HHS Public Access

Author manuscript

Curr Opin Organ Transplant. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Curr Opin Organ Transplant. 2015 October ; 20(5): 506–514. doi:10.1097/MOT.0000000000000232.

Primary graft dysfunction: lessons learned about the first 72 hours after lung transplantation

Mary K. Porteous, M.D[1], **Joshua M. Diamond, M.D., M.S.C.E.**[1,2], and **Jason D. Christie, M.D., M.S.C.E.**[1,2]

[1]Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

[2]Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Abstract

Purpose of Review—In 2005, the International Society for Heart and Lung Transplantation published a standardized definition of primary graft dysfunction (PGD), facilitating new knowledge on this form of acute lung injury that occurs within 72 hours of lung transplantation. PGD continues to be associated with significant morbidity and mortality. This review will summarize the current literature on the epidemiology of PGD, pathogenesis, risk factors, and preventative and treatment strategies.

Recent findings—Since 2011, a number of manuscripts have been published that provide insight into the clinical risk factors and pathogenesis of PGD. In addition, several transplant centers have explored preventative and treatment strategies for PGD, including use of extracorporeal strategies. More recently, results from several trials assessing the role of extracorporeal lung perfusion may allow for much-needed expansion of the donor pool, without raising PGD rates.

Summary—This review will highlight the current state of the science regarding PGD, focusing on recent advances, and set a framework for future preventative and treatment strategies.

Keywords

Primary graft dysfunction (PGD); ischemia-reperfusion injury; lung transplantation; acute lung injury

Introduction

Primary graft dysfunction (PGD) after lung transplantation is a significant source of early morbidity and mortality.(1-7) We will review recently published literature on the epidemiology, pathogenesis, risk factors, prevention, and treatment of PGD.

Corresponding author: Mary Porteous, MD, 839 West Gates, 3400 Spruce Street, Philadelphia, PA 19104, mary.porteous@uphs.upenn.edu.

Conflicts of interest: None

Definition

PGD, a form of acute lung injury occurring within 72 hours of lung transplantation, is characterized by hypoxemia and alveolar infiltrates in the allograft.(2) Early case reports of post-operative allograft dysfunction used different criteria to define the syndrome and referred to it by various names.(4, 8-12) In 2005, the International Society for Heart and Lung Transplantation (ISHLT) standardized the definition of PGD, permitting consistent classification of this clinical syndrome.(2) PGD is now graded based on PaO_2/FiO_2 (P/F) and the presence of bilateral radiographic infiltrates consistent with non-cardiogenic pulmonary edema. The definition also requires exclusion of contributing factors, including hyperacute rejection, pulmonary venous anastomotic obstruction, cardiogenic edema, and pneumonia.(2) The severity of PGD is re-assessed daily up to 72 hours after reperfusion (Table 1). Grade 3 PGD (PGD3) corresponds to a P/F of less than 200 with allograft infiltrates, on ECMO or ventilated with FiO2 above 0.5 and on iNO. (2)

PGD3 occurring at any time point after reperfusion is associated with greater alteration in plasma markers of lung injury and higher 30-day mortality compared to those with lower grades.(1) However, even among patients with PGD3, there is variability in the duration of pulmonary edema and patient mortality,(13) leading to a belief that separate endotypes may be indicative of differences in injury response. In 2015, the ISHLT began a second consensus effort to update and refine the PGD definition; therefore, updated criteria should be forthcoming addressing issues including timing of PGD onset and resolution, precision of the P/F at different levels of FiO_2, single versus bilateral transplants, and differences in grading fibrotic lung disease patients.

Epidemiology and Outcomes

Since 2005, the incidence of PGD3 at a single time point has been reported to be 7.9% and 25%, whereas the incidence of PGD3 at any time point during the first three days is approximately 30%.(1, 5, 7, 14-17) The variability is likely attributable to differences in institutional practices and risk factor distributions.

PGD is associated with significant early and late post-transplant morbidity. Regardless of the time point of grading, PGD3 has been associated with significantly longer hospital length of stay, duration of mechanical ventilation, and 90-day mortality than those with lower grades of PGD.(1, 4, 5, 14, 16, 17) PGD3 present at 48 or 72 hours after transplantation was associated with an 18% increased absolute risk of 90-day mortality(14), with increased mortality persisting at 1, 5, and 10 years after transplantation.(5, 7, 14-17) In addition, all PGD is associated with elevated risk of bronchiolitis obliterans syndrome (BOS), a form of chronic allograft dysfunction.(5, 7, 15, 16) Future mechanistic trials are needed to understand the relationship between PGD and the development of BOS.

Pathogenesis

PGD represents the end result of multiple deleterious mechanisms provoked by donor brain death, mechanical ventilation, procurement, storage, and ischemia reperfusion injury (IRI). IRI refers to sterile inflammation that occurs after substrate supply is restored following a

period of absent blood flow.(18) While many mechanisms are at play, recent studies highlight the importance of the interplay between innate immune activation, epithelial cell injury, endothelial cell dysfunction, and cytokine release(19) (Table 2).

Innate immune activation

Upregulation of innate immune pathways, including toll-like receptors (TLR) and NOD-like receptor (NLR) inflammasome signaling, has been demonstrated in *vivo* animal models following IRI and in human subjects with PGD.(35-38, 40-47) Animal models suggests that IRI occurs in two phases mediated in part by a bimodal response by the innate immune system with donor macrophages and lymphocytes inducing the early phase and recipient monocytes, T-cells, and neutrophils inducing the late phase.(40-47)

A family of innate immune cells, innate lymphoid cells (ILCs) have recently been recognized for their role in inflammation regulation, barrier protection and repair, and host defense. While ILCs have lymphoid morphology, they lack antigen receptors and can be subdivided based on cytokine expression and function.(39, 48, 49) Group 2 ILCs (ILC2) produce type 2 T-helper cell-associated cytokines and have been isolated in both human blood and lung, suggesting a trafficking ability. In animal models, ILC2 cells appear to play an important role in airway epithelial integrity and airway remodeling.(39) Isolation of ILC2 cells in BAL of transplant recipients warrants further exploration of the role of ILCs and other innate immune cells in PGD.(39)

Epithelial cell injury

Numerous markers of epithelial cell injury have also been associated with PGD in both animal and human studies, including receptor for advanced glycation end products (RAGE), type V collage, and plasma clara cell secretory protein. (20-25, 50-52) The role of traumatic brain injury (TBI) in the development of epithelial cell injury and IRI has been of increasing interest as the majority of lungs used for transplantation are procured from patients who have suffered TBI. High-mobility group box protein 1 (HMGB1) is a danger-associated molecular pattern released from necrotic neurons after TBI as well as alveolar macrophages after IRI. HMGB1 binds TLR4 and RAGE, inducing cytokine release and tissue damage. (26, 45) Animal models suggest that HMGB1-induced RAGE activation may contribute to IRI via activation of nuclear factor KB (NF-KB) in epithelial cells and production of IL-17 by natural killer T cells.(26, 45) Elevated levels of HMGB1 from brain-dead donors were associated with lower P/F before and after human lung transplantation.(26, 53) Therapeutic strategies to block RAGE and decrease epithelial cell injury should be explored in recipients of lungs from brain-dead donors post-TBI.

Endothelial dysfunction

Endothelial dysfunction in lung IRI typically results mostly from disruption and restoration of blood flow with minimal contribution from tissue hypoxia and reoxygenation since the lung does not rely on perfusion for oxygenation.(18) Changes in blood flow during ischemia lead to closure of inward rectifying potassium channels and subsequent endothelial cell depolarization.(54-56) This depolarization results in production of reactive oxygen species

(ROS) and nitric oxide,(57-60) increasing cell adhesion molecules for leukocyte adhesion and extravasation and activating NF-KB and other transcription factors.(18, 59, 61) Subsequent reperfusion results in hyperpolization of endothelial cells and activation of a similar cascade as ischemia, the end result of which is further oxidative injury.(62) Future potential targets to modify endothelial activation and decrease PGD may include maintenance of lung perfusion with extracorporeal lung perfusion, administration of potassium channel agonists, and inhibition of ROS production.(18, 63)

In addition to inducing oxidative injury, disruption in blood flow to the lung may disrupt the endothelial cell barrier and induce vascular remodeling. Sphingosine 1-phosphate (S1P) controls endothelial cell tight junction formation and prevents chemotaxis. S1P supplementation prior to reperfusion decreased inflammatory cytokines and improved oxygenation in animal models.(64, 65) Several animal and human studies have demonstrated an association between IRI and mediators of vascular permeability and angiogenesis including vascular endothelial growth factor and angiopoietin-2.(27-30, 66-68) Further trials are needed to explore the role of IRI on endothelial integrity and vascular remodeling.

Chemotaxis and cytokines

In many solid organ models, IRI has been shown to stimulate the release of proinflammatory cytokines as well as chemokines involved in migration of recipient immune cells including IL-1β, IL-6, IL-8, IL-11, interferon (IFN)-gamma, and tumor necrosis factor (TNF) α.(44, 69-78) These findings have been confirmed in human lung observational studies and may represent future therapeutic targets.(31-34)

Clinical risk factors

Many groups have studied PGD risk factors (Table 3).(6, 11, 12, 14, 27, 79-94) Variability in reported risk factors may be attributed to single center studies, differences in peri-operative management between centers, and use of non-standardized PGD definitions; however, several donor, recipient, and procedural risk factors have consistently emerged.

Donor-related clinical risk factors

Reported donor risk factors include age, African American race, female gender, and history of tobacco exposure (Table 3).(6, 14, 80-83, 99) Smoke exposure is hypothesized to increase oxidative injury and thus IRI.(101) In donor lungs deemed unsuitable for transplantation, pulmonary edema and IL-8 were higher in donors who currently smoked although alveolar fluid clearance was lower in donors with greater than 20-pack year history compared to those with a less than 20-pack year history, suggesting a possible dose-related effect.(102) The impact of donor smoke exposure on PGD risk is difficult to ascertain as individual studies use different smoking cut-offs and smoking status is obtained from surrogates. Further studies are needed to clarify the role of donor smoke exposure on PGD. A recent study using registry data suggested that although donor smoke exposure was associated with worse recipient outcomes, the survival probability after receiving such an allograft still exceeded that of remaining on the waiting list.(103)

Donor-acquired risk factors, including prolonged mechanical ventilation, aspiration, and trauma, are potential risk factors without definitive studies demonstrating consistent associations with PGD.(81) Efforts are needed to standardize donor management and to optimize donor-recipient matching.

Recipient-related risk factors

Reported recipient risk factors for PGD include obesity, pulmonary hypertension, and diagnoses of idiopathic pulmonary fibrosis or sarcoidosis (Table 3). (6, 11, 14, 79, 80, 82, 89, 97, 98, 100) After adjustment for multiple risk factors, obese and overweight recipient body mass index (BMI) were independent predictors of PGD. Higher levels of leptin, which regulates adipose tissue mass and has inflammatory properties, were also associated with increased PGD risk and mortality.(97) This supports previous studies showing obesity increases the risk of acute lung injury.(104, 105) While increasing adiposity is associated with PGD, it is unclear how best to assess body composition given a recent study demonstrating that BMI is a poor measure of adioposity.(106) Following identification of a reliable measure of body composition, future studies should evaluate the role of adipokines in cytokine release and PGD.

Peri-operative risk factors

The operative risk factors for PGD reported in multi-center cohort studies are single lung transplant procedure type, prolonged ischemic time, cardiopulmonary bypass (CPB) use, greater than 1L packed red blood cell transfusion volume, use of Euro-Collins preservation solution, and reperfusion FiO_2 greater than 0.4 (Table 3).(12, 14, 82, 87, 89, 90, 95, 96) Oversized allografts have been associated with a decreased risk of PGD3 and improved survival in bilateral lung transplant recipients, especially in non-COPD recipients.(107, 108)

Significant operative variability exists across transplant centers making it difficult to identify consistent operative risk factors. Confounding by disease severity or treatment indication further complicates the study of operative risk factors. Future trials should distinguish between emergent and planned use of cardiopulmonary bypass, evaluate the effect of lower reperfusion FiO_2 on PGD, and explore the mechanisms by which the above operative factors increase PGD risk.

Prevention and treatment

Several agents have been investigated to prevent PGD.(109) While some observational trials of inhaled nitric oxide (iNO) suggest improved clinical outcomes, randomized control trials failed to show a definitive effect of iNO on PGD prevention when used routinely.(110-116) Soluble complement receptor 1 inhibitor, plasminogen activating factor antagonist, and exogenous surfactant demonstrated beneficial effects on surrogates of PGD including A-a gradient.(117-120) A trial of aprotinin failed to detect an effect on PGD risk, and was stopped early out of concern for potential renal toxicity.(121) Clearly, additional trials on PGD prevention are needed.

Therapy for PGD remains largely supportive and is heavily influenced by acute respiratory distress syndrome (ARDS) management strategies. While most centers use low-stretch ventilation, tidal volumes are frequently selected based on recipient characteristics with little consideration given to donor characteristics.(122-124) While there no proven role for iNO in the prevention of PGD, it has been used as salvage therapy for severe allograft dysfunction following transplant and may be useful in patients with refractory hypoxemia post-transplant.(125-127)

Veno-arterial (VA) extracorporeal membrane oxygenation (ECMO) has been studied for refractory hypoxemia and hemodynamic instability after lung transplant.(128-134) Given the link between CPB use and PGD, several groups have evaluated replacing CPB intraoperatively with VA-ECMO with mixed results.(135-137) VA-ECMO has been associated with shorter duration of mechanical ventilation and ICU/hospital length of stay, and lower transfusion requirements, but no statistically significant difference in 90-day mortality.(137) With improvement in ECMO technology, including high performance membranes and coated circuits, veno-venous (VV) ECMO has been increasingly used with similar post-transplant outcomes and survival as VA-ECMO.(131, 135) Several groups have evaluated the use of extracorporeal life support (ECLS) with VV, VA, and arterio-venous ECMO as a bridge to lung transplantation. Future research is needed to identify optimal candidates, standardize management strategies, and understand the impact of ECLS on PGD risk.(138-146)

Future directions

Several commercial devices using extracorporeal lung perfusion technology have been created and differ by pump type, flow type, mobility, perfusate, and presence of an internal ventilator. Extracorporeal lung perfusion has been associated with increased lung utilization, similar or lower risk of PGD, and equivalent 30-day, 1-year, and 3-year survival compared to standard criteria donors.(147-159) Small case series have described similar incidence of acute rejection and respiratory infection up to 1 year after transplant.(150, 153) Use of these technologies for donation after circulatory determination of death have demonstrated similar survival as donation after neurological determination of death.(151) A portable extracorporeal lung perfusion device was created to minimize ischemic time. While case reports of this portable extracorporeal perfusion system are promising,(160, 161) the results of randomized control trials are pending.(162, 163) Extracorporeal lung perfusion may eventually allow safe expansion of the donor pool and serve as a vehicle for testing targeted therapeutics to improve organ quality and decrease PGD risk. Several multicenter prospective trials are underway to further evaluate this technology, with PGD as an endpoint.

Conclusions

PGD is associated with poor short and long-term outcomes. The ISHLT definition has improved our ability to study this clinical syndrome, but further refinement of the definition is still needed and should be forthcoming. Improved understanding of risk factors and pathogenesis will identify potential therapeutic targets to modify the risk of PGD. As the

acceptable recipient pool likely expands secondary to increasing ECLS use, continued safe expansion of the donor pool must follow and may be possible given the promising results seen with extracorporeal lung perfusion.

Acknowledgements

None

Financial support and sponsorship: National Institutes of Health [Grants: T32 HL-007891, K23 HL-121406] and Actelion Entelligence Grant

References

1. Christie JD, Bellamy S, Ware LB, et al. Construct validity of the definition of primary graft dysfunction after lung transplantation. J Heart Lung Transplant. Nov; 2010 29(11):1231–9. PubMed PMID: 20655249. Pubmed Central PMCID: PMC2963709. Epub 2010/07/27. [PubMed: 20655249]
2. Christie JD, Carby M, Bag R, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. Oct; 2005 24(10):1454–9. PubMed PMID: 16210116. Epub 2005/10/08. [PubMed: 16210116]
3. Christie JD, Kotloff RM, Ahya VN, et al. The effect of primary graft dysfunction on survival after lung transplantation. Am J Respir Crit Care Med. Jun 1; 2005 171(11):1312–6. PubMed PMID: 15764726. Pubmed Central PMCID: 2718464. [PubMed: 15764726]
4. Christie JD, Sager JS, Kimmel SE, et al. Impact of primary graft failure on outcomes following lung transplantation. Chest. Jan; 2005 127(1):161–5. PubMed PMID: 15653978. [PubMed: 15653978]
5. Daud SA, Yusen RD, Meyers BF, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. Am J Respir Crit Care Med. Mar 1; 2007 175(5):507–13. PubMed PMID: 17158279. Epub 2006/12/13. [PubMed: 17158279]
6. Whitson BA, Nath DS, Johnson AC, et al. Risk factors for primary graft dysfunction after lung transplantation. J Thorac Cardiovasc Surg. Jan; 2006 131(1):73–80. PubMed PMID: 16399297. Epub 2006/01/10. [PubMed: 16399297]
7. Whitson BA, Prekker ME, Herrington CS, et al. Primary graft dysfunction and long-term pulmonary function after lung transplantation. J Heart Lung Transplant. Oct; 2007 26(10):1004–11. PubMed PMID: 17919620. Epub 2007/10/09. [PubMed: 17919620]
8. Chatila WM, Furukawa S, Gaughan JP, et al. Respiratory failure after lung transplantation. Chest. Jan; 2003 123(1):165–73. PubMed PMID: 12527618. [PubMed: 12527618]
9. Christie JD, Bavaria JE, Palevsky HI, et al. Primary graft failure following lung transplantation. Chest. Jul; 1998 114(1):51–60. PubMed PMID: 9674447. [PubMed: 9674447]
10. Khan SU, Salloum J, O'Donovan PB, et al. Acute pulmonary edema after lung transplantation: the pulmonary reimplantation response. Chest. Jul; 1999 116(1):187–94. PubMed PMID: 10424524. [PubMed: 10424524]
11. King RC, Binns OA, Rodriguez F, et al. Reperfusion injury significantly impacts clinical outcome after pulmonary transplantation. Ann Thorac Surg. Jun; 2000 69(6):1681–5. PubMed PMID: 10892906. [PubMed: 10892906]
12. Thabut G, Vinatier I, Stern JB, et al. Primary graft failure following lung transplantation: predictive factors of mortality. Chest. Jun; 2002 121(6):1876–82. PubMed PMID: 12065352. Epub 2002/06/18. [PubMed: 12065352]
13. Shah RJ, Diamond JM, Cantu E, et al. Latent class analysis identifies distinct phenotypes of primary graft dysfunction after lung transplantation. Chest. Aug; 2013 144(2):616–22. PubMed PMID: 23429890. Pubmed Central PMCID: PMC3734891. Epub 2013/02/23. [PubMed: 23429890]
14. Diamond JM, Lee JC, Kawut SM, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. American journal of respiratory and critical care medicine. 2013; 187(5): 527–34. [PubMed: 23306540]

15. Huang HJ, Yusen RD, Meyers BF, et al. Late primary graft dysfunction after lung transplantation and bronchiolitis obliterans syndrome. Am J Transplant. Nov; 2008 8(11):2454–62. PubMed PMID: 18785961. Pubmed Central PMCID: 2678949. [PubMed: 18785961]

16. Kreisel D, Krupnick AS, Puri V, et al. Short- and long-term outcomes of 1000 adult lung transplant recipients at a single center. J Thorac Cardiovasc Surg. Jan; 2011 141(1):215–22. PubMed PMID: 21093882. [PubMed: 21093882]

17. Prekker ME, Nath DS, Walker AR, et al. Validation of the proposed International Society for Heart and Lung Transplantation grading system for primary graft dysfunction after lung transplantation. J Heart Lung Transplant. Apr; 2006 25(4):371–8. PubMed PMID: 16563963. [PubMed: 16563963]

18. Chatterjee S, Nieman GF, Christie JD, et al. Shear stress-related mechanosignaling with lung ischemia: lessons from basic research can inform lung transplantation. American journal of physiology Lung cellular and molecular physiology. Nov 1; 2014 307(9):L668–80. PubMed PMID: 25239915. Pubmed Central PMCID: 4217004. *This review outlines the mechanisms important in the mechanosignaling cascase that senses changes in blood flow in lung IRI and identifies several potential strategies to reduce IRI in lung transplant recipients. [PubMed: 25239915]

19. Diamond JM, Christie JD. The contribution of airway and lung tissue ischemia to primary graft dysfunction. Curr Opin Organ Transplant. Oct; 2010 15(5):552–7. PubMed PMID: 20693898. [PubMed: 20693898]

20. Briot R, Frank JA, Uchida T, et al. Elevated levels of the receptor for advanced glycation end products, a marker of alveolar epithelial type I cell injury, predict impaired alveolar fluid clearance in isolated perfused human lungs. Chest. Feb; 2009 135(2):269–75. PubMed PMID: 19017890. Pubmed Central PMCID: 2714162. [PubMed: 19017890]

21. Christie JD, Shah CV, Kawut SM, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. Am J Respir Crit Care Med. Nov 15; 2009 180(10):1010–5. PubMed PMID: 19661249. Pubmed Central PMCID: 2778153. [PubMed: 19661249]

22. Pelaez A, Force SD, Gal AA, et al. Receptor for advanced glycation end products in donor lungs is associated with primary graft dysfunction after lung transplantation. Am J Transplant. Apr; 2010 10(4):900–7. PubMed PMID: 20121754. Pubmed Central PMCID: 2871333. [PubMed: 20121754]

23. Bobadilla JL, Love RB, Jankowska-Gan E, et al. Th-17, monokines, collagen type V, and primary graft dysfunction in lung transplantation. Am J Respir Crit Care Med. Mar 15; 2008 177(6):660–8. PubMed PMID: 18174545. Pubmed Central PMCID: 2267340. [PubMed: 18174545]

24. Iwata T, Philipovskiy A, Fisher AJ, et al. Anti-type V collagen humoral immunity in lung transplant primary graft dysfunction. Journal of immunology. Oct 15; 2008 181(8):5738–47. PubMed PMID: 18832733. Pubmed Central PMCID: 2997998.

25. Diamond JM, Kawut SM, Lederer DJ, et al. Elevated plasma clara cell secretory protein concentration is associated with high-grade primary graft dysfunction. Am J Transplant. Mar; 2011 11(3):561–7. PubMed PMID: 21299834. Pubmed Central PMCID: 3079443. [PubMed: 21299834]

26. Weber DJ, Gracon AS, Ripsch MS, et al. The HMGB1-RAGE axis mediates traumatic brain injury-induced pulmonary dysfunction in lung transplantation. Science translational medicine. Sep 3.2014 6(252):252ra124. PubMed PMID: 25186179. ** Blockade of RAGE and neutralization of HMGB-1 attenuated acute lung injury due to TBI in an animal model and elevated donor HMGB-1 was associated with lower P/F after transplantation. These findings suggest that the HMGB-1-RAGE axis plays a role in lung dysfunction after brain death, which has important implications for donation after brain determination of death.

27. Krenn K, Klepetko W, Taghavi S, et al. Recipient vascular endothelial growth factor serum levels predict primary lung graft dysfunction. Am J Transplant. Mar; 2007 7(3):700–6. PubMed PMID: 17250560. Epub 2007/01/26. [PubMed: 17250560]

28. Krenn K, Klepetko W, Taghavi S, et al. Vascular endothelial growth factor increases pulmonary vascular permeability in cystic fibrosis patients undergoing lung transplantation. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Jul; 2007 32(1):35–41. PubMed PMID: 17481912. [PubMed: 17481912]

29. Salama M, Andrukhova O, Hoda MA, et al. Concomitant endothelin-1 overexpression in lung transplant donors and recipients predicts primary graft dysfunction. Am J Transplant. Mar; 2010 10(3):628–36. PubMed PMID: 20055806. [PubMed: 20055806]
30. Diamond JM, Porteous MK, Cantu E, et al. Elevated plasma angiopoietin-2 levels and primary graft dysfunction after lung transplantation. PLoS One. 2012; 7(12):e51932. PubMed PMID: 23284823. Pubmed Central PMCID: 3526525. [PubMed: 23284823]
31. Hoffman SA, Wang L, Shah CV, et al. Plasma cytokines and chemokines in primary graft dysfunction post-lung transplantation. Am J Transplant. Feb; 2009 9(2):389–96. PubMed PMID: 19120076. Pubmed Central PMCID: 2821938. [PubMed: 19120076]
32. De Perrot M, Sekine Y, Fischer S, et al. Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation. Am J Respir Crit Care Med. Jan 15; 2002 165(2): 211–5. PubMed PMID: 11790657. [PubMed: 11790657]
33. Machuca TN, Cypel M, Yeung JC, et al. Protein expression profiling predicts graft performance in clinical ex vivo lung perfusion. Annals of surgery. Mar; 2015 261(3):591–7. PubMed PMID: 25371129. *This article demonstrated that IL-8 and growth-regulated oncogene-α in the extracorporeal lung perfusate distinguished between lungs with a favorable outcome compared to those that developed grade 3 PGD following lung transplantation. [PubMed: 25371129]
34. Moreno I, Vicente R, Ramos F, et al. Determination of interleukin-6 in lung transplantation: association with primary graft dysfunction. Transplantation proceedings. Sep; 2007 39(7):2425–6. PubMed PMID: 17889209. [PubMed: 17889209]
35. Cantu E, Lederer DJ, Meyer K, et al. Gene set enrichment analysis identifies key innate immune pathways in primary graft dysfunction after lung transplantation. Am J Transplant. Jul; 2013 13(7):1898–904. PubMed PMID: 23710539. Pubmed Central PMCID: 3954988. [PubMed: 23710539]
36. Diamond JM FR, Meyer NJ, Shah RJ, Kawut SM, Lederer DJ, Lee J, Ahya VN, Cantu E, Weinacker A, Bhorade S, Lama VN, Orens JB, Sonett J, Wille KM, Crespo M, Weill D, Kohl B, Arcasoy SM, Shah AS, Shah PD, Demissie E, Reynolds JM, Belperio JA, Wilkes D, Ware LB, Palmer SM, Christie JD. Functional TLR4 polymorphisms are associated with lower risk of primary graft dysfunction (PGD) after lung transplantation. The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation. 2012; 31(4):S76.
37. Diamond JM, Lederer DJ, Kawut SM, et al. Elevated plasma long pentraxin-3 levels and primary graft dysfunction after lung transplantation for idiopathic pulmonary fibrosis. Am J Transplant. Nov; 2011 11(11):2517–22. PubMed PMID: 21883907. Pubmed Central PMCID: 3206646. [PubMed: 21883907]
38. Diamond JM, Meyer NJ, Feng R, et al. Variation in PTX3 is associated with primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med. Sep 15; 2012 186(6):546–52. PubMed PMID: 22822025. Pubmed Central PMCID: 3480532. [PubMed: 22822025]
39. Monticelli LA, Sonnenberg GF, Abt MC, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. Nature immunology. Nov; 2011 12(11):1045–54. PubMed PMID: 21946417. Pubmed Central PMCID: PMC3320042. Epub 2011/09/29. [PubMed: 21946417]
40. de Perrot M, Liu M, Waddell TK, et al. Ischemia-reperfusion-induced lung injury. Am J Respir Crit Care Med. Feb 15; 2003 167(4):490–511. PubMed PMID: 12588712. Epub 2003/02/18. [PubMed: 12588712]
41. Eppinger MJ, Jones ML, Deeb GM, et al. Pattern of injury and the role of neutrophils in reperfusion injury of rat lung. The Journal of surgical research. Jun; 1995 58(6):713–8. PubMed PMID: 7791351. Epub 1995/06/01. [PubMed: 7791351]
42. Fiser SM, Tribble CG, Long SM, et al. Lung transplant reperfusion injury involves pulmonary macrophages and circulating leukocytes in a biphasic response. J Thorac Cardiovasc Surg. Jun; 2001 121(6):1069–75. PubMed PMID: 11385373. Epub 2001/06/01. [PubMed: 11385373]
43. Johnston LK, Rims CR, Gill SE, et al. Pulmonary macrophage subpopulations in the induction and resolution of acute lung injury. American journal of respiratory cell and molecular biology. Oct; 2012 47(4):417–26. PubMed PMID: 22721830. Pubmed Central PMCID: PMC3927911. Epub 2012/06/23. [PubMed: 22721830]

44. Naidu BV, Krishnadasan B, Farivar AS, et al. Early activation of the alveolar macrophage is critical to the development of lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg. Jul; 2003 126(1):200–7. PubMed PMID: 12878956. Epub 2003/07/25. [PubMed: 12878956]
45. Sharma AK, LaPar DJ, Zhao Y, et al. Natural killer T cell-derived IL-17 mediates lung ischemia-reperfusion injury. Am J Respir Crit Care Med. Jun 1; 2011 183(11):1539–49. PubMed PMID: 21317314. Pubmed Central PMCID: PMC3137143. Epub 2011/02/15. [PubMed: 21317314]
46. van der Kaaij NP, Kluin J, Haitsma JJ, et al. Ischemia of the lung causes extensive long-term pulmonary injury: an experimental study. Respiratory research. 2008; 9:28. PubMed PMID: 18366783. Pubmed Central PMCID: PMC2335107. Epub 2008/03/28. [PubMed: 18366783]
47. Yang Z, Sharma AK, Linden J, et al. CD4+ T lymphocytes mediate acute pulmonary ischemia-reperfusion injury. J Thorac Cardiovasc Surg. Mar; 2009 137(3):695–702. discussion PubMed PMID: 19258091. Pubmed Central PMCID: PMC2670927. Epub 2009/03/05. [PubMed: 19258091]
48. Artis D, Spits H. The biology of innate lymphoid cells. Nature. Jan 15; 2015 517(7534):293–301. PubMed PMID: 25592534. *This review outlines the structure and function of innate lymphoid cells. [PubMed: 25592534]
49. Monticelli LA, Sonnenberg GF, Artis D. Innate lymphoid cells: critical regulators of allergic inflammation and tissue repair in the lung. Current opinion in immunology. Jun; 2012 24(3):284–9. PubMed PMID: 22521139. Pubmed Central PMCID: PMC3383398. Epub 2012/04/24. [PubMed: 22521139]
50. Haque MA, Mizobuchi T, Yasufuku K, et al. Evidence for immune responses to a self-antigen in lung transplantation: role of type V collagen-specific T cells in the pathogenesis of lung allograft rejection. Journal of immunology. Aug 1; 2002 169(3):1542–9. PubMed PMID: 12133982.
51. Yoshida S, Haque A, Mizobuchi T, et al. Anti-type V collagen lymphocytes that express IL-17 and IL-23 induce rejection pathology in fresh and well-healed lung transplants. Am J Transplant. Apr; 2006 6(4):724–35. PubMed PMID: 16539629. [PubMed: 16539629]
52. Sharma AK, LaPar DJ, Stone ML, et al. Receptor for advanced glycation end products (RAGE) on iNKT cells mediates lung ischemia-reperfusion injury. Am J Transplant. Sep; 2013 13(9):2255–67. PubMed PMID: 23865790. Pubmed Central PMCID: 3776006. [PubMed: 23865790]
53. Weber DJ, Allette YM, Wilkes DS, et al. The HMGB1-RAGE Inflammatory Pathway: Implications for Brain Injury-Induced Pulmonary Dysfunction. Antioxidants & redox signaling. May 14.2015 PubMed PMID: 25751601. Epub 2015/03/10. *This review outlines the studies important to the understanding of the HMGB1-RAGE pathway and its role in lung injury.
54. Chatterjee S, Levitan I, Wei Z, et al. KATP channels are an important component of the shear-sensing mechanism in the pulmonary microvasculature. Microcirculation. Dec; 2006 13(8):633–44. PubMed PMID: 17085424. [PubMed: 17085424]
55. al-Mehdi AB, Ischiropoulos H, Fisher AB. Endothelial cell oxidant generation during K(+)-induced membrane depolarization. Journal of cellular physiology. Feb; 1996 166(2):274–80. PubMed PMID: 8591986. [PubMed: 8591986]
56. al-Mehdi AB, Shuman H, Fisher AB. Oxidant generation with K(+)-induced depolarization in the isolated perfused lung. Free radical biology & medicine. 1997; 23(1):47–56. PubMed PMID: 9165296. [PubMed: 9165296]
57. Al-Mehdi AB, Zhao G, Dodia C, et al. Endothelial NADPH oxidase as the source of oxidants in lungs exposed to ischemia or high K+ Circ Res. Oct 5; 1998 83(7):730–7. PubMed PMID: 9758643. [PubMed: 9758643]
58. Manevich Y, Al-Mehdi A, Muzykantov V, et al. Oxidative burst and NO generation as initial response to ischemia in flow-adapted endothelial cells. American journal of physiology Heart and circulatory physiology. May; 2001 280(5):H2126–35. PubMed PMID: 11299214. [PubMed: 11299214]
59. Wei Z, Costa K, Al-Mehdi AB, et al. Simulated ischemia in flow-adapted endothelial cells leads to generation of reactive oxygen species and cell signaling. Circ Res. Oct 15; 1999 85(8):682–9. PubMed PMID: 10521241. [PubMed: 10521241]

60. Corson MA, James NL, Latta SE, et al. Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. Circ Res. Nov; 1996 79(5):984–91. PubMed PMID: 8888690. [PubMed: 8888690]

61. Sharma AK, Mulloy DP, Le LT, et al. NADPH oxidase mediates synergistic effects of IL-17 and TNF-alpha on CXCL1 expression by epithelial cells after lung ischemia-reperfusion. American journal of physiology Lung cellular and molecular physiology. Jan 1; 2014 306(1):L69–79. PubMed PMID: 24186876. Pubmed Central PMCID: 3920214. ***This study demonstrated that IL-17 and TNF-α induce CXCL1 production in a NADPH oxidase-dependent mechanism. Since CXCL1 is a potent neutrophil attractant, understanding of this mechanism may allow for targeted therapy to attenuate neutrophil infiltration and IRI. [PubMed: 24186876]

62. Olesen SP, Clapham DE, Davies PF. Haemodynamic shear stress activates a K+ current in vascular endothelial cells. Nature. Jan 14; 1988 331(6152):168–70. PubMed PMID: 2448637. [PubMed: 2448637]

63. Kozower BD, Christofidou-Solomidou M, Sweitzer TD, et al. Immunotargeting of catalase to the pulmonary endothelium alleviates oxidative stress and reduces acute lung transplantation injury. Nature biotechnology. Apr; 2003 21(4):392–8. PubMed PMID: 12652312.

64. Okazaki M, Kreisel F, Richardson SB, et al. Sphingosine 1-phosphate inhibits ischemia reperfusion injury following experimental lung transplantation. Am J Transplant. Apr; 2007 7(4):751–8. PubMed PMID: 17391120. [PubMed: 17391120]

65. Stone ML, Sharma AK, Zhao Y, et al. Sphingosine-1-phosphate receptor 1 agonism attenuates lung ischemia-reperfusion injury. American journal of physiology Lung cellular and molecular physiology. Apr 24.2015 ajplung 00302 2014. PubMed PMID: 25910934. Epub 2015/04/26. *Treatment with a sphingosine-1-phosphate receptor agonist decreased pulmonary artery pressure, vascular permeability, and expression of proinflammatory cytokines in a rat model.

66. Abraham D, Taghavi S, Riml P, et al. VEGF-A and -C but not -B mediate increased vascular permeability in preserved lung grafts. Transplantation. Jun 15; 2002 73(11):1703–6. PubMed PMID: 12084990. [PubMed: 12084990]

67. Taghavi S, Abraham D, Riml P, et al. Co-expression of endothelin-1 and vascular endothelial growth factor mediates increased vascular permeability in lung grafts before reperfusion. J Heart Lung Transplant. May; 2002 21(5):600–3. PubMed PMID: 11983551. [PubMed: 11983551]

68. van der Heijden M, van Nieuw Amerongen GP, Koolwijk P, et al. Angiopoietin-2, permeability oedema, occurrence and severity of ALI/ARDS in septic and non-septic critically ill patients. Thorax. Oct; 2008 63(10):903–9. PubMed PMID: 18559364. [PubMed: 18559364]

69. Lemay S, Rabb H, Postler G, et al. Prominent and sustained up-regulation of gp130-signaling cytokines and the chemokine MIP-2 in murine renal ischemia-reperfusion injury. Transplantation. Mar 15; 2000 69(5):959–63. PubMed PMID: 10755557. [PubMed: 10755557]

70. Gerlach J, Jorres A, Nohr R, et al. Local liberation of cytokines during liver preservation. Transplant international : official journal of the European Society for Organ Transplantation. 1999; 12(4):261–5. PubMed PMID: 10460871. [PubMed: 10460871]

71. Oz MC, Liao H, Naka Y, et al. Ischemia-induced interleukin-8 release after human heart transplantation. A potential role for endothelial cells. Circulation. Nov 1; 1995 92(9 Suppl):II428–32. PubMed PMID: 7586450. [PubMed: 7586450]

72. Serrick C, Adoumie R, Giaid A, et al. The early release of interleukin-2, tumor necrosis factor-alpha and interferon-gamma after ischemia reperfusion injury in the lung allograft. Transplantation. Dec 15; 1994 58(11):1158–62. PubMed PMID: 7992355. [PubMed: 7992355]

73. Zhao M, Fernandez LG, Doctor A, et al. Alveolar macrophage activation is a key initiation signal for acute lung ischemia-reperfusion injury. American journal of physiology Lung cellular and molecular physiology. Nov; 2006 291(5):L1018–26. PubMed PMID: 16861385. [PubMed: 16861385]

74. Spahn JH, Li W, Bribriesco AC, et al. DAP12 expression in lung macrophages mediates ischemia/reperfusion injury by promoting neutrophil extravasation. Journal of immunology. Apr 15; 2015 194(8):4039–48. PubMed PMID: 25762783. Pubmed Central PMCID: 4401083. *This study demonstrated the role of alveolar macrophage in production of chemokines involved in leukocyte attraction and extravasation.

75. Krishnadasan B, Naidu BV, Byrne K, et al. The role of proinflammatory cytokines in lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg. Feb; 2003 125(2):261–72. PubMed PMID: 12579094. [PubMed: 12579094]

76. Naidu BV, Woolley SM, Farivar AS, et al. Early tumor necrosis factor-alpha release from the pulmonary macrophage in lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg. May; 2004 127(5):1502–8. PubMed PMID: 15116014. [PubMed: 15116014]

77. Gelman AE, Okazaki M, Sugimoto S, et al. CCR2 regulates monocyte recruitment as well as CD4 T1 allorecognition after lung transplantation. Am J Transplant. May; 2010 10(5):1189–99. PubMed PMID: 20420631. Pubmed Central PMCID: 3746750. [PubMed: 20420631]

78. Kreisel D, Nava RG, Li W, et al. In vivo two-photon imaging reveals monocyte-dependent neutrophil extravasation during pulmonary inflammation. Proceedings of the National Academy of Sciences of the United States of America. Oct 19; 2010 107(42):18073–8. PubMed PMID: 20923880. Pubmed Central PMCID: 2964224. [PubMed: 20923880]

79. Barr ML, Kawut SM, Whelan TP, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part IV: recipient-related risk factors and markers. J Heart Lung Transplant. Oct; 2005 24(10):1468–82. PubMed PMID: 16210118. Epub 2005/10/08. [PubMed: 16210118]

80. Christie JD, Kotloff RM, Pochettino A, et al. Clinical risk factors for primary graft failure following lung transplantation. Chest. Oct; 2003 124(4):1232–41. PubMed PMID: 14555551. Epub 2003/10/14. [PubMed: 14555551]

81. de Perrot M, Bonser RS, Dark J, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part III: donor-related risk factors and markers. J Heart Lung Transplant. Oct; 2005 24(10):1460–7. PubMed PMID: 16210117. Epub 2005/10/08. [PubMed: 16210117]

82. Kuntz CL, Hadjiliadis D, Ahya VN, et al. Risk factors for early primary graft dysfunction after lung transplantation: a registry study. Clinical transplantation. Nov-Dec;2009 23(6):819–30. PubMed PMID: 19239481. Epub 2009/02/26. [PubMed: 19239481]

83. Liu Y, Liu Y, Su L, et al. Recipient-related clinical risk factors for primary graft dysfunction after lung transplantation: a systematic review and meta-analysis. PLoS One. 2014; 9(3):e92773. PubMed PMID: 24658073. Pubmed Central PMCID: PMC3962459. Epub 2014/03/25. *This metanalalysis identified recipient risk factors associated with the development of PGD. [PubMed: 24658073]

84. Nosotti M, Palleschi A, Rosso L, et al. Clinical risk factors for primary graft dysfunction in a low-volume lung transplantation center. Transplantation proceedings. Sep; 2014 46(7):2329–33. PubMed PMID: 25242781. Epub 2014/09/23. *This retrospective study identified clinical risk factors associated with the development of grade 3 PGD in a low-volume center. [PubMed: 25242781]

85. Novick RJ, Bennett LE, Meyer DM, et al. Influence of graft ischemic time and donor age on survival after lung transplantation. J Heart Lung Transplant. May; 1999 18(5):425–31. PubMed PMID: 10363686. Epub 1999/06/11. [PubMed: 10363686]

86. Samano MN, Fernandes LM, Baranauskas JC, et al. Risk factors and survival impact of primary graft dysfunction after lung transplantation in a single institution. Transplantation proceedings. Oct; 2012 44(8):2462–8. PubMed PMID: 23026621. Epub 2012/10/03. [PubMed: 23026621]

87. Allen JG, Lee MT, Weiss ES, et al. Preoperative recipient cytokine levels are associated with early lung allograft dysfunction. Ann Thorac Surg. Jun; 2012 93(6):1843–9. PubMed PMID: 22503850. [PubMed: 22503850]

88. Burton CM, Iversen M, Milman N, et al. Outcome of lung transplanted patients with primary graft dysfunction. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Jan; 2007 31(1):75–82. PubMed PMID: 17134909. [PubMed: 17134909]

89. Fang A, Studer S, Kawut SM, et al. Elevated pulmonary artery pressure is a risk factor for primary graft dysfunction following lung transplantation for idiopathic pulmonary fibrosis. Chest. Apr; 2011 139(4):782–7. PubMed PMID: 20864607. Pubmed Central PMCID: 3071272. [PubMed: 20864607]

90. Felten ML, Sinaceur M, Treilhaud M, et al. Factors associated with early graft dysfunction in cystic fibrosis patients receiving primary bilateral lung transplantation. European journal of cardio-

thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Mar; 2012 41(3):686–90. PubMed PMID: 22345188. [PubMed: 22345188]

91. Shah RJ, Diamond JM, Lederer DJ, et al. Plasma monocyte chemotactic protein-1 levels at 24 hours are a biomarker of primary graft dysfunction after lung transplantation. Translational research : the journal of laboratory and clinical medicine. Dec; 2012 160(6):435–42. PubMed PMID: 22989614. Pubmed Central PMCID: 3500407. [PubMed: 22989614]
92. Lee JC, Christie JD, Keshavjee S. Primary graft dysfunction: definition, risk factors, short- and long-term outcomes. Seminars in respiratory and critical care medicine. Apr; 2010 31(2):161–71. PubMed PMID: 20354929. Epub 2010/04/01. [PubMed: 20354929]
93. Suzuki Y, Cantu E, Christie JD. Primary graft dysfunction. Seminars in respiratory and critical care medicine. Jun; 2013 34(3):305–19. PubMed PMID: 23821506. Pubmed Central PMCID: 3968688. [PubMed: 23821506]
94. Lee JC, Christie JD. Primary graft dysfunction. Proceedings of the American Thoracic Society. Jan 15; 2009 6(1):39–46. PubMed PMID: 19131529. [PubMed: 19131529]
95. Nagendran M, Maruthappu M, Sugand K. Should double lung transplant be performed with or without cardiopulmonary bypass? Interactive cardiovascular and thoracic surgery. May; 2011 12(5):799–804. PubMed PMID: 21297132. Epub 2011/02/08. [PubMed: 21297132]
96. Szeto WY, Kreisel D, Karakousis GC, et al. Cardiopulmonary bypass for bilateral sequential lung transplantation in patients with chronic obstructive pulmonary disease without adverse effect on lung function or clinical outcome. J Thorac Cardiovasc Surg. Aug; 2002 124(2):241–9. PubMed PMID: 12167783. Epub 2002/08/09. [PubMed: 12167783]
97. Lederer DJ, Kawut SM, Wickersham N, et al. Obesity and primary graft dysfunction after lung transplantation: the Lung Transplant Outcomes Group Obesity Study. Am J Respir Crit Care Med. Nov 1; 2011 184(9):1055–61. PubMed PMID: 21799077. Pubmed Central PMCID: 3208644. [PubMed: 21799077]
98. Lederer DJ, Wilt JS, D'Ovidio F, et al. Obesity and underweight are associated with an increased risk of death after lung transplantation. Am J Respir Crit Care Med. Nov 1; 2009 180(9):887–95. PubMed PMID: 19608717. Pubmed Central PMCID: 2773915. [PubMed: 19608717]
99. Oto T, Griffiths AP, Levvey B, et al. A donor history of smoking affects early but not late outcome in lung transplantation. Transplantation. Aug 27; 2004 78(4):599–606. PubMed PMID: 15446321. [PubMed: 15446321]
100. Sommers KE, Griffith BP, Hardesty RL, et al. Early lung allograft function in twin recipients from the same donor: risk factor analysis. Ann Thorac Surg. Sep; 1996 62(3):784–90. PubMed PMID: 8784009. Epub 1996/09/01. [PubMed: 8784009]
101. Lawrence J, Xiao D, Xue Q, et al. Prenatal nicotine exposure increases heart susceptibility to ischemia/reperfusion injury in adult offspring. The Journal of pharmacology and experimental therapeutics. Jan; 2008 324(1):331–41. PubMed PMID: 17947495. Pubmed Central PMCID: 2376745. [PubMed: 17947495]
102. Ware LB, Lee JW, Wickersham N, et al. Donor smoking is associated with pulmonary edema, inflammation and epithelial dysfunction in ex vivo human donor lungs. Am J Transplant. Oct; 2014 14(10):2295–302. PubMed PMID: 25146497. Pubmed Central PMCID: 4169304. **This study demonstrated higher levels of IL-8 and lower rates of alveolar fluid clearance in donor lungs with smoke exposure compared to those without smoke exposure suggesting that exposure to cigarette smoker may affect gas exchange, lung epithelial function and fluid balance. [PubMed: 25146497]
103. Bonser RS, Taylor R, Collett D, et al. Effect of donor smoking on survival after lung transplantation: a cohort study of a prospective registry. Lancet. Aug 25; 2012 380(9843):747–55. PubMed PMID: 22647758. [PubMed: 22647758]
104. Gong MN, Bajwa EK, Thompson BT, et al. Body mass index is associated with the development of acute respiratory distress syndrome. Thorax. Jan; 2010 65(1):44–50. PubMed PMID: 19770169. Pubmed Central PMCID: 3090260. [PubMed: 19770169]
105. Anzueto A, Frutos-Vivar F, Esteban A, et al. Influence of body mass index on outcome of the mechanically ventilated patients. Thorax. Jan; 2011 66(1):66–73. PubMed PMID: 20980246. [PubMed: 20980246]

106. Singer JP, Peterson ER, Snyder ME, et al. Body composition and mortality after adult lung transplantation in the United States. Am J Respir Crit Care Med. Nov 1; 2014 190(9):1012–21. PubMed PMID: 25233138. Pubmed Central PMCID: 4299586. **This study determined that leptin levels were associated with 1-year mortality after lung transplantaiton, while a body mass index of 30-35 was not. Body mass index had poor sensitivity to measure total body fat-defined obesity, suggesting the need to identify a better measure of adioposity. [PubMed: 25233138]

107. Eberlein M, Diehl E, Bolukbas S, et al. An oversized allograft is associated with improved survival after lung transplantation for idiopathic pulmonary arterial hypertension. J Heart Lung Transplant. Dec; 2013 32(12):1172–8. PubMed PMID: 23876630. [PubMed: 23876630]

108. Eberlein M, Reed RM, Bolukbas S, et al. Lung size mismatch and primary graft dysfunction after bilateral lung transplantation. J Heart Lung Transplant. Feb; 2015 34(2):233–40. PubMed PMID: 25447586. Pubmed Central PMCID: 4329253. **This study demonstrated that oversized allograts were associated with a lower risk of grade 3 PGD after bilateral lung transplantation, especially among non-COPD patients. This may have important implications for organ allocation. [PubMed: 25447586]

109. Shargall Y, Guenther G, Ahya VN, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part VI: treatment. J Heart Lung Transplant. Oct; 2005 24(10):1489–500. PubMed PMID: 16210120. [PubMed: 16210120]

110. Bacha EA, Herve P, Murakami S, et al. Lasting beneficial effect of short-term inhaled nitric oxide on graft function after lung transplantation. Paris-Sud University Lung Transplantation Group. J Thorac Cardiovasc Surg. Sep; 1996 112(3):590–8. PubMed PMID: 8800144. Epub 1996/09/01. [PubMed: 8800144]

111. Botha P, Jeyakanthan M, Rao JN, et al. Inhaled nitric oxide for modulation of ischemia-reperfusion injury in lung transplantation. J Heart Lung Transplant. Nov; 2007 26(11):1199–205. PubMed PMID: 18022088. Epub 2007/11/21. [PubMed: 18022088]

112. Meade MO, Granton JT, Matte-Martyn A, et al. A randomized trial of inhaled nitric oxide to prevent ischemia-reperfusion injury after lung transplantation. Am J Respir Crit Care Med. Jun 1; 2003 167(11):1483–9. PubMed PMID: 12770854. Epub 2003/05/29. [PubMed: 12770854]

113. Moreno I, Vicente R, Mir A, et al. Jul-Aug;2009 41(6):2210–2. PubMed PMID: 19715875. Epub 2009/09/01.

114. Tavare AN, Tsakok T. Does prophylactic inhaled nitric oxide reduce morbidity and mortality after lung transplantation? Interactive cardiovascular and thoracic surgery. Nov; 2011 13(5):516–20. PubMed PMID: 21791520. Epub 2011/07/28. [PubMed: 21791520]

115. Thabut G, Brugiere O, Leseche G, et al. Preventive effect of inhaled nitric oxide and pentoxifylline on ischemia/reperfusion injury after lung transplantation. Transplantation. May 15; 2001 71(9):1295–300. PubMed PMID: 11397965. Epub 2001/06/09. [PubMed: 11397965]

116. Yerebakan C, Ugurlucan M, Bayraktar S, et al. Effects of inhaled nitric oxide following lung transplantation. Journal of cardiac surgery. May-Jun;2009 24(3):269–74. PubMed PMID: 19438780. Epub 2009/05/15. [PubMed: 19438780]

117. Keshavjee S, Davis RD, Zamora MR, et al. A randomized, placebo-controlled trial of complement inhibition in ischemia-reperfusion injury after lung transplantation in human beings. J Thorac Cardiovasc Surg. Feb; 2005 129(2):423–8. PubMed PMID: 15678055. Epub 2005/01/29. [PubMed: 15678055]

118. Struber M, Fischer S, Niedermeyer J, et al. Effects of exogenous surfactant instillation in clinical lung transplantation: a prospective, randomized trial. J Thorac Cardiovasc Surg. Jun; 2007 133(6):1620–5. PubMed PMID: 17532965. Epub 2007/05/30. [PubMed: 17532965]

119. Wittwer T, Grote M, Oppelt P, et al. Impact of PAF antagonist BN 52021 (Ginkolide B) on post-ischemic graft function in clinical lung transplantation. J Heart Lung Transplant. Mar; 2001 20(3):358–63. PubMed PMID: 11257563. Epub 2001/03/21. [PubMed: 11257563]

120. Zamora MR, Davis RD, Keshavjee SH, et al. Complement inhibition attenuates human lung transplant reperfusion injury: a multicenter trial. Chest. Jul.1999 116(1 Suppl):46S. PubMed PMID: 10424589. [PubMed: 10424589]

121. Herrington CS, Prekker ME, Arrington AK, et al. A randomized, placebo-controlled trial of aprotinin to reduce primary graft dysfunction following lung transplantation. Clinical

transplantation. Jan-Feb;2011 25(1):90–6. PubMed PMID: 20731686. Epub 2010/08/25. [PubMed: 20731686]

122. Beer A, Reed RM, Bolukbas S, et al. Mechanical ventilation after lung transplantation. An international survey of practices and preferences. Annals of the American Thoracic Society. May; 2014 11(4):546–53. PubMed PMID: 24640938. *This study outlines the variability in mechanical ventilation practices after lung transplantation across centers and highlights the lack of consideration of donor characteristics when selecting tidal volumes. [PubMed: 24640938]
123. Diamond JM, Ahya VN. Mechanical ventilation after lung transplantation. It's time for a trial. Annals of the American Thoracic Society. May; 2014 11(4):598–9. PubMed PMID: 24828803. *This editorial stresses the need for a multi-center trial to standardize mechanical ventilation management after lung transplantation. [PubMed: 24828803]
124. Currey J, Pilcher DV, Davies A, et al. Implementation of a management guideline aimed at minimizing the severity of primary graft dysfunction after lung transplant. J Thorac Cardiovasc Surg. Jan; 2010 139(1):154–61. PubMed PMID: 19909995. [PubMed: 19909995]
125. Adatia I, Lillehei C, Arnold JH, et al. Inhaled nitric oxide in the treatment of postoperative graft dysfunction after lung transplantation. Ann Thorac Surg. May; 1994 57(5):1311–8. PubMed PMID: 8179406. Epub 1994/05/01. [PubMed: 8179406]
126. Date H, Triantafillou AN, Trulock EP, et al. Inhaled nitric oxide reduces human lung allograft dysfunction. J Thorac Cardiovasc Surg. May; 1996 111(5):913–9. PubMed PMID: 8622313. Epub 1996/05/01. [PubMed: 8622313]
127. Macdonald P, Mundy J, Rogers P, et al. Successful treatment of life-threatening acute reperfusion injury after lung transplantation with inhaled nitric oxide. J Thorac Cardiovasc Surg. Sep; 1995 110(3):861–3. PubMed PMID: 7564460. Epub 1995/09/01. [PubMed: 7564460]
128. Aigner C, Wisser W, Taghavi S, et al. Institutional experience with extracorporeal membrane oxygenation in lung transplantation. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Mar; 2007 31(3):468–73. discussion 73-4. PubMed PMID: 17223352. Epub 2007/01/16. [PubMed: 17223352]
129. Bermudez CA, Adusumilli PS, McCurry KR, et al. Extracorporeal membrane oxygenation for primary graft dysfunction after lung transplantation: long-term survival. Ann Thorac Surg. Mar; 2009 87(3):854–60. PubMed PMID: 19231405. Epub 2009/02/24. [PubMed: 19231405]
130. Glassman LR, Keenan RJ, Fabrizio MC, et al. Extracorporeal membrane oxygenation as an adjunct treatment for primary graft failure in adult lung transplant recipients. J Thorac Cardiovasc Surg. Sep; 1995 110(3):723–6. discussion 6-7. PubMed PMID: 7564439. Epub 1995/09/01. [PubMed: 7564439]
131. Hartwig MG, Walczak R, Lin SS, et al. Improved survival but marginal allograft function in patients treated with extracorporeal membrane oxygenation after lung transplantation. Ann Thorac Surg. Feb; 2012 93(2):366–71. PubMed PMID: 21962264. Epub 2011/10/04. [PubMed: 21962264]
132. Meyers BF, Sundt TM 3rd, Henry S, et al. Selective use of extracorporeal membrane oxygenation is warranted after lung transplantation. J Thorac Cardiovasc Surg. Jul; 2000 120(1):20–6. PubMed PMID: 10884650. Epub 2000/07/08. [PubMed: 10884650]
133. Wigfield CH, Lindsey JD, Steffens TG, et al. Early institution of extracorporeal membrane oxygenation for primary graft dysfunction after lung transplantation improves outcome. J Heart Lung Transplant. Apr; 2007 26(4):331–8. PubMed PMID: 17403473. Epub 2007/04/04. [PubMed: 17403473]
134. Xu LF, Li X, Guo Z, et al. Extracorporeal membrane oxygenation during double-lung transplantation: single center experience. Chinese medical journal. Feb 5; 2010 123(3):269–73. PubMed PMID: 20193243. Epub 2010/03/03. [PubMed: 20193243]
135. Bittner HB, Binner C, Lehmann S, et al. Replacing cardiopulmonary bypass with extracorporeal membrane oxygenation in lung transplantation operations. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Mar; 2007 31(3):462–7. discussion 7. PubMed PMID: 17188884. Epub 2006/12/26. [PubMed: 17188884]
136. Ko WJ, Chen YS, Lee YC. Replacing cardiopulmonary bypass with extracorporeal membrane oxygenation in lung transplantation operations. Artificial organs. Aug; 2001 25(8):607–12. PubMed PMID: 11531710. Epub 2001/09/05. [PubMed: 11531710]

137. Machuca TN, Collaud S, Mercier O, et al. Outcomes of intraoperative extracorporeal membrane oxygenation versus cardiopulmonary bypass for lung transplantation. J Thorac Cardiovasc Surg. Apr; 2015 149(4):1152–7. PubMed PMID: 25583107. Epub 2015/01/15. **Patients receiving lung transplantation using extracorporeal membrane oxygenation had a shorter hospital length of stay, lower blood product transfusion requirement, and similar 90-day mortality compared to those on cardiopulmonary bypass after matching for age, transplant indication, and procedure type. This has important implications for peri-operative management of lung transplant recipients. [PubMed: 25583107]

138. Bozso S, Sidhu S, Garg M, et al. Canada's longest experience with extracorporeal membrane oxygenation as a bridge to lung transplantation: a case report. Transplantation proceedings. Jan-Feb;2015 47(1):186–9. PubMed PMID: 25645800. Epub 2015/02/04. *This case report describes the successful use of extracorporeal membrane oxygenation as a bridge to lung transplantation in a patient with stage IV pulmonary sarcoidosis. [PubMed: 25645800]

139. de Perrot M, Granton JT, McRae K, et al. Impact of extracorporeal life support on outcome in patients with idiopathic pulmonary arterial hypertension awaiting lung transplantation. J Heart Lung Transplant. Sep; 2011 30(9):997–1002. PubMed PMID: 21489818. Epub 2011/04/15. [PubMed: 21489818]

140. Kon ZN, Wehman PB, Gibber M, et al. Venovenous extracorporeal membrane oxygenation as a bridge to lung transplantation: successful transplantation after 155 days of support. Ann Thorac Surg. Feb; 2015 99(2):704–7. PubMed PMID: 25639416. Epub 2015/02/03. *This case report describes the use of venovenous extracorporeal membrane oxygenation as a bridge to lung transplantation in a patient recovering from acute respiratory distress syndrome. [PubMed: 25639416]

141. Lehr CJ, Zaas DW, Cheifetz IM, et al. Ambulatory extracorporeal membrane oxygenation as a bridge to lung transplantation: walking while waiting. Chest. May 1; 2015 147(5):1213–8. PubMed PMID: 25940249. Epub 2015/05/06. *This paper reviews the studies using extracorporeal membrane oxygenation as a bridge to lung transplantation and identifies the need for future trials to understand the optimal use of this technology. [PubMed: 25940249]

142. Mohite PN, Sabashnikov A, Reed A, et al. Extracorporeal Life Support in "Awake" Patients as a Bridge to Lung Transplant. The Thoracic and cardiovascular surgeon. Mar 5.2015 PubMed PMID: 25742548. Epub 2015/03/06. *This observational study describes similar one-year survival among those bridged to lung transplantation using extracorporeal membrane oxygenation placed while awake and those placed while sedated.

143. Patil NP, Mohite PN, Reed A, et al. Modified technique using Novalung as bridge to transplant in pulmonary hypertension. Ann Thorac Surg. Feb; 2015 99(2):719–21. PubMed PMID: 25639425. Epub 2015/02/03. *This case report describes the use of Novalung as a bridge to lung transplantation in a patient with pulmonary hypertension. [PubMed: 25639425]

144. Patil NP, Popov AF, Lees NJ, et al. Novel sequential bridge to lung transplant in an awake patient. J Thorac Cardiovasc Surg. Jan; 2015 149(1):e2–4. PubMed PMID: 25454917. Epub 2014/12/03. *This case report describes the use of Novalung as a bridge to lung transplantation in a patient with pulmonary hypertension. [PubMed: 25454917]

145. Schellongowski P, Riss K, Staudinger T, et al. Extracorporeal CO2 removal as bridge to lung transplantation in life-threatening hypercapnia. Transplant international : official journal of the European Society for Organ Transplantation. Mar; 2015 28(3):297–304. PubMed PMID: 25387861. Epub 2014/11/13. *This case series describes the use of extracorporeal CO_2 removal through Novalung for life-threatening hypercapnia as a bridge to lung transplantation. [PubMed: 25387861]

146. Wiktor AJ, Haft JW, Bartlett RH, et al. Prolonged VV ECMO (265 Days) for ARDS without technical complications. ASAIO journal (American Society for Artificial Internal Organs : 1992). Mar-Apr;2015 (2):205–6. PubMed PMID: 25423122. Epub 2014/11/26. *This case report explains the use of extracorporeal membrane oxygenation in a patient with acute respiratory distress syndrome undergoing lung transplantation. [PubMed: 25423122]

147. Aigner C, Slama A, Hotzenecker K, et al. Clinical ex vivo lung perfusion--pushing the limits. Am J Transplant. Jul; 2012 12(7):1839–47. PubMed PMID: 22458511. Epub 2012/03/31. [PubMed: 22458511]

148. Boffini M, Ricci D, Bonato R, et al. Incidence and severity of primary graft dysfunction after lung transplantation using rejected grafts reconditioned with ex vivo lung perfusion. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Nov; 2014 46(5):789–93. PubMed PMID: 25061216. Epub 2014/07/26. *This study demonstrated that grafts reconditioned with extracorporeal lung perfusion had similar rates and severity of PGD as standard grafts. [PubMed: 25061216]

149. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. The New England journal of medicine. Apr 14; 2011 364(15):1431–40. PubMed PMID: 21488765. [PubMed: 21488765]

150. Fildes JE, Archer LD, Blaikley J, et al. Clinical Outcome of Patients Transplanted with Marginal Donor Lungs via Ex Vivo Lung Perfusion Compared to Standard Lung Transplantation. Transplantation. May; 2015 99(5):1078–83. PubMed PMID: 25757211. Epub 2015/03/11. *This study concluded that lungs reconditioned using extracorporeal lung perfusion reconditioned had similar rates of acute rejection, infection, hospital length of stay, and 1-year mortality as lungs deemed acceptable at initial evaluation. [PubMed: 25757211]

151. Machuca TN, Mercier O, Collaud S, et al. Lung transplantation with donation after circulatory determination of death donors and the impact of ex vivo lung perfusion. Am J Transplant. Apr; 2015 15(4):993–1002. PubMed PMID: 25772069. Epub 2015/03/17. *This study demonstrated similar outcomes between lung transplantation after donation after circulatory determination of death and donation after neurologic determination of death. Among lungs transplanted from donation after circulatory determination of death donors, there was a non-statistically significant trend toward shorter hospital length of stay and shorter length of mechanical ventilation if selective extracorporeal lung perfusion was used. [PubMed: 25772069]

152. Sage E, Mussot S, Trebbia G, et al. Lung transplantation from initially rejected donors after ex vivo lung reconditioning: the French experience. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Nov; 2014 46(5):794–9. PubMed PMID: 25061219. Epub 2014/07/26. *This study evaluated the use of extracoporeal lung reconditioning on initially rejected donors and demonstrated that transplantation with these lungs had similar rates of primary graft dysfunction, hospital length of stay, and 1-year mortality compared to conventional donors. [PubMed: 25061219]

153. Tikkanen JM, Cypel M, Machuca TN, et al. Functional outcomes and quality of life after normothermic ex vivo lung perfusion lung transplantation. J Heart Lung Transplant. Apr; 2015 34(4):547–56. PubMed PMID: 25476845. Epub 2014/12/06. *This study concluded that transplantation using allografts reconditioned with extracorporeal lung perfusion had similar mortality, freedom from chronic lung allograft dysfunction, and number of acute rejection episodes as those transplanted from conventional donors. [PubMed: 25476845]

154. Valenza F, Rosso L, Coppola S, et al. Ex vivo lung perfusion to improve donor lung function and increase the number of organs available for transplantation. Transplant international : official journal of the European Society for Organ Transplantation. Jun; 2014 27(6):553–61. PubMed PMID: 24628890. Pubmed Central PMCID: PMC4241040. Epub 2014/03/19. *This paper demonstrated similar rates of grade 3 PGD at 72 hours and survival between allografts reconditioned with extracorporeal lung perfusion and conventional donors and showed that extracorporeal lung perfusion technology could be used to increase the donor pool by 20%. [PubMed: 24628890]

155. Valenza F, Rosso L, Gatti S, et al. Extracorporeal lung perfusion and ventilation to improve donor lung function and increase the number of organs available for transplantation. Transplantation proceedings. Sep; 2012 44(7):1826–9. PubMed PMID: 22974847. Epub 2012/09/15. [PubMed: 22974847]

156. Van Raemdonck D, Neyrinck A, Cypel M, et al. Ex-vivo lung perfusion. Transplant international : official journal of the European Society for Organ Transplantation. Mar.2014 15 PubMed PMID: 24629039. Epub 2014/03/19. *This review analyzed the technique, indications, and outcomes of lung transplantation using extracorporeal lung perfusion.

157. Wallinder A, Ricksten SE, Silverborn M, et al. Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case-control study. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. Jan; 2014 45(1):40–4. discussion 4-5. PubMed PMID: 23666375. Epub 2013/05/15. *Donor lungs

initially deemed unsuitable for transplantation were treated with extracorporeal lung perfusion and then compared to conventional donor lungs that did not require extracorporeal lung perfusion. While lungs treated with extracorporeal lung perfusion demonstrated longer time to extubation and longer median ICU stay, the hospital length of stay was similar. [PubMed: 23666375]

158. Wigfield CH, Cypel M, Yeung J, et al. Successful emergent lung transplantation after remote ex vivo perfusion optimization and transportation of donor lungs. Am J Transplant. Oct; 2012 12(10):2838–44. PubMed PMID: 23009140. Epub 2012/09/27. [PubMed: 23009140]
159. Zych B, Popov AF, Stavri G, et al. Early outcomes of bilateral sequential single lung transplantation after ex-vivo lung evaluation and reconditioning. J Heart Lung Transplant. Mar; 2012 31(3):274–81. PubMed PMID: 22088786. Epub 2011/11/18. [PubMed: 22088786]
160. Warnecke G, Moradiellos J, Tudorache I, et al. Normothermic perfusion of donor lungs for preservation and assessment with the Organ Care System Lung before bilateral transplantation: a pilot study of 12 patients. Lancet. Nov 24; 2012 380(9856):1851–8. PubMed PMID: 23063317. [PubMed: 23063317]
161. Souilamas R, Souilamas JI Jr. Saueressig M, et al. Advanced normothermic ex vivo lung maintenance using the mobile Organ Care System. J Heart Lung Transplant. Jul; 2011 30(7): 847–8. PubMed PMID: 21489815. [PubMed: 21489815]
162. TransMedics. International Randomized Study of the TransMedics Organ Care System (OCS Lung) for Lung Preservation and Transplantation (INSPIRE) 2012. [updated May 11, 2015; cited 2015 June 12]. Available from: https://clinicaltrials.gov/ct2/show/NCT01630434
163. TransMedics. International EXPAND Lung Pivotal Trial (EXPANDLung) 2015. [updated May 11, 2015; cited 2015 June 12]. Available from: https://clinicaltrials.gov/ct2/show/NCT01963780?term=OCS+expand&rank=2

Key points

- While the annual number of lung transplantations is increasing, primary graft dysfunction following lung transplantation remains a significant source of short- and long-term morbidity and mortality.
- The pathogenesis of primary graft dysfunction remains poorly understood, but is likely the end result of multiple deleterious mechanisms provoked by donor brain death, mechanical ventilation, procurement, storage, and ischemia reperfusion injury.
- Better understanding of the role of established clinical risk factors, as well as the emerging data on the importance of innate immunity, epithelial cell injury, endothelial cell dysfunction, and cytokine production in the development of primary graft dysfunction is necessary in order to identify potential high-risk populations and therapeutic targets.
- Future trials should focus on identifying appropriate patients to bridge to transplant with extracorporeal life support and expanding the donor pool with extracorporeal lung perfusion without increasing the risk of primary graft dysfunction.

Table 1

Grading system for PGD(2)

Grade	Pa_{O2}/Fi_{O2}	Radiographic infiltrates consistent with pulmonary edema
0	>300	Absent
1	>300	Present
2	200-300	Present
3	<200	Present

Adapted from Reference(2)

Table 2

Human translational studies exploring the pathogenesis of PGD

Pathway	Human translational studies	Reference
Epithelial cell injury	Higher blood and BAL levels of *RAGE* were associated with PGD	(20-22)
	Preexisting or de novo antibodies against type V collagen were associated with lower P/F ratio and PGD	(23, 24)
	Elevated levels of CC16 were associated with higher odds of PGD	(25)
	Higher donor levels of HMGB1 correlated with lower P/F before and after transplantation.	(26)
Endothelial dysfunction	Grade 3 PGD was associated with higher VEGF levels than lower grades of PGD	(27, 28)
	Increased pretransplant endothelin-1 levels were associated with PGD	(29)
	Angiopoietin-2 levels were elevated in PGD	(30)
Chemotaxis and cytokines	Higher plasma levels of MCP-1, IP-10, and IL-2R were associated with PGD	(31)
	Elevated serum IL-8 levels were associated with PGD	(32, 33)
	Higher blood and BAL levels of IL-6 were associated with grade 3 PGD	(34)
Innate immunity	Upregulation in the expression of genes involved in inflammasome and TLR pathways were found in BAL of patients with grade 3 PGD	(35)
	TLR4 mutations associated with innate immune hyporesponsiveness were associated with a lower PGD risk	(36)
	Higher plasma *PTX3* levels were seen in those with grade 3 PGD	(37)
	Single nucleotide polymorphisms associated with higher levels of *PTX3* were associated with grade 3 PGD	(38)
	Innate lymphoid cells were isolated from BAL of lung transplant recipients	(39)

Adapted from Reference(19)

BAL, bronchoalveolar lavage; CC16, plasma clara cell secretory protein; ET-1, endothelin-1; HMGB1, high-mobility group box-1; IP-10, interferon-inducible protein; MCP-1, monocyte chemotactic protein-1; P/F, PaO_2/FiO_2; PAI-1, plasminogen activator inhibitor-1; PGD, primary graft dysfunction; PTX3, long pentraxin-3; RAGE, receptor for advanced glycation end products; TLR, toll-like receptor; VEGF, vascular endothelial growth factor

Table 3

Clinical risk factors for PGD

Category	Risk factors for Primary Graft Dysfunction
Donor inherent variables	Age>45 or <21yo
	African American race
	Female sex
	Smoke exposure
Donor acquired variables	Prolonged mechanical ventilation
	Aspiration
	Head trauma
	Hemodynamic instability after brain death
Recipient variables	Female gender
	Body mass index>25
	Pulmonary arterial hypertension
	Pulmonary hypertension secondary to parenchymal lung disease
	Idiopathic pulmonary fibrosis
	Sarcoidosis
	Elevated pulmonary arterial pressure at time of surgery
Operative variables	Single lung transplantation
	Prolonged ischemic time
	Use of cardiopulmonary bypass
	Packed red blood transfusion > 1L
	High reperfusion FiO2 > 0.4
	Use of Euro-Collins preservation solution

Adapted from References (6, 11, 12, 14, 32, 79-83, 87, 89, 90, 93-100)