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Exhaled endogenous pulmonary nitric oxide in intubated and ventilated patients with acute respiratory distress syndrome

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Abstract

Purpose: This study aimed to continuously monitor the endogenous production of the fractional exhaled nitric oxide (FeNO), a biomarker of pulmonary inflammation, in intubated and mechanically ventilated patients. The objective was to assess potential changes in FeNO levels between patients with acute respiratory distress syndrome (ARDS) and intubated control patients.

Methods: A prototype based on Optical Feedback Cavity-Enhanced Absorption Spectroscopy (OFCEAS) was developed to detect nitric oxide at very low concentrations (parts per billion range, ppb), with a temporal resolution allowing to monitor respiratory cycles. Real-time FeNO monitoring was conducted, either immediately after anesthesia induction in the control group or within 24 hours after intubation in ARDS patients. In addition to NO, the analyzer simultaneously measured carbon dioxide (CO_2).

Results: Twenty patients were enrolled between January to May 2024. After exclusion of 3 patients, data analysis of 17 patients was performed: 11 control patients and 6 ARDS patients. The OFCEAS analyzer enabled to identify inhalation and exhalation phases through continuous NO and CO_2 monitoring, thereby allowing accurate estimation of FeNO values even when the resolution of respiratory cycle amplitudes was limited. FeNO levels are 4.5 ppb [3.6–5.2] for ARDS patients and 4.7 ppb [3.2–6.0] for controls (values presented as medians with interquartile ranges).

Conclusion: This pilot study shows that, contrary to initial indications from raw data and previous publications, FeNO levels do not differ significantly between ARDS patients and controls. It provides the first demonstration of continuous FeNO monitoring in intubated and mechanically ventilated patients using laser absorption spectroscopy and emphasizes both the potential and the technical challenges of cycle-resolved gas monitoring.

Keywords: exhaled air analysis; nitric oxide; laser spectroscopy; ARDS ; pulmonary inflammation

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1 Introduction

Gasotransmitters are small gaseous molecules produced endogenously and involved in various physiological processes. The best-known gasotransmitters are nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S). Although they are mainly known as toxic gases, they can exhibit cell-protective properties at low concentrations. Gasotransmitters play a role in various respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), obstructive sleep apnea, lung infection, bronchiectasis, cystic fibrosis, primary ciliary dyskinesia, and COVID-19[1]. CO has been shown to prevent ischemia-reperfusion injury in transplanted rat lungs[2]. Monitoring endogenous exhaled CO also appears to be of interest during ex vivo lung perfusion procedures aiming at reconditioning marginal human lung grafts[3]. However, because CO originates from numerous sources in the whole body, its measurement lacks specificity and has limited relevance for assessing lung injury. This work focuses on endogenous NO that is produced by the vascular endothelium and contributes to maintaining physiological arterial and capillary vasodilation. It is synthesized from L-arginine and oxygen by several enzymes known as NO synthases (NOS), one of which is inducible (iNOS), particularly during inflammation[4]. The

cellular activity of eNO is involved in a key pathway : the production of intracellular cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). Its half-life is extremely short (a few seconds) and it dissociates very rapidly in peroxynitrites potentially cytotoxic.

The measurement of the fractional endogenous exhaled NO (FeNO) has proven its clinical use in monitoring asthma patients and detecting exacerbations[1]. FeNO monitoring is being explored in other pulmonary inflammatory diseases. However, the available clinical studies remain preliminary, indicating the need for further investigation[5,6]. During the COVID-19 pandemic, clinical studies reported inconsistent results concerning the interpretation of FeNO. Some investigations reported an inverse correlation between FeNO concentrations and the severity of lung damage in COVID-19 patients[7]. Conversely, Kerget *et al*.[8] showed that, in hospitalized COVID-19 patients, FeNO levels were positively correlated with the extend of parenchymal alterations. Similarly, clinical studies conducted in patients with acute respiratory distress syndrome (ARDS) have led to divergent conclusions. Bret *et al.[9]* reported lower FeNO concentrations in patients with ARDS undergoing mechanical ventilation compared with mechanically ventilated control subjects. In contrast, Tang *et al.[10]* measured FeNO levels in exhaled breath condensates of intubated ARDS patients and observed higher values than those reported in control subjects.

One of the challenges in monitoring gasotransmitters is that extremely low concentrations must be quantified within a gas mixture containing hundreds of compounds[11]. Exhaled CO concentrations do not exceed a few parts-per-million (ppm), whereas FeNO levels are even three order of magnitude lower, with values generally ranging from only a few parts-per-billion (ppb) in healthy patients to a few tens of ppb for asthmatic or COVID-19 patients[5,7,8,12]. Commercial devices designed to measure FeNO primarily rely on two analytical techniques[1,13,14]. In recent

years, electrochemical sensors capable of low-cost measurements have been developed; however, their detection limit is often not good enough for many clinical studies, and their limited reproducibility and accuracy prevent meaningful comparative analyses between different device models[15–17]. The method still considered the gold standard for FeNO measurement is chemiluminescence, providing detection limits around 1 ppb with a response time of about 1 s. Although the sensitivity meets the requirements for FeNO quantification, the temporal resolution is insufficient to resolve respiratory cycles, which represents a major limitation for the type of the study presented here, as demonstrated later. In addition, achieving real-time measurements with chemiluminescence requires gas sampling flows that are too high (> 1 L/min) to be connected directly to a ventilator's gas line without altering the clinical protocol. An alternative to chemiluminescence for achieving highly selective and sensitive measurements is laser spectroscopy. Among the various optical methods developed over the past two decades for the analysis of exhaled air[13,14,18,19], we have chosen a highly sensitive laser-based approach that also provides sufficient temporal resolution to resolve individual respiratory cycles while operating at a low sampling flow rate suitable for online clinical monitoring (0.2 L/min), namely Optical-Feedback Cavity-Enhanced Absorption Spectroscopy[3,20] (OFCEAS).

An OFCEAS analyzer has been specifically developed and optimized for the detection of exhaled NO in clinical applications, allowing to reach a limit of detection of 0.3 ppb in 0.2 s [21,22]. A previous study conducted in a porcine model demonstrated its ability to accurately monitor FeNO in isolated lungs (ex-vivo ventilated and perfused lungs), and showed a correlation between the severity of ischemia–reperfusion injury and FeNO concentrations[22].

This study aims to demonstrate the feasibility of monitoring online NO endogenous production by OFCEAS in patients with acute respiratory distress syndrome (ARDS), a population

for which data remain scarce and for which the available findings can be inconsistent[8–10]. These patients are managed in intensive care units under general anesthesia, intubated, and mechanically ventilated. This work represents the first report of FeNO monitoring by OFCEAS in a clinical setting, particularly with patients in intensive care. The second objective was to determine whether FeNO levels in ARDS patients differ significantly from those of healthy subjects, and whether a correlation can be established between FeNO concentrations and ARDS severity. This study was conducted at Grenoble Hospital (France) and included 20 mechanically ventilated patients. Patients were allocated either to the ARDS group, recruited from the intensive care units, or to the control group, consisting of patients scheduled for thyroid or parathyroid surgery under general anesthesia, all of whom were intubated and mechanically ventilated.

2 Materials and methods

2.1 Ethical regulation issue

This clinical study, named ENOBARDS for "Exhaled Nitric Oxide Biomarker for ARDS", is a monocentric, observational, and non-interventional study approved by the French Personal Protection Committee (CPP) Sud-Ouest et Outre-Mer III (SOOM III) and registered under the French national identifier #2O23.AO23L2.43. The protocol has also been registered on ClinicalTrials.gov under the number NCT06250348.

It is classified under French regulations as an RIPH-3 study ("*Recherche Impliquant la Personne Humaine*", category 3), meaning an observational and non-interventional study as defined in Article L1122 of the French Public Health Code. In emergency situations in which the patient is unable to provide informed consent (as here for ARDS patients, where exhaled NO measurements are performed within the first 24 hours after intubation), French regulations may authorize the initiation of a research protocol without waiting for the consent of the patient or his next of kin. However, consent is actively sought as soon as possible. The patient or his next of kin may retrospectively withdraw consent at any time, in which case the data are removed from the

study. The ENOBARDS study was initially authorized to begin under these conditions by the French CPP with the protocol registered on the ClinicalTrials.gov.

However, after the study had begun, the French Ministry of Health issued a notice specifying a more restrictive interpretation of the regulations governing RIPH studies. As a consequence, our protocol no longer complied with the updated requirements, which now mandate prior authorization from the French Data Protection Authority ("*Commission Nationale Informatique et Libertés*", CNIL) for any project—such as ours—requiring a waiver of informed consent from the patient or next of kin. This regulatory requirement made it more difficult to include ARDS patients in the study. As a result, the number of inclusions dropped drastically, and we were ultimately unable to recruit the full number of patients planned in the protocol within the time available for the study.

2.2 Patient groups

Adult patients from Grenoble Hospital, aged 18 years or older, were recruited into two distinct groups. Control-group patients had no significant medical history (ASA physical status I or II) and no respiratory pathology (including asthma, COPD, or chronic respiratory failure). They were scheduled to undergo thyroid or parathyroid gland surgery under general anesthesia, and were therefore intubated and mechanically ventilated. Information regarding potential participation in the ENOBARDS study was provided during the pre-anesthetic consultation, at least 48 hours before the scheduled surgery date. Informed consent was therefore obtained during this consultation, prior to surgery. Patients in the ARDS group were recruited from the intensive care units (ICU) within the first 24 hours following intubation and initiation of mechanical ventilation. Because of the emergency nature of their respiratory care and the impossibility of obtaining informed consent at the time of measurement, we were initially authorized to perform FeNO measurements within the first few hours after intubation, and then to seek consent from the next of kin (and from the patient himself once he had regained adequate consciousness). This ethical authorization was later amended during the course of the study as previously explained.

2.3 Exhaled air monitoring

2.3.1 OFCEAS analyzer approved for clinical use

Like other laser spectroscopy techniques, OFCEAS provides high sensitivity by achieving a very long effective optical absorption path length through the use of a resonant optical cavity as the sample cell. The effective absorption path length can reach several kilometers, even though the cavity itself is only 0.4 m long. OFCEAS analyzers are robust, compact, highly sensitive and selective, can be operated in situ by non-specialists and do not require periodic calibration[3,12,23–25].

The first OFCEAS prototype designed to meet safety and hygiene requirements for deployment in an operating room or in an ICU was developed in collaboration with the private company AP2E, which exploits the LIPhy team's patent (WO03031949) by commercializing OFCEAS analyzers dedicated to industrial and environmental applications. This instrument was designed to measure exhaled CO and enabled OFCEAS to perform its first measurements in a clinical environment, initially on healthy patients in the operating room[23], and subsequently in a far more demanding context by conducting measurements on ex-vivo lung grafts prior to transplantation at the Foch Hospital transplant center in Paris, France[3].

As part of the study presented here, the OFCEAS medical prototype described in Brenckman *et al*[3] was upgraded for FeNO measurements thanks to recent developments carried out by the LIPhy team, as detailed in Richard *et al*[26]. A risk analysis has been carried out by a specialized and independent company (*SurgiQual institute*™; Meylan; France) allowing the use of this NO analyzer for clinical analysis of exhaled air. The upgrade mainly consisted in shifting the spectral analysis range to exploit intense NO absorption lines in the mid-infrared (using a laser operating at approximately 5.3 µm), which enables a detection limit 0.3 ppb in 0.2 s. This new spectral range also provides access to CO_2 absorption lines, thereby enabling simultaneous measurement of exhaled CO_2 ($FeCO_2$) and NO (Figure 1). Because CO_2 levels are very high (several percent), the signal-to-noise ratio is a hundred times higher than for NO measurements. The CO_2 measurement was essential for correcting the raw NO data, as detailed in the "*Results"* section. The short response time of the analyzer enables the resolution of respiratory cycles at the frequency imposed by the mechanic ventilator.

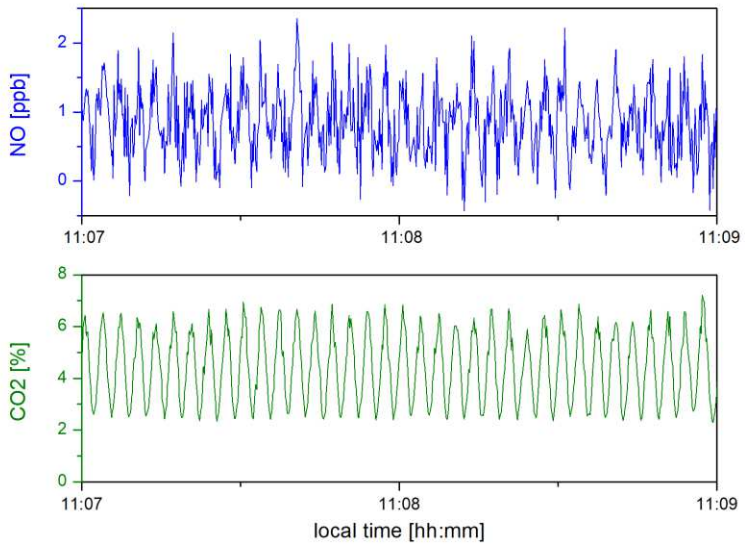


Figure 1: Simultaneous and online measurements by OFCEAS of NO (top) and CO_2 (bottom) endogenously produced by an intubated and mechanically ventilated ARDS patient. The respiratory rate was set to 18 bpm.

2.3.2 Gaz sampling

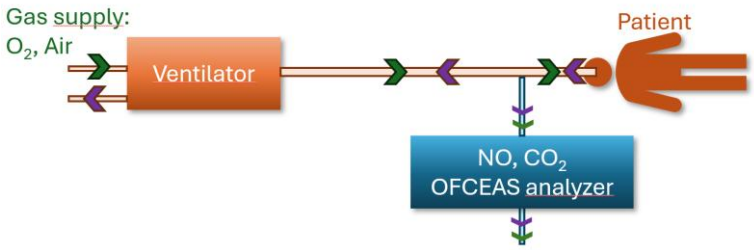


Figure 2: Principle of gas sampling. A controlled flow is continuously extracted from the ventilator circuit by the OFCEAS analyzer during both inspiratory and expiratory phases (indicated by green and purple arrows, respectively).

The OFCEAS analyzer is connected as close as possible to the patient interface of the ventilator (Figure 2). A constant gas flow of 0.2 L/min is withdrawn from the gas ventilator circuit, enabling OFCEAS measurements during both inspiratory and expiratory phases of the respiratory cycle (as shown in Figure 1). This flow rate is a compromise between minimizing gas extraction from the ventilator circuit and achieving a short response time (*i.e* the time required to detect a significant

change in concentration). Indeed, response time is limited by the rinsing time of the sample cell, which is here 0.7 s. This value is derived from the flow rate together with the cell volume (22 cm^3) and its operating pressure (0.1 bar). It is longer than the acquisition time for a single concentration measurement, which is 0.2 s (corresponding to an acquisition frequency of 5 Hz).

In this configuration, the sampled gas represents only a few percent of the total flow managed by the ventilator (ranging between 5 and 11 L/min, depending on the patients). For this reason, no modification of the ventilator settings is required. The parameters are set by the medical staff based on physiological parameters and adjusted to the patient's pathology. All ventilators were set in open breathing circuit, where the exhaled gas is vented out and not re-inhaled. All hospital ventilators are supplied with gas from the same external medical air and oxygen tanks. We verified that these gases contained no significant residual traces of NO, with concentrations close to the instrumental detection limit.

Exhaled NO and CO_2 were continuously monitored over a 5 to 10-minute period for all patients included in the study. In the control group, FeNO measurements were recorded immediately after anesthetic induction and intubation, but before the surgical incision. In the ARDS group, monitoring was initiated as soon as possible after intubation and the start of the mechanical ventilator, and never later than 24 hours post-intubation. The data recorded by the OFCEAS analyzer were then processed as described in the "*Results"* section.

2.4 Clinical parameters

For each patient in both groups, we recorded several types of data. The ventilator settings included tidal volume, respiratory rate (RR), positive end-expiratory pressure (PEEP), inspiratory-to-expiratory ratio (I:E), fraction of inspired oxygen (FiO_2), and end-tidal carbon dioxide ($EtCO_2$). We also collected physiological parameters such as heart rate, systemic blood pressure, and arterial oxygen saturation (SaO_2).

For ARDS patients, we collected additional information. Arterial partial pressure of oxygen (PaO_2) was measured from arterial blood gas samples in order to monitor the PaO_2/FiO_2 ratio at the same time as the FeNO monitoring. We also recorded, for patients in the ARDS group, ventilator-free days, length of stay in the intensive care unit, and survival until hospital discharge.

3 Results

3.1 Patients included in the study

From January to May 2024 we were able to recruit 20 patients into the ENOBARDS protocol. Thirteen patients were enrolled into the control group and seven into the ARDS group. Two patients from the control group were excluded from the analysis: one because of a prior history of COPD, and the other due to unreliable FeNO measurements (likely resulting from an extremely low concentration). One patient in the ARDS group required a very high RR (28 bpm), which was too fast to allow proper monitoring of the respiratory phases, including for CO_2. This patient was therefore excluded of the analysis. The characteristics of the 17 patients included in the analysis are summarized in Table 1, and the flow chart is presented in Figure 3.

	Control group *n* = 11	ARDS group *n* = 6
Age *year* [IQR]	59 [40.5 – 68]	57 [49.5 – 65.3]
Sex Female (%)	6 (55 %)	0 (0%)
Sex Male (%)	5 (45 %)	6 (100%)
Weight *kg* [IQR]	70 [63.5 - 82]	75 [68.3 – 87]
Height *cm* [IQR]	169 [158.5 – 181]	174.5 [173.3 – 178.8]
BMI [IQR]	24.5 [23.3 – 27.6]	24 [21.6 – 27.4]

Table 1: Characteristics of the patients in both groups. Continuous variables are reported as medians and interquartile ranges (IQR). BMI: Body Mass Index. *n*: number of patients.

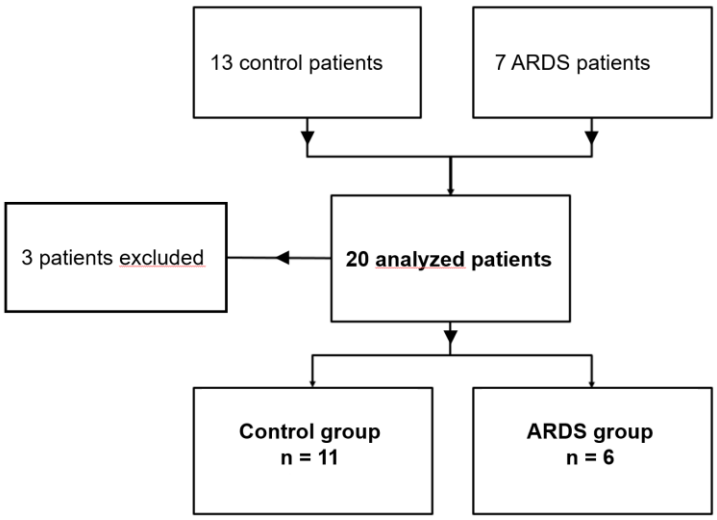


Figure 3: Flow of participants in the ENOBARDS study. Three patients were excluded : one in the control group due to a prior history of COPD, and two due to unreliable measurements (one in each group). *n*: number of patients.

All measurements for patients in the control group were obtained in the same operating room using the same ventilator. Patients with ARDS were admitted to one of the three intensive care units (ICU) at Grenoble hospital and were mechanically ventilated using a distinct ventilator model in each ICU. All patients were ventilated with an inspiratory-to-expiratory ratio of 1:1, except for one ECMO-assisted patient with ARDS, who was ventilated with an I:E ratio of 1:2. The clinical outcomes of the ARDS patients are summarized in Table 2. No significant correlation was found between these parameters and FeNO levels in the ARDS cohort.

Clinical outcomes	ARDS group *n* = 6
PaO2 / FiO2 *mmHg [IQR]*	155.9 [137.6 - 224.8]
Length of oro-tracheal intubation in ICU *days [IQR]*	13.5 [8.8-16,8]

Length of stay in ICU *days [IQR]*	18.5 [12.3-37.5]
Survival until hospital discharge *(%)*	4 (67 %)

Table 2 : Clinical outcomes of the ARDS patients. Continuous variables are reported as medians and interquartile ranges (IQR).

3.2 Raw NO and CO_2 concentration measurements

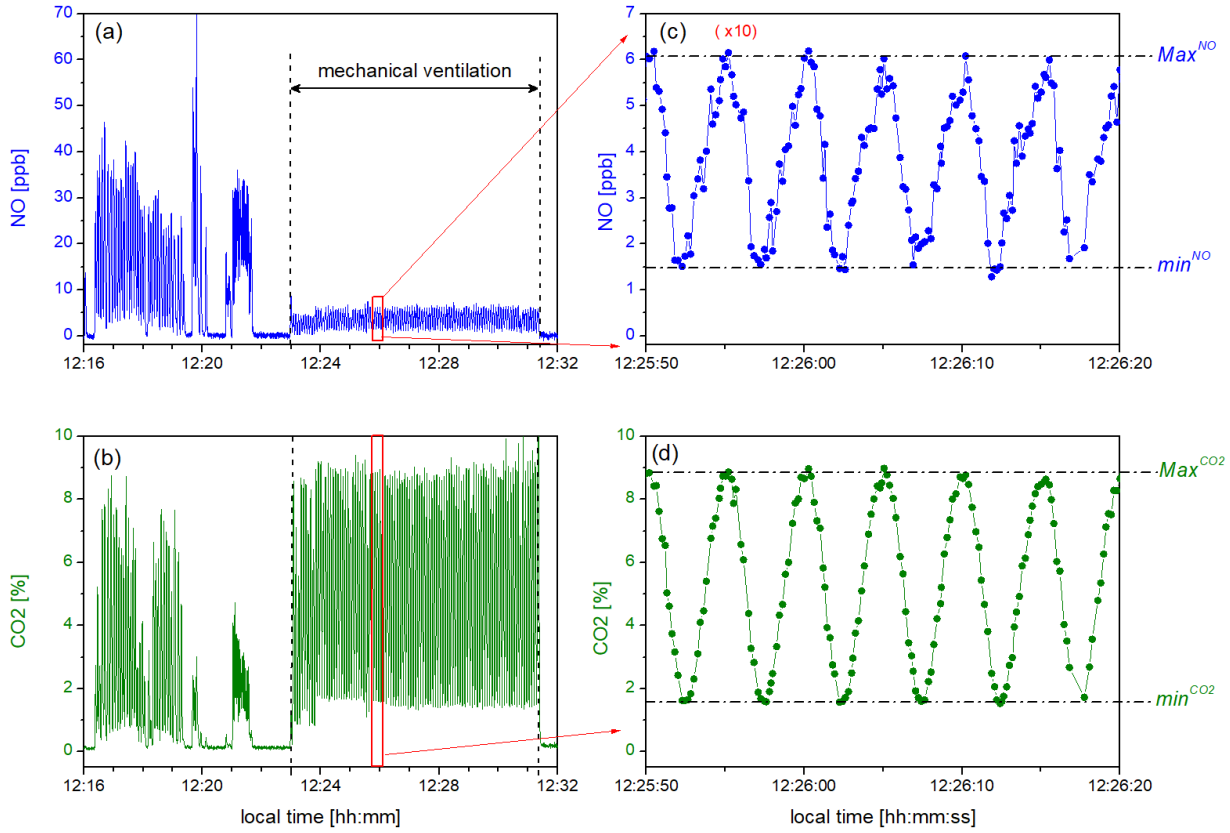


Figure 4: NO (a, c) and CO_2 (b,d) concentrations measured online by OFCEAS for a patient in the control group during mechanical ventilation. The period during which the patient was intubated and mechanically ventilated is indicated by dotted lines in (a) and (b) panels. On the right (c, d) are presented zooms of 30 s long allowing to distinguish 6 respiratory cycles during mechanical ventilation. This is consistent with the RR of 12 bpm set on the ventilator. The I:E ratio was set to 1:1. The averages of the maximum values of the respiratory cycles, Max^{NO} in (c) and Max^{CO2} in (d), and the averages of the minimum values, min^{NO} in and min^{CO2}, are shown as dash-dotted lines. In

the zoomed-in panels, each dot represents a single OFCEAS measurements. Note that for NO zoom (c), the concentration scale has been expanded by a factor 10.

Figure 4 presents an example of NO and CO_2 measurements recorded simultaneously using OFCEAS analyzer in a patient connected to a mechanical ventilator. As expected, NO levels are very different before and after intubation[5]. During the early phase, the patient was ventilated with a face mask (Figure 4a and 3b), resulting in very high NO levels (several tens of ppb), which reflect the predominant contribution from the upper airways and the tracheal production. After intubation (at 12:23 in Figure 4), the measured NO decreases to only a few ppb, as expected for production originating from the lower respiratory airways. A zoom in the OFCEAS measurements clearly reveals respiratory cycles at the RR set by the ventilator (12 bpm, Figure 4c and 3d).

3.3 Procedure for FeNO calculation

During previous clinical measurements performed with an OFCEAS analyzer in healthy volunteers and during ex vivo lung procedures[3,22,23] we quantified endogenous FeCO and FeNO levels by analyzing the amplitude of the respiratory cycles. To achieve this, we developed a dedicated application using a MATLAB® software (Figure 3 in Brenckmann *et al* [3]). It processes series of respiratory cycles to extract the average of the maximum and minimum values, corresponding respectively to the level at the end of the exhalation phase and the baseline level at the end of the inhalation phase. The MATLAB routine was applied to NO and CO_2 measurements acquired in this study. The values, labeled "*Max*" and "*min*", are displayed as dash-dotted lines in measurements plotted in Figure 4c and Figure 4d. This analysis offers the advantage of being insensitive to offset fluctuations, which may arise from residual baseline drift of the OFCEAS analyzer, or in other studies, from variations in trace concentrations in inhaled medical gases.

However, in the context of this study focusing on FeNO measurements in intubated patients, this analysis is no longer sufficient. Unlike in preclinical studies, where it was possible to adjust the ventilator settings and connections, in this clinical study settings were defined by the medical team and we had to adapt to the standardized tubing in use. In addition, one of the specific features of this ENOBOARDS study, is that the RR is high, particularly among patients with ARDS (median 20.5 bpm; IQR [18 – 23.8]) compared with control subjects (median 14 bpm; IQR [12 – 14.5]). By increasing the RR, the duration of the respiratory cycles becomes too short in relation to the gas renewal time between two measurements in the gas cell of the analyzer. In this situation, there is no longer enough time to acquire multiple measurements at the end of exhalation phase or at the end of inhalation phase, which would allow plateaus to be observed, as reported in previous studies conducted at lower RR (Figure 5 in Chollier *et al*[22]). Consequently, although the OFCEAS analyzer can still resolve the respiratory cycles, the recorded minimum and maximum values no longer correspond to the true concentrations at the end of the inhalation and exhalation phases. In particular, the minimum values is not approaching zero (Figure 4), as would be expected given that medical gases and ambient air do not contain significant concentrations of NO or CO_2. Moreover, the gas connections between the ventilator, the patient, and the OFCEAS analyzer introduce additional dead volumes, which can further limit the response time of the measurements even when the RR is sufficiently low to ensure adequate renewal of the sampling cell (as detailed in the "*Discussion*" section).

This smoothing effect due to the gas handling system, together with equal inspiratory and expiratory phase durations (I/E = 1), results in respiratory cycles exhibiting a sinusoidal-like pattern (Figure 1 and Figure 4). For a given patient, higher RR would lead to enhanced smoothing effects, manifested by a reduced oscillation amplitude, whereas the mean concentration value

remains unaffected. Consequently, as the gas circulation system does not allow direct measurement of FeNO and $FeCO_2$, it is easy to show that these can still be deduced from the measured amplitude of the respiratory cycles corrected as follows:

$$FeCO_2 = \eta \times (Max^{CO2} - min^{CO2}) \quad (1)$$

$$FeNO = \eta \times (Max^{NO} - min^{NO}) \quad (2)$$

where the correction factor (η) is identical for both gases because it depends solely on the patient's ventilation parameters, the tubing configuration and the hydraulic characteristics of the OFCEAS analyzer. Given the better signal-to-noise ratio in the CO_2 measurements, this factor is computed based on those measurements. It is determined from the minimum and maximum CO_2 concentration values (respectively min^{CO2} and Max^{CO2}) recorded during mechanical ventilation, as well as the offset value ($Offset^{CO2}$) that is measured immediately after ventilation. The following formula is obtained:

$$\eta = \frac{Max^{CO2} + min^{CO2} - 2 \times Offset^{CO2}}{Max^{CO2} - min^{CO2}} \quad (3)$$

During this clinical study, $Offset^{CO2}$ values were very low (from 0.06 to 0.25%) compared to the concentration levels.

3.4 FeNO values

For each patient, minimum and maximum of CO_2 and NO concentration values are obtained by computing the averaged of the maximum and minimum values derived by the MATLAB routine from CO_2 and NO online measurements. The difference between the maximum and minimum values of each gas defines the mean amplitude of the respiratory cycles recorded online. These uncorrected "raw" data are displayed for NO in Figure 5 ($Max^{NO} - min^{NO}$) using an IQR-based representation for each patient group. We chose to present these intermediate data to support the

Discussion and *Conclusion* sections below. In the ARDS group, the amplitudes were tightly distributed between 1.25 and 1.8 ppb, with a median value of 1.7 ppb. In contrast, in the control group, the amplitudes showed a wider dispersion, ranging from 1.4 to 7.3 ppb, with a median of 2.8 ppb.

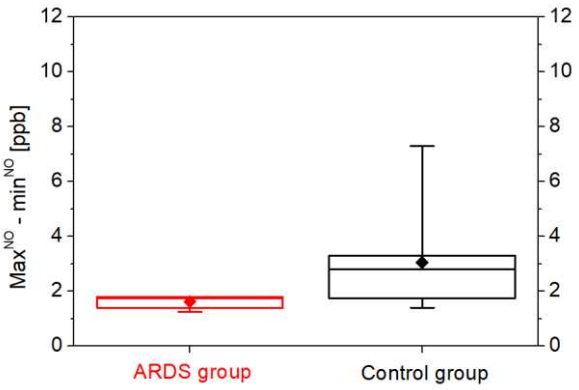


Figure 5: Amplitudes of the raw data of NO respiratory cycles measured online by OFCEAS in the ARDS group (n=6) and the control group (n=11). Measurements are represented as medians and interquartile ranges (IQR). Values are 1.7 ppb [1.4 - 1.8] in the ARDS group and 2.8 ppb [1.75 - 3.3] in the control group.

The FeNO values were then calculated according to equations (2) and (3), and the corresponding results are presented in Figure 6. As expected, because the correction factor exceeds 1, the corrected values are higher than the raw measurements. In addition as the correction factor varies for each patient and depends in particular on the RR (see Figure 7 below and the related discussion), after application of the correction we observe that the difference between the median values of the two groups is no longer significant: 4.5 ppb in the ARDS group and 4.7 ppb in the control group. The dispersion of measurements within each group is greater than in Figure 4, ranging from 2.6 to 5.5 ppb in ARDS patients and from 2.7 to 10.8 ppb in the control group.

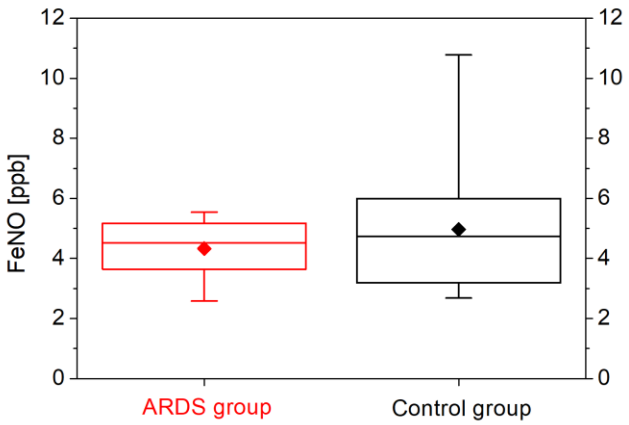


Figure 6: Fraction of expired NO obtained after correction of the raw OFCEAS measurements in both ARDS and control groups. Results represented as medians and interquartile ranges (IQR) are 4.5 ppb [3.6 – 5.2] in the ARDS group and 4.7 ppb [3.2– 6.0] in the control group.

4 Discussion

4.1 Technical challenges in FeNO measurements

The primary challenge in measuring FeNO in intubated patients is to use a device with a detection limit in the sub-ppb range while still resolving respiratory cycles, which requires response times of less than one second. As noted in the introduction, the only commercially available devices that satisfy these requirements, and are approved by regulatory authorities, are high-end chemiluminescence analyzers[13], such as the CLD 88 (Eco Medics AG, 0.1 ppb in 0.5 s) and the NOA 280i (Zysense, 0.5 ppb in 1 s). The chemiluminescence technique relies on detecting light emitted during the oxidation of NO to NO_2 following its reaction with ozone. As a result, it provides an indirect measurement, and constituents of exhaled breath can interfere with the measurement process (primarily CO_2 and H_2O), leading to substantial decreases in the measured NO concentration[27,28]. Moreover, frequent calibration of the instrument is necessary to ensure measurement reliability, particularly to maintain sensitivity at the ppb level.

To address the need for ultra-sensitive NO detection in both medical and atmospheric chemistry applications, several analyzers based on different laser spectroscopy techniques have been developed[18]. To achieve the highest possible sensitivity, several techniques (including the OFCEAS method used in this study) employ an optical resonant cavity as the sample cell to increase the effective absorption path length. Among the most sensitive instruments, Marchenko *et al*[29] developed a cavity enhanced absorption spectrometer (CEAS) with a detection limit of 0.7 ppb of NO for a 1-s integration time. However, the response time is substantially longer because of the large volume of the measurement cell, even when very high flow rates are used (larger than 5 s at a flow rate of 3 L/min). Another laser-spectroscopy instrument based on cavity ring-down spectroscopy (CRDS), developed by Wild *et al*[30], achieved a detection limit of 140 ppt with a 1-s integration time. However, because it relies on an indirect measurement approach (where NO is converted to NO_2 in excess O_3, prior to detection by CRDS), the device can suffer from interferences from other molecular compounds. In addition, it requires periodic calibration using multiple gas standards. Recently, Rollins et al.[31] developed a direct-detection instrument for atmospheric NO measurements based on light-induced fluorescence (LIF), achieving an impressive detection limit of 1 ppt for a 1-s integration time. However, reaching this level of performance requires hourly calibration to maintain the instrument's sensitivity, and a post-correction must be applied to account for water-vapor interference. Because of its strong sensitivity to water concentration, this technique is probably not well suited for analyzing exhaled air, which contains high levels of water vapor.

To the best of our knowledge, although a few clinical applications of laser spectroscopy for exhaled NO have been conducted[13], the OFCEAS spectrometer used in this study is the first instrument capable of monitoring NO respiratory cycles in a clinical environment, particularly in

the demanding context of intubated patients. This OFCEAS device allows online monitoring of FeNO with a detection limit of 0. 0.3 ppb in 0.2 s. This technique has also the advantage of operating at low gas flow rates, thereby preventing any disruption to the clinical ventilation protocols; it does not require regular calibration; and it relies on a direct NO-detection method, which minimizes measurement biases caused by interference from other gaseous species.

A particular challenge in this study arises from the high RR for intubated patients, both in the control group (12 to 18 bpm) and even more so in the ARDS group (18 - 25 bpm, except for one ECMO patient ventilated at 10 bpm). These RR are substantially higher than those reported in previous preclinical and clinical investigations using an OFCEAS analyzer connected to a ventilator. Such studies include experiments in porcine models (RR = 10 bpm in Chollier *et al* [22]), isolated lung measurements (12 bpm in Brenckmann *et al* [23]), and, more specifically, lung graft reconditioning procedures prior to transplantation in patients (7–10 bpm in Brenckmann *et al[3]*). As explained above (section 3.3), in this study, the amplitude of the respiratory cycles is not measured directly because it is smoothed by the gas circulation system rather than by the instrument's response time. To address this limitation, a correction procedure for FeNO respiratory cycle recordings was developed, relying on simultaneous OFCEAS measurements of exhaled CO_2.

4.2 Respiratory cycles monitoring

4.2.1 Insensitivity to zero drift

In this study, achieving absolute measurements in the ppb range requires calibrating the instrument with sub-ppb accuracy, well below the ±10 ppb tolerance typically accepted in most clinical studies[14]. However, producing reference gas samples in this concentration range is extremely challenging: no certified standard cylinders are available, requiring the preparation of dilutions

403 from calibrated cylinders containing higher concentrations (on the order of hundreds of ppb) or
404 the use of permeation tubes. The OFCEAS analyzer was calibrated using a 90-ppb reference gas
405 sample (Air Liquide, France), whose concentration was independently quantified with the
406 OFCEAS instrument via laser spectroscopy (by cavity ring-down measurement according to
407 *Kerstel et al* [32]). This approach enabled a calibration accuracy of about 10% in the 1–20 ppb
408 range, with a linearity of 1%.

409 In this study, the accuracy of FeNO values is limited solely by this calibration, as the
410 calibration of the zero-concentration level was not required owing to the analysis being based
411 exclusively on the respiratory cycle amplitude. Consequently, the accuracy is not affected by
412 baseline fluctuations that arise from the optical setup itself, which lies at the core of the
413 spectroscopy method employed[21]. Over the five months duration of the study, the medical
414 prototype exhibited baseline fluctuations of ±2 ppb in NO concentration. In other prototypes, when
415 applications rely exclusively on absolute concentration measurements (as in environmental
416 monitoring), substantial optimization efforts are undertaken to further reduce this drift [21].

417 Under the ventilation conditions encountered in intubated patients, the breathing excursion
418 amplitudes could not be measured directly, therefore the correction method described above was
419 applied which accounts for the offset value of CO_2 measurements (Eq. 3). This CO_2 baseline value
420 was easily monitored at the end of each measurement, ranging from 0.12 to 0.25%. The uncertainty
421 associated to these values is negligible compared with the amplitude of the respiratory CO_2
422 amplitude (with concentrations ranging from 7 to 11%) and remains below the measurement
423 accuracy described above (10% relative). Consequently, owing to the availability of simultaneous
424 CO_2 measurements, the impact of baseline fluctuations in the OFCEAS analyzer on FeNO
425 measurements is negligible.

426 *4.2.2 Limitations of respiratory cycles measurements*

427 As explained earlier, there are various factors that prevent the full amplitude of respiratory cycles
428 from being recorded. With analyzers having a short acquisition time (0.2 s for the OFCEAS
429 analyzer) relative to the duration of the inhalation or exhalation phases (between 1.2 s and 3 s
430 corresponding to the maximum RR of 25 bpm and the minimum of 10 bpm), the temporal
431 resolution of the cycles is limited solely by the gas flow parameters.

432 For the standalone OFCEAS analyzer, independently of any external connection, the
433 temporal resolution of the measurement is determined by the gas flow and pressure within the
434 analysis cell. These parameters define the volume renewal time (or rising time), which is equal
435 here to 0.7 s (see Section 2.3.2). By applying a conservative factor-of-three margin and considering
436 an I:E ratio of 1, a minimum duration of 4 s is required to ensure full capture of both the inspiratory
437 and expiratory phases. Consequently, the maximum RR compatible with the recording of complete
438 respiratory cycles is 15 bpm. At higher RR, individual respiratory cycles are not fully resolved, as
439 illustrated by the measurements presented in Figure 1 at an RR of 18 bpm. As the RR increases,
440 this smoothing effect becomes more pronounced, as shown in Figure 7, where the correction factor
441 (η) is plotted as a function of RR. By definition (Eq. 3), this factor increases with the magnitude
442 of the difference between the measured amplitude and the true amplitude. The green dotted line in
443 Figure 7 illustrates the behavior of the correction factor for RR values exceeding 15 bpm, clearly
444 showing an increase with RR and, consequently, a degradation of the amplitude measurement.

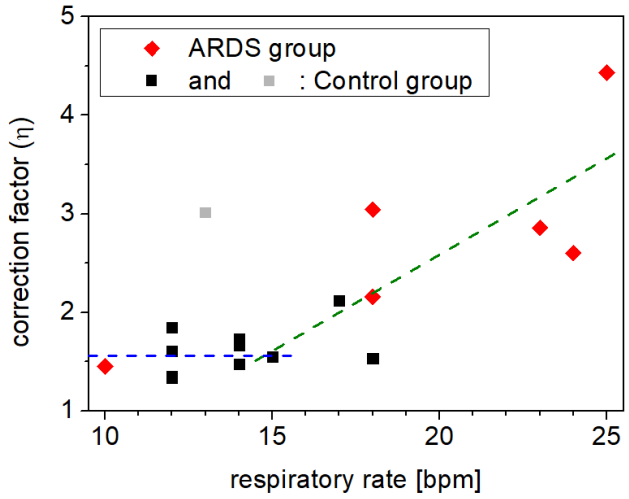


Figure 7: Correction factor applied to derive FeNO values for all patients included in the study as a function of the RR. The blue dotted line denotes the mean value calculated for ventilated patients with an RR ≤ 15 bpm, excluding the patient represented by a gray square. The green dotted line corresponds to the linear regression performed on patients ventilated with an RR ≥ 15 bpm.

For patients ventilated with a RR below 15 bpm, the temporal resolution of the OFCEAS analyzer is sufficient to resolve individual respiratory cycles. Under these conditions, the correction factor is no longer significantly correlated with the RR and remains constant. Nevertheless, its value differs from unity (1.6 in Figure 7), indicating the presence of a residual experimental limitation. We attribute this to the medical tubing and bacterial filters used to connect the patient to the ventilator and to connect the OFCEAS to the ventilation system. This tubing, which extends over several meters, introduces dead space that leads to dilution of exhaled gases into inhaled gases and vice versa. Consequently, the time required to record a complete respiratory cycle is significantly longer than the limit imposed by the OFCEAS instrument's connectors (4 s). For instance, a duration of approximately 8 s is typically required at the end of ventilation for concentration measurements to return to their expected minimum value. Consequently, a RR

462 below 4 bpm would be necessary to fully record complete respiratory cycles, which is completely
463 incompatible with the patient's respiratory requirements. It therefore appears impossible to directly
464 measure the amplitude of respiratory cycles in intubated and mechanically ventilated patients
465 without modifying the tubing configuration used by the medical team.

466 In Figure 7, the data point marked by a gray square has a higher correction factor than
467 expected for a measurement taken at a low RR (13 bpm). It was excluded from the calculation of
468 the mean value indicated by the blue line. This figure shows that patients with ARDS generally
469 exhibit a high correction factor as a consequence of their elevated RR. The correction factor value
470 for control patients is lower, as expected. This explains the difference between the raw NO
471 measurements shown in Figure 5 and the calculated FeNO values in Figure 6.

472 In one particularly severe case of ARDS, veno-arterial Extra-Corporeal Membrane Oxygenation
473 (ECMO) was implemented to support cardiac function and enhance gas exchange. As a result, the
474 ventilator RR was reduced to 10 bpm, since the majority of gas exchange was provided by the
475 ECMO system. In this patient, the correction factor was comparable to the values obtained for
476 control patients.

477 *4.3 FeNO levels in ARDS patients*

478 Metabolism of NO in the context of ARDS is complex[33]. On the one hand, lung inflammation
479 induces physiological alterations as well as coagulopathy and endothelium dysfunction, which
480 result in reduced NO production by constitutive isoforms of NO Synthase (cNOS), namely
481 neuronal NOS (nNOS) and endothelial NOS (eNOS). On the other hand, the activity of the
482 inducible isoform of NO Synthase (iNOS) is upregulated, leading to the production of high NO
483 concentrations, particularly in the alveolar epithelium, where NO levels are usually very low.

The measurement of FeNO in patients with ARDS is particularly challenging, owing not only to the extremely low concentrations encountered, but also to the influence of ventilatory parameter, such as RR and the characteristics of the ventilatory gas delivery line. This undoubtedly contributes to the lack of clinical studies involving patients undergoing mechanical ventilation as compared to a larger number of studies conducted in patient breathing spontaneously.

The results of previously published studies conducted in intubated, mechanically ventilated patients with ARDS are conflicting. Using an enzyme-linked immunosorbent assay (ELISA), Tang *et al* [10] measured NO levels in the exhaled breath condensate of patients with ARDS admitted to the intensive care unit. Their results showed higher NO concentrations in patients with more severe ARDS and higher mortality. In contrast, Bret *et al* [9] assessed FeNO in patients with ARDS using online chemiluminescence measurements and demonstrated that concentrations were lower than those observed in the control group. This latter study was conducted under experimental conditions comparable to those of our study, as it relied on real-time measurements obtained using a highly sensitive analyzer capable of resolving respiratory cycles. FeNO values reported by these authors in the control group (5.5 ± 0.8 ppb [23]) are of comparable magnitude to those observed in our study (median value of 4.7 ppb [3.2– 6.0]; Figure 6). In patients with ARDS, they reported a decrease in FeNO levels, consistent with what we also observe in the raw data (Figure 5). However, whereas their conclusions were based on raw data, we performed additional data processing and corrections, leading to a different conclusion. It is likely that, as in our study, respiratory cycles were not fully resolved, which may have resulted in an underestimation of FeNO. Indeed, although their measurements were performed at a low RR (6 bpm), the respiratory cycles were likely not fully resolved, as no plateau in NO concentration was observed at the end of the expiratory phase (Figures 2 and 4 in Bret *et al* [9]). It should be noted that the ventilatory

settings applied in this 1998 study differ from current lung-protective ventilation strategies recommended for ARDS patients, including lower tidal volumes and higher respiratory rates. The underestimation of FeNO contrasts with our observations in a preclinical model involving intubated and mechanically ventilated pigs, in which a clear expiratory NO plateau was observed (Figure 3 in Chollier *et al* [22]). Consequently, despite differences between the conclusions drawn by Bret et al. and ours, the two studies appear to be consistent.

4.4 Impact of measurement timing and stage of ARDS

Another factor that makes difficult comparisons across different clinical studies is the timing of the measurements with respect to the stage of ARDS, together with the frequent onset of pulmonary edema, which interferes with the accurate quantification of FeNO. For these reasons, our study protocol was restricted to patients intubated for acute respiratory distress syndrome (ARDS) within 24 hours, with the aim of performing measurements at an early stage of lung injury, prior to the development of edema and extensive alveolar flooding. In our previous publication concerning porcine model of lung ischemia-reperfusion injury[22], FeNO measurements were obtained very shortly after the onset of the lesion, within two hours following ischemia–reperfusion. It revealed a threefold increase in FeNO compared with intubated control animals. In the clinical study reported here, measurements were performed at a substantially later time relative to lesion onset. Moreover, pulmonary ischemia-reperfusion injury, although inflammatory in nature, differs from the most common causes of ARDS—particularly those of septic origin—encountered in human clinical practice. We hypothesize that alveolar epithelial damage is already too extensive to allow detection of an early increase in alveolar NO production in response to the inflammatory stimuli. We also assume that capillary endothelial production, which is also typically elevated in inflammatory conditions, is difficult to measure at this stage of ARDS. Indeed, when

measurements are performed within 24 hours after intubation, infiltration of the alveolar–capillary barrier, together with edema formation and alveolar flooding, markedly alters the transfer of endogenous nitric oxide produced by the pulmonary capillary endothelium into exhaled air. These mechanisms may account for the absence of an observed increase in FeNO levels in patients with ARDS. The use of alternative approaches to FeNO assessment, such as the measurement of nitric oxide in exhaled breath condensate as performed by Tang *et al* [10], have yielded different results. This study conducted in China in 2017 reported elevated FeNO concentrations in exhaled breath condensate of mechanically ventilated ARDS patients. A Turkish study published in 2022 described increased FeNO levels in patients with ARDS who were not mechanically ventilated and were therefore likely at a less advanced stage of disease than those included in our study[8].

Finally, it is worth noting that clinical practices regarding the timing of intubation in ARDS are evolving over time and may vary from country to country. The current trend is to perform intubation at a later stage of disease progression, a shift observed since high-flow nasal oxygen therapy has become widely adopted in clinical practice as well as noninvasive ventilation strategies [34]. This trend may explain why FeNO measurements, even when performed within the first few hours after intubation, may nonetheless correspond to a more advanced stage of ARDS and be more strongly influenced by epithelial lung injury and substantial alveolar edema.

5 Conclusion

This study highlights the complexity of measuring FeNO in intubated patients, which arises in part from the technical challenge of developing a device that combines sub-ppb sensitivity with the ability to resolve respiratory cycles, a point of major importance, as previously emphasized. This latter characteristic imposes constraints on the response time of the gas analyzer, which is the primary limiting factor. Clinical parameters may also become limiting, as they cannot be adjusted

for a given clinical gas study and may vary between patients. In particular, the gas connections between the patient and the ventilator, as well as ventilator settings (especially the RR) were identified as limiting factors in the study, even in control patients ventilated at lower RR.

This study demonstrated the feasibility of real-time monitoring NO concentrations in patients intubated and mechanically ventilated. All measurements were performed over several months in various hospital units, during which the device was repeatedly moved and connected to the ventilators by different nurses, who were also responsible for recording NO and CO_2 concentration data. These observations indicates that this medical prototype is suitable for use in clinical trials and does not require specialized staff for data collection. Building on the findings of this study, which highlighted the importance of the OFCEAS analyzer's response time, the instrument could be further optimized for future investigations; Potential improvements include reducing the pressure in the gas analysis cell or increasing the flow within the analyzer without extracting additional gas from the ventilator circuit, for example by introducing a dilution stage with inert gas prior to analysis.

Although online respiratory cycles were not fully resolved, data correction based on simultaneous monitoring of CO_2 concentrations, enabled us to conclude that FeNO levels in patients with ARDS were not significantly different from those observed in the control group. The cohort size is relatively small, in part due to a recent restrictive interpretation of ethical regulations that substantially reduced the number of patients with ARDS compared with the initial protocol. Nevertheless, the findings of this study remain significant, as they do not support several previous reports stating elevated levels of nitric oxide (NO) in the exhaled breath of patients with inflammatory lung diseases, such as asthma[1–3] and bronchiectasis[4].

We compared our results with those of previous studies conducted in patients with ARDS; however, such studies are limited in number and report divergent conclusions. This may be explained by several factors, including clinical ventilation parameters (particularly the RR), the methods used to measure NO concentrations, and differences in data analysis. Previous studies assessing the inter-device comparability of FeNO measurements have concluded that FeNO devices are not interchangeable, with most reported biases leading to an overestimation of FeNO values[14]. We would like to emphasize that issues related to inter-comparison of measurements are not solely attributable to the characteristics of the gas analyzer, but also to various clinical parameters that are not always reported and may be very difficult, if not impossible, to collect, such as a detailed description of the gas connections between the ventilator, the patient, and the gas analyzer. These parameters may, however, become limiting factors for the full resolution of respiratory cycles and, consequently, for the accurate estimation of FeNO levels. Another limitation of the intercomparison is the lack of assessment of ARDS severity and the extent of alveolar flooding at the time of patient intubation.

Measurements reported by Bret *et al.* [9], obtained using a different highly sensitive detection technique (chemiluminescence) but under conditions comparable to ours (patients with ARDS, intubated and mechanically ventilated), are consistent with online measurements of NO concentrations reported here. While these authors concluded, on the basis of their on-line results, that FeNO levels were decreased in the ARDS group compared with the control group, we extended our analysis to account for incomplete monitoring of respiratory cycles. This led to a different conclusion: after correction, no significant difference was observed between the two patient groups.

FeNO monitoring in clinical settings requires further investigation. To record full-amplitude respiratory cycles online, it is necessary to accurately capture the end-expiratory FeNO plateau and the baseline level at end-inspiration. Ideally, such measurements should be performed in clinical contexts that balance patient care priorities with experimental constraints. This implies clinical situations in which a RR below 15 breaths per minute can be tolerated. The ex vivo evaluation of human lung grafts represents a quasi-experimental setting, as mechanical ventilation can be temporarily slowed to enable FeNO measurements without consequences for the graft. In this context, FeNO measurement may provide greater specificity than our previous assessments based on carbon monoxide (CO) monitoring using the OFCEAS analyzer[3]. In addition, measuring FeNO in clinical situations associated with an increased risk of pulmonary inflammation, such as major surgery or sepsis, could be considered to detect early elevations in FeNO in patients who remain clinically stable and are mechanically ventilated at low RR. In such settings, FeNO monitoring could be used to evaluate its predictive value for the subsequent development of severe pulmonary inflammation.

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Disclosures

The authors declare there are no financial interests,or other potential conflicts of interest that have influenced the objectivity of this research.

Code and Data Availability

Data collected as part of the ENOBARDS study, as well as the MATLAB code developed for data processing, will be available upon request.

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