Doctoral thesis

Biomarkers of oxidative stress in acute respiratory distress syndrome in exhaled breath measured online by mass spectrometry

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To my dear family and Hans,
for the time that I stole from them

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List of Abbreviations

AECC: American-European consensus conference
ALI: Acute lung injury
amu: Atomic mass unit
ARDS: Acute respiratory distress syndrome
ARDSNet: ARDS-Network
BAL: Bronchoalveolar lavage
BMI: Body mass index
bpm: Beats per minute
CESAR: Conventional ventilation or ECMO for severe adult respiratory failure
CI: Confidence interval
COPD: Chronic obstructive pulmonary disease
CO_2: Carbon dioxide
CRP: C-reactive protein
CT: Computed tomography
EBC: Exhaled breath condensate
ECLA: Extracorporeal lung assist
ECMO: Extracorporeal membrane oxygenation
EI-MS: Electron impact mass spectrometer
eNose: Electronic nose
FiO_2: Fraction of inspired oxygen
GC-MS: Chromatographic-mass spectrometry
HR: Heart rate
ICU: Intensive care unit
I:E: Ventilation ratio (inspiration/expiration)
iLA: Interventional lung assist
IL: Interleukin
LIS: Lung Injury Score
IL-6: Interleukin 6
IMR-MS: Ion-molecule reaction mass spectrometry
lpm: Liters per minute
LOS: Length of stay
MDA: Malondialdehyde

MDA-P: Malondialdehyde-pentane

MAP: Mean arterial pressure

Min: Minute

MODS: Multiple organ dysfunction syndrome

MS: Mass spectrometry

MSC: Mesenchymal stem cell

MV: Minute volume

m/z: Mass-to-charge ratio

NIH: National Institutes of Health

ORD: Office of rare diseases

$PaO_{2:}$ Arterial partial pressure of oxygen

$PaCO_2$: Arterial partial pressure of carbon dioxide

PBW: Predicted body weight

PCT: Procalcitonin

PCWP: Pulmonary capillary wedge pressure

PEEP: Positive end-expiratory pressure

pH: Measure of the acidity or basicity of an aqueous solution

PiCCO: Pulse-induced contour cardiac output

Pmean: Mean airway pressure

Ppeak: Peak inspiratory pressure

PPLAT: End-inspiratory plateau pressure

PTR-MS: Proton transfer reaction mass spectrometry

ppbv: Parts per billion of volume

pptv: Parts per trillion of volume

PUFA: Polyunsaturated fatty acids

RCT: Randomized controlled trial

RR: Respiratory rate

RR: Relative risk

ROS: Reactive oxygen species

SAPS II: New simplified acute physiology score

SIFT-MS: Selected ion flow tube mass spectrometry

SIRS: Systemic inflammatory response syndrome

SPSS: Statistical Package for the Social Sciences

STSS: Streptococcal toxic-shock syndrome

TBARS: Thiobarbituric acid-reactive substances

TRALI: Transfusion-associated acute lung injury

VILI: Ventilator-induced lung injury

VOCs: Volatile organic compounds

V_T: Tidal volume

vs.: Versus

Abstract

Background: In the setting of acute respiratory distress syndrome (ARDS), activated neutrophils migrate from pulmonary capillaries into the alveolar and interstitial spaces, where they release oxygen free radicals called reactive oxygen species (ROS). ROS play a major role in the pathogenesis of ARDS. The secretion of ROS and other proinflammatory mediators leads to damage to alveolar-capillary membrane, causing increased permeability and pulmonary edema. When ROS interact with lipids from pulmonary cell membranes, a process termed lipid peroxidation takes place, ultimately resulting in the generation of volatile organic compounds (VOCs) such as pentane, malondialdehyde, propionaldehyde, and acetone. Since these compounds are volatile and originate primarily in the lung, they would be expected to be found in exhaled breath earlier than in the blood.

Objectives: The primary endpoint was to determine whether the above-mentioned lipid peroxidation products can be measured online in expiratory air of patients with ARDS and whether their concentrations are higher than in a control group without acute lung injury. Secondary endpoints were to determine i) the systemic inflammatory response measured by C-reactive protein, interleukin-6, and procalcitonin in blood; ii) whether there is a linear association between the concentrations of different VOCs and between VOCs and systemic inflammatory markers, as well as between VOCs and lung injury severity expressed by the Lung Injury Score (LIS) and PaO_2/FiO_2 ratio.

Design: A cross-sectional analysis of VOCs and systemic inflammatory markers within a prospective observational descriptive study of clinical parameters conducted from 2009 to 2011.

Setting: A 20-bed medical-surgical intensive care unit in a university hospital.

Patients: We prospectively enrolled patients with ARDS (within the first 48h of diagnosis) (n=16) and mechanically ventilated patients without acute lung injury (n=14) anytime after admission. ARDS patients were ventilated according to ARDS-Network guidelines. We recorded demographic, physiologic, clinical, radiographic, and biochemical parameters. The severity of the disease at the time of exhaled breath measurement was assessed using the Simplified Acute Physiological Score (SAPS II) and LIS. Extracorporeal lung assist therapy (extracorporeal membrane oxygenation (ECMO) or pumpless interventional lung assist (iLA)) was considered for ARDS patients who did not response to optimal standard treatment.

Intervention: The very low concentration of VOCs in exhaled breath requires a supersensitive method to detect them. To this end, we used ion-molecule reaction mass spectrometry (IMR-MS). This technique enables noninvasive real-time observation of sampling. Controlled alveolar air samples obtained using CO_2 threshold triggering were analyzed by connecting patients to the IMR-MS via a T-piece inserted between the endotracheal tube and the Y-connector of the respirator. Inspiratory air samples were obtained via a T-piece placed directly in the respiratory circuit. Biochemical and biological parameters in blood were analyzed at the time of the mass spectrometry measurement.

Main results: No significant differences in age, sex, body mass index, or comorbidities proportion were found between the two groups. As expected, LIS and SAPS II scores were higher in the ARDS group. In the ARDS group, 8 (50%) patients had severe ARDS defined as PaO_2/FiO_2 <100 mmHg at the time of measurement, and 8 (50%) patients underwent ECMO. The most frequent cause of the development of ARDS was pneumonia with septic shock (94%). ARDS patients had significantly higher concentrations of pentane, malondialdehyde, propionaldehyde, and acetone in expiratory air, as well as significantly higher values for CRP, procalcitonin, and interleukin-6 in blood than the control group. Three significant linear regression models were found between the concentrations of VOCs and between malondialdehyde-pentane and PaO_2/FiO_2 ratio.

Conclusions: The higher concentrations of lipid peroxidation products in alveolar gas samples in ARDS patients may reflect the amount of oxidative stress damage in ARDS. Importantly, the linear association between MDA-P and PaO_2/FiO_2 ratio suggests that an increase in MDA-P might be able to detect disease progression. These biomarkers might be useful for detecting ARDS early, monitoring disease progression, and optimizing treatment. IMR-MS is a novel online noninvasive approach that enables real-time detection of lipid peroxidation products in exhaled breath at the bedside without risk to the patient. Our results are an important step in the continuous monitoring of the dynamic clinical status in critically ill patients.

INTRODUCTION

I. INTRODUCTION

1. Background and current state of knowledge about acute respiratory distress syndrome (ARDS)

ARDS is a very important disease pattern in intensive care medicine due to its severity and high mortality. Although advances in lung-protective strategies (1) have helped improve survival, the mortality rates can still be as high as 30% to 50% (2). This high mortality might be reduced by the identification of suitable biomarkers that allow the early detection of ARDS as well as the monitoring and optimization of treatment.

Oxidative stress triggered by the release of reactive oxygen species (ROS) from activated neutrophils leads to impairment of pulmonary membrane lipids, which plays an important role in the pathogenesis of ARDS. When ROS released by these stimulated neutrophils interact with pulmonary membrane lipids, a process termed lipid peroxidation takes place, ultimately resulting in the generation of volatile organic compounds (VOCs) (Figure 2). Since these compounds are volatile and originate primarily in the lung, they would be expected to be found in exhaled breath earlier than in the blood. Thus, VOCs in exhaled breath are potential biomarkers. The very low concentration of VOCs requires a supersensitive method to detect them. To this end, we used ion-molecule reaction mass spectrometry (IMR-MS), a novel measurement technique that can directly and continuously quantify the simultaneous amount of VOCs in expiratory air resulting from the interaction of free radicals with lipids in the damaged tissue.

a) <u>Definition</u>

ARDS is the severe expression of acute lung injury (ALI). Both are defined as a syndrome of acute onset and lung inflammation with increased pulmonary vascular permeability in the absence of an elevation in the hydrostatic pressure in the pulmonary veins.

The American-European Consensus Conference (AECC) standardized the definitions for ALI and ARDS in 1994, specifying the following diagnostic criteria: a) acute respiratory failure with hypoxemia, defined as arterial partial pressure of oxygen (PaO_2)/ fraction of inspired oxygen (F_iO_2) ratio <300 mmHg for ALI or PaO_2/F_iO2 ratio

<200 mmHg for ARDS, regardless of the level of positive end-expiratory pressure (PEEP), b) bilateral lung infiltrates on chest radiographs, and c) non-clinical evidence of left atrial hypertension or pulmonary capillary wedge pressure (PCWP) ≤ 18 mmHg when measured with a pulmonary artery catheter (3)**.**

b) Diagnosis

The diagnosis of ARDS is based on clinical and radiological information and abnormalities of oxygenation. Thus, an arterial blood gas analysis and chest radiographs allow formal diagnosis by the aforementioned criteria.

According to the AECC definition, pulmonary catheterization is not required for diagnosis and should be used as needed to aid clinical management. Increased intrathoracic pressures from PEEP are often registered by the pulmonary artery catheter and can lead to a false elevation of the actual PCWP. Moreover, high pressures of PCWP may be recorded in patients with ALI/ARDS who have been resuscitated with large volumes of fluid. Several studies have demonstrated that some patients who meet clinical criteria for ARDS may have a PCWP that is above the selected cutoff value of 18 mmHg. Neff et al. (4) showed that 11% of patients meeting chest radiograph and PaO_2/FiO_2 ratio criteria initially had a PCWP >18 mmHg, which would exclude them from being diagnosed with ARDS. However these patients developed a normal PCWP over the course of their illness while still meeting the radiographic and oxygenation requirements for ARDS. Rigid application of the consensus definition may lead to misdiagnoses. Recent evidence showed that the use of pulmonary artery catheters increased costs with no patient benefit in terms of mortality and thus appears unjustified for routine use in ALI (5).

Plain chest radiographs are sufficient to document bilateral alveolar infiltrates in most cases. Several studies have revealed the significant variability that exists in the radiographic interpretation of chest radiographs. Radiologists can consistently assess the chest radiographic component of the lung injury score (LIS), but assessment by other clinicians may introduce significant variations. In one study, chest radiographs from patients with ARDS based on clinical criteria were blindly interpreted by radiologist, anesthesiologists, and critical care physicians (6). There was very good agreement in the radiological score when interpreted by two radiologists (kappa 0.97). However, when chest radiographs were interpreted by anesthesiologists or critical care physicians the

interrater reliability was only fair to poor. Meade et al. (7) demonstrated that formal training in radiographic interpretation significantly increased the interrater reliability and is necessary to achieve adequate levels of agreement for clinical trials (patients enrolled in clinical trials for ARDS).

Although computed tomography (CT) provides more accurate images of the pulmonary parenchyma in ARDS, CT is not widely used to assess ARDS in clinical practice. CT has become a standard method in lung analysis (only between one and three CT slices are needed) to classify the lung parenchyma as normally aerated, hyperinflated, poorly inflated, or not aerated, and this may be extremely important in setting mechanical ventilation (PEEP) and deciding whether to place the patient in the prone position (8). Despite its usefulness, the quantitative CT scan technique never became established in clinical use, maybe due to disadvantages associated with whole lung CT scans.

c) Etiology

The clinical disorders associated with ARDS can be classified into those associated with direct injury to the lung and those that cause indirect lung injury in the setting of a systemic process (Table 1) (3). Arroliga et al. (9) found that the most common cause of ARDS was direct lung injury (pneumonia and aspiration) (76%), followed by non-pulmonary sepsis (18%). Sepsis from a pulmonary source is associated with the highest risk (38%-48%) of progression to ARDS (10-12). Thus, sepsis (and its more severe forms, severe sepsis and septic shock) is the leading cause of the development of ARDS (13). For this reason, the incidence of ARDS is influenced by the underlying clinical condition.

Table 1. Risk factors for ARDS.

Direct lung injury	**Indirect lung injury**
▪ Pneumonia ▪ Aspiration of gastric contents ▪ Pulmonary contusion ▪ Fat emboli ▪ Inhalation injury ▪ Reperfusion pulmonary edema after lung transplantation ▪ Pulmonary vasculitis ▪ Near-drowning	▪ Non-pulmonary sepsis ▪ Major trauma ▪ Acute pancreatitis ▪ Head injury ▪ Severe burns ▪ Multiple transfusions or transfusion-associated acute lung injury (TRALI) ▪ Non-cardiogenic shock

d) Epidemiology: incidence and mortality

The reported incidence of ALI and ARDS varies from 1.5 to 78 cases per 100,000 persons depending on the population studied and the definition of ALI and ARDS used (14).

In a prospective study in the United States including over 1000 patients and performed over 14 months, the crude incidence of ALI was 78.9 per 100,000 person-years and the age-adjusted incidence was 86.2 per 100,000 person-years. Based on these findings, the authors estimate that each year in the United States there are 190,600 cases of ALI, which are associated with 74,500 deaths and 3.6 million hospital days (15).

A large European observational study showed that ALI occurs in 7% of patients admitted to the intensive care unit (ICU) for more than 4 hours and in 16% of all mechanically ventilated patients. Approximately 55% of patients with ALI develop ARDS within three days of admission to an ICU(16).

There is no single, widely accepted definition for rare disease. In the United States, the Rare Disease Act of 2002 defines *rare disease* strictly according to prevalence, specifically "any disease or condition that affects less than 200,000 persons in the United States" or about 1 in 1,500 people. Thus, the Office of Rare Diseases (ORD) of the National Institutes of Health (NIH) lists ARDS as a rare disease. In Japan, the legal definition of a rare disease is one that affects fewer than 50,000 patients in Japan, or about 1 in 2,500 people. The European Commission on Public Health of 2009 defines rare diseases as "life-threatening or chronically debilitating diseases which are of such low prevalence that special combined efforts are needed to address them." The term *low prevalence* is later defined as fewer than 1 in 2,000 people. Based on the results of Rubenfeld et al. (15), ARDS has an age-adjusted incidence of 86 per 100,000 person-years, so it would still be considered a rare disease. However, because of its high lethality and few therapeutic options, research to improve the prognosis of ARDS should be encouraged.

It is often stated that mortality attributable to ARDS has decreased since the initial case descriptions. Phua et al.'s (2) systematic review shows that the overall pooled weighted mortality from studies published between 1984 and 2006 was 44.3% (95% confidence interval (CI), 41.8-46.9). Mortality was higher in observational studies than in randomized controlled trials (RCT) (48.2 vs. 37.5%; $p<0.001$). A small decrease in mortality over time was demonstrated, but the predominant reduction appeared before

the introduction of the standard definition in 1994. Considering only studies performed from 1994 onward, pooled weighted mortality was 44% (95% CI, 40.1-47.5) for observational studies and 36.2% (95% CI, 32.1-40.5) for RCTs. Thus, they concluded that mortality due to ARDS has remained static at 44% for observational studies and 36.2% for RCTs since the publication of the AECC definition of ALI/ARDS in 1994.

Most studies have found that non-survivors of ARDS usually die of non-respiratory causes. Montgomery et al. (17) reported that only 16% of deaths were caused by respiratory failure. In most cases, early death (within in the first 72 hours) is caused by the underlying injury, whereas late death (more than 72 hours after onset) is caused by sepsis. Most deaths occur as a consequence of multiple organ dysfunction syndrome (MODS) (18). Although there is still some controversy about the role of hypoxemia as a prognostic factor, numerous studies support an association between hypoxemia and mortality (19, 20). In the analysis of the patients with ARDS included in the International Study of Mechanical Ventilation (21), it was observed that FiO_2 was independently associated with mortality (odds ratio per 0.1-point increments, 1.77; 95% CI, 1.49-2.11).

e) Treatment

There is no specific treatment for ARDS. The mainstay of treatment is supportive care, mainly to treat the underlying cause and avoid iatrogenic complications, while maintaining adequate oxygenation. Therefore, mechanical ventilation is still the main supportive therapy for ARDS patients. Endotracheal intubation and mechanical ventilation are always necessary to manage the severe hypoxemia in ARDS.

For a long time, the primary goal of ventilation was to increase arterial oxygenation. Meeting this objective involved the use of high FiO_2 and high minute ventilation with correspondingly high tidal volumes (V_T). The volume of aerated lung is reduced because of edema and atelectasis, and lung compliance is decreased due to the stiffening of nonaerated lung regions. Consequently, ventilation with high V_T may produce hyperinflation of the normal aerated lung. Although mechanical ventilation is potentially lifesaving, excessive volume and pressure is associated with several harmful effects and can contribute to the worsening of lung injury; this is called ventilator-induced lung injury (VILI) (22). The consequences of lung overdistention include direct physical damage, with disruption of the alveolar and capillary epithelium, as well as the

induction of an inflammatory response, with the release of inflammatory mediators (23, 24). The nocuous effects of mechanical ventilation may be mediated by localized inflammation and the systemic release of inflammatory cytokines (biotrauma) (25, 26). It is postulated that pulmonary and systemic release of inflammatory mediators occurs only if the lung is primed by a primary inflammatory blow from ALI, suggesting that biotrauma only occurs if mechanical stretch works as a secondary insult to a previously inflamed lung (25, 27). However, it remains controversial whether injurious ventilation *per se*, without preceding lung injury, can initiate cytokine-mediated pulmonary inflammation. Wilson et al. (28) demonstrated in an in vivo mouse model of ALI that high tidal volume in the absence of underlying injury, produced lung injury with an increase in protein and cytokine concentration in BAL. Some evidence suggests that the inflammatory response induced by VILI can have systemic implications, contributing to the pathogenesis of MODS in patients with ARDS (29, 30).

In 1998, Amato et al. (31) reported the first major RCT that showed the benefit of low V_T ventilation in patients with ARDS. This trial compared conventional ventilation (V_T of 12 ml/Kg, low PEEP, and a partial pressure of carbon dioxide (PCO_2) of 35 - 38 mm Hg) vs. protective ventilation strategy ventilation with low V_T (V_T of 6 ml/Kg, high PEEP, and permissive hypercapnia) in 53 patients. The 28-day mortality rate was significantly lower with protective ventilation than with conventional ventilation (38% vs. 71%), but protective ventilation was not associated with a higher rate of survival to hospital discharge.

The importance of VILI in the clinical treatment of ARDS patients was established by the first trial performed by the ARDS-Network (ARDSNet), which compared traditional ventilation treatment (V_T of 12 ml/Kg of predicted body weight (PBW) and a end-inspiratory plateau pressure (PPLAT) of 50 cmH_2O or less) with ventilation with a lower V_T (involved a V_T of 6 ml/Kg of PBW and a PPLAT of 30 cmH_2O or less). The results showed a decrease in hospital mortality in the group treated with lower V_T compared to the group treated with traditional V_T (31% vs. 39.8%, p=0.007) (1). Since then, limitation of V_T to 6 ml/kg of PBW and PPLAT to a maximum of 30 cmH_2O represents the standard for mechanical ventilation of patients with ARDS. In 2004, the ARDS-Net published the results of the effect of PEEP in ARDS. The results suggest that in patients with ALI and ARDS who receive mechanical ventilation with a target V_T of 6 ml/Kg of PBW and PPLAT of 30 cmH_2O, clinical outcomes are similar whether lower (8.3±3.2 cmH_2O) or higher (13.2±3.5

cmH_2O) PEEP levels are used (32). A significant survival benefit has been shown in trials in which conventional ventilation was associated with marked elevations in airway pressures (33). This finding suggests that the benefit of low V_T is a function of plateau pressure. Recent studies found that tidal hyperinflation may occur in some patients despite limiting V_T to 6 ml/kg and PPLAT to 30 cmH_2O (34), so ARDS patients may benefit from V_T reduction even when PPLAT < 30 cmH_2O (35). Terragni et al. (36) demonstrated that using V_T lower than 6 ml/kg PBW in patients who had 28 ≤ PPLAT ≤ 30 cmH_2O during ARDSNet ventilation was associated with significant reductions in inflammatory and morphological markers of VILI.

i) *Rescue therapy: extracorporeal lung assist (ECLA)*

In patients who do not respond to traditional treatment algorithms, few options exist for rescue therapy.

Extracorporeal membrane oxygenation (ECMO) is an intensive treatment that is used in specialized centers to support patients with reversible acute respiratory failure who are unresponsive to conventional therapeutic interventions.

As ECMO requires special training, it is best limited to centers where the professional competence in daily management already exists (37). Therefore, the regionalization in ECMO centers can help ensure an adequate volume of patients to maintain professionals' skills in its application.

ECMO involves removing blood from the patient and circulating it through an artificial lung with a pump, so ECMO can provide pulmonary or cardiac support, depending on the cannula allocation (38). ECMO removes carbon dioxide from venous blood and adds oxygen to it via an artificial membrane lung. Oxygenated blood then returns to the patient via a venous route (jugular vein) in pulmonary support or an arterial route (subclavian artery, femoral artery, or aorta) in cardiac support.

With venoarterial bypass, the extracorporeal pump flow rate determines the systemic perfusion. The pulmonary circulation is bypassed, and arterial blood gases are determined primarily by the efficiency and volume of extracorporeal gas exchange. With venovenous bypass, the extracorporeally treated blood raises the oxygen content and lowers the carbon dioxide (CO_2) content. Gas exchange is augmented by any remaining native pulmonary function, and perfusion depends upon the patient's cardiac output. Venovenous bypass enjoys a number of theoretical advantages over venoarterial

bypass, including the maintenance of pulmonary blood flow, improved myocardial oxygenation, delivery of thrombin or platelet emboli to the pulmonary circulation (as opposed to the systemic circulation in venoarterial bypass), and preservation of the arterial access. Because of these advantages, venovenous bypass has become the preferred mode of ECMO support in patients with respiratory failure.

Reliable predictors to identify adult patients who will benefit from ECMO do not really exist. ECMO may be considered for patients with acute, potentially reversible, life-threatening respiratory failure that is unresponsive to optimal standard treatment. Standard therapy defined by AECC includes supplemental oxygen, PEEP, mechanical ventilation, avoidance of fluid overload, and delivery of care in the ICU setting.

The pumpless arteriovenous system for extracorporeal CO_2 elimination called interventional lung assist (iLA) (NovaLung GmbH, Hechingen, Germany) is another type of ECLA. The transport of the blood through the iLA is passive via the pressure gradient between the femoral artery and femoral vein. CO_2 is eliminated from the blood by diffusion, thus diminishing the respiratory acidosis. Thus far, the indication for iLA is conventionally uncontrollable acidosis (pH < 7.2) due to hypercapnia in acute pulmonary damage, such as ALI or ARDS. The use of iLA as a rescue therapy in ARDS is increasing. It is highly efficient in eliminating CO_2 and allows more protective ventilator settings, ultimately leading to reductions in VILI and remote organ dysfunction (36, 39).

ECMO results in activation of the body's natural defense mechanisms against "nonself" and foreign invasion and activates a systemic inflammatory response (SIRS) (40, 41). The mechanisms of ECMO-related SIRS remain unclear. In a study of venoarterial ECMO in newborn pigs, McIlwain et al. (42) showed that that the concentrations of TNF-α and IL-8 rose faster in plasma than in the peripheral tissues during ECMO, which suggests that rising plasma levels of these cytokines immediately following the initiation of ECMO may not reflect increasing *de novo* tissue synthesis of cytokines but rather the mobilization of preformed cellular stores of inflammatory cytokines.

The hemostatic system represents a major problem in ECMO. Contact with the synthetic surfaces of an extracorporeal circuit activates platelets and the clotting system, inducing high platelet consumption. To avoid loss of platelets and activation of the clotting system, anticoagulation with heparin is necessary. Therefore, bleeding and/or

thrombosis are frequent complications in ECMO patients that require specific treatment and may even necessitate termination of ECMO. Bleeding complications in ECMO have been widely reported; they can be life-threatening, ranging from intracranial hemorrhage to gastrointestinal bleeding, to bleeding from cannula insertion sites (43, 44).

ECMO has been best studied as a treatment for respiratory failure in newborns. Data from three RCT (45-47) support the use of ECMO in neonatal respiratory failure and ECMO is a well-established therapy for severe cases of neonatal respiratory distress syndrome (48-50).

The role of ECMO in adults with respiratory failure remained controversial for a long time despite accumulating evidence. Clinical research on ECMO for severe respiratory failure in adults came to a halt in 1979 after the first RCT conducted by the NIH showed no additive benefit of ECMO to conventional mechanical ventilation in patients with ARDS (51). An interim analysis revealed a high mortality rate (90%) in both groups, and the authors terminated patient recruitment. The interpretation of these results remains problematic due to several factors: several of the 9 centers had no prior ECMO experience, the process of informed consent tended to exclude high risk patients, excessive bleeding among the ECMO patients occurred (average daily blood loss >2L/day), and most patients continued to receive high V_T mechanical ventilation. For all these reasons, the outcomes of this study are unlikely to be accurately generalizable to patients with ARDS receiving current strategies of mechanical ventilation and ECMO.

The second RCT to test ECLA did not take place until thirteen years later, in 1994. Forty patients with ARDS were randomized to extracorporeal CO_2 removal with tracheal insufflation of oxygen or a protocol-driven algorithm of mechanical ventilation alone (52). After enrollment of 40 patients, interim analysis determined that there was less than a ten percent chance of demonstrating a survival difference when extrapolated to the total projected enrollment of 60 patients, and the trial was terminated. Multiple criticisms of this trial have been articulated: the study was insufficiently powered to determine an improvement in survival rate in the ECMO group; the high survival rate in the control group also demonstrates the problems with using historical controls for studies of ECMO in ARDS patients; in this single center trial, the investigators' inexperience may have limited the results.

Although certain centers have reported dramatic survival curves in very ill patient populations, the failure to demonstrate a survival benefit for ECMO over conventional therapy in RCTs and the high level of specific training required for the technique have limited its widespread application in adults.

Given that only two RCTs on ECMO had been conducted in 20 years and that both had considerable problems with validity, the need for further RCTs to evaluate ECMO against conservative management, including low V_T ventilation led to the third RCT. The CESAR (**C**onventional ventilation or **E**CMO for **S**evere **A**dult **R**espiratory failure) was a large multi-center RCT conducted in the United Kingdom from 2001 to 2006; 180 patients were enrolled and randomly allocated to consideration for treatment by ECMO (n=90) or to receive conventional management (n=90). Of note, this was an intention-to-treat analysis and only 68 (75%) of the 90 patients received ECMO. The low V_T ventilation strategy from the ARDSNet study was recommended for all the patients. The primary outcome measure was death or severe disability at 6 months. Fewer patients in the ECMO arm than in the control arm had died or were severely disabled 6 months after randomization (33/90 (36.7%) versus 46/87(52.9%), respectively; relative risk (RR) = 0.69 [95% CI 0.50 to 0.97]; p = 0.030). This was equivalent to one additional survivor for every six patients treated. The authors recommended transferring adult patients with severe but potentially reversible respiratory failure whose LIS score exceeds 3.0 or who have a pH of less than 7.20 on optimum conventional management to a center with an ECMO-based management protocol to improve survival without severe disability (53, 54).

These recent encouraging results support the utility of ECMO at an expert ECMO-center to allow time for injured lung to recover in patients with severe respiratory failure who do not respond to traditional treatment algorithms. This study should lead to wider acceptance of ECMO.

ii) *Novel therapy: mesenchymal stem cell (MSC) therapy. Preclinical evidence*

Stem cell therapy appears to be a promising treatment for many respiratory diseases that have no effective treatment, such as ALI and ARDS. Considering that the repair process and attenuation of both inflammatory and fibrogenic responses are important for the improvement of ALI/ARDS, adequate manipulation of stem cells in the lung parenchyma appears promising.

MSC release several growth factors and anti-inflammatory cytokines that regulate endothelial and epithelial permeability and reduce the severity of the inflammation.

Several preclinical MSC studies using animal models of sepsis, ALI, and ARDS in newborn and mature lungs suggest stem cell therapy might become a treatment option (55, 56). Abreu et al. (57) reviewed the main experimental studies of adult stem cell therapies in Pubmed databases in the past 11 years, and they concluded that there is evidence for beneficial effects of MSC on lung development, repair, and remodeling.

f) Pathogenesis

The exact molecular and cellular basis for ARDS remains unclear more than 40 years after the original description of the syndrome. An intense and dynamic inflammatory response has been implicated in the alveolar and microvascular damage (see Figure 1).

Many experimental studies and several clinical studies suggest that endotoxin, activated leukocytes, endothelial activation, and interaction with a pro-inflammatory cytokine network culminate in extensive oxidative stress, the pathology ultimately underlying ARDS (58). Moreover, many animal studies have demonstrated functional and survival benefits from treatments like anti-endotoxin molecules, neutralizing antibodies against proinflammatory cytokines, neutrophil adhesion molecules, and antioxidants (59).

ARDS is characterized by diffuse alveolar damage that leads to increased permeability of the alveolar-capillary membrane in the early phase, which results in the leakage of edema fluid and plasma proteins from the vasculature into the alveolar spaces and contributes directly to the impairment of gas exchange and loss of lung compliance. The alveolar-capillary membrane is made up of two components: the capillary endothelium and the alveolar epithelium; the function of both is disrupted in ARDS. This injury to the alveolocapillary unit, termed diffuse alveolar damage, has been subdivided into sequentially occurring exudative, proliferative, and fibrotic phases. The initial, exudative phase is characterized by edema. The second, proliferative phase, occurring over the first week, is characterized by proliferation of type II alveolar cells and early deposition of collagen. Some patients progress to a third, fibrotic phase characterized by obliteration of normal lung architecture and diffuse fibrosis (60).

Neutrophils play a key role in ARDS injury. Neutrophils are the predominant cell type in bronchoalveolar lavage (BAL) fluid in ARDS patients (61). Macrophages, monocytes, and neutrophils are recruited to the lung and release proinflammatory mediators such as elastase, collagenase, ROS, growth factors (TGF-β and TGF-α), and cytokines such as tumor necrosis factor-α, interleukin (IL)-1β, IL-4, IL-8,IL-13, amongst others (62). In cases of ARDS with extrapulmonary origins, the release of bacterial proteins and proinflammatory cytokines into circulation are the stimuli for the increase in the permeability of the alveolar endothelium.

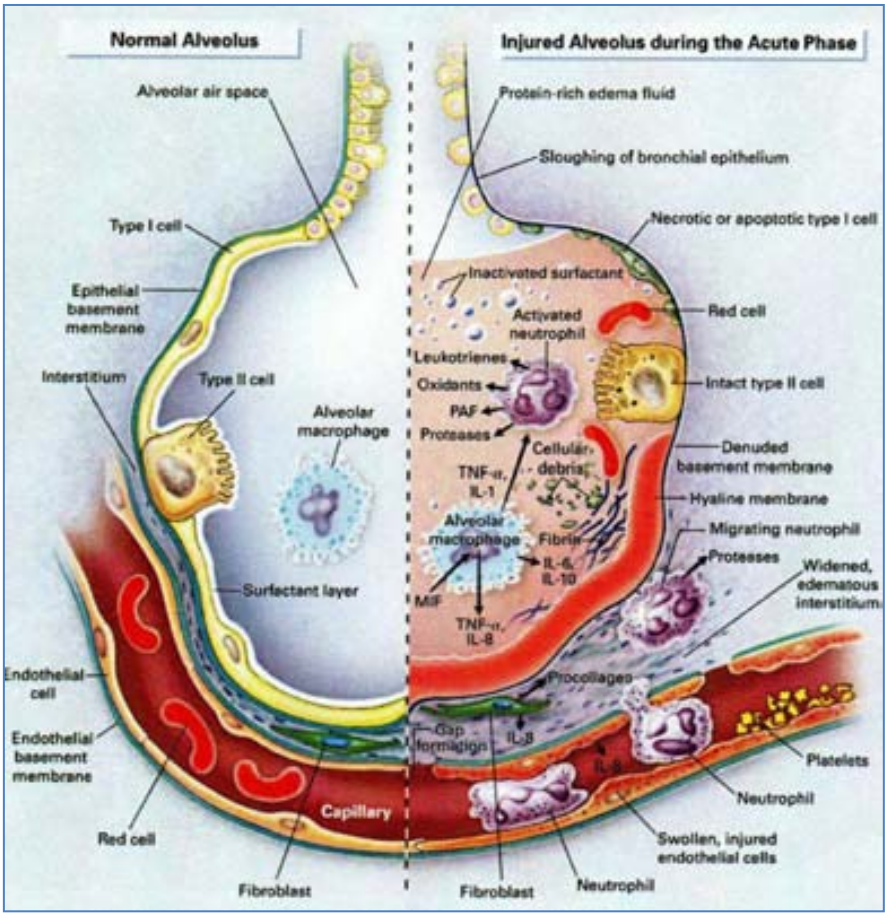

Figure 1. Multiple cellular responses and mediators contribute to alveolar-capillary membrane injury (right-hand side) and the transition from normal alveolar structure and function (left-hand side) in the acute phase of ALI/ARDS. Current concepts of pathogenesis involve multiple molecular factors and imbalance between injurious and reparative signals and pathways. From Ware LB and Mattay MA(63).

i) *Oxidative stress and VOCs*

Oxygen metabolism produces small amounts of ROS through physiological processes such as cellular respiration and inflammatory defense mechanisms (64).

In a non-injured organism there is a balance between the production of ROS and their effective removal by protective antioxidant and scavenger systems such as enzymes (superoxide dismutase, catalase and glutathione peroxidase...), nonenzymatic antioxidants (vitamin C, vitamin E...), and heme-binding proteins (transferrin, haptoglobin, albumin...)(65).

Massive increases in ROS and other radical species and the consequent excessive consumption of antioxidants lead to the loss of this equilibrium and promote cell injury. Whenever the release of ROS takes place in an uncontrolled way, the antioxidant systems will be insufficient to counteract ROS activity and the organism itself will be damaged by oxidative stress.

SIRS, sepsis, or ischemia/reperfusion lead to generation of ROS that may exhaust local or systemic antioxidant defense mechanisms (66). Inflammation and oxidative stress are among the most frequent pathologic states in critical illness (67). The mechanisms underlying these states are poorly understood and treatment remains often merely symptomatic. MODS, which is among the leading causes of morbidity and mortality in critical patients, is a result of oxidative stress (68).

In the setting of infection-induced ALI, activated neutrophils migrate from the pulmonary capillary into the alveolar and interstitial spaces, where they release ROS, reactive nitric oxide species, and cytokines. The secretion of these inflammatory mediators leads to lipid peroxidation of the cellular membrane of the capillary endothelium, which results in damage causing increased permeability and pulmonary edema (69-71).

The release of ROS by stimulated neutrophils leading to the generation of lipid peroxidation plays a major role in the pathogenesis of ARDS (72) (73). The interaction of oxygen free radicals with polyunsaturated fatty acids of membrane lipids (PUFA) produces VOC end-products such as ethane, malondialdehyde (MDA), propionaldehyde (also called propanal) , and pentane. A lipid peroxidation chain reaction is initiated by the removal of an allylic hydrogen atom by ROS. The radical generated in this way is conjugated, peroxidized by oxygen, and undergoes onward reactions (74) (Figure 2).

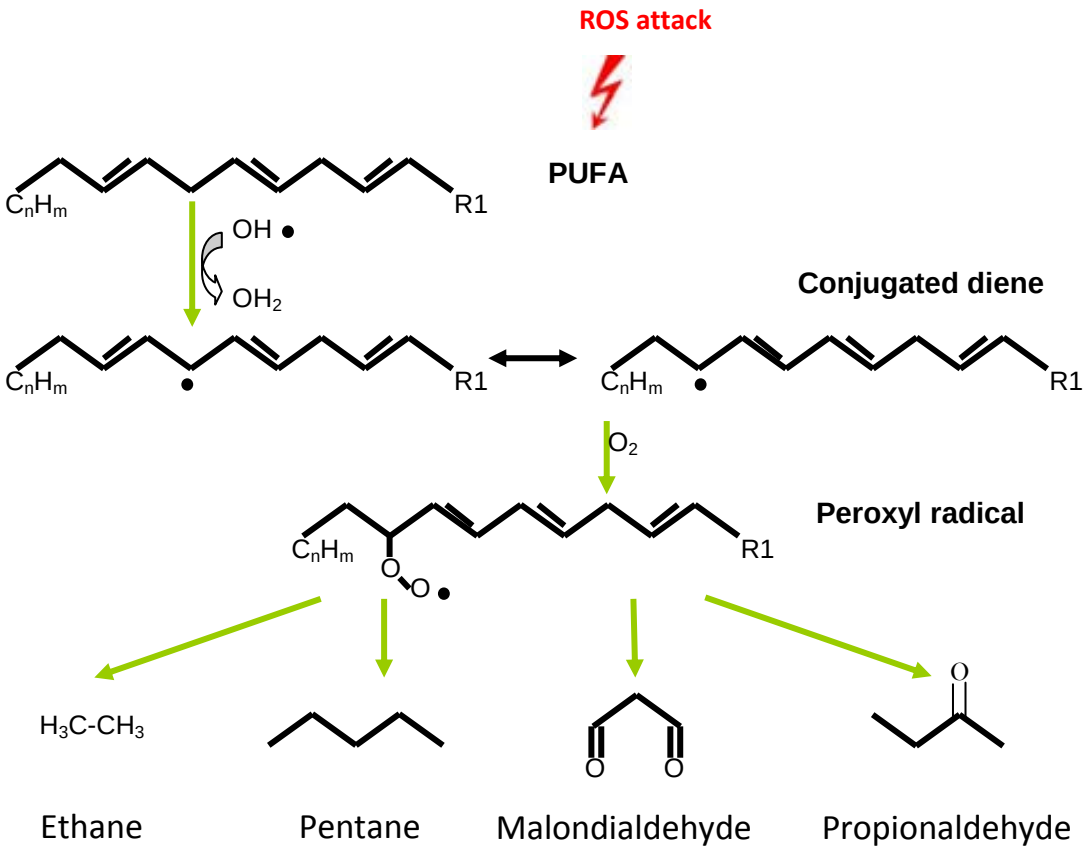

Figure 2. Generation of lipid peroxidation markers due to reactive oxygen species (ROS)-mediated peroxidation of polyunsaturated fatty acids (PUFA). From Miekisch et al. (74)

Omega-3 and omega-6 fatty acids are basic components of cell membranes. Saturated hydrocarbons, such as ethane and pentane, are generated from lipid peroxidation of omega-3 and omega-6 fatty acids, respectively. Due to the physiological ratio of omega−3 to omega−6 fatty acids, four times more pentane than ethane is generated through lipid peroxidation. Along the same pathway, aldehydes like MDA and propionaldehyde are also produced (74). In vitro studies have demonstrated that ethane and pentane are generated when cell cultures are exposed to ROS. Animal and clinical studies have shown a close correlation between clinical conditions with high peroxidative activity and exhalation of ethane and pentane (75). Furthermore, levels of exhaled pentane and ethane correlate well with MDA (76). Pentane may easily be metabolized by hepatic cytochrome P450 enzymes; therefore, extreme caution is warranted in interpreting exhaled pentane concentrations when liver function varies

between patients (77). Hydrocarbons have low solubility in blood and for this reason are released into breath within minutes of their formation in tissues (78).

Isoprene is always present in human breath and is formed along the mevalonic pathway of cholesterol synthesis (74). Experimental evidence shows that isoprene exhalation may be related to oxidative damage to the fluid lining of the lung (79) and the body (80). Lower isoprene levels in patients with ARDS suggests that an impaired cholesterol metabolism may play a role in this illness (81).

Acetone is one of the largest components of human breath. It is produced by hepatocytes via decarboxylation of excess acetyl-CoA. It is derived from the decarboxylation of acetoacetate, from lipolysis, or lipid peroxidation. Acetone can also be formed from oxidation of 2-propanol, a secondary alcohol. The fraction coming from lipid peroxidation is impossible to quantify. Patients with uncontrolled diabetes mellitus show increased acetone concentrations in breath (82).

Acetaldehyde is probably produced by oxidation of endogenous ethanol. One potential endogenous source of ethanol is the intestinal bacterial flora (74).

A new possibly important end-product of lipid peroxidation is propionaldehyde, which to date has only been found in vitro in the hearts of rats subjected to oxidative stress (83) or on ultraviolet-induced skin lipid peroxidation (84).

When using propionaldehyde as a marker of lipid peroxidation, it is necessary to exercise strict caution to avoid contamination from the pre-measurement use of disinfectants containing 1-propanol. Oxidation of 1-propanol during metabolism yields propionaldehyde. Ethanol- or 1-propanol-containing hand disinfectants are widely used for surgical hand antisepsis. Lang et al. (85) showed that no clinically relevant dermal absorption of ethanol and 1-propanol could be observed within 1 h after application; thus, they postulated that the use of the tested formulations containing ethanol and 1-propanol can be considered safe. It is also important to avoid disinfectants containing 2-propanol (also called isopropyl) because the oxidation of 2-propanol during metabolism produces acetone. The blood half-life of 2-propanol after acute intoxication in humans is around 2.5 hours (86) . Given the possible confounding effect of disinfectants on propionaldehyde and on acetone in the exhaled breath, disinfectant use must be closely controlled in breath gas analysis.

The volatile markers pentane, acetone, and isoprene are already detected in exhaled breath (81, 87) as well as in blood (87) of patients. Thus far, however, MDA is detected only in plasma samples (81).

The intensity and type of inflammatory activity seem to affect the generation of the lipid peroxidation products. Scholpp et al. (81) studied 10 healthy controls and 65 critically ill patients. Critically ill patients were divided into 3 groups: ARDS, at risk of developing ARDS, and severe head injury. The patients were also classified according to their infection status using the SIRS and sepsis criteria established by the American consensus conference (88). They determined two plasma peroxidation markers, MDA and thiobarbituric acid-reactive substances (TBARS), and three volatile markers, pentane, isoprene, and acetone. Pentane concentrations were highest in the ARDS group and in SIRS. MDA values were highest in the group at risk of developing ARDS and in sepsis. Since the septic patients were the most severely ill, it seems possible that the intensity of the lipid peroxidation had an impact on the lipid peroxidation products.

Pentane, a volatile substance with poor solubility, is eliminated within a few lung passages. The elimination of MDA is comparably slower and takes place via metabolism. Thus, the breath marker pentane seems to be more sensitive than the serum marker MDA (81).

In the critically ill, lipid peroxidation may be influenced not only by the underlying disease but also by treatments and medical procedures. Ben Baouali et al. (89) found increased levels of MDA in patients during weaning from mechanical ventilation compared to healthy volunteers. Thus, they suggested that mechanical ventilation per se may be responsible for the LIPID PEROXIDATION. However, Scholpp et al. (81) showed that the levels of MDA in mechanically ventilated patients with head injury tended to be even lower than in healthy individuals. The low MDA concentrations in this group are most probably due to the reduced state of metabolism induced by deep sedation and muscle relaxation. Therefore, they postulated that the stress during the weaning phase and not the mechanical ventilation per se caused the elevated MDA values in Ben Baouali et al.'s study. More studies are needed to clarify the relation between mechanical ventilation and lipid peroxidation.

The main components of exhaled human breath (accounting for 99.9%) are nitrogen, oxygen, water vapor, inert gases, and CO_2. The remaining slight fraction consists of trace components. More than 500 of these compounds have been found in exhaled breath (74, 90). These compounds can be divided in two groups according to their origin. Thus, there are the endogenous products released from the body and the exogenous ones, in part absorbed as contaminants from the environment or those which are not absorbed and appear in exhaled breath after the intravenous administration of

drugs (e.g. propofol) (91-97). The endogenous trace compounds include: i) inorganic gases, such as nitric oxide or carbon monoxide released from pulmonary tissue into the gas phase, ii) VOCs such as volatile lipid peroxidation end-products or other VOCs not related to lipid peroxidation, such as acetaldehyde or isoprene released from pulmonary tissue into the gas phase or from extrapulmonary tissue into blood emanating from blood-air exchange in the alveoli (98, 99), and iii) other non-volatile substances such as isoprostanes, peroxynitrite, leukotrienes, and cytokines, amongst others.
Evidence of oxidant activity can be determined in exhaled breath condensate (EBC) (74, 100, 101) and/or in BAL fluid (102).

Some serious problems hinder the quantitative analysis of EBC, such as the unclear relationship between assumed alveolar or airway concentrations in the condensate (103). Moreover, some of these compounds have only limited stability.

BAL has a well-established role in the diagnosis of pulmonary infections, particularly those due to opportunistic organisms in immunocompromised hosts (104) or in ventilator-associated pneumonia in ICU patients (105). Moreover, BAL makes it possible to assess the cellular expression of inflammatory cytokines and growth factors (106).

Pugin et al. (107) analyzed the concentrations of proinflammatory mediators in undiluted pulmonary edema fluids and in plasma in patients with ARDS and compared them to those in patients with hydrostatic lung edema. The results showed that during the early phase of ARDS the lung is the principal site of an intense inflammatory process; this inflammation is mostly limited to the lung and levels of inflammatory mediators in the systemic circulation are low.

VOCs can be generated anywhere in the body and may reflect physiologic or pathologic processes. They are transported via the bloodstream and exhaled through the lung, so they may provide information on the local distribution of inflammatory processes (66). Therefore, in addition to providing information that might help us understand the pathophysiological pathways involved in ARDS, breath-air analysis might be a useful approach to diagnosis.

Critically ill patients can be expected to exhale high concentrations of VOC since pathological conditions are severe. Thus, relationships between VOC and clinical status should be easier to detect than in other patients who are not so ill (66).

Concentrations of VOC in alveolar gas samples are two- to three-times higher than those in mixed expiratory air because they are not diluted by dead-space gas. Moreover, alveolar gas samples have the lowest concentration of contaminants from inspired air (66).

2. Breathomics

Metabolomic analysis provides biochemical profiles of endogenous low-molecular-weight metabolites in biological fluids. Metabolomic analysis applied to exhaled breath condensate is called breathomics.

Concentrations of VOC in exhaled breath are in the range of parts per trillion of volume (pptv) to parts per million of volume (ppmv) (74), so the analysis of these trace compounds in expiratory air requires highly sensitive and specific analytical methods.
A variety of techniques have been used to measure VOC composition of the exhaled air, including chromatographic-mass spectrometry (GC-MS), IMR-MS, and electronic nose (eNose) techniques.

Trace compounds in air can be measured in the range of pptv to ppbv using gas GC-MS or direct mass spectrometry (93, 108, 109). Direct mass spectrometric methods like IMR-MS, proton transfer reaction mass spectrometry (PTR-MS), or selected ion flow tube mass spectrometry (SIFT-MS) have the advantage that measurement results can be obtained in real-time online without the need for the preconcentration procedures that are necessary in traditional GC-MS methods. Although IMR-MS's fast reaction time, onsite application, and continuous registration bring it closer to clinical applicability, GC-MS is still considered the gold standard for the detection, separation, and identification of VOCs.

Enose integratively capture complex VOC mixtures using an array of different sensors that have individual sensitivity and specificity for VOCs. Electronic nose analysis of breath provides a unique fingerprint of exhaled metabolites, called a breath-print. These breath-prints can be used for diagnostic and monitoring purposes that do not require the identification of individual molecular constituents (110).

The analysis of VOCs in exhaled air enables noninvasive real-time observation of biochemical processes in the body. VOC in the expiratory air of mechanically

ventilated ARDS patients can be quantified online using IMR-MS and may reflect the amount of tissue damage caused by ROS (111, 112).

a) Ion-molecule reaction mass spectrometry

The IMR-MS system used in this study (V&F Analyse und Messtechnik GmbH, Absam, Austria) was originally designed to measure trace gas components in industrial fields.

The IMR-MS system consists of two mass spectrometry systems: A conventional electron impact mass spectrometer (EI-MS) and an ion-molecule-reaction mass spectrometer coupled with quadrupole mass spectrometer. The latter provides a highly sensitive method for sampling organic and inorganic compounds in exhaled breath (95, 113-116). The system is portable and can be used for both online and offline measurements (114, 117). No preconcentration technique was necessary because MS is sensitive enough to detect concentrations in the nmol/l range.

In contrast to EI-MS, IMR-MS largely avoids molecular fragmentation by soft ionization of breath sample molecules through low-energy primary ions. Three compounds (acetaldehyde, acetone, and isoprene) were directly calibrated using the calibration gas supplied by the manufacturer. We calibrated using a reference test gas containing a known concentration of these substances (acetaldehyde 1000 ppbv, acetone 1010 ppbv, and isoprene 990 ppbv). The remaining compounds, propionaldehyde and malondialdehyde-pentane, were indirectly calibrated to the sensitivity of isoprene. This semiquantitative calibration procedure is widely used in multicomponent analytical devices. Previously published limits of detection were acetaldehyde 2.2 ppbv, acetone 3.9 ppbv, ethanol 6.8 ppbv, and isoprene 2.6 ppbv (95).

A schematic diagram of the IMR-MS is shown in Figure 3. In the IMR-MS analyzer, the principle of ion-molecule reactions is applied on the interaction of positively charged atomic ions with neutral sample gas molecules. Two body collision processes result in the formation of product ions whenever the ionization potential of the sample molecule is less than the potential energy of the incoming primary ion and hence the entropy of the process becomes positive. The excess energy of the binary reaction is first stored in the product ion and then it is either statistically distributed in internal degrees of freedom (electron vibration, bond oscillations) or is used up to break the weakest bond of the ionized molecule, leaving an ion with a lower molecular

weight. Differences between the ionization potentials of primary ions and those of product ions may result in the rupture of the bonds and hence in a fragment ion with a lower molecular weight. Depending on the desired ionization energy, krypton, mercury, or xenon can be used as the source of the primary ions (section I, Figure 3). For the present study, mercury ions generated out of mercury vapor were used as primary ions.
The IMR ionization method can use the atomic mass scale to detect different molecules that have the same molecular weight. For example, acetaldehyde and CO_2 have the same mass of 44 amu (atomic mass unit). The ionization potential of mercury is 10.4 eV, so the mercury beam does not ionize CO_2 (13.8 eV) but it does ionize acetaldehyde (10.2 eV). Switching different ion beams (and hence energy levels) takes only 400 ms. The instrument uses two octopole systems (section II, Figure 3) operated at high frequencies to store both primary and product ions in a confined volume against their coulomb repulsion and transmit ions to the quadrupole mass analyzing section. The quadrupole mass separator (section III, Figure 3), driven by direct current and alternating current, operates as an electromagnetic filter according to a parametric resonance to a specific mass-to-charge ratio. At a given alternating-to-direct current ratio, only one specific mass of ions experiences a stabile trajectory through the quadrupole. A secondary electron multiplier (section IV, Figure 3) may generate as many as 10^8 electrons for each incoming ion. This allows the generation of an electrical pulse strong enough to be accepted by a computer counting system. The pulse rate represents the concentration of the molecular species in the gas sample brought to the instrument. The sample gas (inlet B, section II, Figure 3) is transferred to the instrument in a 2.5 m long heated capillary system (Silcosteel®, Restek, Bellefonte, PA, USA) at flow rates of 50 ml/min. Via a second capillary system, a constant pressure controller feeds a stable amount (1.5 ml/min) of the sample gas into the high vacuum ionization section. Gas response times to concentration changes are 50 ms, the switching time between different mass-to-charge ratios is below 10 ms, and the gas dead time (transfer time of gas through the capillary) is 2 seconds (95). Dwell time per mass analyzed is 300 ms with a mass resolution of 1 unit.

The compounds in expiratory air are measured in absolute concentrations of ppbv.

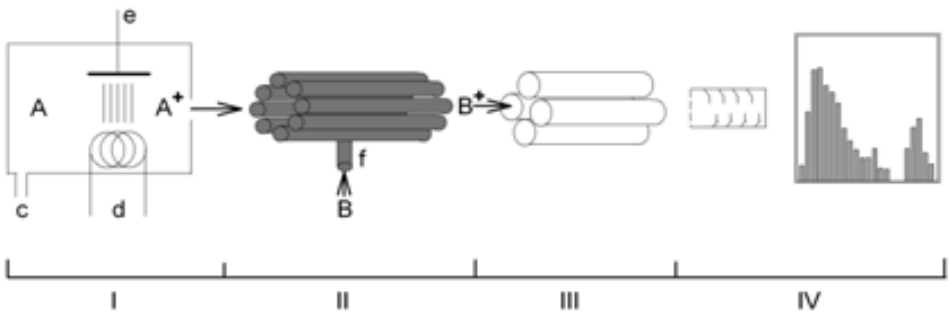

Figure 3. Schematic illustration of the IMR-MS. Section I: region of primary ion generation (A^+) by electron-impact ionization; c, inlet for primary ion rare gas (A; krypton, mercury, or xenon); d, heated filament for electron emission; e, electrode. Section II: region of interaction between primary ion (A^+) and analytical gas (B) surrounded by octopole field; f, inlet for analytical gas. Section III: quadrupole field mass selection unit for analytical gas ions (B^+). Section IV: secondary electron multiplier detection and signal processing unit.

3. Innovation and scientific relevance

The two predominant trends in ARDS research aim to determine:

1- How to optimize mechanical ventilation to avoid VILI.

2- The pathophysiological pathways of ARDS to improve understanding of the inflammatory cascade to enable diagnostic markers to be found and specify treatments to be developed.

This project is focused on the second approach.

This study is relevant two main ways:

1- This study provides information about lung tissue damage by oxidative stress in patients with ARDS:

The results improve our understanding of the inflammatory cascade that takes place when the lung is injured and our knowledge of the mechanisms involved in lung damage and repair. This better understanding of the inflammatory response can facilitate the search for specific diagnostic markers and the development of specific treatments that can lead to better outcomes.

Early recognition of ARDS is important in determining outcome. The prognosis depends partly on the nature and prompt management of the precipitating condition. We cannot always have an impact on the nature of the cause (e.g., in TRALI or trauma), but we can act quickly and purposefully. To date, the diagnosis of ARDS has depended on clinical and radiologic criteria. If we find molecular diagnostic markers, they could be valid not only for the diagnosis of ARDS, but they could also very important for the early detection of patients at-risk of developing ARDS and for the evaluation of the process, and thus for estimation of the prognosis.

There is still no specific treatment for ARDS. Apart from treating the underlying cause of ARDS, when possible, ARDS therapy consists of supportive mechanical ventilation while protecting the lung from VILI and avoiding secondary insults. There is no treatment that targets the mechanisms involved in the development of ARDS. Research on the pathophysiological mechanisms might help us find specific treatments to interfere in the processes underlying the development of ARDS. Furthermore, the appropriate biomarkers could also be used for monitoring and optimizing treatment.

2- This study helps to define the usefulness of IMR-MS in identifying markers in exhaled breath:

- It provides information about the method of using gas analyses of expiratory air for continuous noninvasive monitoring, as well as specific experience in the novel technique, IMR-MS.
- This noninvasive method might also be used to improve our understanding of the pathophysiological mechanisms involved in other lung diseases or even in pulmonary infections (identify patterns associated to ventilator-associated pneumonia).
- This system might also be applied in patients with spontaneous ventilation.
- Further studies could then use this method to monitor the effects of therapeutic interventions on oxidative stress and lipid peroxidation.
- The findings will also provide useful additional information about other noninvasive or minimally invasive methods (e.g., eNose, EBC, or BAL).

HYPOTHESIS AND OBJECTIVES

II. HYPOTHESIS AND OBJECTIVES

1. Formulation of the hypothesis

We hypothesized that the concentration of lipid peroxidation products (MDA, pentane, propionaldehyde, and acetone) is higher in the expiratory breath of ARDS patients than in that of controls without lung injury.

2. Endpoints

a) Primary endpoint

The primary aim of the study is to determine and assess the lung oxidative stress response by measuring the concentration of VOCs in the expiratory air of ARDS patients and compare it to the control group.

b) Secondary endpoints

a) To determine the systemic inflammatory response measured by C-reactive protein (CRP), interleukin-6 (Il-6), and procalcitonin (PCT) in blood in both groups.

b) To determine whether there is a linear association between the concentrations of different VOCs.

c) To determine whether there is a linear association between VOCs and systemic inflammatory markers, as well as between VOCs and lung injury severity expressed by the Lung Injury Score (LIS) and PaO_2/FiO_2 ratio.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

1. Study Design

We conducted a cross-sectional analysis of VOCs and systemic inflammatory markers within a prospective observational descriptive study of clinical parameters.
We measured systemic inflammatory markers in blood and potential VOCs in the exhaled breath of ARDS and control patients by online IMR-MS. Clinical parameters were recorded at the time of measurement and until hospital discharge when appropriate.
One year after hospital discharge, we evaluated the one-year mortality in the two groups.

2. Pilot study

In 2008, a pilot study was done in the Department of Anesthesiology at the Hospital Großhadern (Ludwig-Maximilians-University of Munich) to assess the technical and clinical feasibility of the proposed project and the study protocol.
The lipid peroxidation products MDA, pentane, and propionaldehyde were measured online by IMR-MS in expiratory and inspiratory air of 7 patients with ARDS, 5 of whom were on ECMO). Postoperatively ventilated patients served as a control group (n = 5).
In this pilot study, MDA, pentane, and propionaldehyde were significantly higher in patients with ARDS than in control group patients.

3. Sample size

On the basis of this pilot study, we calculated 28 patients would have to be included in the present study (14 patients in each group) to enable us to detect a difference of 1.5 units ± 1 unit (expiratory/inspiratory ratio of VOCs) between ARDS patients and controls at a type I error of 5% and a power of 80% (beta error 20%)(Mann-Whitney-U test).

4. Recruitment of patients

This study was conducted from 2009 to 2011 in a 20-bed medical-surgical intensive care unit at the University Hospital Großhadern (Department of Anesthesiology, Ludwig-Maximilians-University of Munich).
This Department of Anesthesiology is one of the largest anesthesiology departments in Europe; it is a reference center for ARDS treatment and is one of the six ECMO-centers in Germany.
The study protocol complied with the Helsinki Declaration and was approved by the local ethics committee of the Ludwig-Maximilians-University of Munich (protocol no. 089/04). A legal representative or a close relative provided written informed consent for each patient. Additionally, all patients who recovered and regained consciousness with adequate cognitive functioning gave their consent to the study retrospectively.
All consecutive eligible patients were included.

5. Comparison groups

a) Inclusion and exclusion criteria

i) *ARDS group:*

- ✓ Inclusion criteria

  Patients were eligible for inclusion if they met all the following criteria:

  a) Mechanically ventilated patients with ARDS according to the American-European Consensus Conference (3) who were admitted to the ICU.
  b) Within the first 48 hours of diagnosis.
  c) Age: 16-85 years.

  When the criteria were met, informed consent was obtained.

- ✓ Exclusion criteria:

  a) Pregnancy.
  b) Malignancy, defined as metastatic cancer or hematologic malignancy.

c) Severe chronic respiratory disease: chronic restrictive, obstructive, neuromuscular, or pulmonary vascular disease resulting in severe exercise restriction, e.g. unable to climb stairs or carry out household tasks.

d) Chronic alcohol abuse.

e) Limitation of therapy.

f) Acute brain injury, defined as traumatic brain injury or acute ischemic stroke.

g) Cardiac surgery

h) Refusal to provide consent.

ii) *Control group:*

- ✓ <u>Inclusion criteria</u>

  Patients were eligible for inclusion if they meet all the following criteria:

  a) Mechanically ventilated ICU patients without ALI at any time after admission.
  b) Chest radiograph without alveolar infiltrates.
  c) Hemodynamic stability, defined as systolic blood pressure >90 mmHg after fluid resuscitation with or without the need for vasopressors afterward.
  d) Age: 16-85 years

  When the criteria were met, informed consent was obtained.

- ✓ Exclusion criteria:

  a) Pregnancy

  b) Malignancy, defined as metastatic cancer or hematologic malignancy

  c) Severe chronic respiratory disease: chronic restrictive, obstructive, neuromuscular or pulmonary vascular disease resulting in severe exercise restriction, e.g. unable to climb stairs or carry out household tasks.

  d) Chronic alcohol abuse.

  e) Limitation of therapy.

  f) Acute brain injury, defined as traumatic brain injury or acute ischemic stroke.

g) Cardiac surgery

h) Refusal to provide consent.

iii) *Rationale for the exclusion criteria*:

During traumatic brain injury and acute ischemic stroke (112, 118, 119) as well as in cardiac surgery (120, 121), potentially specific VOCs might be released and therefore might act as confounders in our study. Severe chronic lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), or pulmonary hypertension are linked to chronic or acute inflammation. As lipid peroxidation is a basic mechanism of inflammatory processes, lipid peroxidation markers would probably be increased in these diseases (112).

b) Considerations

We restricted the use of cutaneous antiseptic solutions and disinfectants (e.g., for catheterization, the care of open wounds, or surgery) within 4 hours prior to measurements. For this reason, surgical patients were not eligible until 4 hours after surgery. Moreover, the person doing the MS measurements was not allowed to use hand disinfectants one hour beforehand.

c) Indications for ECLA therapy

ECLA (ECMO and iLA) was considered for ARDS patients who did not response to optimal standard treatment defined by AECC.

ECLA was administered at the attending physicians' discretion. The rough indications are described below. More details about the evidence-based therapy for severe ARDS based on an algorithm-guided approach reported by Deja el al. (122) are available in the appendix, Figures 11 and 12.

i) *Indications for ECMO*

ECMO was indicated in patients with reversible underlying disease when, despite protective ventilation strategy (V_T= 4-6 ml/Kg of PBW):

- PaO2/FiO2 ratio < 70-80 mmHg
- Uncontrollable acidosis (pH<7.2)

ii) *Indications for iLA*

iLA was indicated in patients with reversible underlying disease when, despite protective ventilation strategy (V_T= 4-6 ml/Kg of PBW):

- Uncontrollable acidosis (pH<7.2)
- PaO2/FiO2 ratio >100 mmHg
- Hemodynamic stability defined as need for noradrenalin < 2mg/h

6. Measurements

a) Sampling of airway gas

Alveolar air samples were analyzed online by direct mass spectrometry system, the IMR-MS. In the ARDS group, the analyses were done within the first 48 hours of the diagnosis of ARDS; in the control group, they were done at any time after admission.
We measured the concentrations of the following lipid peroxidation molecules: malondialdehyde–pentane (MDA-P), propionaldehyde, and acetone. Furthermore, acetaldehyde and isoprene were also analyzed as standard markers in the exhaled breath.

i) *Method*

Before the airway gas measurements, the system was directly or indirectly calibrated for the molecules of interest, as described in section 1.1.6 (IMR-MS).

As part of the standardized airway gas sampling procedure, ventilation parameters were kept constant 60 min prior and during airway gas sampling.

Carbon molecular sieves are affected by high water content in the samples. Since the water content of samples can be very high when active humidifiers are used, the humidifier was removed 10 minutes before the measurements.

For expiratory gas sampling, a sterilized stainless steel T-piece was placed between the end of the endotracheal tube and the Y-piece of the respirator circuit, and connected to the heated capillary system of the IMR-MS. While the IMR-MS was connected to the respiratory circuit, the electron impact MS integrated into the IMR-MS allowed simultaneous CO_2 detection, and the expiratory air CO_2 concentration was simultaneously recorded. The simultaneous measurement of CO_2 concentration enables the identification of the alveolar phase during expiratory gas measurements. Controlled alveolar sampling was enabled by a software tool that used this CO_2 signal to identify the end-expiratory phase of respiration, triggering the simultaneous data acquisition of IMR-MS measurements and transmitting the results to an end-expiratory data file. Before each measurement, the CO_2 threshold was set to 70% of maximum measured CO_2 concentration during a preceding 2 min observation time. When the capnogram exceeded the preset value (CO_2 threshold), the measurements of the concentrations of the molecules in this period were recorded, as long as the actual measured CO_2 concentration fell below the preset CO_2 value during the respiratory cycle, point of time where an automatic switching off took place. See Figure 4 below.

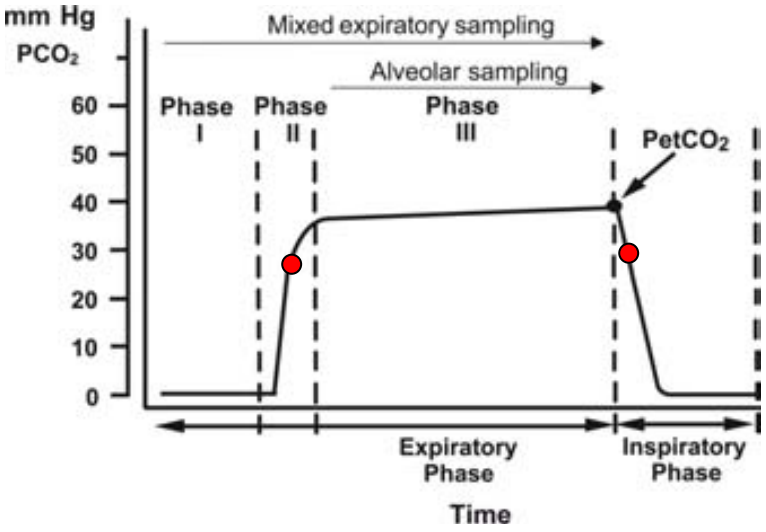

Figure 4. Schematic diagram of a normal capnogram. Phase I is the first expiratory stage, representing the anatomical dead space that would not contain CO_2 and VOCs. Phase II reflects the appearance of CO_2. Gas sampled during this phase typically contains a mixture of alveolar and dead space air. Phase III reflects a minimal increase in CO_2 concentration as the result of alveolar emptying. This phase is referred to as the alveolar plateau. $PetCO_2$ is the terminal portion of exhaled CO_2, which reveals the actual end-tidal concentration. The red dots mark the switching points of the measurement. (From Miekisch et al. (123)).

Inspiratory gases were analyzed through a T-piece placed directly in the respiratory circuit.

Airway gas was continuously sampled at a rate of 50 ml/min and analyzed during a period of 20 minutes in the expiratory phase and 5 minutes in the inspiratory phase. Since only the alveolar gas sample was suitable for the expiratory measure and furthermore the expiratory time is reduced in the ARDS group, the expiratory recording time must be four times as long as the inspiratory recording time to achieve the same number of valid cycles.

The mean concentration of each molecule in each phase was calculated. To reduce the likelihood of surface adhesion of VOCs, the T-piece is small (total surface area exposed to respiratory gas = 2.4 cm^2).

Concentrations of compounds in expiratory mass were measured by the mass spectrometer in absolute concentrations (ppbv) and were converted to nmol/L (Conversion factor: 25.5 ppbv corresponds to 1 nmol/L at body temperature, pressure, saturated conditions).

Both MDA and pentane molecules have the identical atomic mass of 72 amu and so far they are not distinguishable by IMR-MS. During ionization by the IMR-MS both

MDA and pentane occasionally lose one hydrogen atom, resulting in peak measurements for both molecules after ionization of mass-to-charge ratio (*m/z*) 71 and *m/z* 72, depending on whether or not they lost a hydrogen atom. For this reason the results of these two molecules are expressed as MDA-P m/z 71 or 72.

The elimination and intake of VOCs depends on the alveolar concentration gradients of the substances and on the ventilation/perfusion ratio in the lung. Due to the complexity of pulmonary adsorption and exhalation of VOCs, these effects are not linear. Thus, we decided to report inspiratory values together with expiratory values rather than to correct expiratory values.

b) Blood samples

The following biochemical and biological parameters were analyzed for both groups at the time of the mass spectrometry measurement: serum electrolytes, prothrombin time, platelet count, hemoglobin, leukocytes, bilirubin, urea, creatinine, glucose, CRP, IL-6, and PCT. A blood gas analyzer (Rapidlab®1265, Siemens) was used to determine respiratory parameters (PaO_2, $PaCO_2$, bicarbonate, and pH) and lactate.

7. Data collection

Patients were followed until hospital discharge. Patients who were transferred to another hospital for rehabilitation were also followed completely.
At least one year after the hospital discharge, all patients were telephoned to determine the one-year mortality.
Data on demographic, physiologic and radiographic characteristics, coexisting comorbidities, medications and complications were recorded.
The following list contains all the variables recorded in detail:

- Chest radiographs from patients were reported by radiologists.
- Demographic data: Age, gender, weight, ideal weight, height. Body mass index (BMI) was computed.
- Transfer from another hospital.

- Comorbidities or risk factors: Diabetes mellitus, obesity, arterial hypertension, mild-to-moderate chronic respiratory disease (when severe, it was a criterion for exclusion), and a miscellaneous group comprising previously undefined comorbidities that must be considered due to their importance (anorexia, hypothyroidism, chronic pancreatitis, epilepsy, and osteoporosis).
- Causes of ARDS. If sepsis, the presumed site of infection and microorganism were recorded and blood cultures and semiquantitative cultures of endotracheal aspirates were obtained.
- Patients with PaO_2/FiO_2 ratio < 100 mmHg were classified in a subgroup with severe ARDS.
- Existence of SIRS, sepsis, or septic shock.
- Event surgery before the measurement and how many hours before.
- Need for ECLA and related variables (length of treatment, blood flow, gas flow, and complications) anytime during the ICU stay.
- ICU length of stay (LOS) and hospital LOS.
- ICU mortality, hospital mortality, 28-day mortality, 90-day mortality, and one-year mortality.
- Cause and location of death.
- The severity of disease at the time of the exhaled breath measurement was assessed using the New Simplified Acute Physiological Score (SAPS II) (124) and the Lung Injury Score (LIS) (125). Our SAPS II scores were generated without assigning points for the estimated state of consciousness, as this seemed to be largely speculative in deeply sedated patients. See the appendix for a description of the SAPS II.
- Punctuation in the Ramsay Scale (126). See the appendix for a description of the scale.
- Patients were monitored for signs of failure of nonpulmonary organs and systems in the first 24 hours after inclusion:
  - ✓ Circulatory failure was defined as a systolic blood pressure ≤ 90 mm Hg or the need for treatment with any vasopressor.
  - ✓ Coagulation failure was defined as a platelet count ≤ 80,000/ mm^3.
  - ✓ Hepatic failure was defined as a serum bilirubin concentration ≥ 2 mg/dl.
  - ✓ Renal failure was defined as a serum creatinine concentration ≥ 2 mg/dl.

- Renal replacement therapy (yes or no) anytime during the ICU stay.
- Corticosteroids (yes or no) at the time of the mass spectrometry measurement. When yes, type and dose.
- Prone position (yes or no).
- Vital signs at the time of the mass spectrometry measurement: heart rate (HR); systemic systolic, diastolic, and mean blood pressure (MAP); body temperature and urine output.
- Respiratory parameters at the time of the mass spectrometry measurement: Ventilator mode, days of mechanical ventilation, tracheotomy, PaO_2, FiO_2, $PaCO_2$, bicarbonate, pH, respiratory rate (RR), inspiration/expiration ratio (I:E), minute volume (MV), PEEP, peak inspiratory pressure (Ppeak), mean airway pressure (Paw), V_T, ideal V_T (=V_T (ml)/PBW (Kg)). Lung compliance was computed.
- Respiratory complications, defined as reintubation or barotrauma defined as clinical relevant pneumothorax requiring pleural drainage at at anytime while the patient was intubated.
- Need for vasopressors. When yes, type and dose.
- Need for hemodynamic catheter (pulmonary artery catheter or Pulse-induced Contour Cardiac Output (PiCCO)).
- Blood and biologic parameters: serum electrolytes, prothrombin time, platelet count, hemoglobin, leukocytes, bilirubin, urea, creatinine, glucose, CRP, IL-6, PCT and lactate.
  - ✓ We considered values of CRP $\leq$0.05 mg/dl, PCT $\leq$ 0.5 ng/ml, IL-6 $\leq$ 5.9 pg/ml and Lactate < 2 mmol/l to be within the normal range.

8. Statistical methods

We used the Kolmogorov-Smirnov test to determine whether the descriptive variables were normally distributed and verified the results by drawing histograms and boxplots. Nominal and ordinal data are expressed as frequencies, continuous normal data are expressed as mean ± standard deviation, and continuous non-normal data are expressed as median and interquartile (25^{th} and 75^{th} percentile) range.

To evaluate differences between the two groups, we used parametric tests when the data were normally distributed and non-parametric tests when they were not. Continuous variables were analyzed by Student's t-test or Mann-Whitney-U test and categorical variables were analyzed by the chi-square test or Fisher´s exact test.

To make comparisons of VOCs within the groups, we used the Wilcoxon signed-rank test.

The level of significance was set at $p < 0.05$. All tests were two tailed.

Although this is an explorative study, we decided to submit the analysis of the VOCs to a test for multiple comparisons. As the Bonferroni correction is substantially conservative and the threshold too stringent for our study type, we chose the Bonferroni-Holm method. The Bonferroni-Holm was applied for the expiratory breath VOCs, for the comparisons within the groups and for the inflammatory markers.

To determine the relationship among the VOCs and between the VOCs and systemic inflammatory markers (CRP, PCT, and IL-6), LIS, and PaO_2/FiO_2 ratio, we used a simple linear regression model, validated by residual analysis, including a check of the normality of the residuals, homoscedasticity of the response variable, and presence of outliers/influential data points. If appropriate, data were transformed to improve the model.

Statistical analyses were done using the software packages R 2.11.1 (127) and SPSS (Statistical Package for the Social Sciences) version 20.0. (128).

9. Bias and confounding

The control group was made up of intubated patients without lung injury to control for the potential confounding effects of mechanical ventilation. Since the difference between ARDS patients and healthy patients is not only the illness, but also the therapeutic intervention (mechanical ventilation), this study was performed with intubated patients as control group.

None of the patients refused to participate in the study and none dropped out of the study. All patients were completely followed until hospital discharge, even the patients who were transferred to other hospitals for rehabilitation or prolonged weaning. For the one-year mortality survey, two patients were not found.

A precise protocol was defined for the study. Study subjects were recruited according to established criteria, and the protocols for the exhaled breath analysis, blood analysis, and recording of parameters was followed in strict detail.

To avoid interobserver variability, a single person was responsible for recruitment.

RESULTS

IV. RESULTS

1. Baseline Characteristics

a) ARDS group (n=16)

Nine (56%) of the ARDS patients had comorbidities; the most frequent was non-severe chronic obstructive pulmonary disease (COPD).

Thirteen (81%) of the ARDS patients were admitted from other hospitals.

The LOS in the ICU was 37 ± 33.5 days and the LOS in the hospital was 70.1 ±69.5 days.

The most frequent cause of the development of ARDS was pneumonia with septic shock (n = 15; 94%)**.** One patient also had streptococcal toxic-shock syndrome (STSS) and another also had an incarcerated umbilical hernia. One patient developed ARDS following multiple trauma.

Three different types of pneumonia affected the patients:

1-Community-acquired pneumonia: 66.7% (n=12)

2- Nosocomial pneumonia or hospital-acquired pneumonia: 13.3% (n=2)

3- Aspiration pneumonia: 6.6% (n=1)

Table 2 reports the pathogens found in the semiquantitative cultures of endotracheal aspirates.

The most frequent microorganism was *Streptococcus pneumoniae* (n=4, 26.7%). In three patients the endotracheal aspirate cultures were negative.

Table 2. Types of pneumonia and their causative germs.

Types of pneumonia	**Germ**
Community-acquired	*Streptococcus pneumoniae* (4 cases) *Streptococcus pyogenes* *Beta-hemolytic streptococci* (STSS) *Staphylococcus aureus* *Serratia marcescens, Pseudomonas aeruginosa* *Citrobacter, Klebsiella pneumoniae* *Herpes simplex* (2 cases)
Nosocomial	*Human respiratory syncytial virus*

All ARDS patients were ventilated in pressure-control mode and all were in the prone position except one patient with multiple trauma who could not lie on his abdomen due to a vacuum pack temporary abdominal closure.

No patients received neuromuscular blockers and all scored 6 on the Ramsay sedation scale.

Respiratory complications occurred in 25%, one patient was reintubated after self-extubation and three presented pneumothorax requiring pleural drainage due to barotrauma. Eight patients (50%) needed a hemodynamic catheter (pulmonary artery catheter in 5 and PiCCO in 3).

Eight patients (50%) were being treated with venovenous ECMO at the time of measurement; the median length of ECMO treatment was 29.7 (9.3-22) days. ECMO blood flow (oxygenation) and gas flow (decarboxylation) were individually adjusted to achieve adequate oxygenation (PaO_2 = 60-70 mmHg) and decarboxylation (pH 7.3-7.45). The ECMO system was set at a blood flow rate (=flow through the ECMO circuit) of 3.3±0.52 liters per minute (lpm) and sweep gas (= the oxygen line attached to the top of the oxygenator) rate of 6.3±1.4 lpm. Four more patients were treated with ECLA after the measurement, two patients with ECMO and two with iLA. Altogether, 12 patients in the ARDS group required ECLA. The overall complication rate of ECLA was 25% (3 of 12 patients: 2 under ECMO, 1 under iLA). One developed ischemia in the right leg from the iLA arterial catheter, which was quickly exchanged and no permanent disability occurred. In one of the patients receiving ECMO, the ECMO

jugular catheter was displaced; it was immediately replaced with another one and there were no clinical implications or sequelae. Another patient receiving ECMO developed a lethal intracranial hemorrhage.

Table 3 reports the PaO_2/FiO_2 values for patients in the ARDS group. Because ECMO improves oxygenation, for the 8 patients receiving ECMO at the time of measurement, we provide the PaO_2/FiO_2 values both at the time of measurement of VOCs and prior to starting ECMO. According to the PaO_2/FiO_2 values, 13 patients (81%) had severe ARDS (PaO_2/FiO_2 ratio <100 mmHg) at the very beginning. Eight (50%) of the ARDS patients had severe ARDS at the time of mass spectrometry measurement.

Table 3. PaO_2/FiO_2 values at the time of measurement of VOCs and before starting ECMO in the ARDS group.

PaO_2/FiO_2 at the measurement time	**PaO_2/FiO_2 before starting ECMO**
186	-
98	60
108	53
193	43
131	68
104	-
151	40
90	70
45	-
75	-
56	-
71	-
141	30
92	40
37	-
154	-

Three (19%) patients underwent surgery before the exhaled breath measurement. See Table 4.

Table 4. Surgery before the exhaled breath measurement in ARDS group.

Patient	**Reason for surgery**	**Type of surgery**	**Time from surgery to measurement**
1	Double lung surgery	Closing the thorax	24h
2	Incarcerated hernia	Bowel resection	120h
3	Trauma	Vacuum-pack temporary abdominal closure	20h

The 28 day-mortality, 90 day-mortality, ICU mortality, and hospital mortality were 25% (n=4), 31% (n=5), 31% (n=5) and 37.5% (n=6), respectively. The one-year mortality remained the same as hospital mortality, 37.5%. For the subgroup of ARDS patients treated with ECLA, the hospital mortality was 41.7% (5/12).

Five patients died in the ICU: four patients died of MODS and one died of massive intracranial hemorrhage. Because the intracranial hemorrhage occurred while the patient was on ECMO, this death was considered iatrogenic. Another patient died in the general ward of late tracheostomy hemorrhage. Details of the mortality are reported in Table 5.

Table 5. Chronological mortality among the consecutive eligible ARDS patients included.

Patients	**Cause of ARDS**	**Germ in lung**	**ECMO at time of death**	**Day of death**	**Place of death**	**Cause of death**
1	Pneumonia	*Herpes simplex*	Yes	135	ICU	MODS
2	Pneumonia	*Human respiratory syncytial virus*	Yes	20	ICU	MODS
3	Incarcerated umbilical hernia and pneumonia	*Streptococcus pneumoniae*	No	67	Ward	Late tracheostomy hemorrhage
4	Pneumonia	*Staphylococcus aureus*	Yes	14	ICU	MODS
5	STSS-Pneumonia	*hemolytic streptococci*	Yes	7	ICU	Massive intracranial hemorrhage
6	Polytrauma	No germ	No	3	ICU	MODS

MODS: multiple organ dysfunction syndrome; ICU: Intensive Care Unit; STSS: streptococcal toxic-shock syndrome.

b) Control group (n=16)

Nine (64%) of the control patients had comorbidities; the most frequent was arterial hypertension.

One (7%) control patient was admitted from another hospital.

The LOS in the ICU was 8.6±8.8 days and the LOS in the hospital was 28.1±23.7 days.

All the patients underwent surgery before the exhaled breath measurement (time from surgery to measurement=28.4 ± 25 h). Most were patients admitted to the ICU after neck and throat surgery requiring postoperative intubation and mechanical ventilation for unstable airways in the absence of major lung pathology.
Table 6 summarizes the reasons for hospital admission and the type of surgery.

Table 6 . Reasons for hospital admission and type of surgery in the control group.

Reasons for admission	**Type of surgery**
Mouth abscess	Drainage
Tracheomalacia	Plastic enlargement
Tracheal stenosis	Resection
Epiglottic abscess	Drainage
Pharyngeal carcinoma, 3 cases	Resection
Gonarthritis	Knee replacement
Vertebral fracture	Internal fixation
Pelvis fracture, 2 cases	Osteosynthesis
Prostate carcinoma	Prostatectomy
Pheochromocytoma	Adrenalectomy
Obstructive sleep apnea syndrome	Tonsillectomy

Only one patient had sepsis; none had septic shock. Eight (57%) patients had SIRS.
The patients were ventilated in different ventilatory modes (pressure control, volume control, and pressure support). Neuromuscular blockers were administered and Ramsay sedation scale scores ranged from 2 to 5. There were no respiratory complications.
The hospital mortality was 0% and the one-year mortality remained unchanged.

2. Outcomes and endpoints

a) Comparison of the populations of the two groups

A total of 30 patients (ARDS, n = 16; control, n = 14) underwent expiratory air analysis with IMR-MS. All 16 ARDS patients met the North American-European definition of ARDS and all patients from both groups fulfilled the inclusion criteria.

There were no significant differences between the two groups with respect to age, sex, BMI, or comorbidities proportion, so the groups were demographically comparable.

The physiologic index of the severity of respiratory dysfunction (PEEP level, PaO_2/FiO_2, and LIS) and the SAPS II scores were, as expected, higher in patients with ARDS than in control patients. Note that the SAPS II scores did not include the points corresponding to the Glasgow coma scale and thus the SAPS II score in the ARDS group could be underestimated. Table 7 summarizes the demographic and severity characteristics of the two groups.

Table 7. Demographic and severity characteristics and mortality. (An expanded version of this table showing the difference of the means, standard error, and 95% confidence interval for the continuous variables is found in the appendix, Table 19.).

Characteristic	ARDS	Control	P-value
Age[*]	40.5 ± 16.2	46.5 ± 22.1	0.17[‡]
Male/Female	62.5%/37.5%	57%/43%	0.77[$]
BMI[*]	30.0 ± 10.8	26.3 ± 8.0	0.29[‡]
Comorbidities	56%	64%	0.66[$]
SIRS	100%	57%	0.005[#]
Sepsis	94%	7%	<0.0005[$]
Septic shock	94%	0%	<0.0005[$]
Number of organ failures[Ω] (including respiratory failure)	5 (3-5)	2 (2-2.25)	<0.0005[o]
SAPS II[*] score	34.3±12.6	18.4 ±6.5	<0.0005[‡]
LIS [*]score	3.6±0.32	0.9±0.4	<0.0005[‡]
28 day-mortality	25% (n=4)	0%	0.1[#]
90 day-mortality	31% (n=5)	0%	0.04[#]
ICU mortality	31% (n=5)	0%	0.03[#]
Hospital mortality	37.5% (n=6)	0%	0.019[#]
One-year mortality	37.5% (n=6)	0%	0.019[#]

ARDS: Acute respiratory distress syndrome; BMI: body mass index; SIRS: systemic inflammatory response syndrome; SAPS II: Simplified acute physiological score; LIS: Lung injury score.
[*] Mean value ± standard deviation
[Ω]Median (interquartile range=25th-75th percentiles)
[‡] Student's t-test
[$] Chi^2 test
[#] Fisher's exact test
[o]Mann-Whitney- U test

The lung compliance was 25.0 ±13.6 ml/cmH_20 and all the patients had opacities involving at least 3 quadrants (three quadrants in 50% had and four quadrants in 50%). In the ARDS group, the mean time under mechanical ventilation was 39 ±40 days with a FiO_2 of 0.8±0.20 and PEEP of 18±2 cmH_2O. The resultant PaO_2/FiO_2 ratio was 108±47. All patients were ventilated under permissive hypercapnia with the consequent

respiratory acidosis. Seven (44%) ARDS patients simultaneously presented lactic acidosis.

ARDS patients were ventilated in pressure–control mode with a maximum Paw of 28 cmH_2O. The resultant V_T was according to ARDSNet.

Ventilator settings with resulting gas parameters are reported in Table 8.

Table 8. Ventilator settings and resulting gas parameters. (An expanded version of this table showing the difference of the means, standard error, and 95% confidence interval for the continuous variables is found in the appendix, Table 20.).

Parameter	**ARDS**	**Control**	**P-Value**
Days MV	38.9±39.6	6.2±8.4	0.005
PaO_2 (mmHg)	77.6±23.4	101.6±29.4	0.019
FiO_2	0.80±0.20	0.34±0.06	<0.0005
PaO_2/FiO_2 (mmHg)	108.2±47.4	309.8±116.0	<0.0005
CO_2 (mmHg)	51.9±10.4	37.98±7.3	<0.0005
pH	7.33±0.1	7.45±0.07	0.002
RR (breath/min)	15.8±8.9	14.4±7.0	0.64
PEEP (cmH_2O)	17.7±2.3	7.5±2.1	<0.0005
Ppeak (cmH_2O)	32.5±5.9	20.6±3.8	<0.0005
Pmean (cmH_2O)	25.2±2.8	11.5±3.2	<0.0005
V_T (ml)	377.4±202.3	600.6±142.9	0.002
Ideal V_T (ml/kg PBW)	5.7±2.4	9.3±3.3	0.001
MV (l/min)	6.2±4.8	7.8±2.6	0.28
I:E	1.35±0.8	0.66±0.3	0.004

Mean values ± standard deviation; Student's t-test.

ARDS: Acute respiratory distress syndrome; SE: Standard error; DOM: Difference of means; 95% CI: 95% Confidence interval; MV days: days under mechanical ventilation; PaO_2: Arterial partial pressure of oxygen; FiO_2: Fraction of inspired oxygen; $PaCO_2$: Arterial partial pressure of carbon dioxide; RR: Respiratory rate; PEEP: Positive end-expiratory pressure; Ppeak: Peak inspiratory pressure; Pmean: Mean airway pressure; V_T: Tidal volume; PBW: Predicted body weight; MV: Minute volume; I:E: Ventilation ratio (inspiration/expiration).

All ARDS patients received hydrocortisone (100 mg bolus injection followed by infusion of 10 mg/h) for septic shock. Five (35.7%) control patients received either a bolus of hydrocortisone (100 mg) or a bolus of prednisolone (1000 mg) for surgical aggression to the throat.

All patients in the ARDS group required volume resuscitation and noradrenalin infusion, so all had at least two organ failures (respiratory and circulatory). In the control group, all patients required volume resuscitation and nine (64%) required noradrenalin infusion. Hepatic failure was present in 11 (69%) (bilirubin= 4.2 (2.8-13.5 mg/dl) of the ARDS patients and in only two (12%) of the controls (bilirubin=2.15 mg/dl). Renal replacement therapy was needed in 10 (62.5%) ARDS patients during the ICU stay and in two of them it was present at the time of measurement.

See the Tables 9 and Table 10 below for the number of organ failures, and hemodynamic and blood parameters.

Table 9. Number of organ failures including respiratory failure

Group	**Number of organ failures**				
	1	**2**	**3**	**4**	**5**
ARDS	0%	6.3% (n=1)	37.5% (n=6)	0%	56.3% (n=9)
Control	35.7% (n=5)	42.9% (n=6)	7.1% (n=1)	14.3 (n=2)	0%

Table 10. Hemodynamic and blood parameters, and medication. (An expanded version of this table showing the difference of the means, standard error, and 95% confidence interval for the continuous variables is found in the appendix, Table 21.).

Parameter	**ARDS**	**Control**	**P-Value**
Lactate (mmol/l)	5.2±6.5	1.2±0.3	0.04
Noradrenalin ((µg/kg/min)	0.43±0.41	0.08±0.06	0.003
HR (bpm)	98±19	76±30	0.02
MAP (mmHg)	83±11	81±18	0.69
Prothrombin time (%)	66±16	91±14	<0.0005
Platelet count (g/l)	78938±69110	140429±58453	0.01
Hemoglobin (g/dl)	10.4±1.1	10.5±2.1	0.80
Leukocytes (g/l)	14.2±13.8	12.2±5.9	0.63
Bilirubin (mg/dl)	7.5±10.2	1.0±0.6	0.02
Creatinine (mg/dl)	2.0±1.1	1.1±0.3	0.004
Glucose (mg/dl) *	146 (77-191)	104 (92-115)	0.26
Cortisone	87.5%	35.7%	0.003

Mean values ± standard deviation; Student's t-test.
* Median (interquartile range=25^{th}-75^{th} percentiles); Mann-Whitney-U test.
ARDS: Acute respiratory distress syndrome; SE: Standard error; DOM: Difference of means; 95% CI: 95% Confidence interval; NA: Noradrenalin; bpm: beats per minute; MAP: Mean arterial pressure.

b) Endpoints

i) *Primary endpoint*

The sample size was calculated to detect a difference in the expiratory/inspiratory ratio of VOCs of 1.5 ± 1 units between the ARDS and control groups. However, in the interim between the design of the study and the analysis of the results, we changed our approach for dealing with the inspiratory values. Therefore, although the expiratory/inspiratory ratio is not represented in the results, the calculation was done and the premise was large fulfilled.

ARDS patients had significantly higher concentrations of acetone, propionaldehyde, and MDA-P (*m/z 71* and *m/z 72*) in expiratory air compared with control patients (Figure 5 and Table 11). We want underline that certain expiratory values of VOCs, mainly acetone, fluctuate greatly within subjects of the ARDS group. No significant differences in the inspiratory values of VOCs were found between the control group and the ARDS group, except for MDA-P *m/z* 71. Results of the online measurement of VOCs in inspiratory and expiratory air are shown in Figure 5 and in Tables 11 and 12.

Within the ARDS group, all expiratory air substance concentrations were significantly higher than the corresponding inspiratory air substance concentration ($p \leq 0.001$, Wilcoxon signed-rank test, with Bonferroni-Holm adjustment). The same applies for substance concentrations found within the control group ($p < 0.01$) with the exception of *m/z* 72 ($p = 0.08$, Wilcoxon signed-rank test, with Bonferroni-Holm adjustment).

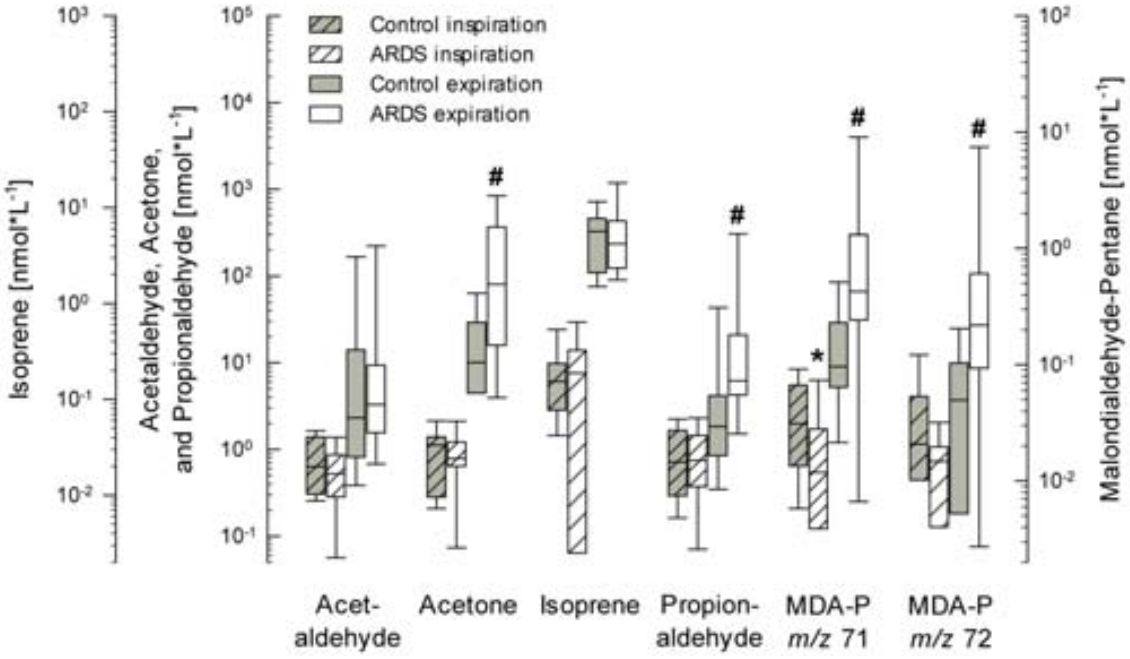

Figure 5. Volatile organic compounds detected in expiratory and inspiratory air of ARDS and control patients. ARDS patients exhaled significantly more acetone, propionaldehyde, and malondialdehyde-pentane (*m/z 71* and *m/z* 72) compared to control patients. $^{\#}p \leq 0.01$, Mann-Whitney-U-test with Bonferroni-Holm adjustment. *p <0.05, Mann-Whitney-U-test.

Table 11. Expiratory VOCs in nmol/l unit.

VOCs	Expiratory		P-value
	ARDS	**Control**	
Acetaldehyde	3.28 (1.56-9.33)	2.31 (0.82-14.05)	0.561
Acetone	103.36 (19.68-434.94)	10.08 (4.45-29.21)	0.004*†‡
Isoprene	4.18 (2.35-7.24)	5.63 (2.09-7.74)	0.803
Propionaldehyde	6.17 (4.25-20.91)	1.83 (0.85-4.17)	0.010*†
MDA-P *m/z 71*	0.43 (0.24-1.31)	0.10 (0.06-0.23)	0.001*†‡
MDA-P *m/z 72*	0.22 (0.09-0.60)	0.05 (0.005-0.10)	0.002*†‡

Median (25^{th}-75^{th} percentiles);Mann-Whitney-U-test
*No multiple comparisons adjustment; $p<0.05$
†Bonferroni-Holm adjustment; $p<\alpha/k$ for the smallest p-value, successively $\alpha/k-1$
‡Bonferroni adjustment; $P<0.008$ ($\alpha/6$)

Table 12. Inspiratory VOCs in nmol/l unit.

VOCs	Inspiratory		P-value
	ARDS	**Control**	
Acetaldehyde	0.52 (0.29-0.90)	0.63 (0.31-1.39)	0.34
Acetone	0.79 (0.66-1.22)	1.13 (0.28-1.39)	0.38
Isoprene	0.19 (-0.01-0.32)	0.15 (0.08-0.23)	0.84
Propionaldehyde	0.75 (0.37-1.45)	0.72 (0.29-1.66)	1.00
MDA-P *m/z 71*	0.01 (0.004-0.03)	0.03 (0.01-0.07)	0.04#
MDA-P *m/z 72*	0.01 (0.004-0.02)	0.02 (0.01-0.05)	0.10

Median (25^{th}-75^{th} percentiles); #$p<0.05$, Mann-Whitney-U-test.

Table 13 shows the proportions of inspiratory substances for each compound analyzed in the control and ARDS groups.

Table 13. Proportion of inspiratory to expiratory concentrations.

VOCs	Inspiratory proportion [(C_{insp}/C_{exp})*100]	
	Control patients	**ARDS patients**
Acetaldehyde	29.1 (4.7-91.1)	10 (3.7-36.5)
Acetone	7.1 (2.8-12.1)	0.5 (0.1-2.8)
Isoprene	3.1 (1.2-11.2)	4.5 (1.1-9.2)
Propionaldehyde	34.5 (7.3-81.1)	7.8 (3.5-12.9)
MDA-P m/z 71	37.8 (4.8-98.9)	3.3 (1-10.3)
MDA-P m/z 72	85 (35.1-171.2)	5.2 (0.5-15.8)

The values calculated from the raw data in ppbv units are expressed as medians (95% CI). VOCs: Volatile organ compounds; Cinsp: Inspiratory concentration, Cexp: Expiratory concentration.

In the subgroup analysis of VOCs in ARDS patients treated with (n=8) and without (n=8) ECMO, we noted a significant difference in exhaled isoprene (ECMO: 7.2 (4.5-14.1) nmol/l, vs. nonECMO: 2.7 (2.0- 4.0) nmol/l; p=0.01). Actually, levels of all VOCs except MDA-P were higher in the ECMO subgroup than in nonECMO subgroup, although this difference was significant only for isoprene. In addition, ECMO-treated ARDS patients had a significantly lower MV of ventilation (ECMO: 2.8 (2.0-3.4) l/min vs. nonECMO: 8.0 (7.1-10.8) l/min; p<0.001) and lower ideal V_T (ECMO: 4.4 (4.0-5.3) ml vs. nonECMO: 6.2 (5.1-7.4) ml; p=0.015), but we found no significant differences in PEEP (ECMO: 17.1±2.0 cmH_20 vs. nonECMO: 18.3 ± 2.7 cmH_20; p= 0.35) or in Paw (ECMO: 24.6 ± 2.7 cmH_20 vs. nonECMO: 25.8 ± 2.9 cmH_20; p= 0.43).

No significant differences in expiratory VOCs were found between patients with severe ARDS (PaO_2/FiO2 < 100; n = 8) at the time of measurement and those with nonsevere ARDS (PaO_2/FiO2 > 100; n = 8).

We found no association between levels of VOCs and mortality (p>0.05).

ii) *Secondary endpoints*

Compared to control group patients, ARDS patients had significantly higher values for CRP, PCT, and IL-6 in blood.

Figure 6 and Table 14 show the levels of inflammatory markers in blood in ARDS and control group patients.

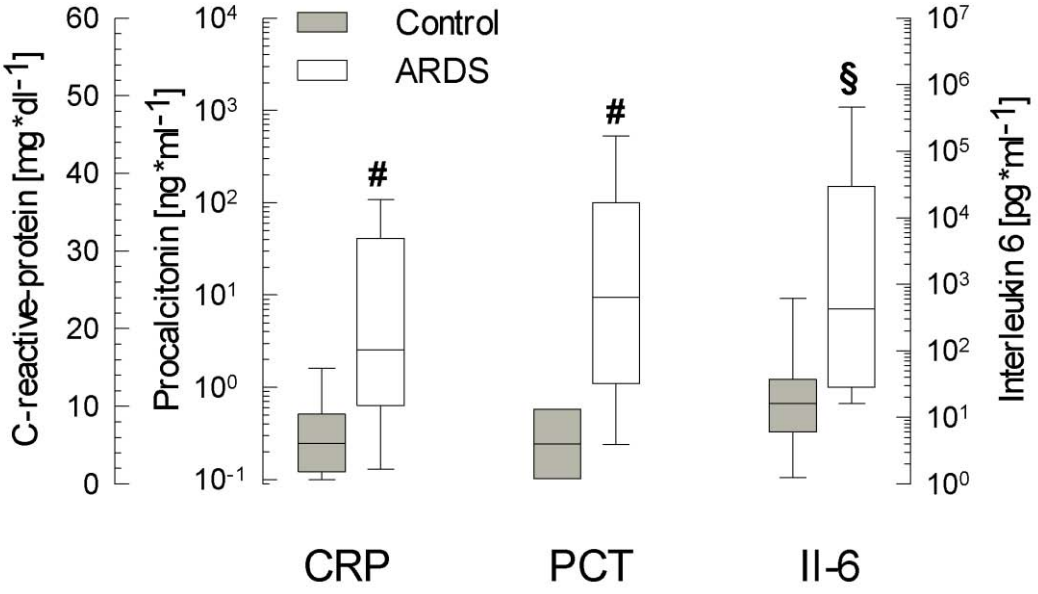

Figure 6. Inflammatory markers C-reactive-protein (CRP), procalcitonin (PCT), and interleukin 6 (Il-6) analyzed in blood. Inflammatory markers were significantly higher in ARDS patients than in control group patients. $^{\#}p < 0.01$, $^{\S}p<0.05$, Mann-Whitney-U test without multiple comparisons adjustment.

Table 14. Inflammatory markers C-reactive-protein (CRP), procalcitonin (PCT), and interleukin 6 (Il-6) analyzed in blood.

Parameter	ARDS	Control	P-value
PCT (ng/ml)	9.4 (1.1-100.1)	0.2 (0.1-0.4)	0.002*†‡
CRP (mg/dl)	17.3 (10.1-31.6)	5.2 (1.6-9.0)	0.002*†‡
Il-6 (pg/ml)	430.0 (28.5-29338.5)	34.3 (16.0-64.7)	0.026*†

Mann-Whitney-U-test, median values (25^{th}-75^{th} percentiles)
*No multiple comparisons adjustment; $p<0.05$
†Bonferroni-Holm adjustment; $p<\alpha/k$ for the smallest p-value, successively α/k-1
‡Bonferroni adjustment; $P<0.016$ ($\alpha/3$)

We performed 15 linear regression models between VOCs. In three models, a linear association was found between the concentrations of the VOCs:

- ✓ Linear regression model for log(acetone) vs. log(acetaldehyde). Results are shown in Figure 7 and in Table 15.

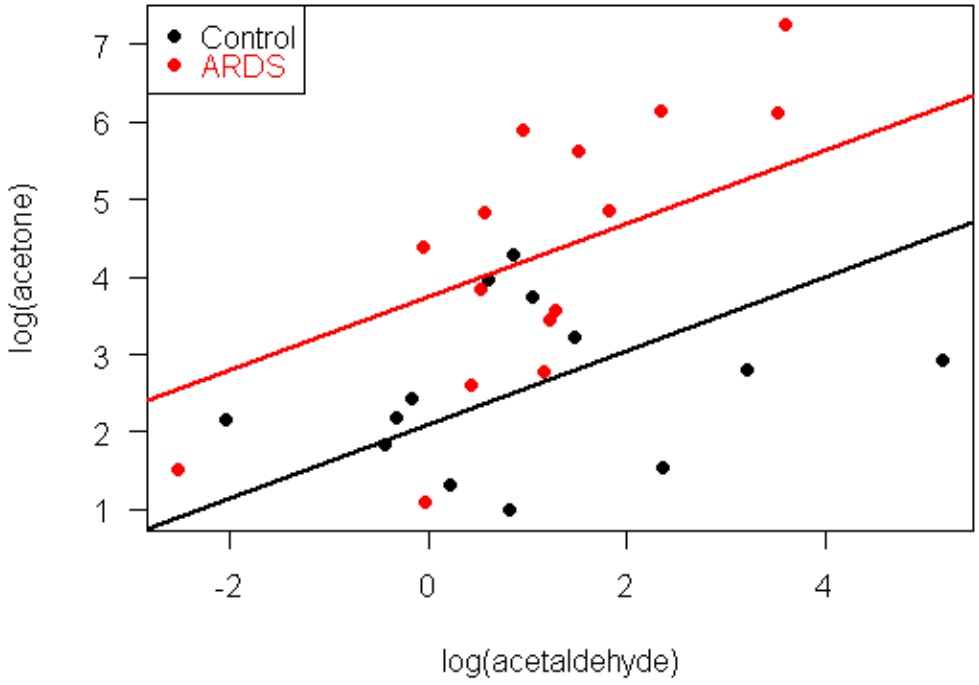

Figure 7. Linear regression model for log(acetone) vs. log(acetaldehyde)

Table 15. Summary of log(acetone) vs. log(acetaldehyde) linear fit

Coefficients	Estimate	SE	T-value	P-value	df	adj R^2	P-value
Intercept	2.104	0.390	5.391	$1.36x10^{-5}$	25	0.418	0.0004
log(acetaldehyde)	0.474	0.154	3.077	0.005			
Group	1.646	0.491	3.346	0.003			

SE: Standard error; df: Degrees of freedom; adj R^2: Adjusted R-squared

In control patients, for each increase in acetaldehyde of 1 unit in the logarithmic scale, the logarithm of acetone increases 0.47 units. However, the mean level of acetone is 1.65 units higher in ARDS patients than in control patients. Since adjusted R^2 estimates the model's goodness of fit or the adequacy of the linear model, we can postulate that 42% of the variation in acetone is attributable to acetaldehyde (adj R^2= 0.418).

- ✓ Linear regression model for isoprene vs. acetone. Results are shown in Figure 8 and in Table 16.

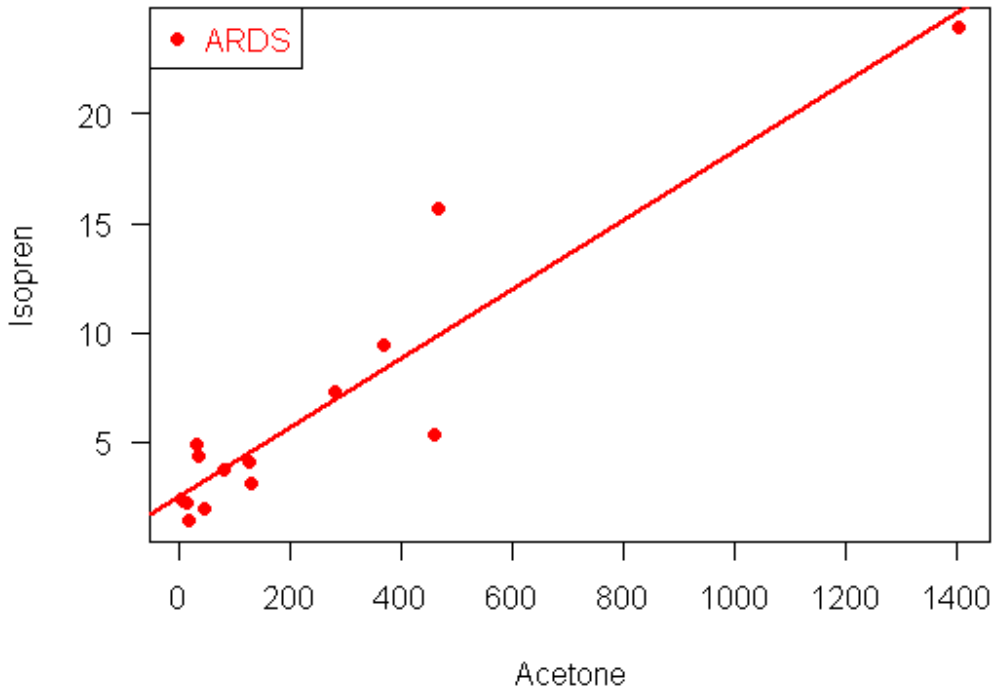

Figure 8. Linear regression model for isoprene vs. acetone

Table 16. Summary of isoprene vs. acetone linear fit

Coefficients	Estimate	SE	T-value	P-value	df	adj R^2	P-value
Intercept	2.491	0.702	3.545	0.00359	13	0.863	3.434×10^{-7}
Acetone	0.016	0.002	9.455	3.43×10^{-7}			

SE: Standard error; df: Degrees of freedom; adj R^2: Adjusted R-squared

In the ARDS group, for each increase in acetone of one unit, isoprene increases 0.016 units; 86% of the variation in isoprene is attributable to acetone (adj R^2= 0.863). In the control group, linear regression found no association.

✓ Model log(MDA *m/z* 72) vs. log(MDA *m/z* 71). Results are shown in Figure 9 and in Table 17.

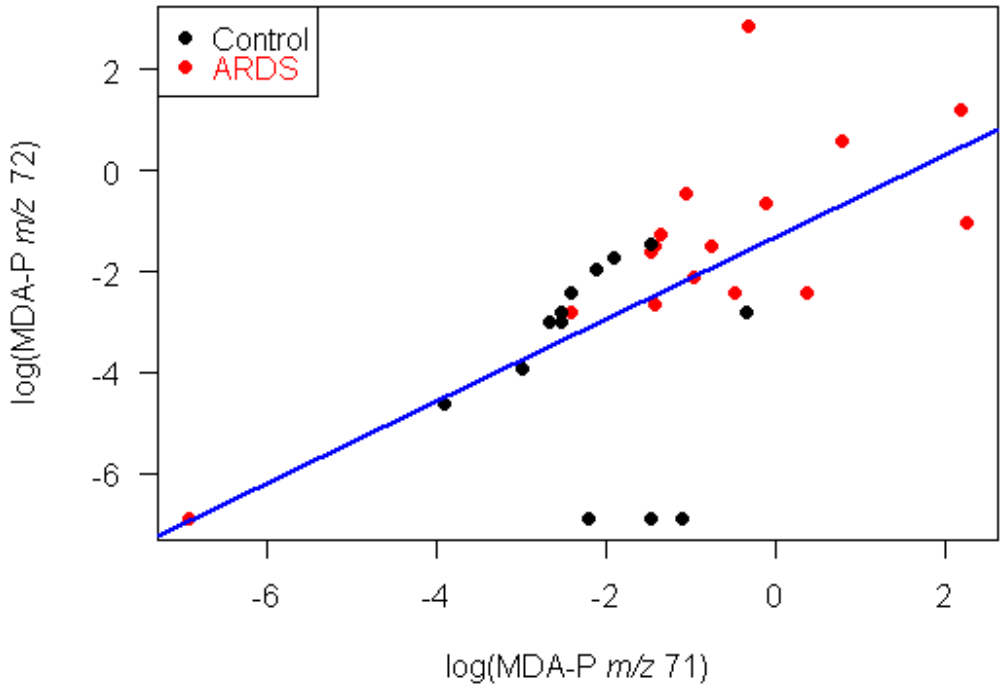

Figure 9. Linear regression model for log(MDA *m/z* 72) vs log(MDA *m/z* 71)

Table 17. Summary of log(MDA *m/z* 72) vs. log(MDA *m/z* 71) linear fit

Coefficients	Estimate	SE	T-value	P-value	df	adj R^2	P-value
Intercept	-1.310	0.442	-2.964	0.006	28	0.370	0.0002
Log(MDA-P *m/z* 71)	0.816	0.192	4.249	0.0002			

SE: Standard error; df: Degrees of freedom; adj R^2: Adjusted R-squared

For each increase in MDA-P *m/z* 71 of one unit in the logarithmic scale, the logarithm of MDA-P *m/z* 72 increases 0.816 units; 37% of the variation in MDA-P *m/z* 72 is attributable to MDA-P *m/z* 71 (adj R^2= 0.370).

We fitted 30 linear regression models to assess the relationship between lung oxidative stress response expressed by VOCs and systemic inflammatory markers (CRP, PCT and IL-6) as well as between VOCs and lung injury severity expressed by LIS and PaO_2/FiO_2 ratio.

The only linear association found was between MDA *m/z* 71 and PaO_2/FiO_2 ratio.

- ✓ Model log(MDA *m/z* 71) vs. log(PaO_2/FiO_2). Results are shown in Figure 10 and in Table 18.

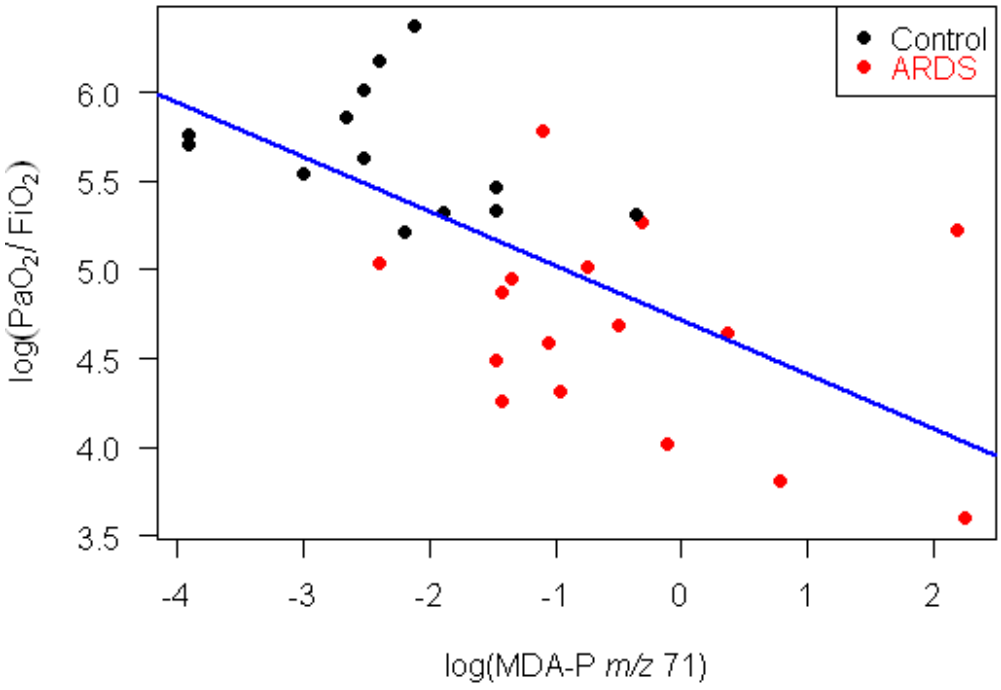

Figure 10. Linear regression model for log(PaO_2/FiO_2) vs. log(MDA *m/z* 71).

Table 18. Summary of log(PaO_2/FiO_2) vs. log(MDA *m/z* 71).

Coefficients	Estimate	SE	T-value	P-value	df	adj R^2	P-value
Intercept	4.714	0.135	34.955	$2x10^{-6}$	27	0.402	0.0001
MDA-P *m/z* 71	-0.306	0.06884	-4.452	0.0001			

SE: Standard error; df: Degrees of freedom; adj R^2: Adjusted R-squared

For each increase in MDA *m/z* 71 of one unit in logarithmic scale, the logarithm of PaO_2/FiO_2 decreases 0.30 units; 40% of the variation in PaO_2/FiO_2 is attributable to MDA *m/z* 71 (adj R^2= 0.402).

DISCUSSION

V. DISCUSSION

1. Critical evaluation of the methods employed and comparison of results with the literature

ARDS is a severe illness caused by diverse etiological factors with different insult systems but with the common denominator that the inflammatory cascade is activated, resulting in lung injury.

Sepsis is the leading cause in the development of ARDS, (13) and this was true in our population as well. Our ARDS population was very homogenous, predominantly consisting of patients with severe ARDS caused by pneumonia with septic shock, so we must be cautious in extrapolating our conclusions because they might not hold true for patients with ARDS of differing etiologies.

In demographic terms, our ARDS and control groups were comparable. However, there were significant differences in the severity of illness as expressed by the SAPS II score, LIS, and the invasiveness of respiratory therapy applied. Furthermore, all but one of the ARDS patients fulfilled the criteria for sepsis and septic shock at the time of the measurements, whereas only one patient in the control group had sepsis. On the day of the measurements, our ARDS patients had a mean LIS score of 3.6, PaO_2/FiO_2 ratio of 108 mmHg, PEEP of 18 cmH_2O, and a SAPS II score of 34. Previous studies in ARDS patients reported LIS scores ranging from 2.7 to 3.8, PaO_2/FiO_2 ratios from 60 to 192 mmHg, PEEP ranging from 7 to 19 cmH_2O, and SAPS II scores ranging from 35 to 50 (53, 129-131). Thus, our ARDS is comparable with others for the severity of lung injury as expressed by the LIS score, the PaO_2/FiO_2 ratio, and the PEEP level. On the other hand, it seems that our SAPS II score of 34 is only comparable to the SAPS II of 35 reported in an influenza A (H1N1)-ARDS population treated with venovenous ECMO (129), and our population seems to be less critically ill than those of Verzilli et al. (131) and Osman et al. (130) with SAPS II scores of 44 and 50, respectively. However, it is important to remember that our SAPS II scores were generated without assigning points for the estimated state of consciousness as this seemed to be largely speculative in case of deeply sedated patients. Furthermore, half of our ARDS patients (n=8) were being treated with venovenous ECMO at the time of measurement and that improved their PaO_2/FiO_2 ratio, which is also part of the SAPS II score. If we use the number of organ failures as an indicator of severity, our ARDS

patients were sicker than those in the first ARDSNet RCT (4.1±1.1 vs. 2.8±1.1 organ failures, respectively) (1). Since all control patients had undergone surgery before the measurement, most needed norepinephrine to correct hypotension due to general anesthesia, so presumably the real hemodynamic failure is overestimated. As all control patients were also mechanically ventilated and we could not evaluate their true respiratory function, they were all considered to have respiratory failure. Thus, the number of organ failures and SAPS II values in the control group are definitely overestimated.

The hospital mortality of our ARDS patients was 37.5%, which is comparable to that reported in other studies including ARDS patients treated with venovenous ECMO: 37% and 41% (53, 129). The mortality in our ARDS population was practically identical to that in the ECMO arm in the CESAR trial (37%), which supported the trend to lower mortality in an intention-to-treat ECMO population with severe ARDS (Murray score >3) in comparison to conventional management (53%). For the one-year mortality survey, two patients (one in the control group and one in the ARDS group) were not found. Since the number of lost to follow-up is small and equitably distributed, this problem can be handled by non-informative censoring, which means that their loss is not related to the event (death). Using this approach, the one-year mortality remains unchanged from the hospital mortality in both groups. Davidson et al. (132) showed that there was no difference in the long-term mortality rate between ARDS patients and that of matched controls after adjusting for age, risk factor for ARDS, comorbidity, and severity of illness.

The important role of ROS in the pathogenesis of ARDS was shown in the 1980s. BAL from ARDS patients showed increased activity of neutrophil granulocytes and evidence of oxidant activity by reduced alpha-1-proteinase inhibitor (102, 133). Direct evidence of the role of ROS was gained with the detection of increased hydrogen peroxide within the EBC of ARDS patients (72). In our current understanding of the pathogenesis of ARDS, neutrophil-mediated lung injury plays an important role by releasing ROS, proteases, and leukotrienes (63). Here, ROS react with the allylic hydrogen atom of polyunsaturated lipids, initiating a reactive cascade that leads to the production of saturated hydrocarbons (ethane and pentane) or aldehydes (MDA, propionaldehyde)(123, 134). To date, clinical studies have shown that plasma lipid peroxidation products increase in patients at risk for ARDS and in those with established ARDS (73, 112). Furthermore, pentane concentrations in exhaled breath are

higher in ARDS patients than in healthy volunteers (ARDS: 1.0 nmol/l vs. volunteers: 0.12 nmol/l, $p< 0.05$), whereas plasma levels of MDA (ARDS: 0.55 ng/ml vs. volunteers: 0.38 ng/ml) are not different (112). To our knowledge, our study is the first to directly measure the well-known lipid peroxidation end-products MDA and pentane in exhaled breath. As stated above, both MDA and pentane have the same mass, 72 amu, and cannot therefore be differentiated from each other. During ionization with mercury in IMR-MS, MDA and pentane occasionally lose one hydrogen atom. This results in peak measurements for both molecules at *m/z* 71 and 72. Our finding of MDA-P concentrations of 0.43 nmol/l at *m/z* 71 and 0.22 nmol/l at *m/z* 72 corresponds well to previous reports with 1.0 nmol/l pentane measured in exhaled breath in ARDS patients (81), 0.33-0.55 nmol/l in septic patients (81, 87), and 0.45 nmol/l in a mixed group of ventilated patients measured using gas chromatographic techniques (135). Therefore, we consider our direct measurement method for MDA-P reliable.

Sepsis and septic shock are the leading causes of the development of ARDS in our population, so sepsis is a possible confounder in our study because VOCs increase with increasing inflammatory status (81). Thus, we cannot discern the respective roles of ARDS and of sepsis in the release of VOCs. From our results, we can affirm that ARDS induced by sepsis results in higher levels of MDA-pentane than is found in an ill population without lung injury. Scholpp et al. (81) found that pentane concentrations in exhaled breath were significantly higher in the ARDS group (1.00 nmol/l) and in the group of patients at risk of developing ARDS (0.49 nmo/l) than in healthy individuals (0.12 nmol/l). They found that plasma MDA was higher in septic patients (0.62 ng/ml) than in patients without inflammation (0.33 ng/ml, $p<0.05$). In contrast, no differences in the concentration of pentane in exhaled breath were found when comparing septic patients (0.55 nmol/l) with patients without inflammation (0.12 nmol/l) (81) or ventilated septic patients (0.33 (0.00-0.97) nmol/l) with ventilated non-septic (0.028 (0.00-0.72) nmol/l) patients (87). Although evidence is lacking, these results suggest that MDA might be more related to sepsis and pentane might be more related to ARDS.

Pentane concentrations may be influenced by hepatic metabolism via cytochrome P450 enzymes. Cytochrome P450 is a family of isozymes responsible for the biotransformation of many drugs and molecules via oxidation. These enzymes are heme-containing membrane proteins, which are located in the smooth endoplasmic reticulum of several tissues. Although most of these isozymes are located in the liver, metabolism also occurs in the kidneys, skin, gastrointestinal tract, and lung (136).

Hepatic failure occurred in 69% of the patients in the ARDS group compared to 12% in the control group; therefore, pentane values might be biased.

Aldehydes are considered diagnostic markers of lipid peroxidation given that the breakdown of PUFAs induced by ROS leads to the generation of aldehydes in general. Cordi et al. (83) found increased acetaldehyde and propionaldehyde in an isolated heart perfusion model of oxidative stress and Moeskops et al. (84) reported ultraviolet-induced skin lipid peroxidation-associated release of acetaldehyde and propionaldehyde. As acetaldehyde is generated by alcohol dehydrogenase-catalyzed oxidation of ethanol, the ingestion of ethanol results in an increase in baseline expiratory air acetaldehyde values, which ranged from 0.2 to 0.6 nmol/l in the abstinent group and from 5 to 50 nmol/l after ethanol ingestion (98, 137). In our measurements, exhaled acetaldehyde was markedly increased in both the ARDS and control groups compared to values obtained in abstinent persons. As the intestinal bacterial flora is a potential source of endogenous ethanol (138), the acetaldehyde expression in our study is probably produced by oxidation of endogenous ethanol.

To our knowledge, our study is the first to use propionaldehyde as a potential volatile biomarker for lipid peroxidation in the expiratory air of ARDS patients. Our finding of increased propionaldehyde concentrations in the exhaled breath in ARDS patients suggests this compound might be useful as a marker of lipid peroxidation. Unfortunately, no data on expiratory air measurements of propionaldehyde are available to compare our results with. Given the possible confounding effect of disinfectants containing 1- and 2-propanol on propionaldehyde and on acetone in exhaled breath, we strictly controlled their use before MS measurement. These disinfectants are often used prior to surgery; although three ARDS patients and all control patients had undergone surgery before the measurements, the minimum time from surgery to measurement was 4 hours, well over the reference times of 2.5 hours and 1 hour considered safe for 2-propanol and 1-propanol, respectively. We therefore conclude that the increases in propionaldehyde in our study population are the result of lipid peroxidation rather than due to the use of disinfectants; this conclusion is strengthened by the observation that propionaldehyde levels were higher in ARDS patients even though surgery was less frequent in the ARDS group and the period from surgery to measurement was longer.

The two major endogenous sources of acetone are the decarboxylation of acetoacetate from lipolysis or lipid peroxidation (112) and the oxidation of 2-propanol(139). Scholpp et al. (81) found expiratory acetone was 50.0 (19.6-72.3) nmol/l

in ARDS patients compared to 33.2 (20.8-38.6) nmol/l in a control group, although the difference was not significant. Schubert et al. (135) found in a mixed critical care population acetone values of 45.2 nmol/l. In contrast, expiratory acetone was 103.4 nmol/l in our ARDS patients and 10.1 nmol/l in our controls, and this difference was significant ($p<0.01$). These differences are possibly explainable by the high stress-induced metabolism (identified by increased inflammatory markers) to which patients are subjected in the very early phase of ARDS. However, a direct comparison on the basis of inflammatory systemic markers is not possible, as these data are not available in the above-mentioned studies. There is a linear correlation between blood acetone concentrations and food intake and glucose metabolism (140-142). No significant differences in the glucose levels were found between the two groups.

Isoprene, one of the main hydrocarbons produced endogenously by mammals during cholesterol biosynthesis, is typically found in concentrations around 100 ppbv (4 nmol/l) in the exhaled breath of healthy adults (95, 135, 143-145). Scholpp et al. (81) found lower expiratory isoprene values in ARDS patients (2.18 nmol/l) than in healthy volunteers (5.99 nmol/l); similarly, Schubert et al. (146) also found lower isoprene values in ARDS patients than in patients without ARDS [9.8 (8.2-21.6) vs 21.8 (13.9-41.4) nmol/m2 per min [median (95% confidence interval)], $p = 0.04$]. These findings suggest that impaired cholesterol metabolism (decreased cholesterol biosynthesis) may play a role in the development of ARDS by impairing membrane repair (139, 146, 147). However, we found no differences in expiratory isoprene between ARDS patients (4.18 nmol/l) and our mechanically ventilated controls (5.63 nmol/l). One possible explanation for this discrepancy might be the use of ECMO in eight of our patients with a reduced minute volume. Isoprene elimination is critically dependent on pulmonary ventilation due to isoprene's lipophilic behavior and low Henry constant (the ratio of the concentration of a chemical substance in air to the concentration in an aqueous solution at equilibrium that is used as a qualitative measure of the volatility of the substance) (148) so ECMO might lead to systemic isoprene accumulation and higher isoprene concentrations in the breath of these patients. The median isoprene concentration in patients in our ARDS group who were not treated with ECMO was 2.7 nmol/l, which is comparable to the value 2.18 nmol/l mentioned above. It seems that the level of isoprene increased in the patients receiving ECMO (7.18 nmol/l) and consecutively the isoprene value for the entire ARDS group increased to 4.18 nmol/l. Our findings could not support the experimental evidence that isoprene exhalation may be related to

oxidative lung (79) and body (80) damage. Although the only difference that was significant was for isoprene, all VOCs except MDA-P were higher in the non-ECMO subgroup. Thus, we can affirm that ECMO-related SIRS induced by contact with the synthetic surfaces of the extracorporeal circuit did not play a significant role in the expression of VOCs in our ARDS population.

When analyzing VOCs in exhaled breath, the concentrations of inspiratory substances become possibly critical because they can influence expiratory concentrations. However, since ARDS patients required a FiO2 of 0.80±0.20, the proportion of ambient air is low, so the influence of inspiratory substances concentrations is very low. Nevertheless, the possible impact of inspiratory substances must be considered. Approaches to dealing with this issue include the subtraction of inspired from expired concentrations and pre-sampling ventilation of purified air. However, both methods suffer from limitations. In the first approach, the relationship between inspiratory and expiratory substance concentrations is not necessarily linear. In the second approach, although it has been suggested the lung can be washed out in approximately 4 min if the subject breathes pure air, the washout of the whole body may take days or weeks depending on the molecule in question. Therefore, the use of purified air is limited by uncertainties in the time required for washout and this approach is impracticable in ventilated critically ill patients (149-152). Schubert et al. (151) recommended caution in interpreting expiratory substance concentrations when blood substance levels are unknown and inspiratory substance concentrations are greater than 5% of the expiratory substance concentration. However, this recommendation does not take into account that ambient air substance concentrations may vary due to location and time, and high inspiratory air concentrations might preclude numerous substances from being used as breath markers. In a more practical approach, Risby et al. (152) recommend accepting inspiratory substance concentrations up to 25% of expiratory substance concentrations, since the study subject may not be in a steady state with the environment. Thus, we decided to report inspiratory values together with expiratory values instead of performing corrections on expiratory values. The data obtained in this study seem to justify this approach of reporting inspiratory and expiratory values together, as the inspiratory proportion of substance concentrations was higher in our less critically ill control group than in our ARDS patients, indicating that compounds in exhaled breath become interpretable in the context of a specific disease state. The inspiratory proportion of nearly all the molecules is higher in the control group than in

the ARDS group because the expiratory values of the VOCs are very low in the control group since they have only marginal lipid peroxidation compounds. Thus, we postulate that it might not be possible to interpret lipid peroxidation products in less critical patients without lung injury, because these compounds are present in very low concentrations in the exhaled breath. When the patient develops ARDS, the lipid peroxidation markers in the expiratory air increase and the inspiratory proportion becomes valuable (<25%), as the inspiratory concentration remains nearly the same.

Regarding the significant associations we found in the linear regression models, it is important to point out the linear association between MDA-P *m/z* 71 and PaO_2/FiO_2, which suggests that an increase in the level of this VOC could be useful for detecting disease progression. The linear regression model for log(MDA-P *m/z* 72) vs. log(MDA-P *m/z* 71) is explained insofar as both VOCs arise from the same two molecules but with different *m/z.* The linear associations demonstrated between log(acetone) vs. log(acetaldehyde) and between isoprene vs. acetone suggest the hypothesis that these compounds might share some intermediate pathways. Linear regression found no association between isoprene and acetone in the control group. We found no pathophysiological explanations for this difference between groups in this model.

Procalcitonin can be used as a marker of severe sepsis and generally correlates well with the degree of sepsis, although levels of procalcitonin in the blood are very low (153). Procalcitonin has the greatest sensitivity (85%) and specificity (91%) for differentiating patients with SIRS from those with sepsis, when compared with CRP, IL-2, IL-6, IL-8, and TNF-alpha (154). It represents a good biological diagnostic marker for sepsis, severe sepsis, or septic shock, but it can also be increased in non-septic systemic inflammatory response, as can occur after surgery or trauma without obvious infection (155). As our all but one of the patients in our ARDS population had pneumonia with septic shock, we cannot discern to what extent the increase in PCT is attributable to sepsis and to what extent it is attributable to the inflammatory stimulus of ARDS.

Il-6 is secreted by T-cells and macrophages to stimulate the immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. Pugin et al. (107) reported that the very early phase of ARDS is associated with a sequential alveolar secretion of proinflammatory cytokines that chemoattracts circulating neutrophils to the alveolar space and promotes inflammatory

cell secretion. Thus, the alveolar space is the principal site of delivery of proinflammatory cytokines whereas the levels of inflammatory mediators in the systemic circulation remain low. Although these authors did not evaluate Il-6 so we cannot directly assume that this premise is also valid for Il-6, their results are reliable to postulate that the inflammation in the early phase is mostly limited to the lung. In our study, Il-6 concentrations in blood were higher in ARDS patients than in controls. Nevertheless, the increase might be not completely representative of the inflammatory status to which the lung is subjected. Thus, based on these pathophysiological findings, the online monitoring of the exhaled breath measured by IMR-MS might play a crucial role in the very early phase since direct pulmonary input could be immediately evaluated.

Under the premise that biotrauma might occur only if mechanical stretch acts as a secondary injury to a previous inflamed lung (25, 27), ARDS patients would be the ideal candidates for developing biotrauma. Although our ARDS population was ventilated according to the ARDSNet recommendations, recent studies have shown that V_T lower than 6 ml/Kg PBW is associated to a significant reduction in inflammatory and morphological markers of VILI (36), so we cannot exclude a certain degree of VILI expression in our VOCs values.

No data about the role of corticoids in VOCs expression are available in the literature.

Unlike other alveolar sampling techniques such as gas chromatography, EBC, or BAL,a direct mass spectrometry system such as the IMR-MS enables noninvasive online measurement of exhaled breath at bedside in real time. Furthermore, unlike other techniques, MS is sensitive enough to detect concentrations in the nmol/l range, so no preconcentration or condensing techniques are necessary. However, direct or indirect calibration of the targeted gases is necessary before the measurements. Calibration is a methodical process that is easy to perform once learned. The time required for calibration depends on the number of direct calibrations to be performed; approximately 5 minutes are needed for each gas calibration.

2. Limitations of the study and future studies

One limitation of our study is that our ARDS patients were more severely ill than our controls. However, close matching for severity coupled with no ALI and no alveolar infiltrates in the chest radiograph in the control group was not possible because the more severe the illness is, the higher the probability of lung injury.

Another limitation is that we did not attempt to systematically match patients in the two groups for age, sex, and BMI because matching is very time consuming and would not have been feasible in a single-center study, since the ARDS is uncommon. Nevertheless, in the end there were no significant differences between the two groups for these variables.

Another problem with our study is the confounding effect of sepsis: sepsis might cause the release of specific VOCs (87), making it impossible to determine to what extent VOCs are due to ARDS and to what extent they are due to sepsis. However, as sepsis is the main cause of ARDS (13), it is difficult to obtain an ARDS group without sepsis because the other causes of ARDS (fat emboli, pulmonary contusion, massive blood transfusions, burns, inhalation injury…) are rare. Clarifying the sepsis component would require a prospective multicenter study with two groups: ARDS with sepsis vs. ARDS without sepsis. The results of such a study could provide an approximation of the relative roles of sepsis and ARDS in the production of VOC.

The early appearance of significantly higher VOCs in exhaled breath samples of patients at risk for developing ARDS could be an important prognostic indicator for the development of ARDS. Further studies including a group of patients at risk of developing ARDS are needed.

Finally, as few studies have yet to use IMR-MS, our data cannot be compared with those from studies carried out with the same technique. This means that our results must be compared with studies that used substantially different technical approaches, so although our results are in the same direction, normalization to some generally accepted standard procedure is lacking.

CONCLUSIONS

VI. CONCLUSIONS

This study is the first to use online mass spectrometry to measure lipid peroxidation markers in the expiratory air of ARDS patients.

IMR-MS allowed the online measurement of the lipid peroxidation markers MDA-P, acetone, and propionaldehyde as well as acetaldehyde and isoprene in the expiratory air of mechanically ventilated patients with ARDS.

Until now, MDA in ARDS patients has only been measured in blood. As IMR-MS is not able to differentiate pentane from MDA because the two compounds have the same amu, on basis of in vitro studies, we know that pentane and MDA are both detectable at *m/z* 71 and 72. Thus, we can postulate that this is the first study of an indirect measure of MDA in the expiratory air of ARDS patients.

In addition to the well-established lipid peroxidation products MDA, pentane, and acetone, we were also able to measure propionaldehyde in vivo.

Expiratory concentrations of lipid peroxidation markers malondialdehyde-pentane, acetone, and propionaldehyde were higher in ARDS patients than in critically ill patients without lung injury; these findings may reflect the amount of pulmonary and systemic oxidative stress damage produced by ARDS originating from pneumonia with sepsis. We also found significantly higher values for valid biochemical blood markers such as CRP, PCT, and Il-6 in the ARDS group, and these findings about systemic markers strengthen our findings about markers in expiratory breath.

Importantly, the linear association between MDA-P and PaO2/FiO2 suggests that an increase in MDA-P might be able to detect disease progression.

Determining VOC in exhaled breath by mass spectrometry is a novel online noninvasive approach that enables real-time detection of lipid peroxidation products in exhaled breath at the bedside. This method of breath analysis represents no risk to the patient and the exhaled breath measurements can be repeated as often as necessary.

Our results are an important step in the continuous monitoring of the dynamic clinical status in critically ill patients, because they show that it is possible to obtain immediate information about the local or systemic inflammatory response.

IMR-MS online measurement of lipid peroxidation products could be useful in identifying disease, detecting disease progression, or monitoring the response to therapeutic interventions.

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VII. REFERENCES

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APPENDIX

VIII. APPENDIX

1. Statistical analysis

a) **Table 19.** Demographic, and severity characteristics (expended version).

Characteristic	ARDS	Control	Difference of means	SE of DOM	95% CI of DOM		P- value
					Inferior	Superior	
Age	40.5 ± 16.2	46.5 ± 22.1	6	7.0	-8.4	20.4	0.17
Body mass index	30.0 ± 10.8	26.3 ± 8.0	3.7	3.5	-3.4	10.9	0.29
SAPS II score	34.3±12.6	18.4 ±6.5	-15.9	3.6	-23.4	-8.5	0.000
LIS score	3.6±0.3	0.9±0.4	-2.7	0.1	-3.0	-2.5	0.000

Mean values ± standard deviation; Student's t-test.
ARDS: Acute respiratory distress syndrome; SE: Standard error; DOM: Difference of means; 95% CI: 95% Confidence interval; SAPS II: Simplified acute physiological score; LIS: Lung injury score.

b) **Table 20.** Ventilator settings and resulting gas parameters (expended version).

Respiratory parameter	ARDS	Control	Difference of means	SE of DOM	95% CI of DOM		P-value
					Inferior	Superior	
MV days	38.9±39.6	6.2±8.4	-32.7	10.2	-54.1	-11.2	0.005
PaO_2 mmHg	77.6±23.4	101.6±29.4	24.0	9.7	4.2	43.8	0.019
FiO_2	0.80±0.20	0.34±0.06	-0.45	0.05	-0.56	-0.34	0.000
PaO_2/FiO_2	108.2±47.4	309.8±116.0	201.6	33.2	131.5	271.8	0.000
$PaCO_2$ mmHg	51.9±10.4	37.98±7.3	-13.9	3.3	-20.8	-7.1	0.000
pH	7.33±0.1	7.45±0.07	0.1	0.3	0.05	0.18	0.002
RR (breaths/min)	15.8±8.9	14.4±7.0	-1.4	3.0	-7.5	4.7	0.64
PEEP cmH_2O	17.7±2.3	7.5±2.1	-10.2	0.8	-11.9	-8.5	0.000
Ppeak cmH_2O	32.5±5.9	20.6±3.8	-11.9	1.8	-15.7	-8.2	0.000
Pmean cmH_2O	25.2±2.8	11.5±3.2	-13.8	1.2	-16.2	-11.3	0.000
V_T (ml)	377.4±202.3	600.6±142.9	223.3	64.9	90.4	356.1	0.002
Ideal V_T (ml/kg of PBW)	5.7±2.4	9.3±3.3	3.7	1.0	1.5	5.8	0.001
MV (liters/min)	6.2±4.8	7.8±2.6	1.6	1.4	-1.4	4.5	0.28
I:E	1.35±0.8	0.66±0.3	-0.7	0.2	-1.1	-0.2	0.004

Mean values ± standard deviation; Student's t-test.

ARDS: Acute respiratory distress syndrome; SE: Standard error; DOM: Difference of means; 95% CI: 95% Confidence interval; MV days: days under mechanical ventilation; PaO_2: Arterial partial pressure of oxygen; FiO_2: Fraction of inspired oxygen; $PaCO_2$: Arterial partial pressure of carbon dioxide; RR: Respiratory rate; PEEP: Positive end-expiratory pressure; Ppeak: Peak inspiratory pressure; Pmean: Mean airway pressure; V_T: Tidal volume; PBW: Predicted body weight; MV: Minute ventilation; I:E: Ventilation ratio (inspiration/expiration).

c) **Table 21.** Hemodynamic, and blood parameters and medication (expended version).

Parameter	ARDS	Control	Difference of means	SE of the DOM	95% CI of DOM		P-value
					Inferior	Superior	
Lactate (nmol/l)	5.2±6.5	1.2±0.3	-4.0	1.9	-7.8	-0.1	0.04
NA (µg/kg/min)	0.43±0.41	0.08±0.06	-0.35	0.10	-0.57	-0.14	0.003
Heart rate (bpm)	98±19	76±30	-22	9.0	-40.3	-0.05	0.02
MAP (mmHg)	83±11	81±18	-2	5.4	-13.3	8.9	0.69
Prothrombin time (%)	66±16	91±14	25	5.4	13.4	35.7	0.000
Platelet count (g/l)	78938±69110	140429±58453	61491	23561.3	13227.9	109754.2	0.01
Hemoglobin (g/dl)	10.4±1.1	10.5±2.1	0.1	0.6	-1.1	1.4	0.80
Leukocytes (g/l)	14.2±13.8	12.2±5.9	-2.0	4.0	-10.1	6.2	0.63
Bilirubin (mg/dl)	7.5±10.2	1.0±0.6	-6.5	2.6	-12.0	-1.1	0.02
Creatinine (mg/dl)	2.0±1.1	1.1±0.3	-0.9	0.3	-1.5	-0.3	0.004

Mean values ± standard deviation; Student's t-test.
ARDS: Acute respiratory distress syndrome; SE: Standard error; DOM: Difference of means; 95% CI: 95% Confidence interval; NA: Noradrenalin; bpm: beats per minute; MAP: Mean arterial pressure.

2. Background information

a) Ramsay sedation scale (126)

1=anxious and agitated or restless, or both
2=co-operative, oriented, and calm
3=responsive to commands only
4=exhibiting brisk response to light glabellar tap or loud auditory stimulus
5=exhibiting a sluggish response to light glabellar tap or loud auditory stimulus
6=unresponsive

b) Simplified Acute Physiology Score II

SAPS II stands for New Simplified Acute Physiology Score, which is a system for classifying the severity of disease, one of several ICU scoring systems. The SAPS II is used to assess the severity of illness in intensive care patients and to predict the risk of hospital mortality. The SAPS II includes 17 variables: 12 physiology variables, age, type of admission (scheduled surgical, unscheduled surgical, or medical), and three underlying disease variables (acquired immunodeficiency syndrome, metastatic cancer, and hematologic malignancy)(124).

c) Lung Injury Score (LIS) or Murray score

The LIS grades the severity of lung injury on the basis of PaO_2/FiO_2, compliance, PEEP and chest radiograph (125).

1. Chest radiograph		
No alveolar consolidation		0
Alveolar consolidation confined to 1 quadrant		1
Alveolar consolidation confined to 2 quadrant		2
Alveolar consolidation confined to 3 quadrant		3
Alveolar consolidation in all 4 quadrant		4
2. Hypoxemia score		
PaO_2/FiO_2	≧300	0
PaO_2/FiO_2	225-299	1
PaO_2/FiO_2	175-224	2
PaO_2/FiO_2	100-174	3
PaO_2/FiO_2	＜ 100	4
3.PEEP score		
PEEP	≦ 5 cm H_2O	0
PEEP	6-8 cm H_2O	1
PEEP	9-11 cm H_2O	2
PEEP	12-14 cm H_2O	3
PEEP	≧ 15 cm H_2O	4
4. Respiratory system compliance score		
Compliance	≧80 ml/cmH_2O	0
Compliance	60-79 ml/cmH_2O	1
Compliance	40-59 ml/cmH_2O	2
Compliance	20-39 ml/cmH_2O	3
Compliance	≦ 19 ml/cmH_2O	4

Parameters:

(1) chest radiograph evaluated for alveolar consolidation
(2) ratio of the partial pressure of oxygen in arterial blood to the inspiratory fraction of oxygen
(3) PEEP level
(4) Respiratory compliance

Score= The sum of all the parameters/Number of parameters

Score 0: No lung injury
Score 0.1-2.5: mild to moderate lung injury
Score >2.5: Severe lung injury

d) ACCP/SCCM Consensus Conference: Definitions for sepsis and organ failure (88)

Systemic Inflammatory Response Syndrome (SIRS)
SIRS represents the systemic inflammatory response to a variety of severe clinical insults. The response is manifested by two or more of the following conditions:

- Temperature >38°C or <36°C
- Heart rate > 90 beats/min
- Respiratory rate > 20 breaths/min or PaCO2 <32 torr (<4.3kPa)
- WBC > 12,000 cells/mm3, <4,000 cells/mm3, or >10% immature (band)forms

Sepsis
The systemic response to infection.
This systemic response is manifested by two or more of the above-mentioned SIRS criteria as a result of proven or suspected infection.

Severe Sepsis
Sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status.

- Hypotension
  A systolic blood pressure of <90 mm Hg or a reduction of >40 mm Hg from baseline in the absence of other causes for hypotension

Septic shock
Severe sepsis with hypotension, despite adequate fluid resuscitation, along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status. Patients who are on inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured.

Multiple Organ Dysfunction Syndrome (MODS)
Presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.

e) Evidence-based therapy of severe ARDS: an algorithm-guided approach

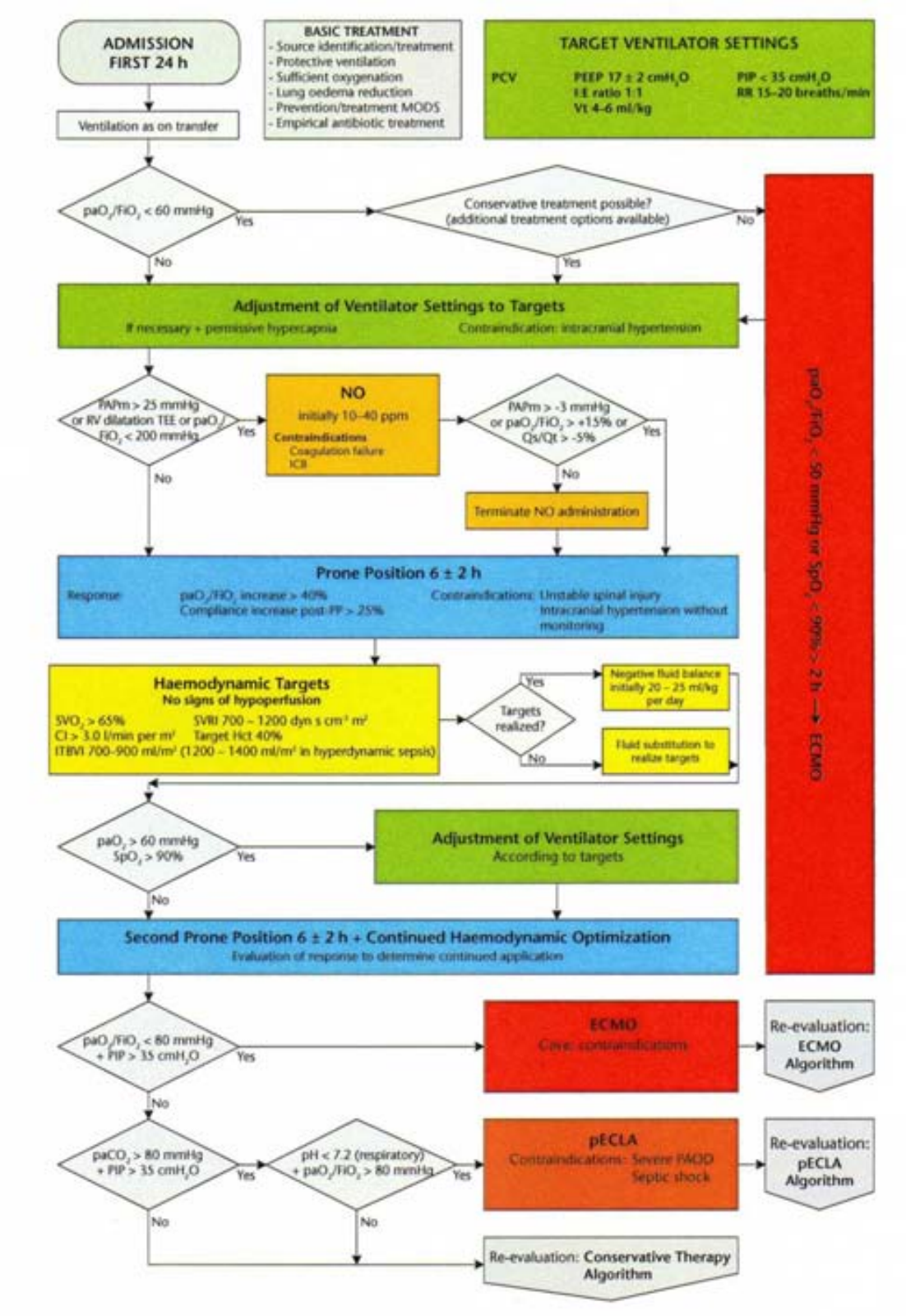

Figure 11 . The "Admission Algorithm" applies to first 24h of treatment and employs all advanced treatment options for patients with acute ARDS.

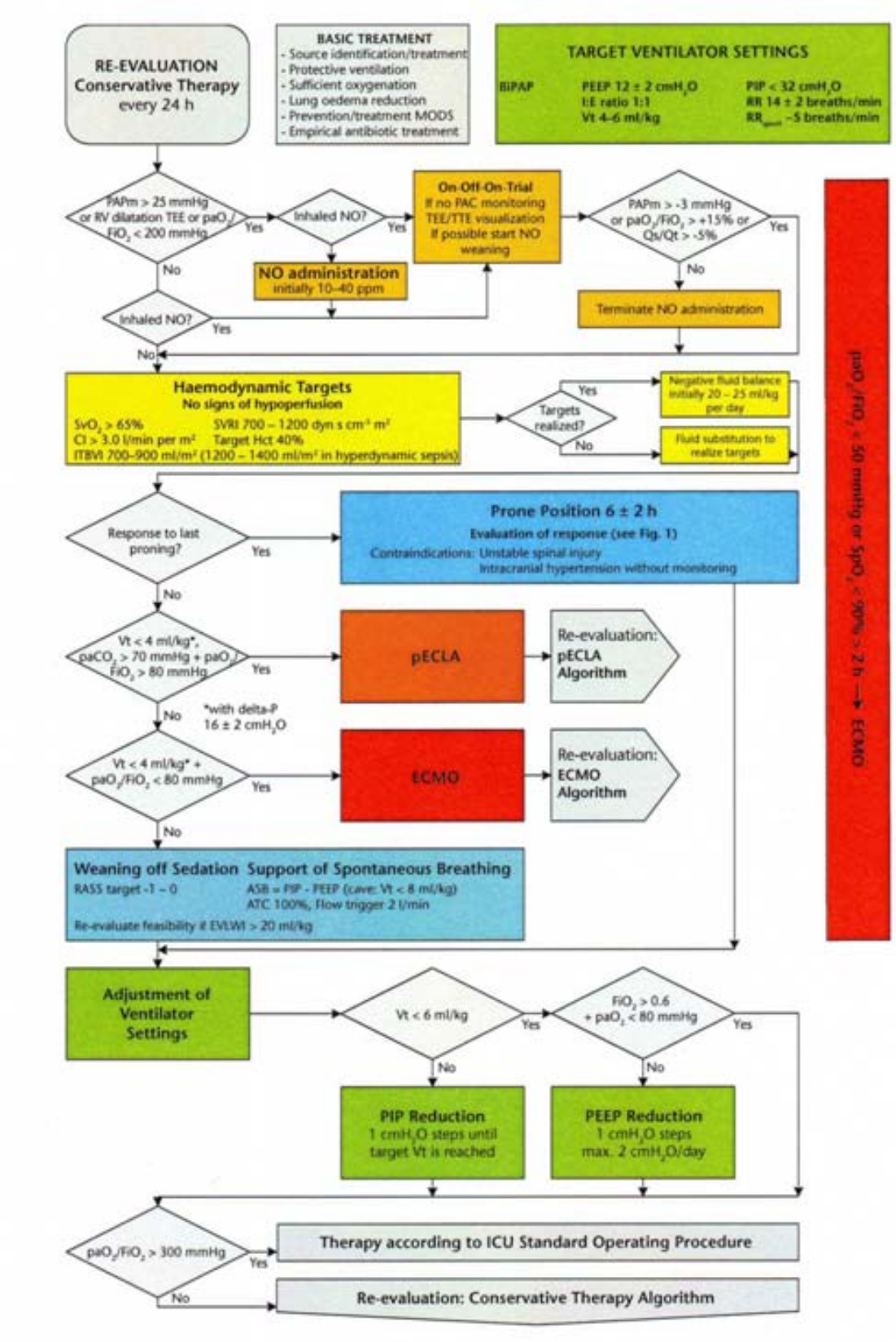

Figure 12. The "Re-evaluation Conservative Therapy" algorithm applies for patients with acute ARDS that do not fulfil the criteria for ECLA.