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Please cite this article as: Heijnen NFL, Hagens LA, van Schooten F-J, et al. Breath octane and acetaldehyde as markers for ARDS in invasively ventilated patients suspected to have VAP. *ERJ Open Res* 2022; in press (https://doi.org/10.1183/23120541.00624-2021).

This manuscript has recently been accepted for publication in the *ERJ Open Research*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

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Breath octane and acetaldehyde as markers for ARDS in invasively ventilated patients suspected to have VAP

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Author's contributions:
All authors contributed to the study concept and design. AM, NFLH, RMS performed the data collection. NFLH performed the data analysis and wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.
Funding:
The authors received a grant from Health Holland and the Dutch Lung Foundation for the DARTS study. This grant is co-financed by Philips Research. They had no role in the data analysis and did not influence this manuscript in any way.

Running head: Exhaled breath markers for ARDS in VAP

Word count: 2583

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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 curve of the ROC (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 100000 count change (95% CI: 1.02 – 1.13), p=0.01). A combined model with octane and acetaldehyde showed a high specificity and low sensitivity (respectively, 90% and 40.6%), 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.

**Words:** 298 (max. 300 words)

**Keywords:** Exhaled breath; ARDS; metabolomics; critically ill; ventilator-associated pneumonia
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 characterized by acute onset of non-cardiogenic pulmonary edema resulting in hypoxemia 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 like 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 edema, especially in the early stages of ARDS, and notably in 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). Early diagnosis, however, is key for selecting patients who might benefit most from specific treatments, as opposed to diagnosing when severe pulmonary alveolar edema is already present. Without a diagnostic test capturing and reflecting involved pathophysiological mechanisms, it remains challenging to diagnose ARDS.

Exhaled breath is one of the suggested biomarkers for early diagnosis. It contains volatile organic compounds (VOCs), of which the composition 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 shown to vary based on the presence of certain lung disorders (e.g., asthma, pneumonia, lung cancer) (8–11). They can potentially serve as non-invasive 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 hours after initiation of invasive ventilation (12). However, a small number of patients with competing diseases (like pneumonia or cardiogenic pulmonary edema) were included as control in that study (respectively, 3 out of 101 and 4 out of 101), making it difficult to estimate the diagnostic accuracy when competing diseases or ARDS mimickers are present.

Patients with VAP frequently fulfill the criteria for ARDS and have a higher attributable mortality for VAP and ARDS combined than for VAP alone (13). As a multiplicity of pathophysiologic 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 à priori chance of ARDS. We hypothesize that the concentration of octane and acetaldehyde differs between patients suspected of ventilator-associated pneumonia (VAP) 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 analyzed exhaled breath with new diagnostic modalities to diagnose ventilator-associated pneumonia (VAP) (14). Between 2009 and 2012, this prospective observational cohort study was performed at the mixed intensive care units (ICUs) 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. Parts of the data were previously used and described to report about the use of the electronic nose and volatile organic compounds (VOCs) 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 hours 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 5 healthy male volunteers and 10 mechanically ventilated patients without lung injury or VAP-suspicion (8 males and 2 females; age 53 years (mean ± 20 SD)), 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 mechanically ventilation ≥ 48 hours when fulfilling the VAP criteria of the CDC (16, 17): 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/μL, and/or left shift or leukopenia < 3000/μL; (3) more than 10 leukocytes per high power field in Gram stain of endotracheal aspirate; (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 (ICO) and/or quantitative culture results of ≥10^4 cfu/ml. 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 (5L) was connected to the expiratory limb of the Draeger® Evita XL ventilator (Lübeck, Germany). This closed setup prevented pollution of the samples by the environment and allowed for safely filling 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 by using a mouthpiece.
The content of the bag was transported on stainless steel two-bed desorption tubes (Carbograph 1TD/carbopack X, Markes International, Llantrisant, Wales, UK) by a vacuum pump (VWR International, France) within 1 hour. The trapped VOCs on the carbon adsorbent were measured by 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 was pre-processed. Pre-processing of the data reduces the influence of artefacts on the VOC signal by denoising, baseline correction, alignment, normalization, and scaling of the data (20). Missing values were imputed with the low value of 10000 counts.

**Statistical analysis**

Demographic and clinical patient characteristics were compared between patients with and without ARDS. Differences between groups were tested with the Student’s 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 non-parametric Mann-Whitney U-test and depicted as log₂ fold difference violin plot normalized 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 (AUC-ROC) curve. An AUC of 0.6-0.7 was considered as poor, 0.7-0.8 as fair, 0.8-0.9 as good, and 0.9-1.0 as excellent. Cutoffs of 90% sensitivity and specificity were extrapolated with corresponding negative
predictive value (NPV) and positive predictive value (PPV). Finally, the AUC-ROC curves of acetaldehyde and octane combined were stratified for the severity of hypoxemia using the PaO$_2$/FiO$_2$ 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

Ninety-eight 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 sequential organ failure scores (SOFA-score, mean: 8 ± 2.0 SD) and a lower PaO$_2$/FiO$_2$-ratio (PF-ratio, mean: 181 mmHg ± 48 SD) compared to patients who did not (SOFA-score, mean: 6 ± 3.1 SD, p = 0.01; PF-ratio, mean: 225 mmHg ± 84 SD, p = 0.007). In addition, a higher in-hospital mortality rate was observed in patients with ARDS: 22 (68.8%) versus 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: ≈28E4 (12E4 to 190E4)) compared to the healthy controls (median: ≈7.7E4 (7.6E4 to 10E4), p = 0.04) and VAP-suspected patients without ARDS (median: ≈21E4 (5.5e4 to 39E4), p = 0.03; Figure 1, Table E1/E2). In logistic regression analysis, the area under the acetaldehyde peak was associated with the presence of ARDS (OR: 1.06 per 100000 count change (95% CI: 1.02 – 1.13), p = 0.01). The area under the octane peak did not significantly differ between groups.
(Figure 1, Table E1/E2) and was not associated with ARDS (OR: 1.06 per 100000 count change (95% CI: 0.90 – 1.26), 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 cutoff of 90% sensitivity corresponded with a specificity of 22.7%, an NPV of 83.3% and PPV of 36.3%. A cutoff of 90% specificity corresponded with a sensitivity of 40.6%, an NPV of 75.9% and PPV of 68.4%.

Stratification of all patients based on PaO2/FiO2 categories as used in the Berlin definition showed an AUROCC of 0.67 (95% CI: 0.47-0.87) for respectively the mild ARDS category (200 mmHg < PaO2/FiO2 ≤ 300 mmHg) and 0.67 (95% CI: 0.50-0.84) for the moderate ARDS category (100 mmHg < PaO2/FiO2 ≤ 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 positive predictive value 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 oxidized (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 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 ion (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 (non-biological) possible explanations for the difference in diagnostic accuracy. First, sampling methods were slightly different from the original report. Bos et al. 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 (12, 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 hours of admission and start of mechanical ventilation (12). As part of the VAP-definition, our study required a minimal mechanical ventilation duration of 48 hours 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 generalized 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, like additional patient (history) characteristics: duration of mechanical
ventilation until sampling, duration of admission, and scores like the APACHE II score at admission, and lung injury prediction (LIPS)-score. Second, we did not measure the concentrations of the biomarkers but rather relied on a semi-quantitative 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 PaO2/FiO2 at the moment of sampling was available instead of the poorest PaO2/FiO2 in the last 24 hours, which would be more representative.

Our results highlight several knowledge gaps in the use of exhaled breath analysis as diagnostic test in the 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 prioritized, as GC-MS machines are complex and expensive to handle. Zhou et al. 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 (35). The development of a non-invasive 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 positive predictive value. 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.
# Tables

**Table 1:** Demographics and clinical characteristics of VAP-suspected patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No ARDS</th>
<th>ARDS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>n=66</td>
<td>n=32</td>
<td>0.43</td>
</tr>
<tr>
<td>Age in years (median [IQR])</td>
<td>64 [53, 73]</td>
<td>65 [58, 69]</td>
<td>0.79</td>
</tr>
<tr>
<td>Admission by diagnosis group, n (%)</td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>15 (22.7)</td>
<td>6 (18.8)</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>14 (21.2)</td>
<td>13 (40.6)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>11 (16.7)</td>
<td>2 (6.2)</td>
<td></td>
</tr>
<tr>
<td>Hematologic</td>
<td>10 (15.2)</td>
<td>8 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Neurologic</td>
<td>9 (13.6)</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Orthopedic/trauma</td>
<td>5 (7.6)</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (3.0)</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Severe sepsis, n (%)</td>
<td>20 (30.3)</td>
<td>15 (46.9)</td>
<td>0.17</td>
</tr>
<tr>
<td>VAP*, n (%)</td>
<td>24 (36.4)</td>
<td>8 (25.0)</td>
<td>0.37</td>
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</tbody>
</table>

Characteristics at time of BAL

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No ARDS</th>
<th>ARDS</th>
<th>p-value</th>
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<tbody>
<tr>
<td>SOFA-score (mean (SD))</td>
<td>6 (3.1)</td>
<td>8 (2.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>PaO2/FiO2 -ratio (mean (SD))</td>
<td>225.2 (83.8)</td>
<td>180.9 (48.2)</td>
<td>0.007</td>
</tr>
<tr>
<td>ARDS severity**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>11 (34.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>20 (62.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>1 (3.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Outcome

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No ARDS</th>
<th>ARDS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU-mortality, n (%)</td>
<td>24 (36.4)</td>
<td>19 (59.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>In-hospital mortality, n (%)</td>
<td>29 (43.9)</td>
<td>22 (68.8)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*VAP confirmed by BAL-results. **ARDS severity according to the Berlin definition: mild PaO2/FiO2 200-300 mmHg with PEEP ≥ 5 cm H2O, moderate PaO2/FiO2 100-200 mmHg with PEEP ≥ 5 cm H2O and severe ≤ 100 mmHg with PEEP ≥ 5 cm H2O.

SOFA = Sequential Organ Failure Assessment Score at time of BAL

BAL = Broncho-alveolar lavage. VAP = Ventilator associated pneumonia.
References


29. Wong RKM, Pettit AI, Quinn PA, Jennings SC, Davies JE, Ng LL. Advanced glycation end products stimulate an enhanced neutrophil respiratory burst mediated through the activation of cytosolic phospholipase A2 and generation of arachidonic Acid. *Circulation* 2003;108:1858–64.


**Figures**

**Figure 1:** $\log_2$ fold difference in octane and acetaldehyde normalized to healthy controls. The violin plot depicts the median fold difference and interquartile ranges (IQR) relative to the healthy controls, with the horizontal line representing zero. Octane did not significantly differ between groups. Acetaldehyde was significantly higher in VAP-suspected patients with ARDS compared to healthy controls ($p=0.04$) and VAP-suspected patients without ARDS ($p=0.03$) (Table E1; E2).
Figure 2: Receiver operating characteristics curves for (A) (combinations of) octane and acetaldehyde and (B) octane-acetaldehyde combined per ARDS severity oxygenation classification (Mild ARDS: PaO$_2$/FIO$_2$ 200-300 mmHg with PEEP ≥ 5 cm H$_2$O and Moderate ARDS: PaO$_2$/FIO$_2$ 100-200 mmHg with PEEP ≥ 5 cm H$_2$O).
Online Data Supplement

Breath octane and acetaldehyde as markers for ARDS
In invasively ventilated patients suspected to have VAP

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Content:
Supplemental tables (2)
Supplemental figures (0)
**Supplemental tables**

**Table E1:** Median and interquartile range of area under the octane and acetaldehyde peak per group.

<table>
<thead>
<tr>
<th></th>
<th>Octane</th>
<th>Acetaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>110134 [77742, 161637]</td>
<td>77211 [76246, 100720]</td>
</tr>
<tr>
<td>Ventilated control</td>
<td>270050 [211928, 333942]</td>
<td>96438 [82693, 139994]</td>
</tr>
<tr>
<td>No ARDS</td>
<td>158865 [72042, 258962]</td>
<td>210733 [54654, 390877]</td>
</tr>
<tr>
<td>ARDS</td>
<td>180797 [102936, 345789]</td>
<td>277206 [118514, 1902631]</td>
</tr>
</tbody>
</table>

**Table E2:** Comparison of both octane and acetaldehyde area under the peak between groups.

<table>
<thead>
<tr>
<th></th>
<th>Octane</th>
<th>Acetaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ventilated control</td>
<td>No ARDS</td>
</tr>
<tr>
<td>Healthy control</td>
<td>0.014</td>
<td>0.51</td>
</tr>
<tr>
<td>Ventilated control</td>
<td>0.047</td>
<td>0.22</td>
</tr>
<tr>
<td>No ARDS</td>
<td></td>
<td>0.28</td>
</tr>
</tbody>
</table>

Depicted numbers represent the p-values.