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Post-transplant bronchiolitis obliterans

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Post-transplant bronchiolitis obliterans. A. Boehler, M. Estenne. ©ERS Journals Ltd 2003.
ABSTRACT: Over the last decade, improvements in surgical techniques, lung preservation, immunosuppression, and management of ischaemia/reperfusion injury and infections have made intermediate-term survival after lung transplantation an achievable goal. However, chronic allograft dysfunction in the form of bronchiolitis obliterans remains a major hurdle that threatens both the quality of life and long-term survival of the recipients. It affects up to 50–60% of patients who survive 5 yrs after surgery, and it accounts for >30% of all deaths occurring after the third postoperative year.

This article discusses the alloimmune-dependent and -independent risk factors for bronchiolitis obliterans, the current understanding of the pathogenesis of bronchiolitis obliterans based on results of animal and human studies, the clinical staging of the complication, strategies that may contribute to the prevention and/or early detection of bronchiolitis obliterans, and suggestions for future research.
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Over the last decade, improvements in surgical techniques, lung preservation, immunosuppression, and management of ischaemia/reperfusion injury and infections have contributed to increase the 1-yr patient survival after lung transplantation (LTx) to 70–80% [1]. After successful reduction of early complications, chronic allograft dysfunction has become the major obstacle to long-term survival. A shift in the nature of complications from early to late graft dysfunction has been similarly observed after transplantation of other solid organs [2]. Bronchiolitis obliterans (BO) after LTx was first described in 1984 at Stanford University, Stanford, CA, USA, in heart-lung transplant recipients who showed a progressive decline in forced expiratory volume in one second (FEV1) [3]. Lung biopsies from these patients showed intraluminal polyps comprised of fibromyxoid granulation tissue and plaques of dense submucosal eosinophilic scar. Obliteration of small airways by these lesions produces progressive airflow obstruction, often accompanied by recurrent lower respiratory tract infection.

BO and its clinical correlate bronchiolitis obliterans syndrome (BOS) affect up to 50–60% of patients who survive 5 yrs after surgery, irrespective of the type of transplant procedure [1, 4–6]. This incidence is high compared with that of chronic graft dysfunction in recipients of liver, heart or kidney transplants (table 1). The time between transplantation and onset of BOS can range from a few months to several years, but in most series, the median time to diagnosis is 16–20 months. In most patients, BOS is a progressive process that responds poorly to augmented immunosuppression, and

it accounts for >30% of all deaths occurring after the third postoperative year [1]. Survival at 5 yrs after the onset of BOS is only 30–40%, and survival at 5 yrs after transplantation is 20–40% lower in patients with than in patients without BOS [7]; this difference widens as postoperative follow-up increases. After single LTx, survival after BOS onset is longer in recipients with emphysema compared with recipients with idiopathic pulmonary fibrosis [8].

Clinical presentation

Clinical symptoms at onset are unspecific or even absent, and many patients only present with an asymptomatic fall in FEV1. However, some patients have an acute presentation with an initial episode of acute rejection or infection that does not respond to treatment. Later in the disease course, symptoms often include repeated episodes of bacterial infection, followed by permanent airway colonisation with pathogenic bacteria and fungi (*e.g. Pseudomonas* and *Staphylococcus* spp., and *Aspergillus fumigatus*). During the first postoperative months, nonspecific bronchial hyperreactivity may be present in patients who will develop BOS later [9]. It is important to emphasise that from the clinical point of view, BOS is a very heterogeneous condition. This probably reflects the variety of risk factors and underlying mechanisms that may be involved (see below). The rate of progression of the disease, as assessed by the decline in FEV1, may show the following: 1) a sudden onset with rapid decline of lung

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Table 1.–Chronic allograft dysfunction in solid organ transplantation

Organ	Pathological features	Incidence 5 yrs after transplant %
Liver	Vanishing bile duct syndrome	5
Kidney	Glomerular sclerosis/tubular atrophy	30
Heart	Coronary artery sclerosis	20
Lung	Bronchiolitis obliterans	50–60

function; 2) an insidious onset with a slow, progressive decline over time; and 3) an initial rapid decline in FEV1 followed by a prolonged period of stability [10]. The latter pattern, however, is not very common as <20% of affected patients are free of functional degradation at 2 yrs after diagnosis [11].

The risk for progression increases with the number of episodes of acute rejection within 6 months of surgery, and seems to be more pronounced in patients who develop BOS before the second postoperative year [12]. In a recent study of 151 patients with BOS [13], 75% had a sudden onset of BOS (pattern 1) and 25% had a chronic onset (pattern 2). Acute onset of BOS occurred earlier relative to surgery than chronic onset, and carried a worse prognosis (median survival after diagnosis 29 *versus* 58 months). Acute onset of BOS was significantly associated with acute rejection during the first 6 months (though no such event was recorded for many patients), and was often triggered by an acute rejection or infection episode. There is evidence that the number of respiratory infections and the aggressiveness with which they are treated also impact on BOS progression [11]. For example, in the authors' experience, patients who show a very slow decline in FEV1 (pattern 2) generally have few or no respiratory infection and/or airway colonisation.

The presence of BOS negatively affects not only survival but also health-related quality of life [14]. Patients with BOS show less energy and physical mobility, and more depressive symptoms compared with their counterparts without this complication. BOS is also associated with substantial additional healthcare costs, in particular for hospitalisations and medications [15].

Risk factors

Many factors have been reported as risk factors for BOS [16]. However, quality of data is often a problem, because almost all existing information derives from retrospective studies with no control groups and/or reflects the experience of single centres. Numbers are small and often difficult to interpret. In some cases, risk factors appear to have been more important in the earlier years of LTx, *e.g.* cytomegalovirus (CMV) infection (see below). Risk factors reported in the literature are designated as probable and potential risk factors in table 2.

Alloimmune-dependent factors

The bulk of evidence suggests that BO is mediated by an immunological injury directed toward epithelial and endothelial cells. This is supported by several studies that have shown that acute rejection histology, characterised by perivascular and/or peribronchial infiltration of activated lymphocytes into graft tissue, is a statistical risk factor for BOS; the risk increases when the acute rejection is histologically severe or when it persists or recurs after treatment [11, 16–21]. Three or more episodes of acute rejection within the

Table 2.–Risk factors for bronchiolitis obliterans syndrome (BOS)

Probable risk factors for BOS	Potential risk factors for BOS
Acute rejection	CMV infection (without pneumonitis)
Lymphocytic bronchitis/bronchiolitis	Community respiratory virus infection
	Donor antigen-specific reactivity
CMV pneumonitis	Medical noncompliance
Anti-HLA pretransplantation	Gastro-oesophageal reglux
	Older donor age combined with longer graft ischaemic time

CMV: cytomegalovirus; HLA: human leukocyte antigen.

first 6–12 months after surgery increase the risk of developing BOS by 3–4 times [11]. Similarly, lymphocytic bronchitis and bronchiolitis in the absence of acute perivascular rejection and infection have been shown to predate BOS in a significant number of patients [19, 21, 22]. Acute rejection later in the postoperative period is another important risk factor [12, 23]; in one study [23], ~50% of patients treated for acute rejection beyond 6 months after transplantation went on to develop BOS within 3 months of treatment for rejection. In these patients, the absence of a long intervening time period suggests that acute rejection may lead directly to airway obliteration.

However, many patients with acute rejection do not develop BOS, and some patients with BOS have never experienced acute rejection [13]. Therefore, the relationship between acute rejection and BOS appears to be complex and may depend on several factors, such as the time after transplantation, whether acute rejection histology occurs in a symptomatic or asymptomatic patient and on the intensity of therapy provided. In a recent study, A3 or multiple A2 rejection episodes occurred in only 13% and 7% of the 96 patients studied, respectively, and their occurrence did not predict BOS. SWANSON *et al.* [24] suggested that the use of intense induction and maintenance immunosuppression and of aggressive treatment of acute rejection may uncouple the association between early acute rejection and BOS.

Several lines of evidence support the concept that alloreactivity directed towards human leukocyte antigens (HLA) is involved in the pathogenesis of BOS. Patients with anti-HLA antibodies pretransplantation have an increased incidence of acute rejection and BOS [25]. The bronchoalveolar lavage (BAL) fluid of stable lung transplant recipients shows a persistent increased percentage of CD8+ T-cells, which suggests subclinical ongoing allogeneic stimulation [26]. Expression of class-I and class-II HLA antigens by bronchial epithelial cells is upregulated during chronic rejection [27–30]. Identification of anti-HLA antibodies directed towards these antigens in previously unsensitised recipients is associated with, or may even precede, the development of BOS [31, 32]. In addition, reactivity of bronchoalveolar [33] and peripheral blood [34] lymphocytes in patients with BOS is generally directed towards donor-specific class-I HLA antigens. Despite these observations and single-centre reports of an association between HLA mismatching and BOS [35], analysis of the United Network for Organ Sharing/International Society for Heart and Lung Transplantation (ISHLT) database [36] and a recent review of the published literature [16] do not support HLA mismatching as an established risk factor for chronic rejection. This is probably related to the fact that very few patients have two or less HLA mismatches because no attempt at HLA matching is made.

Persistent donor antigen-specific reactivity of recipient T-lymphocytes has reportedly led to increased rates of BOS,

and conversely donor-specific hyporeactivity (generally associated with peripheral blood allogeneic microchimerism) has been reported to be protective in some [37, 38], but not in all [39], studies. Preliminary experience from the Pittsburgh (PA, USA) transplant group has shown that the infusion of donor bone marrow in combination with LTx increases donor cell chimerism and donor-specific hyporeactivity, and is associated with a lower incidence of BOS [40]. In this study, two of 22 patients (9%) who received bone marrow infusion developed BO and/or BOS *versus* five of 12 control patients (42%); the significance of these results, however, is limited by the small number of patients studied and the short duration of the follow-up.

Alloimmune-independent factors

Although BOS is thought to be mediated by an alloimmunological injury, it is likely that nonalloimmunological inflammatory conditions also play a role. The lung is constantly exposed to inhaled agents, such as antigens, toxins and irritants, which promote local inflammation. In addition, the allograft is particularly susceptible to inhaled exogenous infectious agents [41–43]. Bacterial and fungal infections are not known to contribute directly to the pathogenesis of BOS, though they may increase the risk of acute rejection [21]. In contrast, CMV-related illness has been implicated in chronic vascular rejection of nonpulmonary solid organ transplants, and in some studies CMV pneumonitis was correlated with the development of BOS [12]. In the ISHLT registry [1], CMV mismatch is reported as a risk factor for 1- and 5-yr mortality. However, the impact of CMV on the development of BOS remains controversial, possibly due to several factors, such as the matching of CMV-seronegative recipients with CMV-seronegative donors, the prospective monitoring of CMV antigenaemia as a surveillance technique for CMV infection, the use of prophylactic or pre-emptive antiviral treatments and changes in the immunosuppressive regimen, all of which may mask potential associations [44]. Several centres have reported a decreased risk of CMV in the development of BOS, either a decreased incidence or a delayed onset, after the use of CMV prophylaxis [45–47].

Community respiratory virus infections, including respiratory syncytial virus, parainfluenza virus, adenovirus, and influenza A and B occur frequently in lung transplant recipients [48, 49]. Although these viruses have not been unequivocally associated causally with BOS, their involvement cannot be excluded. In the study by BILLINGS *et al.* [48], patients with community virus infections involving the lower respiratory tract were predisposed to high-grade BOS development.

The role of airway ischaemia in the pathogenesis of BOS is uncertain. Ischaemia may occur as a result of two mechanisms. First, chronic ischaemia due to interruption of the bronchial artery supply after reimplantation of the graft is a potential facilitator of subsequent small airway injury, however, the fact that bronchial artery revascularisation at the time of surgery has not significantly reduced the prevalence of BOS argues against the role of chronic airway ischaemia [50]. Secondly, "cold ischaemia" that occurs during the time interval between organ procurement and organ transplantation has been suggested to increase the risk of death and chronic graft dysfunction after LTx. In the study by NOVICK *et al.* [51], the effect of ischaemia time on the incidence of BOS was magnified as donor age increased, suggesting that the susceptibility to ischaemic injury at the time of organ procurement is dependent on additional intrinsic donor characteristics.

Recently, gastro-oesophageal reflux disease (GERD) has been reported to be associated with BOS. GERD is common after LTx, in part due to intraoperative injury to the vagal nerve and medication-induced gastroparesis. Afferent denervation of the airways also diminishes the chronic cough associated with GERD. Transplant recipients may thus be insensitive to microaspiration that may promote chronic inflammation and bacterial infections in the lower airways, and hence may be a risk factor for BOS [52]. Antireflux surgery (fundoplication) in transplant patients may improve lung function [52–54].

Medication noncompliance may represent an important, but often underestimated, risk factor for chronic rejection [55, 56]. For example, 12 months after kidney transplantation, the rate of compliance with a multidrug immune suppressive regimen was only 48% [56]. Although similar studies are not available in lung transplant recipients, medication noncompliance should be considered as a potential risk factor for BOS.

Several additional factors have been proposed as risk factors for BOS, but convincing data to support their role is lacking [16]. These factors include a history of smoking or asthma in the donor, donor age, head injury as cause of death, reperfusion injury, primary pulmonary hypertension as recipient primary disease, organising pneumonia, and genotypic suceptibility related to cytokine gene polymorphisms [57, 58].

Pathogenesis

The histopathological features of BO suggest that injury and inflammation of epithelial cells and subepithelial structures of small airways lead to excessive fibroproliferation due to ineffective epithelial regeneration and aberrant tissue repair [59]. In parallel with the concept of "injury response" that has been proposed to explain chronic dysfunction of other organ allografts [60], the evolving concept is that BO represents a "final common pathway" lesion, in which various insults (see above) can lead to a similar histological result. Accordingly, the cellular mechanisms discussed here may all contribute to the development of BO, irrespective of the nature of the initial injury and offending agent.

Animal models

Experimental studies have used the orthotopic LTx model and the heterotopic airway transplantation model. The first model consists of an orthotopic left single LTx performed in small animals like rats [61] and large animals like pigs [62]. It is mostly used to study acute events, since chronic changes such as BO occur infrequently in this model [62–64]. In contrast, the heterotopic airway transplantation model leads to consistent and reproducible airway obliteration and produces a histological lesion, which is very similar to that of human BO. The model, which was developed in the early 1990s in mice [65] and rats [66, 67], is technically less demanding than the orthotopic model and can be produced in larger numbers [68]. Although the heterotopic airway transplantation model has proved very useful to improve the understanding of the pathogenesis of BO, it differs from the clinical situation in several aspects. First, the transplanted airways are not primarily revascularised but depend on neovascularisation, and therefore, are exposed to pronounced ischaemia. In this model, however, airway ischaemia alone does not lead to airway obliteration [69]. Secondly, clinical BO is a chronic process that usually starts a few months after

surgery. In contrast, airway obliteration occurs within a few weeks after transplantation in the airway transplantation model, in particular when no immunosuppression is given and when allografts from fully major histocompatibility complex-mismatched animal strains are used.

Histologically, the heterotopically transplanted trachea shows an initial ischaemic phase [69], followed by marked lymphocytic infiltration with complete epithelial loss, and then by fibrous obliteration of the airway lumen. In the tracheal allograft model, injury to the epithelium is immune-mediated, but interestingly, nonimmune-mediated injury to the epithelium of tracheal isografts (e.g. produced by protease digestion) may also lead to airway obliteration [70]. In both the allograft and the isograft models, the loss of epithelium plays a pivotal role in this process. Thus, epithelial replacement in denuded isografts can significantly reduce airway obliteration [71]. After the epithelium is lost, thickening and fragmentation of the basement membrane can be observed [72].

The loss of basement membrane integrity will allow lymphocytes to infiltrate the tracheal mucosa, producing a histological picture that closely resembles the lymphocytic bronchitis and bronchiolitis seen in humans. Lymphocytic inflammation of the airway thus seems to be a precursor of BO [69]. Infiltrating cells include CD4+ and CD8+ T-cells (with a higher proportion of CD8+ cells early on [67, 73]), natural killer cells and macrophages [74], and later, myofibroblasts [73]. Recipient T-cells will recognise donor major histocompatability complex class-I and class-II alloantigens by both direct and indirect pathways [75, 76]. T-cell activation requires co-stimulatory signals. It has been shown that cellular infiltration and airway obliteration highly depend on host CD40 ligand and to a lesser degree on CD28 [72]; the interaction of CD28 with its ligand B7-2, but not B7-1, is involved in upregulating proinflammatory and T-helper (Th) cell type-1 cytokine responses [77]. Consistent with this, treatment with cytotoxic T-lymphocyte antigen 4-immunoglobulin G (CTLA4-IgG), which blocks the CD28/B7 co-stimulatory pathway, delays epithelial injury and attenuates obliterative changes. When administration of CTLA4-IgG and FTY720 (which induces T-cell apoptosis and sequestration of circulating mature lymphocytes) are combined, the integrity of both epithelium and airway lumen is maintained [78].

Mediators involved in the process include a strong and persistent Th1-type response with upregulation of interferon-γ and interleukin (IL)-2 accompanied by a mild upregulation of Th2 cytokines, such as IL-4 and IL-10 [79]. The Th1 response persists even after completion of the airway obliteration, indicating ongoing immune stimulation [80]. CC as well as CXC chemokines and their receptors play an important role in the recruitment of intragraft leukocytes. RANTES (regulated upon activation, normal T-cell expressed and secreted), a chemoattractant for memory T-cells, monocytes and eosinophils, has been shown to be highly expressed in mononuclear cells infiltrating the tracheal allograft [80]; the use of a neutralising anti-RANTES antibody decreases the number of CD4+ infiltrating T-cells and prevents airway obliteration [81]. Similarly, monocyte chemoattractant protein (MCP)-1 acting through its receptor CCR2 is a potent chemoattractant for mononuclear cell. Loss of MCP-1/CCR2 signalling significantly reduces mononuclear cell recruitment and later airway obliteration [82].

Typical cells involved in the fibroproliferative phase are fibroblast-like cells that express type-III collagen messenger ribonucleic acid (mRNA) [83]. Mediators include profibrotic cytokines, such as platelet-derived growth factor (PDGF), fibroblast growth factor, transforming growth factor (TGF)-β, insulin-like growth factor (IGF)-1 and endothelin (ET)-1 [84]. These cells and mediators promote extracellular matrix deposition, proliferation of smooth muscle cells, angiogenesis and excessive fibroproliferation.

In addition to its contribution to the identification of several cellular mechanisms involved in BO, the animal models have been used to understand how CMV infection may enhance BO [85], to test protocols of orally induced tolerance [63] and to assess the effectiveness of novel immunosuppressive agents (and other compounds) in the prevention of airway obliteration [86–92].

Human studies

As in the animal model, there is evidence that damage to the airway epithelium plays a key role in the cascade of events leading to human BOS. As noted above, indirect allorecognition of donor HLA class-I peptides in patients with BOS may lead to sensitisation of T-cells [34] and production of anti-HLA class-I antibodies [31]. These antibodies, in turn, may induce proliferation of airway epithelial cells in vitro [93]. Furthermore, non-HLA antibodies directed against airway epithelial cells are found in some patients with BOS, and binding of such antibodies to epithelial cells may upregulate growth factors like TGF-β [94].

In patients with BOS, bronchial epithelial cells overexpress the Ki-67 antigen [30] (which is a proliferation marker) and co-stimulatory B7 molecules [95]. As a result of epithelium destruction, there is a decline in Clara cell function and protein production with decreased concentration in BAL fluid [96]. This reduction may render the bronchiolar epithelium more sensitive to oxidative stress (see below), and promote both inflammation and fibroproliferation. The bronchial epithelium is also an important source of chemokines that attract neutrophils (see below).

Endobronchial biopsies show that the bronchial epithelium of patients with BOS contains increased numbers of dendritic cells (DC) [97, 98], with a higher proportion of antigen-presenting cells and a lower proportion of "suppressor" macrophages. In addition, these DC express co-stimulatory molecules of the B7 family (in particular the B7-2), which are capable of inducing optimal T-cell stimulation [97]. Thus, epithelial DC in lung transplant recipients presumably activate local and systemic immune responses, which may contribute to the process of chronic rejection.

With BOS development, BAL neutrophilia (and eosinophilia) increase [99–102] above levels seen in stable patients [26, 101, 103, 104]. However, whether this is a specific feature of BOS or whether it reflects concomitant airway infection is still debated. The increased neutrophilia is also found in induced sputum [105] and in lung tissue [30]. Besides the physiological function of clearing invading microorganisms, activated neutrophils have a large potential to cause damage to lung tissue through the generation of reactive oxygen species (ROS) and the release of proteases [106]. Markers of granulocyte activation, such as the oxidative enzymes myeloperoxidase and eosinophil cationic protein, may be detected in BAL fluid months before the clinical onset of BOS [107–111]. Lung transplant recipients without BOS already have a compromised antioxidant status [112], but the oxidative stress substantially increases when BOS develops [109, 110]. The increased oxidative stress may simply reflect neutrophil influx in the airways, but iron overload caused by microvascular leakage may be an additional mechanism [113, 114]. BOS is also associated with impaired antiprotease activity, evident from decreased concentrations of BAL secretory leukocyte protease inhibitor [109]. Furthermore, unopposed neutrophil elastase activity is frequently found in lung transplant recipients, usually in association with

endobronchial bacterial infection in the context of BOS [111]. At present, it is not entirely clear whether the increased oxidative stress and impaired protease/antiprotease balance should be merely considered as markers of BOS or whether they may be directly involved as pathogenic mechanisms.

The important role played by neutrophils in the pathogenesis of human BOS differs from what is seen in animal models where graft infiltration is mainly of lymphocytic origin (see above). This difference may be explained by several factors. First, in the rat, circulating blood lymphocyte concentrations are higher (40–80% of total leukocyte count) than in humans. Secondly, the animals are kept under strict sterile conditions and the subcutaneously placed allograft has no contact to the environment; as a result, the primary injury leading to airway obliteration is alloimmune in nature, and hence driven by lymphocytes. In contrast, bronchial infection or aspiration, which typically elicit neutrophilic infiltration, may be present in the clinical setting. Thus, unlike what is seen in human BOS (see below), macrophage inflammatory protein-2, which is the functional rodent correlate of human IL-8 and has potent neutrophil-chemoattractant properties, is upregulated only in the first few days after transplantation [80].

One of the major mediators of airway inflammation in human BOS is IL-8, a member of the CXC chemokine family and a key chemoattractant and activating factor for neutrophils. IL-8 is produced by bronchial epithelial [115] and smooth muscle cells [116], and its concentration in BAL fluid of BOS patients highly correlates with airway neutrophilia [99, 102, 107, 115, 116]. Attraction of neutrophils and eosinophils may also be induced by ET-1, which is upregulated during bacterial infections in lung transplant recipients [117]. ET-1 has profibrotic properties and is involved in the airway remodelling of several inflammatory diseases. As in the heterotopic airway model in animals, increased BAL concentrations of MCP-1 [99], RANTES [102] and growth factors (PDGF [118], IGF-1 [119], and TGF-β [115, 120–122]) are found in human BOS. These profibrotic cytokines are responsible for an increased fibroblast-proliferative activity in BAL supernatant [123]. In addition to the effects of growth factors, the "fibrolytic" activity of IL-1 is inhibited by increased levels of IL-1-receptor antagonist, resulting in a local profibrotic environment [123].

Summary

Taken together, accumulated experience from human and animal studies suggests that alloimmune and/or nonalloimmune injury to the airway epithelium triggers a massive influx of inflammatory cells through the fragmented basement membrane, and the secretion of proinflammatory cytokines (IL-2, IL-6, tumour necrosis factor (TNF)-α) and chemokines (IL-8, RANTES, MCP-1) by epithelial cells, T-cells, activated macrophages and smooth muscle cells (fig. 1). In human BOS, this leads to attraction and accumulation of activated neutrophils; these cells promote production of additional cytokines and chemokines that amplify cell recruitment, and release large amounts of ROS and toxic proteases that produce further airway injury. Macrophages typically produce profibrotic cytokines that elicit attraction and proliferation of fibroblasts, leading to extracellular matrix deposition and proliferation of smooth muscle cells. Thus, after an initial epithelial injury, ineffective epithelial regeneration and massive inflammation eventually produce aberrant tissue repair with scar tissue obliterating the airway lumen.
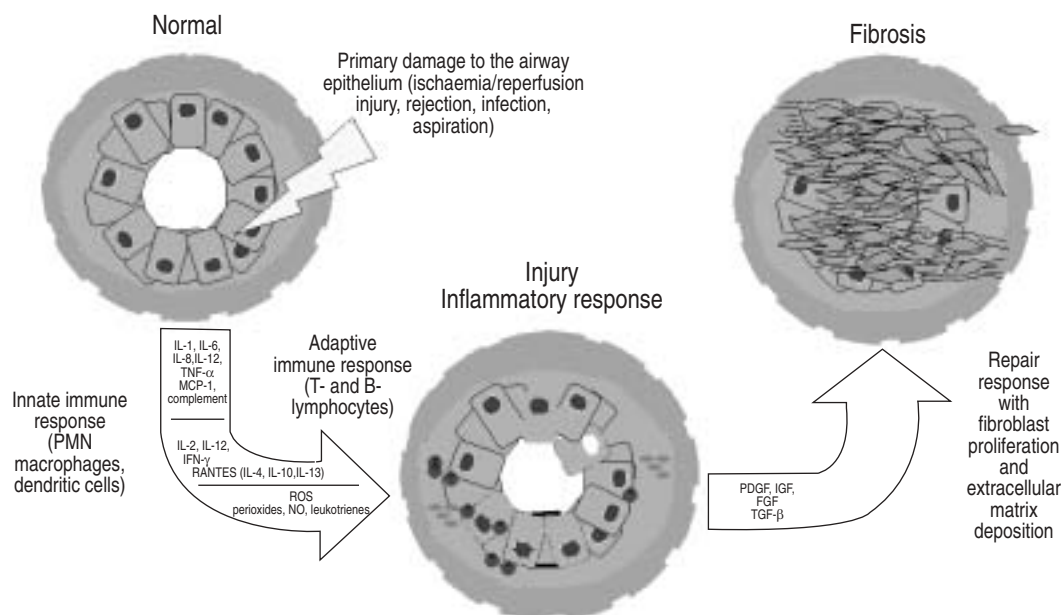

Fig. 1. – Primary damage to the lung allograft leads to activation of the innate immune system, which by interaction of dendritic cells and T-lymphocytes is followed by activation of the adaptive immune system. Injury to the airway epithelium and loss of epithelium leads to repair mechanisms finally ending in intrabronchiolar scar formation. PMN: polymorphonuclear; IL: interleukin; TNF: tumour necrosis factor; MCP: monocyte chemoattractant protein; IFN: interferon; RANTES: regulated on activation, T-cell expressed and secreted; ROS: reactive oxygen species; NO: nitric oxide; PDGF: platelet-derived growth factor; IGF: insulin-like growth factor; FGF: fibroblast growth factor; TGF: transforming growth factor.

Staging

The diagnosis of BO is based on histology, but a histological proof is often difficult to obtain using transbronchial lung biopsies. Therefore, in 1993, a committee sponsored by the ISHLT proposed a clinical description of BOS based on changes in FEV1 [124]. The aims were to provide a classification system for airway disease after LTx that did not rely on histopathological findings, was sensitive and specific, relied on diagnostic techniques available to all lung transplant physicians, and was relatively simple to understand and apply. For each patient, a stable post-transplant baseline FEV1 was defined as BOS stage 0; in patients who experienced a decrease in FEV1, progressive stages of BOS, from 1–3, were defined according to the magnitude of the decrease (table 3). The functional alteration has to be irreversible (*i.e.* be present for a period of $\geqslant 3$ weeks) and not be explained by other conditions that may alter graft function; when such conditions are found, the diagnosis of BOS can only be made if the functional alteration persists after appropriate treatment [17].

Although this classification system has been adopted by transplant centres worldwide as a useful descriptor of chronic allograft dysfunction, concern has been raised regarding its ability to detect small changes in pulmonary function. This concern recently led to formulation of a revised classification system for BOS [17], which includes a new "potential BOS" stage (BOS 0-p) defined as a decrease in forced mid-expiratory flow rates (FEF25–75%) and/or FEV1 (table 3). The rationale for including FEF25–75% comes from studies that showed that this variable deteriorates before FEV1 at the onset of BOS [100, 125, 126]. The new BOS 0-p stage is meant to alert the physician and to indicate the need for close functional monitoring and for in-depth assessment using surrogate markers for BOS (see below).

Most data supporting the usefulness of monitoring the FEF25–75% have been obtained in recipients of heart-lung and bilateral-lung, rather than single-lung grafts. In the latter, the presence of the native lung may make interpretation of functional changes more difficult. Disease progression in the native lung and complications affecting this lung may contribute to a change in overall lung function. In patients with emphysema, progressive hyperinflation of the native lung may produce clinical and functional changes that resemble those produced by BOS [127]. These confounding factors may explain, at least in part, that in a recent retrospective study performed in single-lung transplant recipients, FEF25–75%

was shown to be a very sensitive, but not specific, indicator of the subsequent development of BOS [128].

The classification system for BOS has proved useful to categorise patients according to the degree of chronic graft dysfunction, and has allowed transplant centres to use a common language to compare results and therapy from their programmes. However, the implication for an individual patient to be categorised in a given stage is less clear because different patients may have vastly different patterns of BOS acquisition and subsequent progression (see above). This is the reason why the classification system is not intended to be used to construct treatment algorithms and make therapeutic decisions. Such algorithms would need to include informations on the patient risk factors, history of rejection and infection, previous and current immunosuppression, and pattern of BOS onset and progression [129].

Prevention and early detection

To the extent that current therapies work to stop or slow down the progression of BOS, they do so mostly by an anti-inflammatory, and not an antifibrotic effect. Therefore, they are more likely to be effective in the early stage of BOS. For this reason, various parameters have been evaluated to determine whether they may be useful as early markers of a fall in graft performance.

Surveillance transbronchial biopsy

Several studies have shown that surveillance transbronchial biopsy (TBB) performed during the first postoperative months may show acute rejection histology in 22–73% of clinically and physiologically stable patients [24, 130, 131]. Similarly, a recent study that used home monitoring of FEV1 and FEF25–75% *via* the internet to detect acute rejection (and infection) found a sensitivity of only 63% because many episodes detected by surveillance TBB were not associated with significant functional changes [132]. Thus, the performance of surveillance TBB in the first months after surgery provides a means to detect and treat clinically silent rejection episodes, and may dictate the use of a more intense maintenance immunosuppression. This strategy may eventually prove useful to uncouple the association between acute rejection and BOS [24]. In addition, BANDO *et al.* [18] have reported that BO resolved or stabilised in 87% of patients in whom the diagnosis was made by surveillance TBB (*i.e.* who were asymptomatic and in BOS stage 0 at the time of diagnosis). However, no information on the long-term functional evolution of these patients was provided, and the apparent resolution of BO histology may simply reflect a sampling error on subsequent biopsies. Whether or not early initiation of augmented immunosuppression may slow down progression of the disease, both consistently and in the long term, thus remains to be established.

Pulmonary function

A potential limitation of the staging system proposed by the ISHLT is that hospital spirometry may be performed infrequently, especially in patients who live at great distances from transplant centres. This limitation, however, may be overcome by the use of home spirometry with telemetric transmission of functional data to the transplant centre [133]. Alterations in the distribution of ventilation in peripheral airways may also contribute to the early detection of BOS.

Table 3. – Bronchiolitis obliterans syndrome (BOS) classification system

	1993 Classification	2002 Classification	
	FEV1 $\geqslant 80\%$ of baseline	FEV1 $>90\%$ of baseline and FEF25–75% $>75\%$ of baseline	BOS 0
		FEV1 81–90% of baseline and/or FEF25–75% $\leqslant 75\%$ of baseline	BOS 0-p
BOS 1	FEV1 66–80% of baseline	FEV1 66–80% of baseline	BOS 1
BOS 2	FEV1 51–65% of baseline	FEV1 51–65% of baseline	BOS 2
BOS 3	FEV1 $\leqslant 50\%$ of baseline	FEV1 $\leqslant 50\%$ of baseline	BOS 3

FEV1: forced expiratory volume in one second; FEF25–75%: forced mid-expiratory flow rates.

Two recent prospective studies in heart-lung and bilateral-lung recipients have shown that the slope of the alveolar plateau for nitrogen or helium obtained during single-breath washouts (that reflects the heterogeneity of ventilation distribution) may increase up to several months before the criteria for BOS 0-p are met [100, 125]. Finally, the presence of nonspecific bronchial hyperreactivity may also precede BOS. In a recent longitudinal study that included 111 patients undergoing bilateral LTx, a positive methacholine challenge at 3 months after transplantation was associated with the development of BOS, with a positive predictive value of 72% [9]. This observation may be related to the fact that, in transplanted subjects, methacholine-induced bronchoconstriction involves the small airways [134].

Bronchoalveolar lavage

At least three reports have shown that BAL neutrophilia may predate the spirometric criteria defining BOS 1 [100, 102, 116], and cross-sectional studies of patients with BOS have demonstrated increased concentrations of various cytokines in the BAL (see above) [99, 102, 107, 115, 116, 118, 119]. These alterations, as well as the increase in BAL fibroblast-proliferative activity [123], may precede the functional alteration of BOS, suggesting that overexpression of cytokines may be predictive of BOS onset. However, the overlap of concentrations found in patients with and without BOS, the fact that measurements of these markers are not currently available at most transplant centres, and the cost of such measurements are all expected to limit their clinical application.

Induced sputum

Sputum induction, a noninvasive method to measure inflammation of the lower respiratory tract, was recently evaluated in patients with BOS [105]. Similarly to BAL findings, increased neutrophil counts have been found. In addition, matrix metalloproteinase (MMP)-9 and the ratio of MMP-9 to its inhibitor have been found to be elevated in BOS patients; MMP-9 correlated negatively with $FEV1$ values and positively with sputum neutrophils and TNF-α [135]. The usefulness of induced sputum analysis for the early detection of BOS remains to be investigated in larger studies.

Exhaled breath condensate

Volatile and nonvolatile markers originating from the respiratory tract can be measured in breath condensate [136]. To date, eicosanoids, products of lipid peroxidation, vasoactive amines, nitric oxide (NO)-related products, ammonia, hydrogen ions and cytokines have been measured, and there is hope that this method may contribute to the early detection of BOS in the future. Only exhaled carbonyl sulphide as a marker of acute rejection [137] and leukotriene B$_4$ as a marker of infection [138] have been described in the lung transplant population.

Exhaled nitric oxide

Exhaled nitric oxide (eNO) concentration, which has been proposed as a noninvasive marker of airway inflammation, may be useful in the early detection of BOS. It is elevated in patients with lymphocytic bronchiolitis and in patients with BOS, in particular at the onset of the functional deterioration (BOS 1). Concentrations of eNO correlate with the expression of inducible NO synthase in the bronchial epithelium and with the percentage of neutrophils in BAL [139–141]. These data indicate that eNO reflects the degree of airway inflammation in lung transplant recipients [142], but the extent to which eNO may predict the development of BOS in an individual patient remains to be established.

Computed tomography

The presence of air trapping on expiratory high-resolution computed tomography (CT) is an accurate indicator of the bronchiolar obliteration underlying BOS. In patients with BOS, the pulmonary lobules that have normal airways increase in density during the expiratory phase, while areas with obstructed airways cannot empty and remain radiolucent. Studies in adults and children have shown that the sensitivity of air trapping for enabling the diagnosis of BOS and BO ranges 74–91% while the specificity ranges 67–94% [143, 144]. This variability may be accounted for by differences in the technique used to quantify the extent of air trapping, and by the fact that some studies included both heart-lung or bilateral-lung and single-lung transplant recipients [144]. Interestingly, in the study by BANKIER *et al.* [143], five of the six patients with initial false-positive findings (with significant air trapping but an $FEV1$ >80% of baseline) later developed BOS, which suggests that expiratory CT may contribute to the early detection of the condition. Conversely, air trapping has a very high negative predictive value (>90%), *i.e.* a low score of air trapping in a patient with declining lung function makes the diagnosis of BOS very unlikely. The extent to which these results may apply to recipients of single-lung grafts is unknown.

Clinical management

The various approaches to the treatment of BOS have been described in detail in an earlier chapter. Several issues may deserve attention in the decision-making process of selecting a specific treatment for BOS. As discussed previously, it is likely that BOS represents a heterogeneous syndrome, with alloimmune and nonalloimmune mechanisms predominating to variable degrees in individual patients. To the greatest extent possible, lung transplant physicians should attempt to discern these differences and individualise therapy. For example, it may be more appropriate to have an aggressive therapeutic attitude in patients with rapid, as opposed to slow, BOS onset. Many patients with BOS suffer from recurrent bacterial, viral and fungal infections that further compromise lung function and often become the proximate cause of death [41–43]. Therefore, vigorous efforts to identify and treat infections are warranted during exacerbations of respiratory illness in recipients with BOS. It is also likely, although unproven, that aggressive immunosuppressive treatment of BOS predisposes to intercurrent bronchopulmonary infections; in patients with repeated infections, some physicians regard decreasing immunosuppression as an option. It is clear that the infectious risk must be factored into the risk-benefit analysis of augmented immunosuppression.

Current therapies, when effective, will necessarily preserve the most lung function if they are employed early in the evolution of the disease process. In this regard, it is possible that lung recipients with risk factors for chronic rejection, such as prior episodes of acute rejection, CMV pneumonitis, lymphocytic bronchiolitis, or anti-HLA antibodies, may benefit from intensified immunosuppressive therapy, even

prior to observing a decrease in pulmonary function (or while in BOS 0-p) [24]. However, this approach is rarely taken by lung transplant physicians due to the relatively low predictive value of currently recognised risk factors and the known risks of increased immunosuppression. In addition to the infectious risk mentioned above, progressive kidney insufficiency and bone fractures will inevitably alter patient survival and/or quality of life. Therefore, an important goal for future research will be to validate the surrogate markers described above in longitudinal studies, and to develop more sensitive and specific noninvasive biomarkers of the risk for progression to BOS.

Suggestions for future research

Post-transplant BO has become the major hurdle to long-term survival after LTx. Although considerable information has been gained in the understanding of the pathogenesis of this complication, its prevalence has not substantially decreased over time and it remains the first cause of late death in lung transplant recipients. This is due to the fact that, to date, no treatment has proved efficient to reverse established BOS, or even to slow down the progression of the functional deterioration. The following are some suggestions for future research.

Bronchiolitis obliterans syndrome classification

The term BOS is currently used to qualify chronic allograft dysfunction (whatever the underlying cause/mechanism). The staging system has proved useful to categorise patients according to the degree of functional impairment and has allowed transplant centres to use a common language to compare results from their programmes. However, from the therapeutic point of view, the use for an individual patient to be diagnosed in any particular BOS category is not established, because patients in a given stage may have vastly different patterns of disease progression. Both the cause of BOS and the rate of functional decline, which are not currently included in the classification, may have important implications when making decisions about treatment [129].

Risk factors

Most data regarding risk factors for BOS come from retrospective studies. Longitudinal studies evaluating all suspected risk factors should be undertaken in a sufficiently large number of patients to reach the statistical power needed to assess the relative risk associated with each factor. Among others, the following factors should be studied: 1) preoperative characteristics of recipient (primary disease, HLA sensitisation) and donor (cause of death, age, history of asthma and smoking); 2) matching of donor and recipient sex, CMV status, and HLA antigens; 3) presence and severity of ischaemia/reperfusion injury; 4) episodes of CMV infection, acute rejection and community respiratory viral infection; and 5) presence of gastro-oesophageal reflux disease. In these studies, specific attention should be given to clarify the relationship between acute rejection and subsequent BOS development.

Pathogenesis

A number of abnormalities have been observed in the BAL of patients with BOS, but they do not necessarily directly contribute to the pathogenesis of BOS, i.e. they may merely be disease markers. In addition, the variable pathogenic mechanisms and time course of BOS have not been correlated with differences at the level of cellular and molecular markers. The evolving technologies of functional genomics and proteomics, which allow simultaneous comparisons of large numbers of mRNA or protein species between individuals or over time in a single individual, will likely yield a more comprehensive and informative view of the development of bronchiolar fibrosis.

Early detection

Longitudinal studies using several potential early markers (lung function, BAL analysis, analysis of exhaled gases and breath condensate, CT etc.) should be performed to assess the time course of changes and their ability to predict future decreases in lung function. The validity of some markers (e.g. $FEF_{25-75\%}$, air trapping on CT) remains to be established in recipients of single-lung transplants.

Treatment

Prospective studies are needed to determine if changes (and which changes) in immunosuppressive regimens made at the onset of bronchiolitis obliterans syndrome may alter the subsequent evolution of the process. Developing effective antifibroproliferative therapies (based on either novel immunosuppressive agents or cytokine/chemokine antagonists [81, 84, 120]) may well eventually be the best treatment option for bronchiolitis obliterans syndrome.

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