Bronchiolitis Obliterans After Lung Transplantation*
A Review

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Abbreviations: BO=bronchiolitis obliterans; BOOP=bronchiolitis obliterans organizing pneumonia; BOS=bronchiolitis obliterans syndrome; CMV=cytomegalovirus; HLA=human leukocyte antigen; MMF=mycophenolate mofetil; TBB=transbronchial biopsy

As a result of improvements in surgical techniques, immunosuppression, and postoperative management, the current 1-year survival rate following lung transplantation is 71%, and 46% of transplant recipients will survive at least 5 years. Although infections, medication-related adverse events, and malignancies remain important complications, prolonged survival is limited predominantly by bronchiolitis obliterans (BO), a condition that was associated with transplantation more than a decade ago.2-5 Data suggest that at least 50% of lung transplant recipients surviving at least 3 months will develop BO.6 BO is not only responsible for death, but may lead to substantial disability, morbidity through infections, and morbidity secondary to augmented immunosuppression in attempts to halt progression.

The terminology for BO has been refined with increasing experience and understanding of this disorder. The term “bronchiolitis obliterans” is used in “The Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart and Lung Rejection” of the International Society for Heart and Lung Transplantation,7 and in its revised form.8 It describes the histologic features of what is assumed to be chronic lung allograft rejection. The histologic hallmarks of BO are scar formation and fibrosis in small airways, which are often accompanied by intimal thickening and sclerosis of vessels (chronic vascular rejection).

The diagnosis of BO requires tissue obtained from either transbronchial biopsies (TBBs) or an open lung biopsy. However, the diagnostic evaluation by TBB may lead to underdiagnosis of BO because of sampling error, the patchy character of the process, and other coexisting pathologies. Repeated sampling by open lung biopsy, a more reliable method of diagnosis, is unrealistic. Therefore, in 1993, an expert group from the International Society for Heart and Lung Transplantation created a clinically applicable system for the staging of chronic rejection after lung transplantation.9 In addition to the preexisting histologic grading system,7 a classification of chronic pulmonary graft dysfunction based on an objective clinical marker was proposed. The FEV1 has been found to be a reliable and consistent indicator of graft function and was selected for use in scoring chronic allograft dysfunction. The term bronchiolitis obliterans syndrome (BOS) describes the deterioration of graft function after lung transplantation not explained by acute rejection, infection, and problems of the bronchial anastomosis. The BOS grading system requires the establishment of a posttransplant baseline value of FEV1 (average of the two best measurements taken 3 to 6 weeks apart) and is divided into four categories (Table 1). The system has been used widely and is commonly reported in clinical studies of chronic lung allograft rejection.

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INCIDENCE AND PREVALENCE

At Stanford University, the prevalence of clinically or histologically diagnosed BO in lung transplant recipients surviving longer than 3 months was 68%. The incidence within the first year after transplantation was 28% and the actuarial freedom from BO was 67, 64, 42, and 15% at 1, 2, 3, and 5 years after lung transplantation. Long-term data indicate that the cumulative risk of BO may reach 60% after 5 to 10 years after transplantation. Some reports indicate that the prevalence might be higher in children. The Pittsburgh lung transplant experience was associated with a mean time to the occurrence of BO after transplantation of 434±422 days, with a wide range of 60 to 2,058 days. Whereas most of the patients developed BO within the first two years after surgery, the others remained at risk, including those who survived beyond 5 years. Survival at 4 years posttransplant was about 40% for recipients developing BO vs more than 80% for patients without this complication. It appears that between 25 and 40% of recipients will die directly or indirectly (infections) from BO.

HISTOLOGY

The histopathologic features of posttransplant BO have been described in various reports. The hallmark is an organizing inflammatory response centered on the respiratory and terminal bronchioles, and mature collagen is a key requirement for the pathologic diagnosis of BO. The collagen may totally or subtotally fill the lumen of the bronchiole or may lie in a subepithelial location. The mature collagen may be accompanied by the chronic inflammatory infiltrate and by proliferating fibroblasts and extracellular matrix. The lesions are patchy in distribution and may be heterogeneous in the temporal stage of injury. If inflammatory cells are present, the lesion is considered active, characterized by a mononuclear infiltrate consisting of lymphocytes, plasma cells, and monocytes. This bronchiolar infiltrate is perivascular and transmural and may cause ulceration of the bronchiolar mucosa. If inflammatory cells are absent, the lesion is considered inactive.

The Lung Rejection Study Group from the International Society for Heart and Lung Transplantation recommended in its 1995 modification of the 1990 working formulation of pulmonary allograft rejection that distinguishing between subtotal and total forms of BO by TBB is not worthwhile. An emphasis was placed on the differentiation of the relative intensity of the inflammatory infiltrate in the BO lesions, dividing them into active and inactive lesions. Active BO lesions consist of proliferation of submucosal or intraluminal fibrous tissue accompanied by mononuclear infiltrative cells (Fig 1) whereas infiltrates are absent in inactive lesions (Fig 2). In addition, the Study Group recommended in the revised formulation that the presence and intensity of combined large and small airway inflammation should be noted histologically and recognized as a possible precursor of BO. Nonfibrosing lymphocytic inflammation of the airways, termed lymphocytic bronchitis/bronchiolitis, may precede, accompany, or follow acute rejection, and has been associated with an increased risk of subsequent BO. Hence, whereas BO is by definition confined to the small airways, the entire bronchial tree may be involved.

Ongoing acute rejection presenting with perivascular mixed lymphocytic infiltrates can coexist with BO in the same biopsy specimens and most centers treat acute rejection in the context of BO in the same way they would treat it in the absence of BO.

Chronic vascular pathology may occur concomi-

**Figure 1.** Active BO with a bronchiolus showing luminal narrowing by a mild and diffuse submucosal increase of connective tissue, and a mixed mononuclear cell infiltrate (hematoxylin-eosin, original ×200).
tantly with BO.\textsuperscript{20} It is characterized by vascular intimal fibroproliferation and sclerosis.\textsuperscript{24} The pathologic appearance is very similar to the proliferative vascular sclerosis of other solid organ allografts and involves large elastic and smaller muscular vessels (Fig 3). The impact of these vascular changes on the outcome after lung transplantation cannot be estimated because the chronic airway changes are the dominant factor affecting survival.

Bronchiolitis obliterans organizing pneumonia (BOOP), characterized as a clinicopathologic entity in nontransplant patients,\textsuperscript{25} has also been noted in lung transplant recipients\textsuperscript{7,15,23,26-28} and may have an incidence of up to 10\%.\textsuperscript{29} BOOP is histologically distinct from BO. BOOP is characterized by nodules of granulation tissue located within the lumen of small airways which extend into the alveolar ducts and alveoli (Fig 4). The lesion may result from acute injury due to acute rejection, infection, aspiration, or other causes. Treatment is usually determined by the etiology of BOOP. Some investigators have suggested that BOOP is predictive of subsequent BO,\textsuperscript{26,29} whereas others could not find such a correlation.\textsuperscript{27}

**Pathogenesis**

Graft rejection has been traditionally classified by its histopathologic pattern as acute or chronic. Acute rejection is orchestrated primarily by helper T-lymphocytes, which recognize donor major histocompatibility complex epitopes and secrete cytokines stimulating proliferation of cytotoxic T lymphocytes. These cells in turn cause injury to the graft. During acute rejection, donor-specific alloreactive lymphocytes have been found in BAL fluid and transbronchial biopsies.\textsuperscript{29-32} In addition, antibody-dependent processes appear to play a role in rejection.\textsuperscript{33,34}

In all solid organ allografts, chronic rejection is histologically characterized by a common picture of fibrous obliteration of endothelialized or epithelialized luminal structures. Rejection of liver allografts is manifested by disappearance of bile ducts (vanishing bile duct syndrome).\textsuperscript{35} Chronically rejecting kidneys demonstrate glomerular sclerosis and tubular atrophy.\textsuperscript{36} Cardiac transplant rejection is associated diffuse concentric narrowing of the coronary arteries.\textsuperscript{18,37} Chronic lung allograft rejection manifests as BO. The key factors for triggering chronic rejection are still a matter of discussion. Although acute and chronic rejection are distinct from a mechanistic point of view and present with different histopathologic pictures, the frequency, intensity, and duration of acute rejection episodes are thought to be correlated with subsequent chronic rejection.\textsuperscript{38-42} Thus, the obstructive lesions characteristic of chronic re-

**Figure 2.** Inactive BO showing complete fibrous luminal obliteration without residual epithelium. The elastic lamina and smooth muscle layer are partially disrupted (Elastica van Gieson, original ×150).

**Figure 3.** Chronic vascular rejection: artery with asymmetrical fibrointimal thickening, mural mononuclear inflammatory cell infiltrate, and a thrombus (hematoxylin-eosin, original ×200).

**Figure 4.** BOOP: focus of BOOP with fibroblast plugs obliterating bronchiolar lumen and peribronchiolar air spaces (Elastica van Gieson, original ×150).
jection are thought to progress through a cascade of events. Repetitive endothelial or epithelial injury is followed by repair and by proliferation of smooth muscle cells or other mesenchymal cells like fibroblasts, finally leading to luminal obliteration.\textsuperscript{43-45} Clonal expansion of T cells, driven by a limited number of immunodominant epitopes, has been documented to be prevalent in the circulation and in the BAL fluid of lung transplant recipients with BO.\textsuperscript{46} It is suggested that these cognate, cellular immune responses play an important role in the pathogenesis of BO.\textsuperscript{46} In BO, injury to epithelium coincides with a defective regenerative reaction of the mucosa and stimulation of a fibroobliterative reaction.

While cellular cytotoxicity is one cause of loss of airway epithelium in the allograft lung, another contributing factor may be the deficient capacity of the airway epithelium to regenerate and heal after episodic or transient injury.\textsuperscript{47} Several types of molecules have been shown to be involved in this process.\textsuperscript{45,48} Inflammatory cytokines, released by lymphocytes and macrophages, and eicosanoids are present in chronically rejecting organs as well as in acute rejection episodes. The detection of adhesion molecules like E-selectin in TBB tissue was associated with acute rejection and subsequent BO.\textsuperscript{49} Insulin-like growth factor-I, platelet-derived growth factor, transforming growth factor-\( \beta \), and basic fibroblast growth factor have been reported to be involved in the molecular cascade in chronic rejection.\textsuperscript{45,50}

Two working hypotheses have evolved to explain the etiology of chronic rejection. First, the process is thought to be primarily an antigen-dependent phenomenon influenced by early immunologic injury such as acute rejection and continuing host alloresponsiveness. The second hypothesis is that alloantigen-independent factors—such as prolonged ischemia, surgical manipulation, reperfusion injury, recurrent infection, and aspiration—may lead to the progressive changes.\textsuperscript{51} The concept of “response to injury”\textsuperscript{52,53} combines these hypotheses and establishes a linkage between nonspecific injury, acute rejection, and long-term outcomes. Clinical evidence in different solid organ transplants underscores the association of chronic rejection with nonspecific injury and acute rejection. It is suggested that injury induces a stereotyped injury response, which promotes immune recognition and makes immunologic injury more likely. This response represents a new form of injury that initiates the injury response again, becoming self-propagating. At a certain point, chronic rejection presumably is programmed for irreversibility, even without any further immune response.\textsuperscript{52} These concepts may have an important impact on future therapeutic approaches to BO, as they implicate intervention strategies against different phases in the evolution of this lesion.

From observations and investigations, a hypothesis for the development of the obliterator bronchiolar lesions can be proposed. During the initial injury, the airway epithelium is partly or completely lost and the subepithelial basement membrane shows breaks and focal thickening.\textsuperscript{54,55} Recovery depends on effective repopulation of the air-bronchial interface by epithelial cells.\textsuperscript{56} Regeneration occurs from the stem cells in the small airways (Clara cells).\textsuperscript{57,58} Initially, the denuded surface is covered by provisional matrix proteins like fibronectin and fibrin, which will be resorbed after reconstitution of the epithelium. However, in some bronchioli, myofibroblasts migrate through gaps in the basement membrane into the provisional matrix and begin to deposit connective tissue, leading finally to the obliteration of the airway lumen.

**Animal Models of BO**

Animal transplant models have added substantially to the understanding of chronic allograft rejection. As outlined above, acute rejection is believed to be one of the most important precursors for later chronic rejection. To investigate this particular aspect of BO, an animal model, as a stable system with fewer confounding factors than are present in the setting of human transplantation, would be of great value. In a mouse model of skin and cardiac allografts, blocking T-cell-dependent immune responses (by simultaneously blocking CD40 and CD28 pathways) not only prolonged long-term survival of fully allogeneic skin grafts, but also inhibited the development of chronic vascular rejection of the cardiac allografts.\textsuperscript{59} BO-like lesions can be reproduced in a rodent model of heterotopic airway transplantation.\textsuperscript{60-62} In this model, the acute phase of lymphocytic airway infiltration is a precursor of late airway obliteration in allografted animals, whereas isografted animals do not develop lymphocytic infiltration nor obliterator airway lesions; this underscores the immune-mediated mechanism in these lesions.\textsuperscript{62} Daily high-dose cyclosporine has been shown to prevent airway epithelial loss and the fibroproliferative response in this model.\textsuperscript{63,64} On the other hand, the epithelium was not preserved when the immunosuppressive drug was given only at a low dose, and the fibroproliferative reaction was poorly controlled. Similar results were obtained in a single lung transplant model in rats using cyclosporine for 3 days after surgery.\textsuperscript{65} Although the lung was not rejected in the initial
phase, 6 months later the signs of chronic rejection were documented histologically, implicating a subclinical smoldering alloimmune injury after cessation of cyclosporine.

Are manifestations of chronic rejection reversible? To answer this question, chronic rejecting rat kidney allografts were retransplanted back into the donor rat strain sequentially after engraftment to remove the graft organ from ongoing host immune response. It was shown that retransplantation beyond a critical timepoint resulted in progressive chronic rejection even in the absence of a host immune response. In the same way, graft coronary arteriosclerosis progressed without ongoing immunoreaction. A similar study confirmed these results in the setting of chronic airway rejection in the airway transplant model, airway transplantation back to the donor strain after a certain period could not inhibit the ongoing process to total airway obliteration. In addition, it has been demonstrated that other forms of injury might lead to the same functional and morphologic findings as in chronic rejection after allotransplantation. In all these experiments, alloimmune injury was excluded by isogenic transplantation or autotransplantation. Severe initial ischemia of kidney isografts and intraoperative manipulation of naive kidneys resulted in changes closely mimicking chronic allograft rejection.

Ischemic and toxic injury appear to induce a stereotyped inflammatory response with increased MHC class I and II expression, increased interstitial dendritic cells, and increased messenger RNA levels of interferon-γ, interleukin-2, interleukin-10, granulocyte-macrophage colony-stimulating factor, and transforming growth factor-β. The same pattern of reaction has been described during acute allograft rejection as well. Growth factors have been shown to be important in the fibroproliferative phase of chronic allograft rejection. Obliterative airway lesions have also been produced in heterotopic tracheal isografts injected with platelet-derived growth factor or basic fibroblast growth factor, suggesting that these factors have the potential to induce airway fibroproliferation even in the absence of an immune response.

**Risk Factors**

**Risk Factors for Nontransplant BO**

BO can be caused by many different factors in a variety of nontransplant settings. The development of BO has been described after inhalational injury with thionyl chloride, zinc chloride, and smoke; after infections with respiratory syncytial virus, parainfluenza virus, influenza virus, adenovirus, measles virus, and *Mycoplasma pneumoniae*; after drug treatment with d-penicillamine and tiopronine; after aspiration of charcoal; after irradiation; after working in the animal feed industry; after persistent occlusion of large airways; together with Wegener's granulomatosis; as a congenital condition following presumed intrauterine viral infection; in association with ulcerative colitis; and recently, in an epidemic outbreak after consumption of the vegetable *Sauropus androgynus*.

The common denominator in the aforementioned list of BO risk factors is their shared potential for injury to the bronchiolar epithelium. During the repair process, excessive proliferation of granulation tissue may consequently lead to narrowing or obliteration of the airway lumen.

**Risk Factors for Posttransplant BO**

Risk factors for the development of BO after transplantation have been sought since the early descriptions of disorder. One of the difficulties associated with the earlier reports resides in the inconsistent definition of BO itself and the relatively late publication of a clinical definition for BOS. Another important point may be the difficulty in recognizing an association between potential risk factors and BO in a single transplant center because of the small number of patients. Pooling of data and multicenter studies would be useful in advancing knowledge about BOS.

The major risk factor for BO appears to be acute rejection. In the Papworth experience, the number of acute rejection episodes experienced in the first 6 months after transplantation had a significant impact on subsequent BO, with those who experienced three or more episodes being at a higher risk than those who had one or two episodes. The Pittsburgh experience confirmed that the frequency of acute rejection episodes was associated with an increased risk for BO, but also suggested that the degree of rejection played a role. Accordingly, patients with one or more episodes of moderate to severe acute rejection (at least grade 3) were more likely to develop BO.

Other potential risk factors have been identified. Clinically stable recipients with persistent donor-specific alloreactivity in their BAL lymphocytes have an increased incidence of subsequent BO. On the other hand, it has been shown that high levels of peripheral blood allogeneic microchimerism correlated with donor antigen-specific hyporeactivity, and that both parameters correlated with a BO-free survival. The occurrence of lymphocytic bronchiolitis on histologic examination of lung tissue specimens also may represent a risk factor for BO.
These findings provide a rationale for performing surveillance TBBs in order to detect occult acute rejection (at least grade 2), which occurs in about 20% of patients. However, to date, it has not been shown that treatment of clinically silent acute rejection (ie, no symptoms, stable lung function) decreases the incidence of BO. Nevertheless, it is reasonable to assume that there might be a subgroup of patients who, if left untreated, will progress from clinically silent to clinically overt acute rejection or to a higher histologic degree of rejection. According to pathologic investigations, patients with persistent or worsening grade A2 acute rejection tend to have more large and small airway inflammation, larger numbers of eosinophils and plasma cells in their biopsies, and airway and airspace granulation tissue. In addition, at least one report suggests that preclinical early diagnosis of BO by surveillance biopsies and early therapy is associated with a response to treatment.

Associations between BO and preceding infectious episodes have been investigated. In the early transplant experience, late bacterial pneumonia and Pneumocystis carinii infection were associated with the development of BO. However, the same group found that these infectious complications were not associated with BO when analysis was limited to patients who received transplants after 1988, a finding that could potentially be a result of a decreased incidence of infections pursuant to the prophylactic use of antibiotics. The authors suggested that eliminating risk factors may indeed reduce the prevalence of BO.

Many centers have reported that cytomegalovirus (CMV) pneumonitis is a significant risk factor for BO. However, other centers have been unable to document such a relationship. Human CMV infection is believed to promote allograft rejection through different mechanisms, including the production of several proinflammatory cytokines, increased expression of major histocompatibility complex and adhesion molecules, and molecular mimicry. The introduction of ganciclovir prophylaxis and the use of CMV-negative blood products for CMV-negative recipients has led to a reduction in the incidence of CMV disease and related mortality. In the Stanford experience, ganciclovir prophylaxis produced a significant delay in the development of BO compared with historic control subjects. Duncan and colleagues compared ganciclovir and acyclovir to prevent CMV infections after lung transplantation and showed a decreased prevalence of BO during the first year after transplantation (17 vs 54%; p<0.033) in the patients given ganciclovir.

Other viral respiratory infections may also occur in lung transplant recipients. However, the viruses are not always identified and the numbers are therefore too small to assess whether or not a correlation with BO exists. One study showed that the onset of BO was seasonal and correlated with the peak season for different respiratory viruses, suggesting that these viral infections may trigger the development of BO. This observation is in accordance with the clinical observation that previously stable lung transplant patients present with BO just after recovering from an acute respiratory viral infection.

The role of airway ischemia as a risk factor for BO and consequently the direct revascularization of the transplanted airways remains controversial. Whereas Bando et al noted that airway ischemia within the first 14 days after transplant had an effect on later BO, Scott and colleagues did not find a significant relationship. Initial ischemic injury occurs starting with the interruption of bronchial artery circulation at transplantation. Some groups therefore recommend reanastomosis of the bronchial arterial circulation with the recipient’s mammary artery or to the recipient’s ascending aorta. In a series, the incidence of BO was 33% after 3 years. Additional ischemia might be caused by the primary immune response, presumably directed against endothelial antigens. Immune-mediated endothelial cell injury may then lead to occlusive artheriopathy resulting in epithelial ischemia. The epithelium injured by ischemia might express more HLA class II surface antigens and then initiate a second immune-mediated injury against epithelial cells.

HLA matching and its potential relationship to BO was investigated by several groups, but the issue remains controversial. One or more mismatches at the HLA-DR and the HLA-B loci were risk factors for early severe acute rejection, according to one center’s experience, whereas donor HLA-DR type was a risk factor for early onset of BO at another center. On the other hand, HLA-DR match or mismatch at one locus and HLA-B mismatch at two or fewer loci were also associated with BO in another study. One explanation for these contradictory results might be HLA-restricted immunologic mechanisms against different antigens, which then initiate an immunologic injury. Therefore, enhanced HLA matching in the presence of such presumed antigens might be a disadvantage. This has been suggested in the setting of CMV and chronic rejection after liver transplantation.

Several other minor risk factors have been explored. The evidence regarding a potential relationship between postoperative GI dysmotility with reflux and aspiration and BO has been conflicting, and only case reports or small studies have been reported. Mechanical factors potentially involved in
the development of BO include posttransplant denervation of the lung with consequent reduction of local defense mechanisms like coughing, and the reduction of mucociliary clearance rendering the lung more vulnerable to aspirated particles and inhaled pollutants.

Factors not correlated to the incidence of BO have been age of recipient and donor (in adults), sex, ABO blood group, the recipient's underlying disease, donor ischemic time, and the occurrence of diffuse alveolar damage after transplantation.10,39

CLINICAL SYMPTOMS AND SIGNS

The original descriptions of BO in heart-lung transplant recipients reported shortness of breath and chronic productive cough as major symptoms.2 Such early reports may have reflected findings of advanced disease with complicating bacterial infection or bronchiectasis. Today, BO is often diagnosed earlier due to close monitoring with pulmonary function studies and a high level of awareness.

Symptoms and signs of early BO are nonspecific. A decrease in FEV1 may occur before any clinical symptoms appear. Perhaps the earliest finding is a decline in flow rates at low lung volumes, such as forced expiratory flow at 25 to 75% of vital capacity. Most commonly, the initial symptom is the gradual onset of progressive dyspnea and a decline in exercise tolerance. Some patients may complain of fatigue and a nonproductive cough. BO may develop in previously stable patients who acquire what appears to be a bacterial or viral airway infection and thereafter show an irreversible decline in FEV1. Infrequently, BO first presents as asthmatic bronchitis that initially responds to bronchodilators and oral corticosteroids.23 In the early stages of BO, the physical examination is unremarkable, although occasionally crackles and wheezes at the lung bases are audible.122

Episodes of increased cough and purulent sputum accompanied by positive sputum cultures or actual pneumonia may complicate the underlying process.14 Over time, the airways of most of the affected patients become colonized with bacterial and occasionally fungal organisms. Staphylococcus, Pseudomonas, other Gram-negative organisms, or Aspergillus spp may be found and can lead to recurrent lung infections.14

While BO is defined as a disease limited to the small airways, involvement of large airways is common, especially in the advanced course of the disease. Bronchiectasis documented by high-resolution CT is a well recognized phenomena associated with BO.123 Bronchiectasis may represent a consequence of rejection, infection, or a combination of both.

**Diagnostic Procedures**

Pulmonary Function Tests

After surgery, bilateral lung transplant recipients and heart-lung transplant recipients demonstrate normally shaped flow-volume curves. In contrast, single lung transplant recipients continue to show a pathologic flow-volume loop pattern depending on the underlying disease in the remaining native lung.124,125 A sustained decline in FEV1 of at least 20% compared to baseline (calculated as \([\text{current FEV1} - \text{baseline FEV1}] / \text{baseline FEV1}] \times 100\) in the absence of acute rejection or infection is a useful clinical surrogate for the pathologic abnormalities characteristic of BO. The degree of decline has been used to stage BOS (Table 1).

The first clinical sign of BO is often a decrease in flow rates at low volumes. Documentation of forced expiratory flow at 25 to 75% of vital capacity values of less than 70% predicted appears to be a sensitive marker for the onset of BO.126 The Stanford group demonstrated that a decline in the forced expiratory flow at 25 to 75% of vital capacity, to less than 70% predicted, occurred nearly 4 months earlier than the 20% decline in FEV1. Similar diagnostic information can be found by measuring specific airway conductance.127 In single lung transplant recipients, the combination of 133Xe-radiospirometry and dynamic spirometry may provide selective information on graft function and may show graft dysfunction at an early time.128 Maximal inspiratory and expiratory flow-volume loops help to differentiate BO from stenosis of the bronchial anastomosis.129 Response to bronchodilators has been shown to be predictive of subsequent BO with a sensitivity of 51% and specificity of 81%.130

With progression of the disease, a decrease in volumes2,131 and of diffusing capacity in combination with a further decline in flow rates is noted. Remarkably, gas exchange remains nearly normal until late in the course of BO. Hypoxemia and hypercapnia invariably develop with end-stage disease.15

Radiology Studies

The chest radiograph is normal in the early stages of BO. As disease progresses, peripheral decreased vascular markings, slight volume loss, and subsegmental atelectasis may be seen.123 Later findings, such as nodular or patchy alveolar densities, usually represent complicating infections.132 Pleural-based densities in the mid to upper lung zones are findings in advanced stages of the disease after isolated lung transplantation and represent histologically bland fibrosis.15

Peripheral cylindrical bronchiectasis associated with BO has been seen on high-resolution CT123 and
is suggestive of small airway obstruction. However, it cannot be regarded as an early sign of BO, as it occurs in only 14% of patients before the decline in FEV₁. Mosaic perfusion has been described in BO of different etiologies and is probably sensitive in the detection of posttransplant BO. It is characterized by decreased attenuation in a geographic distribution reflecting reduced blood flow due to reflex vasoconstriction in hypoventilated areas. Hypoventilation in BO is caused by bronchiolar inflammation and fibrosis. Air trapping on expiratory CT scans (lung regions retaining air during exhalation remain more lucent than normal lung regions do) is another sensitive radiologic indicator of BO (Fig 5). Whereas many of these findings are sensitive, they are not specific. Therefore Ikonen and colleagues combined several high-resolution CT findings using a scoring system. The CT changes consisted of central bronchial dilatation at upper and lower fields, peripheral bronchial narrowing, diminution of peripheral vascular markings, hyperlucency (overinflation), mosaic appearance of the lung parenchyma, perivascular or peribronchial infiltrations, thickening of septal lines, volume contraction, and volume expansion. When five positive findings were used as a minimum score, the sensitivity for detection of BO was 93% and the specificity was 92%.

Ventilation/perfusion scanning in BO usually reveals a decline in ventilation that is most prominent in the peripheral parts of the lung. In patients with BO after single lung transplantation for primary pulmonary hypertension, the decline in ventilation with well preserved perfusion often results in severe ventilation/perfusion mismatch.

**Bronchoscopy and Lung Biopsy**

In the clinical setting, BAL has not as yet been useful in the detection of BO. Neither total cell counts nor cell differentials in the BAL fluid are diagnostic. Increased lymphocyte cell counts are often obtained in acute rejection in contrast to BO. An increased neutrophil count has been reported in BO. However, increased neutrophils may reflect sampling in larger airways, which could indirectly be a sign of occluded smaller distal airways. The most consistent clinical observation from BAL in patients with BO is the markedly reduced fluid return. While its utility in BO is questionable, BAL—with or without TBB—remains the method of choice to diagnose the pulmonary infections that often complicate BO.

TBB is incorporated into diagnostic protocols of all lung transplant programs. TBB is extremely useful in the diagnosis of acute rejection, with a sensitivity of 80% and a specificity of nearly 100%. However, the sensitivity of TBB in the detection of BO varies from center to center, ranging from 15 to 78%. The specificity ranges from 75 to 93%. The sensitivity of TBB in the diagnosis of BO depends upon sample size (number and size of tissue fragments), sectioning and staining variables (number of levels examined, stains used), and probably on interpreter variables (pathologists’ familiarity with the diagnosis and diagnostic threshold). As the sensitivity of TBB in diagnosing BO is somewhat variable, a sustained decline in FEV₁ in the absence of infection and acute rejection is sufficient for a diagnosis of chronic rejection, but the disorder is then classified as BOS. Open lung biopsy, with a sensitivity of 100%, is the gold standard and should be considered in atypical cases in which other diagnoses are possible and potentially treatable.

**Clinical Course of BO**

Three distinct patterns of presentation and progression in the clinical course of BO have been described. The first pattern is characterized by a rapid onset followed by a relentlessly progressive course, usually leading to death due to respiratory failure within 1 year of diagnosis. The second pattern is characterized by a similar rapid onset and initial rapid decline, but stabilization of lung function follows. The third pattern is characterized by an insidious onset and chronic deteriorating course. At the time of diagnosis, there are no parameters to predict the different courses. After diagnosis of BO,
the mean survival of all affected patients was 66, 44, 37, and 10% after 1, 3, 5, and 10 years, respectively. The main complications of BO are superimposed infections, which are responsible for about 60% of BO-related deaths, and progressive hypoxemia and hypercapnia.

**TREATMENT**

In general, the range of available treatment options for established BO is narrow and disappointing. Complete return of lung function in cases of established BO has been described only anecdotally. To date, the best possible result is often a stabilization of lung function. As previously stated, the natural course of BO varies among affected patients. The variability of course and the frequency of concomitant airway infections further complicate evaluation of the success of different therapies.

**Short-term Heightened Immunosuppression**

Perhaps the most common initial approach is to apply an aggressive augmentation of immunosuppression for a limited time. The rationale for this therapy is based on the presumption of an active inflammatory process that is potentially reversible. It is unlikely that dense scar tissue will be affected by any therapy or that augmented immunosuppression is targeted against any remaining active lesions.

Although no protocol is universally accepted for the treatment of BO, augmentation of corticosteroids and cytolytic therapy have been used as initial therapies in the past. Occasionally, presumed acute rejection (ie, an abrupt decline of a previously stable lung function, absence of infection, and nondiagnostic or inadequate histologic examination) may be the initial manifestation of BO; it is treated with augmented steroids. Methylprednisolone, 0.5 to 1 g IV daily for 3 consecutive days, is followed by a 3- to 4-week tapering course of prednisone back to baseline. While a second course of augmented corticosteroids might have been administered years ago, if the FEV$_1$ fails to improve within approximately 4 weeks, antilymphocyte antibody preparations now are often used next. The optimal duration of antilymphocyte therapy is unclear, but generally varies between 7 and 14 days. In two separate studies, nine of 10 and seven of 15 patients achieved a stabilization of lung function for at least 6 months with antithymocyte globulins. Paradis and colleagues reported that 31% of their patients showed stabilization of lung function after one course of either steroids or antilymphocyte antibodies, whereas the remaining patients required at least three courses of treatment. Therefore, they concluded that patients should receive a minimum of three courses of augmented immunosuppression before treatment is accepted as not efficacious.

One notable problem with these short-course therapies is the failure to maintain improvements or stabilization of lung function. A relapse is defined clinically as a significant decline in pulmonary function or histologically as active bronchiolitis in biopsy specimens after previously inactive results.

**Methotrexate**

A study from Toronto reported successful stabilization of lung function with prolonged once-weekly oral methotrexate in seven out of 10 patients in whom treatment with multiple courses of steroids or antilymphocyte products had already failed to control ongoing BO.

**Tacrolimus**

Tacrolimus as a substitute for cyclosporine was shown anecdotally to stabilize the course of two patients with BO. A large randomized trial of 133 patients compared tacrolimus to cyclosporine as first-line immunosuppressive therapy. Patients receiving tacrolimus had fewer acute rejection episodes per 100 patient-days (0.85 ± 0.72) compared with the patients given cyclosporine (1.09 ± 0.72), although the difference did not reach significance (p = 0.07). Significantly fewer patients given tacrolimus developed BO (13 of 60, or 21.7%) compared with patients receiving cyclosporine (19 of 50, or 38%) (p = 0.025). However, significantly more patients in the tacrolimus group also developed fungal infections. This observation suggested that the relative degree of overall immunosuppression was higher in the tacrolimus group, thereby explaining the lower incidence of acute rejection and BO.

A study on the effect of conversion from cyclosporine to tacrolimus in 15 patients with established BO showed a significant reduction in the monthly decline in FEV$_1$ after administration of tacrolimus (1.1 vs 5.3%; p = 0.002).

**Mycophenolate Mofetil**

Mycophenolate mofetil (MMF) has been demonstrated to decrease the incidence of acute rejection in cardiac and renal transplant recipients as compared with azathioprine. It blocks the de novo pathway of purine synthesis, which is essential for lymphocytes, inhibiting T- and B-cell proliferation and antibody formation. Whereas MMF is an effective inhibitor of acute rejection, its impact on chronic rejection is less clear. Results in an animal model of allograft arteriosclerosis have shown that
MMF inhibited the proliferation of smooth muscle cells, crucial for the development of intimal thickening in the vascular media.\textsuperscript{164,165} This effect was achieved mainly by suppression of the inflammatory lymphocytes. On the other hand, MMF failed to prevent obliterative airway lesions in rat tracheal allografts at doses reported to be effective in preventing arteriosclerosis in rat aortic allografts.\textsuperscript{60} Clinical studies in human lung transplant recipients are under way; preliminary data suggest that MMF is superior to azathioprine in decreasing the incidence of acute lung allograft rejection.\textsuperscript{166-168} In a case report, a recipient with BO showed a prompt increase in lung function with MMF, 3 g/day, whereas a dose of 2 g/day was not effective.\textsuperscript{169} Therefore, dosage adjustments of MMF may be important when treating refractory rejection or BO.

**Inhaled Immunosuppressive Drugs**

Targeted delivery of immunosuppressive agents directed to the graft has been applied in experimental systems for years.\textsuperscript{170-173} The rationale for targeted delivery was to achieve higher local levels of the drug with less systemic toxicity. Additionally, it has been hypothesized that the drug may act along pathways not accessible from the systemic route alone.

In an experimental lung transplant model, bronchial mucosal blood flow was documented to be decreased markedly during acute rejection episodes.\textsuperscript{174} It is therefore possible that alterations in blood flow may occur in chronic lung rejection.\textsuperscript{175} Hence, inhalation of immunosuppressive agents could potentially have effects beyond what would be expected with systemic delivery alone. Recently, two trials with aerosolized cyclosporine were published.\textsuperscript{176,177} Inhaled cyclosporine was effective in a small group of patients with refractory acute rejection,\textsuperscript{176} and in seven of nine patients with active BO.\textsuperscript{177} Adverse drug effects consisted of severe cough, which warranted cessation of aerosolized cyclosporine in three of the nine patients. Although systemic absorption is low, measurable blood levels of cyclosporine can be detected following inhalation of cyclosporine. Therefore, it cannot be excluded that cyclosporine’s beneficial effect on BO was due to increased systemic immunosuppression rather than by a local effect.\textsuperscript{23}

Inhaled corticosteroids play an important and well-known role in the treatment of asthma, a chronic inflammatory condition of the airways. Although inflammation of the airways is also an important feature of early BO, inhaled steroids have not been studied systematically for BO. In a placebo-controlled, randomized trial, patients who experienced an acute rejection episode that resistant to systemic steroids received inhaled budesonide; an improvement in lung function and a nonsignificant reduction in acute rejection and BO were noted.\textsuperscript{178} In another study, a transplant recipient with histologically documented BO received inhaled fluticasone propionate in a double-blind, randomized, controlled trial. An improvement in FEV\textsubscript{1} was noted while the patient received fluticasone.\textsuperscript{179}

**Other Immune-Modulating Therapies**

Other immune-modulating treatment strategies used against BO include extracorporeal photopheresis,\textsuperscript{180} plasmapheresis,\textsuperscript{181} total lymphoid irradiation,\textsuperscript{12,182} and allogeneic bone marrow transplantation to achieve chimerism.\textsuperscript{183} To date, the numbers of patients treated with these strategies are too small to be conclusive. Extracorporeal photopheresis has been approved for use in cutaneous T-cell lymphoma and appears to be effective for this indication. Extracorporeal photopheresis has been used on an experimental basis for solid organ transplant rejection, although the mechanism of action is not fully understood. Animal models suggest that there might be a vaccination effect of photomodulated cells against pathogenic T-cell clones.\textsuperscript{184} A small study in three patients with BO showed stabilization of lung function for at least 6 to 23 months in all of the patients.\textsuperscript{180} Two other reports described stabilization or improvement of FEV\textsubscript{1} in five of nine patients and four of six patients with BO, respectively.\textsuperscript{185,186}

Plasmapheresis has been used in acute rejection after heart and kidney transplantation, but to date, only one published report of its use in BO exists.\textsuperscript{181} Nepomuceno et al.\textsuperscript{181} documented stabilization of FEV\textsubscript{1} in two of four patients after plasmapheresis. In a nonrandomized, uncontrolled trial, total lymphoid irradiation appeared to be successful in treating recurrent or refractory acute rejection after lung transplantation.\textsuperscript{187} Nevertheless, four of six patients later developed BO. In another study appearing in abstract form only, total lymphoid irradiation was used in 20 patients to treat established BO and was associated with a decrease in the rate of lung function decline.\textsuperscript{182} In both studies, total lymphoid irradiation was generally well tolerated.

Bone marrow cells of donor origin circulating in the recipient (bone marrow microchimerism) have been shown to be associated with a reduced incidence of late acute rejection episodes.\textsuperscript{96,183} Donor cell chimerism can be facilitated by infusion of donor bone marrow during lung transplantation.\textsuperscript{185} In the Pittsburgh experience,\textsuperscript{183} lung transplantation combined with donor bone marrow infusion in 20 patients led to an increased donor-specific hyporeac-
tivity compared with control patients. The observation period, however, is still too brief to determine an effect on the development of BO.

It is extremely difficult to draw definitive conclusions about the effectiveness of the aforementioned interventions for the treatment of BO. The majority of reports consist of anecdotal, single-center, non-randomized, and uncontrolled investigations with relatively small sample sizes. In addition, it appears that most reports indicate stabilization or slowing of the rate of loss of lung function. It is unclear whether this represents a therapeutic benefit or simply the natural history of BO in these subjects.

Retransplantation

Retransplantation remains the ultimate therapeutic option in BO. Due to the inferior graft survival compared with the first transplant and the shortage of lung allografts, questions have been raised as to whether retransplantation should continue to be performed. Recent results contradict suggestions that BO recurs in an accelerated manner after retransplantation. However, early mortality after retransplantation is clearly higher than after primary transplantation, due to an increased incidence in infections. The most important single predictor of survival after retransplantation appears to be ambulatory status of the recipient immediately before reoperation. Also associated with survival were identical match of ABO blood group and absence of donor-recipient CMV status mismatch. Retransplant recipients surviving the first 3 postoperative months have a medium-term prognosis similar to that of patients after a first-time procedure.

At the present time, the results of treatment of established BO are not very encouraging. Emphasis should be placed on prevention of events known to be associated with high risk of development of BO. Because acute rejection is an identified culprit, improved immunosuppressive strategies are warranted that will hopefully lessen the likelihood of BO.

Future Therapeutic Options

Most of the interest in the treatment of BO has concentrated on the inhibition of the alloimmune response. More recently developed drugs (eg, 15-deoxyspergualin, rapamycin, leflunomide) also attack the late fibroproliferative response that leads to the BO lesion. In an animal model of allograft arteriosclerosis, 15-deoxyspergualin was able to prevent chronic rejection by reducing the number of activated macrophages and inhibiting the synthesis of several growth factors in the vascular wall. However, it was not found to be a potent inhibitor of airway obliteration in the rat tracheal allograft model.

Rapamycin, a structural analogue of tacrolimus, seems to downregulate not only the response to T-cells to interleukin-2, but also to inhibit growth factor-induced mesenchymal cell proliferation.

Future treatment strategies may include (1) small molecules such as antisense nucleotides, which interfere with the migration of mesenchymal cells into the airway lumen and the deposition of extracellular matrix proteins; (2) temporary overexpression of immune-modulatory cytokines; or (3) blocking antibodies to inflammatory cytokines.

CONCLUSION

BO occurs in more than half of lung transplant recipients who survive for more than 5 years and is the leading cause of death in the late posttransplant period. Our understanding of BO has been enhanced by studying quite reliable and reproducible animal models of obliterative airways disease that are likely to be reasonable surrogates of BO. Prevention of BO is the most useful direction for therapeutic interventions, particularly as it relates to the early diagnosis and treatment of acute rejection episodes during the first few months following lung transplantation. Antiviral strategies may also have a role in preventing or delaying the development of BO. Once BO or BOS is established, aggressive measures directed towards treatment of infectious episodes and increased immunosuppression with minimal adverse events appear to be important and may modestly improve outcomes. However, based on the available clinical data to date, there is no immunosuppressive strategy that has been shown to reliably improve lung function significantly in patients with BOS. Furthermore, at best there appears to be a stabilization or decrease in the rate of decline in lung function following augmented immunosuppressive regimens. Hopefully, further investigation of the pathogenesis of BO and progress in immunobiology will bring a solution for this devastating process in the near future.

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