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Bronchoalveolar Lavage as a Tool to Predict, Diagnose, and Understand Bronchiolitis Obliterans Syndrome

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Abstract

Bronchiolitis obliterans syndrome (BOS), a condition of irreversible small airway fibrosis, is the principal factor limiting long-term survival after lung transplantation. Bronchoscopy and bronchoalveolar lavage (BAL), techniques central to lung transplant clinical practice, provide a unique opportunity to interrogate the lung allograft during BOS development and identify potential disease mechanisms or biomarkers. Over the past twenty years, numerous studies have evaluated the BAL cellular composition, cytokine profiles, and protein constituents in lung transplant recipients with BOS. To date, however, no summative evaluation of this literature has been reported. We developed and applied objective criteria to qualitatively rank the strength of associations between BAL parameters and BOS in order to provide a comprehensive and systematic assessment of the literature. Our analysis indicates that several BAL parameters, including neutrophil count, interleukin-8, alpha defensins, and MMP-9, demonstrate highly replicable associations with BOS. Additionally, we suggest that considerable opportunity exists to increase the knowledge gained from BAL analyses in BOS through increased sample sizes, covariant adjustment, and standardization of BAL technique. Further efforts to leverage analysis of BAL constituents in BOS may offer great potential to provide additional in-depth and mechanistic insights into the pathogenesis of this complex disease.

Keywords

Bronchoalveolar Lavage; Bronchiolitis Obliterans Syndrome; Lung Transplantation

Introduction

Lung transplantation is a viable short-term therapy for many advanced lung diseases. Long-term outcomes, however, remain limited with a median 5-year survival rate of only 50% (1). Bronchiolitis obliterans syndrome (BOS), a condition of progressive small airways fibrosis manifested by increasing airflow limitation, is the most widely described form of chronic lung allograft dysfunction (CLAD) and is a principal factor contributing to poor long-term survival (2). Despite its clinical significance, the mechanisms leading to BOS remain poorly understood (3).

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Because lung transplant recipients regularly undergo bronchoscopy as part of their clinical care, examination of the cellular composition, cytokine profile, and protein constituents in bronchoalveolar lavage (BAL) fluid provides a unique window into the microenvironment of the lung allograft. Since the initial description of BOS, numerous studies have been published in which BAL fluid was examined from lung transplant recipients with this condition. To date, however, no summative evaluation of this body of literature has been reported. The purpose of this article is to apply objective criteria to evaluate the methodological quality of each study linking BAL parameters to BOS in order to provide an overall assessment of the strength of correlation between any specific BAL parameter and BOS. We then comment specifically on the most replicable associations with BOS and how they have impacted our understanding of disease pathogenesis. Finally, we highlight opportunities to enhance future studies and gain further insights into BOS and emerging CLAD phenotypes.

Study Identification and Appraisal

On June 30, 2012, a PubMed search was conducted using the search terms “Lung Transplant” AND “Bronchoalveolar Lavage,” and results were subsequently limited to articles published between 1991 and 2012. Using this technique, 1,176 articles were identified. The study title and abstract were manually reviewed and articles not relevant to the study of BAL in BOS were excluded based on the following hierarchy: not focused on lung transplant (n=419), non-human study (n=157), not focused on BOS (n = 449), not utilizing BAL (n = 46), and full text article not available for review (n=1). We also chose to exclude articles that focused exclusively on analysis of BAL parameters associated with gastroesophageal reflux disease (e.g., bile acids, pepsin), given that this topic was recently reviewed elsewhere (4) [n = 4]. The remaining 100 articles were read and categorized with respect to study design, number of patients included, follow-up time, and BAL parameter(s) assessed.

In order to determine which BAL parameters were the most reliable, each parameter was qualitatively ranked based on its replicability across independent studies and on the individual trial designs. Parameters fell into one of three broad categories largely based on sample size and inter-study agreement. A parameter was considered *highly* replicable if at least three studies found it to be significant, in which each study featured greater than 30 subjects (at least 15 with BOS). A parameter was considered *moderately* replicable if it was found to be significant in two studies following the criteria described above, or in three or more studies including >= 20 subjects (at least 10 with BOS). A parameter was considered *weakly* or *non*-replicable if it was assessed in only one study, assessed in studies providing disparate results, or assessed in studies not meeting the aforementioned methodological criteria. The majority of studies describing high and moderately replicable parameters accounted for or omitted concurrent infection and acute rejection. Notably, although follow-up time is an important consideration in evaluating study strength, heterogeneity in reporting precluded its inclusion in these objective criteria.

Overview of Studies

Applying the above criteria, we identified 100 articles describing 176 distinct parameters in the BAL of lung transplant recipients with BOS. Commonly identified BAL parameters were subclassified into the following major categories: cellular composition, cytokine and chemokine profiles, innate immune components, matrix metalloproteinases, and markers of oxidative stress. Considering all available studies, the overall median sample size was 39.5, and the median number of patients with BOS was 13. Table 1 outlines the median sample size and other characteristics with respect to each individual BAL parameter category. While

the majority of studies featured strictly cross-sectional designs, 36% included a longitudinal component which examined the trajectory of parameters over time. Of the 176 parameters described, our analysis identified 7 as highly replicable, 13 as moderately replicable, and 156 as weakly or non-replicable. A summary of high and moderately replicable BAL constituents in BOS is outlined in Table 2, and the remaining weakly or non-replicable parameters are summarized in Supplementary Tables 1 and 2.

BAL Cell Composition

By far the neutrophil is the most predominant cell type in the BAL fluid of patients with BOS, whether described as a percentage of total cells or in absolute cell count. Thirty-six out of the 38 studies examining neutrophils found them to be elevated in association with BOS (5–42). More convincingly, the degree of BAL neutrophilia increases in correlation with increasing BOS stage (9). Going beyond a simple association, several studies have attested the value of elevated BAL neutrophil counts prior to the onset of BOS as a factor useful in predicting its future development. For example, Neurohr et al. followed 63 patients for three years post-transplant and demonstrated that neutrophil proportions greater than 20% predicted BOS onset a median of 232 days post-bronchoscopy (19). Similarly, Slebos et al. showed that a percentage of neutrophils of at least 16% predicted future BOS, while Reynaud-Gauber, et al. reported a threshold of 24% (23, 24, 28).

These data suggest that neutrophils play a central role in the development or progression of BOS. More recently, it has also been proposed that BAL neutrophilia can be used to identify a subphenotype of CLAD, neutrophilic reversible airway dysfunction (NRAD), which may be responsive to treatment with azithromycin (43). While further studies are necessary to better understand the role of neutrophils in promoting epithelial injury and subsequent fibrosis, the concept of using a BAL parameter to identify disease subtypes that respond differentially to treatment is intriguing and represents a priority for further research.

Beyond neutrophils, a variety of other cell types have been evaluated for their association with BOS. Given the alloimmune basis for acute lung allograft rejection, several studies have examined lymphocyte subpopulations in BOS BAL (28, 37, 44–50). While the levels of total lymphocytes, CD4+ cells, and CD8+ cells were noted to have only weakly or non-replicable associations with BOS, there is growing interest in the protective role of FOXP3+CD4+ T-regulatory (T-reg) cells and their ability to control the immune response and reduce local inflammation. Indeed, T-regs were significantly reduced in the BAL from lung transplant recipients with BOS (45, 46). The proportion of T-regs has also been shown to be predictive of BOS susceptibility, with levels less than 3.2% significantly predicting the development of BOS within two years post-transplant (45). Furthermore, Gregson et al. demonstrated that CCR7+ T-regs, which have central memory functions, were decreased to an even greater extent in BOS BAL(46).

More recently, significant attention has been paid to multipotent donor-derived resident lung mesenchymal stromal cells (MSCs), defined as cells that express stem cell markers CD73, CD90, and CD105 in the absence of hematopoietic markers, and their potential role in promoting the small airway fibrosis typical of bronchiolitis obliterans. Badri et al. demonstrated a two-fold increase in MSCs in the BAL from lung transplant recipients with BOS, and this important finding has since been replicated in two additional studies (51–53). Moreover, MSCs isolated from the BAL of BOS patients have been shown to have a more proliferative and profibrotic phenotype than those isolated from stable lung transplant recipients (52–54). These findings not only highlight the importance of MSCs in promoting or sustaining a fibrogenic milieu, but also represent a unique translational approach to

leverage cells from the BAL for ex vivo culture to provide additional mechanistic insights into disease pathogenesis.

BAL Cytokines, Chemokines, and Growth Factors

While BAL levels of a variety of cytokines and chemokines have been evaluated, interleukin-8 (IL-8) has been the most striking and consistent parameter associated with BOS. IL-8 is a pro-inflammatory cytokine and potent neutrophil chemotractant released primarily by alveolar macrophages and epithelial cells. Eighteen out of the 20 studies examining IL-8 have shown it to be elevated in the BAL of lung allograft recipients with BOS (5, 10–12, 18, 19, 23, 25–31, 35, 55–59). Additionally, multiple longitudinal studies have demonstrated that IL-8 begins to increase up to one year prior to clinical BOS diagnosis, suggesting that IL-8 plays a role in BOS development (19, 23, 27).

Building upon the previous results that considered cellular components in BOS, eight studies have shown IL-8 levels to correlate strongly with BAL neutrophil counts (10, 12, 18, 19, 23, 26, 28, 35). In a well-designed cohort of 86 patients, DiGiovine et al. demonstrated that IL-8 levels were positively correlated with percent neutrophils with an overall correlation coefficient of 0.85. Furthermore, the authors performed chemotaxis experiments and confirmed that IL-8 accounts for at least a portion of neutrophil chemotactic activity (10). These observations provide compelling evidence that IL-8 is a driving force for neutrophil influx into the lung allograft.

The CC chemokines, also involved in inflammation, have received considerable attention as well. Of these chemokines, CCL2, also known as monocyte chemotractant protein-1 (MCP-1), has provided the most striking and highly replicable association with BOS. Along with recruiting monocytes, MCP-1 attracts memory T-cells and dendritic cells, and may contribute to the differentiation of monocytes into fibrogenic macrophages (60, 61). All six of the studies examining MCP-1 found it to be elevated in the BAL of lung transplant recipients with BOS (23, 27, 56, 59, 62, 63). In addition, Reynaud-Gaubert et al. demonstrated elevated MCP-1 prior to clinical BOS diagnosis (23).

Also noteworthy are moderately replicable cytokines and chemokines, including interleukin-6 (IL-6), endothelin-1 (ET-1), monokine induced by gamma interferon (MIG, also known as CXCL9), interferon gamma induced protein 10 (IP-10, also known as CXCL10), and Regulated Upon Activation Normal T-Cell Expressed and Secreted (RANTES, also known as CCL5). Although studies involving these cytokines have not been reported as consistently, each has been described as elevated in cross-sectional studies (23, 27, 44, 52, 59, 64, 65) and several were demonstrated to be elevated prior to BOS onset in longitudinal studies (23, 27, 63, 64).

Interestingly, despite a growing interest in interleukin-17 (IL-17) in the development of lung transplant rejection, only weakly replicable evidence supports its role as a BAL biomarker of BOS (29, 59, 63). It is possible that assessment of different IL-17 subtypes may have contributed to disparate findings among these studies. Additionally, as is the case with many cytokines and chemokines, IL-17 levels may be near the lower limit of detection by traditional assays, and alternative approaches that consider tissue staining or flow cytometry-based methods to examine intracellular cytokine production might ultimately prove more valuable.

BAL Innate Immune Components

Increasingly, the innate immune system is recognized to play a key role in instructing host adaptive immunity and contributing to the development of allograft rejection. For this

reason, several components important to innate host defense, including antimicrobial peptides (alpha defensins and secretory leukoprotease inhibitor [SLPI]), surfactant proteins, and neutrophil elastase have been assessed in the BAL from lung allograft recipients with and without BOS and these results have substantiated a role for the innate immune system in this complex process.

To date, alpha defensins, primarily neutrophil-derived antimicrobial peptides, are the innate immune elements holding the most highly replicable BOS association, with multiple studies reporting an increase in their levels in the BAL of patients with established BOS (5, 66–69). Nelsestuen et al, demonstrated alpha defensin levels 10 to 100 times greater in allograft recipients with BOS compared to stable post-transplant patients. This increase was substantially higher than the corresponding increase in neutrophil counts, suggesting that increased alpha defensins are more than simply a reflection of concomitant neutrophilia. Furthermore, the authors showed that alpha defensin levels begin to increase in the BAL up to 15 months prior to BOS diagnosis. Since alpha defensins have been shown to induce epithelial and fibroblast proliferation and increase collagen expression, they may play a plausible role in initiating a fibrotic response to allograft infection or other exogenous insults (66, 70–72).

Beyond alpha defensins, the associations of SLPI, surfactant protein A (SP-A), and neutrophil elastase with BOS has been evaluated with moderately replicable results. Out of the four studies assessing SLPI, three found diminished levels in the BAL of lung transplant recipients with BOS. In addition, Neurohr et al. described decreased SLPI in stable patients who subsequently developed BOS (5, 14, 19, 73). The surfactant proteins are similarly reduced in association with BOS, and a reduction in SP-A in particular may predict future BOS onset (74–76). In contrast to these results, neutrophil elastase has been noted to be increased in the BAL. These findings are, however, pathophysiologically compatible, as neutrophil elastase is known to facilitate SLPI degradation (14, 21, 73, 77).

Matrix Metalloproteinases

The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes, some of which are thought to contribute to extracellular matrix remodeling. MMPs also display proteolytic activity, and are able to break down several of the lung's structural proteins, including gelatins, collagens, fibronectin, and elastin (78, 79). While several MMPs have been evaluated in the BAL of lung transplant recipients, the most striking is MMP-9.

MMP-9 is a gelatinase, and contributes to the migration of inflammatory cells through the endothelial layer (80, 81). Nine studies clearly showed MMP-9 to be elevated in the BAL of patients with BOS using multiple methodologies (protein array analysis, specific ELISA, gelatin zymography, and immunocytochemical staining) in well-constructed cohorts absent of concurrent infection or acute rejection (6, 15, 55, 57–59, 73, 76, 82). In addition, Ramirez et al. demonstrated MMP-9 elevation an average of 140 days prior to BOS onset, indicating that MMP-9 plays a relatively early role in matrix remodeling (57). Notably, multiple studies have also shown MMP-9 levels to correlate strongly with neutrophils, suggesting that neutrophil influx during BOS could contribute to MMP-9 elevation (15, 73). Other MMPs and their counterparts, the tissue inhibitors of metalloproteinases, have also been evaluated, but their associations with BOS have not been as striking or consistent.

Oxidative Stress

In addition to defensins and MMPs, activated neutrophils release reactive oxygen species (ROS). ROS can invoke epithelial injury, deplete antioxidant defenses, and stimulate the release of pro-inflammatory cytokines (83). One of the most prominent ROS is

myeloperoxidase, shown in six distinct studies to be elevated in the BAL of lung allograft recipients with BOS (7, 10, 14, 25, 26, 59). In addition, Riise et al. described elevated myeloperoxidase up to five months prior to BOS diagnosis, indicating that oxidative stress may contribute to early epithelial injury and cytokine release (25).

Along with specific ROS, several studies have focused on general indicators of oxidative stress, including levels of glutathione, lipid peroxidation, and oxidized metathione. One of the most compelling oxidative stress measures is glutathione, a prominent antioxidant. The reduced form of glutathione has been shown to be decreased in association with BOS, while the oxidized form is significantly increased (7, 26, 84). Similarly, Madil et al. showed increased lipid peroxidation in a well-defined cohort of 58 patients, 35 of which had BOS. The authors also demonstrated lipid peroxidation and oxidized glutathione to be even higher in the BAL of patients with severe BOS compared to mild BOS (84). Oxidized metathione was described as increased in two studies, and was also shown to correlate significantly with increased myeloperoxidase, further indicating that neutrophil-derived ROS contributes to oxidative stress (7, 14). Together, these consistent results suggest a net increase in oxidative stress in association with BOS.

BAL Parameters in the Context of Emerging CLAD Phenoytpes

In general, it remains uncertain how the BAL parameters evaluated to date relate to emerging subphenotypes of CLAD, for example NRAD and restrictive allograft syndrome (RAS). Additional complexity is imposed given the variable approaches to identify these novel phenotypes as well as ambiguity regarding the exact relationship of NRAD or RAS to BOS. For example, NRAD is typically described to *precede* BOS, while in some cases RAS may develop *after* BOS onset (43, 85). Although the majority of studies included in this review predate the description of NRAD and RAS, both of these new disease subtypes are heralded by airflow limitation and thus are likely represented to some extent within these prior patient cohorts. In fact, such phenotypic heterogeneity may have contributed to variability in observed results, and this is of particular interest with respect to BAL neutrophilia.

Only two of the 100 articles identified for this review specifically stratified patients according to one of these emerging CLAD phenotypes (59, 76). In the first, Verleden et al demonstrated that BAL from patients with NRAD had greater cytokine and growth factor variability than BAL from patients with non-neutrophilic BOS (59). This lends pathophysiologic credibility to the concept that NRAD may be an important antecedent inflammatory state central to the establishment of airways fibrosis and BOS. In the second, Kosanam et al performed proteomic analysis comparing three patients with RAS to four stable lung recipient controls (76). Interestingly, several of the BAL parameters differentially expressed in the RAS group paralleled those found to be highly or moderately replicable within the current review, including an increase in MMP-9 and myeloperoxidase and a decrease in SP-A, suggesting that some similarities may exist among all forms of CLAD. Moving forward, longitudinal studies with rigorous CLAD phenotyping will be necessary to fully elucidate the BAL profiles and important mechanistic overlaps or distinctions between BOS, NRAD, and RAS.

Methodological Limitations of Current Studies

In evaluating the current studies that consider BAL parameters in BOS, several common limitations emerge, as summarized in Table 3. Perhaps most notable are small sample sizes, lack of control for potential confounders, and absence of detailed methods related to BAL collection and handling. Several aspects of BAL technique, such as the anatomic site of lavage, the amount of fluid and number of aliquots instilled, return volume, and timeliness

of transport and suspension in appropriate culture media have been shown to affect the recovery of cellular and acellular alveolar constituents(86).

Recently, an American Thoracic Society committee performed an extensive literature review related to BAL standardization and provided key recommendations to limit BAL variability; including targeting a total instilled volume of 100 to 300mL in 3 to 5 aliquots at a consistent anatomic site. Furthermore, they offer a detailed report on sample processing methods that can by employed to decrease inconsistency in the detection of cellular elements in particular(86). Efforts to adhere to these newly developed recommendations and improve on the other methodological limitations highlighted here may lead to more reproducible observations in future studies examining BAL parameters in BOS.

Conclusions and Future Directions

Examination of the cellular composition, cytokine profile, and protein constituents in bronchoalveolar lavage (BAL) fluid has not only provided a unique window into the microenvironment of the lung allograft, but has also suggested plausible pathobiologic mechanisms that contribute to the development and progression of BOS. Interestingly, by considering the collective core of most replicable parameters and their relationships to each other, a plausible scenario develops which highlights a central role for neutrophils and their inflammatory products in BOS (Figure 1).

It is likely that sustained epithelial injury from a myriad of post-transplant insults (ischemia-reperfusion injury, pulmonary infection, cellular or humoral acute rejection) leads to release of IL-8 and other pro-inflammatory cytokines, which serve to activate the airway epithelium and promote the influx of inflammatory cells, including neutrophils. These activated neutrophils then release MMPs, defensins, and ROS that result in matrix degradation, fibroblast proliferation, depletion of antioxidant defense, and, ultimately, airway remodeling and fibrosis.

While this is one plausible mechanism suggested by the most reproducible BAL observations; BAL data are best interpreted within the context of information derived from non-BAL related clinical studies of BOS and experimental models of lung allograft rejection. Collectively, these lines of research have established that humoral immunity, innate immunity and autoimmunity in addition to cellular immunity contribute to BOS development (3). Within this framework, it is interesting that several reproducible BAL changes identified in our review support such diverse mechanisms. For example, changes in RANTES and IP-10 could affect auto as well as alloreactive T-cells and alterations in clara cell secretory protein or SP-A could influence monocyte and macrophage function. It is also important to note that although many of the most replicable BAL parameters can be mechanistically related to neutrophils, they can also function in other ways. IL-8, for instance, is recognized as a potent neutrophil chemotractant, but also promotes angiogenic activity that may be important during the fibroproliferative phase of BOS (87).

Although these studies of BAL analysis have provided interesting insights into the mechanisms underlying BOS, there are many opportunities to enhance the quality of the data being generated. Future research examining BAL from lung transplant recipients with BOS should include large sample sizes, rigorous clinical phenotyping, post-transplant time matching, standardized BAL collection and handling techniques, and control for confounding factors such as acute rejection and infection. Studies should also consider how parameters vary with newly identified CLAD subphenotypes (e.g. NRAD and RAS), and how they change across longitudinal prospective patient cohorts. Furthermore, positive associations should be validated in independent populations, and this will likely require collaboration between multiple transplant centers.

In conclusion, over the last twenty years numerous studies have analyzed BAL as a tool to predict, diagnose, and understand BOS. Methodological concerns notwithstanding, these studies have provided important insights into disease pathogenesis. As the quality of studies continues to improve, BAL analysis can not only be leveraged to gain further insight into BOS pathogenesis, but can also serve to generate biomarkers that may be incorporated into models to predict disease development or treatment response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BOS	Bronchiolitis obliterans syndrome
CLAD	Chronic lung allograft dysfunction
BAL	Bronchoalveolar lavage
NRAD	Neutrophilic reversible airway dysfunction
T-reg	T-regulatory cell
MSC	Mesenchymal stromal cell
IL-8	Interleukin-8
MCP-1	Monocyte chemotractant protein-1
IL-6	Interleukin-6
IL-13	Interleukin-13
IL-17	Interleukin-17
ET-1	Endothelin-1
MIG	Monocyte induced by gamma interferon
IP-10	IFN-inducible protein 10
RANTES	Regulated upon activation normal T-cell expressed and secreted
SLPI	Secretory leukoprotease inhibitor
SP-A	Surfactant protein A
MMP	Matrix metalloproteinase
ROS	Reactive oxygen species
RAS	Restrictive allograft syndrome

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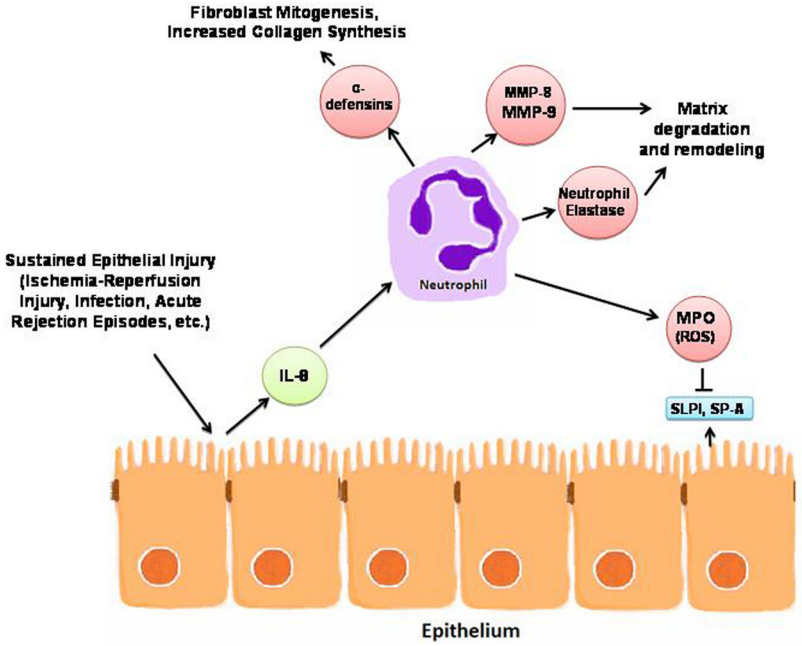

Figure 1.
Proposed central pathobiologic role of neutrophils in BOS as supported by BAL studies

Table 1

Overview of Studies Examining BAL from Lung Transplant Recipients with BOS.

BAL Parameter*	Number of Published Studies	Sample Size, median (min-max)	Subjects with BOS, median (min-max)	Longitudinal Component	Explicit adjustment for acute rejection and infection
Cell Composition	50	53.1 (14 – 194)	15.8 (3 – 61)	16 (32%)	31 (62%)
Cytokines and Chemokines	43	53 (9 – 194)	16.5 (3– 54)	22 (51%)	25 (58%)
Innate Immune Components	23	48.2 (16 – 121)	19.8 (6 – 56)	7 (30%)	12 (52%)
Matrix Metalloproteinases	9	28 (20 –72)	12.5 (7 – 34)	3 (30%)	7 (70%)
Markers of Oxidative Stress	11	39.2 (22 – 86)	14.6 (5 – 35)	2 (18%)	6 (55%)

*Among the 100 total BAL studies, some examined multiple biomarkers and are thus included in more than one category.

Table 2

Significant BAL Parameters with Highly or Moderately Replicable Associations with BOS.

Parameter	Direction of Association	HIGH	MOD.
Cell Composition			
Neutrophils (5–42)	↑	■	
T-regulatory cells (45, 46)	↓		■
Mesenchymal Stromal Cells (51–53)	↑		■
Cytokines, Chemokines, Growth Factors			
IL-6 (17, 27–29, 31, 57, 59, 88)	↑		■
IL-8 (5, 10–12, 18, 19, 23, 25–31, 35, 55–59).	↑	■	
ET-1 (52, 64)	↑		■
CCL2/MCP-1 (23, 27, 56, 59, 62, 63)	↑	■	
CCL5/RANTES (18, 23, 56, 59)	↑		■
MIG/CXCL9 (63, 65)	↑		■
IP-10/CXCL10 (44, 65)	↑		■
Innate Immune Components			
SP-A (74–76)	↓		■
SLPI (5, 14, 19, 73)	↓		■
CCSP (20, 68, 69)	↓		■
Neutrophil Elastase (14, 21, 73, 77, 89)	↑		■
Alpha Defensins/HNPs (5, 66–69)	↑	■	
Matrix Metalloproteinases			
MMP-9 (6, 15, 55, 57–59, 73, 76, 82)	↑	■	
Oxidative Stress			
Myeloperoxidase (7, 10, 14, 25, 26, 59)	↑	■	
Oxidized/Reduced Glutathione (7, 26, 84)	↑	■	
Methionine Sulfoxide (7, 14)	↑		■

Table 3

Common Methodological Concerns in Studies Examining BAL in BOS.

Methodological Concerns
Small sample size
Cross-sectional design
Inconsistent time-matching post-transplant in cases and controls
Insufficient follow-up post-transplant
Inconsistent adjustment for concurrent infection or acute rejection
Variation in BAL collection, processing, and normalization