TESIS DOCTORAL

NUEVAS PERSPECTIVAS EN TRASPLANTE DE PULMÓN

Jordi Riera del Brío

Muss es sein? Es muss sein!

Ludwig van Beethoven, 1826

Milan Kundera, 1984

Índice

Agradecimientos

Todo aquello que existe lo hace en un contexto. La existencia de esta tesis doctoral es el resultado de numerosos factores. El buen científico lucha por que la incertidumbre que genera la infinitud de relaciones se vea minimizada con una categorización de estas, tratando de identificar aquéllas que han tenido más peso en la generación de lo estudiado. En este apartado destaco aquellos pilares sobre los que se ha construido este trabajo:

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Mi concepción de la labor médica es de continua evolución hacia la excelencia en tres ámbitos: la clínica, la docencia y la investigación. Pretendo que el trabajo que he realizado para dar este primer paso en este último apartado no cese y me permita seguir fluyendo en el cauce de esta bendita profesión.

Prólogo

La presente tesis doctoral por compendio de publicaciones se ha organizado estructuralmente de acuerdo con las recomendaciones recogidas en el Marco Regulador de Estudios de Doctorado RD 1393/2007 de la Universitat Autònoma de Barcelona. Según el punto II.18.2 de dicho marco, se recomienda que la tesis contenga como apartados una introducción en la que se presenten los trabajos y se justifique la unidad temática de la tesis; un resumen global de los resultados y la discusión de los mismos; unas conclusiones finales; y una copia de los trabajos ya publicados admitidos por la Comissió d'Estudis de Postgrau.

Se han añadido, además, dos artículos de revisión en relación con el tema de la tesis escritos por el doctorando y publicados durante el período de elaboración de la misma. Estos artículos completan y amplían el contenido de los dos artículos originales que conforman la tesis, por lo que se ha considerado pertinente incorporarlos como anexos a la misma.

Abreviaturas

DPI: Disfunción primaria del injerto.

ECMO: Oxigenación con membrana extracorpórea.

F_IO_2: Fracción inspiratoria de oxígeno.

PaO_2: Presión arterial de oxígeno.

ProADM: Proadrenomedulina.

I. INTRODUCCIÓN

El trasplante de pulmón es la medida terapéutica de elección en cierta población de pacientes con insuficiencia respiratoria crónica en estadío terminal. La supervivencia a corto plazo tras un trasplante pulmonar ha ido mejorando en los últimos años pero sigue siendo menor que la de otros trasplantes de órgano sólido. Esto es debido a que ciertas complicaciones pueden entorpecer la evolución clínica del receptor de trasplante de pulmón en el postoperatorio inmediato.

Una de ellas es la disfunción primaria del injerto (DPI). Se trata de la complicación más frecuente y está asociada a una elevada morbimortalidad. Fisiopatológicamente se define como la presencia de una inflamación alveolar secundaria a múltiples factores y en la que el proceso de daño por isquemia-reperfusión juega un papel principal. Sin embargo, clínicamente es una entidad con una definición poco precisa basada en la presencia de infiltrados pulmonares bilaterales en la radiografía de tórax, ausencia de evidencia de un componente hidrostático en el edema pulmonar y cierto grado de hipoxemia, siendo la disfunción primaria grado tres aquella que indica una peor oxigenación. Asimismo, se definen cuatro puntos en el tiempo en los que puede aparecer la entidad: al ingreso, a las 24 horas, a las 48 horas y a las 72 horas. Esta inespecífica definición hace que existan notables diferencias entre los trabajos que estudiaron la relación de la DPI con la mortalidad. Estudios recientes han tratado de identificar biomarcadores específicos de esta entidad que afinaran mejor su definición, optimizando así su relación con las diferentes variables de resultado. La adrenomedulina es un péptido cuyos niveles plasmáticos se encuentran elevados en determinados procesos patológicos. Su expresión se ve inducida por señales relacionadas con distintas lesiones tisulares como citoquinas proinflamatorias, productos bacterianos, la hipoxia y el estrés oxidativo. Se ha observado que la molécula tiene un efecto inmunomodulador atenuando la inflamación, evitando la apoptosis y disminuyendo la permeabilidad vascular. En estudios experimentales se ha observado que la administración de adrenomedulina atenúa el daño por isquemia-reperfusión en el infarto de miocardio y mejora la lesión pulmonar aguda inducida por lipopolisacárido. La proadrenomedulina (proADM) es un péptido cosintetizado en relación 1:1 con la adrenomedulina. La monitorización de este biomarcador ofrece ventajas ya que tiene una vida media más larga, carece de bioactividad y no se une a proteínas. El primero de los artículos es el resultado de un estudio prospectivo que tenía como objetivo identificar la relación entre la proADM, la DPI y la mortalidad precoz en receptores de trasplante de pulmón.

Otra entidad que puede complicar el postoperatorio de trasplante de pulmón es la infección respiratoria. La población de receptores de trasplante de pulmón está predispuesta a sufrir infección respiratoria debido a la pérdida de sistemas de defensa básicos durante la cirugía, un nivel de inmunosupresión elevado y el contacto directo del injerto con el ambiente. Estudios previos han mostrado resultados heterogéneos en cuanto al impacto real que tienen la neumonía y la traqueobronquitis asociadas a ventilación mecánica. En los últimos años los protocolos de inmunosupresión y de profilaxis antibiótica en el postoperatorio inmediato de trasplante de pulmón han ido optimizándose, lo que probablemente haya cambiado la repercusión que tienen estas entidades en las variables de resultado tras la cirugía. Por otro lado, dado el mayor riesgo de infección, estos pacientes reciben con frecuencia combinaciones

de antibióticos y pautas de tratamiento más largas, lo que modifica los hallazgos microbiológicos en comparación con otros pacientes. Por último, determinados factores presentes en esta población, como son la disfunción diafragmática y la paresia gástrica, podrían predisponer a la aparición de neumonía o traqueobronquitis. El segundo artículo es el resultado de un estudio retrospectivo de la repercusión real de estas entidades en el trasplantado pulmonar, de los patógenos que más frecuentemente generan la infección y del estudio de los factores de riesgo asociados a la aparición de estas dos entidades.

II. JUSTIFICACIÓN DE LA UNIDAD TEMÁTICA DE LA TESIS

Ambos trabajos de investigación tratan sobre las dos complicaciones postoperatorias del trasplante de pulmón que tienen más relevancia clínica, ofreciendo nuevo conocimiento que, además, tiene trascendencia a nivel clínico. Por un lado se ha identificado un nuevo biomarcador que podría optimizar la definición, facilitar el diagnóstico y afinar en el pronóstico de la DPI. Por otro lado, se han identificado factores de riesgo importantes para la aparición de neumonía y traqueobronquitis asociadas a ventilación mecánica, así como los patógenos que más frecuentemente las originan, lo que tiene repercusión en cuanto a su prevención y su tratamiento. Por último, los trabajos de revisión adjuntados como anexos tratan dos aspectos novedosos relacionados con el trasplante de pulmón. El primero es una puesta al día en DPI y su relación con factores de riesgo, biomarcadores y medidas terapéuticas; y el segundo supone una actualización sobre el uso de la oxigenación con membrana extracorpórea (ECMO) en el perioperatorio del trasplante de pulmón.

III. HIPÓTESIS, OBJETIVOS Y MÉTODOS

Estudio 1. Papel de la proADM en la DPI en los pacientes receptores de trasplante de pulmón.

Hipótesis: Los niveles plasmáticos de proADM medidos en el postoperatorio de trasplante de pulmón están en relación con los diferentes grados de DPI y con la mortalidad precoz.

Objetivos:

1. Principal: Identificar la relación entre los niveles plasmáticos de proADM medidos en el primer, segundo y tercer día del postoperatorio con los distintos grados de disfunción primaria y la capacidad de oxigenación del injerto, medida los mismos días, mediante el índice presión arterial de oxígeno/fracción inspiratoria de oxígeno (PaO_2/F_IO_2).
2. Secundario: Evaluar la asociación entre estos niveles de proADM y la mortalidad en la unidad de cuidados intensivos.

Métodos: Se diseñó un estudio prospectivo donde se recogieron los datos clínicos de todos los receptores de trasplante de pulmón ingresados en la unidad de cuidados intensivos en el postoperatorio inmediato entre Septiembre de 2011 y Mayo de 2013. Se empleó la definición de la *International Society for Heart and Lung Transplantation* (ISHLT) para DPI [1]. La radiografía de tórax fue evaluada por dos investigadores diferentes. Se recogieron muestras de sangre a las 24, 48 y 72 horas de la cirugía en tubos EDTA. Estos tubos fueron inmediatamente centrifugados, extrayendo el plasma que fue congelado y posteriormente analizado. La medición de niveles de proADM se realizó mediante inmunofluorescencia. Se empleó el coeficiente de correlación de Spearman para identificar la relación entre los niveles de proADM y el índice PaO_2/F_IO_2. Se estudió la relación entre los niveles de proADM y la DPI empleando regresión logística. En el análisis multivariado se incluyeron variables que podrían suponer factores de confusión en el estudio de esta relación. Estas variables fueron la edad y el sexo del donante y del receptor, el tipo de trasplante, el diagnóstico de base del receptor, el uso de circulación extracorpórea en la cirugía, el tiempo de isquemia de el/los injerto/s y la presencia de transfusión. Se empleó el área bajo la curva ROC para comparar la precisión pronóstica de mortalidad en la unidad de cuidados intensivos de los niveles de proADM y del grado tres de DPI a las 72 horas. Se realizó una validación interna de los resultados mediante *bootstrap resampling*.

Estudio 2. Infecciones respiratorias asociadas a ventilación mecánica tras trasplante de pulmón.

Hipótesis: La infección respiratoria asociada a ventilación mecánica que acontece en el postoperatorio inmediato de trasplante de pulmón está asociada a peores variables de resultado.

Objetivos:

1. Principal: Evaluar el efecto de la neumonía y la traqueobronquitis asociadas a ventilación mecánica en el tiempo de estancia en la unidad de cuidados intensivos y en el hospital y con la mortalidad en la unidad de cuidados intensivos.
2. Secundario: Identificar los gérmenes causantes de dichas infecciones y los factores de riesgo de aparición de las mismas.

Métodos: Se diseñó un estudio retrospectivo donde se recogieron los datos clínicos de todos los receptores de trasplante de pulmón ingresados en la unidad de cuidados intensivos en el postoperatorio inmediato entre Enero de 2010 y Diciembre de 2012. Se siguieron las recomendaciones de la *Infectious Diseases Society of America* para la definición de neumonía asociada a ventilación mecánica. Para el diagnóstico de traqueobronquitis asociada a ventilación mecánica se emplearon los criterios propuestos en publicaciones previas [2]. Así se hizo también para las definiciones de gérmenes multirresistentes [3,4]. Siguiendo la línea de anteriores publicaciones, determinados hallazgos ecocardiográficos definieron la presencia de paresia frénica [5]. Para la definición de paresia gástrica se utilizaron criterios clínicos y radiográficos empleados en trabajos previos [6]. Para el estudio de los factores de riesgo para el desarrollo de neumonía asociada a ventilación mecánica se empleó un análisis multivariado. Teniendo en cuenta los resultados del análisis univariado, se incluyeron las siguientes variables: paresia gástrica, paresia frénica, colonización previa del donante y colonización previa del receptor.

IV. RESUMEN GLOBAL DE LOS RESULTADOS

Estudio 1. Papel de la proADM en la DPI en los pacientes receptores de trasplante de pulmón.

- Se incluyeron en el estudio 100 pacientes.
- El único grado de DPI relacionado con una mayor mortalidad en la unidad de cuidados intensivos fue el grado tres a las 72 horas.
- Los niveles plasmáticos de proADM estaban relacionados con el índice de oxigenación PaO_2/F_IO_2 en los tres cortes de tiempo.
- Los niveles plasmáticos de proADM medidos a las 24 y a las 72 horas fueron significativamente más elevados en los pacientes que sufrieron DPI grado tres a las 72 horas en comparación con el resto de la población. Las dos asociaciones se mantuvieron estadísticamente significativas al introducir en el análisis factores de riesgo de aparición de DPI.
- Los niveles plasmáticos de proADM medidos a las 48 y a las 72 horas fueron significativamente más elevados en los pacientes que fallecieron en la unidad de cuidados intensivos.
- El área debajo de la curva ROC de la disfunción primaria grado tres a las 72 horas para predecir la mortalidad en la unidad de cuidados intensivos fue optimizada añadiendo al modelo la de los niveles de proADM medidos a las 72 horas.

Estudio 2. Infecciones respiratorias asociadas a ventilación mecánica tras trasplante de pulmón.

- Se incluyeron en el estudio 170 pacientes.
- Los pacientes que sufrieron infección respiratoria asociada a ventilación mecánica estuvieron más tiempo con ventilación mecánica y más tiempo en la unidad de cuidados intensivos y en el hospital.
- La mortalidad fue más elevada en los pacientes que sufrieron neumonía asociada a ventilación mecánica, no así en los pacientes con traqueobronquitis asociada a ventilación mecánica.
- *Pseudomonas aeruginosa* fue el germen que más frecuentemente causó infección asociada a ventilación mecánica. En el caso de la neumonía, 8/12 fueron *Pseudomonas aeruginosa* multirresistente.
- Se encontró que la paresia gástrica fue un factor de riesgo independiente para el desarrollo de neumonía asociada a ventilación mecánica, asociación que se mantuvo al ajustar el análisis por los días de ventilación mecánica. Asimismo, se evidenció que la paresia gástrica y la paresia frénica fueron factores de riesgo para el desarrollo de traqueobronquitis asociada a ventilación mecánica.

V. **Estudio 1**

Primary graft dysfunction and mortality following lung transplantation: A role for proadrenomedullin plasma levels

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Primary Graft Dysfunction and Mortality Following Lung Transplantation: A Role for Proadrenomedullin Plasma Levels

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Primary graft dysfunction (PGD) after lung transplantation (LT) is a heterogeneous syndrome that comprises clinical presentations with diverse grades of severity. Proadrenomedullin (proADM) levels may be associated with PGD and may enhance its relationship with outcomes. We prospectively included 100 LT recipients. Plasma levels of proADM were measured at 24, 48 and 72 h after admission to the intensive care unit (ICU). We assessed their relationship with PGD grade and ICU mortality. Fifty patients (50%) presented grade 3 PGD at ICU admission. Twenty-two patients (22%) developed grade 3 PGD at 72 h, the only grade associated with higher mortality (odds ratio 6.84, 95% confidence interval [CI] 1.47–38.44). ProADM levels measured at 24 h (3.25 vs. 1.61 nmol/L; p = 0.016) and 72 h (2.17 vs. 1.35 nmol/L; p = 0.011) were higher in these patients than the rest of the population. When we added the individual predictive utility of grade 3 PGD at 72 h for ICU mortality (area under the curve [AUC] 0.72, 95% CI 0.53–0.90) to that of ProADM at 72 h, the predictive value of the model improved (AUC 0.81, 95% CI 0.65–0.97). Higher levels of proADM measured following LT are associated with grade 3 PGD at 72 h. ProADM enhances the association of this entity with mortality.

Abbreviations: ADM, adrenomedullin; APACHE, Acute Physiology and Chronic Health Evaluation; AUC, area under the curve; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; F_IO_2, fraction of inspired oxygen; G, grade; ICU, intensive care unit; LT, lung transplantation; OR, odds ratio; PaO_2, partial pressure of arterial oxygen; PGD, primary graft dysfunction; proADM, proadrenomedullin; ROC, receiver operating characteristic; T0, time of ICU admission; T24, 24 h from ICU admission; T48, 48 h from ICU admission

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Introduction

Primary graft dysfunction (PGD) is a form of inflammatory lung edema that impairs early graft function within the first 72 h after lung transplantation (LT) (1). Its complex pathogenic mechanisms revolve around the ischemia–reperfusion injury (2), which produces epithelial and endothelial damage secondarily to the release of inflammatory mediators. It is the most common complication after LT, although its reported frequency varies between 11% and 57% according to current literature. This variability is due to the rather nonspecific clinical definition of the condition, based on the presence of hypoxemia and lung infiltrates on radiographic images at four time points after surgery (3). Recent investigations have aimed to identify biomarkers that may enhance the relationship of the different grades of PGD and the outcomes, mainly, early mortality and graft function (4,5). Adrenomedullin (ADM) is a 52–amino acid peptide with immune-modulating, metabolic and vasodilator activity. Its expression is inducible by shear stress, oxidative stress and hypoxia (6,7). It also reduces endothelial permeability (8), protects against apoptosis (9) and attenuates organ injury in sepsis models (10). Moreover, an infusion of exogenous ADM significantly attenuates myocardial ischemia–reperfusion injury (11) and improves lipopolysaccharide-induced acute lung injury in rats (12). ADM may have a role in the development of early graft failure in LT recipients, but this possibility has not been investigated to date. Midregional proadrenomedullin (proADM) is cosynthesized with ADM; it offers the advantage of a longer half-life, lack of bioactivity and lack of protein binding and thus is more suitable for evaluation.

The hypothesis of the present study was that the proADM plasma levels measured following LT may be related to early graft function and mortality. The primary objective was

to establish the relationship between proADM plasma levels measured at days 1, 2, and 3 after LT with early oxygenation and with the various grades of PGD. The secondary objective was to evaluate the association of this biomarker with early mortality.

Materials and Methods

The study was approved by the ethics committee of the Vall d'Hebron University Hospital (reference code PR_AG_279-2013). Informed consent was obtained from the relatives of each patient enrolled in the cohort.

Study population and end points

Adult patients consecutively admitted to the intensive care unit (ICU) of the Vall d'Hebron University Hospital after LT between September 2011 and May 2013 were prospectively included in the study. Donor selection, graft procurement, surgical technique and postoperative management all proceeded in accordance with our standard transplant protocol, which has been described elsewhere (13). All clinical data were collected prospectively by the investigators. Plasma samples were obtained 24, 48 and 72 h after ICU admission. The primary end points were early allograft function and various grades of PGD. The secondary end point was mortality in the ICU. The follow-up period ended at ICU discharge.

Determination of PGD grade

The PGD grade was determined using the International Society for Heart and Lung Transplantation consensus definition (1). Chest x-ray images and arterial blood gases were assessed at the time of ICU admission and at 24, 48 and 72 h after transplantation. Two different physicians examined chest x-ray images to assess the presence of diffuse infiltrates in the transplanted lungs. The severity of PGD was graded according to the ratio of partial pressure of arterial oxygen (PaO_2) to fraction of inspired oxygen (F_iO_2). Grade 3 PGD was defined as a PaO_2/F_iO_2 ratio of <200 mmHg.

Measurement of plasma biomarkers

Blood samples were collected in sterile ethylenediaminetetraacetic acid tubes (Vacutainer; Becton Dickinson, Cockeysville, MD). Tubes were immediately centrifuged at 915,642 g for 10 min at 4 °C, and serum was kept refrigerated at −86 °C until assayed. ProADM was measured in serum using an immune time-resolved amplified cryptate emission technology assay (MR-proADM Kryptor; Brahms GmbH, Hennigsdorf, Germany) with a functional assay sensitivity of 0.12 nmol/L. This assay is based on a monoclonal antibody against Katacalcin. Antibodies bind to the Katacalcin sequence of precursor molecules.

Statistical analysis

Continuous variables were expressed as mean and standard deviation or median and interquartile range. Categorical variables were expressed as frequencies and percentages. Continuous variables were compared with the Student t-test or the Wilcoxon rank sum test, as appropriate. Differences between categorical variables were assessed with the chi-square test or Fisher's exact test, as appropriate. A two-sided $p < 0.05$ was considered statistically significant. For the association between PGD and the PaO_2/F_iO_2 ratio, Spearman's rank correlation coefficient was used. The relationship of proADM levels with PGD was evaluated using logistic regression. We assessed donor and recipient age and sex, type of LT, pretransplant diagnosis, cardiopulmonary bypass, total ischemic time and transfusion as potential confounders. The inclusion of these variables in the multivariate analysis was based on previous literature, avoiding overfitting (i.e. limiting the inclusion of one variable for every 5–10 cases) and taking into account the exploratory results of the univariate analysis. We computed the predicted probability of the proADM levels for ICU mortality by fitting logistic regression models; we then created a receiver operating characteristic (ROC) curve and calculated the area under the curve (AUC) and corresponding 95% bootstrap confidence interval (CI). We also included grade 3 PGD within 72 h as a categorical variable in the model. After logistic regression was fitted, ROC curves were drawn and compared for grade 3 PGD at 72 h and the combination of grade 3 PGD at 72 h and proADM at 72 h (14). Internal validation was performed by bootstrap resampling (1000 samples). All analyses were performed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population

Overall, 100 consecutive LTs were assessed. Patient characteristics are summarized in Table 1. Grade 3 PGD was present in 50% of the population at ICU admission. At 72 h, PGD was identified in 22% of patients. Grade 3 PGD at 72 h was the only grade associated with ICU mortality, with an odds ratio of 6.84 (95% CI 1.47–38.44); it had an AUC of 0.72 (95% CI 0.53–0.90) for predicting ICU mortality (Figure 1).

Early graft function

A statistically significant association between proADM levels and the PaO_2/F_iO_2 ratio at 24, 48 and 72 h was found, with p values of 0.004 (24 h), 0.003 (48 h) and 0.001 (72 h) (Figure 2).

Primary graft dysfunction

Plasma levels of proADM measured at 72 h were higher in patients with grade 3 PGD at 72 h than in patients with less severe grades (2.17 vs. 1.35 nmol/L; $p = 0.011$) (Figure 3). Patients in whom grade 3 PGD was present at 72 h had higher levels of proADM measured at 24 h than the rest of the population (3.25 vs. 1.61 nmol/L; $p = 0.016$) (Figure 4). This association remained significant when common risk factors for PGD were controlled individually, except for recipient age and total ischemic time (Table 2). The variation of proADM levels from 24 to 72 h (ΔproADM) was calculated, but no differences in this parameter were found between the two groups (−0.52 vs. −0.35 nmol/L; $p = 0.7$).

Mortality

Plasma levels of proADM measured at 48 and 72 h were significantly higher in the patients who died in the ICU (3 vs. 1.7 nmol/L [$p = 0.04$] and 2.2 vs. 1.4 nmol/L [$p = 0.02$], respectively). In contrast, no differences were found when we compared the value of ΔproADM (0.54 vs. −0.49 nmol/L; $p = 0.27$).

ProADM measured at 72 h had an AUC of 0.76 (95% CI 0.62–0.90) for predicting ICU mortality. Adding individual predictive utility for ICU mortality of grade 3 PGD at 72 h to

Table 1: Donor and recipient characteristics stratified by primary graft dysfunction status at 72 hours

Variables	All (n = 100)	No PGD (n = 77)*	PGD (n = 22)*	p value
Recipient age, years	55 (48–60)	55 (50–61)	52 (46–57)	0.080
Recipient sex, female, %	41	38	50	0.427
Donor age, years	53 (45–62)	53 (43–62)	52 (48–62)	0.708
Donor sex, female, %	44	47	36	0.534
Transplant type, bilateral, %	51	51	54	0.898
Recipient diagnosis, %				0.533
COPD	37	39	29	
Pulmonary fibrosis	54	53	57	
Cystic fibrosis	4	4	5	
Pulmonary arterial hypertension	5	4	9	
APACHE II score	19 (16–23)	19 (16–23)	19 (17–24)	0.621
Urgent lung transplant, %	9	6	18	0.108
CPB, %	26	19	45	0.028
Time on bypass, min	128 (108–180)	150 (115–175)	117 (90–180)	0.455
Ischemic time, min	302 (260–365)	320 (265–365)	325 (285–410)	0.470
Packed red blood cell transfusion, %	54	49	73	0.089
Platelet transfusion, %	9	10	4	0.679
Fresh frozen plasma transfusion, %	17	18	14	0.756
Mechanical ventilation days	15 (3–32)	6 (2–30)	32 (18–64)	<0.001
Diaphragmatic dysfunction, %	37	33	50	0.225
T0 G3 PGD, %	50	39	86	<0.001
T24 G3 PGD, %	33	18	82	<0.001
T48 G3 PGD, %	25	12	73	<0.001
ICU days	21 (7–38)	11 (6–37)	37 (26–70)	<0.001
ICU mortality, %	9	4	23	0.013

Continuous variables are expressed as median (interquartile range), and categorical variables are expressed as percentages. Wilcoxon rank sum and chi-square tests were used for comparisons of continuous and categorical variables, respectively. APACHE, Acute Physiology and Chronic Health Evaluation; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; G, grade; ICU, intensive care unit; PGD, primary graft dysfunction; T0, time of ICU admission; T24, 24 hours from ICU admission; T48, 48 hours from ICU admission.
*The sum is not 100 because one patient died before 72 hours.

that of proADM measured at 72 h improved the predictive value of the model (AUC 0.81, 95% CI 0.65–0.97) (Figure 1), although the difference was not statistically significant ($p = 0.08$).

Exploring the best predictive utility of proADM for ICU mortality in patients with grade 3 PGD at 72 h, we found that a value at 72 h >1.72 nmol/L was associated with ICU mortality, with sensitivity of 75% and specificity of 90%. Furthermore, survivors whose levels of proADM measured at 72 h were >1.72 nmol/L spent more time on the ventilator (32.5 vs. 3 days; $p < 0.001$) and in the ICU (38 vs. 8 days; $p < 0.001$) compared with patients with lower levels.

Discussion

This study provides the first report of a robust association between plasma levels of proADM and early graft function and mortality in LT recipients. Higher levels of this biomarker measured at 24 h were associated with grade 3 PGD at 72 h, which has been related to higher ICU mortality. Furthermore, proADM measured at 72 h had considerable individual discriminatory ability for ICU mortality, and its addition improved the predictive utility of grade 3 PGD at 72 h.

Measuring proADM following LT may help clinicians evaluate the severity of the complex insult manifested as PGD and its consequences for mortality. The definition of PGD has major limitations due to its low specificity for predicting outcomes. The only grade that has been shown to be associated with early mortality is grade 3 occurring at late time points, especially at 72 h (5). Interestingly, proADM at 72 h had better predictive utility for ICU mortality than grade 3 PGD at 72 h, and predictive utility was considerably increased by the combined use of the two items, although the difference did not reach statistical significance. Furthermore, we found that time on the ventilator and time in the ICU were longer in LT recipients with high levels of proADM measured at 72 h. Substantial evidence already exists regarding the value of proADM as a mortality predictor in patients with sepsis (15), in patients presenting at the emergency department with dyspnea (16), in patients with community-acquired pneumonia (17) and in those with chronic obstructive pulmonary disease (18). Shah *et al* demonstrated that the addition of biomarkers of epithelial injury and cell adhesion to clinical PGD grade added significant predictive value compared with PGD grade alone for 90-day mortality, with an AUC of 0.76 (95% CI 0.65–0.87) (4). It may well be worth measuring all of these biomarkers, at least in those LT recipients who suffer severe graft dysfunction during the

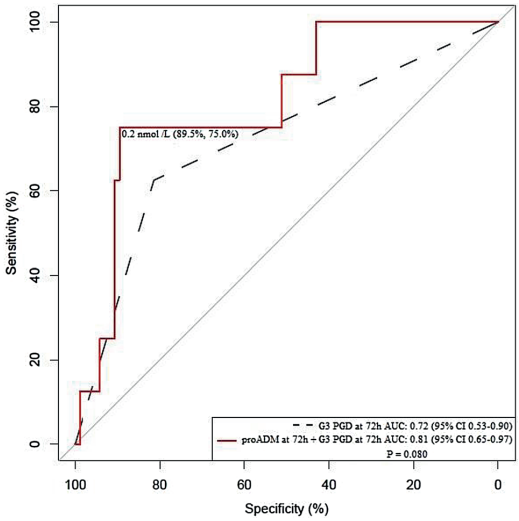

Figure 1: Two receiver operating characteristic curves are shown. The first expresses the predictive utility of grade 3 Primary Graft Dysfunction at 72 h for mortality in the intensive care unit (dashed line), with an area under the curve of 0.72. The second represents the predictive utility of the addition of proadrenomedullin measured at 72 h to that of Primary Graft Dysfunction at 72 h (solid line), with an area under the curve of 0.81. The difference between the two values was substantial, though not statistically significant ($p = 0.080$). To test this difference a chi square test was used. For a value of proadrenomedullin of 0.2 nmol/L in patients with grade 3 Primary Graft Dysfunction at 72 h, the sensitivity and specificity estimates were 75.0% and 89.5%, respectively. AUC, area under the curve; G, grade; h, hours; PGD, primary graft dysfunction; proADM, proadrenomedullin.

first 3 days after surgery, to optimize the predictive utility of the clinical PGD grade for early mortality.

A considerable number of LT recipients have severe grades of PGD at ICU admission following surgery (19,20). These patients may need high levels of respiratory support during the first hours of the postoperative period; however, not all patients will suffer the condition at 72 h (1,21). Measuring proADM at 24 h may help identify patients who will have the condition at 72 h. This finding has implications for the management of these patients. LT recipients who suffer severe hypoxemia in the first hours after surgery and who have higher levels of this biomarker may benefit from specific changes in their management to minimize graft inflammation, such as more aggressive negative fluid balance, higher doses of corticosteroids or ultra–lung-protective ventilation connecting the patient to an extracorporeal membrane oxygenation system. At present, however, these are hypotheses that must be confirmed in further studies.

The secretion of ADM, the precursor of proADM, has been shown to be stimulated by hypoxia. This may explain the relationship found between proADM levels and early graft function. Furthermore, this lung-produced molecule has the property of decreasing endothelial permeability and attenuating ischemia–reperfusion injury. One might speculate that LT recipients who suffer a higher inflammatory insult before, during and after surgery may secrete higher levels of proADM during the first postoperative days to limit the magnitude of the inflammatory response and tissue damage. Similarly, a previous study showed that the plasma levels of the anti-inflammatory cytokine IL-10 were increased in the first postoperative hours in LT recipients with severe grades of reperfusion edema (22). The higher the graft inflammation, the higher the proADM levels. This may explain the association of proADM plasma levels with early mortality; early severe inflammation of the graft has been associated with this fatal outcome.

The stimulation of proADM by inflammatory processes and its relationship with ischemia–reperfusion injury may be the reason for its increased levels at 24 h in patients suffering grade 3 PGD at 72 h, whose poor clinical evolution may be due to a real inflammatory insult. Other patients labeled as suffering severe grades of PGD during the first 2 days may have other causes of hypoxemia such as cardiogenic

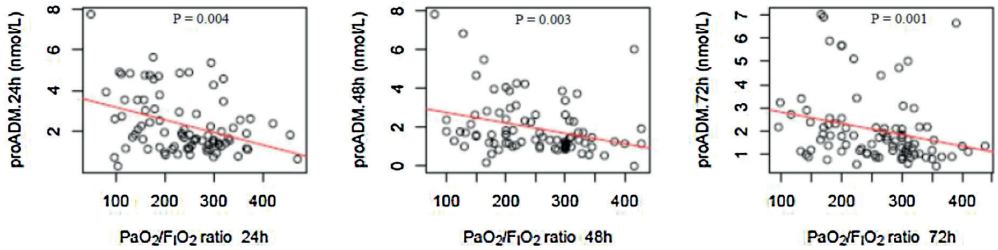

Figure 2: Association between proADM plasma levels and the PaO_2/F_iO_2 ratio at 24, 48, and 72 hours. All patients are represented by a circle. The p value reported is from Spearman's rank correlation coefficient. F_iO_2, fraction of inspired oxygen; h, hours; PaO_2, partial pressure of arterial oxygen; proADM, proadrenomedullin.

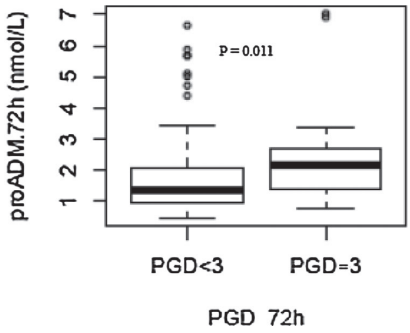

Figure 3: Comparison of the levels of proADM measured at 72 hours in patients with and without grade 3 PGD at 72 hours. The horizontal line indicates the median concentration. The upper and lower limits of the box indicate the interquartile range. Circles represent outliers. The p value reported is from the Wilcoxon rank sum test. h, hours; PGD, primary graft dysfunction; proADM, proadrenomedullin.

edema, atelectasis, or other conditions associated with a ventilation/perfusion mismatch.

Our study has certain limitations that should be noted. The investigation was performed at a single center with a specific immunosuppressive protocol, which may have interfered with the kinetics of the inflammatory biomarkers. The results should be validated in a well-designed multicenter study. Furthermore, we did not measure the proADM plasma levels before surgery, so ΔproADM (variation from these levels to those at 72 h) could not be calculated. This parameter would have added more important information; however, the evidence found with absolute values was sufficient.

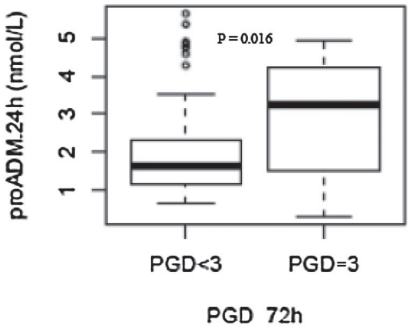

Figure 4: Comparison of the levels of proADM measured at 24 hours in patients with and without grade 3 PGD at 72 hours. The horizontal line indicates the median concentration. The upper and lower limits of the box indicate the interquartile range. Circles represent outliers. The p value reported is from the Wilcoxon rank sum test. h, hours; PGD, primary graft dysfunction; proADM, proadrenomedullin.

Table 2: Odds ratios of the association between grade 3 primary graft dysfunction (PGD) at 72 hours and proadrenomedullin levels measured at 24 hours, adjusted in logistic regression models for common risks factors for PGD

Model	OR (95% CI)	p value
Unadjusted base model	1.63 (1.14–2.36)	0.010
Adjusted by		
Lung disease	1.79 (1.20–2.78)	0.010
CPB	1.51 (1.03–2.22)	0.030
Transplant type	1.87 (1.24–2.92)	<0.001
Recipient age	1.27 (0.77–2.14)	0.350
Recipient sex	1.62 (1.14–2.35)	0.010
Donor age	2.20 (1.15–4.90)	0.030
Donor sex	1.65 (1.15–2.41)	0.010
Total ischemic time	2.07 (1.01–4.98)	0.070
Packed red blood cell transfusion	1.59 (1.11–2.33)	0.010
Platelet transfusion	1.62 (1.13–2.36)	0.010
Fresh frozen plasma transfusion	1.63 (1.14–2.39)	0.010

CI, confidence interval; CPB, cardiopulmonary bypass; OR, odds ratio.

In conclusion, higher levels of proADM measured following LT are associated with the most severe grade of PGD and with early mortality. Our findings may have implications for prognosis in LT recipients and may be relevant to future research into PGD.

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Drs. Rello and Roman have served as consultants and speakers for Astellas. Dr. Rello has received grants and participated in the speakers bureau for Thermo Fisher. The other authors have no conflicts of interest to disclose.

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VI. **Estudio 2**

Ventilator-associated respiratory infection following lung transplantation

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Ventilator-associated respiratory infection following lung transplantation

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ABSTRACT The medical records of 170 adult patients who underwent lung transplantation between January 2010 and December 2012 were reviewed to assess the incidence, causative organisms, risk factors and outcomes of post-operative pneumonia and tracheobronchitis.

20 (12%) patients suffered 24 episodes of ventilator-associated pneumonia. The condition was associated with mean increases of 43 days in mechanical ventilation and of 35 days in hospital stay, and significantly higher hospital mortality (OR 9.0, 95% CI 3.2–25.1). *Pseudomonas aeruginosa* (eight out of 12 patients were multidrug-resistant) was the most common pathogen, followed by *Enterobacteriaceae* (one out of five patients produced extended-spectrum β-lactamases). Gastroparesis occurred in 55 (32%) patients and was significantly associated with pneumonia (OR 6.2, 95% CI 2.2–17.2). Ventilator-associated tracheobronchitis was associated with a mean increase of 28 days in mechanical ventilation and 30.5 days in hospital stay, but was not associated with higher mortality (OR 1.2, 95% CI 0.4–3.2). *Pseudomonas aeruginosa* (six out of 16 patients were multidrug resistant) was the most common pathogen, followed by *Enterobacteriaceae* (three out of 14 patients produced extended-spectrum β-lactamase). Patients with gastroparesis also had more episodes of ventilator-associated tracheobronchitis (40% *versus* 12%, p<0.001).

In conclusion, ventilator-associated pneumonia following lung transplantation increased mortality. Preventing gastroparesis probably decreases the risk of pneumonia and tracheobronchitis. Multidrug-resistant bacteria frequently cause post-lung-transplantation pneumonia and tracheobronchitis.

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VAP following lung transplantation increases mortality and gastroparesis increases the incidence of VAP http://ow.ly/CeRgp

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Conflict of interest: Disclosures can be found alongside the online version of this article at erj.ersjournals.com

Introduction

Lung transplantation has become a standard of care for selected patients with end-stage non-malignant disabling lung disease [1]. Post-lung-transplant survival has improved markedly in recent years, but due to a variety of complications mortality remains higher than in other solid organ transplants, such as heart, kidney or liver. Infection is among the most common complications and is associated with high mortality rates [2]. Certain specific circumstances make the lung graft highly vulnerable to respiratory infection [3]. Donor conditions, such as decreased consciousness and mechanical ventilation, increase the risk of bacterial colonisation and infection, which may be subclinical. In addition, recipients are often colonised prior to transplant by pathogens that may be reactivated after surgery. Contact of the graft with the environment, inadequate lymphatic drainage and the effects of nerve damage during transplantation on airway clearance all increase the risk of lung infection. Post-operative gastroparesis can delay gastric emptying and thereby increase the risk of gastro-oesophageal reflux, aspiration and respiratory infection [4, 5]. In addition, ventilator-associated tracheobronchitis (VAT) and ventilator-associated pneumonia (VAP) may complicate the post-operative course and may have severe consequences in this highly immunosuppressed population. In the past few years, protocols for immunosuppression and antibiotic prophylaxis have been developed [6] and antimicrobial resistance has increased [7], and all this may have changed the impact of respiratory infections on the outcomes of lung transplant recipients. There are also inconsistencies in the definitions of pneumonia and tracheobronchitis, which may partly explain the conflicting results between studies of the impact of post-lung-transplant respiratory infection [3, 8–10].

Our hypothesis for the present study was that post-lung-transplant pneumonia and tracheobronchitis are associated with worse outcomes. The primary objective was to assess their impact on intensive care unit (ICU) and hospital length of stay, and on survival. Secondary objectives were to identify their causative organisms and their potential risk factors.

Methods

The study took place in the ICU of the Vall d'Hebron University Hospital (Barcelona, Spain). It is a mixed medical and surgical unit with 32 beds in a tertiary hospital with 1100 beds. The study was approved by the ethics committee of the hospital (study PR_AG_279-2013). We retrospectively reviewed the medical records of consecutive adult patients who were admitted to the ICU after lung transplantation between January 2010 and December 2012. We assessed hospital stay, survival, pathogens and risk factors. The follow-up period ended 1 year after ICU admission. The ethics committee considered that informed consent of the patients was not necessary due to the retrospective and non-interventional nature of the study.

Lung transplant preparation, procedure and post-operative care

Lungs were obtained *via en bloc* extraction and immediately immersed in a solution for rapid cooling, transport and storage (Perfadex; Vitrolife, Gothenburg, Sweden). Single lung transplants were performed *via* anterior thoracotomy and double lung transplants *via* bilateral anterior thoracotomy with transverse sternotomy (clamshell approach). Prior to lung reperfusion, 500 mg of intravenous methylprednisolone was administered. Extracorporeal circulation was only used if respiratory or haemodynamic instability might impede the lung transplant procedure.

Antibiotic prophylaxis comprised of ceftazidime and amoxicillin-clavulanate for 5 days. Microorganism-based surveillance of patients on the waiting list was undertaken before surgery. If any microorganism was isolated prior to surgery, appropriate post-operative antibiotic therapy was selected. Nebulised amphotericin and *i.v.* ganciclovir were given as prophylaxis against *Aspergillus* species and *Cytomegalovirus*, respectively. *Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole was started on post-operative day 30. The general VAP prophylaxis consisted of head-of-bed elevation, and selective oropharyngeal and digestive-tract decontamination with tobramycin and colistin. Subglottic drainage tubes were not used.

During the first few days following lung transplantation, patients were monitored *via* a Swan-Ganz heart catheter. Negative fluid balance was carefully maintained to avoid lung oedema and maintain adequate renal perfusion. Lung protective mechanical ventilation with low tidal volumes (<7 $mL \cdot kg^{-1}$ of ideal body weight) and airway pressures (plateau pressure <30 cmH_2O) was scheduled with low doses of sedatives (target Ramsay sedation scale of 2) and early extubation if the patient was stable. A 30-min T-tube test was always performed before extubation. If the first test failed, sedation was resumed and a daily 30 min T-tube trial was started. When the patient failed the test repeatedly or had to be re-intubated, early tracheostomy was performed. If diaphragmatic paresis was suspected, thoracic echography was performed to evaluate the diaphragm movement. Physiotherapy and early mobilisation were started as soon as the patient was stabilised. If refractory hypoxaemia developed, the patient was placed in the prone position to optimise oxygenation. If pulmonary hypertension developed, nitric oxide (5–20 ppm) was added to the

inhaled gas. No extracorporeal support was used in these cases. Enteral feeding was usually started on the second post-operative day. If gastroparesis was diagnosed, the patient received parenteral feeding and gastric pro-kinetic drugs. Routine pre-operative evaluation of gastroparesis was not performed.

No induction immunosuppression was used. Tacrolimus (serum target range 10–15 $ng \cdot mL^{-1}$) and methylprednisolone (1 $mg \cdot kg^{-1} \cdot day^{-1}$ for the first 5 days followed by 0.3 $mg \cdot kg^{-1} \cdot day^{-1}$) were used as standard immediate post-operative immunosuppressant. On post-operative day five mycophenolate (1–2 g per day) was started. If acute cellular rejection was diagnosed, a 3-day course of high-dose *i.v.* methylprednisolone (5–10 $mg \cdot kg^{-1} \cdot day^{-1}$) was administered. If antibody-mediated rejection was diagnosed, treatment with plasmapheresis, *i.v.* immunoglobulin and rituximab was started.

Data collection and definitions

Data were compiled from the hospital's patient data management system.

Pneumonia was defined as new or progressive radiographic opacity, $\geq 10^4$ $CFU \cdot mL^{-1}$ in bronchoalveolar lavage (BAL) fluid and at least two of the following: fever >38°C, leukocytosis (≥15 000 $cells \cdot mm^{-3}$) or leukopenia (<4000 $cells \cdot mm^{-3}$) and purulent secretions. Late-onset pneumonia was defined when it occurred after >4 days of admission [11]. When associated with mechanical ventilation, it was defined as VAP. When VAP was suspected, a fibrobronchoscopy was performed and BAL was obtained. Galactomannan in the BAL was always measured and *Aspergillus* was investigated using cultures and PCR. No routine surveillance for sampling patients was implemented.

Tracheobronchitis was defined as fever >38°C, new or increased sputum production, $\geq 10^6$ $CFU \cdot mL^{-1}$ in sputum suctioned from the endotracheal tube, and no new pulmonary opacities on chest radiographs [12]. When associated with mechanical ventilation, it was defined as VAT.

Multidrug-resistant *Pseudomonas aeruginosa* was defined as resistance to at least three of the following antibiotics classes: antipseudomonal penicillins, antipseudomonal oxyimino-β-lactams, fluoroquinolones, aminoglycosides and carbapenems [13]. *Enterobacteriaceae* producing extended-spectrum β-lactamase were analysed as a subgroup [14].

Phrenic paresis was defined as difficulty in weaning from the ventilator and echographic signs of paresis. An echogram of diaphragm function was performed at 3.5–5.0 MHz, with a 10 MHz phased-array probe [15]. Diaphragm excursion was evaluated by displacement in the M mode. The lower limit of diaphragm excursion was considered to be 9 mm in females and 10 mm in males. The thickening fraction was calculated with the M mode, as follows.

Thickening fraction = (thickness at end-inspiration – thickness at end-expiration)/thickness at end-expiration

A thickening fraction >20% was considered normal. Qualitative discriminations were made between reduced and paradoxical inspiratory movement [15].

Gastroparesis was defined as the presence of three criteria: 1) abdominal discomfort with enteral nutrition; 2) gastric-contents aspiration volume (measured every 6 h) >500 mL or 150–500 mL at two consecutive measurements [16]; and stomach dilation on radiographs. All patients with gastroparesis received gastric pro-kinetic drugs.

Statistical analysis

Continuous data are reported as median (interquartile range (IQR)). Categorical data are reported as n (%). Differences between categorical variables were assessed with the Chi-squared test or Fisher's exact test, as appropriate. Continuous variables were compared with the t-test or the Mann–Whitney test, as appropriate. A two-sided $p<0.05$ was considered statistically significant. Multivariate analysis for pneumonia was conducted *via* stratified analysis and logistic regression. Variables included in the analysis were selected based on previous bibliography, avoiding over fitting (thus limiting the inclusion of one variable for each five to 10 cases), and taking into account the exploratory results of the univariate analysis. All analyses were performed with statistical software (PASW Statistics 19.0; SPSS, Chicago, IL, USA).

Results

Patients

A total of 170 consecutive lung transplants were assessed (table 1). The median (IQR) duration of mechanical ventilation was 12.5 (2.0–37.2) days and 5 (2.0–25.0) days for the patients without phrenic paresis. The median (IQR) ICU stay and hospital stay were 18.0 (6.0–38.7) days and 40.0 (28.0–60.0) days, respectively. 17 (10%) patients died in the ICU and hospital mortality was 15%. 18 (11%) patients were prioritised for urgent transplantation, and eight (5%) were on mechanical ventilation prior to lung

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TABLE 1 Characteristics and global outcomes of the population

Subjects n	170
Age years	54 (46–59)
Males	63 (37)
Type of lung transplant	
Bilateral	96 (56)
Right lung	34 (20)
Left lung	40 (24)
Indication for bilateral lung transplant	
COPD	55 (32)
Cystic fibrosis	13 (8)
IPAH	12 (7)
IPF	6 (4)
Other	10 (6)
Indication for unilateral lung transplant	
IPF	66 (39)
COPD	3 (2)
Other	5 (3)
APACHE II score	18.9 (8–37)
Urgent lung transplant	18 (11)
On mechanical ventilation prior to lung transplant	8 (5)
Cardiopulmonary bypass	45 (26)
Ischaemic time min	240 (190–289)
Re-thoracotomy	10 (6)
Mechanical ventilation days	12.5 (2–37)
Tracheostomy	79 (46)
T48 Grade 3 PGD	43 (25)
T72 Grade 3 PGD	37 (22)
Time in ICU days	18 (6–39)
Time in hospital days	40 (28–60)
ICU mortality	17 (10)
Hospital mortality	25 (15)

Data are presented as median (interquartile range) or n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease; IPAH: idiopathic pulmonary arterial hypertension; IPF: idiopathic pulmonary fibrosis; APACHE: Acute Physiology and Chronic Health Evaluation; PGD: primary graft dysfunction; ICU: intensive care unit; T48: 48 h from ICU admission; T72: 72 h from ICU admission.

transplant. 40.6% of the cohort had an Acute Physiology and Chronic Health Evaluation II score between 20 and 30.

Incidence of respiratory infections

All the episodes developed in mechanically ventilated patients. There were 24 episodes of VAP in 20 (12%) patients. 21 episodes were late onset and the median (IQR) onset was 33 (13–46) days after lung transplant. Other details of these episodes are summarised in table 2. There were 55 episodes of VAT in 36 (21%) patients and the median onset was 25 (12–37) days. Six patients (five of them with gastroparesis) suffered episodes of both VAT and VAP. There were 5.2 episodes of VAP and 11.7 episodes of VAT per 1000 ventilator-days.

Figure 1 shows the flowchart of all the screened patients that developed any new lung infiltrates not related to primary graft dysfunction. Four necropsies were performed in the 14 patients with noninfectious pulmonary opacities that died. Signs of humoral rejection, thromboembolism, acute respiratory distress syndrome and pulmonary haemorrhage were reported.

Risk factors for respiratory infections

60 (35%) patients had phrenic paresis and 55 (32%) had gastroparesis. Gastroparesis was more common in patients who had undergone bilateral transplantation than in those who had undergone unilateral transplant (40% *versus* 22%, p=0.01). The same was true for phrenic paresis (31% *versus* 25%, p=0.04). Both gastroparesis and phrenic paresis were associated with more days of ventilation and longer ICU and hospital stay (table 3). Compared with the rest of the population, patients with gastroparesis had more episodes of VAT (40% *versus* 12%, p<0.001) and VAP (26% *versus* 5%, p<0.001). Similarly, patients with

TABLE 2 Features of the 24 episodes of ventilator-associated pneumonia

Patient	Age years	Lung disease	Lung transplantation	Cultures prior to lung transplantation		Day of diagnosis	Microorganism	Antibiotics at diagnosis	Leukocytes $\times 10^9$ cells·L^{-1}	Temperature °C	F_{IO_2}	Chest radiograph	Purulent secretions	Time to death days	Gastroparesis	Most relevant abdominal symptoms/ signs related with gastroparesis
				Donor	Recipient											
1	59	COPD	Bilateral			21	*E. cloacae* ESBL	None	4–15	>38	0.6	2/4	Yes		Yes	Abdominal distension and pain, CT scan with stomach dilation
						52	*P. aeruginosa*	None	4–15	>38	1	2/4	Yes			
2	59	COPD	Bilateral		*S. maltophilia*	16	Multi-resistant *P. aeruginosa* and *E. cloacae*	None	4–15	>38	0.3	1/4	Yes[#]		Yes	Vomiting, stomach dilation on radiograph
3	62	COPD	Bilateral	*S. aureus*		12	Multi-resistant *P. aeruginosa*	None	>15	>38	0.5	1/4	Yes		Yes	Vomiting and abdominal pain, stomach dilation on radiograph
4	50	COPD	Bilateral			28	Multi-resistant *P. aeruginosa*	Meropenem	>15	>38	0.7	3/4	Yes	33	Yes	Abdominal distension, stomach dilation on radiograph
5	55	COPD	Bilateral	*K. pneumoniae*		19	*A. fumigatus*	Pipe-tazo Nebulised colistin	>15	<38	0.5	1/4	Yes	162	No	
6	49	COPD	Bilateral		*P. aeruginosa*	15	Unknown[¶]	Cefepime Nebulised colistin	>15	<38	0.4	1/4	Yes[+]		Yes	Abdominal distension and pain, CT scan with stomach dilation
7	64	COPD	Bilateral			44	Multi-resistant *P. aeruginosa*	None	>15	<38	0.4	1/4	Yes[#]	190	Yes	Abdominal distension, CT scan with intestinal dilation
8	57	COPD	Bilateral			65	Unknown[¶]	None	4-15	>38	0.4	1/4	Yes[+]		No	
9	45	COPD	Bilateral			21	MRSA	None	4-15	>38	0.6	2/4	Yes		Yes	Abdominal distension, diarrhoea and stomach dilation on radiograph
10	56	COPD	Right	*S. aureus*		16	*P. aeruginosa*	Ceftazidime Amoxi-clav	4–15	>38	0.6	2/4[§]	Yes	81	Yes	Abdominal distension, stomach dilation on radiograph
						31	*S. maltophilia*	Tigecycline Nebulised colistin	4–15	>38	1	3/4[f]	Yes			
						70	Multi-resistant *P. aeruginosa*	Meropenem Nebulised colistin	4–15	>38	0.6	3/4[f]	Yes			

Continued

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TABLE 2 Continued

Patient	Age years	Lung disease	Lung transplantation	Cultures prior to lung transplantation		Day of diagnosis	Microorganism	Antibiotics at diagnosis	Leukocytes $\times 10^9$ cells·L^{-1}	Temperature °C	F_{IO_2}	Chest radiograph	Purulent secretions	Time to death days	Gastroparesis	Most relevant abdominal symptoms/ signs related with gastroparesis
				Donor	Recipient											
11	18	CF	Bilateral	*Aspergillus* spp.	*S. maltophilia* and MRSA	15	*P. aeruginosa*	Pipe-tazo	>15	<38	0.5	1/4	Yes		Yes	Vomiting and abdominal pain, stomach dilation on radiograph
12	62	Histiocytosis X	Bilateral	*M. tuberculosis*	*S. aureus* and *Haemophilus* spp.	13	Unknown[¶]	Anti-TB drugs	>15	<38	0.8	1/4	Yes		Yes	Nausea and vomiting, abdominal distension
13	57	LAM	Bilateral	*S. aureus*	*S. aureus*	0	*S. aureus*	Amoxi-clav Aztreonam	>15	>38	0.5	2/4	Yes	8	No	
14	46	IPAH	Bilateral	*S. aureus*		13	Unknown[¶]	Cefepime Nebulised colistin	>15	<38	0.7	1/4	Yes		Yes	Abdominal distension, diarrhoea, stomach dilation on radiograph
15	46	IPF	Bilateral	*S. aureus*	Multi-resistant *P. aeruginosa*	55	Multi-resistant *P. aeruginosa*	Nebulised colistin	>15	<38	0.5	2/4	Yes	133	Yes	Vomiting and abdominal pain
						106	Multi-resistant *P. aeruginosa*	Amikacine Nebulised colistin	4–15	>38	0.5	1/4	Yes			
16	65	IPF	Left	*C. freundii*		33	*K. pneumoniae* and *E. cloacae*	None	>15	<38	0.7	2/4[§]	Yes		Yes	Abdominal distension, diarrhoea
17	65	IPF	Left	*Haemophilus* spp.		164	Multi-resistant *P. aeruginosa*	Nebulised colistin	>15	<38	0.5	2/4[§]	Yes	171	Yes	Nausea and abdominal distension, diarrhoea, CT scan with stomach and colic dilation
18	49	IPF	Right	*S. aureus*	*S. aureus*	0	*S. aureus*	None	<4	<38	0.8	1/4[##]	Yes	42	Yes	Abdominal distension, CT scan with stomach and gallbladder dilation
19	31	CLAD	Right			0	*E. aerogenes*	None	>15	>38	0.5	1/4[§]	No[+]		No	
20	46	IPF	Left	*E. coli* and *S. pneumoniae*		5	*P. aeruginosa*	Cefotaxime	>15	<38	0.7	1/4[§]	Yes	26	No	

Chest radiography data is presented as quadrants with opacities. F_{IO_2}: inspiratory oxygen fraction; COPD: chronic obstructive pulmonary disease; ESBL: extended-spectrum β-lactamase; CT: computed tomography; Pipe-tazo: piperacillin-tazobactam; Amoxi-clav: amoxicillin-clavulanate; CF: cystic fibrosis; TB: tuberculosis; LAM: lymphangioleiomyomatosis; IPAH: idiopathic pulmonary arterial hypertension; IPF: idiopathic pulmonary fibrosis; CLAD: chronic lung allograft dysfunction; *E. cloacae*: *Enterobacter cloacae; P. aeruginosa*: *Pseudomonas aeruginosa*; *S. maltophilia*: *Stenotrophomonas maltophilia*; *S. aureus*: *Staphylococcus aureus*; *K. pneumoniae*: *Klebsiella pneumoniae*; *A. fumigatus*: *Aspergillus fumigatus*; MRSA: methicillin-resistant *S. aureus*; *M. tuberculosis*: *Mycobacterium tuberculosis*; *C. freundii: Citrobacter freundii*; *E. aerogenes: Enterobacter aerogenes*; *E. coli*: *Escherichia coli*; *S. pneumoniae*: *Streptococcus pneumoniae*. [#]: dyspnoea; [¶]: no histopathological evidence of acute rejection; [+]: rales; [§]: native quadrants; [f]: native and graft quadrants; [##]: graft quadrants.

phrenic paresis had more episodes of VAT (32% *versus* 15%, p<0.001) and showed a trend toward more episodes of VAP (18% *versus* 8%, p=0.050).

Neither donor nor recipient colonisation was significantly associated with a higher incidence of ventilator-associated respiratory infection (OR 1.9 (95% CI 0.8–4.3) and OR 1.9 (95% CI 0.8–4.9), respectively), regardless of type of transplantation (unilateral or bilateral) (table 4).

None of the underlying diseases were associated with the development of VAP or VAT. The 13 patients with cystic fibrosis were colonised before lung transplantation, but their incidence of ventilator-associated respiratory infection was similar to the rest of the population (23% of VAT and 7.6% of VAP). Mechanical ventilation prior to transplantation was not associated with a higher risk of post-operative pneumonia (25% *versus* 11%, p=0.23) or tracheobronchitis (22% *versus* 13%, p>0.99).

When gastroparesis, phrenic paresis and recipient and donor colonisation were included in a one-step multivariate logistic regression, only gastroparesis was an independent risk factor for VAP (OR 6.2, 95% CI 2.2–17.2), and when this analysis was adjusted for days of mechanical ventilation the association was still significant (OR 3.1, 95% CI 1.1–9.5; p=0.04).

Pathogens

The top three pathogens in the patients with VAP were *Pseudomonas aeruginosa* (eight out of 12 patients were multidrug-resistant), followed by *Enterobacteriaceae* (one out of five patients produced extended-spectrum β-lactamase) and *Staphylococcus aureus* (one out of three patients were methicillin resistant) (fig. 2). *P. aeruginosa* (six out of 16 patients were multidrug-resistant) was also the most frequent pathogen in patients with VAT, followed by *Enterobacteriaceae* (three out of 14 patients produced extended-spectrum β-lactamase) and *Staphylococcus aureus* (one out of eight patients was methicillin resistant) (fig. 3). There were no documented episodes of viral pneumonitis.

Patient outcomes

VAP was significantly associated with longer mechanical ventilation (+43 days), ICU stay (+42.5 days) and hospital stay (+35 days) (table 5), as was VAT (+28, +27.5 and +30.5 days, respectively). VAP was also significantly associated with higher probability of ICU death (OR 10.4, 95% CI 3.4–32) and hospital death (OR 9.0, 95% CI 3.2–25.1). Figure 4 shows the Kaplan–Meier curve for survival in patients with VAP. In contrast, VAT was not associated with worse mortality (ICU death: OR 1.21, 95% CI 0.4–3.2).

Gastroparesis was associated with a trend toward worse mortality in the ICU (OR 2.62, 95% CI 0.95–7.21) and in the hospital (OR 1.80, 95% CI 0.76–4.29), but phrenic paresis was not (table 3). No differences in mortality were found comparing single *versus* bilateral lung transplantation (13.5% for single and 15.6% for bilateral; p>0.20). The same was true when comparing patients aged >55 years *versus* the rest of the population (15.4% and 14.1%, respectively; p>0.20).

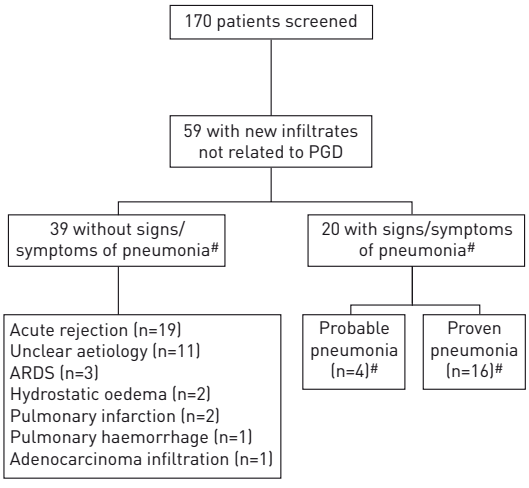

FIGURE 1 Flowchart of patients with new lung infiltrates not related to primary graft dysfunction (PGD). ARDS: acute respiratory distress syndrome. #: following the International Society of Heart and Lung Transplantation criteria.

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TABLE 3 Outcomes of lung transplant recipients with or without gastroparesis or phrenic paresis

	With gastroparesis	Without gastroparesis	p-value	With phrenic paresis	Without phrenic paresis	p-value
Subjects n	55	115		60	110	
Mechanical ventilation days	39 (29–57)	4 (1–22)	<0.001	34 (7–57)	4 (1–25)	<0.001
Time in ICU days	44 (32–65)	8 (5–26.2)	<0.001	38 (12–62)	8 (5–27)	<0.001
Time in hospital days	64 (45–80)	31 (24–48)	<0.001	60 (37–80)	32 (25–48)	<0.001
Ventilator-associated tracheobronchitis	22 (40)	14 (12)	<0.001	19 (32)	17 (15)	<0.001
Ventilator-associated pneumonia	14 (26)	6 (5)	<0.001	11 (18)	9 (8)	0.050
ICU mortality	9 (16)	8 (7)	0.056	6 (10)	11 (10)	>0.99
Hospital mortality	11 (20)	14 (12)	0.12	9 (15)	16 (15)	0.94

Data are presented as median (interquartile range) or n (%), unless otherwise stated. ICU: intensive care unit.

Discussion

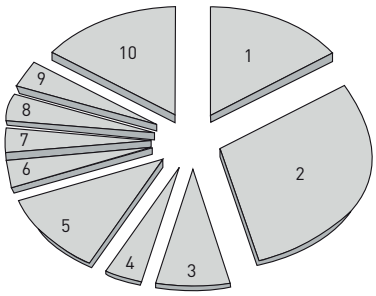
This is the first study to report a robust association between post-lung transplant VAP and ICU and hospital mortality. Both VAP and VAT increased costs, by increasing mechanical ventilation and ICU and hospital stays. Multidrug-resistant bacteria were common causative organisms of ventilator-associated respiratory infections. Interestingly, there was a strong association between gastroparesis and VAP, a finding that has obvious implications for prevention.

Previous studies found that the rate of infectious complications is higher in lung transplant recipients than in recipients of other solid organs, and that infections accounted for >20% of mortality in the first 30 days following lung transplantation [9]. Bacterial pneumonia occurs most frequently in the early post-operative phase and, excluding primary graft dysfunction, is the major cause of new lung infiltrates in this period [17]. Nevertheless, attributable mortality is controversial [17–19]. In the present study we found a marked association between VAP and mortality. In contrast, a recent investigation of the clinical response to antimicrobial therapy in ICU patients with pneumonia (ventilator-associated in 77.1% of the cases) after lung or heart–lung transplantation found no impact of the condition on ICU mortality [10]. However, a recent meta-analysis reported attributable mortality associated with VAP >60% in surgical patients [20] and larger previous studies in lung transplant recipients found that post-operative pneumonia significantly increased mortality [3, 9]. The discrepancies between these studies may be attributed to differences in immunosuppression protocols, antibiotic regimens and microbiology patterns in the study centres. Moreover, there are no gold standard criteria for the definition of VAP [21], particularly in lung transplant recipients, and the differences in the definitions between the studies may be crucial in the interpretation of the results. We used the criteria of the American Thoracic Society/Infectious Diseases Society of America [11] which are divided into clinical, radiological and microbiological criteria, as in the definition proposed by the International Society of Heart and Lung Transplantation (ISHLT) consensus statement [22]. For the diagnosis of tracheobronchitis we also applied criteria that fulfil those proposed by the ISHLT. Using these criteria, a recently published prospective, observational study concluded that immunocompromised patients were at a higher risk for ventilator-associated respiratory infection likely to increase ICU and

TABLE 4 Pre-operative colonisation and ventilator-associated respiratory infection in lung transplant recipients

	Donor colonised	Donor not colonised	p-value	Recipient colonised	Recipient not colonised	p-value
VAT						
Unilateral transplant	17	21	0.73	20	19	0.91
Bilateral transplant	21	25	0.75	19	29	0.33
VAP						
Unilateral transplant	6	3	0.52	0	4	>0.99
Bilateral transplant	14	17	0.84	17	14	>0.99
Hospital mortality	20	10	0.07	19	11	0.21

Data are presented as %, unless otherwise stated. VAT: ventilator-associated tracheobronchitis; VAP: ventilator-associated pneumonia.



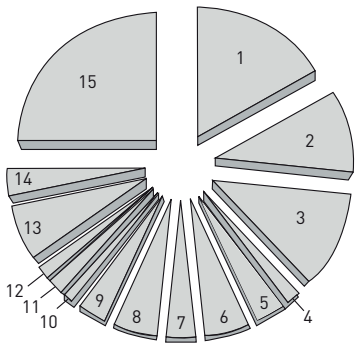
		Total n=26#
1	*Pseudomonas aeruginosa*	4 (15)
2	Multidrug-resistant *Pseudomonas aeruginosa*	8 (31)
3	Methicillin-susceptible *Staphylococcus aureus*	2 (8)
4	Methicillin-resistant *Staphylococcus aureus*	1 (4)
5	*Enterobacter* species	3 (12)
6	ESBL-producing *Enterobacteriaceae*	1 (4)
7	*Klebsiella pneumoniae*	1 (4)
8	*Stenotrophomonas maltophilia*	1 (4)
9	*Aspergillus* species¶	1 (4)
10	Other	4 (15)

FIGURE 2 Pathogens in lung transplant recipients that caused ventilator-associated pneumonia. ESBL: extended-spectrum β-lactamase. #: polymicrobial in two cases; ¶: aspergilloma.

hospital length of stay [23]. Similarly, and in agreement with other studies [18, 24], we found VAT to be significantly associated with longer mechanical ventilation, and ICU and hospital stay, but not with mortality.

Multidrug-resistant bacteria are common in lung transplant recipients [10, 25]. In our patients, multidrug-resistant *P. aeruginosa* was the most frequent microorganism in the patients with VAP, and was also common in those with VAT. Lung transplant recipients receive diverse antibiotics in the post-operative period, which may favour the development of multidrug-resistant pathogens. This fact should be taken into account in the choice of empiric antibiotic therapy for respiratory infection developing in this population.

Gastroparesis was associated with a higher incidence of VAP and worse ICU and hospital mortality. Berkowitz *et al.* [26] observed that obliterative bronchiolitis was more frequent in patients with gastroparesis than in nonsymptomatic patients. Curiously, those authors reported that no patients had symptoms immediately after surgery. In contrast, but in agreement with our findings, Smith *et al.* [27] found 11 early abdominal complications in 75 lung transplant recipients, including four cases of nonobstructive ileus. This gastrointestinal hypodynamism is thought to be secondary to vagus nerve injury during surgery, and immunosuppression in a population with an above-average incidence of pre-operative oesophageal dysmotility [28]. These conditions promote gastro-oesophageal reflux, which increases the risk of aspiration in lung transplant recipients who also have impaired cough ability and impaired mucociliary clearance. Reflux and aspiration probably contribute to chronic lung dysfunction [4, 26, 29, 30], and anti-reflux surgery has been proposed in order to improve lung graft function [5, 31, 32]. In lung transplant and heart–lung transplant recipients there is an association between reflux and respiratory infections [33]. In 1990, Reid *et al.* [4] recommended studying gastric emptying in heart–lung transplant recipients who



		Total n=60#
1	*Pseudomonas aeruginosa*	10 (17)
2	Multidrug-resistant *Pseudomonas aeruginosa*	6 (10)
3	Methicillin-sensitive *Staphylococcus aureus*	7 (12)
4	Methicillin-resistant *Staphylococcus aureus*	1 (2)
5	*Escherichia coli*	2 (3)
6	*Serratia marcescens*	3 (5)
7	*Enterobacter* species	2 (3)
8	ESBL-producing *Enterobacteriaceae*	3 (5)
9	*Proteus* species	2 (3)
10	*Klebsiella pneumoniae*	1 (2)
11	*Morganella morganii*	1 (2)
12	*Stenotrophomonas maltophilia*	1 (2)
13	*Streptococcus pneumoniae*	4 (7)
14	*Aspergillus* species	2 (3)
15	Other	15 (25)

FIGURE 3 Pathogens in lung transplant recipients that caused ventilator-associated tracheobronchitis. ESBL: extended-spectrum β-lactamase. #: polymicrobial in five cases.

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TABLE 5 Outcomes of lung transplant recipients with and without ventilator-associated respiratory infection

	No infection	VAT	VAP
Subjects n	114	36	20
Mechanical ventilation days	4 (1–23.2)	32 (25–58)[#]	47 (31.2–113.5)[#]
Time in ICU days	8 (5–27)	35.5 (28.2–64)[#]	50.5 (38–115)[#]
Time in hospital days	32 (25.2–48.5)	62.5 (43.2–80)[#]	67 (45–126.2)[¶]
ICU mortality	6 (5.2)	3 (8.3)[+]	8 (40)[¶]
Hospital mortality	9 (7.9)	6 (16.6)[+]	10 (50)[¶]

Data are presented as median (interquartile range) or n (%), unless otherwise stated. VAT: ventilator-associated tracheobronchitis; VAP: ventilator-associated pneumonia; ICU: intensive care unit. [#]: p<0.0001; [¶]: p=0.001; [+]: p-value nonsignificant.

had recurring pulmonary sepsis. In 1991, AUGUSTINE *et al.* [34] identified gastrointestinal complications as a risk factor for respiratory infection in heart–lung transplant recipients. VOS *et al.* [35] found an association between bile acid aspiration and *P. aeruginosa* airway colonisation after lung transplantation. But, to our knowledge, the present study is the first to find a significant association between gastroparesis and VAP in the immediate post-lung transplant period. The trend we found of worse mortality in the patients with gastroparesis may be due to the higher incidence of VAP. Studies are needed on measures to prevent aspiration, such as fundoplication, continuous post-operative gastric suctioning, duodenal feeding and pro-kinetic drug-based nutrition protocols. Moreover, given the high incidence of multidrug-resistant organisms causing respiratory infections, investigations into the effects of selective digestive tract decontamination on this incidence would increase the currently available evidence in this field.

Diaphragmatic paresis is another repercussion of nerve injury during lung transplantation [36]. In agreement with previous reports [37], we found that it was associated with longer mechanical ventilation, and ICU and hospital stay. Patients with diaphragmatic paresis had more episodes of ventilator-associated respiratory infections, but the multivariate analysis showed that it was not an independent risk factor for VAP. In addition, in accordance with previous studies, there was no difference in mortality between patients with diaphragmatic paresis and those without the condition [36, 37]. Further studies comparing patients with *versus* without diaphragmatic paresis would be of interest.

Also in accordance with previous studies [10, 38, 39], pre-transplant colonisation was not associated with a higher incidence of ventilator-associated respiratory infection and patients with cystic fibrosis (despite the fact that they were all colonised, frequently by multidrug-resistant organisms) did not present more episodes of VAT or VAP than the rest of the population. The same was true for all the other underlying diseases. Mechanical ventilation prior to transplant was also not a significant risk factor for post-transplant pneumonia. All these findings are probably due to the adequate effect of antibiotic prophylaxis based on

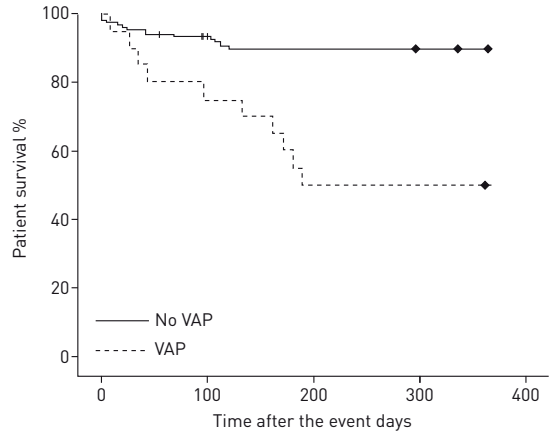

FIGURE 4 Kaplan–Meier graph of 1-year survival in patients with and without post-lung-transplant ventilator-associated pneumonia (VAP). p<0.001 *via* log rank test.

close microorganism surveillance before surgery. Also, *Aspergillus* was effectively prevented by nebulisation of amphotericin B.

The limitations of the present study are inherent to its retrospective nature. In addition, data derived from a single centre may limit the generalisation of the results to centres that may have substantially different management of lung transplant recipients. We did not evaluate the presence of gastro-oesophageal reflux in the patients with gastroparesis. However, the methods used to diagnose gastro-oesophageal reflux have only moderate sensitivity/specificity and can be invasive and expensive [40] so, similar to previous studies [16], we assumed that with the criteria used for the gastroparesis definition we would find an association with post-lung-transplant respiratory infections. Finally, the small sample size may have led to a type II error and may preclude some statistical comparisons. Nevertheless, the association between gastroparesis, VAP and outcomes was robust and has important clinical implications.

In conclusion, preventing VAP after lung transplant may improve survival. The association between gastroparesis and VAP may open up a new avenue for preventing pneumonia, thus reducing mortality and lowering costs. Treatment of ventilator-associated respiratory infections should cover multidrug-resistant bacteria. Preventing VAT may also decrease costs.

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VII. **DISCUSIÓN**

Ambos estudios aportan novedades relacionadas con las complicaciones del postoperatorio de trasplante de pulmón.

1. Los niveles de proADM medidos a las 24 horas son más elevados en aquellos pacientes con DPI grado tres a las 72 horas, entidad asociada a una mayor mortalidad en la unidad de cuidados intensivos.

La DPI es una entidad derivada de un proceso fisiopatológico complejo resultante de distintos factores, entre los que destaca el proceso de isquemia-reperfusión del injerto. La definición de esta entidad es altamente inespecífica [7]. Está basada en la presencia de infiltrados pulmonares bilaterales de la radiografía de tórax, la evidencia de ausencia de disfunción del ventrículo izquierdo y la presencia de hipoxemia. Según el nivel de hipoxemia se definen cuatro grados de disfunción, siendo el grado tres aquél con una cifra de PaO_2/F_iO_2 por debajo de 200. También se especifican cuatro puntos en el tiempo para su definición: al ingreso en la unidad de cuidados intensivos, a las 24 horas, a las 48 horas y a las 72 horas. Numerosos trabajos de investigación han estudiado la asociación de esta entidad con variables de resultado como función del injerto y mortalidad. El grado que se ha visto relacionado con la mortalidad en el postoperatorio es el grado tres medido a las 72 horas [8], lo cual se confirmó en nuestra población. Sin embargo, la mitad de los pacientes cumplen criterios de DPI al ingreso, y solo un 22% cumplirá esos criterios a las 72 horas. Por tanto, identificar a las 24 horas aquellos que presentarán disfunción primaria grado tres a las 72 horas permitiría modificar el algoritmo de tratamiento con el objetivo de minimizar el daño pulmonar, como por ejemplo la aplicación de una ventilación mecánica ultraprotectora permitida mediante la conexión del paciente a un sistema de oxigenación con membrana extracorpórea, modificaciones en el tratamiento con corticoides o el inicio precoz de tratamiento diurético.

2. Los niveles de proADM medidos a las 72 horas optimizan la relación de la DPI grado tres a las 72 horas con la mortalidad en la unidad de cuidados intensivos.

Dado lo inespecífico de la definición de DPI, su relación con las variables de resultado, en especial la mortalidad, varía entre los estudios. Por esta circunstancia, investigaciones previas han tratado de encontrar biomarcadores que optimizaran la relación entre esta patología y la mortalidad. Recientemente Shah y colaboradores encontraron que añadiendo el nivel de marcadores de daño epitelial, como ICAM-1 y sRAGE, a la definición de disfunción primaria se aumentaba su capacidad de predicción de mortalidad a los 90 días hasta llegar a un AUC de 0.76 (95% CI, 0.65‑0.87) [9]. En nuestro modelo, la capacidad individual de predicción de mortalidad en la unidad de cuidados intensivos de la disfunción primaria fue optimizada con la adición de los niveles de proADM medidos a las 72 horas hasta llegar a un AUC de 0.81 (95% CI 0.65-0.97). Esto ofrece beneficios tanto en el ámbito clínico, donde permite establecer un pronóstico más afinado, como en el ámbito de la investigación donde se ofrece un nuevo biomarcador para trabajos relacionados con la DPI.

3. La neumonía asociada a ventilación mecánica en el postoperatorio de trasplante de pulmón se asocia a una mayor mortalidad. Los gérmenes multirresistentes, especialmente *Pseudomonas aeruginosa*, son patógenos frecuentes.

La repercusión de la neumonía en el postoperatorio de trasplante de pulmón varía en las diferentes publicaciones [10-13]. La disparidad de estos resultados es debida a diferencias en

los regímenes de inmunosupresión, profilaxis antibiótica y patrones microbiológicos entre los centros. Asimismo, existe disparidad en la definición de la patología, cuestión difícil en el receptor de trasplante de pulmón que con frecuencia presenta infiltrados radiológicos e hipoxemia que pueden ser atribuidos a diferentes lesiones pulmonares. Empleando la definición de la *American Thoracic Society/Infectious Diseases Society of America*, observamos que aquellos pacientes que sufrieron neumonía asociada a ventilación mecánica tenían una mortalidad superior al resto de la población. Este hallazgo coincide con resultados de estudios previos [11,12] y tiene repercusión a nivel clínico. La sospecha de neumonía asociada a ventilación mecánica en el postoperatorio de trasplante de pulmón exige el inicio precoz de antibioticoterapia con cobertura para gérmenes multirresistentes, especialmente *Pseudomonas aeruginosa*. Nuestros resultados en cuanto a los patógenos causantes de neumonía asociada a ventilación mecánica es coherente con estudios previos donde se observó que esta población está colonizada con frecuencia por bacterias multirresistentes [13,14].

4. La traqueobronquitis asociada a ventilación mecánica en el postoperatorio de trasplante de pulmón se asocia a más días de ventilación mecánica, estancia en la unidad de cuidados intensivos y hospitalaria, pero no con más mortalidad.

Este es el primer estudio que investiga los efectos de la traqueobronquitis que aparece en el postoperatorio de trasplante de pulmón. Esta entidad se asocia a un aumento del gasto sanitario por aumento de los días de ventilación mecánica y tiempo de estancia intrahospitalaria. Estos hallazgos coinciden con los encontrados en otras investigaciones de los efectos de la traqueobronquitis en pacientes no trasplantados que reciben ventilación mecánica [15].

5. La paresia gástrica es un factor de riesgo para la aparición de neumonía asociada a ventilación mecánica.

La paresia gástrica es una de las complicaciones que pueden aparecer tras un trasplante de pulmón. Esta entidad favorece el reflujo gastroesofágico y la aspiración, lo que se ha visto relacionado con disfunción crónica del injerto [16] y colonización bacteriana [17]. Sin embargo, este es el primer estudio en el que se ha encontrado una relación entre gastroparesia y neumonía asociada a ventilación mecánica en el postoperatorio inmediato de trasplante de pulmón. Esto justificaría la aplicación de medidas de prevención de la aspiración como la colocación de una sonda nasoyeyunal o la aplicación de un protocolo de nutrición con drogas estimulantes de la cinética intestinal. Asimismo, la funduplicatura, que se ha propuesto como medida de prevención de la disfunción crónica del injerto asociada a aspiración, podría ser una opción quirúrgica que minimizara el riesgo de aparición de neumonía asociada a ventilación mecánica.

6. La paresia gástrica y la paresia frénica son factores de riesgo para la aparición de traqueobronquitis asociada a ventilación mecánica.

Tanto la paresia gástrica como la frénica pueden complicar el postoperatorio de trasplante de pulmón. Se ha encontrado una relación entre estas entidades y la aparición de traqueobronquitis asociada a ventilación mecánica. La gastroparesia favorece la aparición de reflujo y aspiración lo que predispone a la colonización y a la infección respiratoria. Por otro lado, la paresia diafragmática dificulta el proceso de destete de la ventilación mecánica lo que aumentaría el riesgo de sufrir traqueobronquitis. Sin embargo, no se observó asociación entre paresia diafragmática y neumonía en el análisis multivariado.

7. Definición, patogénesis y tratamiento de la DPI.

Se ha investigado la relación entre DPI y determinados biomarcadores de procesos relacionados con su patogénesis (de daño epitelial, de adhesión celular, de activación plaquetaria, de fibrinólisis y de inflamación) con el objetivo de optimizar su definición y tratando de encontrar un tratamiento específico de esta patología [9]. Sin embargo, hasta la fecha, no se ha encontrado ningún tratamiento específico, siendo esencial en paciente con DPI el mantenimiento de la oxigenación mediante medidas de soporte adecuadas, como la ventilación mecánica protectora y, eventualmente, la ECMO.

8. Aplicación de la ECMO en el perioperatorio de trasplante de pulmón.

El uso de ECMO en el paciente crítico se ha simplificado en los últimos años gracias a avances tecnológicos notables, lo que ha mejorado los resultados tras la aplicación de esta medida de soporte [18]. Actualmente, existe evidencia de los beneficios de la aplicación de la ECMO en el perioperatorio de trasplante de pulmón, tanto como método de soporte que permite el puente al trasplante [19], como en casos de hipoxemia refractaria en el postoperatorio [20]. A pesar de que se necesitan estudios bien diseñados que aporten evidencia más rotunda sobre los beneficios de la técnica, resultados publicados por centros de referencia sugieren que todo centro con un programa de trasplante de pulmón ha de tener la posibilidad de aplicar la técnica para ofrecer todas las posibilidades terapéuticas a los pacientes antes, durante y después del trasplante.

VIII. **CONCLUSIONES**

1. Los niveles plasmáticos de proADM en el postoperatorio inmediato del trasplante de pulmón tienen valor pronóstico al estar asociados con el grado más grave de DPI y con la mortalidad precoz.
2. La medición de niveles plasmáticos de proADM a las 24 horas del trasplante permite predecir aquellos que sufrirán DPI grado tres a las 72 horas, entidad asociada a una mayor mortalidad precoz.
3. La prevención de neumonía asociada a ventilación mecánica en el postoperatorio del trasplante de pulmón puede disminuir la mortalidad precoz tras la cirugía.
4. El tratamiento empírico de las infecciones respiratorias asociadas a ventilación mecánica en el postoperatorio del trasplante de pulmón ha de cubrir gérmenes multirresistentes.
5. La prevención de gastroparesia disminuiría la probabilidad de aparición de neumonía y traqueobronquitis asociadas a ventilación mecánica.
6. La prevención de paresia frénica disminuiría la probabilidad de aparición de traqueobronquitis asociada a ventilación mecánica.

IX. **PROYECTOS FUTUROS**

- Tras los resultados del primer trabajo de investigación que compone esta tesis, se ha diseñado un estudio prospectivo de análisis de determinados biomarcadores, incluyendo proADM, en trasplantados pulmonares con reingreso en la unidad de cuidados intensivos. También se realizará un estudio multicéntrico sobre los datos epidemiológicos y de resultados de esta población de pacientes.
- Dados los resultados de asociación entre el biomarcador proADM y la DPI, se ha analizado la relación de otro biomarcador, la procalcitonina, con esta entidad. Los niveles de procalcitonina se elevan en procesos infecciosos pero hay literatura que sugiere un aumento de los niveles en el postoperatorio inmediato de trasplante hepático y cardíaco en pacientes sin infección.
- Teniendo en cuenta los resultados parciales derivados del segundo estudio sobre los efectos de la paresia frénica en el postoperatorio de trasplante de pulmón, se ha realizado un análisis de sus efectos en la aparición de infecciones respiratorias, tiempo de estancia hospitalaria, mortalidad y repercusión en la función del injerto a largo plazo.
- Siguiendo la línea de investigación sobre las complicaciones del postoperatorio del trasplante de pulmón, se han analizado los resultados de la aplicación del decúbito prono en receptores con disfunción primaria e hipoxemia refractaria. Existen numerosos estudios mostrando buenos resultados con la aplicación de ECMO como método de soporte, pero no existe literatura que analice los resultados de la aplicación de esta maniobra posicional en esta población.
- Se está diseñando un estudio prospectivo en trasplantados pulmonares sobre el papel del DNA libre del injerto circulante en el receptor con inflamación pulmonar, particularmente sobre su uso como biomarcador que ayude a discriminar si un episodio de insuficiencia respiratoria es motivado por infección o por rechazo.
- Se ha llevado a cabo un estudio multicéntrico retrospectivo sobre la evolución postoperatoria del receptor de trasplante de pulmón teniendo en cuenta complicaciones como la DPI, la infección respiratoria y la paresia frénica.

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XI. **Anexo 1. Artículo de revisión**

Primary graft dysfunction in lung transplant recipients: definition, pathogenesis and clinical management

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Primary graft dysfunction in lung transplant recipients: definition, pathogenesis and clinical management

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Primary graft dysfunction (PGD) is a form of inflammatory lung edema that hinders respiratory function recovery within the first 72 hours after lung transplantation. The criteria for its definition are unspecific and current investigations are trying to identify elements that may be added to these criteria and, thus, enhance the relation between the definition and the short-term and long-term outcomes. PGD has complex pathogenic mechanisms that revolve around the ischemia-reperfusion injury. In the recent years new knowledge has been generated on this field. Biomarkers may help on prevention, early diagnosis and monitoring of the pathology. Furthermore, an accurate screening of the risk factors of the donor and the recipient for PGD, an adequate management of the donor before lung explant and a cautious storage and transport of the graft before implantation may reduce the possibilities of PGD occurrence. When it develops, the therapy is predominantly focused on supportive care and lung-protective ventilation. The present article reviews the published literature about PGD. Firstly its current definition is examined. Then, the main evidence about the physiopathology of PGD, particularly about its risk factors and related biomarkers, is summarized. Finally the clinical management, based principally on prevention and supportive measures, is discussed.

KEY WORDS: Transplants - Lung transplantation - Biological markers.

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Lung transplantation (LT) is the standard treatment for certain patients with end-stage respiratory disease. The number of patients that receive a lung graft over the world is constantly increasing over the years.[1] Survival in the immediate postoperative period has markedly improved in the last years, but particular complications contribute to a higher mortality compared to other solid organ transplants. One of the most feared complications following LT is the primary graft dysfunction (PGD). Its reported frequency varies among studies due to variations on its definition. It has a great impact on survival and allograft function and several investigations about its pathogenesis have been conducted in order to better understand the underlying mechanisms that trigger its development, which may help to identify a target for a specific treatment. However, to date, its treatment consists only on supportive measures.

Definition

Primary graft dysfunction is a form of acute lung injury that clinically manifests

TABLE I.—*2005 ISHLT Primary Graft Dysfunction Classification.*

Grade at T0, T24, T48, T72	Chest x-ray consistent with diffuse lung edema	Pa_{O2}/F_IO_2*	Exceptions
0	–	Any	
1	+	>300	Nasal cannula or F_IO_2 <0.3
2	+	200-300	
3	+	<200	ECMO or NO with F_IO_2 >0.5 on MV

* Should be measured on PEEP 5 cmH_2O at F_IO_2 of 1.0 on MV.
ECMO: extracorporeal membrane oxygenation support; F_IO_2: fraction of inspired oxygen; ISHLT: International Society for Heart and Lung Transplantation; MV: mechanical ventilation; NO: nitric oxide; PaO_2: partial arterial oxygen tension (mmHg).

within the first few days after allograft reperfusion in lung transplant recipients. It is characterized by nonspecific alveolar damage, poor lung compliance and hypoxemia. Different terms have been used in the literature to define this heterogeneous entity. Post-reperfusion edema, ischemia-reperfusion injury, early graft dysfunction and reimplantation lung edema are some of the labels utilized to define PGD. This disparity led to significant variability in the studies about the incidence and consequences of PGD which hindered reproducibility and generalizability of their results. The International Society for Heart and Lung Transplantation (ISHLT) Working Group on PGD tried to standardize its definition. A consensus document with the diagnostic criteria was published in 2005.[2] These criteria include the relation between the arterial partial pressure of oxygen and the inspired oxygen fraction (PaO_2/F_IO_2) and the chest X-ray pattern, within the first 72 hours after LT. Considering these variables, PGD is classified in three different grades of severity (Table I) assessed at four time points: at intensive care unit (ICU) admission or within the first six hours (T0 h), within 24 h of admission (T24 h), within 48 h of admission (T48 h) and/or within 72 hours of admission (T72 h). One of the objectives of this definition was to facilitate research on this syndrome and subsequent studies have verified the validity of its association with clinical outcomes and biologic markers of lung inflammation.[3, 4] However, it is still a vague definition that comprehends a wide range of organ dysfunction and may be an overly extensive instrument for predicting specific outcomes. Shah *et al.* recently identified two main subphenotypes, with distinct outcomes, within a group of patients with grade 3 PGD, those with severe lung dysfunction that remains persistent at day 3, and those with a transient dysfunction that resolves or attenuates on day 1 or 2.[5] It seems that the subgroup that is particularly associated with worse mortality and graft function is grade 3 PGD at 72 hours. Patients that fulfill the criteria at any other grade and/or time point may have less severe impact on outcomes. Several variations of the definition have been proposed with the aim of optimizing it.[6-9] Interestingly, PGD has many features in common with the acute respiratory distress syndrome (ARDS).[10] In 2012, an international expert panel reviewed the ARDS definition, addressing limitations of the original one.[11] Some of these limitations are also inherent to the current PGD definition and some of the changes proposed for the new ARDS definition may be evaluated for its potential inclusion in the current list of PGD criteria (Table II). Moreover, some of these changes, mainly those related with risk factors and biomarkers, have been already proposed in the literature as potential criteria that may enhance the PGD definition.[12, 13] Further studies trying to refine it are currently ongoing.

Pathogenesis

Several studies have been designed to recognize the specific mechanisms that are involved in the development of PGD. Unfortunately, the exact process that progresses from the diagnostic of brain death in the donor to the first hours after graft reper-

TABLE II.—*Changes on the ARDS Definition and Potential Implications on PGD criteria.*

AECC Definition	AECC limitation	Addressed in the Berlin definition	Questions to address in the PGD definition
Acute onset	No definition of acute	Time frame specified	Is PGD occurring at T0, T24h and/or T48h associated with worse outcomes?
PaO_2/F_IO_2 <300 mmHg	ALI misinterpreted as PaO_2/F_IO_2 =201-300, leading to confusing ALI/ARDS term	Three Mutually exclusive subgroups of ARDS by severity. ALI term removed	Is grade 1 or 2 PGD associated with worse outcomes?
PaO_2/F_IO_2 <300 mmHg (regardless of PEEP)	Inconsistency of PaO_2/F_IO_2 ratio due to the effect of PEEP and/or F_IO_2	Minimal PEEP level added across subgroups. F_IO_2 effect less relevant in severe ARDS group	Does PEEP change oxygenation significantly?
Bilateral infiltrates observed on frontal chest radiograph	Poor interobserver reliability of chest radiograph interpretation	Chest radiograph criteria clarified. Example radiographs created	Does the division in quadrants help in PGD grading of severity, especially in SLT?
PAWP <18 mmHg when measured or no clinical evidence of left atrial hypertension	High PAWP and ARDS may coexist. Poor interobserver reliability of PAWP and clinical assessments of left atrial hypertension	PAWP requirement removed. Hydrostatic edema not the primary cause of respiratory failure. Clinical vignettes created to help exclude hydrostatic edema	Does high PAWP coexist in some cases of PGD?
No defined risk factors	Not formally included in definition	Included. When none identified, need to objectively rule out hydrostatic edema	Does the absence of a combination of risk factors (*e.g.,* donor smoking, recipient obesity, use of CPB) exclude, in some cases, the presence of PGD?

AECC: American-European Consensus Conference; ALI: acute lung injury; CPB: cardiopulmonary bypass; F_IO_2: fraction of inspired oxygen; PaO_2: partial arterial oxygen tension (mmHg); PAWP: pulmonary artery wedge pressure; PEEP: positive end expiratory pressure; PGD: primary graft dysfunction; SLT: single lung transplant.

fusion is complex and has not been clearly recognized. The ischemia following organ procurement and the subsequent reperfusion injury are considered to be the main mechanisms leading to the characteristic sterile inflammation of the lung. The cold ischemic storage has deleterious effects on the graft.[14] Oxidative stress resulted from the nonhypoxic lung ischemia mainly affects the endothelium,[15] the sodium (Na^+/K^+-ATPase) pump inactivation causes cell swelling, the intracellular calcium overload potentiates the damaging effects of free radicals on the mitochondria [16] and the iron release activates platelet aggregation.[17] The consequences of this bridging step may be attenuated by measures such as an adequate perfusion solution or the *ex vivo* transport system. Although the explanted lungs continue to consume oxygen in the alveoli, and so they maintain some aerobic metabolism during the storage period,[18] this ischemic phase still has damaging consequences. Adhesion molecules upregulation causes donor leukocyte extravasation into the lung tissue,[19] a prothrombotic milieu favors microvascular thrombosis,[20, 21] and proinflammatory cytokines released in the graft induce macrophage and neutrophil chemotaxis and T cell proliferation. This recruitment of recipient leukocytes triggers a delayed phase of reperfusion injury that may perpetuate lung tissue damage.[22] Hence, it seems that the reperfusion injury shows two phases, a neutrophil-independent first period during the first hours with an important role of macrophages,[23] and a neutrophil and lymphocyte mediated delayed phase.[24] This

bimodal pattern may be related to the differences on the outcomes found between patients with transient grade 3 PGD and those with persistent grade 3 PGD at 72 h.[5]

Conditions other than the ischemia-reperfusion injury have been found to play an important role in the process. The diagnosis of PGD may include different pathological entities with the same clinical manifestation, due in part to its unspecific definition. That means different risk factors, diverse markers of evolution and dissimilar targets for specific treatment.

Risk factors

A number of risk factors associated to PGD development have been investigated, but to date conflicting results have been yielded. This may be explained by the heterogeneity of the studies, most of them retrospective and developed in single centers, together with inconsistencies in the PGD definition. The evidence about the relation of the different risk factors with PGD is summarized in Table III. They can be classified into three groups. First those related with the donor (inherent or acquired), second those related with the recipient and finally those associated with the perioperative period.

Although there is a significant heterogeneity among studies, it seems that recipient's age is not associated with PGD. In contrast, donor age over 50 years may be a risk factor for PGD within 72 h.[25-27] This risk may be increased when the donor's age is over 65 years.[28] This association could be explained by age-related changes in the lung such as a decreased elastic recoil, an increased alveolar size and loss of alveolar surface area and an impaired repair capacity of lung epithelial cells.[29, 30] Moreover, levels of the anti-inflammatory cytokine IL-10 have been found to be inversely correlated with donor age, what may be associated with a more susceptibility for graft injury in lungs form older donors. Conflicting results have been published regarding the association of younger donors (<21 years) with reperfusion lung injury.[25-28]

Gender combinations have important effects on outcomes in lung transplant recipients with higher risk for 90 d mortality for female donor/male recipient and lower risk for female donor/female recipient.[29] Regarding PGD, it seems that female recipients have increased risk to suffer it. The explanation for this finding is not clear. If we look at the differences in the hormonal milieu, estrogen has been found to be protective while testosterone may have damaging effects,[30, 31] which is not coherent. Furthermore, pre-menopausal and post-menopausal recipients have the same risk for PGD. There is evidence of gender-specific differences in the innate immune response,[32] but to date there's no strong evidence to link it with this predisposition to suffer PGD. More likely, humoral immunity may have a role in this differential risk. Female patients may have higher levels of anti-HLA antibodies at transplantation, due basically to prior pregnancies, and this may increase the risk for PGD development.[33]

Regarding the donor race, conflicting results have been published about African American being a risk factor for PGD [25] or not.[26, 36] African Americans recipients are at higher risk of PGD.[26, 34-36] Differences in endothelial cell responses to stimuli in this population could play an important role in this higher incidence.

Genetic determinants of the recipient may modify injury and repair responses. Investigations about the impact of the innate immunity on PGD have reported interesting results,[37] particularly a recently published study about the implication of the Prostaglandin E_2 (PGE_2) pathway on the PGD development.[38] Gene set enrichment analysis (GSEA) may utilize biological information (*e.g.*, differences in BAL gene expression prior to transplantation and after the procedure) to be analyzed and monitored with an interpretable result. A pretransplant risk stratification based on underlying genotypes and biomarkers profiles would improve postoperative outcomes.[39, 40] Further research on this field is warranted.

Primary pulmonary hypertension (PPH) as a risk factor for PGD has been extensively

TABLE III.—*Evidence on the association of the risk factors and PGD.*

Risk factor	Association with PGD	References
Age	Recipient: not associated with PGD. Heterogeneity among the studies. Donor: risk of PGD increased when donor age > 50 years and greater if donor age > 65 years.	Sommers 1996,[80] King 2000,[41] Thabut 2002,[42] Christie 2003,[25] Pilcher 2005,[81] Burton 2007,[70] Krenn 2007,[44] Kuntz 2009,[26] Fang 2011,[34] Felten 2012,[61] Allen 2012,[72] Shah 2012,[35] Samano 2012,[27] Baldwin 2013 [28]
Gender	Recipient: female associated with PGD. Only one study in patients with cystic fibrosis found tendency to less PGD in women (Felten 2012: OR 0.42, 95% CI 0.18, 0.99). Donor: Female associated with PGD.	Thabut 2002,[42] Christie 2003,[25] Whitson 2006,[43] Burton 2007,[70] Krenn 2007,[44] Kuntz 2009,[26] Fang 2011,[34] Felten 2012,[61] Allen 2012,[72] Shah 2012,[35] Samano 2012,[27] Diamond 2013 [73]
Race	Recipient: african American associated with PGD. Lowest incidence of PGD in white race.	Christie 2003,[25] Kuntz 2009,[26] Fang 2011,[34] Shah 2012,[35] Diamond 2013 [73]
Innate immunity	Recipient genetic variants in PTX3, NLR, TLR4, TLR9 and CD14 are associated with altered PGD risk. Particular variants of PGE_2 associated with different incidence of PGD.	Cantu 2013,[37] Diamond 2014 [38]
Underlying diagnosis	PPH, Sarcoidosis and IPF associated with PGD. COPD and suppurative diseases with the lowest risk for PGD.	Boujoukos 1997,[82] King 2000,[41] Thabut 2002,[42] Christie 2003,[25] Whitson 2006,[43] Burton 2007,[70] Krenn 2007,[44] Kuntz 2009,[26] Allen 2012,[72] Shah 2012,[35] Diamond 2013 [73]
Pulmonary arterial pressure at transplant	Higher mean PAP in PGD patients. Underlying diagnosis may have a role in this association. Heterogeneity among the studies.	King 2000,[41] Christie 2003,[25] Krenn 2007,[44] Fang 2011,[34] Allen 2012,[72] Shah 2012,[35] Diamond 2013 [73]
Body mass index	High BMI (>25) associated with PGD.	Burton 2007,[70] Kuntz 2009,[26] Lederer 2011,[83] Felten 2012,[61] Diamond 2013 [73]
Donor smoking	Associated with PGD. Measurement bias determining donor smoking history.	Christie 2003,[25] Botha 2006,[84] Whitson 2006,[43] Felten 2012,[61] Bonser 2012,[85] Samano 2012,[27] Diamond 2013 [73]
Donor cause of death	Conflicting results. Traumatic cause of brain death may be associated with PGD.	Sommers 1996,[80] Fisher 1999,[57] Kuntz 2009,[26] Diamond 2013 [73]
Single or bilateral	Not associated with PGD. Some studies (Kuntz 2009) found that PGD was more frequent in SLT.	King 2000,[41] Thabut 2002,[42] Christie 2003,[25] Oto 2006,[7] Whitson 2006,[43] Krenn 2007,[44] Kuntz 2009,[26] Fang 2011,[34] Allen 2012,[72] Shah 2012,[35] Samano 2012,[27] Diamond 2013 [73]
Thoracic size mismatch	Conflicting results among studies. Undersized allografts associated with PGD.	Kuntz 2009,[26] Eberlein 2012 [77]
Ischemic time	Prolonged ischemic time associated with PGD.	Sommers 1996,[80] King 2000,[41] Thabut 2002,[42] Christie 2003,[25] Kuntz 2009,[26] Felten 2012 [61]
Preservation solution	Intracellular-type preservation solutions associated with PGD.	Soccal 2000,[86] Thabut 2001,[63] Rabanal 2003,[64] Kuntz 2009 [26]
Use of cardiopulmonary bypass	Associated with PGD. Heterogeneity among the studies.	King 2000,[41] Thabut 2002,[42] Whitson 2006,[43] Krenn 2007,[44] Fang 2011,[34] Nagendran 2011,[71] Felten 2012,[61] Allen 2012,[72] Shah 2012,[35] Samano 2012,[27] Diamond 2013 [73]
Transfusion	Greater amount of packed RBCs and plasma transfused in patients with PGD.	Fang 2011,[34] Felten 2012,[61] Diamond 2013 [73]

BMI: body mass index; COPD: chronic obstructive pulmonary disease; IPF: idiopathic pulmonary fibrosis; PAP: pulmonary arterial pressure; PGD: primary graft dysfunction; PPH: primary pulmonary hypertension; RBC: red blood cells; SLT: single lung transplantation.

reported in the literature.[25, 26, 41-44] One reasonable explanation is the increased shear stress on the vascular endothelium caused by the acute reduction of afterload of the functionally-hypertrophied right ventricle.[45] However, although secondary pulmonary hypertension may be also a risk factor for PGD,[26, 34] some studies found that what increases the risk of PGD is the disease state of PPH, rather than just the presence of pulmonary hypertension.[25, 43, 46] Idiopathic pulmonary fibrosis is also associated with PGD probably due to alterations in the inflammatory balance after ischemia-reperfusion injury [47] and to the relative oversize of the lung allograft in a chronically contracted chest wall.[48]

A strong association between elevated recipient body mass index (BMI) and PGD has been reported by various studies. The exact mechanistic link is still unclear. Obesity may affect the cytokine regulation during the period of ischemia-reperfusion. Studies with obese mice models have showed that this population has higher levels of cytokines, such as IL-6 and TNF-α, following myocardial reperfusion.[49, 50] This particular condition in the cytokine milieu of the obese lung transplant recipients may predispose them to suffer a higher incidence of PGD than the rest of the population. Furthermore, obese patients are susceptible to suffer atelectasis of pulmonary dependent areas,[51] due to a low transpulmonary pressure in these regions, what significantly impairs oxygenation. This potential cause of hypoxemia in a lung transplant recipient that very frequently has some grade of pulmonary edema and chest x-ray opacities may fulfill the required criteria and be included as PGD. In this sense, also recipients of single lung transplantation (SLT) have less pulmonary reserve and worse oxygenation indexes in the postoperative period than recipients of bilateral grafts, what may explain the reported association between PGD and SLT in some studies.[26] Whether SLT is an independent risk factor for PGD must be confirmed in further studies.

Donor smoking has been found to be associated with grade 3 PGD.[52] The definition of smoking donor may be inaccurate due to the difficulty to obtain precise information from the donor relatives, what may cause significant measurement bias. Nevertheless, donors with recent smoke exposure may have higher proportion of proinflammatory lymphoid cells in their BAL than non-smokers, which may explain this association.[53] Also the ischemia-reperfusion injury seems to be exacerbated by prior nicotine exposition.[54]

Traumatic cause of brain donor death has been described as a risk factor for PGD. Acute brain injury is associated with increases in IL-8 levels and neutrophil infiltration of the lung tissue [55, 56] which may have a role in the occurrence of pulmonary edema after lung transplantation. However, this increase of cytokines may be also present in non-traumatic brain injury [57] and a recent study found no influence of the mode of death in the PGD occurrence,[36] so further studies are needed to confirm the association of traumatic brain donor death with recipient PGD.

Size differences between the recipient thorax and the donor grafts have been found to be associated with PGD,[77] although contrary results have been also reported.[26] Recipients with undersized allografts may receive relative higher tidal volumes in the postoperative period and this may cause ventilator-induced lung injury [78] what enhances pulmonary edema.[79]

Various publications have evidenced that PGD is more frequent in patients with long periods of graft ischemic time.[26, 58-61] In this transient period is of special interest the solution used for preservation. The Eurocollins "intracellular" preservation solution has been shown to be associated with PGD.[62-64] Extracellular-type preservation solutions may be associated with better lung preservation than intracellular-type solutions. This may be explained in part by the fact that they contain low K^+ and high Na^+ concentrations which favors a better recovery of the sodium pump function, inactivated in the hypothermic storage.[65, 66] Also other parameters such as storage temperature, volume of flush solution and pressures dur-

ing flush delivery, are crucial to maintain a good graft function after reperfusion.[67-69]

The use of cardiopulmonary bypass (CPB) may be associated with an increased risk of PGD, but this is still controversial.[27, 34, 35, 41-44, 61, 70-73] Other factors may contribute to this association, such as a higher pulmonary arterial pressure prior to surgery and more blood transfusion during it in the population of patients supported with CPB. However, the use of the technique may independently be a risk factor for PGD by increasing the release of cytokines and by triggering the coagulate cascade.[74-75] Similarly, transfusion has been proposed as a risk factor for PGD [34, 61, 73] but important confounders such as the cause of transfusion requirement may play an important role in this association. Nevertheless, the already demonstrated link between acute lung injury and transfusion [76] warrants well designed prospective studies about the relationship of blood transfusion and PGD.

Biomarkers

The association between PGD and various biomarkers has been the issue of several studies. The main findings about biomarkers on PGD and the conclusions of the most important papers to date about this association are summarized in Tables IV and V, respectively. These investigations have basically focused on molecules involved in the relationship between endothelium, epithelium and leukocytes. The research on biomarkers is of special interest to better understand the complex mechanisms in the pathogenesis of PGD. This may facilitate the identification of targets for a potential specific treatment. This is the case of the pre-existing lung-associated self-antigens (SAgs), such as antibodies to k-alpha-1 tubulin and collagen type V, that may be detected in higher rates in patients with certain lung diseases such as IPF and CF. They are associated with a higher risk for the development of PGD and their removal prior to transplantation may decrease the incidence of PGD.[87] Also the blockade of some proinflammatory cytokines, such as IL-8, and adhesion molecules, such as the intercellular adhesion molecule-1 (ICAM-1) and P-selectin, prior to reperfusion may decrease neutrophil infiltration and lung injury.[88-90] Biomarkers may be also used to monitor the evolution and the severity of the disease.[56, 91, 92] This is of particular interest when the supportive measures may in some cases enhance the lung injury, which is the case of the mechanical ventilation (MV).[78, 93] Finally, the levels of some biomarkers have been used to enhance the definition of PGD.[13] Interestingly, it seems that some of these biomarkers, such as plasma RAGE and ICAM-1, may predict more accurately short-term outcomes than the current PGD definition.[12, 13] Ongoing investigations on biomarkers in patients with PGD would increase the currently available evidence in this field.

Clinical management

Unfortunately, to date, there's no specific treatment for PGD. There are two key points in its management that may have impact in the repercussion of the disease. First, it must be prevented by adjusting selection of donors and matching with receptors taking into account the risk factors commented above, by improving the preoperative management of the recipient and the donor, and by optimizing the storage techniques of the graft. Secondly, if PGD develops, supportive therapies must focus on assuring an adequate gas exchange avoiding an increment of the lung injury.

Prevention

The prevention of PGD after lung transplantation starts with an adequate management of the support measures of the donor. Brain death induces an important imbalance of the homeostatic regulation with profound dysfunction of the endocrine function and a marked inflammatory reaction that may have impact on the lung causing neurogenic pulmonary edema and inflammatory acute lung injury.[120] Bronchoalveo-

TABLE IV.—*Summary of the most important information about the association of biomarkers and PGD.*

Biomarker	Description and association with PGD	References
ICAM-1	Marker of cell adhesion. Higher plasma levels associated with mortality in ARDS. Higher levels in PGD.	DeMeester 1996 [89]. Flori 2003 [94]. Colombat 2004 [95]. Calfee 2007 [12]. Covarrubias 2007 [96]. Calfee 2009 [97]. Shah 2012 [13]
P-selectin	Marker of cell adhesion and platelet activation. Increased levels in ARDS. Higher levels in PGD at 72h.	Naka 1997 [90]. Colombat 2004 [95]. Kawut 2009 [98]
sRAGE	Marker of epithelial injury. Higher plasma levels associated with longer ICU stay and duration of mechanical ventilation after lung transplant. Higher levels associated with PGD	Calfee 2007 [12]. Christie 2009 [99]. Pelaez 2010 [100]. Shah 2012 [13]
CC16	Clara Cell Secretory Protein. Marker of epithelial injury. Lower levels in ALI than in cardiogenic pulmonary edema. Lower levels in BOS. Higher levels in smoke exposition. Higher levels in PGD in non-IPF patients.	Diamond 2011[112]
SP-D	Marker of epithelial injury. Higher plasma levels associated with mortality in ARDS. Associated with PGD in IPF patients receiving single lung transplant.	Hartl 2006 [101]. Sims 2011 [102]. Shah 2012 [13]
KL-6	Marker of epithelial injury. Associated with mortality in ARDS. Elevated in fibrotic lung diseases. Specific marker of bronchiolitis obliterans. Not associated with PGD.	Ishizaka 2004 [103]. Sato 2004 [104]. Calfee 2007 [12]
Protein C	Marker of impaired coagulation. Low levels in ARDS and PGD. Lower levels associated with PGD.	Ware 2003 [105]. Christie 2007 [106]. Shah 2012 [13]
vWF	Marker of platelet activation. Not associated with PGD.	Covarrubias 2007 [96]
PAI-1	Marker of impaired fibrinolysis. Increased levels in ARDS. Low levels protective against ischemia-reperfusion injury. Higher levels associated with PGD.	Christie 2007 [106]. Lau 2009 [107]. Shah 2012 [13]
Ang2	Induces increased endothelial permeability. Increased levels in ARDS. Associated with PGD.	Diamond 2012 [108]
VEGF	Regulator of vascular permeability and angiogenesis. Preoperative levels higher in grade 3 of PGD.	Taghavi 2002 [109]. Krenn 2007 [44]
ET-1	Regulator of vascular permeability. Pretransplant ET-1 mRNA overexpression in donors associated with elevated pretransplant serum ET-1 in recipients contribute to PGD development.	Taghavi 2002 [109]. Salama 2010 [110]
IL-6	Marker of inflammation. Associated with mortality in ARDS. Higher in PGD at 48h and 72h. Higher levels in BAL and blood in PGD at 12h.	Mathur 2006 [91]. Moreno 2007 [111] Calfee 2007 [12]. Hoffman 2009 [92]
IL-8	Chemokine-promoting neutrophil migration and activation. Associated with mortality in ARDS. Donor lungs with high levels associated with PGD.	Fisher 2001[55]. De Perrot 2002 [56]. Mathur 2006 [91]. Calfee 2007 [12]
IL-2R	Lymphocyte receptor of IL-2. Marker of inflammation. Higher levels in PGD.	Hoffman 2009 [92]
IL-13	Marker of inflammation associated with allergic lung disease. Lower levels in PGD at 72 h	Hoffman 2009 [92]
IFN- α	Marker of inflammation. Lower levels in PGD at 24h, 48h and 72h	Hoffman 2009 [92]
MCP-1	Marker of inflammation. Monocyte chemotactic protein. Implicated in myocardial I/R injury. Higher levels in PGD.	Hoffman 2009 [92]
PTX3	Central innate immune mediator. Marker of inflammation. Increased in ARDS and acute myocardial infarction. Higher levels in lung transplant recipients with IPF and PGD.	Diamond 2011 [112]
anti-col(V) Abs	Antibodies to tissue-restricted self-antigen Collagen V. Involved in the pathogenesis of BOS. Patients with pre-existing Abs are at increased risk for PGD.	Bobadilla 2008 [113]. Iwata 2008 [114]. Bharat 2010 [115] Tiriveedhi 2013 [116]
K-alpha1- tubulin Abs	Antibodies to an epithelial surface gap junction cytoskeletal protein Kα1T. Involved in the pathogenesis of BOS. Patients with pre-existing Abs are at increased risk for PGD.	Goers 2008 [117]. Bharat 2010 [115]. Tiriveedhi 2013 [116]
IP-10	Implicated in myocardial I/R injury. Key role in early injury after cardiac and kidney transplantation. Higher levels in PGD, peaking at 24 h postoperatively.	Hoffman 2009 [92]

Abs: antibodies; ALI: acute lung injury; ARDS: acute respiratory distress syndrome; BAL: bronchoalveolar lavage; BOS: bronchiolitis obliterans syndrome; IBP: idiopathic pulmonary fibrosis; PGD: primary graft dysfunction.

TABLE V.—*Biomarkers and PGD. Main conclusions of the most relevant studies.*

Study	Biomarkers	Main findings
Fisher 2001 [55]	IL-8	High levels of IL-8 in the donor's BAL associated with severe early graft dysfunction and with early recipient mortality.
Mathur 2006 [91]	IL-6. IL-8. IL-10. TNFα	Higher levels of IL-6, IL-8 and IL-10 in patients with PGD. In PGD, levels of TNFα and IL-10 were significantly higher in the systemic versus the pulmonary arterial samples.
Moreno 2007 [111]	IL-6	Higher levels in BAL and blood in PGD at 12h. At 24h and 48h higher levels but not significant.
Christie 2007 [106]	PAI-1. Prot C	Lower levels of Prot C and higher levels of PAI-1 are associated with PGD.
Calfee 2007 [12]	sRAGE. ICAM-1. KL-6. IL-6. IL-8	Higher levels of sRAGE predicted duration of MV and ICU LOS. sRAGE had better prognostic value for these outcomes than the clinical diagnosis of PGD. No biomarker associated with PGD (grades 0 vs 1-3).
Covarrubias 2007 [96]	ICAM-1. vWF	Higher levels of ICAM-1 associated with PGD independent from clinical variables except PAP prior to transplant. vWF not associated with PGD.
Bobadilla 2008* [113]	col(V)-specific IL-17	Anti-col(V) immune status before transplantation associated with PGD.
Iwata 2008* [114]	anti-col(V) Abs	Higher levels associated with PGD.
Christie 2009 [99]	sRAGE	Higher levels associated with PGD, blood product transfusion and use of cardiopulmonary bypass.
Lau 2009* [107]	PAI-1	Preventing fibrin deposition may reduce inflammation and PGD.
Hoffman 2009 [92]	ILs# Eotaxin. IP-10. MIG. MCP-1. MIP-1α. MIP-1β. RANTES. TNF-α. IFN-α. IFN-γ. GM-CSF. IL-1Ra. IL-2R	MCP-1 and IP-10 associated with PGD. IL-2R higher in PGD. IL-6 higher in PGD at 48h and 72h. IL-13 lower in PGD at 72h. IFN-α lower in PGD at 24h, 48h and 72h. Marked reduction in both case and control plasma levels of TNF-α, IL-1β and IL-2
Kawut 2009 [98]	P-selectin	Higher levels associated with PGD at 72h.
Pelaez 2010 [100]	sRAGE	Higher risk for PGD in recipients of organs with high levels of sRAGE prior to explant. Increased sRAGE levels in BAL of patients with PGD.
Bharat 2010 [115]	Abs to. Collagen I. Collagen V. k-alpha-1 tubulin	Presence of pretransplant antibodies increases the risk of PGD and bronchiolitis obliterans on long-term follow-up.
Sims 2011 [102]	SP-D	Higher levels of SP-D in IPF awaiting LT. After LT, SP-D levels influenced by single vs bilateral. SP-D not associated with PGD.
Diamond 2011 [112]	PTX3	Elevated levels associated with PGD in lung transplant recipients with IPF but not COPD.
Diamond 2011 [118]	CC16	Elevated levels associated with PGD in non-IPF patients.
Diamond 2012 [108]	Ang2	Elevated levels associated with PGD, similarly to ARDS.
Shah 2012 [13]	ICAM-1. sRAGE. SP-D. Protein C. PAI-1	Biomarkers at 24h more useful than biomarkers at 6h. PAI-1 and sRAGE had the greatest individual discriminant ability for PGD. ICAM-1 and PAI-1 predictive utility for 90-d mortality. Combinations of ICAM-1 with sRAGE or PAI-1 had predictive utility for 90-d mortality, exceeding concurrent clinical PGD grading.
Bastarache 2012 [119]	Estradiol	Higher estradiol levels at 24 h were associated with an increased risk of PGD within 72h in male patients. No relationship between estradiol levels and PGD in females.

*Experimental studies. #IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17. Abs: antibodies; ARDS: acute respiratory distress syndrome; BAL: bronchoalveolar lavage; COPD: chronic obstructive pulmonary disease; GSEA: gene set enrichment analysis; IPF: idiopathic pulmonary fibrosis; LOS: length of stay; LT: lung transplantation; PGD: primary graft dysfunction; ICU: intensive care unit; MV: mechanical ventilation; PAP: pulmonary arterial pressure.

lar lavage concentrations of neutrophils and IL-8 have been shown to be predictive of early graft failure and mortality after lung transplantation.[55] A high-dose treatment with corticoids may improve oxygenation at organ recovery by reducing this inflammatory reaction.[121] However, randomized clinical trials using methylprednisolone in brain-dead donors contradict this hypothesis.[122] Also a recently published randomized trial failed to demonstrate that treatment with nebulized albuterol decreases pulmonary edema in brain-dead organ donors.[123] Pulmonary dysfunction in the brain-dead organ donor is also associated with incidents not related to brain death *per se* such as aspiration pneumonia, contusion, and ventilator-induced-lung-injury (VILI). Antibiotics should be used if aspiration is suspected and bronchoscopy and endobronchial suctioning may be performed regularly to assess the airways and remove mucous plugs. It is very important to implement a protective MV strategy in these patients.[124] Low-tidal-volume ventilation may avoid an added injury to the already inflamed lung. There is no robust evidence to recommend any other ventilation maneuver such as high positive end-expiratory pressure (PEEP) levels or recruitment maneuvers. Any measure focused on evaluating or maintaining the gas exchange may take into account the importance of limiting the VILI.

Experimental studies have been published investigating the effects of certain molecules on the different pathogenic mechanisms that are involved in the ischemia-reperfusion lung injury. Calcium channel blockers administered to the donor before lung retrieval may prevent endothelial damage.[125] Deferoxamine, an iron chelator, was found to be associated with better lung function in a swine model of heart-lung transplantation.[126] Also agents with effects on the inflammation and coagulation cascades, such as selectin inhibitors,[127] C1-esterase inhibitors,[128] matrix metalloproteinase inhibitor,[62] anti-IL-8 antibodies,[88] endothelial cadherin antagonist,[129] nebulized prostacyclin and rolipram [130] and mast cell membrane stabilizers [131] showed beneficial effects decreasing reperfusion injury, edema and early graft dysfunction. These results encouraged investigators to study the effects of some of these molecules in human LT. A multicenter, randomized, double-blinded, placebo-controlled trial in 59 lung transplant recipients studied the effects of the inactivation of C3a and C5a convertases before reperfusion of the first allograft.[132] The study was underpowered to be able to statistically demonstrate important differences between the case and the control groups. Moreover, no differences were found between the groups in the oxygenation and the chest X-ray score at admission. Contrarily, another study that investigated the effects of a platelet activating factor antagonist on graft function observed a significant improvement in the alveolar-arterial oxygen difference during the first 12 hours after reperfusion and better chest X-ray score in the group receiving the antagonist, when compared with the control group.[133] After promising results of experimental investigations, a randomized prospective study in 29 patients observed protective effects of exogenous surfactant instilled into donor lungs before retrieval *via* bronchoscopy.[134] These beneficial preventive effects of exogenous surfactant on PGD occurrence were also observed by another group of investigators in a retrospective study.[135] Well-designed multicenter studies are justified to confirm these good results. In animal models, prophylactic inhaled nitric oxide (iNO) was associated with lower reperfusion injury.[136] However, clinical studies failed to confirm this preventive effect on PGD.[137, 138] Primary graft dysfunction is a multifactorial injury process and a specific pathogenic-targeted prevention may be difficult to find. That may explain the variability of the results of the use of these therapies on lung transplant recipients. However, more well-designed multicenter trials are needed to continue evaluating the potential clinical impact of all these preventive strategies as they are developed.

The *ex vivo* lung perfusion (EVLP) system is an evaluation and reconditioning technique for suboptimal grafts. It is applied fol-

lowing lung explant and consists of a ventilator and a circuit with centrifugal pump, oxygenator, heat-exchanger and leucocyte filter. The lung is perfused with a normothermic, hyperoncotic, acellular serum that dehydrates it and this, combined with recruitment maneuvers, allows an improvement of the function of marginal grafts. Moreover, it keeps the lung in a physiologic status prior to implantation with maintained normal metabolic activity. Recent investigations have reported less incidence of PGD in patients that received a graft preconditioned with EVLP [142] and larger multi-center studies are currently ongoing (http://clinicaltrials.gov/ct2/show/NCT01630434). Furthermore, the system may offer a platform to evaluate particular circumstances that could have great impact on postoperative graft function and it permits the study of potential preconditioning and protective measures, such as the administration of mesenchymal stem cells [143] and decellularization techniques [144] focused on decreasing the incidence of PGD. This is a fertile field for further well designed studies.

Specific treatment

When PGD is established, iNO therapy is generally used when hypoxemia and/or elevated pulmonary arterial pressure may compromise the patient's stability. Investigations of small case series (<32 patients) of patients with PGD have reported an association of iNO treatment with better clinical outcomes.[140] Moreover, iNO may lower the interleukin concentration in blood and alveoli.[141] However, to date there are no randomized studies to support its routinely use for treatment in patients with PGD.

Experimental studies have reported that PGE_1 (intravenously or aerosolized) in the treatment of severe PGD appears to be helpful.[139] However, currently, there are no well-designed randomized trials to support its use in humans. The results of the recent investigations on the PGE_2 pathway and its association with PGD [38] indicate that treatments or preventive therapies focused on prostaglandins may be interesting strategies for the future.

Supportive therapies

Current therapy for PGD is predominantly focused on supportive care and lung-protective ventilation.[60] When PGD is established, it is essential to avoid further damage on the graft.[94] Similarly to the recommendations for the ARDS ventilating management,[78] protective ventilation with low tidal volume is mandatory. As pointed above, when the graft is smaller than the recipient thorax, tidal volume may be adjusted taking into account the height of the donor. Also plateau pressure may be limited at least below 30 cmH_2O to protect the parenchyma and peak pressure may be also closely monitored in order to protect sutures. The level of PEEP may be individualized considering oxygenation, hemodynamics, peak and plateau pressures and potential leaks of air. Although the lung edema of PGD is basically inflammatory, negative fluid balance may help to improve gas interchange. Neuromuscular blockade may be necessary for a short period of time if refractory hypoxemia is persistent and there's a high risk of barotrauma. However, this treatment might be resumed as soon as possible to minimize the risk of ICU-acquired weakness. Early mobilization and physiotherapy are crucial. When oxygenation improves, early extubation might be considered. However, other conditions such as myopathy, poor lung compliance or phrenic paresis, might hinder the weaning from the ventilator and, in case the MV is withdrawn, they may compromise respiratory function. If acute respiratory failure develops after extubation, high-flow oxygen through nasal cannula may be considered before reintubation.

Extracorporeal membrane oxygenation (ECMO) is a temporary artificial support that may be necessary in cases of refractory hypoxemia. Large case reports have evidenced that the early institution of ECMO in lung recipients with severe PGD is associated with good recovery of the respira-

tory function, with acceptable medium term survival but worse large term survival when compared to that of patients who do not require ECMO.[145-147] In the last years, the system has been simplified and the latest generation of ECMO devices is associated with less frequent complications. Other particular features of the management of the patient on ECMO are also very important for the success of the technique. A high-specialized, high-experienced unit of ECMO support may be of interest to improve the outcomes.[148] It is essential to find an accurate protocol with well-defined criteria to identify the patient that may benefit from its implementation. It is also important to identify the most appropriate timing for the therapy institution.[145] It should be also of interest to define the conditions that would make either veno-arterial (VA) or veno-venous (VV) mode more appropriate as each mode has its own advantages and disadvantages when applied in the postoperative period of LT. The VA approach reduces pulmonary vascular flow which could modulate the endothelial activation and aggravation of pulmonary edema secondary to reperfusion injury.[146] However, with VA ECMO bronchial anastomotic healing could be worsened as bronchial artery perfusion is excluded after LT.[149] Moreover, the VV mode increases aortic oxygen saturation, reduces the risk of systemic embolism and cannulation is relatively easier. Therefore, many authors recommend implementing the VV approach in case of hemodynamic stability or mild hemodynamic instability.[147] Finally, the adjustment of MV parameters should be standardized as well as the antimicrobial regimen which could reduce the risk of developing a dangerous complication such as sepsis.[147, 150]

Conclusions

The current criteria for the diagnosis of PGD have a low specificity. Recent studies are trying to find new criteria to enhance the relation between the definition of PGD and its associated outcomes. Patients with persistent severe PGD at 72 hours have worse outcomes than the other subgroups. This may be due to a bimodal pattern of the pathogenesis of the disease, with a worse repercussion of the delayed phase which is mediated by recipient's neutrophils and lymphocytes. A strict surveillance of the risk factors of the recipient, the donor and those related with the surgery may decrease the incidence of PGD. Of particular interest are the recent investigations about the genetic patterns associated with a particular predisposition for the disease. Also investigations on biomarkers are adding important information that may modify the current definition of PGD, its monitoring measures and its standard management. To date it has no specific treatment and its management is based on supportive measures, such as protective ventilation, nitric oxide and VV ECMO.

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XII. **Anexo 2. Artículo de revisión**

Extracorporeal Membrane Oxygenation in the Perioperative Care of Lung Transplantation

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Extracorporeal Membrane Oxygenation in the Perioperative Care of Lung Transplantation

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Abstract: Extracorporeal membrane oxygenation (ECMO) is a temporary artificial support in cases of ineffective oxygenation due to severe lung dysfunction, severe circulatory failure, or both. Lung transplantation (LTx) has become a life-saving procedure for patients suffering from end-stage lung diseases. Its indications have progressively broadened over time and outcome has steadily improved. Unfortunately, still a considerable number of patients die on the waiting list before transplantation. Moreover, postoperative complications can be life threatening. The present article reviews the published literature about the implementation of ECMO in the perioperative care of the LTx patient. This progressively developed technique of vital support is a feasible therapeutic option in cases of terminal respiratory failure before transplant, thus being a bridge to it, and in the management of severe immediate postoperative complications. First, we present a historic view of the role of ECMO support in acute respiratory failure, with critical discussion of the only 3 published clinical trials on ECMO in adult patients. Then, the interactions between ECMO and LTx are examined. Larger case series about ECMO bridging to LTx are reviewed, with particular focus on the *awake ECMO* strategy. Finally, evidence for ECMO support in the intraoperative and postoperative care of LTx is discussed.

Key Words: ECMO, extracorporeal membrane oxygenation, lung transplantation, bridge to transplant, primary graft dysfunction

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"All truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as being self-evident."

Arthur Schopenhauer

ECMO SUPPORT FOR ADULTS WITH SEVERE ACUTE RESPIRATORY FAILURE. HOPE OR CLINICAL EVIDENCE?

Extracorporeal membrane oxygenation (ECMO) temporarily assures gas exchange by replacing respiratory function totally or partially, making use of the venovenous (VV) mode. If needed, hemodynamic support can also be provided applying the venoarterial (VA) mode. More than 35 years have passed since Dr Bartlett and colleagues successfully used a bedside cardiopulmonary bypass (CPB) to support Esperanza's life.[1] Since then, several studies have addressed finding the precise role of the technique in respiratory and hemodynamic support of the critically ill patient.

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In the neonatal population, extracorporeal life support (ECLS) has been extensively incorporated in the treatment of pulmonary arterial hypertension (PAH) or severe respiratory failure. Dr Bartlett et al[2] published the first large experience with ECMO application in newborns in 1982. They found that in a cohort of 45 neonates in whom the mortality rate was thought to be as high as 90%, ECMO support decreased this number to 45%. Seven years later, a phase I, prospective, randomized study on newborn infants with severe persistent PAH and respiratory failure was stopped because of the superiority of ECMO in this patient population (6/10 babies in the conventional therapy group vs. 9/9 in the ECMO group survived).[3] These results were confirmed later in a large clinical trial involving 185 mature newborn infants.[4] The UK Collaborative ECMO Trial Group compared ECMO with conventional therapy in newborns with severe respiratory failure (oxygenation index ≥ 40) showing a survival benefit for the ECMO treated neonates with a number needed to treat of 4 (risk ratio of 0.55; 95% confidence interval, 0.39-0.77).

Despite the satisfactory preliminary results of ECMO on newborns, the extrapolation of these results to the adult population was clearly limited by the obvious differences in patient characteristics and pathophysiology of the underlying conditions prompting its use. The first successful use of bedside ECLS in an adult patient was reported in 1972.[5] Other encouraging reports followed this study.[6,7] Nonetheless, the first multicenter prospective study in adults failed to show any survival benefit of ECMO over conventional therapy.[8] In that study, 90 patients with severe acute respiratory distress syndrome (ARDS) were randomized to either conventional mechanical ventilation (MV) or MV supplemented with partial VA bypass. Survival rates were found to be similar in both arms (9.5% ECMO vs. 8.3% control). However, this trial had important limitations. The chosen mode of ECMO support was only VA, through the femoral vessels, which is associated with more complications than the VV mode. Moreover, 6 of the 9 centers that collaborated in this trial lacked any prior ECMO experience, which also could be associated with more ECMO-related complications (eg, significant bleeding, that had an average rate of 3.8 L on the first day of treatment). Further, duration of high-pressure ventilation before randomization averaged 9.6 days, which could directly affect survival of ECMO patients. Finally, ECMO was removed when no improvement was observed after 5 days, which excluded the feasible possibility of late clinical recovery.[9,10] This recovery was less expected as the ECMO group did not receive any lung-protective ventilation, because this current practice of ventilator support was unknown at that time.

The second randomized controlled clinical trial comparing ECMO therapy with conventional management in adult patients with severe ARDS was published in 1994.[11] In this case, 40 patients with severe ARDS were randomized to either a lung rest strategy with low-frequency positive-pressure ventilation plus carbon dioxide removal ($ECCO_2R$) or conventional positive-pressure ventilation. The study did not find

significant differences in survival at 30 days with these 2 strategies (33% $ECCO_2R$ vs. 42% control; $P = 0.8$). However, the $ECCO_2R$ group had particularities that could explain these results. It is well known that the capacity of this ECMO strategy to ensure a correct oxygen transfer is unreliable; therefore, aggressive MV parameters, with higher levels of airway pressures, were present in that group. Moreover, the rate of ECMO-related complications (thrombosis, hemorrhage) requiring treatment discontinuation was high. Probably, inexperience with $ECCO_2R$ management contributed to this high rate of complications. Clearly, patient selection could be improved, and the use of this new technology was at the beginning of the learning curve, but these trials froze the interest in ECMO to support adult patients with severe respiratory failure, and the technique was abandoned worldwide for decades.

Meanwhile, intensivists became aware that intermittent positive-pressure ventilation (IPPV) caused significant deleterious effects on the already diseased lung with ARDS.[12–14] This renewed the interest in ECMO as an alternative method to support these patients while allowing the lung to rest until recovery. In addition, ECMO conception and construction improved significantly. More biocompatible systems with lesser need of anticoagulation were designed, complexity of the systems was minimized, and progress in the efficacy of the cannula, oxygenator, and pump was also achieved.[15] These technological developments and the inertia of knowledge about the adverse effects of the nonphysiological IPPV impelled the publication of favorable small series in patients with ECMO support.[16–22] Finally, in 2009, Peek et al[23] published a multicentre randomized controlled trial (the CESAR trial) that tried to firmly establish this intuited evidence. A total of 180 adult patients with severe acute respiratory failure were enrolled and randomly allocated to receive conventional management (n = 90) or an ECMO-based protocol (n = 90). Survival at 6 months without disability was higher in the ECMO group than in the control group (63% vs. 47%; risk ratio 0.69; 95% confidence interval, 0.05-0.97). This was the first clinical trial favorable to ECMO support in adult patients with ARDS. However, important limitations must be considered regarding this study. A pragmatic design was chosen; therefore no standardized protocol was implemented in the control group. In fact, treatment with a lung-protective strategy was significantly more frequent in the ECMO group than in the control group. Further, 25% of the patients randomized to the ECMO-based protocol were in fact managed successfully with conventional treatment without needing extracorporeal assistance (82% of whom survived). Finally, the reproducibility of these results could be limited due to the criteria for patient selection, a key point in every successful ECMO program. In the CESAR study (as in the majority of ECMO protocols) the definition of "potentially reversible respiratory failure" lacks consistency, and the evaluation of this "reversibility" depends on the clinician's judgment, which is frequently associated with subjectivity-related errors. Thus, reproducibility of this work would be very difficult to attain. Nevertheless, this impressively executed study, despite its limitations, lightly tipped the balance toward the benefits of ECMO application in hypoxemic emergencies, renewing the interest in this technique.

Similarly to what happened in the poliomyelitis epidemic in Copenhagen in 1952,[24] the 2009 novel swine-origin influenza A (H1N1) virus infection determined new and demanding conditions with a high incidence of young adults with severe ARDS, frequently needing nonconventional respiratory support. In this context, ECMO was used as a rescue therapy in many cases, and several studies were published explaining different experiences.[25–33] The latest generation of ECMO devices, simpler and safer, was used on these patients, and, interestingly, hospital survival ranged from 44% to 92%, in a population in which mortality was expected to be much higher. One of the largest reports was recently published by Noah et al.[32] Of the 80 patients referred to an ECMO center, 69 received ECMO, and, remarkably, hospital mortality for matched non–ECMO-referred patients was approximately twice that of the ECMO-referred patients. These results are in line with other reports from expert centers such as the Karolinska ECMO center, which reported a survival of 92% in H1N1 patients with ECMO respiratory support.[28] Experienced centers supported these patients with VV ECMO usually with a double lumen cannula in the right jugular vein, with lung-resting parameters on the ventilator, and by trying to maintain the patient in the awake state as much as possible.

These good outcomes encouraged many centers to incorporate this technically demanding but life-saving technique in their protocols. Moreover, the indications for ECMO have recently been extended. However, the correct identification of those patients who will benefit from its application remains the main issue that needs to be addressed. Nowadays, a well-designed clinical trial is mandatory to better answer the crucial questions about "in whom, how, and when" ECMO should be applied.

ECMO SUPPORT IN THE PERIOPERATORY SETTING OF LUNG TRANSPLANTATION (LTx)

In this general context, ECMO support has been proposed as a valuable tool in the perioperative care of LTx, either as a bridge therapy or to treat intraoperative or postoperative complications.

Half a century has passed since the first human LTx was carried out.[34] Nowadays, LTx has become a standard of care for selected patients with end-stage, disabling lung disease.[35] The indications for LTx have expanded over the years and currently include a wide range of severe respiratory diseases.[36] Referral for LTx has progressively increased, with >2500 LTx procedures carried out worldwide in 2011 (http://www.ishlt.org/registries/quarterlyDataReport.asp). The introduction of new technologies and strategies has increased the number of available organs. The recently developed ex vivo perfusion system[37] and the emerging source of donation after cardiac death[38] allow for more organs to become available. Gentle ventilation of potential donors might also increase availability of lungs suitable for transplant.[39,40] Even so, the number of patients listed for LTx largely exceeds the number of available transplantable organs; therefore, the mortality of patients on the waiting list is still high[41]: in 2009, the prevalence of death of this population in North America was 6% (http://www.srtr.org/annual_reports/2010/1203_can-gender_lu.htm). However, this mortality varies as do the eligibility criteria to be included in the waiting list among the different countries. In Spain, with a constantly increasing number of patients included (249 inclusions in 2010), the mortality of adult patients awaiting LTx was 3.7% in year 2010 (http://www.catib.org/imgdb/archivo_doc469.pdf).

Adequate listing criteria is key if an LTx program is meant to be successful. Controversy exists in the eligibility of wait-listed patients who suffer an exacerbation of their chronic disease or in whom a concurrent pathology aggravates their fragile respiratory function, needing critical care. Of particular controversy is the suitability of LTx for ventilator-dependent patients. Although LTx in ventilator-dependent patients was previously discouraged, many programs maintain selected patients on their active waiting list. The US experience with

ventilated patients who were transplanted from 1987 until 2008 has been published with reasonable outcomes: 1-year and 2-year survival of 62% and 57%, respectively, compared with 79% and 70% for unsupported patients.[42] MV is a potential cause of ventilator-associated pneumonia[43] and ventilator-induced lung injury, thus enhancing lung damage. Moreover, in the mechanically ventilated patients, prolonged immobilization combined with corticosteroids and diaphragmatic weakness leads to a difficult process of weaning from the ventilator, increasing the rate of associated complications.[44] These deleterious effects of conventional MV, which could result in unsuitability for LTx or in a hazardous postoperative course, could be minimized if ECMO is implemented before transplantation, especially with the *awake ECMO* strategy. In addition, intrathoracic pressure rises when IPPV is started, and this hinders management of patients with PAH.[45] ECMO support, with different modes or approaches, could increase the number of PAH patients unmanageable with conventional support to be eventually bridged to LTx. However, ECMO therapy historically has been a relative contraindication to LTx because patients supported with the technique were considered too ill to afford the intervention. This premise is currently changing as some encouraging results with ECMO support as a bridge to LTx have been published in the last 20 years in studies that are discussed below.

Moreover, ECLS could be of interest not only in the preoperative period. The first case of ECMO support in the perioperative period of LTx was published by Veith in 1977.[46] Intraoperative ECMO support offers important advantages against CPB, especially when it is prolonged into the early postoperative period. One of the main reasons for the use of intraoperative CPB is the avoidance of volume overflow of the first implanted lung. However, CPB needs full systemic anticoagulation and is associated with intense systemic inflammatory response, which could increase the permeability of the implanted lungs in the postoperative period, impairing lung function. Once CPB is discontinued, the transplanted lungs are exposed to the complete cardiac output (CO). Particularly in patients with PAH, this can have a profound negative impact on early allograft function, and in some cases results in a need for aggressive ventilation with high tidal volumes and pressures. All these factors can be avoided by the use of intraoperatively and postoperatively prolonged ECMO support, most commonly with the VA mode. ECMO offers the potential to a controlled reperfusion of the transplanted lung(s), titrating extracorporeal flow, which reduces CO, relieving immediate right ventricular demand, and allowing smooth recovery of the transplanted lung when the therapy is prolonged into the postoperative period. This could decrease the incidence of one of the most feared complications in the postoperative period of LTx: the primary graft dysfunction (PGD). PGD is a form of acute lung injury developing in the immediate postoperative period. It is characterized by diffuse alveolar damage on pathology, but the pathogenesis of the disease remains unclear. It remains a diagnosis of exclusion, and differential diagnosis requires consideration of other diseases with a similar clinical presentation, such as hyperacute and acute rejection, infection, venous anastomosis complication, and cardiogenic pulmonary edema. This complication leads to severe early and long-term adverse consequences in LTx recipients. Mortality associated with PGD is still high and, in severe cases, is a leading cause of death in the perioperative period, with a short-term mortality of 30% to 40%.[47,48] Treatment of this condition is essentially supportive. Detrimental effects of IPPV in the acutely injured lung necessarily lead to implementation of protective strategies in the ventilator management in these patients. However, extremely severe allograft injury in some cases makes it difficult to achieve adequate tissue oxygenation, without exceeding the recommended limits. In those cases partial or total ECMO support allows the clinician to decrease all the ventilator parameters that could cause the undesirable ventilator-induced lung injury.

ECMO AS A LIFE-SAVING BRIDGE TO LTx. A LAST OPTION?

After the first experience with ECMO support in the perioperative period of LTx,[46] the first ECMO application as a bridge to LTx was published in 1985 by the Toronto Lung Transplant Group in a paraquat-poisoned patient in whom sequential bilateral LTx was performed.[49] The therapy allowed adequate oxygenation of the patient, who could be transplanted twice, although, unfortunately, death occurred from a cerebrovascular accident 93 days after the first LTx.

Although controversy about ECMO application in adult population stalled its implementation as a rescue therapy for acute respiratory failure,[8,11] the Hannover group tried to offer it as an alternative therapeutic strategy to bridge patients to LTx, despite the inherent difficulties of the primitive technology available in the early 90s. Their first 2 cases of successful bridging to LTx were published in 1991.[50] In the first case, VA ECMO was instituted during 8 hours in the postoperative day (POD) 11 of right LTx, because of severe hypoxia, hypercarbia, and metabolic acidosis, despite MV support. The situation was desperate but the technique allowed rapid stabilization and retransplantation. The patient was finally discharged from hospital on POD 93. In the second case, VA ECMO was implemented in a complicated postoperative course of a single right LTx. After 232 hours on ECMO, the patient could finally be retransplanted and weaned from ECMO in the operative table. She ultimately died on POD 159 because of progressive pulmonary failure due to interstitial fibrosis. The same group, 2 years later, published their results of 3 further ARDS-affected patients on ECMO in whom native lungs did not recover and needed LTx.[51] They were supported with VV ECMO for 5 to 12 days and successfully bridged to LTx with a good outcome in 2 of them (the third patient died because of severe multiorgan failure). These short series proved that extracorporeal support as a bridge to LTx was feasible and should not be systematically regarded as a contraindication to it.

Nevertheless, reluctance to transplant patients in such severe conditions was still the norm, defied by some centers that continued accumulating experience and publishing their results. The Vienna group reported their results of ECMO support in the perioperative period of LTx in patients with PAH.[52] Two of the 17 transplanted patients underwent VA ECMO as a bridge to LTx. One was on ECMO for 3 weeks before transplantation with good postoperative outcome (mean follow up of 18 ± 11.4 mo), and the other was resuscitated and bridged with ECMO for 1 week until transplantation, but severe hypoxic cerebral damage was detected and he died at 5 months posttransplantation. A larger report from the Hannover group described excellent results in ECMO bridging to LTx with a pumpless arteriovenous $ECCO_2R$ (iLA).[53] Twelve high-urgency recipients were connected to the system and 10 (83%) were successfully bridged to LTx. Two of them died before being transplanted because of severe multiorgan failure. Mean duration of iLA support was 15 ± 8 days and the system significantly decreased the $PaCO_2$ levels, but it did not increase oxygenation. One-year survival of the transplanted patients

was high (80%). This excellent outcome could be related to the less advanced condition before iLA support (PO_2/FIO_2 before iLA was 135 ± 33), which could be considered in this case an effective support therapy in patients with reasonable oxygenation capacity.

Several small case series of patients undergoing ECMO as a bridge to LTx were published afterward with good overall results.[54–68] Interestingly, in some cases, patients were on ECMO without MV support, using small doses of sedatives and maximizing the potential to improve conditions to face LTx, in an *awake ECMO* strategy. Different bridging modalities were implemented in these studies: VA, VV, or iLA depending on the severity of lung disease. With these encouraging results, the idea that ECMO should no longer be considered as an absolute contraindication for LTx started to spread. In the last 2 years, 5 additional reports have been published trying to add more evidence to confirm this perception. The first one investigated the early outcomes of patients ECMO-bridged to LTx in 2 Scandinavian transplant centers between 2005 and 2009.[69] Sixteen patients were supported with ECMO and 13 (81%) underwent LTx. One patient could be weaned from ECMO before LTx and the technique was continued during the surgical procedure in 4 cases (30%). Only 1 patient died 82 days after LTx (1-y survival of 92%). These outstanding results were probably influenced by the implementation of modern devices and the accuracy of the entry criteria of 2 experienced LTx centers. The counterpoint, however, was the high incidence of postoperative morbidity, with an occurrence of 33% of critical illness myopathy. This resulted in a long intensive care stay (28 ± 18 d after LTx), long hospital stay (total hospital stay of 86 ± 45), and long rehabilitation time. The authors proposed the *awake ECMO* approach as an alternative strategy to diminish this condition. In 2011, the Pittsburg group reported on their experience between March 1991 and October 2010 with 17 patients who were supported with ECMO before intervention, using a matched non-ECMO group as a control.[70] One-year overall survival and allograft function were comparable between both groups, even though the ECMO group had significant perioperative morbidity. The median duration of support was much shorter than other reports (Table 1), a fact that is probably an important determinant of the good outcomes in this series. Also, the short time of pre-ECMO MV (median of 36 h) may have contributed to the results. In this regard, it should be emphasized that there was a significant improvement in the survival of patients supported since 2005, as compared with the patients in the 90s, which is a consequence of the accumulated experience and development of the different parts of the ECMO equipment. It is worth noting that in 3 patients running VV ECMO, a double lumen catheter inserted in the jugular vein was implemented. This catheter is the consequence of the aforementioned improvement in ECMO technology and has special interest as it offers high efficiency with only-1-site cannulation, which allows the patient to move freely. This is of particular interest in the *awake ECMO* strategy that provides particular advantages in the LTx outcomes. The main benefit of this concept is the avoidance of the several deleterious effects of MV and sedation.[13,43,44]

In this sense, the Hannover group has recently reported their experience between August 2008 and March 2011 with 26 patients awaiting LTx who were supported with ECMO while awake, completely substituting the conventional MV by the extracorporeal support.[71] These patients were breathing spontaneously, received active physiotherapy, and could eat and drink. Thus, they could be much better conditioned for transplantation than patients on MV. In this study, a historical group of 34 patients treated with MV before LTx was identified and compared with the *awake ECMO* group. Survival at 6 months after LT was significantly higher in the *awake ECMO* group (80% vs. 50%; $P = 0.02$). Further, this group needed less days on MV after surgery (means of 37 vs. 14 d; $P = 0.04$) and less days in the intensive care unit (means of 39 vs. 18 d; $P = 0.07$) and in hospital stay (means of 67 vs. 38 d; $P = 0.06$). Mortality before LTx was similar between the 2 groups (23% vs. 29%; $P = 0.58$). In the ECMO group, 7 patients (27%) required secondary intubation, and, interestingly, this group had a post-LTx survival rate of only 43% after a 6-month follow-up time. In contrast, in the MV group, ECLS had to be instituted in 18 patients (53%). VV approach could not contribute sufficiently to an adequate oxygenation and the VA mode may lead to a competition between the nonoxygenated native CO and the oxygenated blood flow coming from the arterial cannula, dividing the circulation into a "blue" and a "pink" part in the upper and the lower body, respectively. ECMO modes and the incidence of switching between them in these 5 studies are summarized in Table 1. In patients with PAH, the VA mode is preferred, as a significant reduction of pulmonary arterial pressure is beneficial.[56] This could efficiently substitute IPPV as a method of support for wait-listed patients with PAH in whom noninvasive measures are insufficient. A novel approach in decompensated PAH patients has been proposed by the groups from Toronto and Hannover. It consists of a pumpless lung assist device implemented in patients with PAH and cardiogenic shock as a bridge to thoracic organ transplantation.[74] Remarkably, this method could also be implemented with an *awake ECMO* strategy. In

TABLE 1. Mode of ECMO, Median of Days on ECMO, Incidence of Mode Switching, and 1-Year Survival of the ECMO-bridged Transplanted Patients

References	VA Mode, n (%)	VV Mode, n (%)	Days on ECMO, Median (Range)	Switched, n (%), Cause	1-Year Survival (%)
Hämmäinen et al[69]	6 (46)	7 (54)	12 (1-59)	3 (42), RVF	92
Bermudez et al[70]	9 (53)	8 (47)	3.2 (1-49)	0	74
Fuehner et al[71]	9 (45)	11 (55)	9 (1-45)	3 (27), refractory hypoxemia	80 (6 mo)
Dellgren et al[72]	2 (22)	7 (88)	8 (2-59)	2 (28), RVF	67
Lang et al[73]	12 (35)	19 (56) iLA: 3 (9)	4.5 (1-63)	VV → VA: 2 (10) iLA → VV: 1 (33) iLA → VA: 1 (33) Cause: NS	60

ECMO indicates extracorporeal membrane oxygenation; iLA, interventional lung assist; NS, not specified; RVF, right ventricular failure; VA, venoarterial ECMO; VV, venovenous ECMO.

TABLE 2. Incidence and Causes of Death in Patients on ECMO Before Transplantation

References	Period	Total Patients on ECMO to LTx [n (n/year)]	Patients Dead Before LTx, n (%)	Cause of Death
Hämmäinen et al[69]	2005-2009	16 (3.2)	3 (19)	All: Sepsis and MOF
Bermudez et al[70]	1991-2010	20 (2)	3 (15)	1: Severe brain hypoxia previous to ECMO 2: Circuit thrombosis with inadequate ECMO flow 3: Multiple antibodies, unlikely to suitable donor
Fuehner et al[71]	2008-2011	26 (6.5)	6 (23) MV group: 29%	4: Sepsis and MOF 1: CA during cannulation 1: CA during oxygenator membrane exchange
Dellgren et al[72]	2005-2010	11 (1.8)	2 (18)	All: Sepsis and MOF
Lang et al[73]	1998-2011	38 (2.9)	4 (10)	All: Sepsis and MOF

CA indicates cardiac arrest; ECMO, extracorporeal membrane oxygenation; MOF, multiorgan failure; MV, mechanical ventilation.

this sense, the Michigan group has recently published an experimental study implementing an interatrial shunt and VV ECMO in a model of sheep with acute right ventricular failure obtaining an improvement of ventricular function when right to left atrial shunt was >20%, while maintaining normal arterial blood gases.[75] Thus, percutaneous atrial septostomy and *awake VV ECMO* strategy would be of interest as an alternative to VA ECMO in patients with PAH and right ventricular failure.

The fourth large series, published by Dellgren et al,[72] reported their results on 11 patients who were placed on ECMO to bridge them to LTx from April 2005 to November 2010. Nine patients were transplanted. The authors stressed the fact that they often accepted borderline donors to diminish the time on ECMO and with the objective to anticipate LTx. Still, they found a 1-year survival of 67%.

Finally, in the fifth large report, the Vienna group reviewed their 13-year experience with ECMO bridging to LTx from 1998 to 2011.[73] Thirty-eight patients underwent ECMO support to bridge to LTx. Four patients (10%) died before being transplanted because of septic multiple organ failure. Special attention should be paid to infection prevention, as sepsis-related multiple organ failure is the main reason for death in patients with ECMO support waiting for a compatible allograft (Table 2). From the transplanted patients, 76% were discharged from the hospital and 1-year survival was 60%, which was significantly worse when compared with all other LTx cases within the same period of time (60% vs. 80%; $P = 0.003$). As reported by the authors, mortality of the patients ECMO-bridged to LTx is expected to be 100% without LTx. In this study, ECMO support was maintained in 94% of patients, 41% with inguinal VA cannulation, 44% with central VA cannulation, and 8% with VV running. As the common institutional strategy, in 70% of the cases, VA ECMO support was prolonged into the postoperative period with a median support of 2 days.

INTRAOPERATIVE ECMO: BETTER FEATURES THAN CPB?

The Vienna group summarized their experience on intraoperatory ECMO implementation between January 2001 and January 2006.[54] Of the 306 LTx patients, 27 received CPB intraoperative support and ECMO was installed in 130 cases. One hundred and twelve were placed on ECMO before the intervention, whereas in 18 cases (5.8%) ECMO had to be urgently initiated during the procedure. One-year survival of the intraoperative ECMO group was 74.2%, slightly higher than that of the CPB group (65.9%; $P = 0.41$). Central cannulation was preferred as vein drainage is better and groin morbidity is increased in these patients. Nonetheless, extrathoracic cannulation is also feasible.[73]

However, different results have been published by Bittner et al[76] when comparing CPB (7 patients) with heparin-bonded low-dose heparin ECMO support (8 patients) in LTx surgery from 2003 to 2005. In this study, ECMO support was clearly associated with worse outcomes after LTx surgery. Patients under ECMO showed an increased rate of early posttransplant viral infection and sepsis, augmented incidence of severe graft ischemia/reperfusion, extended ventilator times, and lower 1-year survival.

Another main benefit of ECMO over CPB is the potential to extend support beyond the operation itself, which might be of special interest in patients with intraoperative high Oxygenation Index or elevated pulmonary artery pressure and right ventricle dysfunction. The Austrian group also published their experience with ECMO application in the intraoperative and the early postoperative periods of 17 patients with PAH.[52] They reported a perioperative mortality of 5.9% and a 1-year survival of 88.2%. In 14 patients (82.3%) extracorporeal support was maintained for a median of 12 hours into the postoperative period. The mean pulmonary artery pressure was reduced to 29 (±3.4) from 66 (±15) mm Hg before transplantation and the functional performance of the transplanted lungs was excellent [arterial oxygen pressure measured 2 h after weaning from ECMO of 157 (±28) mm Hg with inspired oxygen fraction of 0.4 on the ventilator].

Therefore, ECMO support could be safely implemented during LTx and it may be of interest in the early postoperative period to avoid the development of pulmonary edema and the progression to PGD, particularly in patients with PAH. However, more evidence is still needed to better identify the subgroup of patients in whom the risk-benefit balance is clearly favorable for this technique.

ECMO SUPPORT IN PGD: DESPERATE MEASURE? LAST RESORT?

PGD is thought to be associated with ischemia-reperfusion injury and other inflammatory events that induce increased capillary permeability. The deteriorated ability to sustain oxygenation and ventilation usually leads to implementation of maximal MV support. However, this method of support could exacerbate pulmonary inflammation. ECMO has been proposed as an alternative therapy in cases of PGD.[76]

TABLE 3. ECMO Criteria for Primary Graft Dysfunction. Frequency of VV and VA Modes and Incidence of Switching

References	Period	Criteria to ECMO	VA Mode Switching	VV Mode Switching
Dahlberg et al[84]	1997-2002	Not uniform	93% peripheral 7% central No switching	0% —
Wigfield et al[83]	1991-2004	Stated individual considering: PaO_2/FiO_2 Static compliance Respiratory status after ventilator optimizing Amount of hemodynamic support	41% peripheral 59% central No switching	0% —
Bermudez et al[82]	1991-2006	Oxygenation and organ perfusion not maintained through conventional methods: VM ($PaO_2 < 60$ mm Hg with $FiO_2 > 80\%$) NO Paralysis High PEEP Vasopressors	25% peripheral 20% central 19% to VV	55% 3% to VA
Hartwig et al[80]	2001-2009	Not specific. Considered if: PIP 35 cm H_2O FiO_2 0.6 Copious pulmonary edema	0% —	100% No switching

ECMO indicates extracorporeal membrane oxygenation; NO, nitric oxide; PEEP, positive end expiratory pressure; PIP, peak inspiratory pressure.

A number of case reports have described successful management of patients utilizing ECMO after LTx.[50,51,77] Fischer et al[78] reviewed the outcomes of the 151 patients (adult and pediatric) with ECMO support for PGD registered in the ELSO archive from January 1987 to December 2005. Ninety-one patients (62%) recovered but only 42% survived until hospital discharge. The predominant ECMO mode used in the adult population was VV, but with a relatively low frequency of 44%. In retrospect, one would expect a higher incidence of VV ECMO application as it has clinical advantages and is related to fewer complications than the VA mode and in the case of PGD only respiratory support should be enough.

Hartwig et al[79] compared the VV and the VA modes in the extracorporeal support for PGD. Of the 23 patients with PGD supported with ECMO in the period between March 1992 and June 2004, the VA mode was chosen in 15 (65%). The VV support was associated with a higher 30-day survival (88% vs. 6%) and less incidence of ECMO-related complications. The same group has recently published their short-term and long-term outcomes of patients with PGD supported with ECMO, comparing them with those of a non-ECMO group.[80] In the period from November 2001 to December 2009, 28 patients required VV ECMO support for severe PGD. ECMO weaning was successful in 28 cases (96%) and 30-day survival was 82%, compared with 97% in the non-ECMO group. However, the ECMO group developed a significant early decrement in a 5-year graft survival of 49%, compared with 61% in the non-ECMO group. Moreover, pulmonary function was considerably worse in the ECMO group (peak forced expiratory volume in 1 s: 58% in ECMO vs. 83% in non-ECMO; $P = 0.001$). These findings are not unexpected, as severe PGD is historically associated with high mortality[47,48] and pulmonary dysfunction.[81] But, interestingly,

TABLE 4. Features of the 3 Different Modes of ECMO Support in Primary Graft Dysfunction

	VA	VV	iLA
Oxygen delivery capacity	++	+	+/−
CO_2 removal capacity	++	++	++
PAP decrease	++	+	+/−
Pulmonary flow	+/− Bronchial anastomotic healing worsened	= Oxygenated	=
Aortic saturation*	++ If no CO +/− If CO preserved and no lung oxygenation	+	=
Hemodynamic support	++	+†	=
Particular disadvantages	Ischemia distal to arterial cannula Risk of system emboli	Recirculation	Distal ischemia to arterial cannula if pumpless AV
Particular advantages	Could be implemented intraoperatively and continued in the PO for PGD prevention	Highly oxygenated blood to the lungs System pulsatility preserved	No systemic anticoagulation needed

++, high; +, medium; +/−, low; =, equal.
*Femoral artery cannulation.
†Not supported but improved.
AV indicates arteriovenous; CO, cardiac output; ECMO, extracorporeal membrane oxygenation; PGD, primary graft dysfunction; PO, postoperatory.

the reported 30-day survival is much higher when compared with that of previous studies.

The Pittsburg group analyzed their outcomes after ECMO use for severe PGD, in the period from March 1991 to March 2006, reporting a 30-day survival of 56% and a 5-year survival of 33%.[82] Survival was similar for the patients supported with VA or VV ECMO. Wigfield et al[83] reported a 30-day survival of 74.6% and a 3-year survival of 36% in the period between 1991 and 2004. Dahlberg et al[84] published their results in a cohort of PGD ECMO-supported patients from 1997 through 2002. The 90-day survival was 60% and the 2-year survival was 46%. In this study, pulmonary function was not significantly different from the overall group (peak forced expiratory volume in 1 s at 2 y: 63% in ECMO vs. 68% in non-ECMO; $P = 0.11$). These discordances between studies could be explained by the fact that the cohorts covered different periods, with different levels of ECMO technology development,[85] and also to different practice of ECMO support (Table 3).

Although the best approach should be to prevent PGD by implementing ECMO early in the postoperative course in high-risk patients, an accurate protocol with well-defined criteria should be established to identify the most appropriate timing for the therapy in LTx patients who develop PGD.[84,86] Features of each ECMO mode for PGD support are summarized in Table 4. The VA approach reduces pulmonary vascular flow, which could modulate the endothelial activation and pulmonary edema secondary to reperfusion injury.[83] However, with VA ECMO bronchial anastomotic healing could be worsened as bronchial artery perfusion is excluded after LTx.[87] Moreover, the VV mode increases aortic oxygen saturation, reduces the risk of systemic embolism and cannulation is relatively easier. Therefore, many authors recommend implementing the VV approach in case of hemodynamic stability or mild hemodynamic instability.[80,82,88] Finally, the adjustment of MV parameters should be standardized as well as the antimicrobial regimen, which could reduce the risk of developing a dangerous complication such as sepsis.[80]

Although the mode and the timing should be better defined in further studies, it seems that there is enough evidence to propose ECMO support not as a last resort option, but as an alternative method of support in lung grafts suffering from severe PGD. However, the cost benefit ratio of the use of ECMO in this group of patients is still difficult to determine. A prospective, multicenter study would provide better evidence about the benefits of ECMO in PGD after LTx.

A MATTER OF TIME

Extracorporeal support is a tool to gain time, maintaining life while awaiting a resolution of the underlying disease. Time is what is vital for LTx wait-listed patients with exacerbated severe pulmonary chronic disease needing invasive support before the arrival of a compatible graft. Optimal care during this waiting period is essential to improve the patient's general condition before surgery. The *awake ECMO* strategy, as a substitute for MV, allows a vital conditioning of the patient during this critical period. Intraoperatively, ECMO can support patients during LTx (as a substitute of CPB) and it can be maintained during the postoperatory time, preventing the emergence of PGD. The conventional approach in the case of severe respiratory insufficiency related to PGD after LTx has deleterious effects in the already injured pulmonary parenchyma, and those effects are worsened if IPPV is maintained for long. There is enough evidence to propose ECMO as an alternative method of support in these patients, partially or totally, sustaining respiratory function and minimizing ventilator-induced injury. However, we still need further well-designed studies to adequately identify the subgroups of patients that could benefit from this technique.

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