Chapter

# Cytokine Biomarkers as Indicators of Primary Graft Dysfunction, Acute Rejection, and Chronic Lung Allograft Dysfunction in Lung Transplant Recipients: A Review

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## Abstract

Lung transplantation is well accepted form of treatment for end-stage lung disease in selected patients. The number of lung transplants performed worldwide has increased annually with chronic obstructive pulmonary disease being the leading cause. The morbidity and mortality in the early period are due to nonspecific primary graft dysfunction (PGD) and acute lung rejection (ALR). Chronic lung allograft dysfunction (CLAD) is the cause of long-term complications following lung transplantation and seen in almost half of the patient during the first 5 years. Activation of pro- and anti-inflammatory cytokines and chemokines has been described during various phases of lung transplantation recovery. We reviewed the literature for cytokine activity associated with PGD, ALR, and CLAD. This review aims to summarize the specific associations between bronchoalveolar lavage (BAL) and plasma cytokine levels and the association of PGD, ALR, and CLAD.

**Keywords:** cytokines, lung transplant, primary graft dysfunction, acute rejection, chronic lung allograft dysfunction

## 1. Introduction

The incidence of lung transplantations worldwide has increased annually with chronic obstructive pulmonary disease being the leading cause [1]. From 2009 to June 2016, the median survival of primary lung transplantation was 6.5 years [2]. The frequency of at least one treated acute rejection episode occurring within 1 year posttransplantation is around 27% [2]. Bronchiolitis obliterans syndrome (BOS), a phenotype of chronic lung rejection, is currently one of the most significant long-term complications of lung transplantation with a 5-year follow-up incidence of 41.5% [2].

Primary graft dysfunction (PGD) complicates lung transplant outcomes. PGD is a common early complication of lung transplantation that often occurs in the first

72 h posttransplantation [3]. PGD has also been indicated as a risk factor for the development of BOS [4].

Acute lung rejection (ALR) in lung transplant recipients is a major cause of early complication and death [5]. It is a major risk factor for the development of BOS [6]. BOS is the most common manifestation of chronic lung allograft dysfunction (CLAD) and is characterized by subepithelial fibrosis of small cartilaginous airways leading to partial or total occlusion [7].

PGD, ALR, and CLAD all have been associated with pro- and anti-inflammatory cytokine and chemokine expressions. This review aims to summarize the specific associations between bronchoalveolar lavage (BAL) and plasma cytokine levels and the development of PGD, ALR, and CLAD.

## 2. Methods

PubMed was explored using MeSH terms "lung transplantation," "cytokines," "biomarkers," "acute rejection," "chronic allograft dysfunction," and "primary graft dysfunction." Inclusion criteria consisted of studies through May 2018 that provided information on plasma and/or BAL cytokines and acute rejection, chronic rejection, or primary graft dysfunction in lung transplant recipients. Prospective, retrospective, and review articles were included. The references of searched articles were also examined for potential studies to include. We focused on the following cytokines: interleukin (IL)-1a, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, and IL-17; interferon-gamma (IFN-y); tumor necrosis factor-alpha (TNF-a); transforming growth factor-beta (TGF-b); and monocyte chemotactic protein (MCP)-1.

## 3. Primary graft dysfunction

PGD typically occurs within the first 72 h posttransplantation and is identified as ischemia-reperfusion injury with pulmonary edema that presents as increasing hypoxia in the affected patient [3].

Lung transplantation, and any other major surgeries, constitutes massive damage to patient tissues.

TNF-α is one of the first cytokines to be released into circulation from such an injury, peaking in serum concentration around 1 h after the beginning of injury. IL-6, IL-8, and IL-10 are expressed and released in circulation shortly after, with peaks in concentration between 2 and 4 h after injury. Additionally, if injury severity increases, there is an associated shift away from a cell-mediated response to a humoral immune response [8].

Macrophage-associated cytokines IFN-y, TNF-a, and MCP-1 have all been strongly associated with PGD development in lung transplant recipients. Bharat and associates identified elevated serum IFN-γ in PGD positive patients [9]. Early release of TNF-α was associated with early hemodynamic failure posttransplantation [10]. In another study, elevated systemic TNF-α concentrations were associated with PGD development [11]. MCP-1, a macrophage chemotactic agent, has demonstrated a strong role in PGD. Shah and associates measured plasma MCP-1 at various time points in lung transplant recipients. They found elevated MCP-1 levels at 24 h posttransplantation were associated with PGD grade 3. These results attested to the importance of monocyte chemotaxis in PGD [12]. Another group of authors found similar results with elevated serum MCP-1 in PGD positive lung transplant recipients [13]. INF-γ is a potent activator of macrophages. Elevations in IFN-γ

along with increases in MCP-1, a strong monocyte chemotactic agent, suggest that ischemia-reperfusion injury increases macrophage activation.

Macrophage activation leads to release of pro-inflammatory cytokines, including IL-6 and IL-8. PGD is linked to concomitant increases in IL-6 and IL-8 in lung transplant recipients. Early hemodynamic failure posttransplantation was associated with increases in both IL-6 and IL-8 [10]. A different study had similar results, in which IL-6 and IL-8 were both elevated in patients with PGD [11]. Moreno and associates found elevated BAL and blood IL-6 and IL-8 in patients with PGD. They are subsequently treated with inhaled nitric oxide, which lowered IL-6 and IL-8 and also decreased PGD incidence [14]. Increases in IL-6 often occur as a result of upstream macrophage-induced activation of Th1 immunity. In addition to macrophage activation, neutrophil chemotaxis from IL-8 upregulation is associated with increased PGD incidence. Increases in other pro-inflammatory cytokines caused by macrophage activation lead to pulmonary vasoconstriction and increased pulmonary vascular permeability, precipitating hemodynamic instability characteristic of PGD.

## 4. Acute lung rejection

In the weeks to months following transplantation, the allograft recipient's T-cell-mediated immunity intensifies, potentially leading to the development of ALR. ALR is understood to be originally caused by mismatched MHC recognition and adaptive immune response [15].

Acute lung rejection is precipitated by the adaptive T-cell response. MHC mismatch and the adaptive immune response are associated with T-cell activation and differentiation, which is facilitated by IL-2 [16]. It is expected that IL-2 would be increased in acute rejection; however the literature is conflicting on its association with lung rejection. Jordan and associates analyzed the serum of 17 lung transplant recipients and found serum IL-2 significantly elevated in patients with acute rejection confirmed; however, Moudgil and associates found no correlation between IL-2 levels and acute rejection in lung transplant recipient [17, 18]. In addition to IL-2, IL-15 is a cytokine derived from stromal cells that behaves similarly to IL-2 in terms of biological function and is involved in T-cell chemoattraction to allografts [23]. Bhorade and associates measured IL-15 levels in BAL fluid of lung transplants and found that IL-15 was significantly elevated in patients experiencing acute rejection when the patients were given anti-CD25 monoclonal antibodies [19]. This study along with the evidence for IL-2 activation suggests the potential importance of IL-2 and IL-2 receptors in ALR immune responses.

T helper (Th) cells orchestrate the immune response and are divided into two subsets, Th1 and Th2 cells. T-cell differentiation into Th1 cells leads to increased expression of IFN-γ by Th1 cells. IFN-γ is involved in many important immune mechanisms and is a main component of the Th1 immune response, as it is a strong activator of macrophage-mediated antimicrobial and antitumor activity [20]. Its role in ALR is supported by a study measuring IFN-γ in BAL fluid of lung transplantation patients, which found IFN-γ levels were significantly elevated in early acute rejection [18]. IL-12 is a known mediator of interferon-gamma expression [21]. D'ovidio and associates found IL-12 in BAL fluid elevated in acute rejection patients, which suggests it influences IFN-γ in ALR [22]. Ultimately, IFN-γ activation of macrophages induces pro-inflammatory cytokine release to cause inflammation.

IL-1, IL-6, and TNF-α are all acute phase pro-inflammatory cytokines that occur in most disease states and are secreted by activated macrophages to induce

inflammation. IL-1, which consists of both IL-1a and IL-1b, is a ubiquitous cytoplasmic cytokine that is associated with a plethora of disease states, including allograft rejection [23]. This family is associated with general acute phase reactions. Because the IL-1 family has been linked to several disease states, it is no surprise that lung transplant rejection bears an association to its expression. Specifically, Patella and associates recently found BAL IL-1β elevated in acute rejection episodes [24]. In another study, Rizzo and associates found significant increases in IL-1a and IL-1b expressions from alveolar macrophages of acute lung rejection patients compared to patients without acute rejection [25]. IL-6 is another acute phase marker and pro-inflammatory cytokine that is involved in hematopoiesis and immune regulation [26]. Its role in immunity is similar to that of IL-1 cytokines, which leads it to also be elevated in acute rejection. The literature supports this claim. Whitehead and associates also found IL-6 significantly elevated in the BAL of acute lung rejection patients [27]. Patella and associates examined IL-6 in BAL samples of lung transplant recipients and found IL-6 to be higher in acute rejection cases [24]. The last of the acute phase cytokines is TNF-a. TNF-α has been associated with many disease processes, including infections, septic shock, and allograft rejection [28]. Hodge and associates found TNF-α was elevated in BAL CD4+ and CD8+ cells in acute lung rejection cases [29]. Magnan and associates measured TNF-α in alveolar macrophages and lung transplant recipients and found increased TNF-α in acute rejection [30].

In addition to acute phase cytokines, IL-8 is a known mediator of inflammation and neutrophil chemotaxis [31]. Its role in ALR, however, is minor. A recent study found no association between IL-8 and acute rejection [22].

Along with Th1, Th2 differentiation occurs with IL-2 activation of naive T cells. In addition, Th2 cell differentiation is activated by IL-4, a cytokine normally released by mast cells and basophils [32]. The literature is currently conflicting on the role of IL-4 in acute lung rejection. Whitehead and associates found BAL IL-4 elevated in acute lung rejection patients compared to patients without rejection [27]. On the other hand, another study looking at pro-inflammatory cytokine expression in lung transplant recipients found no difference in BAL, plasma, or bronchial brushing IL-4 levels between acute rejection and stable patients [29]. Based on conflicting literature, the Th2 response may not have a significant role in acute lung rejection.

The Th1 response is regulated by anti-inflammatory cytokines. IL-10 is an anti-inflammatory cytokine that is involved in immune response regulation and limiting of immune destruction to host tissues [33]. Patella and associates found that IL-10 was actually elevated in acute rejection cases compared to stable patients [24]. This evidence suggests IL-10 is elevated in an attempt to limit inflammation in ALR.

Monocyte and macrophage activity is strongly associated with activation of the Th1 response and is responsible for secretion of pro-inflammatory cytokines. IL-17, also known as IL-17A, is released by Th17 cells and induces monocytes and stromal cells to produce cytokines in addition to stimulating granulopoiesis. It is also involved in the pathogenesis of several autoimmune diseases [34]. In a study analyzed IL-17 mRNA and protein levels in BAL samples of lung transplant recipients, the authors found both IL-17 mRNA and protein levels significantly elevated in acute lung rejection [35]. MCP-1, also known as CCL-2, is a chemokine with strong mononuclear cell chemotaxis properties involved in chronic inflammation [36]. Belperio and associates evaluated BAL fluid from lung transplant recipients and found increased levels of MCP-1 in acute rejection cases compared to stable patients [37]. The role of MCP-1 and IL-17 suggest that mononuclear immune cell regulation occurs concomitantly to the Th1 response in ALR.

## 5. Chronic lung allograft dysfunction

Airway inflammation is the main contributor to CLAD. CLAD encompasses many manifestations of chronic rejection, including BOS and RAS (restrictive allograft syndrome). Currently, it is characterized by a decrease in $FEV_1$ and/or FVC by at least 20% compared to baseline, which is determined as a mean of two optimal postoperative measurements taken 3 weeks apart [38].

Pro-inflammatory cytokines IL-1, IL-6, and TNF-α are all upregulated in CLAD. Firstly, IL-1 has been studied in the setting of chronic rejection in lung transplantation. Suwara and associates studied cytokine expression in BAL fluid of lung transplant recipients with respect to different phenotypes of CLAD. They found IL-1a and IL-1b were elevated in lymphocytic bronchiolitis and persistent airway neutrophilia cases [39]. Verleden and associates also analyzed BAL fluid cytokines in different chronic lung rejection phenotypes and found IL-1b was significantly elevated in neutrophilic BOS and RAS episodes compared to stable patients [40]. In persistent airway neutrophilia, a specific phenotype of CLAD, BAL IL-6 was found to be significantly elevated [39]. Verleden and associates studied cytokine expression in BAL fluid of lung transplant recipients and found that IL-6 levels were elevated in RAS patient and correlated with survival among lung transplantation patients with RAS [40]. Lastly, TNF-α has been linked to CLAD. Suwara and associates studied cytokine expression in the context of several CLAD phenotypes. They found that BAL TNF-α levels were increased in patients with primary airway neutrophilia [39]. Additionally, Bharat and associates measured serum cytokines in patients with and without BOS after lung transplantations. They found that IL-10 *decreased* threefold during the onset of BOS [41]. This evidence suggests that inflammation in the absence of regulation may contribute to airway inflammation in CLAD which likely arises from uninhibited pro-inflammatory cytokines.

Pro-inflammatory cytokine expression in CLAD may be a result of increased monocyte/macrophage chemotaxis. IFN-y, which activates macrophages to induce inflammation, has been indicated in chronic lung rejection. Hodge and associates found that, compared to BOS patients, stable lung transplant recipients displayed significant reductions in blood IFN-γ levels [42]. Both IL-17 and MCP-1, which are macrophage-recruiting cytokines, have been indicated in CLAD. MCP-1 was found elevated in patients before and during BOS indicating elevated MCP-1 posttransplantation is predictive of BOS [13]. Fisichella and associates found increases in BAL IL-17 as an indicator of early onset BOS [43].

Unlike ALR, neutrophil-associated airway damage is strongly associated with CLAD development. IL-8 is known to facilitate neutrophil chemotaxis and has shown to be involved in chronic rejection among lung transplant recipients. DiGiovine and associates first established the contribution of IL-8 expression to airway neutrophilia and BOS development [44]. BAL IL-8 levels in lung transplantation patients were elevated in neutrophilic BOS and RAS compared to stable patients in a recent study [40]. Elssner and associates found that IL-8 mRNA expression from bronchial cells was significantly elevated in BOS cases compared to stable patients [45].

The activity of IL-12 in CLAD is also contrary to ALR. IL-12 appears to attenuate the development of CLAD, specifically BOS. Meloni and associates measured BAL cytokines in 44 lung transplant recipients and identified significant decreases in IL-12 to be correlative with BOS development [46]. Krenn and associates determined that azithromycin administration in lung transplant recipients reduced overall fibrosis and kept IL-12 levels from decreasing [47]. The authors remarked on the future significance of macrolide therapy in reduction of BOS development

through effects on IL-12. The Th2 cytokine IL-4 has also shown to contribute to CLAD. Kastelijn and associates measured serum IL-4 levels in lung transplant recipients and found IL-4 levels were significantly lower in patients with BOS than BOS-negative patients [48]. The importance of IL-12 as a negative regulator as well as the potential role of IL-4 in CLAD indicates that the Th1 response may be downregulated in CLAD.

Chronic inflammation from persistent airway damage eventually leads to airway remodeling. TGF-β is an anti-inflammatory cytokine involved in tissue remodeling and scar formation [49]. Several studies have correlated TGF-β with the development of chronic lung rejection episodes, including El-Gamel and associates who discovered elevated TGF-β levels in biopsies in patients with BOS [50]. Elssner and associates studied BAL fluid and respiratory epithelial lining fluid in lung transplant recipients and found that BOS patients had elevated TGF-β levels in both samples [45]. Another study correlated TGF-β levels with BOS, which validated the author's claims that the biological role of TGF-β in tissue repair may also lead to airway fibrosis and obliteration [51].

## 6. Conclusions

The literature contains ample evidence on cytokines as biomarkers in lung transplantation outcomes. PGD is augmented by IFN-y, IL-6, IL-8, TNF-a, and MCP-1. This could be explained by monocyte involvement and inflammatory changes during ischemia-reperfusion injury. IL-1b, IL-6, IL-10, IL-15, and IFN-γ appear to be strong indicators to supplement the diagnosis of acute rejection in lung transplant recipients. These cytokines are linked to a Th1 immune response associated with acute inflammation. IL-1b, IL-6, IL8, IL-15, IL-17, IFN-γ, and TGF-β are significant contributors to chronic lung allograft dysfunction. IL-12 has also shown to attenuate chronic lung rejection. CLAD appears to be more associated with inflammation and airway neutrophil chemotaxis.

The role of cytokines requires more controlled studies in order for diagnostic characteristics to be attributed. That being said, cytokines and chemokines in primary graft dysfunction, acute rejection, and chronic allograft dysfunction are promising markers of future diagnostic tests and targets of therapies to ultimately improve outcomes and survival in lung transplant recipients.

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