

# Diagnosis of acute respiratory distress syndrome by exhaled breath analysis

Lieuwe D. J. Bos<sup>1,2</sup>

<sup>1</sup>Department of Respiratory Medicine, <sup>2</sup>Department of Intensive Care, University of Amsterdam, Academic Medical Center, Amsterdam, The Netherlands

Correspondence to: Lieuwe D. J. Bos. Meibergdreef 9, Room: M0-127, 1105AZ Amsterdam, the Netherlands. Email: l.d.bos@amc.uva.nl.

**Abstract:** The acute respiratory distress syndrome (ARDS) is a complication of critical illness that is characterized by acute onset, protein rich, pulmonary edema. There is no treatment for ARDS, other than the reduction of additional ventilator induced lung injury. Prediction or earlier recognition of ARDS could result in preventive measurements and might decrease mortality and morbidity. Exhaled breath contains volatile organic compounds (VOCs), a collection of hundreds of small molecules linked to several physiological and pathophysiological processes. Analysis of exhaled breath through gas-chromatography and mass-spectrometry (GC-MS) has resulted in an accurate diagnosis of ARDS in several studies. Most identified markers are linked to lipid peroxidation. Octane is one of the few markers that was validated as a marker of ARDS and is pathophysiologically likely to be increased in ARDS. None of the currently studied breath analysis methods is directly applicable in clinical practice. Two steps have to be taken before any breath test can be allowed into the intensive care unit. External validation in a multi-center study is a prerequisite for any of the candidate breath markers and the breath test should outperform clinical prediction scores. Second, the technology for breath analysis should be adapted so that it is available at a decentralized lab inside the intensive care unit and can be operated by trained nurses, in order to reduce the analysis time. In conclusion, exhaled analysis might be used for the early diagnosis and prediction of ARDS in the near future but several obstacles have to be taken in the coming years. Most of the candidate markers can be linked to lipid peroxidation. Only octane has been validated in a temporal external validation cohort and is, at this moment, the top-ranking breath biomarker for ARDS.

**Keywords:** Acute respiratory distress syndrome (ARDS); breath analysis; metabolomics

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## Acute respiratory distress syndrome (ARDS)

The ARDS is a complication of critical illness that is characterized by acute onset, protein rich, pulmonary edema (1). The clinical diagnosis is established based on the radiological, physiological and clinical criteria summarized in the Berlin definition (2): (I) acute onset of hypoxemia characterized as a  $\text{PaO}_2/\text{FiO}_2$  below 300 mmHg; (II) bilateral opacities on chest radiography consistent with pulmonary edema; and (III) cardiac dysfunction is insufficient explanation for the previous findings. ARDS is a frequently encountered problem on intensive care units,

with about 10% of the patients fulfilling the criteria (3). Hospital mortality remains about 40%, despite improvements in ventilatory support (3).

The management of patients with ARDS currently includes all measurements taken for the general critically ill ICU population in combination with adjustments of the ventilator settings to reduce additional lung injury via mechanical ventilation (4). In patients with more severe hypoxemia ventilator induced lung injury can be further reduced through prone positioning or neuromuscular blockade (5,6). None of the many pharmacological interventions that have been tested have shown any benefit

in survival for those treated with the drug up to now (7). The non-superiority of these interventions may be explained by two facts inherent to the diagnostic criteria for ARDS. First, ARDS is a biologically heterogeneous syndrome. This is supported by the plethora of pathological diagnoses that can be made in post-mortem studies of the lung of patients with ARDS (8,9). Furthermore, biological data from living ARDS patients suggest that there are at least two subtypes of patients, with different biological profiles and clinical characteristics (10-12). Second, ARDS is only diagnosed when the lungs are already flooded with edema. This is fundamental to the use of hypoxemia and chest radiography as diagnostic criteria. Pharmacological interventions frequently aim to limit the formation of pulmonary edema, but might be less effective when edema has already formed. Thus, while the definition is very well equipped for the epidemiological description of the syndrome it might not be suited for the selection of patients that might benefit most from specific treatments.

In this review, we will review the possibility of using breath analysis as an early diagnostic tool for ARDS. There is still limited data on the diagnostic accuracy of breath tests, but we will provide a rationale on why the technique required further investigation.

### Prediction and/or early diagnosis of ARDS

Prediction and early diagnosis both involve the quantification of the risk of an individual patient on the development of ARDS, before the patient fulfills the clinical criteria. The main difference between prediction and early diagnosis is that the former aims to assess the probability of ARDS in patients who do not show any signs of the disease, while the latter identifies pathophysiological phenomena that facilitate an earlier diagnosis.

The lung injury prediction score (LIPS) has been the best effort up to now to construct a prediction tool for ARDS based on clinical characteristics (13). It performed well in terms of discrimination in an internal and external validation cohort and reached an area under the receiver operating characteristics curve of around 0.80 (13). However, in several other studies where the tool was used to select patients at risk for ARDS the predicted probability did not correspond to the observed probability, with a much lower prevalence than expected (14,15). The combined results show that prediction based on clinical variables alone might be insufficient and that it might need to be combined with early diagnosis (and thus detection of

pathophysiological changes).

Biomarkers in plasma might be suitable candidates as markers for early diagnosis of ARDS as plasma is easily obtained and can be analyzed quickly. Angiopoietin-2 was found to be higher in patients in the emergency room who fulfilled the criteria for ARDS in subsequent days than in those who did not (16). More importantly, it added to the diagnostic accuracy of LIPS with a combined area under the ROC curve of 0.84. However, promising the results of this study, the study population was small and the findings are not yet reproduced independently, so the diagnostic accuracy of angiopoietin-2 requires careful consideration and it cannot be implemented into clinical practice at this moment.

Broncho-alveolar lavage (BAL) fluid might contain more information on the pathophysiological processes leading to ARDS than plasma. Indeed, landmark studies from the previous century suggest that inflammation is limited to the lung in the early phases of ARDS and that compartmentalization is only lost after severe pulmonary damage has occurred (17,18). However, the logistics of obtaining BAL fluid, or any other alveolar sample for that matter, are too difficult to use as an early diagnostic tool in patients at high risk for ARDS.

### Exhaled breath analysis

The exchange of oxygen and carbon dioxide is the primary function of the lung. These molecules, together with nitrogen and water, attribute for almost all the volume in breath. However, upon deeper inspection breath contains hundreds of lower abundance compounds present in the range of part-per-trillion to part-per-million. Many of these molecules are volatile organic compounds (VOCs); organic molecules produced through the human metabolism or by the microbiome (19), others are inhaled from the environment or degradation products of medications or toxins. VOCs in the exhaled breath have been linked to glucose metabolism (20), cholesterol metabolism (21), lipid peroxidation (22), bacterial growth (23,24) and malignant cell growth (25).

The major advantage of exhaled breath analysis over analysis of biomarkers in fluids are: (I) the material is easily obtained; (II) the sampling procedure can be repeated endlessly; (III) the detection method can be performed bedside after the exact biomarker is known or when only the pattern of molecules is studied and not the individual molecules; and (IV) the results are available (almost) without

delay. As we explore these advantages, several reservations should be made on their application, which also highlight the disadvantages of breath analysis.

Exhaled breath is easy to obtain as we exhale about two thirds of the time. However, as recently has been summarized in a technical standard from the European Respiratory Society, breath needs to be collected using the same protocols with regard to flow, volume, resistance and sampling materials in each patient (26). This results in practical problems as patients with shortness of breath cannot exhale the same way as healthy volunteers or even hospitalized patients without pulmonary symptoms do. Therefore, the sampling method should be optimized for the target population and not for healthy subjects.

The sampling procedure can be repeated frequently, but each time breath has to be analyzed on-line or it has to be stored (19). Storage can be done for a short period of time in special bags, which limit the contamination of the sample (27). For longer storage, the VOCs have to be loaded onto a storage tube filled with an absorbent material such as Tenax (28,29). Another advantage of storage on an absorption tube is that the sample can be concentrated by, for example desorbing a volume of 1L of VOCs from exhaled breath into 10 mL of nitrogen gas. The major disadvantage of storage is that the molecular composition of the mixture will change and that it might not represent the clinical situation anymore, where an answer is required in minutes rather than days. For biomarker discovery studies, storage and transport of a sample to an analytical chemistry facility is typically necessary, but this further complicates the direct application of results into clinical practice.

The detection of a VOC is possible at the bedside, but only after the target VOC is known. Untargeted analysis of exhaled breath requires metabolomics platforms such as gas-chromatography and mass-spectrometry (GC-MS); laborious machines that require expertise for operation. When discovery studies provide one or several target molecules other analytical methods can be used to detect those at the bedside [such as compact GC, laser spectroscopy based methods or selective biochemical sensors; for a review on the techniques see reference (19,26)]. An alternative method for the detection of profiles of VOC is electronic nose (eNose) (30). eNoses do not separate, identify or quantify individual VOCs, but rather respond to the mixture of gasses. It received its name after the similarities with mammalian smell; we don't smell one molecule but recognize mixtures and couple those patterns to conclusions. The eNose works in a similar way; it is

trained to recognize patterns of "smells" and couples those to specific disease states. If new mixtures are presented to the eNose, it will compare those to the already learned patterns and provide a probability of diagnosis. eNoses have been successfully trained in the recognition of several pulmonary disease states (31-33).

In theory, the results of a breath test can be readily available at the bedside. However, the current technological readiness level makes the analysis of each sample cumbersome and very time consuming as GC-MS analysis requires extensive calibration and the statistical analysis is highly sophisticated. A bedside diagnostic result can only be provided when the three previous steps are harmonized and have led to acceptable results (*Figure 1*).

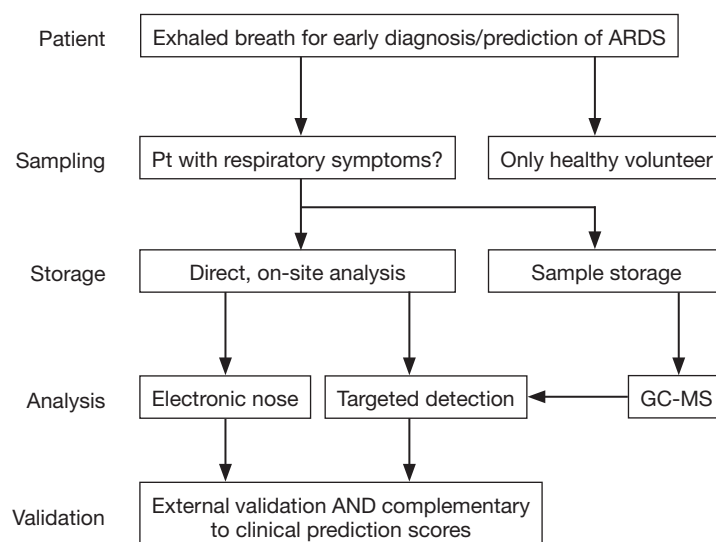
### Breath sampling in intubated and mechanically ventilated patients

Multiple sampling methods have been used for breath collection in the ICU, ranging from a manually operated switch on the expiratory valve (34,35), automated CO<sub>2</sub> regulated alveolar sampling (36), continuous breath collection with a side stream connector close to the endotracheal tube (37) and through an endotracheal suction catheter (38). These methods have not been compared head-to-head for the diagnosis of ARDS. Based on physiological arguments, it is reasonable to prefer CO<sub>2</sub> based methods for sampling alveolar air for the detection of blood-borne VOCs (19,26). For ARDS however, it is not established if this provides more information on top of analyses of the whole exhaled breath (including the dead space portion).

### Exhaled breath metabolites of interest

#### *Isoprene*

Isoprene was proposed as an exhaled breath marker for ARDS in 1998 (34). It was hypothesized that isoprene was produced through cholesterol metabolism and experimental data showed that these pathways were also altered in patients with ARDS (34,39). However, isoprene has never been validated as a diagnostic marker afterwards and a recent study could not replicate the results (40). Other groups have suggested that isoprene originates from muscle cells rather than cholesterol metabolism under most circumstances and this may be a huge confounder (41). It may be speculated that isoprene was a marker when ARDS patients were still regularly paralyzed and the contribution



**Figure 1** Challenges in exhaled breath analysis for the early diagnosis or prediction of ARDS. ARDS, acute respiratory distress syndrome; GC-MS, gas-chromatography and mass-spectrometry.

of muscle activity was minimal, but that this signal was diluted with 21<sup>st</sup> century sedation protocols and decreased use of muscle paralysis.

### Alkanes

Alkanes in exhaled breath are known to originate from lipid peroxidation (42). Ethane, the smallest alkane, was identified as a marker of oxidative stress more than 50 years ago (43). Since, we have learned that peroxidation of different fatty acids results in different profile of alkanes (42). Octane was shown to be predictive of ARDS in a cohort of ventilated ICU patients and this result was validated in a temporal external validation cohort (40). Only the peroxidation of oleic acid results in the production octane (42,44). Observational studies have shown that oleic acid and lipid peroxidation are increased in plasma of patients with ARDS (45), which might explain why only octane is increased rather than all alkanes. Furthermore, oleic acid is a well-known compound to experimentally induce ARDS in animals and has profound effects on oxidative stress itself (46-48).

### Acetaldehyde

Acetaldehyde was identified as a marker of ARDS in the same study that provided octane as a marker (40). Acetaldehyde has been linked to bacterial metabolism

*in vitro* (23), but airway colonization was not a confounding factor. Several recent studies suggest the enrichment of *Enterobacteriaceae* in the lungs of ARDS patients and it could be speculated that these communities contribute to the exhaled concentration of acetaldehyde (49,50). Alternatively, acetaldehyde can be produced by leukocytes (51), but little is known about the VOCs produced by, for example, activated neutrophils.

### Ethylene

Ethylene is a well-established marker of lipid peroxidation and its production is independent of the type of unsaturated fatty acid that is oxidized (52). Ethylene was not directly linked to ARDS development in clinical studies but was increased during events known to cause oxidative stress during cardiac surgery and systematic inflammatory response syndrome (53,54). Interestingly, ethylene is a very early marker of oxidative stress and seems to increase almost instantaneously, making it more suitable for monitoring purposes.

### Other

Exhaled breath was studied in several animal models that can be regarded surrogates for acute lung injury (55). LPS and mechanical ventilation were used as “hits” to provoke acute lung injury. A large range of changes was found in the breath of the injured rats, when compared to control

animals (56,57). Most of these molecules can be linked to changes in metabolism of amino acids or oxidative stress. However, the exact molecules do not match those found in human experiments. It can be hypothesized that rodents have, for example, a different composition of fatty acids in their lungs and therefore produce other lipid peroxidation products, but this theory is highly speculative. The discordant findings might also just represent the difference between ARDS in humans and experimentally induced lung injury in rodents (55).

## Contaminants of breath

### *Anesthetics*

Anesthetics such as propofol, isoprene and sevoflurane are detectable in the exhaled breath of patients who have been exposed to these molecules. Propofol is administered intravenously and passes the blood-air barrier in the lungs. The concentration of propofol in breath is proportional to the blood concentration (58-60). The concentrations are quite low (parts-per-billion) and do not disturb the rest of the exhaled breath signal. Inhaled anesthetics on the other hand can be omnipresent in exhaled breath, even when not administered anymore at the moment of sampling. Sevoflurane has been found in the breath of health care professionals working in the operating rooms (61). Even days after surgery, sevoflurane can still be found in high parts-per-billion concentration. Furthermore, as it is very volatile it typically presents at the beginning of the chromatogram and may shift retention times of many compounds with similar chromatographic properties (personal communication). The inability of eNoses to separate the anesthetic signals from disease specific signals might explain why the diagnostic accuracy of these machines is so much lower in critically ill patients than in the spontaneously breath individuals (62).

### *Tubing*

The ventilator circuit contains many types of plastics all of which may release volatiles that might disturb the exhaled breath signal. A thorough check of the background signal is needed before exhaled breath analysis can be set up (37). The endotracheal tube also contributed to the collection of exhaled VOCs, most notably through the addition of cyclohexanone (63).

## Towards a clinical application of a breath test

Several steps have to be taken before any breath test can be used for the diagnosis and/or prediction of ARDS. First, all the markers require further validation in a truly independent multi-center cohort. Such a cohort should be of sufficient size to allow for the quantification of confounding and moderating effects of ventilator settings, comorbidities and colonization of the respiratory tract. The exhaled breath test should also be compared to other techniques for ARDS prediction, such as plasma biomarkers and clinical scores like the LIPS (64). The additive value of a breath test to the LIPS should also be evaluated.

Second, many of the detection methods are too laborious at the moment. This means that the machine required for detection of the molecule are too expensive, require specialized personal, have considerable down-time and results take too long for an intensive care setting. Special attention should be given towards the development of an on-site analytical device. Ideally, the machine could be operated by a nurse and provide rapid results—very similar to the decentralized blood gas analyzers that are commonly used nowadays. A second possibility is the integration of the breath analyzer into the ventilator, providing continuous results. Continuous exhaled breath monitoring in mechanically ventilated patients has been tried with an eNose, but gave very difficult to interpret results (65,66). Also, the technological challenges are more profound when the breath analyzer has to be integrated into the ventilator circuit and the costs will increase due to the necessity of one breath analyzed per patient. It would also require the ventilator company to acquire the technology for continuous breath analysis and the technology would only be available for those patients who are ventilated with the machine from that particular company. It seems that a decentralized lab option is more feasible in the near future than the integration of a breath analyzer into the ventilator circuit, although this might be possible on the longer term.

## Conclusions

The currently available evidence suggests that exhaled breath contains several molecules that may serve as diagnostic or predictive biomarkers for ARDS. Most of the candidate markers can be linked to lipid peroxidation. Only octane has been validated in a temporal external validation cohort and is, at this moment, the top-ranking breath



biomarker for ARDS. Thorough independent validation has to be performed for all identified potential biomarkers and the breath tests have to evolve to qualify for clinical use. A decentralized breath analyzer that can be operated by nurses is probably the most feasible scenario in the near future.

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### Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

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