Breath octane and acetaldehyde as markers for acute respiratory distress syndrome in invasively ventilated patients suspected to have ventilator-associated pneumonia

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Shareable abstract (@ERSpublications)
Octane and acetaldehyde had poor discriminative accuracy for ARDS in a population suspected to have VAP. Future research should take into account the potential overlap in exhaled breath signal between patients with ARDS and competing diseases such as VAP. https://bit.ly/3KOrq7H

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Abstract

Rationale The concentration of octane and acetaldehyde in exhaled breath has good diagnostic accuracy for acute respiratory distress syndrome (ARDS). We aimed to determine whether breath octane and acetaldehyde are able to distinguish the presence and absence of ARDS in critically ill patients suspected to have ventilator-associated pneumonia (VAP).

Methods This is a secondary analysis of a prospective observational study into exhaled breath analysis using gas chromatography–time-of-flight mass spectrometry. Difference in the relative abundance of octane and acetaldehyde in exhaled breath was compared between patients with and without ARDS using the Mann–Whitney U-test and the association was quantified using logistic regression. The discriminative accuracy of octane and acetaldehyde, alone or in combination, was calculated using the area under the receiver operating characteristic curve (AUROCC).

Results We included 98 patients, of whom 32 had ARDS and 66 did not. The area under the acetaldehyde peak was higher in patients with ARDS (p=0.03), and associated with the presence of ARDS (OR 1.06 per 100 000 count change, 95% CI 1.02–1.13 per 100 000 count change; p=0.01). A combined model with octane and acetaldehyde showed a high specificity and low sensitivity (90% and 40.6%, respectively), with a low accuracy (AUROCC 0.65, 95% CI 0.53–0.78).

Conclusion Patients suspected to have VAP with ARDS had a higher acetaldehyde concentration in exhaled breath than patients suspected to have VAP without ARDS. However, in this patient population, discrimination of these breath biomarkers for ARDS was poor, indicating the difficulty of translating diagnostic tests between clinical settings.

Introduction

Acute respiratory distress syndrome (ARDS) is a severe form of acute lung injury with a high mortality and both short- and long-term morbidity. It is characterised by acute onset of noncardiogenic pulmonary oedema resulting in hypoxaemia which can be triggered *via* multiple pathways, as depicted by the wide variety of risk factors for ARDS [1, 2]. In the absence of validated diagnostic biomarkers, surrogates such

as clinical, physiological and radiographic characteristics are combined in the current Berlin definition for ARDS and used at the bedside to determine whether or not a patient has ARDS [3]. This introduces challenges, as radiographic criteria have poor interobserver reliability concerning the presence of alveolar oedema, especially in the early stages of ARDS, and notably in the presence of pre-existing lung pathology or ARDS mimicry disorders [4]. Not surprisingly, post-mortem pathological tissue findings show only moderate correlations with the currently used ARDS classification [5, 6]. However, early diagnosis is key for selecting patients who might benefit most from specific treatments, as opposed to diagnosing when severe pulmonary alveolar oedema is already present. Without a diagnostic test capturing and reflecting involved pathophysiological mechanisms, diagnosis of ARDS remains challenging.

Exhaled breath is one of the suggested biomarkers for early diagnosis. It contains volatile organic compounds (VOCs), the composition of which is influenced by 1) local cellular metabolism (endogenous origin); 2) environment/bacteria (exogenous origin); and 3) systemic processes (systemic origin) [7]. Exhaled breath signals have been shown to vary based on the presence of certain lung disorders (*e.g.* asthma, pneumonia, lung cancer) [8–11]. They can potentially serve as noninvasive metabolic biomarkers for multiple disorders by reflecting their pathophysiological and physiological processes, with a relatively easy and highly available sampling potential as their main advantage. A previous study showed that the concentration of octane and acetaldehyde in exhaled breath can be used to accurately identify patients with ARDS in a mixed group of mechanically ventilated critically ill patients, within 24 h after initiation of invasive ventilation [12]. However, a small number of patients with competing diseases (such as pneumonia or cardiogenic pulmonary oedema) were included as controls in that study (three out of 101 and four out of 101, respectively), making it difficult to estimate the diagnostic accuracy when competing diseases or ARDS mimickers are present.

Patients with VAP frequently fulfil the criteria for ARDS and have a higher attributable mortality for VAP and ARDS combined than for VAP alone [13]. As a multiplicity of pathophysiological mechanisms can lead to ARDS [1, 2], it is possible that some of them overlap. This raises the question whether breath octane and acetaldehyde can discriminate between the presence and absence of ARDS in these patients with a higher *a priori* chance of ARDS. We hypothesise that the concentration of octane and acetaldehyde differs between ventilator-associated pneumonia (VAP)-suspected patients with and without ARDS, and that both breath biomarkers have a good diagnostic accuracy for diagnosing ARDS in this population.

Methods

Study design and ethical considerations

This was a secondary analysis of a study which analysed exhaled breath with new diagnostic modalities to diagnose VAP [14]. Between 2009 and 2012, this prospective observational cohort study was performed at the mixed intensive care units of a university-based tertiary hospital (Maastricht University Medical Center+, Maastricht, the Netherlands). The local institutional review board approved the study protocol and waived the requirement to obtain informed consent. Previously, parts of the data have been used and described in reports on the use of the electronic nose and volatile organic compounds in exhaled breath to diagnose VAP [14, 15].

Population

The original study included adult mechanically ventilated patients suspected of VAP, who underwent a diagnostic bronchoalveolar lavage (BAL) [14]. In this analysis, a subset of patients with available chest radiographs taken within 12 h before the moment of sampling and complete profiles of both BAL and gas chromatography–time-of-flight mass spectrometry (GC–tof-MS) were included. No additional exclusion criteria were used. In addition, complete profiles of GC–tof-MS from five healthy male volunteers and 10 mechanically ventilated patients without lung injury or VAP-suspicion (eight males and two females; mean±SD age 53±20 years) were used to serve as control subjects. Both the healthy volunteers and mechanically ventilated patients originate from the initial study dataset. Being clinically suspected of VAP was defined as having received mechanical ventilation for ⩾48 h when fulfilling the VAP criteria of the Centers for Disease Control and Prevention [16, 17], which were having a new, persistent or progressive infiltrate on chest radiograph, and meeting three or more of the following criteria: 1) rectal temperature >38.0°C or <35.5°C; 2) leukocytosis >10 000 cells·μL^{-1}, and/or left shift or leukopenia <3000 cells·μL^{-1}; 3) >10 leukocytes per high-power field in Gram stain of endotracheal aspirate; and 4) a positive culture of endotracheal aspirate. The diagnosis was confirmed when BAL fluid analysis results showed a presence of ⩾2% cells containing intracellular organisms and/or quantitative culture results of ⩾10^4 CFU·mL^{-1}. The presence of ARDS at the moment of sampling was retrospectively scored by two trained researchers using the Berlin definition [3]. A third trained independent researcher was consulted to reach consensus in case of conflicting scores.

Sample collection

The BAL fluid samples were obtained, using standard clinical protocol, on the same day as the patient fulfilled the criteria of being clinically suspected of VAP [14, 18]. Prior to the BAL procedure, sequential organ failure assessment (SOFA) scores and exhaled breath samples were collected.

For the sampling procedure of exhaled breath in ventilated patients [14, 15], a sterile Tedlar bag (5 L) was connected to the expiratory limb of the Evita XL ventilator (Draeger, Lübeck, Germany). This closed setup prevented pollution of the samples by the environment and allowed for safe filling of the bags with patients' exhaled breath without suction. At the end of the sampling procedure, the valve of the bag was closed, preserving the sample. In healthy volunteers, the same type of Tedlar bags was filled using a mouthpiece.

The content of the bag was transported on stainless steel two-bed desorption tubes (Carbograph 1TD/Carbopack X; Markes International, Llantrisant, UK) by a vacuum pump (VWR International, Paris, France) within 1 h. The trapped VOCs on the carbon adsorbent were measured using GC–tof-MS [19]. The area under peak of octane and acetaldehyde was manually extracted from the chromatogram based on the retention time and mass recognition by a trained laboratory technician after the raw GC–tof-MS data were pre-processed. Pre-processing of the data reduces the influence of artefacts on the VOC signal by denoising, baseline correction, alignment, normalisation and scaling of the data [20]. Missing values were imputed with the low value of 10 000 counts.

Statistical analysis

Demographic and clinical patient characteristics were compared between patients with and without ARDS. Differences between groups were tested with the t-test, the Mann–Whitney U-test and the Chi-squared test, as appropriate. The area under the peak of exhaled octane and acetaldehyde was compared between healthy controls, ventilated controls, and patients with and without ARDS using the nonparametric Mann–Whitney U-test and depicted as $\log_2$ fold difference violin plot normalised to the healthy controls. Logistic regression analysis ("lrm" package) was used to assess the association between octane and acetaldehyde and the presence of ARDS in patients suspected to have VAP. The discriminative accuracy, sensitivity and specificity of exhaled octane and acetaldehyde was assessed by the area under the receiver operating characteristic curve (AUROCC). An AUROCC of 0.6–0.7 was considered poor, 0.7–0.8 fair, 0.8–0.9 good and 0.9–1.0 excellent. Cut-offs of 90% sensitivity and specificity were extrapolated with corresponding negative predictive value (NPV) and positive predictive value (PPV). Finally, the AUROCC curves of acetaldehyde and octane combined were stratified for the severity of hypoxaemia using the arterial oxygen tension (P_{aO_2})/inspiratory oxygen fraction (F_{IO_2}) categories defined in the Berlin definition in a sensitivity analysis. A p-value of 0.05 was considered statistically significant. All analyses were performed in R version 3.6.2 (www.r-project.org) using the R-studio interface.

Results

98 patients were included in the analysis, of whom 32 (33%) fulfilled the definition of ARDS at the moment of sampling and 66 (67%) did not. Patients fulfilling the definition of ARDS had higher SOFA scores (mean±SD 8±2.0) and a lower P_{aO_2}/F_{IO_2} ratio (mean±SD 181±48 mmHg) compared to patients who did not (SOFA score 6±3.1, p=0.01; P_{aO_2}/F_{IO_2} ratio 225±84 mmHg, p=0.007). In addition, a higher in-hospital mortality rate was observed in patients with ARDS: n=22 (68.8%) *versus* n=29 (43.9%) without ARDS (p=0.04; table 1).

The area under the acetaldehyde peak was higher in VAP-suspected patients with ARDS (median $\approx 28\times10^4$, interquartile range 12×10^4 to 190×10^4) compared to the healthy controls ($\approx 7.7\times10^4$, 7.6×10^4 to 10×10^4; p=0.04) and VAP-suspected patients without ARDS ($\approx 21\times10^4$, 5.5×10^4 to 39×10^4; p=0.03) (figure 1 and supplementary tables E1 and E2). In logistic regression analysis, the area under the acetaldehyde peak was associated with the presence of ARDS (OR 1.06 per 100 000 count change, 95% CI 1.02–1.13 per 100 000 count change; p=0.01). The area under the octane peak did not significantly differ between groups (figure 1 and supplementary tables E1 and E2) and was not associated with ARDS (OR 1.06 per 100 000 count change, 95% CI 0.90–1.26 per 100 000 count change; p=0.4).

Acetaldehyde and octane in exhaled breath both had poor discriminative accuracy for ARDS. The AUROCC for acetaldehyde was 0.63 (95% CI 0.51–0.76) and for octane 0.57 (95% CI 0.45–0.69). Combining octane and acetaldehyde improved the AUROCC marginally: 0.65 (95% CI 0.53–0.78; figure 2a). A cut-off of 90% sensitivity corresponded with a specificity of 22.7%, an NPV of 83.3% and PPV of 36.3%. A cut-off of 90% specificity corresponded with a sensitivity of 40.6%, an NPV of 75.9% and PPV of 68.4%.

TABLE 1 Demographics and clinical characteristics of patients with suspected ventilator-associated pneumonia

	No ARDS	ARDS	p-value
Patients	66	32	
Patient characteristics			
Male	45 (68.2)	25 (78.1)	0.43
Age, years	64 (53–73)	65 (58–69)	0.79
Admission by diagnosis group			0.17
Cardiovascular	15 (22.7)	6 (18.8)	
Respiratory	14 (21.2)	13 (40.6)	
Gastrointestinal	11 (16.7)	2 (6.2)	
Haematological	10 (15.2)	8 (25.0)	
Neurological	9 (13.6)	1 (3.1)	
Orthopaedic/trauma	5 (7.6)	1 (3.1)	
Other	2 (3.0)	1 (3.1)	
Severe sepsis	20 (30.3)	15 (46.9)	0.17
Ventilator-associated pneumonia[#]	24 (36.4)	8 (25.0)	0.37
Characteristics at time of BAL			
SOFA score	6±3.1	8±2.9	0.01
P_{aO_2}/F_{IO_2} ratio	225.2±83.8	180.9±48.2	0.007
ARDS severity[¶]			
Mild		11 (34.4)	
Moderate		20 (62.5)	
Severe		1 (3.1)	
Outcome			
ICU mortality	24 (36.4)	19 (59.4)	0.05
In-hospital mortality	29 (43.9)	22 (68.8)	0.04

Data are presented as n, n (%), median (interquartile range) or mean±SD, unless otherwise stated. ARDS: acute respiratory distress syndrome; BAL: bronchoalveolar lavage; SOFA: Sequential Organ Failure Assessment at time of BAL; P_{aO_2}: arterial oxygen tension; F_{IO_2}: inspiratory oxygen fraction; ICU: intensive care unit. [#]: confirmed by BAL results; [¶]: according to the Berlin definition: mild P_{aO_2}/F_{IO_2} 200–300 mmHg with positive end-expiratory pressure (PEEP) ⩾5 cmH_2O; moderate P_{aO_2}/F_{IO_2} 100–200 mmHg with PEEP ⩾5 cmH_2O; severe P_{aO_2}/F_{IO_2} ⩽100 mmHg with PEEP ⩾5 cmH_2O.

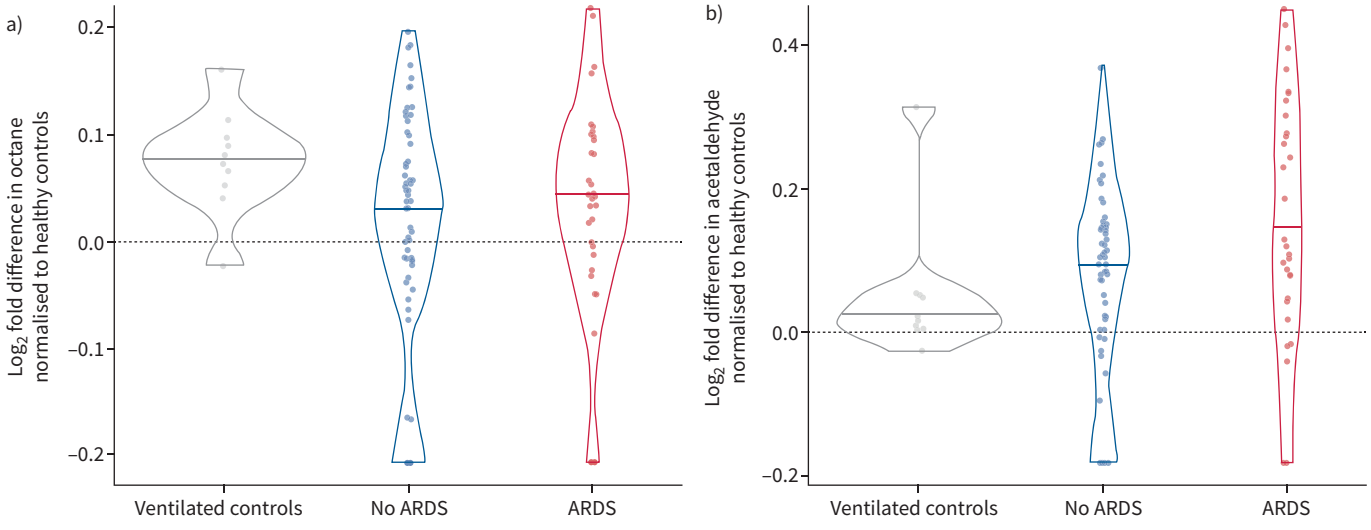

FIGURE 1 Log_2 fold difference in a) octane and b) acetaldehyde normalised to healthy controls. The violin plot depicts the median fold difference and interquartile ranges relative to the healthy controls, with the horizontal line representing zero. a) Octane did not differ significantly between groups. b) Acetaldehyde was significantly higher in ventilator-associated pneumonia (VAP)-suspected patients with acute respiratory distress syndrome (ARDS) compared to healthy controls (p=0.04) and VAP-suspected patients without ARDS (p=0.03) (supplementary tables E1 and E2).

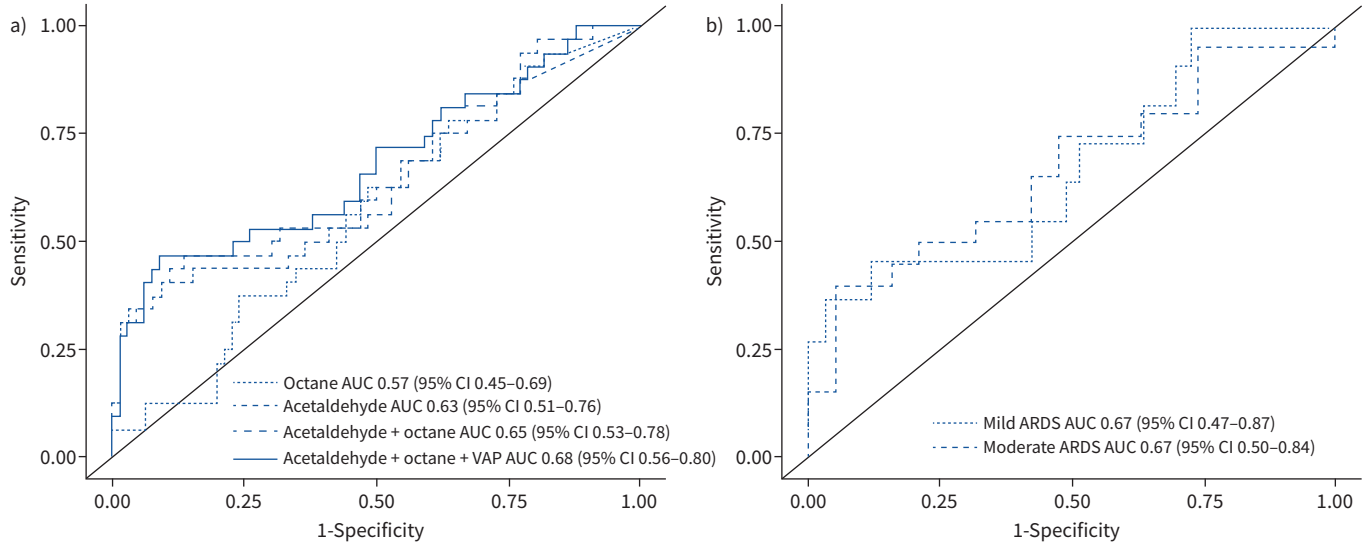

FIGURE 2 Area under the curve (AUC) of the receiver operating characteristic for a) (combinations of) octane and acetaldehyde and b) octane–acetaldehyde combined per acute respiratory distress syndrome (ARDS) severity oxygenation classification (mild ARDS: arterial oxygen tension (P_{aO_2})/inspiratory oxygen fraction (F_{IO_2}) 200–300 mmHg with positive end-expiratory pressure (PEEP) ⩾5 cmH_2O; moderate ARDS: P_{aO_2}/F_{IO_2} 100–200 mmHg with PEEP ⩾5 cmH_2O).

Stratification of all patients based on P_{aO_2}/F_{IO_2} categories as used in the Berlin definition showed an AUROCC of 0.67 (95% CI 0.47–0.87) for the mild ARDS category (200 mmHg<P_{aO_2}/F_{IO_2}⩽300 mmHg) and 0.67 (95% CI 0.50–0.84) for the moderate ARDS category (100 mmHg<P_{aO_2}/F_{IO_2}⩽200 mmHg; figure 2b).

Discussion

In this study, we performed exhaled breath analysis of patients who were suspected to have VAP, and show that the exhaled concentration of acetaldehyde, but not octane, is associated with the presence of ARDS. However, the diagnostic accuracy of both octane and acetaldehyde was poor. Combining acetaldehyde and octane slightly increased the diagnostic accuracy and a high exhaled concentration of both volatile metabolites allowed for a high PPV for ARDS as indicated by a high specificity.

To understand the difference in diagnostic accuracy between this study and the original report we need to evaluate the biochemical origin of the investigated VOCs. Alkanes (octane included) in exhaled breath are products of fatty acid oxidation (lipid peroxidation), which can be induced by reactive oxygen species. The composition of alkanes depends on the types of fatty acids being oxidised [21, 22]. Octane, in particular, is associated with the oxidation of oleic acid, which is increased in plasma of patients with ARDS [23–25]. The origin of acetaldehyde, an aldehyde, can be linked to multiple mechanisms: bacterial metabolism *in vitro* [10], hepatic ethanol metabolism by alcohol dehydrogenase [26], and as a product of leukocytes [27]. Specifically, neutrophils are proposed to produce acetaldehyde by the alternative pathway of amino acid oxidation involving myeloperoxidase, hydrogen peroxidase and chloride ions [27]. This implies that both volatile metabolites can be linked to inflammatory pathways and oxidative stress, which in turn are related, as inflammation can trigger reactive oxygen species *via* cytokine production, and the other way around [28–30]. It can be postulated that these exhaled breath biomarkers capture similar underlying biological mechanisms, since oxidative stress and inflammatory processes both occur in VAP and ARDS, thereby decreasing the discriminative accuracy of octane and acetaldehyde in our analysis.

There are also other (nonbiological) possible explanations for the difference in diagnostic accuracy. First, sampling methods were slightly different from the original report. Bos *et al*. [12] sampled exhaled breath directly onto a sorbent tube, whereas we used a Tedlar bag to collect exhaled breath before storing it onto a sorbent tube [14]. It is unknown whether using a specific sampling technique is associated with different results or decreases repeatability. Second, in the original report the samples of critically ill patients were obtained within 24 h of admission and start of mechanical ventilation [12]. As part of the VAP definition, our study required a minimal mechanical ventilation duration of 48 h to be suspected of VAP regardless of the other criteria [14]. ARDS and other critical illnesses evolve over time with different phases and ARDS is often already present at the start of mechanical ventilation, so it could be possible that exhaled breath

signals differ similarly over time. The evolution over time of octane and acetaldehyde is currently unknown. However, an experimental rat model into a different exhaled breath marker for oxidative stress (pentanal) showed that there seems to be a dynamic correlation between pentanal and lung injury during mechanical ventilation over time [31].

Our study has several strengths. First, by selecting only patients who were suspected to have VAP, we included a category of patients that was lacking in the original evaluation of the diagnostic accuracy of exhaled breath octane and acetaldehyde. Results for other types of pneumonia (community-acquired pneumonia and hospital-acquired pneumonia) can not necessarily be generalised to VAP, and *vice versa*, because of differences in developmental timing, causative pathogens, host response, and treatment [32–34]. Second, ARDS was diagnosed as accurately as possible by performing the chest radiography assessment in triplicate. Third, instead of performing a discovery of new biomarkers, we validated markers that were already described in the literature. This way, we limited false discovery. However, our study also has some important limitations. First, the retrospective nature of data due to the secondary analysis caused certain information to be missing, such as additional patient (history) characteristics: duration of mechanical ventilation until sampling, duration of admission, and scores such as the Acute Physiology and Chronic Health Evaluation (APACHE) II score at admission, and lung injury prediction score (LIPS). Second, we did not measure the concentrations of the biomarkers but rather relied on a semiquantitative surrogate of the concentration by using the area under the peak obtained by gas-chromatography and mass-spectrometry. Third, ARDS was scored based on the Berlin definition as the best alternative in the absence of the tissue histopathology (current gold standard). This might have introduced some bias, as only the P_{aO_2}/F_{IO_2} at the moment of sampling was available instead of the poorest P_{aO_2}/F_{IO_2} in the last 24 h, which would be more representative.

Our results highlight several knowledge gaps in the use of exhaled breath analysis as diagnostic test in intensive care. Effects of disease evolution and resolution, time and natural history of breath biomarkers should be elucidated and are essential to progress in this area. Additionally, the development and validation of a bedside test should be prioritised, as GC-MS machines are complex and expensive to handle. Zhou *et al.* [35] have taken an important first step by developing a small bedside test device able to identify different peaks in exhaled breath of critically ill patients. The development of a noninvasive diagnostic test reflecting the pathophysiological mechanism could improve diagnosing ARDS by increasing the sensitivity in early stages. This could improve clinical trial selections and the development of pharmacotherapeutic therapies.

Conclusion

Patients suspected to have VAP and ARDS showed the highest exhaled breath concentration of acetaldehyde, but there was no difference in exhaled octane. The diagnostic accuracy of a model that incorporated both exhaled acetaldehyde and octane had poor performance, although a high concentration of both biomarkers could identify ARDS with a high PPV. Future research should focus on validating the discriminative accuracy of these breath biomarkers to diagnose ARDS, while considering ARDS mimickers such as VAP and other pulmonary diseases.

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