

DIAGNOSIS AND MANAGEMENT OF BRONCHIOLITIS OBLITERANS SYNDROME: AN
INTERNATIONAL ISHLT/ATS/ERS CLINICAL PRACTICE GUIDELINE

ON-LINE SUPPLEMENT

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1. IS BOS THE BEST TERM TO USE FOR DELAYED, PERSISTENT LUNG ALLOGRAFT DYSFUNCTION?

The term BOS is used to connote delayed allograft deterioration secondary to the onset and persistence of airflow obstruction as manifested by a significant decline ($\geq 20\%$ from baseline value) in FEV₁. However, not all patients in whom a decline in FEV₁ and/or airflow obstruction develops have BOS, and OB may be present in allografts that do not display a significant pattern of airflow obstruction that meets previously defined criteria for the diagnosis of BOS (1,2). Several confounding conditions can also cause delayed allograft dysfunction (see TABLE 4 in Executive Summary). Some of these entities may be reversible, and non-BOS causes of allograft dysfunction and FEV₁ decline should be excluded as diagnostic possibilities when the diagnosis of BOS is made. These conditions include infection, acute rejection, disease recurrence, anastomotic complications, native lung hyperinflation in single lung transplantation (SLT) and a number of conditions that cause decreases in both the vital capacity (VC) and the FEV₁ (e.g., an increase in body mass index, muscular weakness, pleural effusion, etc.) without a decrease in the FEV₁/VC ratio. Restrictive allograft syndrome (RAS) has been recently identified as a form of lung allograft dysfunction that can cause an irreversible decline in FEV₁ to $< 80\%$ of baseline, but RAS is characterized by restrictive physiology and evidence of peripheral lung allograft fibrosis (3). It should be recognized, however, that these non-BOS conditions can also coexist with OB and must not necessarily be considered as a sole cause of delayed allograft dysfunction when detected. Additionally, delayed loss of allograft function should not be assumed to be irreversible and due to OB without excluding other causes of significant lung function decline.

The use of the term “CLAD” (chronic lung allograft dysfunction) has introduced some confusion in terminology for post-transplant delayed decline in allograft function. This term has been used in a variety of ways in the published literature and has been used interchangeably with the terms BOS and chronic rejection. The committee recognized that a precise definition is needed for the term CLAD.

2. DO DISTINCT BOS PHENOTYPES EXIST?

The identification of patient groups with specific attributes or patterns of disease that differ from the entire cohort of patients with BOS may allow the recognition of specific risk factors, pathogenetic disease mechanisms, and/or strategies for treatment and prevention. Many alloimmune and non-alloimmune factors have been associated with the development of BOS, including donor-recipient mismatching (4,5), infection with a variety of pathogens including viruses, bacteria, and fungi (6-11), episodes of acute

cellular rejection (12-14) lymphocytic bronchitis/bronchiolitis (15), reperfusion injury/primary graft dysfunction (16,17), and type of immunosuppressive regimen (18,19). Additionally, BOS has been associated with bronchoalveolar lavage (BAL) neutrophilia (20,21), persistent infection (9), gastroesophageal reflux (GER) (22,23), and various indicators of compartmentalized immune activation including innate immune mechanisms (24), autoimmune activation (25), or perturbed immune regulation (26,27). However, distinct phenotypes of BOS that are based upon specific risk factors or other parameters have not been definitively established.

Distinct clinical phenotypes of timing and course of BOS have been suggested in the literature. The cumulative incidence of BOS at 5 years post-transplant may be as high as 80% (28-31). The International Society of Heart and Lung Transplantation (ISHLT) Registry data of over 10,000 recipients followed from 1994 to 2006 reveal an incidence of 25% at 2.5 years and 50% at 5.6 years post-transplant (31). More recent data show a prevalence of 75% in patients who have survived to 10 years post-transplant (32). The onset and clinical course of BOS may be gradual over months to years but an abrupt onset with rapid decline also occurs (1,33,34). Median survival for recipients with acute onset BOS was noted to be 29 months vs. 58 months for later, chronic onset BOS (33). Burton et al. (30) found that progression of BOS from lower to higher grade increases the risk of mortality up to 3-fold, and a rapid decline in FEV1 of >20% has been associated with worse prognosis (1). Additionally, Brugiere et al. (34) found that recipients with early-onset BOS had lower mean FEV1, need for supplemental oxygen, and poorer graft survival than those with later-onset BOS. These observations suggest that recipients with early onset BOS are at risk of a more rapid decline in lung function and higher incidence of graft failure and death as compared to late-onset BOS. However, some patients with rapidly declining lung function may stabilize despite an initial rapid onset and loss of lung function (35).

Other phenotypes can be suggested on the basis of potential etiology (e.g. PGD-associated graft injury, detection of abnormal GER), bronchoscopic observations (e.g. BAL neutrophilia), or response to therapy (e.g. azithromycin-responsive). However, investigations to date still have not clearly established specific phenotypes on the basis of potential causes, specific biomarkers, or response to specific therapeutic interventions. A possible exception is a phenotype of recipients with significant BAL neutrophilia who respond to azithromycin therapy (36,37) such that FEV1 improves and the recipient no longer meets the spirometric criterion of depressed FEV1. These patients appear to have a reversible, BOS-like syndrome associated with BAL neutrophilia, and the recently published, randomized prospective clinical trial conducted by Vos et al. (38) suggested that prophylactic administration of azithromycin initiated shortly after transplantation suppressed the development of this syndrome. It has been suggested that

azithromycin responsiveness may identify a phenotype that characterized by increased neutrophils in BAL, while lack of response to azithromycin may characterize a fibroproliferative OB phenotype (36).

WHAT MAJOR RISK FACTORS FOR BOS HAVE BEEN IDENTIFIED?

Although BOS has been associated with many risk factors, data are incomplete or sparse for many of these associations. The risk factors discussed below have reasonably good data to support their role as potential causes or contributors to OB/BOS.

Primary graft dysfunction

PGD affects 10-25% of all lung transplants and is a leading cause of early morbidity and mortality (39-42). PGD is a form of acute lung injury that is thought to be largely due to the effects of the periods of ischemia and reperfusion that occur during donor lung procurement and implantation. PGD is defined on the basis of the PaO₂/FiO₂ ratio and the presence or absence of chest radiograph infiltrates assessed daily up to 72 hrs (Table 1) (39). A number of studies have not consistently linked PGD to BOS (17,43-46), but recent studies support a link between PGD and BOS (16,47,48). Daud et al. (16) used ISHLT consensus definitions for PGD and found a convincing association of PGD grade with increased risk of developing BOS Stage 1. More recent analysis of outcomes by this group identified a direct relationship between PGD severity at 24, 48, and 72 hours post-transplant and the risk of BOS (49), and PGD Grade 3 at all 3 time points was associated with the highest risk of developing BOS (RR 3.31 for grade 3 at 24 hrs). Interestingly, although PGD has been identified as a risk for BOS, a similar relationship of PGD with acute rejection or LB has not been observed (16).

Acute cellular rejection

Acute rejection (AR) after lung transplantation is defined as the presence of perivascular lymphocytic infiltrates (Grade A rejection) or the presence of small airway mononuclear infiltrates termed lymphocytic bronchiolitis (LB) (Grade B rejection) on transbronchial lung biopsy (TBLB) specimens. This classification system of acute rejection, developed by The Lung Rejection Study Group and endorsed by the Board of the International Society for Heart and Lung Transplantation, is based on the intensity of the infiltrates which are graded in the following manner Grade A0 (none), A1 (minimal), A2 (mild), A3 (moderate), A4 (severe), BO (none), B1R (low grade), B2R (high grade), BX (ungradeable) (50).

Several studies that have identified Grade A acute rejection as a risk factor for the development of BOS, and Burton et al. (51) have shown via surveillance bronchoscopies that the risk of recurring AR is significantly increased following an initial episode of AR. Most of these studies have found that AR

remains a major risk factor for the development of BOS when time dependent Cox regression models were constructed and multivariate analyses accounted for other clinical events (13,14,28,44,52,53). One systematic review revealed that seven studies showed a significant association of acute rejection with at least 320 cases of BOS (55). Both late acute rejection (13,14,44) and increasing frequency and severity of acute rejection (13,44,52) have been found to be risk factors for BOS.

The significance of minimal AR (Grade A1) rejection for BOS risk after lung transplantation has been unclear. The majority of earlier studies have shown that increasing frequency and grade of acute rejection are highly associated with the development of BOS (13,44,51,52). Hopkins et al. (56) have reported that minimal AR remains relatively prevalent on surveillance transbronchial biopsies up to 2 years post-transplant. Minimal AR was recently identified as a risk factor for the development of BOS, and one study has shown that patients with multiple episodes of Grade A1 rejection have an earlier onset of BOS. A second study subsequently showed that a single episode of A1 rejection was independently associated with the progression to BOS by univariate Cox regression analysis and subsequent multivariate models (45). Lastly, two studies suggested that augmenting immunosuppression for A1 AR may be associated with a decreased risk of developing BOS (56,57).

Grade B rejection has also been identified as a potential risk factor for the development of BOS (13,15,44,53,58). The grading of LB remains more controversial due to the wider variation in scores between different pathologists, potential coexistence of infections and inadequate sampling of small airways in TBLB. Nevertheless, recent investigations have identified LB as a major risk factor for BOS (15,51), and it has been suggested that this increased risk may be due to its association with BAL neutrophilia (59). Husain and colleagues showed that a higher cumulative score of Grade B rejection was highly associated with the development of BOS (13). Recently, Glanville and colleagues showed that the highest Grade B score was associated with BOS by rigorous multivariable Cox proportional hazards analysis (15).

Humoral rejection & anti-HLA antibodies

Multiple retrospective studies and one single-center prospective study suggest that an association between pre-transplant and de novo anti-HLA antibodies and the development of BOS exists (60-64). The development of BOS can lag for more than a year from the time when such alloantibodies are detected (62). However, a retrospective study showed no difference in acute rejection (AR) (episodes/first 100 days) (2.1 vs. 1.9), BOS (38.9% vs. 31.2%) and 1- and 3- year survival rates for 21 of 247 lung transplant

recipients with panel reactive antibodies (PRA) positivity >10% as compared to those with low PRA values (65).

Diagnostic criteria for antibody-mediated rejection (AMR) after lung transplant remain controversial, and the ISHLT revision of the nomenclature of lung rejection could not reach a consensus on the histologic hallmarks of AMR in lung transplantation (50). However, the National Conference of AMR in Solid Organ Transplantation working group proposed a general classification that includes the detection of circulating donor-specific antibodies, evidence of C4d deposition in the allograft, allograft tissue pathology consistent with AMR, and evidence of allograft dysfunction (66). However, C4d and C3d staining is not specific for lung transplant associated-AMR; C4d and C3d staining has been demonstrated in primary graft dysfunction, allograft infections and in normal-appearing allograft lung tissue obtained from routine surveillance TBLB (67,68). The treatment of pre-sensitized or de novo produced anti-HLA antibodies in lung transplant recipients have only been published in case reports and small single-center series, and any possible effect of various treatments for AMR on the risk of developing BOS remains unknown.

If increased donor-specific antibody levels are detected in the context of declining lung function consistent with presentation of BOS, biopsy specimens obtained via TBLB can be examined to detect histopathological evidence of AMR (necrotizing septal capillary injury involving the interalveolar septae accompanied by intra-alveolar fibrin and hemosiderin deposition with septal fibroplasia as well as the presence of intracapillary macrophages, with acute lung allograft injury), which may be seen with or without the presence of C4d positive staining.

Gastroesophageal reflux

Multiple studies have reported a high prevalence of abnormal GER in patients with advanced lung disease (22,69-75), and GER with presumed aspiration has been linked to both subacute and chronic lung allograft dysfunction (76-89). Additionally, acid reflux may worsen following transplantation (90). Sweet et al. (70,71) have reported that although a majority of patients who undergo transplant evaluation have some evidence of significant abnormal GER, clear symptoms of GER are frequently absent. Although gastroparesis and esophageal dysmotility may also exist among patients undergoing lung transplant evaluation, the prevalence of these disorders is less clear. Despite varying definitions of abnormal GER with some studies measuring only acid and not non-acid reflux, a consistent association has been observed between the presence or severity of abnormal GER and an increased risk for BOS. A negative correlation was found between increasing severity of acid reflux (as measured by 24 hour pH study) and

post-transplant FEV1 (80). Additionally, the presence of non-acid reflux as measured by impedance testing was reported to increase the risk for BOS nearly 3-fold (87), and GER has been linked to collagen V sensitization and BOS (91). GER and aspiration of bile acids have been linked to BOS (83). Bile acids in BAL fluid have been found to be increased in cross-sectional studies of patients with BOS (83,84,92), and the presence bile acids in BAL fluid significantly increased the risk of BOS onset (83,92) as well as a poor response to azithromycin therapy (93). Recent studies in animal models of lung transplantation suggest that gastric aspiration might enhance allorecognition and promote lung allograft rejection (94,95), and Cantu et al. (81) reported that early post-transplant fundoplication was associated with greater freedom from subsequent BOS.

Viral infection

Infection caused by community-acquired respiratory viruses (CARV) and the β -herpes viruses can cause serious infection and complications in lung transplant recipients. More than 75% of solid organ transplant recipients develop primary or reactivation infection with cytomegalovirus (CMV), a member of the β -herpes virus family, following transplantation (96). In the early years of lung transplantation CMV pneumonitis occurred in up to 50% of recipients, especially in D+R- (donor-positive, recipient-negative) recipients and those with more intense immunosuppression. A large number of studies have linked pulmonary CMV infection to BOS (14,28,54,97-99). The prevalence of CMV seroconversion antibodies in the general population increases with advancing age such that more than 90% of immunocompetent individuals beyond age 80 years will be seropositive vs. approximately 40% of young adults (100). Primary infection is followed by latency, not eradication in the normal host (101). CMV infection engages both the innate and adaptive components of immunity and causes upregulation of HLA class I and class II antigens on epithelial cells (102,103), and it stimulates and augments the generation of allogeneic immune responses and pro-inflammatory cytokines (102,104). ISHLT Registry data have shown that survival over the first 5 years post-transplant is significantly worse for recipients receiving a lung from a positive donor (D+R- or D+R+) vs. recipients who receive lungs from seronegative donors (D-R+ or D-R-) (105), suggesting that de novo infection with the donor CMV strain is a significant risk factor for decreased post-transplant survival.

Prophylactic and preemptive strategies to prevent and/or treat CMV infection have had a considerable impact on the incidence of CMV disease in lung transplant recipients (106-109). Relatively small, retrospective studies of peri-operative ganciclovir prophylaxis showed a decrease in CMV disease, and ganciclovir prophylaxis also appeared to delay the onset of BOS (7,110,111). Additionally, Weigt et al. (6) found that increased levels of the CCL2 chemokine in BAL fluid during episodes of CMV

pneumonitis predicted subsequent development of BOS. A recent, prospective single-center study found a CMV pneumonitis incidence of 21% within 6 months of transplant in a cohort of 231 recipients despite short-course prophylaxis of high-risk recipients, and CMV pneumonitis significantly increased the risk of BOS (HR 2.19) and diminished survival (HR 1.89) (112). Additionally, a prospective, randomized 11-center trial of 3 vs. 12 months of post-transplant valganciclovir prophylaxis for D+R-, D+R+, and D-R+ recipients showed significantly greater CMV infection (64% vs. 10%), CMV disease (32% vs. 4%), and disease severity for 3 vs. 12 months of therapy without any significant difference in rates of acute rejection, opportunistic infection, CMV UL97 ganciclovir-resistance mutations, or adverse events (113).

Infection with other β -herpes viruses can complicate post-transplant outcome. These include Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella zoster virus (VZV), and human herpes viruses 6 (HHV-6) and 7 (HHV-7). A prospective cohort study of 385 lung transplant recipients linked repetitive detection of EBV DNA in peripheral blood with the development of BOS (114). Additionally, small studies have associated HHV-6 and HHV-7 with BOS (115,116), although a recent prospective study of 93 recipients receiving prolonged antiviral prophylaxis did not identify an association of β -herpes virus replication or viral load with the development of acute rejection or BOS (117).

The CARV virions include influenza A and B, respiratory syncytial virus (RSV), parainfluenza viruses, rhinoviruses, enteroviruses, adenoviruses, human metapneumovirus, human coronavirus, and human bocavirus. Infections with CARV can be asymptomatic, cause mild symptoms, cause significant respiratory tract disease, or lead to acute respiratory insufficiency and death. Recovery of virus during infections suspicious for CARV can range from 34 to 66% (8,118,119). Numerous retrospective investigations have linked CARV infections with BOS in up to 60% of affected lung transplant recipients (8,120-124). More recent prospective studies have also found symptomatic CARV infection to be a significant risk for BOS (125,126). However, a single-season prospective study by Milstone et al. (119) did not identify a significant association of CARV infection with subsequent graft dysfunction, although another single-season case-control study by Kumar et al. (8) showed that 18% of recipients with symptomatic CARV infection developed subsequent BOS vs. none of the control group. In a large retrospective study of nearly 576 pediatric recipients, however, an association of CARV infection occurring within the first year post-transplant with BOS was not identified at one year post-transplant, although a significant impact on survival was observed (127). Additionally, a smaller single-center retrospective study of 55 pediatric recipients did not identify a significant correlation of CARV infection and mortality or chronic allograft dysfunction (128).

Bacterial infection

Bacterial infections may trigger, amplify or promote persistence of lung allograft inflammatory and allo- or autoimmune responses and may pose an increased risk for BOS when such infections occur. Post-transplant bacterial infection is exceedingly common in recipients with prior septic lung disease (CF and non-CF bronchiectasis) and is a leading cause of death in recipients with established BOS. Transient bacterial airway colonization can significantly increase BAL neutrophils and other indicators of lung inflammation (129). Botha et al. (9) examined 155 consecutive lung transplants and reported that *de novo* allograft colonization with *Pseudomonas aeruginosa* was strongly associated with developing BOS within 2 years of transplant (23.4% colonized vs. 7.7% non-colonized).

Vos et al. (130) reported that persistent *Pseudomonas* colonization was an even greater risk for BOS than *de novo* colonization, and Gottlieb et al. (131) found that persistent allograft colonization with *Pseudomonas* in a cohort of 59 patients with CF significantly increased the prevalence of BOS. Additionally, a retrospective, cross-sectional, case-control study of 24 stable bilateral lung transplant recipients showed that BAL bile acid levels, neutrophils, and IL-8 levels correlated significantly with *Pseudomonas* colonization (132), suggesting that the presence of abnormal GER and microaspiration may be a risk factor for colonization of human lung allografts by *Pseudomonas*.

Fungal infection

Invasive fungal infections are an important cause of morbidity and mortality in lung transplant recipients. Despite recent advances and the widespread use of antifungal prophylaxis, the cumulative incidence of fungal infections in organ transplant recipients at one year was 8.6% in a large multicenter prospective study (133). This rate of fungal infection was comparable to that of mismatched allogeneic hematopoietic stem cell transplant recipients (134), and *Aspergillus* infection was observed in 80% of cases followed by *Candida* species for solid organ recipients (135). Over half of the cases of invasive aspergillosis in lung recipients occurred a year after lung transplantation, and mortality in patients with invasive aspergillosis was 29% at 12 weeks (135).

Two retrospective cohort studies have specifically looked at the association of fungal infection or disease and the subsequent development of invasive fungal infections in lung transplant recipients (10,11). In one study of 160 lung transplant recipients from 1990-2005, the role of various infections, including fungal pneumonia or pneumonitis, was evaluated in patients surviving greater than six months (10). Fungal pneumonia or pneumonitis was noted to be an independent predictor of BOS with a hazard ratio of 2.1 (95% CI 1.1-4.0) for early (0-100 days post-transplant) and 1.5 (95% CI 1.1-1.9) for late (\geq one year) fungal pneumonia on multivariate analysis (10). However, this study was prone to evaluation bias as

surveillance bronchoscopies were not routinely performed on recipients, which may have led to significant overestimation of hazard ratios (10). Fifty-four recipients developed *Aspergillus* colonization prior to a BOS diagnosis in another retrospective analysis of 201 lung transplant recipients (11). In the multivariate Cox regression analysis, *Aspergillus* colonization was independently associated with the subsequent development of BOS (HR=1.81; 95% CI 1.03-3.19) and BOS-associated mortality (HR=2.57; 95% CI 1.19-5.55). Additionally, recipients with new or persistent *Aspergillus* colonization after developing BOS had increased risk of progression to Stage 3 BOS or death. Interestingly, pre-transplant *Aspergillus* colonization did not predispose to post-transplant colonization in this study. However, all patients with pre-transplant *Aspergillus* colonization received 6 months of antifungal prophylaxis.

Autoimmunity

It has recently become apparent that *de novo* immune responses to self-antigens (e.g. myosin or vimentin in heart transplantation, collagen IV or VI in renal transplantation, and collagen V or K-alpha-1-tubulin in lung transplantation) can develop post-transplantation and may be induced by alloimmune responses (136,137). Hagedorn et al. (138) have reported that sera from patients with higher grades of BOS contain autoantibodies that react to a number of self-antigens. T cells that specifically recognize collagen V have been shown to induce peribronchiolar and perivascular inflammation (139), and these responses can be suppressed by CD4+CD45RChigh regulatory T cells (140,141) that likely account for the suppression of rejection responses and airway pathology via the collagen V-induced oral tolerance that has been observed in animal models of lung transplantation (142-144). These responses appear to be IL-17/Th17 cell-dependent (25,141,145) as observed for non-transplant-related autoimmune disorders, and IL-10-producing regulatory T cells (suppressor IL-10 T cells) that are dependent on the presence of regulatory CD4+25+ and may suppress collagen V autoimmune sensitization decline along with loss of these regulatory T cells in peripheral blood of human lung transplant recipients who develop chronic rejection (146,147). Additionally, collagen V sensitization has been associated with primary graft dysfunction (148-150). A prospective study that monitored peripheral blood mononuclear cell responses in 54 lung transplant recipients over a 7-yr period showed a strong correlation of collagen V-specific responses with the incidence (HR 5.4 for BOS-1, HR 9.8 for BOS-2) and severity of BOS (25), and induction of collagen V reactivity has been associated with abnormal GER and the development of BOS (91). Additionally, Saini et al. (151) found a strong correlation of the appearance of donor-specific anti-HLA antibodies with the detection of antibodies directed against self-antigens (collagen V and K- α 1 tubulin) in a retrospective analysis of 42 lung transplant recipients with BOS.

BAL Neutrophilia

A persistent increase in BAL neutrophils has been identified as a predictor of mortality (152), and numerous studies have shown evidence of neutrophil recruitment and activation when BAL was performed in recipients with acute rejection, infection, and/or BOS (152-155). Zheng et al. (156) observed early and persistent BAL neutrophilia in recipients who developed BOS as compared to stable patients who remained BOS-free, and BAL neutrophilia was most intense if it was also associated with concomitant lower tract infection. Neurohr et al. (20) found that increased BAL neutrophils, especially a BAL neutrophil percentage $\geq 20\%$ (hazard ratio 3.57), was predictive of subsequent BOS with a median onset at 232 days following bronchoscopy (which was performed in clinically “stable” patients 3-12 months post-transplant). Additionally, Schloma et al. (157) also reported that increased BAL neutrophils were associated with early onset BOS. Subsequent investigations by Gottlieb et al. (37) and Vos et al. (36) have also linked BAL neutrophilia to BOS and have demonstrated that a subset of patients with BAL neutrophilia ($\geq 15\text{-}20\%$) can have a significant clinical response to the administration of azithromycin such that pulmonary function improves enough to no longer meet spirometric criteria for BOS, a phenomenon that has been termed “neutrophilic reversible allograft dysfunction (NRAD).” Whether a significant degree of histopathologic OB is associated with NRAD is currently unknown.

HOW CAN AN EARLY AND ACCURATE DIAGNOSIS OF BOS BE MADE?

Lung function testing

Spirometry is easily performed and has been adopted as the test of choice to monitor recipients for allograft dysfunction following lung transplantation. Additionally, it can be easily used daily in the outpatient setting and, hence, may detect the onset of FEV1 decline earlier than clinic spirometry, which is often performed infrequently and is associated with additional costs (158). Because the onset of graft dysfunction can be insidious (1,33,34) spirometric measurements may take some time to reach the threshold of BOS Stage I or even Stage 0-p. Consequently, the diagnosis of BOS is often made later in the course of the disease process, and this may in part explain the lack of efficacy for therapies that have been used to treat BOS. An early and accurate diagnosis of progressive allograft dysfunction leading to BOS may be beneficial by allowing the initiation of potentially effective treatment options earlier in the course of disease and thereby preserving allograft function and even possibly reversing function loss.

Several studies have evaluated the predictive value of the ISHLT BOS grading system including the BOS-0p threshold that was adopted to assist in earlier detection of BOS onset (159-161). These studies suggest that the FEV1 criterion for BOS-0p (10-19% decrease in FEV1) may provide useful information that predicts the subsequent development of BOS in both single and bilateral lung transplant recipients.

Lama et al. (161) found that the probability of testing positive for BOS-0p by the FEV1 criterion was 71% at two years before the onset of BOS and the specificity of the FEV1 criterion was 93% in single lung transplant (SLT) recipients. Interestingly, a higher specificity and predictive ability was shown by this criterion for SLT recipients with underlying restrictive physiology. Hachem and colleagues (160) similarly describe a sensitivity of 74% and a specificity of 86% for the BOS-0p FEV1 threshold in predicting the subsequent development of BOS in bilateral lung transplants. Stage BOS-0p has also been defined by a $\geq 25\%$ decrease in forced expiratory flow, mid-expiratory phase (FEF₂₅₋₇₅). Assessment of the predictive ability of this criterion has had mixed results. Lama and colleagues (161) found that the FEF₂₅₋₇₅ criterion lacked sensitivity and specificity as the sole predictor for BOS in single lung transplant recipients. However, Nathan (159) and colleagues found a 80% sensitivity and 82.6% specificity of the FEF₂₅₋₇₅ criterion in 43 single lung transplant recipients. Differences between these two studies may be related to different statistical techniques, sample size, and follow up time. Hachem et al. (160) found that the FEF₂₅₋₇₅ criterion had a 78% sensitivity but only a 44% specificity in bilateral lung transplant recipients. Although BOS is classically considered to be associated with an obstructive pattern of lung function decline, more recent data suggest that FEV1 and FVC can decline together (2) suggesting a restrictive ventilatory defect may be observed, which is not consistent with the current definition of BOS and may identify a subset of patients with RAS rather than BOS.

The 6-minute walk test (6-MWT) has proven to be a very useful instrument for detecting exertional oxyhemoglobin desaturation, the need for supplemental oxygen requirements on exertion, and disease stability vs. progression via determination of walk distance and oxyhemoglobin saturation (SpO₂) profile (162,163). However, parameters other than lung function can have an impact on 6-MWT results. These include cardiac function, muscle strength, conditioning, or technical problems and/or lack of adherence to strict methodological protocols. Although centers may use this test to monitor lung transplant recipients in the clinical post-transplant setting, there is a paucity of published data using the 6-MWT for screening and serial monitoring for allograft dysfunction. Nathan et al. (164) retrospectively examined the 6-MWT in 42 patients with BOS and found that walk distance was a strong predictor of survival and performed better than spirometry.

Thoracic imaging

Although posterior-anterior routine chest radiographs (CXR) are frequently performed and useful to identify and screen for significant abnormalities and complications post-transplant, it is generally recognized that the CXR is relatively insensitive and non-specific, and CXR imaging is a poor predictor of early allograft rejection or BOS (165,166). High-resolution CT scanning (HRCT) can detect numerous

changes associated with acute rejection such as interlobular septal thickening, ground-glass opacities, consolidation, and volume loss, but the HRCT has limited accuracy in the diagnosis or severity grading of acute rejection (167). HRCT imaging in BOS can show bronchial wall thickening, mosaic attenuation, air trapping, small nodular and linear branching opacities in a bronchiolar distribution, and bronchiectasis (168). Leung et al. (169) obtained inspiratory and expiratory HRCT in 21 (11 with biopsy-proven OB, 10 without BOS) transplant recipients. Expiratory images showed air trapping in 91% of recipients with OB and had a specificity of 80% and diagnostic accuracy for BO of 86%. Bankier et al. (170) examined a series of heart-lung transplant recipients and found that an air-trapping threshold of 32% distinguished recipients with BOS vs. those without with a sensitivity of 83% and specificity of 89%. In contrast, Lee et al. (171) examined 7 recipients with biopsy-proven OB and 21 with normal lung function and biopsy findings and found a lower sensitivity of 74%, specificity 67%, and accuracy 71%. Similarly, Berstad et al. (172) followed 40 consecutive recipients for a median of 3 years post-transplant and found that air trapping on HRCT had a sensitivity of 77%, specificity 74%, positive predictive value 68%, and negative predictive value 81%. Lastly, Konen et al. (173) examined consecutive HRCTs in 52 recipients (26 with BOS) and found a lower sensitivity for air trapping (44%) and mosaic attenuation (20%) with early BOS but specificities of 100% and 96% respectively. Early studies of (3)He-MRI suggest that this modality may be more sensitive than HRCT for detecting OB, but larger studies are needed to determine the clinical utility of imaging with MRI using rare gases (174-176).

Bronchoscopy

Bronchoscopy with TBLB and BAL for lung transplant recipients has been developed as a means of detecting infection and/or allograft rejection, which can both be occult, with the purpose of providing appropriate therapy that can prevent loss of allograft function and OB, and TBLB is the key procedure for detecting the presence of acute rejection, LB, or other parenchymal abnormalities (177,178). Burton et al. (178) have demonstrated that surveillance TBLB can retrieve tissue sample that show changes consistent with OB. In this study, OB was not detected until after 3 months post-transplant, but the number of patients with changes of OB as well as other forms of tissue fibrosis steadily increased over a 2-year time period post-transplant. Concomitantly obtained BAL fluid can be analyzed for evidence of significant infection and to determine the BAL immune cell profile (179,180). The majority of transplant centers perform surveillance bronchoscopy to facilitate optimal recipient management and intervene with appropriate therapy if significant occult abnormalities are detected (181,182). However, many centers do not routinely perform surveillance bronchoscopy, and no clinically significant difference in post-transplant outcomes including survival was identified in one single-center, retrospective study for a cohort of recipients subjected to scheduled surveillance biopsies vs. a cohort for which only clinically indicated

biopsies were performed (183). No adequately powered, multi-center, randomized trials of surveillance versus clinically-mandated TBLB to provide firm evidence of a strong risk-benefit ratio for either strategy have been reported in the literature. Data from centers that have either abandoned surveillance procedures (184) or perform them only for clinical trials (183,185) have been confounded by the use of historical controls or small mismatched cohorts. Furthermore, operator performance has not been analyzed critically as a determinant of risks associated with these procedures. Nonetheless, it is acknowledged that high volume bronchoscopy centers report low rates of morbid sequelae (186) with some exceptions (187), and a comprehensive safety protocol can minimize the risk of complications (188). Surveillance bronchoscopy with serial BAL sampling may detect early persistent neutrophilia that may be amenable to azithromycin treatment, which may suppress the neutrophilia and prevent FEV1 decline with progression to BOS (36,38).

Predictors of BOS

The identification and validation of early markers that detect early, subclinical BOS have the potential to allow early interventions that may prevent or lessen progressive declines in allograft function. Persistent BAL neutrophilia (see above) and the presence of bile acids in BAL (23,83,93) have been linked to risk for the development of BOS (see above). Numerous other biomarkers in respiratory secretions, peripheral blood, or exhaled gas have been examined and proposed as potential markers and/or predictors of OB (26,154,189-210). Other potential predictors such as detection of bronchial hyperresponsiveness (212,213), altered patterns of ventilation distribution (154,214), or advanced imaging with magnetic resonance (174-176,215) may detect early BOS, but these have not been validated as having adequate predictive power. Although many potentially valuable markers of evolving BOS have been studied to date, none have been demonstrated to reliably predict impending allograft dysfunction prior to the onset of changes in pulmonary function. Additional research is needed to identify reliable markers of BOS.

Diagnostic approach

When clinically stable lung transplant recipients develop symptoms (e.g. dyspnea, cough, fatigue) and/or signs (decline in FEV1 on home spirometry or at clinic visit follow-up evaluation), the symptoms and signs may indicate allograft dysfunction. A common approach to such patients is to initiate clinical evaluation in the outpatient setting that is followed by specific testing (imaging, confirmatory spirometry, and bronchoscopy as indicated) to identify a specific cause or causes of lung function decline (see Figure 1 in the main guideline). Non-OB/BOS causes must be ruled out before a definite diagnosis of BOS can be made. If a non-OB/BOS cause is identified but lung function does not significantly improve and FEV1 decline meets criteria for BOS, OB may also be present in the allograft and contributing to functional

decline. If BOS appears to be the cause of lung function decline, treatment approaches discussed in the next section can be considered, which will depend somewhat on the BOS stage and the tempo of lung function decline.

Special considerations in children

Because children under 4 years of age are generally unable to perform spirometry, making a diagnosis of chronic allograft dysfunction can be considerably more challenging. Prior to age 4 spirometry results are often unreliable, and the availability of normative reference data is limited. Therefore, for young children, the FEV1 and FEF₂₅₋₇₅ measurements that are required for the diagnosis of BOS-p or BOS may be unattainable or unreliable. Although BOS criteria do not exist for infants, infant pulmonary function techniques (using external compression vests) can provide information regarding the presence of airflow obstruction. These tests require anesthesia, specialized equipment and experience; therefore, such testing cannot be performed as frequently as spirometry (216,217). For older children who are capable of performing spirometry, to account for growth, the most recent BOS grading scheme revision recommends that percent predicted values, rather than absolute measurements, be used for calculating the BOS score (218), but this approach has yet to be validated. Thus, for infants and to a certain extent older children, diagnosis of chronic allograft dysfunction by pulmonary function criteria remains problematic.

Transbronchial biopsies (TBBx) are also more challenging in small children, particularly in infants and toddlers. Their small airways limit the size of endoscopes used for bronchoscopy and therefore the size of the suction channel. Most pediatric endoscopes have suction channels that are 1.2 mm in diameter (compared to 2.0 mm or greater for larger scopes); forceps that fit into these smaller channels significantly restrict the ability to retrieve sufficient tissue to diagnose rejection. Moreover, sufficient airway tissue is rarely present in such biopsies to allow assessment of airway inflammation or fibrosis (50,219).

Thus, modalities used in adults to establish a clinical or histologic diagnosis of chronic allograft dysfunction are unavailable or suboptimal in infants and small children. For this reason, many pediatric centers add alternative lung function measurements or imaging modalities such as ventilation/perfusion scanning and inspiratory/expiratory HRCT scanning to enhance the ability to detect the presence of airflow obstruction. Similarly, pediatric centers are more likely to use surgical lung biopsy to confirm a diagnosis of suspected OB, if it is suspected on the basis of these multiple non-invasive testing modalities, to support decisions regarding therapy (220).

HOW SHOULD BOS BE TREATED?

Intensified immunosuppression

Because BOS is generally equated with chronic rejection, intensified immunosuppression has been viewed as a logical therapeutic strategy, and the administration of high-dose corticosteroids is a standard treatment measure for newly detected AR (221,222).

Although increased doses of corticosteroids are usually the initial approach to treating acute rejection, there are no data to support intensified doses of corticosteroids as an effective therapy for BOS. Ross et al. (223) reported that no recipient of a cohort of 10 patients with evolving BOS showed any response to repetitive courses of high-dose methylprednisolone (Evidence Table 3). Chronic high-dose corticosteroid administration is associated with numerous, significant adverse side effects and has not been shown to benefit patients with BOS.

Two small case series (224,225) have suggested that cytolytic agents may slow the rate of FEV1 decline, but occult AR was not adequately ruled out, and the data are not sufficient to support the use of cytolytics. Aerosolized cyclosporine has been examined at one institution in patients with histologic evidence of OB (226,227); a beneficial effect on survival was suggested, but these results have not been replicated elsewhere. Other approaches that have suggested benefit include the addition of methotrexate (228,229), addition of cyclophosphamide (230), addition of mycophenolate (231,232) or mycophenolate plus tacrolimus (233), or addition of sirolimus (234,235).

Switching from CSA to tacrolimus has been reported to slow lung function loss by a number of investigators who published case series analyses (19,223,34,236-243). However, no prospective, randomized studies have been performed to support this switch.

Other approaches to augment immunosuppression include total lymphoid irradiation (TLI) and extracorporeal photopheresis (ECPP). These therapies attempt to reduce the numbers of sensitized lymphocytes that may be driving immunologically-mediated chronic rejection that may be causing BOS. Limited data (244-251) suggest that ECPP may reverse, stabilize or decrease the rate of decline of lung function in some patients with BOS. However, the mechanism of action is unclear but may lead to modification of host responses such as the induction of a clone-specific anti-lymphocyte immune response, altered cytokine production, and/or induction of regulatory T-cells. ECPP is generally well-

tolerated, albeit expensive but risks include infectious complications and/or bone marrow suppression (252).

Limited results with TLI have also been published (253-255), and these case series (total recipients=54) suggested that TLI slowed the rate of decline in FEV1 for some patients who could tolerate TLI and completed the majority of their treatments. Fisher et al. (254) reported significant reduction in rate of decline in FEV1 for 27 recipients who completed at least 80% of the radiation fractions (pre-TLI 123 ml/month decline vs. 25 ml/month decline post-TLI), and major adverse effects included bone marrow suppression and infection.

In summary, data from various studies suggest that intensifying immunosuppression may slow the rate of FEV1 decline in BOS, but these data have not been generated by randomized prospective trials, and predictions of clinical response vs. failure cannot be made. Also the variable natural history of BOS may confound interpretation of the efficacy of any intervention that augments immunosuppression.

Azithromycin

Macrolides and the novel azalide, azithromycin, have been shown to suppress IL-8 production and neutrophil recruitment for a variety of disorders characterized by bronchial inflammation and progressive airway damage and destruction (256).

The effects of azithromycin on lung function decline in BOS have been examined by a number of centers, and beneficial effects have been reported for approximately 35-40% of treated recipients (36-38,257-263). Complete reversal of FEV1 decline may occur in some patients, and patients with BAL neutrophilia appear to represent a subset of patients that are particularly likely to respond to azithromycin therapy (36,37,261). Additionally, Benden et al. (264) reported a beneficial effect of clarithromycin, although Dhillon et al. (265) reported that survival and BOS-free survival for a cohort of patients who “routinely” received clarithromycin did not differ from that of control groups. A retrospective cohort study by Jain et al. (263) of 78 patients treated with azithromycin showed a survival advantage via univariate analysis that was most pronounced for recipients in whom treatment was initiated during Stage 1 BOS, and multivariate analysis showed an association of azithromycin therapy with a significant reduction in risk of death (adjusted hazard ratio of 0.23, p=0.01). Similarly, Vos et al. (36) retrospectively found an increase in FEV1 of $\geq 10\%$ after 3-6 months in 40% of patients with BOS treated with azithromycin, and this response correlated with BAL neutrophilia. Lastly, a single-center, randomized, prospective, double-blind study of azithromycin versus placebo initiated upon recovery from the lung

transplant procedure demonstrated a protective effect of azithromycin in preventing BOS over an observation period of 2 years, and a substantial number of patients who initially received placebo and developed BOS responded to salvage therapy with azithromycin (38).

Detection and treatment of GER

As discussed under risk factors above, abnormal GER is highly prevalent in patients with advanced lung disease and in lung transplant recipients (22,69-75), and it has been implicated as a risk factor for BOS (69,76-90). Many of these studies used only esophageal pH probes to detect reflux, which has somewhat limited sensitivity and specificity (266), but more recent studies also used impedance in addition to pH to detect weakly acid or non-acid reflux as well as acid reflux.

Proximal gastrointestinal tract motility studies and pH/impedance testing can be used to diagnose motility abnormalities and abnormal acid and/or non-acid GER (269), but a true gold standard for detecting abnormal GER combined with penetrance into the lung is lacking. Examination of BAL for markers of aspiration (e.g. oil red O staining and determination of a lipid index, BAL fluid pepsin, BAL fluid bile acids) has been reported as useful for the detection of microaspiration of refluxed gastroesophageal material (22,23,268). Given the absence of a “gold standard” for the detection of abnormal GER and microaspiration, additional studies correlating BAL markers of aspiration with GER and BOS are needed to facilitate selection of recipients with BOS who might benefit from interventions such as laparoscopic fundoplication.

Blondeau et al. (23) reported that treatment with proton-pump inhibitor agents did not affect non-acid reflux and gastric aspiration. Nissen fundoplication can be performed with reasonable safety on lung transplant candidates with advanced lung disease or lung transplant recipients with documented abnormal GER (74,79,81,269-278) and may prevent reflux and aspiration of gastric secretions (277). Davis et al. (79) reported that 16 of 26 patients diagnosed with BOS in whom abnormal GER was detected via esophageal pH probe underwent laparoscopic Nissen fundoplication and subsequently improved with 13 of 16 no longer meeting criteria for BOS. Additionally, Cantu et al. (81) published a retrospective analysis of 457 lung transplant recipients in which the incidence of abnormal GER was 76%; a small subgroup of 14 patients who underwent fundoplication within 90 days post-transplant had significantly improved freedom from BOS at 1 and 3 years post-transplant. Burton et al. (274) performed fundoplication on 21 recipients with clinically confirmed abnormal GER at a mean of 768 days post-transplant; GER symptoms significantly improved, but one peri-operative death occurred and progression to BOS Stage 1 was not altered, although a decreased likelihood to progress to Stage 2 or 3 was

suggested. Fisichella et al. (275) compared safety and efficacy of laparoscopic fundoplication for 29 consecutive LTX recipients and found no difference in outcomes when compared with 23 non-LTX patients and no mortality. This group also showed that anti-reflux surgery was associated with reduced BAL pepsin levels (277). Hoppo et al. (74) reported significant improvement in FEV1 in 20 of 22 LTX recipients following anti-reflux surgery, and surgery was well-tolerated in the entire cohort of 24 patients, and no operative mortality or significant morbidity occurred. Finally, Hartwig et al. (276) prospectively collected data on 297 LTX recipients and reported that LTX recipients with abnormal GER via pH testing attained lower peak allograft lung function at 1 year post-LTX, but early fundoplication appeared to preserve allograft function.

Unfortunately, no prospective, randomized controlled clinical trials of fundoplication for BOS have been reported to validate these results. Nonetheless, the committee felt that minimally invasive fundoplication performed by expert surgeons may stabilize a decline in lung function associated with the presence of GER.

Retransplantation

Lung retransplantation may be the only treatment option for BOS that is refractory to other forms of treatment. A number of single-center retrospective analyses and case series on retransplantation were identified (279-285). Actuarial 1- and 3-year survival rates have improved significantly from 47% and 33%, respectively, in the 1990s to 60% and 49% in the modern period (284,285).

Outcomes for retransplantation for BOS appear to be better than outcomes for PGD or airway complications. Outcomes following retransplantation for BOS in carefully selected patients (ambulatory patients evaluated via the same selection process used for first-time transplantation) are now expected to approach those of first-time lung transplants if performed by experienced centers (280,286). However, ethical questions regarding access to the scarce resource of donor lungs (taking utility and equity into consideration) must be considered carefully in each case.

ARE PREVENTIVE STRATEGIES EFFECTIVE?

Type of Immune Suppression

Most lung transplant recipients are maintained on triple immunotherapy in the form of a CNI (tacrolimus or CSA), an antimetabolite (mycophenolic acid or azathioprine) and corticosteroids (gradually tapered to low dose). The data for this is mostly derived from other forms of solid organ transplantation. However,

this approach is generally accepted for lung transplant recipients, as it is well recognized that the lungs are the most immunogenic of the solid organs and have a greater propensity to develop both acute and chronic allograft rejection. The best forms and combinations of maintenance therapy are unknown.

There has been one multicenter study of CSA compared to tacrolimus published to date (287). CSA or tacrolimus were administered in conjunction with induction therapy and mycophenolate mofetil (MMF) in a 2-center, randomized, prospective, open-label trial. There was no apparent difference in survival seen between the two groups, but median follow-up was less than one and a half years. This relatively short duration precluded any analysis of BOS incidence; however there was a trend towards more episodes of acute rejection in the CSA group. A second single center prospective study comparing CSA to tacrolimus in patients maintained on azathioprine and corticosteroids has also been reported (288). Although this study showed fewer episodes of LB and AR, only a trend towards less BOS-0p was observed for group that received tacrolimus.

MMF has been compared to azathioprine in two prospective, randomized controlled studies (289,290). The first of these included only 6 months of follow-up and did not show any difference between the two groups in the primary endpoint of biopsy-proven acute allograft rejection (289). The second of these studies had a longer duration of follow-up. In addition to CSA and steroids, patients also received induction therapy (290). Although there was a trend towards improved survival at one year in the MMF group, this was lost at 3 years with no difference in the incidence of BOS at this later time point.

In one of the largest randomized, double-blind, multicenter trials to date in lung transplant recipients (n=213), azathioprine was compared to everolimus (291). BOS-free patients on maintenance immunosuppression were randomized to continue azathioprine or have azathioprine substituted by everolimus at 3 months. The study met the primary endpoint with fewer episodes of efficacy failure (significant decline in FEV1, allograft loss, death, or lost to followup) at 12 months (21.8% vs. 33.9%; $p = 0.046$) and better preservation of lung function in the everolimus group. However this apparent benefit was lost at 24 months, and the everolimus group had a higher incidence of treatment discontinuations and side-effects. Another large, multi-center, randomized trial (292) in which lung transplant recipients could receive everolimus (open-label) in addition to their CNI to reduce their CNI exposure versus continuing on their standard CNI regimen without everolimus, use of everolimus was associated with a protective effect on renal function, but an effect on BOS was not reported.

Although type of immune suppression (induction and/or chronic maintenance) may have an impact on the incidence and severity of BOS if manifestations of alloimmune rejection (AR, LB, AMR) are adequately suppressed, there are no adequately powered, prospective randomized clinical trials that have definitively identified a strategy (e.g. tacrolimus and mycophenolate) that is superior to another (e.g. CSA and azathioprine). However, preliminary results from a multi-center, randomized trial (EAILTx) of tacrolimus versus CSA (plus mycophenolate and prednisone in both arms) suggest that the relative risk of developing BOS is greater with CSA (2.00; 95% confidence interval 1.046-3.838, $p=0.036$) although survival did not differ between the two groups (293). Another recently published multi-center, randomized study (AIRSAC trial) evaluated sirolimus versus azathioprine added to tacrolimus-based immunosuppression, and no significant difference in the incidence of BOS was found for sirolimus versus azathioprine (294). Many other changes in donor organ preservation, surgical technique, and post-operative management are significant confounders that would need to be controlled for such studies to provide reliable results, and multi-center trials would be required to provide adequate numbers of randomized subjects.

Allograft surveillance and treatment of minimal acute rejection

Surveillance bronchoscopy has been performed by a majority of lung transplant centers to detect occult acute rejection and/or infection (176). However, there are no adequately powered, multi-center, randomized trials of scheduled, intermittent surveillance plus clinically mandated TBLB versus only clinically mandated TBLB to provide firm evidence of a strong risk-benefit ratio for either strategy. The decision to perform surveillance TBLB must balance the known risks (177) with the putative benefit of early and effective therapy for occult AR or LB (178,295) and infection (296). The case for surveillance biopsies hinges on the concept of the preservation of lung function and a reduction in morbid sequelae with the early detection and implementation of therapy for occult events that may increase the risk of developing BOS. The frequency and duration of bronchoscopic surveillance after lung transplantation has not been standardized or adequately addressed. If a surveillance program is implemented, it is likely to be most useful during the periods of highest risk for AR, which generally spans the first 6 to 12 months following transplantation. Single centers have provided longitudinal data of risks associated with the severity and frequency of AR (13) and LB (15) as determined by surveillance procedures. However, no study has definitively demonstrated that such an approach results in significant functional preservation or improvements in overall survival (297). Indeed there is evidence to suggest that acute vascular rejection is not an independent risk factor for BOS when detected and treated in a surveillance program (15). Data from centers that have either abandoned surveillance procedures (184) or perform them only for clinical trials (183,185) have been confounded by the use of historical controls or small mismatched cohorts. Furthermore, operator performance has not been analyzed critically as a determinant of risks associated

with these procedures. Nonetheless, it is acknowledged that high volume bronchoscopy centers report low rates of morbid sequelae (186) with some exceptions (187).

Other strategies

Infection prophylaxis has undoubtedly had a significant impact on outcomes, especially prophylaxis/suppression of CMV infection as discussed above. However, a clear cut impact in reducing BOS incidence and/or severity has not been demonstrated via robust randomized controlled trials. Epidemiological data strongly suggest that various infections – viral, bacterial, and fungal – likely play a role in predisposing recipients to subsequent development of BOS, but published evidence from clinical investigations that link infections to BOS onset remains weak.

As discussed above, abnormal GER and microaspiration have been strongly linked to allograft dysfunction, and these are present in a substantial number of patients with advanced lung disease and may worsen following their transplant. The identification of patients with significant GER appears to be important in management decisions and assessing risk for post-transplant allograft complications. However, a definitive marker of GER combined with microaspiration that identifies patients at significant risk for associated allograft injury and dysfunction and in whom clinical intervention such as fundoplication should be recommended remains elusive. Only retrospective studies have linked prophylactic fundoplication for recipients with GER to improved outcome and decreased incidence and/or severity of BOS.

The recently published prospective study of azithromycin when administered shortly after transplantation (38) suggests that the early administration of azithromycin as a chronic maintenance therapy can significantly decrease the risk of developing BOS. Induction of graft tolerance without significantly impairing immune defenses would be an ideal strategy for prevention of BOS, but the committee could not identify any clinical trials in humans of the induction of allograft tolerance and prevention of BOS. Although disruption of the bronchial circulation at the time of transplant has been proposed as a significant risk factor for the development of BOS (298), we could not identify any adequately powered, prospective randomized controlled trials that demonstrate any effect of bronchial arterial revascularization on BOS incidence.

Glossary of terms:

AMR – antibody-mediated rejection

OB – obliterative bronchiolitis

BOS – bronchiolitis obliterans syndrome
CARV – community-acquired respiratory virus
CF – cystic fibrosis
CMV – cytomegalovirus
CSA – cyclosporin A
CXR – routine postero-anterior and lateral chest x-ray
FEV1 – forced expiratory volume in one second
FEF25-75 – forced expiratory flow (average from 25-75% vital capacity)
VC – vital capacity
GER – gastroesophageal reflux
HLA – human leukocyte antigen
HRCT – high-resolution thoracic computed tomographic scan
ISHLT – International Society for Heart and Lung Transplantation
CLAD – chronic lung allograft dysfunction
BAL – bronchoalveolar lavage
LB – lymphocytic bronchiolitis
TBLB – transbronchial lung biopsy
MHC – major histocompatibility complex
MRI – magnetic resonance imaging
RSV – respiratory syncytial virus
6-MWT – 6-minute walk test
RR – risk ratio
TLI – total lymphoid irradiation
ECPP – extra-corporeal photopheresis

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Table 1. ISHLT PGD Grading System.

Grade	PaO₂/FiO₂	Radiographic infiltrates consistent with pulmonary edema
0	>300	Absent
1	>300	Present
2	200-300	Present
s3	<200	Present