

## Original Article

## Interleukin 8 Concentrations in Donor Bronchoalveolar Lavage: Impact on Primary Graft Failure in Double Lung Transplant

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## ABSTRACT

**Background and Objective.** The purpose of this study was to determine concentrations of interleukin 8 (IL-8) in the bronchoalveolar lavage (BAL) fluid from donor lungs and assess the role of IL-8 levels in the development of primary graft failure.

**Patients and Methods.** Twenty patients who received a double lung transplant were studied. A series of data, including BAL fluid concentrations of IL-8, were collected for the donors. Data collected for the recipients included arterial blood gases after 6, 24, and 48 hours, and intubation time. Patients with a ratio of PaO<sub>2</sub> to the fraction of inspired oxygen (FiO<sub>2</sub>) of less than 300 during the first 48 hours were diagnosed with primary graft failure. IL-8 levels were determined by enzyme-linked immunosorbent assay. Associations between the donor variables and IL-8 concentrations were evaluated using the Spearman rank correlation coefficient ( $\rho$ ) and the Mann-Whitney test for categorical and continuous variables, respectively. Logistic regression was used for multivariate analysis.

**Results.** Fifteen of the 20 donors were men. The cause of brain death was trauma in 9 donors, 7 were smokers, 13 required inotropic support, and pathogens were isolated in the BAL fluid of 18. The median age was 35 years (interquartile range [IQR], 23.5-51.25 y), the median ventilation time was 1 day (IQR, 1-2 d), the median PaO<sub>2</sub>/FiO<sub>2</sub> was 459.5 (IQR, 427-510.25), and the median IL-8 concentration in BAL fluid was 49.01 ng/L (IQR, 7.86-94.05 ng/mL).

Ten of the recipients were men and the median age was 48.43 years (IQR, 25.4-56.81 y). The median ischemic time was 210 minutes (IQR, 176.25-228.75 min) for the first lung and 300 minutes (IQR, 273.75-333.73 min) for the second lung. The median PaO<sub>2</sub>/FiO<sub>2</sub> ratio for the implant at 6, 14, and 48 hours was 329 (IQR, 190.25-435), 363.5 (IQR, 249-434.75), and 370.5 (IQR, 243.25-418.25), respectively. The median intubation time was 39.5 hours (IQR, 19.25-68.5 h) and the correlation with IL-8 values was positive: higher IL-8 concentrations in BAL fluid correlated with longer ventilation times (Spearman rank correlation,  $P=0.007$ ;  $\rho=0.583$ ). Five patients developed primary graft failure; IL-8 concentrations were significantly higher in these patients than in those whose grafts did not fail (Mann-Whitney test,  $P=0.003$ ).

**Conclusion.** High IL-8 concentrations in donor BAL fluid lead to longer ventilation time in the recipients and favor the development of primary graft failure after lung transplant.

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### Interleucina-8 en el lavado broncoalveolar del donante y su impacto en la disfunción primaria del injerto en el trasplante bipulmonar

## RESUMEN

**Introducción y objetivo.** El propósito del estudio es conocer el papel que las concentraciones de interleucina-8 (IL-8) en el lavado broncoalveolar (BAL) del donante pulmonar desempeñan en el desarrollo de la disfunción primaria del injerto pulmonar (DPIP).

**Pacientes y método.** Se ha analizado a 20 pacientes que recibieron un trasplante bipulmonar. Se recogió una serie de datos del donante, incluida la concentración de IL-8 en el BAL. Del receptor se registraron los valo-

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res gasométricos a las 6, 24 y 48 h del implante, así como el tiempo de intubación. Los pacientes con un cociente presión arterial de oxígeno ( $\text{PaO}_2$ )/fracción inspiratoria de oxígeno ( $\text{FiO}_2$ ) inferior a 300 mmHg durante las primeras 48 h se clasificaron como pacientes que habían desarrollado DPIP. La determinación de IL-8 se realizó mediante enzimoanálisis. La posible asociación entre las variables de los donantes y las concentraciones de IL-8 se evaluó mediante la *r* de Spearman y el test de Mann-Whitney, según las variables fueran continuas o categóricas. En el análisis multivariante se utilizó la regresión logística.

**Resultados.** De los 20 donantes estudiados, 15 eran varones. En 9 la causa de la muerte cerebral fue traumática, 7 eran fumadores activos, 13 precisaron soporte inotrópico y en 18 se aislaron gérmenes en el BAL. La mediana de edad fue de 35 años (rango intercuartílico [RIC]: 23,5-51,25), el tiempo de ventilación asistida fue de un día (RIC: 1-2), el cociente  $\text{PaO}_2/\text{FiO}_2$  fue 459,5 (RIC: 427-510,25) y la concentración de IL-8 obtenida en el BAL fue de 49,01 ng/l (RIC: 7,86-94,05).

En cuanto a los receptores, 10 eran varones y la mediana de la edad fue de 48,43 años (RIC: 25,4-56,81). El tiempo de isquemia para el primer pulmón fue de 210 min (RIC: 176,25-228,75) y para el segundo, 300 min (RIC: 273,75-333,73). El cociente  $\text{PaO}_2/\text{FiO}_2$  a las 6; 24, y 48 h del implante fue de 329 (RIC: 190,25-435), 363,5 (RIC: 249-434,75) y 370,5 (RIC: 243,25-418,25), respectivamente. El tiempo de intubación fue de 39,5 h (RIC: 19,25-68,5) y su correlación con los valores de IL-8 resultó positiva, ya que, cuanto mayor era la concentración de IL-8 en el BAL, más tiempo de ventilación asistida precisó el paciente (test de Spearman,  $p = 0,007$ ;  $r = 0,583$ ). Desarrollaron DPIP 5 pacientes, en los que la concentración de IL-8 fue significativamente mayor que en quienes no presentaron dicho cuadro clínico (test de Mann-Whitney,  $p = 0,003$ ).

**Conclusión.** Las concentraciones elevadas de IL-8 en el BAL del donante condicionan un mayor tiempo de ventilación asistida en el receptor de trasplante y favorecen el desarrollo de DPIP.

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## Introduction

Primary graft failure continues to be the main cause of perioperative and early mortality in lung transplant recipients.<sup>1</sup> This acute lung injury occurs in the immediate perioperative period as a result of a series of events inherent in lung transplants. Its clinical manifestations include severe hypoxemia, and x-ray studies show alveolar infiltrates (pulmonary edema). The patient must be kept intubated and mechanically ventilated with high levels of oxygen and nitric oxide. Intubation favors lung infection, sepsis, and subsequent multiple organ failure in the transplant patient.<sup>2</sup>

Primary graft failure has been associated almost exclusively with ischemic injuries that occur during preservation of the lung and its subsequent reperfusion in the recipient. The mechanism of ischemia-reperfusion lung injury is highly complex and is not yet fully understood. It is the result of the interaction between potent inflammatory mediators and various cell types.<sup>3,4</sup> Among the mediators, the interleukins (IL), particularly IL-8, appear to play an important role in the development of primary graft failure.

Lung transplantation, however, is a complex process, suggesting that its success or failure may be conditioned by other factors, including the donor. Fisher et al<sup>5</sup> showed that high concentrations of IL-8 in the bronchoalveolar lavage (BAL) fluid from ideal lung donors correlate significantly with primary graft failure; that is, there may be subclinical inflammation (not revealed by x-ray or blood gas analysis) that conditions the subsequent course of the transplant patient.

The objective of our study was to determine IL-8 concentrations in fluid from BAL of the donor lung and to assess the role of IL-8 levels in the development of primary graft failure.

## Patients and Methods

### Patients

This prospective study enrolled 20 patients who underwent a sequential double lung transplant in La Fe University Hospital, Valencia, Spain between November 2005 and December 2006.

All the lung donors were ideal, according to generally accepted criteria.<sup>6</sup> The lungs were extracted in accordance with the protocol of La Fe University Hospital, Valencia, Spain and were preserved using a low-potassium solution at 4°C.

BAL was performed immediately before implantation by instilling 20 mL of saline solution into both main bronchi. All the

samples were processed and stored at  $-80^\circ\text{C}$  for subsequent analysis.

In the immediate postoperative period, arterial blood gas values (ratio of  $\text{PaO}_2$  to the fraction of inspired oxygen [ $\text{FiO}_2$ ]) were recorded at 6, 24, and 48 hours after implantation. Recipient intubation time in this period was also noted. Patients with a  $\text{PaO}_2/\text{FiO}_2$  ratio of less than 300 during the first 48 hours were diagnosed with primary graft failure once acute rejection, obstruction of venous anastomosis, cardiogenic pulmonary edema, and pneumonia had been ruled out.

### Determination of Interleukin 8

All the samples were processed and stored at  $-80^\circ\text{C}$  for subsequent analysis. IL-8 levels were determined by enzyme-linked immunosorbent assay (ELISA, R&D Systems, Inc, Minneapolis, Minnesota, USA).

### Study Variables

- The variables collected for the donors were sex, age, cause of death, smoking history, intubation time (days),  $\text{PaO}_2/\text{FiO}_2$  ratio, inotropic support, bacteria culture, and IL-8 concentrations in BAL fluid.
- The variables collected for the recipients were sex, age,  $\text{PaO}_2/\text{FiO}_2$  ratio at 6, 24, and 48 hours, intubation time (hours), and primary graft failure.

### Statistical Analysis

Donor and recipient characteristics were described using the usual statistics for central tendency and dispersion (median and interquartile range [IQR]). Associations between the donor variables and IL-8 concentrations were evaluated using the Spearman rank correlation coefficient ( $\rho$ ) and the Mann-Whitney test for continuous and categorical variables, respectively. Logistic regression was used for multivariate analysis. Statistical significance was established at  $P$  less than .05 for all analyses.

## Results

### Donors

Fifteen donors were men and 5 were women. The median age was 35 years (IQR, 23.5-51.25 y). The cause of brain death was

trauma in 9 cases and nontrauma in 11 cases. Seven donors were active smokers at the time of death. Ventilation time was 1 day (IQR, 1-2 d). The median PaO<sub>2</sub>/FiO<sub>2</sub> ratio was 459.5 (IQR, 427-510.25). Thirteen donors required inotropic support to maintain hemodynamic stability. Pathogens were isolated in the BAL fluid of 18 donors. The median IL-8 concentration in BAL fluid was 49.01 ng/mL (IQR, 7.86-94.05 ng/mL).

**Recipients**

Ten of the patients were men and 10 were women. The median age was 48.43 years (IQR, 25.4-56.81 y). The lung disease that made the transplant necessary was of septic origin in 8 cases and nonseptic origin in the remaining 12 cases. A sequential double lung transplant procedure was performed in all cases, with a median ischemic time of 210 minutes (IQR, 176.25-228.75 min) for the first lung and 300 minutes (IQR, 273.75-333.73 min) for the second lung. The PaO<sub>2</sub>/FiO<sub>2</sub> ratios at 6, 24, and 48 hours after transplant were 329 (IQR, 190.25-435), 363.5 (IQR, 249-434.75), and 370.5 (IQR, 243.25-418.25), respectively.

**Relationship Between Interleukin 8 Levels and the Clinical Characteristics of the Donor, Intubation Time of the Recipient, and the Development of Primary Graft Failure**

No association was found between the clinical characteristics of the donor and levels of IL-8 in the BAL fluid (Table 1).

The median recipient intubation time was 39.5 hours (IQR, 19.25-68.5 h) and the correlation with IL-8 values was positive: higher IL-8 concentrations in BAL fluid correlated with longer ventilation times (ρ=0.583, P=.007) (Figure 1).

Five patients developed primary graft failure; 2 of these died in the immediate postoperative period (Table 2). These patients had received lungs from donors in whom the IL-8 concentrations in BAL fluid were significantly higher than in donated lungs transplanted to

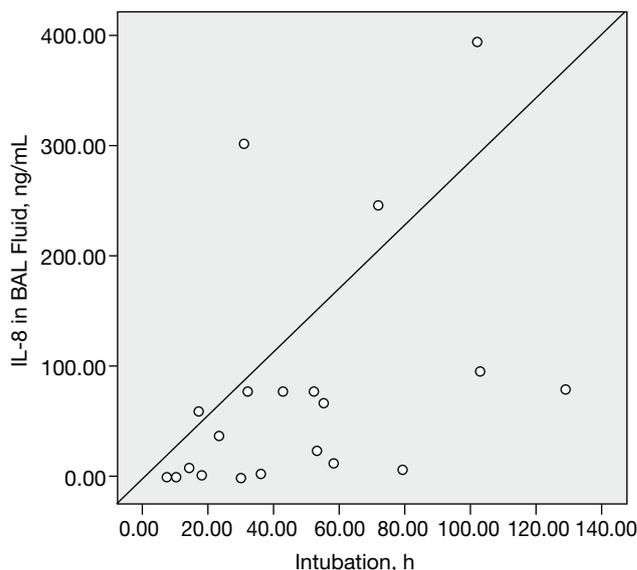
patients who did not develop primary graft failure (Mann-Whitney test, P=.003) (Figure 2).

Concentration of IL-8 in the BAL fluid was the only variable for which the correlation was maintained in multivariate analysis using a binary logistic regression model, though significance was limited (P=.07). The following regression equation was established: -5.079 + (BAL × 13.16).

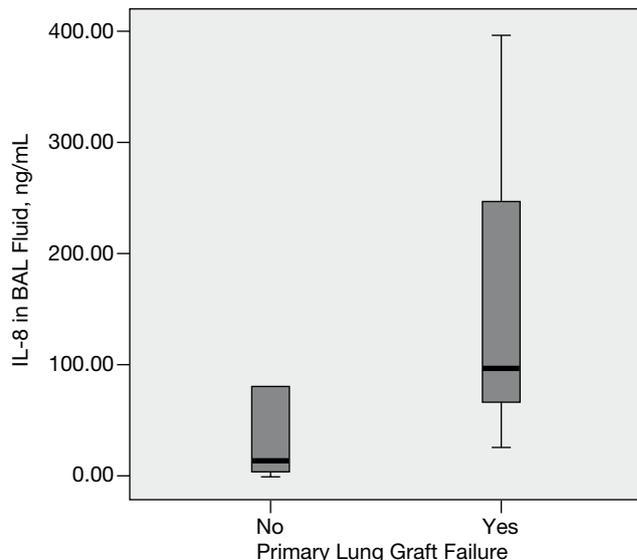
**Discussion**

This study has shown that high IL-8 concentrations in the BAL fluid from donor lungs are associated with longer ventilation time in the transplant patient and favor the development of primary graft failure after lung transplant.

Obtaining organs for transplant—particularly lungs—is very difficult. Current criteria for selecting lung donors are based on



**Figure 1.** Concentration of interleukin 8 (IL-8) in the donor bronchoalveolar lavage (BAL) fluid and recipient intubation time (Spearman rank correlation, ρ=0.538, P=.007).



**Figure 2.** Concentration of interleukin 8 (IL-8) in the donor bronchoalveolar lavage (BAL) fluid and primary graft failure (Mann-Whitney U test, P=.032).

**Table 1**  
Concentrations of Interleukin 8 in Bronchoalveolar Lavage Fluid and Clinical Characteristics of the 20 Donors

Characteristics	IL-8, Median (IQR), ng/mL	P <sup>a</sup>
<b>Categorical variables</b>		
Men (n=15)	24.48 (4-79.09)	.445
Women (n=5)	79.09 (31.58-162.74)	
Nonsmokers (n=13)	79.09 (16.42-87.54)	.211
Smokers (n=7)	9 (2.74-60.43)	
Brain death due to trauma (n=9)	24.48 (1.99-79.09)	.456
Brain death not due to trauma (n=11)	60.43 (9-96)	
Inotropic support (n=13)	37.6 (6.18-87.54)	.817
No inotropic support (n=7)	60.43 (2.7-79.09)	
Positive BAL culture (n=18)	42.45 (3.68-83.31)	.674
Negative BAL culture (n=2)	58.34 (37.6-79.09)	
<b>Continuous variables</b>		
Age		.202 <sup>c</sup>
Intubation time		.615 <sup>c</sup>
PaO <sub>2</sub> /FiO <sub>2</sub> ratio		.186 <sup>c</sup>

Abbreviations: BAL, bronchoalveolar lavage; FiO<sub>2</sub>, fraction of inspired oxygen; IQR, interquartile range.

<sup>a</sup>IL-8 levels were analyzed with the Mann-Whitney U test. Continuous variables were analyzed with the Spearman rank correlation test.

**Table 2**  
Concentrations of Interleukin 8 in Donor Bronchoalveolar Lavage Fluid and Development of Primary Graft Failure After Double Lung Transplants

Recipients (n=20)	IL-8, Median (IQR), ng/mL	P <sup>a</sup>
Primary graft failure (n=5)	96 (45.99-320.33)	.039
No primary graft failure (n=15)	12.9 (2.74-79.09)	

Abbreviation: IQR, interquartile range.

<sup>a</sup>Mann-Whitney U test.

clinical data such as age, smoking, and arterial blood gases, radiology and bronchoscopy findings, and physical examination of the lung on extraction. Most of these criteria, however, have not been rigorously evaluated and are based more on clinical impressions than on solid evidence.<sup>7</sup> Although marginal donors may provide acceptable results,<sup>6</sup> primary graft failure continues to be a serious problem even when donors are classified as ideal.<sup>8</sup>

Primary graft failure has been associated almost exclusively with ischemic injuries that occur during the preservation and subsequent reperfusion of the lung. Although the mechanism of ischemia-reperfusion lung injury is not yet fully understood, it is highly complex, the result of the interaction between potent inflammatory mediators and different cell types.<sup>3,4</sup> IL-8 is a potent proinflammatory mediator in the activation and recruitment of neutrophils at the sites where acute inflammatory processes occur and, together with other interleukins, appears to play an important role in the development of primary graft failure. High levels of proinflammatory cytokines in the immediate post-transplant period have been shown to correlate significantly with the development of primary graft failure.<sup>9-11</sup>

Lung transplantation, however, is a complex process, and it would not be surprising if its success or failure were affected by other factors, including the donor. Many studies have analyzed the impact of donor characteristics on the transplant outcome.<sup>6</sup> Few, however, have examined the biological status of the donor lung and its impact on primary graft failure.

Some authors have found a significant correlation between blood concentrations of specific cytokines and the hormone depletion that takes place after brain death.<sup>12</sup> An acute lung injury histologically characterized by neutrophilic pulmonary infiltrates, identical to that found in primary graft failure, has been reported in patients with isolated brain injury; this has led some authors to question the inflammatory state of the lung in organ donors.<sup>13</sup> Fisher et al,<sup>5</sup> after analyzing 12 lungs before extraction and 15 ischemic lungs, showed that high concentrations of IL-8 in the BAL fluid of ideal lung donors influenced the subsequent development of primary graft failure. A recent study by Kaneda et al<sup>14</sup> described a model for predicting perioperative mortality in which the IL-6/IL-10 ratio obtained by analyzing messenger RNA from donor lung biopsies affected mortality. In our study, high levels of IL-8 in BAL fluid had an impact on mechanical ventilation time. Furthermore, patients who developed primary graft failure showed significantly higher levels of IL-8.

In conclusion, our limited experience leads us to agree with Fisher et al<sup>5</sup> that certain donors classified as ideal may have subclinical lung injuries that are expressed by the elevated BAL fluid concentrations of IL-8 that develop with ischemia and reperfusion of the graft. This may partly explain why, despite the effort to achieve ideal lung

preservation,<sup>15</sup> primary graft failure continues to affect lung transplant outcomes. It is therefore necessary to develop new strategies that allow biological evaluation of the donor lung before implantation.<sup>14</sup> Also needed are treatment options that minimize the effect of the inflammatory cascade in the period immediately after transplantation.<sup>16</sup>

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