Oxidative lung injury correlates with one-lung ventilation time during pulmonary lobectomy: a study of exhaled breath condensate and blood

José García-de-la-Asuncióna,*, Eva García-del-Olmo b, Jaume Perez-Griera c, Francisco Martí a, Genaro Galan a, Alfonso Morcillo a, Richard Wins d, Ricardo Guijarro e, Antonio Arnau a, Benjamín Sarriá e, Miguel García-Raimundo a and Javier Belda a

a Department of Anaesthesiology and Critical Care, Hospital Clínico Universitario de Valencia, Fundación Investigación Clínico de Valencia, Instituto de Investigación Sanitaria (INCLIVA), Valencia, Spain
b Department of Thoracic Surgery, Consorcio Hospital General Universitario de Valencia, Valencia, Spain
c Laboratory of Biochemistry, Hospital Clínico Universitario de Valencia, Valencia, Spain
d Department of Thoracic Surgery, Hospital Clínico Universitario de Valencia, Valencia, Spain
e Department of Pharmacology, University of Valencia, Valencia, Spain

* Corresponding author. Department of Anaesthesiology and Critical Care, Hospital Clínico Universitario de Valencia, Fundación Investigación Clínico de Valencia-INCLIVA, Av. Blasco Ibañez 17, 46010 Valencia, Spain. Tel: +34-96-3862653; fax: +34-96-3987831; e-mail: josegarcia delaasuncion@gmail.com. (J. García-de-la-Asunción).

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Abstract

OBJECTIVES: During lung lobectomy, the operated lung is collapsed and hypoperfused; oxygen deprivation is accompanied by reactive hypoxic pulmonary vasoconstriction. After lung lobectomy, ischaemia present in the collapsed state is followed by expansion–reperfusion and lung injury attributed to the production of reactive oxygen species. The primary objective of this study was to investigate the time course of several markers of oxidative stress simultaneously in exhaled breath condensate and blood and to determine the relationship between oxidative stress and one-lung ventilation time in patients undergoing lung lobectomy.

METHODS: This single-centre, observational, prospective study included 28 patients with non-small-cell lung cancer who underwent lung lobectomy. We measured the levels of hydrogen peroxide, 8-iso-PGF2α, nitrites plus nitrates and pH in exhaled breath condensate (n = 25). The levels of 8-iso-PGF2α and nitrites plus nitrates were also measured in blood (n = 28). Blood samples and exhaled breath condensate samples were collected from all patients at five time points: preoperatively; during one-lung ventilation, immediately before resuming two-lung ventilation; immediately after resuming two-lung ventilation; 60 min after resuming two-lung ventilation and 180 min after resuming two-lung ventilation.

RESULTS: Both exhaled breath condensate and blood exhibited significant and simultaneous increases in oxidative-stress markers immediately before two-lung ventilation was resumed. However, all these values underwent larger increases immediately after resuming two-lung ventilation. In both exhaled breath condensate and blood, marker levels significantly and directly correlated with the duration of one-lung ventilation immediately before resuming two-lung ventilation and immediately after resuming two-lung ventilation. Although pH significantly decreased in exhaled breath condensate immediately after resuming two-lung ventilation, these pH values were inversely correlated with the duration of one-lung ventilation.

CONCLUSIONS: During lung lobectomy, the operated lung is collapsed and oxidative injury occurs, with the levels of markers of oxidative stress increasing simultaneously in exhaled breath condensate and blood during one-lung ventilation. These increases were larger after resuming two-lung ventilation. Increases immediately before resuming two-lung ventilation and immediately after resuming two-lung ventilation were directly correlated with the duration of one-lung ventilation.

Keywords: One-lung ventilation • Oxidative lung injury • 8-Isoprostane • Hydrogen peroxide • Nitrites and nitrates • Exhaled breath condensate

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INTRODUCTION

Patients undergoing lung lobectomy are at risk for developing severe lung injury because the operated lung is completely collapsed and hypoperfused for a period of time. A collapsed lung is also hypoperfused due to reactive hypoxic pulmonary vasoconstriction, which optimizes gas exchange and helps to prevent arterial hypoxaemia in response to alveolar hypoxia [1, 2]. Additionally, the production of reactive oxygen species (ROS) increases during tissue hypoxia, particularly at two sites in the mitochondrial respiratory chain (Complex I and Complex III) [3, 4]. This increase contributes to additional vasconstriction [3, 5] and may cause mitochondrial dysfunction via oxidative damage. Thus, in patients undergoing lobar resection, relative ischaemia during lung collapse is followed by expansion–reperfusion and injury attributed to the production of ROS [6, 7].

To date, few investigations have assessed these issues in detail. For example, increases in hydrogen peroxide (H$_2$O$_2$) levels in exhaled breath condensate (EBC) and urinary malondialdehyde levels were larger in lobectomy patients than in pneumonectomy patients after pulmonary resection (30 min and 1 day after surgery) [8]. An increase in malondialdehyde blood levels due to lung re-expansion in lung cancer patients after lung resection has been associated with adverse cardiovascular effects [9, 10]. In patients undergoing video-assisted thorascopic surgery with one-lung ventilation (OLV), resuming two-lung ventilation (TLV) induced massive superoxide production, which was associated with lung re-expansion and re-oxygenation [11]. A recent investigation [12] showed that ROS stimulate lymphatic metastasis via the epithelial-to-mesenchymal transition and lymphangiogenesis in the tumour microenvironment. Correlation between serum ROS levels and nodal extension may assist the development of new diagnosis and treatment strategies [12].

EBC collection is a non-invasive method for obtaining samples from the lower respiratory tract. EBC contains a large number of biomarkers, including isoprostanes, nitrogen oxides and H$_2$O$_2$ [13]; pH changes in EBC have been reported in patients undergoing lung lobectomy for cancer [13, 14]. The isoprostanes (such as 8-iso-PGF$2\alpha$) are a family of products from arachidonic acid that are generated through the non-enzymatic action of ROS. Increased blood levels of 8-isoprostane have thus been considered a reliable index of in vivo lipid peroxidation due to their chemical stability [13, 15, 16]. Nitrites (NO$^−_2$) and nitrates (NO$^−_3$) are end products of nitric oxide (NO) and peroxynitrite anion metabolism that are present in the fluid, lining the epithelium of the respiratory tract. NO and superoxide anion (O$_2^−$) react to form peroxynitrite anion, which is a powerful oxidant [13, 17]. H$_2$O$_2$ is a volatile ROS produced from the conversion of superoxide anion to H$_2$O$_2$ by superoxide dismutase; it is released from inflammatory and epithelial cells of the respiratory system and reflects oxidative injury to the lungs [8, 13].

The aim of the current study was to investigate whether oxidative injury occurs during OLV and after resuming TLV in lung cancer patients undergoing lung lobectomy. We undertook the first evaluation of whether OLV duration directly correlates with the levels of oxidative-stress markers in both EBC and blood samples at specific times. We also determined whether the levels of H$_2$O$_2$ (a pro-oxidant) directly correlate with increases in the levels of 8-iso-PGF$2\alpha$ and NO$_2^−$ + NO$_3^−$ in EBC, or whether these levels indirectly correlate with the ratio of partial oxygen pressure (PaO$_2$) to fraction of inspired oxygen (FiO$_2$), a parameter for detecting impaired intrapulmonary gas exchange and oxygenation.

MATERIALS AND METHODS

Study subjects

Twenty-eight patients (24 men and 4 women) were scheduled for elective lung lobectomy for clinical stage I (7 patients) or stage II (21 patients) non-small-cell lung cancer. Patients were prospectively and observationally studied in a single centre. Patient age ranged from 40 to 81 years. All patients were admitted to the post-surgical intensive care unit within 24 h of lobectomy. The exclusion criteria included previously received radiotherapy or chemotherapy, ingested antioxidant vitamins, systemic active infections and body temperature >38°C. The Ethics Committee at Hospital Clínico Universitario de Valencia (Spain) approved the study protocol. Informed consent was obtained from each patient.

Anaesthesia and lung ventilation

Sodium thiopental (3–5 mg/kg) and fentanyl (2 μg/kg) were introduced intravenously. After the induction of anaesthesia, endotracheal intubation was performed with a double-lumen tube (37 or 39 French as appropriate) and facilitated with rocuronium bromide (0.6 mg/kg). Intubation was adjusted via a fibre-optic bronchoscope after the patient was turned to the lateral decubitus position. Anaesthesia was subsequently maintained with sevoflurane (2%), fentanyl and rocuronium bromide.

During TLV, the lungs were ventilated in volume-controlled mode for a tidal volume of 8 ml/kg ideal body weight, a respiratory rate of 12–14 breaths/min and an inhaled:exhaled ratio of 1 : 2. During OLV, the non-dependent lung was collapsed and open to the air, whereas the dependent lung was ventilated with a tidal volume of 6 ml/kg, a respiratory rate of 14–18 breaths/min and an inhaled:exhaled ratio of 1 : 2 to maintain an end-tidal CO$_2$ <40 mmHg. Positive end-expiratory pressures of 5–7 cmH$_2$O and FiO$_2$ 0.5 were used during TLV and OLV to maintain O$_2$ saturation >92%. FiO$_2$ was increased momentarily only when O$_2$ saturation fell below 92% and when the recruitment manoeuvres to the ventilated lung were not effective.

Before anaesthesia induction, a thoracic epidural catheter was inserted for postoperative pain management. Standard anaesthesia monitoring parameters, including arterial oxygen saturation, invasive blood pressure in the radial artery, heart rate and end-tidal CO$_2$ were measured during anaesthesia. All patients were transferred to the post-surgery intensive care unit, where they remained for at least 24 h after surgery and received oxygen (FiO$_2$ 0.5) through a facemask.

Collection of exhaled breath condensate

After anaesthesia induction, air expired from 25 of 28 patients was collected through a cold-collecting system which consisted of a double-walled glass chamber cooled with wet ice and connected to the expiratory port of the ventilator. Approximately, 2 ml of EBC was collected at five time points. The samples were immediately aerated with argon (350 ml/min for 10 min [13, 14]) and stored at −80°C until analysis. EBC samples were collected from all patients at specific time points: T0, preoperatively (baseline); T1, during the 20 min of OLV immediately preceding the resumption of TLV; T2, during the 20 min immediately after resuming TLV; T3,
60 min after resuming TLV and T4, 180 min after resuming TLV. EBC pH was measured in a 0.4-ml aliquot with a blood gas analyser (ABL 88 Flex apparatus, Radiometer, Copenhagen, Denmark).

**Blood sampling**

Arterial blood samples were collected from all patients at five time points: T0, preoperatively (baseline); T1, during OLV, 5 min before resuming TLV; T2, 5 min after resuming TLV; T3, 60 min after resuming TLV and T4, 180 min after resuming TLV. Blood samples were collected (BD Vacutainer PST II tubes with lithium heparin, BD-Plymouth, UK) from a radial artery line and then centrifuged at 3000 rpm for 12 min. Plasma was separated and stored at −80°C until analysis. Arterial blood pH, PaO2, partial carbon dioxide pressure (PaCO2) and PaO2/FiO2 ratio (a useful parameter for detecting impaired intrapulmonary gas exchange and oxygenation) were analysed with an ABL 88 Flex apparatus (Radiometer).

**Determination of the levels of oxidative-stress markers in exhaled breath condensate and blood**

The levels of 8-iso-PGF2α, a stable prostaglandin-like product formed from arachidonic acid by the action of ROS, were measured using a competitive enzyme immunoassay (ELIA kit from Cayman Chemical Co., Ann Arbor, MI, USA). Less than half of total plasma 8-iso-PGF2α is present as a free acid; most of it is esterified into phospholipids. Therefore, determination of the total plasma levels of 8-iso-PGF2α requires alkaline hydrolysis prior to enzyme immunoassay [18].

In vivo, the final products of NO metabolism are NO2− and NO3−. The relative proportion of these products is variable and cannot be predicted with certainty. Thus, the best index of total NO production is the sum of NO2− and NO3−. Total NO2− + NO3− levels were measured by converting NO3− to NO2− using nitrate reductase. The Griess reagent was used to convert NO2− into a compound that was measured with a colorimetric assay at 540 nm (nitrate/nitrite colorimetric assay kit, Cayman Chemical Co.) [19].

H2O2 is unstable in EBC; therefore, after collection, aliquots were rapidly frozen at −80°C until analysis [13]. H2O2 levels were measured within 1 week after sample collection via the oxidation of ferrous ions to ferric ions by H2O2 under acidic conditions (a colorimetric assay kit, Cayman Chemical Co). Ferric ion binds the dye xylene orange to form a stable complex, which can be measured at 595 nm.

**Statistical analysis**

Data are expressed as mean ± standard deviation. Normality was assessed with the Shapiro-Wilk test. Data were parametric and normally distributed. One-way analysis of variance for repeated measures (time course: T1–T4 vs T0) was performed to assess the effect of OLV time and subsequent lung re-expansion on the levels of oxidative-stress markers in EBC and blood. When the comparisons were significant, post hoc multiple comparisons with Tukey’s test (or the Games–Howell test for unequal variances) were used to compare values between sampling times. Correlations between total time of lung collapse and the levels of several oxidative-stress markers (8-iso-PGF2α, NO2− + NO3−, H2O2), PO2/FiO2 ratio and pH from EBC and arterial blood samples were analysed using Pearson’s correlation coefficient (r). Results were considered statistically significant when P < 0.05. Statistical analysis was performed using the SPSS statistical software, version 16.0 (SPSS, Inc., Chicago, IL, USA).

**RESULTS**

Demographic, preoperative functional parameters and clinical data from the intraoperative period are summarized in Table 1. Mean values during surgery were as follows: temperature, 35.9 ± 0.5°C; arterial pressure, 82 ± 6 mmHg; heart rate, 80 ± 19 beats/min. No patients had difficulty maintaining lung isolation during the operation. It was not necessary to implement intermittent TLV by decreasing O2 saturation during OLV. Only 6/28 patients (21.4%) intermittently required more than 50% oxygen during OLV. All patients were extubated without incident at the end of surgery. No epidural analgesia failures occurred in any patient.

During the first 24 h of admission to the postoperative intensive care unit, the average patient body temperature was 37.36 ± 0.33°C, with an average white blood cell count of 9750 ± 3700 leukocytes/ml. Arterial blood gas analysis of 4/28 patients (14%) revealed PaO2/FiO2 ratios <200 (the mean ratio for all patients was 254 ± 56). Chest radiography of 5/28 patients (18%) during the first 24 h indicated varying degrees of pulmonary infiltrate on the side of the operated lung. Cardiac arrhythmia (atrial fibrillation) was observed in 7/28 patients (25%) at some point during their stay in the intensive care unit. No patients developed sepsis or died during their stay in the intensive care unit.

| Table 1: Demographic and clinical data during the intraoperative period |
|-----------------------------|-----------------------------|
| **Age (years)** | 64.5 ± 10.2 |
| **Sex (male/female)** | 24/4 |
| **Body mass index (kg/m²)** | 26.2 ± 4.1 |
| **Current smokers (n)** | 9 |
| **Ex-smokers (n)** | 17 |
| **Never smoked** | 2 |
| **Preoperative FEV1 (l)** | 2.11 ± 0.64 |
| **Preoperative FVC (l)** | 2.80 ± 0.72 |
| **Preoperative FEV1/FVC** | 0.77 ± 0.23 |
| **Side (right/left)** | 17/11 |
| **Location** | Right upper lobe 14 | Right middle lobe 1 | Right lower lobe 2 | Left upper lobe 9 | Left lower lobe 2 |
| **Histology** | Adenocarcinoma 10 | Squamous carcinoma 17 | Other 1 |
| **OLV time (min)** | 113 ± 39 |
| **Surgery duration (min)** | 152 ± 37 |
| **Intraoperative fluid load (l)** | 1.40 ± 0.33 |
| **Intraoperative urine (l)** | 0.55 ± 0.32 |

Values are expressed as mean ± standard deviation (n = 28).

FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; OLV: one-lung ventilation.
Table 2: Time course of arterial blood gas parameters during lung lobectomy

<table>
<thead>
<tr>
<th>Time points</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
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</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.37 ± 0.04</td>
<td>7.36 ± 0.03</td>
<td>7.35 ± 0.04</td>
<td>7.37 ± 0.03</td>
<td>7.37 ± 0.04</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>36.4 ± 3.4</td>
<td>39.0 ± 2.4*</td>
<td>37.9 ± 2.8</td>
<td>37.4 ± 1.3</td>
<td>37.0 ± 3.4</td>
</tr>
<tr>
<td>PaO₂</td>
<td>170 ± 31</td>
<td>136 ± 31***</td>
<td>151 ± 33</td>
<td>146 ± 32*</td>
<td>147 ± 26*</td>
</tr>
<tr>
<td>PaO₂/FiO₂</td>
<td>329 ± 70</td>
<td>245 ± 80***</td>
<td>284 ± 74</td>
<td>293 ± 65</td>
<td>295 ± 52</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (n = 28). Differences relative to baseline (T0) were assessed with pairwise comparisons with post hoc analysis of variance.
P: partial oxygen pressure; FiO₂: fraction of inspired oxygen; PaCO₂: partial carbon dioxide pressure.
*P < 0.05.
**P < 0.01.
***P < 0.001.

Table 2 summarizes arterial blood gas parameters from all patients at various points during lobectomy. PaCO₂ values were significantly higher (P < 0.05) at time point T1 during OLV than at T0 (baseline). Relative to T0, PaO₂ decreased at T1, T3 and T4, particularly T1 (during OLV, P < 0.001), and remained low at T3 and T4 (P < 0.05). PaO₂/FiO₂ was significantly lower at T1 (P < 0.001) than at T0.

The time course of the levels of oxidative-stress markers and pH values in EBC and in arterial blood samples appears in Fig. 1. In EBC samples, the levels of 8-iso-PGF2α, a stable end product of the oxidation of cellular phospholipids, increased at T1-T4 relative to T0, particularly at T2 and T3 after lung re-expansion (P < 0.001). The levels of NO₂⁻ + NO₃⁻ in EBC also significantly increased at T1 and T2 relative to T0 (P < 0.05 and P < 0.001, respectively). Significant increases in EBC H₂O₂ levels occurred at T2 relative to T0 (P < 0.05). We detected a significant and inverse correlation (r = −0.3926; P = 0.0470) between preoperative FEV₁ values and H₂O₂ levels in EBC at T0. EBC pH significantly decreased at T2 (P < 0.05). 8-iso-PGF2α levels in arterial blood were significantly higher at T1-T3 than at T0 (P < 0.05, P < 0.001 and P < 0.01, respectively); however, these levels were approximately half the levels detected in EBC samples. The blood levels of NO₂⁻ + NO₃⁻ were significantly higher at T2 than at T0 (P < 0.01), but blood levels were approximately double those in EBC.

The levels of all oxidative-stress markers in EBC samples were significantly and positively correlated with total duration of lung collapse at T1 and similarly after lung re-expansion at T2 (8-iso-PGF2α, P < 0.001; NO₂⁻ + NO₃⁻, P < 0.05; H₂O₂, P < 0.01; Fig. 2A-C). A significant decrease in EBC pH occurred at T2 (Fig. 1D), and pH was inversely correlated with OLV duration at T1 and T2 (P < 0.05; Fig. 2D).

In arterial blood, 8-isoprostanate levels and OLV duration at T1 were positively correlated (r = 0.443; P < 0.05) and this correlation was stronger (r = 0.567; P < 0.01) after lung re-expansion (at T2; Fig. 3A). For NO₂⁻ and NO₃⁻, correlation with OLV duration was significant (r = 0.467; P < 0.05) only at T2 (Fig. 3B). In arterial blood, PaO₂/FiO₂ ratio and pH were inversely correlated with the duration of total lung collapse at T1 and T2: PaO₂/FiO₂ (at T1, r = −0.525, P < 0.01 and at T2, r = −0.505, P = 0.01) and pH (at T1, r = −0.369, P < 0.05; and at T2, r = −0.452, P < 0.05) similarly at T1 and T2 (Fig. 3C and D). To our knowledge, these are the first observations of such inverse correlations.

We detected a positive correlation between the levels of H₂O₂ (a potent pro-oxidant formed by the dismutation of superoxide radicals) and the levels of 8-iso-PGF2α (which reflect lipid peroxidation and lung damage) in EBC at T1 during lung collapse (r = 0.389; P < 0.05), but not during lung re-expansion at T2 (Fig. 4A). EBC levels of NO₂⁻ + NO₃⁻ (end products of NO and peroxynitrite anion metabolism) were even more strongly correlated (r = 0.528; P < 0.01) with H₂O₂ levels at T1 (Fig. 4B). However, H₂O₂ levels (Fig. 4C) and 8-iso-PGF2α levels (Fig. 4D) in EBC were significantly and inversely correlated with PaO₂/FiO₂ ratio in arterial blood at T2; these correlations were particularly strong with H₂O₂ levels (P < 0.531; P < 0.01), suggesting a disturbance in oxygen transport induced by oxidative damage to the alveolar membrane.

DISCUSSION

The pathogenesis of lung injury after lobectomy with OLV appears to be multifactorial, including effects from surgical manipulation, lung collapse with hypoxic vasoconstriction followed by lung re-expansion and reperfusion with large releases of radical superoxides and cytokine production in the operated and dependent lungs [8-11]. Therefore, patients undergoing lung resection and OLV for more than 1 h can suffer cardiac arrhythmia due to ROS generation during reperfusion [10]. Some experimental studies have found a basic role of ROS in the pathogenesis of pulmonary hypertension, which may underlie post-resectional lung oedema and increase the risk, at least in part, of acute lung injury/acute respiratory distress syndrome [20, 21].

Here, 24 h after lung lobectomy, we detected arterial blood gas PaO₂/FiO₂ < 200 in 4/28 patients (14.3%) wearing an oxygen mask (FiO₂ 0.5), indicating acute lung injury. Moreover, chest radiography revealed infiltrates in the operated lung of these patients; these lungs were collapsed during OLV for periods that were longer than the mean (113 ± 39 min) duration of OLV. Seven patients suffered cardiac arrhythmia (atrial fibrillation), with 5/7 patients experiencing a collapse time longer than the mean. In the present investigation, the complications observed within 24 h of admission to the postoperative intensive care unit were comparable with those reported in previous studies [10, 22] that associated the degree of oxidative stress with major adverse effects after lung resection.

During OLV, the operated lung remains atelectatic as well as hypoperfused because only a small fraction of pulmonary blood flow passes through the lung. After lung re-expansion, the operated lung may suffer ~50% reduced perfusion due to vasoconstriction induced by microatelectasis and hypoxic exposure.
Simultaneously, during OLV, the ventilated lung may experience significant hyperperfusion and hyperinflation and begin to undergo diffuse alveolar damage [23]. Table 2 (and Fig. 3C and D) contains a complete description of arterial blood gases throughout the surgical process, data that were not previously reported. We observed an increase in PaCO₂ at T1 during OLV due to hypoventilation. PaO₂ decreased at all time points, particularly T1, during OLV; thus, PaO₂/FiO₂ values were lower at T1 than at T0 (see Table 2). These results seem to reflect a ventilation/perfusion mismatch during and after OLV in both dependent and non-dependent lungs (see Fig. 3C). After re-expansion, the lung may experience reduced perfusion due to microatelectasis, but during OLV, the dependent lung undergoes diffuse alveolar disturbances due to hyperperfusion and hyperinflation [23].

EBC collection is a non-invasive method for sampling the lower respiratory tract in humans, usually through a refrigerated device. EBC contains many mediators and during exhalation, molecules and water directly diffuse from fluid covering alveoli and airways into microdroplets of condensed water vapour [13]. Our study of EBC uncovered an increase in 8-iso-PGF2α produced in vivo by the peroxidation of arachidonic acid at T1–T4, especially at T2 and T3. These results indicate that direct oxidative injury from ROS begins during the ischaemic period at T1 and quickly increases at T2–T3. 8-iso-PGF2α levels in EBC were approximately double those in blood plasma (see Fig. 1A and E), possibly due to peroxidation of arachidonic acid in cells of the alveolar membrane (in close proximity to fluid covering the airspaces) and rapid diffusion of 8-iso-PGF2α into EBC.
NO synthesized from L-arginine by NO synthase is present in various inflammatory and structural cell types within the airway, such as alveolar epithelial cells, vascular endothelial cells and smooth muscle cells. NO and superoxide anion react to form peroxynitrite, a powerful oxidant. NO$_2^-$ and NO$_3^-$ are end products of NO and peroxynitrite metabolism [13, 17]. The levels of NO$_2^-$ + NO$_3^-$ in EBC increased at T1 and T2 in a pattern similar to that of 8-iso-PGF2α levels in EBC, reflecting oxidative injury that began during the ischaemic period (T1) and increased after lung re-expansion (T2). The levels of NO$_2^-$ + NO$_3^-$ in EBC were approximately half the levels detected in blood plasma (see Fig.1B and F), possibly because the main source of NO and superoxide anion was the pulmonary capillary endothelium (with more production than in alveolar epithelia). In addition, NO may induce selective pulmonary vasodilatation with improved perfusion of ventilated alveoli. However, this phenomenon has been shown to aggravate lung damage via peroxynitrite formation and nitrosative stress [17].

H$_2$O$_2$ can be released from both inflammatory and epithelial cells and produced by superoxide dismutase through conversion of the superoxide anion. However, this phenomenon has been shown to aggravate lung damage via peroxynitrite formation and nitrosative stress [17].

In the current study, EBC pH decreased during the onset of lung reperfusion at T2 (after resuming TLV) (see Fig. 1D). This acidification was accompanied by an inverse correlation between pH and total time of lung collapse; this correlation was stronger during the lung re-expansion period (Fig. 2D) and may be associated with neutrophil activation and increased oxidative injury. EBC acidification is associated with the exacerbation of several inflammatory airway pathologies, neutrophil activation and oxidative stress [13, 19].

We also demonstrated simultaneous increases in blood levels of the oxidative-stress markers 8-iso-PGF2α and NO$_2^-$ + NO$_3^-$ (Fig. 1E and F), with patterns similar to those found in EBC (Fig. 1A and B) and were positively correlated with OLV duration and this correlation was stronger after lung re-expansion (Fig. 3A and B).

We observed a significant increase in oxidative injury during OLV, although this increase was greater after resuming TLV due to the extensive production of superoxide anions. Therefore, oxidative lung injury after hypoxic insult is biphasic, starting with a hypoxic period during lung collapse and worsening during lung re-expansion/re-oxygenation. Although seemingly paradoxical, ROS are generated under conditions of ischaemia or lowered...
Figure 3: Pearson correlation coefficients ($r$) between total time of lung collapse (min) and the levels of oxidative-stress markers, PaO$_2$/FiO$_2$ ratio and pH in arterial blood samples during OLV, 5 min before resuming TLV (T1; blue circles and blue lines) and 5 min after resuming TLV (T2; red circles and red lines). (A) 8-iso-PGF$_2\alpha$. (B) NO$_2^−$ + NO$_3^−$. (C) PaO$_2$/FiO$_2$ ratio. (D) pH. $P < 0.05$ was considered statistically significant ($n = 28$). EBC: exhaled breath condensate; OLV: one-lung ventilation; TLV: two-lung ventilation; PaO$_2$: partial oxygen pressure; FiO$_2$: fraction of inspired oxygen; NO$_2^−$: nitrites; NO$_3^−$: nitrates.

Figure 4: Pearson correlation coefficients ($r$) of the levels of oxidative-stress markers (in EBC) with H$_2$O$_2$ or with PaO$_2$/FiO$_2$ ratio during OLV, immediately before resuming TLV (T1; blue circles and blue lines) and immediately after resuming TLV (T2; red circles and red lines). (A) H$_2$O$_2$ and 8-iso-PGF$_2\alpha$. (B) H$_2$O$_2$ and NO$_2^−$ + NO$_3^−$. (C) H$_2$O$_2$ and PaO$_2$/FiO$_2$ ratio. (D) 8-iso-PGF$_2\alpha$ and PaO$_2$/FiO$_2$ ratio. $P < 0.05$ was considered statistically significant ($n = 25$). EBC: exhaled breath condensate; OLV: one-lung ventilation; TLV: two-lung ventilation; PaO$_2$: partial oxygen pressure; FiO$_2$: fraction of inspired oxygen; NO$_2^−$: nitrites; NO$_3^−$: nitrates; H$_2$O$_2$: hydrogen peroxide.
oxygen. Cytochromes in the mitochondrial respiratory chain pass into a redox-reduced state, allowing the transfer of electrons to molecular oxygen and producing large amounts of superoxide anions [24]. It is important that we uncovered significant correlations between the levels of several markers of oxidative damage and the total time of lung collapse in both EBC and blood samples because these correlations support the hypothesis of a direct relationship between the total time of lung collapse and the severity of oxidative damage that occurs during lung collapse and subsequent reperfusion in the expanded lung. Thus, it is critical to shorten the time of lung collapse to minimize pulmonary oxidative damage and to prevent complications after lung lobectomy.

EBC samples also displayed at T1-positive relationships among the pro-oxidant effect of $H_2O_2$ levels and the formation of $8$-iso-PGF$_2\alpha$ and $NO_2^- + NO_3^-$, indicating that oxidative injury began during ischaemia and lung collapse (Fig. 4A and B). However, $H_2O_2$ and 8-iso-PGF$_2\alpha$ levels in EBC at T2 were significantly and inversely correlated with $PaO_2/FiO_2$ ratio in arterial blood (Fig. 4C and D), highlighting a difficulty in oxygen transport that is likely due to alterations in the alveolar capillary wall. These findings underscore the need for further studies of pertinent pathophysiological mechanisms and the need to develop new therapeutic strategies, such as remote ischaemic preconditioning, in order to prevent oxidative stress and lung injury during lung lobectomy [22, 25].

In conclusion, cancer patients undergoing lung lobectomy suffer a critical increase in ROS formation; here, the levels of markers of oxidative and nitrosative stress increased simultaneously in EBC and in blood during lung collapse and OLV. However, these levels increased more after the resumption of TLV and were directly correlated with the duration of total lung collapse and OLV. These findings may explain, at least partly, the acute lung injury that occurs in some patients after pulmonary lobectomy.

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