Diagnosis of pulmonary injury and infection by exhaled breath analysis
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Chapter 6.

Exhaled breath metabolomics as a non-invasive diagnostic tool for ARDS

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Abstract

There is a need for biological markers in the acute respiratory distress syndrome (ARDS). Exhaled breath contains hundreds of metabolites in gas phase, some of which reflect (patho-)physiological processes. We aimed to determine the diagnostic accuracy of metabolites in the exhaled breath for ARDS.

Breath from ventilated ICU-patients (n=101) was analyzed using gas-chromatography and mass-spectrometry during the first day of admission. ARDS was defined by the Berlin definition. Training and a temporal validation cohort were used.

23 patients in the training cohort (n=53) had ARDS. Three breath metabolites, octane, acetaldehyde and 3-methylheptane, could discriminate between ARDS and controls with an area under the receiver operating characteristics curve (ROC-AUC) of 0.80. Temporal external validation (19 ARDS, n=48) resulted in a ROC-AUC of 0.78. Discrimination was insensitive to adjustment for severity of disease, a direct or indirect cause for ARDS, comorbidities or ventilator settings. Combination with the lung injury prediction score increased the ROC-AUC to 0.91 and improved net reclassification by 1.17.

Exhaled breath analysis showed good diagnostic accuracy for ARDS, which was externally validated. These data suggest that exhaled breath analysis qualifies for the diagnostic assessment of ARDS.
**Introduction**

As stated in the updated and improved consensus criteria for acute respiratory distress syndrome (ARDS), a valid and reliable definition for ARDS is considered essential for clinical management and to facilitate enrolment of consistent patient phenotypes into clinical trials [1]. The presently used Berlin criteria are empirically selected clinical, radiological and physiological variables [1]. This definition is highly suitable for epidemiological studies but show a moderate correlation with post-mortem pathological findings [2]. and ARDS can still be mistaken for pneumonia or cardiogenic pulmonary edema (CPE), and vice versa [2, 3]. Therefore there is need for molecular markers to group phenotypes more objectively and consistently [4].

A diagnostic molecular marker should improve classification on top of the pre-test probability of disease [5, 6]. In the case of ARDS, risk factors and risk modifiers have been thoroughly assessed, externally validated and incorporated into the Lung Injury Prediction Score (LIPS) [7, 8]. Discovery and validation of biological markers that reflect the pathophysiological mechanisms underlying lung injury may allow for improved diagnosis before the clinical definition of ARDS is met [9].

Metabolomics can provide an integrated view of upstream physiological, genomic, transcriptomic and proteomic data by assessment of the composition of metabolite mixtures in biological material [10-12]. Exhaled breath contains metabolites that are volatile [13]. Importantly, opposed to exhaled breath condensate collection, analysis of volatile organic compounds (VOCs) in gas phase does not rely on analysis of soluble markers. Instead, volatile metabolites can be trapped onto a sorbent tube and detected using gas–chromatography and mass–spectrometry (GC–MS) [14, 15]. Exhaled VOCs can be of systemic origin, can be produced in the lung (e.g. under influence of oxidative stress and inflammatory response) or can be the result of bacterial metabolism [16-21]. Pre–clinical studies provide evidence that lung injury induces changes in exhaled metabolites [17, 22]. Therefore, it is timely to validate the diagnostic accuracy of exhaled metabolomics in ARDS, by using a training set as well as a validation set of patients and controls [23].
We hypothesized that exhaled breath analysis by GC–MS can accurately diagnose ARDS in ventilated ICU–patients. We aimed to investigate the accuracy, reproducibility and robustness of this diagnosis and the classification of patients with CPE and pneumonia using exhaled breath analysis. This was done by following international guidelines on validating diagnostic accuracy as provided by STARD [24] (Table E1). Finally, we studied the classification performance for ARDS of exhaled breath analysis when combined with the a–priori risk, represented by the LIPS.

**Methods**

*Ethical approval and informed consent*

The institutional review board of the Academic Medical Center, Amsterdam, The Netherlands, decided that the study did not fulfil the criteria for medical research as stated in the Dutch ‘Law on medical research’ because of the non-invasiveness and absence of burden of examining exhaled air (IRB: 10.17.0729). It was judged by the institutional review board that exhaled breath should be analyzed without informed consent of the patient. This trial was registered at the Dutch Trial Register (NTR 2750, www.trialregister.nl).

*Design, subjects and setting*

This was a prospective single centre cohort study. All patients admitted to the ICU, with the exception of cardiopulmonary surgery patients, were screened between December 2011 and April 2013. The only inclusion criterion was mechanical ventilation within the first 24 hours of ICU-admission. The only exclusion criteria were previous ICU admission or mechanical ventilation. Patient who were included before June 2012 entered the training cohort and the remaining patients were used for temporal external validation [25]. Patients were categorized into four groups: controls, ARDS, pneumonia without ARDS and CPE.

*Clinical diagnosis of ARDS*

A team of trained clinical research fellows prospectively scored the presence of ARDS [26], which was later re–evaluated according to the new Berlin definition [1]. All observers were trained on several occasions.
before the start of the study. All assessors had attended meetings in which clinical case vignettes were discussed and had at least 6 months of work experience [27].

**Competing diagnoses**

The diagnosis of community– or hospital–acquired pneumonia consisted of adapted Center for Disease Control–criteria and a post–hoc likelihood of infection was scored (none, possible, probable or proven; see figure E1 and table E2 in the supplementary material) [27, 28]. In contrast to ARDS, the diagnosis of CPE required that the findings (acute onset, bilateral infiltrates and PaO2/FiO2 ratio < 300) were fully explained by cardiac dysfunction based on echocardiography [1].

**Exhaled breath analysis**

Exhaled breath was sampled and analyzed by standardized methodology that was previously published [29]. In short, breath was collected through a disposable side-stream connection for 10 minutes and VOCs were stored on a sorbent tube. These tubes were analyzed by means of GC-MS.

**Statistical analysis**

Differences between the groups were compared using the Mann–Whitney U or Kruskal–Wallis test for continuous variables and chi–square for categorical variables. Data was summarized using the median and 25–75th percentile for skewed variables and with mean and 95%–confidence interval (CI) for normally distributed variables and with count and percentage for categorical variables. All analyses were performed in R statistics using the R–studio interface [30]. P–values below 0.05 were considered significant.

The metabolomics data were analysed using an a priori data analysis plan (figure 1) that followed the latest recommendations for metabolomics [31, 32] and is described per step here and in the results section. The first step was feature selection and training of algorithm. To select the most relevant features, the training cohort was repeatedly split into a 2/3 training set and a 1/3 test set. 1000 iterations of sparse–partial least square (SPLS) logistic regression analysis (K=3, eta=0.9) were performed. This is a
method for penalized feature selection and is a form of regression that can select predictive variables and limit false discovery in situations where large number of independent variables are investigated in low numbers of individuals [33]. The five most frequently chosen ion-fragments (number of co-variates in a model is number of included patients divided by 10 [34]) were selected. A logistic regression model was fitted using the selected ion-fragments in the training cohort. We will refer to the result of that model as the “exhaled breath signal”. The logistic regression model was applied to the complete training cohort and the area under the receiver operating characteristic curve (ROC–AUC), with bootstrapped 95% confidence interval (95%–CI) was reported. The second and third step were to test the reproducibility and the temporal external validation by applying the logistic regression model to another breath sample, obtained at the same time point in the patients of the training cohort and to a new group of patients, respectively, and compare the ROC–AUC. The calibration of the predicted and observed probability of group membership was visualized in a correlation plot and displayed as figure E4 in the supplementary material. Step four was to perform a subgroup analyses for pulmonary/non-pulmonary and mild/moderate/severe ARDS. Step five was to investigate the diagnostic accuracy of the exhaled breath signal for competing diagnoses. Step six was a sensitivity analysis for comorbidities (chronic respiratory disease, malignancies and diabetes mellitus), bacterial growth in respiratory samples, ventilator settings and measures of severity of disease (Acute Physiology and Chronic Health Evaluation (APACHE) II and Simplified Acute Physiology Score (SAPS) II). Finally, the net reclassification improvement [6] of the combination with LIPS improves classification was investigated.

False–discovery and over–fitting was avoided by using a temporal external validation cohort [31, 32], with a required sample size of 18 ARDS patients to find 90% sensitivity with a lower confidence limit of 0.55 (i.e. statistical significance) [35].
Figure 1: Statistical analysis plan

Data after pre-processing

<table>
<thead>
<tr>
<th>#</th>
<th>Group</th>
<th>Ion-fragments (Abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ARDS</td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

Matrix of abundances

Selection of peaks

SPLS

Model Fitting

Logistic regression

1. VOC
   - Octane M/Z 84 -2.03
   - Octane M/Z 85 7.02
   - Octane M/Z 114 3.65
   - Acetaldedhyde M/Z 44 0.49
   - 3-methylheptane M/Z 84 0.87

β = regression coefficient per selected ion-fragment, SPLS = Sparse partial least square.

Training 2/3

“Exhaled breath signal”

Result logistic regression

Int. Validity

Apply to Training set

Reproducibility

Apply to other Tenax

Ext. Validity

Apply Validation set

Figure 2: Patient inclusion

300 Screened

140 Not eligible

89 Excluded

42 MV before

9 Logistics

1 Objection

7 Missed

160 Eligible

Training

53 Included

25 Control

23 ARDS

3 Pneumonia

2 CPE

Validation

48 Included

27 Control

19 ARDS

0 Pneumonia

2 CPE

Top: Inclusion chart

Bottom: Plan for statistical analysis, β = regression coefficient per selected ion-fragment, SPLS = Sparse partial least square.
<table>
<thead>
<tr>
<th>Table 1: Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong> (52)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Male (yes)</strong></td>
</tr>
<tr>
<td><strong>APACHE II</strong></td>
</tr>
<tr>
<td><strong>SAPS II</strong></td>
</tr>
<tr>
<td><strong>Admission type</strong></td>
</tr>
<tr>
<td><strong>Medical</strong></td>
</tr>
<tr>
<td><strong>Elective surgery</strong></td>
</tr>
<tr>
<td><strong>Emergency surgery</strong></td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
</tr>
<tr>
<td><strong>COPD</strong></td>
</tr>
<tr>
<td><strong>Other respiratory</strong></td>
</tr>
<tr>
<td><strong>Malignancy</strong></td>
</tr>
<tr>
<td><strong>DM</strong></td>
</tr>
<tr>
<td><strong>Pmax (cmH₂O)</strong></td>
</tr>
<tr>
<td><strong>Pplateau (cmH₂O)</strong></td>
</tr>
<tr>
<td><strong>PEEP (cmH₂O)</strong></td>
</tr>
<tr>
<td><strong>Vt/IBW (ml/kg)</strong></td>
</tr>
<tr>
<td><strong>Minute volume (l/min)</strong></td>
</tr>
<tr>
<td><strong>PaCO₂ (kPa)</strong></td>
</tr>
<tr>
<td><strong>PaO₂ (kPa)</strong></td>
</tr>
<tr>
<td><strong>FiO₂ (%)</strong></td>
</tr>
<tr>
<td><strong>PaO₂/FiO₂ (mmHg/%)</strong></td>
</tr>
<tr>
<td><strong>LIPS</strong></td>
</tr>
<tr>
<td><strong>ICU Mortality</strong></td>
</tr>
</tbody>
</table>

Continuous variables are expressed as median (25th to 75th percentile). Categorical variables are expressed as number (percentage).
Results

Subjects

Three hundred patients were screened, of whom 140 were not eligible and 59 met exclusion criteria (see figure 2). Thus, 101 patients were included. No adverse events were reported during or shortly after breath collection. Forty-two (42%) patients fulfilled the clinical definition for ARDS [1], 29 patients were classified as having mild ARDS, and 13 and 2 patients as moderate and severe ARDS, respectively (Table 1). 30 patients had ARDS due to a pulmonary cause (pneumonia (n=22), aspiration (n=2), other (n=6)) and 12 due to a non-pulmonary cause (sepsis (n=8), pancreatitis (n=1), other (n=3)). 52 (52%) patients did not fulfil the definition for ARDS; these patients served as control patients. Competing diagnoses were pneumonia (3 patients) and CPE (4 patients). None of the control patients progressed towards ARDS during the first three days of ICU-admission.

Feature selection and training of algorithm

Twenty-three patients with ARDS and 25 controls were included in the training cohort (Table E4 in the supplementary material shows baseline characteristics for this cohort). Five ion–fragments generated by the mass–spectrometer, which originated to three VOCs: octane (Chemical Abstract Service (CAS) registry number: 111–65–9), acetaldehyde (CAS: 75–07–0) and 3–methylheptane (CAS: 589–81–1), were selected by the algorithm (figure 1 and supplementary material) and were used as predictive variables in a logistic regression model (Table E3). Figure E2 and E3 in the supplementary material show the relative abundance of the fragments between patients with and without ARDS in the training cohort. The diagnostic accuracy of this exhaled breath signal for ARDS in the training–cohort was good (ROC–AUC: 0.80 [95%–CI: 0.66–0.92]; figure 3, dashed line).

Reproducibility

The exhaled breath signal derived from the 3 VOCs was reproducible using another breath sample taken from the same patient population within 10 minutes from the other sample (ROC–AUC: 0.78 [95%–CI: 0.65–0.91]; figure 3, dotted line).
Temporal external validation

19 patients with ARDS and 27 controls were included in the validation cohort (Table E5 in the supplementary material shows baseline characteristics for this cohort). The exhaled breath signal from the same 3 VOCs showed moderate to good diagnostic accuracy in a new cohort of patients (AUC–ROC: 0.78 [95%–CI: 0.65–0.91]; figure 3, solid line). Figure E4 in the supplementary material shows the relative abundance of the 5 fragments selected by the algorithm between patients with and without ARDS in the temporal external validation cohort.

Subgroup analyses

The logistic regression function was not different between patients with ARDS due to a pulmonary and a non–pulmonary hit (p = 0.24, figure E6 in the supplementary material). Discrimination of both pulmonary and non–pulmonary ARDS from controls was also not different (ROC–AUC: 0.75 [95%–CI: 0.63–0.86] and 0.80 [95%–CI: 0.63–0.96] for pulmonary and non–pulmonary ARDS, respectively, test between ROC–AUCs: p=0.63 [36]).

The exhaled breath signal was not different between patients with mild or moderate/severe ARDS (p = 0.21, figure E6 in the supplementary material) and was not correlated with the PaO₂/FiO₂ (Spearman’s correlation: 0.18, p=0.27). Discrimination between ARDS and controls was also not different (test between ROC–AUCs: p = 0.19) between mild (ROC–AUC: 0.80 [95%–CI: 0.70–0.90]) and for moderate/severe ARDS (ROC–AUC: 0.65 [95%–CI: 0.44–0.86]).

Competing diagnoses

Discrimination of cardiopulmonary edema and pneumonia patients (not having ARDS) from ARDS was excellent (ROC–AUC: 0.91 [95%–CI: 0.80–1.0] and 0.90 [95%–CI: 0.81–1.0], for CPE and pneumonia, respectively).
**Figure 3:** Discrimination by breath analysis

**Figure 4:** Breath analysis and LIPS

Left: The diagnostic accuracy of breath analysis for ARDS was similar in the training and temporal external validation cohort. The dashed line shows the ROC-curve for the training cohort, the dotted line for reproducibility and the solid line for the validation cohort.

Right: The addition of breath analysis to the lung injury prediction score improves classification. The dashed line shows the ROC-curve for exhaled breath analysis, the dotted line for LIPS and the solid line for the combination.
Sensitivity analysis

The influence of co–variates on the association between exhaled breath and ARDS was assessed by comparing the log odds–ratio of the signal derived from the 3 VOCs \(4.5 \times 10^{-3} \) for ARDS in an unadjusted logistic regression model to the log odds-ratio found in a logistic regression model adjusted for the co–variates. The log odds–ratio was not sensitive to changes in comorbidities \(4.7\), bacterial growth in respiratory samples \(4.5\), maximal inspiratory pressure \(4.8\), positive end–expiratory pressure \(4.9\), inspired oxygen fraction \(4.6\), ratio of pulmonary arterial oxygen tension to inspired oxygen fraction \(5.7\), smoking \(4.7\), alcohol consumption \(4.7\), APACHE II–score \(4.3\) and SAPS–II score \(4.2\).

Combination with LIPS improves classification

The LIPS score alone showed moderate/good discrimination between ARDS and controls (ROC–AUC: \(0.78 \times 10^{-3} \) in the combined training and validation cohort. Discrimination improved when combined with the exhaled breath signal (ROC–AUC: \(0.91 \times 10^{-3}\), \(p=0.001\) vs. LIPS alone, \(p=0.001\) vs. exhaled breath signal alone; figure 4). The net reclassification improvement was \(1.17 \times 10^{-3}\) \((p<0.001\).

Discussion

This study shows that exhaled breath analysis provides good diagnostic accuracy for ARDS in ventilated ICU–patients. Discrimination was reproducible and the diagnostic performance was similar after temporal external validation. The exhaled breath diagnosis of ARDS was not influenced by severity of illness, ventilator settings or the examined comorbidities. Furthermore, the diagnostic accuracy increased when the exhaled breath analysis was combined with the LIPS. These findings indicate that exhaled breath analysis qualifies as diagnostic tool for the assessment of ARDS. Clinical application of this technology will be facilitated by further elevation of the diagnostic accuracy.

The described ROC–AUC of \(0.80\) for distinguishing ARDS patients from other ventilated ICU–patients is higher than most previously described
biological markers, such as interleukins, surfactant proteins and selectins [37]. In contrast to most other studies, we followed an untargeted approach for biomarker discovery. Our data extend previous ‘omics’ approaches using plasma or broncho-alveolar lavage (BAL) in diagnosing ARDS. A recent study that focused on metabolomics reported that the metabolite profile is altered in plasma of patients with sepsis–induced lung injury [38]. Previously, we argued that in the case of ARDS, preferably the lung should be assessed [15]. Indeed, experimentally induced lung injury promotes profound metabolomic changes in BAL and lung tissue [39, 40].

Taken together, our findings demonstrate that metabolomic profiling in ARDS is not only effective in plasma and BAL, but that exhaled breath also provides accurate diagnostic information on the altered metabolites in ARDS.

We identified three volatile biological markers of ARDS: octane, acetaldehyde and 3–methylheptane. However, we observed no differences in exhaled isoprene concentrations between patients with and without ARDS (figure E7 in the supplementary material), as reported in a pioneer paper by Schubert et al.[19]. The two studies differ on two major points: the conceptual approach and timing of patient selection. Concerning the first, we did not a–priori specify what compounds may be associated with ARDS, whereas the previous study was hypothesis-driven by fociussing on exhaled acetone, isoprene and pentane. Second, our patients were included within 24 hours after ICU–admission, thereby early in the development of ARDS, whereas the patients in the previous study were included later during the course of disease. At the time, isoprene was linked to cholesterol metabolism and neutrophil activity but recent evidence connects isoprene to muscle activity [41]. In ICU-patients, muscle activity is affected by ICU-acquired weakness, coma, sedation and delirium. Thus, we could not reproduce the findings of the only previously published study on breath biomarkers for ARDS, which may be due to methodological differences or due to imbalances in confounding variables between the two groups in that study.

ARDS was associated with higher concentrations of octane in the exhaled breath. This VOC contributed most strongly to the diagnostic model. Octane is an end product of lipid–peroxidation, one of the degenerative processes caused by oxidative stress [42, 43]. Oxygen tension is known
to influence production of other hydrocarbons (e.g., pentane) but this did not apply to octane in our study as the sensitivity analysis showed that the log-odds ratio of the exhaled breath signal did not change when corrected for the inspired oxygen fraction. This may be explained by a difference in substrate of the peroxidation process; pentane is the result of peroxidation of n-6 PUFA, linoleic acid or arachidonic acid, while the proposed origin of octane is oleic acid [44]. The latter may not be as dependent on oxygen tension as the former, as suggested by our results. Of note, octane is also present in the breath of healthy individuals, but at very low concentrations [45], and increases due to smoking [46], but this did not influence the diagnostic accuracy in our study.

We also found that higher concentrations of acetaldehyde and 3-methylheptane in the exhaled breath were predictive for ARDS. A variety of bacterial strains can produce acetaldehyde \textit{in vitro} [16]. Bacterial colonization of the lung occurs frequently in ventilated ICU patients and this could have explained the association between ARDS and acetaldehyde. However, the findings that the sensitivity analysis showed no effects of airway colonization on the log–odds ratio for the exhaled breath signal and that patients with unilateral pneumonia included in this study were classified as not having ARDS suggest that bacterial metabolism is not a likely cause for the increased acetaldehyde concentration observed in patients with ARDS in this cohort. Acetaldehyde is also a product of leukocytes [47], and as neutrophil infiltration is a hallmark of ARDS [48] this may be a more appropriate explanation of the difference between patients with ARDS and patients without ARDS. Branched hydrocarbons like 3-methylheptane were suspected to be produced through lipid–peroxidation, similar to octane. However, alkenes and alkynes cannot result from lipid–peroxidation because there are no branched polyunsaturated fatty acids in the body [44]. To our knowledge, there have not been any reports on an alternative pathway for branched hydrocarbon production despite their association with many oxidative diseases, such as lung cancer [49]. This finding illustrates the hypothesis–generating aspect of metabolomics and deserves exploration. By and large, the exhaled breath signal appears to be mainly influenced by (pulmonary) oxidative stress and is further balanced by reflection of inflammatory or infectious processes and an as yet unidentified pathway. Their strength in assessing
ARDS appears to be their combination, which should be reflective of their partly independent metabolic pathways.

Our findings show that exhaled breath analysis provides information on the presence or absence of ARDS and this is complementary to pre-test risk assessment by the LIPS. This may not be unexpected as the LIPS consists of risk factors, whereas exhaled breath analysis is based on biochemical alterations that occur during ARDS. Hence, a patient can have a very high a-priori risk for disease (LIPS), but in absence of the biochemical presentation we may still conclude the patient probably does not have the condition (yet). In line with that, if a patient exhibits the biochemical profile with a negligible risk for disease such patient probably does not have the condition either. Our data provide the evidence that the combination of exhaled breath analysis with the LIPS can be highly suitable for identification of patients with ARDS.

Our study may have a number of strengths. First, the complex mixture of exhaled breath was profiled and the features most strongly linked to ARDS were selected, following the latest recommendations for metabolomics [31, 32]. The diagnostic accuracy of these biological markers was externally validated in a newly recruited population. Second, the influence of disease severity, phenotype and potential confounders was investigated thoroughly. Third, exhaled breath was obtained within 24 hours after ICU-admission, in the early phase of disease. Finally, we reported these findings strictly following STARD-guidelines to allow for optimal assessment of bias (Table E1).

Nevertheless, several limitations should also be noted. First, the consensus criteria for ARDS are excellent for epidemiological research but may not entirely cover the underlying pathophysiology as meant to be identified using biological markers. Tissue histopathology as a gold standard would have been preferable, but understandably we could not obtain that in this study. Correlation with other markers of ARDS could have added to our understanding, but the design of this study did not allow additional sampling of blood or to perform broncho-alveolar lavages. Future studies could correlate breath profiles with changes in markers of ARDS. Second, we included only mechanically ventilated patients. Therefore, the generalizability of our results to spontaneous breathing patients remains
to be established. Additional studies are also needed to evaluate the clinical application of exhaled breath analysis for biological monitoring of ARDS progression or resolution and to evaluate the influence of previous intubations for mechanical ventilation, like development of lung injury [50] or airway colonization [51, 52]. Third, we found that cardiopulmonary edema and unilateral pneumonia patients could be distinguished from ARDS patients. However, the number of included patients with these diagnoses was very low. This may be explained by the rapid increase of non–invasive mechanical ventilation as an alternative for intubation in these patients. Therefore, although we know that the exhaled breath signal is different in patients between pneumonia or CPE and ARDS, the accuracy of discriminating these patients cannot be quantified precisely.

The prevalence of ARDS was around 40% in our study, which is surprisingly high compared to previous reports [53]. Several factors contribute to this finding. First, ventilated post-surgical patients are not admitted to the ICU routinely in our hospital and when they are admitted because of complications, ARDS is more likely to develop. Second, the included population was severely ill, as exemplified by the high APACHE II score and high mortality. Third, we assessed ARDS prospectively, which could have resulted in the diagnosis of ARDS in patients that would have been missed retrospectively.

The present data suggest that exhaled breath analysis may be used to diagnose ARDS. This study represents a required step towards use of exhaled breath analysis into clinical practice [24]. Next, the predictive value of the observed biological markers should be evaluated in a prospective study including patients that are at risk for ARDS, possibly also including spontaneous breathing patients. Subsequently, the diagnostic accuracy of the combination of risk factors and multiple biological markers should be further improved. In the long run, a move from the diagnostic accuracy paradigm towards a test validation paradigm might be justified [54]. This would allow for the comparison of added value of several index–tests, including exhaled breath analysis, in clinical decision–making. Finally, a bedside test should be developed especially for the detection of VOCs, plausibly octane, acetaldehyde and 3–methylheptane, in exhaled breath of ICU–patients. An array of sensors or rapid mass–spectrometric techniques may be used for that [13]. This may also allow for continuous

**Conclusion**

Exhaled breath analysis showed good diagnostic accuracy for diagnosing ARDS. Discrimination with controls was reproducible and externally validated. The exhaled breath diagnosis of ARDS was not influenced by severity of illness, ventilator settings or comorbidities. Notably, the diagnostic accuracy increased when the exhaled breath analysis was combined with the LIPS. These data suggest that exhaled breath analysis qualifies for the diagnostic assessment of ARDS.

**Online supplement**

For the *online supplement*, please use the following link, or scan the 2D code with your mobile phone.

http://erj.ersjournals.com/content/suppl/2014/04/17/09031936.00005614.DC1/Bos_ERJ_GCMSforARDS_SupplementaryMaterial_R1_1.pdf

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