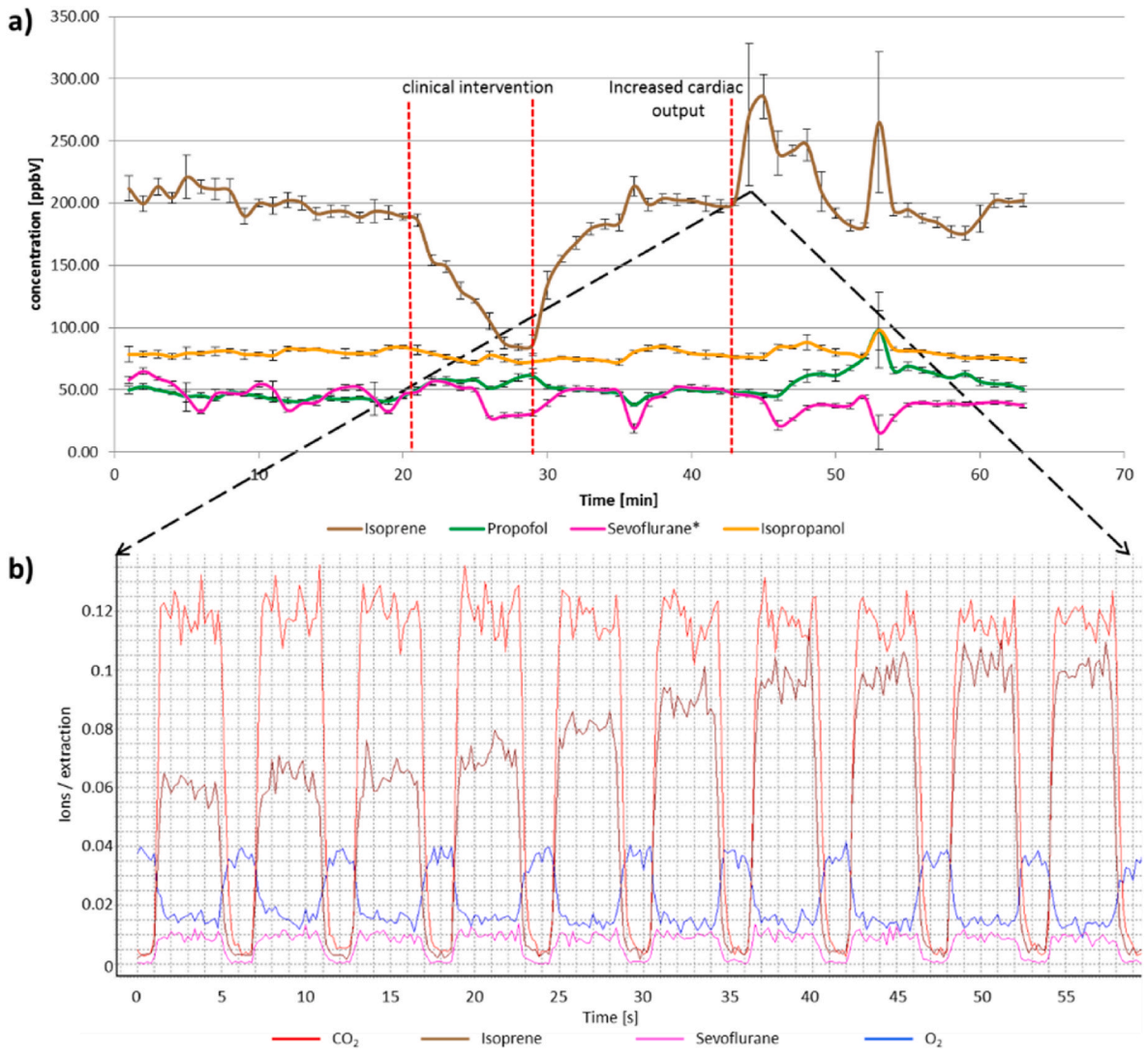

Fig. 7. Changes in breath profiles of a ventilated ICU patient during recruitment of the lung (clinical intervention) and administration of catecholamines (a). Note that induced concentration changes of selected marker compound occur in a time frame of seconds-minutes (b). Reprinted with permission from Trefz et al. Anal. Chem. 2013, 85, 21, 10321–10329. Copyright 2013 American Chemical Society.

substance profiles are not constant but may reflect very different effects. One unique feature of any VOC/substance emission through living organisms is the highly dynamic and quickly changing character of breath composition. In clinical environments, conditions are further complicated through high amounts of confounding factors such as high concentrations of disinfectants in ambient air and unforeseeable effects of actual or recent exposition to various exogenous substances.

Single point analyses cannot take into account the fundamentally dynamic nature of exhaled substance profiles and sufficiently reflect the kinetics of exogenous contaminants/confounders and are, therefore, prone to erroneous interpretation. This holds especially true when single point analyses are applied for primary diagnostic purposes. These disadvantages cannot be overcome by means of any statistics as complex and sophisticated these algorithms may be.

Setups of clinically oriented studies must, therefore, take into account these dynamic effects, and the numerous confounders occurring in the clinical environment. In addition, substance origins, physiochemical properties of exhaled substances, and clinical relevance in terms of already existing tests have to be assessed properly.

As dynamic VOC profiling provides valuable information on kinetics of markers and confounders or even the expression of genetic or enzymatic variability, it offers huge and so far unexplored potential for physiological, metabolic, therapeutic and environmental monitoring. Driven by new and innovative technologies such as real time mass spectrometry and highly specific sensor systems, future applications may range from home care to ICU monitoring.

CRediT authorship contribution statement

Wolfram Miekisch: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pritam Sukul:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Jochen K. Schubert:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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