# Endothelin-1 is a useful biomarker for early detection of bronchiolitis obliterans in lung transplant recipients

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**Objectives:** Bronchiolitis obliterans (BO) is a severe complication limiting long-term survival after lung transplantation. To date, no cure exists for BO, and the mechanisms leading to BO are not well understood. Endothelin-1 (ET-1) is a potent mitogenic and profibrotic peptide produced by pulmonary vascular endothelial cells that play a role in the pathophysiology of lung allograft dysfunction. Whether ET-1 could predict BO syndrome (BOS) development is unknown.

**Methods:** Transbronchial biopsy specimens and serum and bronchoalveolar lavage were obtained from 30 lung transplantation patients with and 30 without BOS at 3 points. The serum and bronchoalveolar lavage ET-1 concentrations were measured by enzyme-linked immunosorbent assay, and the ET-1 mRNA expression in the transbronchial biopsy specimens was examined using real-time polymerase chain reaction.

**Results:** The pretransplant ET-1 serum concentrations were greater in the patients with BOS ($P = .02$); and ET-1 mRNA was significantly upregulated in the lung grafts of those with versus those without BOS at 3 and 12 months after transplant ($P = .01$). At 3 and 12 months after transplantation, the ET-1 concentrations were significantly elevated in the serum ($P < .01$ and $P < .0001$, respectively) and bronchoalveolar lavage ($P < .01$ and $P = .02$, respectively) of patients with compared with those without BOS. On logistic regression analysis, the pretransplant and 3-month post-transplant serum ET-1 level predicted for BOS (odds ratio, 1.01; 95% confidence interval, 1.004–1.025; $P < .007$; odds ratio, 2.9; 95% confidence interval, 1.01–8.52; $P < .001$). The serum ET-1 level at 12 months was diagnostic for BOS (odds ratio, 3.9; 95% confidence interval, 1.42–10.80; $P = .008$).

**Conclusions:** Elevated serum ET-1 concentrations were predictive of BOS, and the assessment of circulating ET-1 might be beneficial in diagnosing and monitoring BO. (J Thorac Cardiovasc Surg 2010;140:1422-7)

Bronchiolitis obliterans syndrome (BOS) remains the major cause of late graft loss in lung transplant recipients.[1] BOS affects 18% to 57% of lung allograft recipients within the first 5 years after transplantation. It also accounts for more than 30% of all deaths after the third post-transplant year.[1,2] Histologically, BOS is characterized by obliterative bronchiolitis and, physiologically, by deterioration of pulmonary function and a decrease in the forced expiratory volume in 1 second ($FEV_1$).[3,4] Although the $FEV_1$ is the main diagnostic tool of BOS, transbronchial biopsy (TBB) remains the reference standard. However, TBB is invasive and difficult to perform repeatedly in patients with impaired pulmonary function. More importantly, obtaining tissue by TBB that will allow a conclusive diagnosis of BO might be problematic. Also, false-negative histologic results can occur despite the declining pulmonary function.

The pathogenesis of BOS remains unclear, although repeated injury to the allograft by ischemia reperfusion, acute rejection, inflammation, alloreactivity directed toward HLA antigens and infections, particularly cytomegalovirus (CMV) infection, have been thought to contribute to BOS development.[3,5] These assaults are followed by responsive neutrophil and lymphocyte infiltration of the allograft, with secondary activation of various cytokines and chemokines, which contribute to inflammatory cell infiltration and BOS progression.[6] Endothelin (ET)-1 is one of the main cytokines and is expressed in the endothelium, smooth muscle cells, airway epithelium, and alveolar macrophages of the lung.[7] Evidence has suggested that ET-1 is involved in many pulmonary diseases, including pulmonary fibrosis[8] and acute respiratory distress syndrome.[9] Moreover, studies using animal models have indicated that ET-1 might have an effect on the development of BO.[10,11]

We have previously reported that ET-1 mediates increased vascular permeability and edema formation in lung grafts before reperfusion and primary graft dysfunction,

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**Abbreviations and Acronyms**

BAL = bronchoalveolar lavage
BO = bronchiolitis obliterans
BOS = bronchiolitis obliterans syndrome
CI = confidence interval
CMV = cytomegalovirus
ET-1 = endothelin-1
$FEV_1$ = forced expiratory volume in 1 second
OR = odds ratio
PCR = polymerase chain reaction
TBB = transbronchial biopsy

a common risk factor for BOS.[12,13] Others have shown that antagonizing ET-1 in experimental lung transplantation prevents fibrous airway obliteration.[14] In the present study, we addressed the utility of ET-1 in the prediction of BOS development in lung transplant recipients.

## PATIENTS AND METHODS

### Patients

The ethics committee of the Medical University of Vienna approved the present study. A total of 60 patients who had undergone lung transplantation between 2006 and 2009 and who have given informed consent to participate were enrolled. The demographic data and the most relevant characteristics of the patients are listed in Table 1. The serum samples were obtained shortly before transfer to the operating room. Moreover, TBB, serum, and bronchoalveolar lavage (BAL) samples were obtained at 3 and 12 months after transplantation during routine clinical follow-up.

All samples were coded and snap frozen. All patients were routinely followed up weekly in the first 3 weeks, monthly during the first year, and every 2 months for the second year after transplantation, or when clinically indicated. The surveillance protocol involved the patients' history, physical status, pulmonary function tests, blood tests, and bronchoscopy with BAL and TBB. All TBB and BAL samples were examined histologically by an independent pathologist.

### BOS Diagnosis and Classification

The average of the 2 greatest pulmonary function measurements obtained at least 3 weeks apart during the post-transplant course was used to establish the baseline $FEV_1$ and the forced expiratory flow at 25% to 75% of the vital capacity. BOS was then classified, according to the International Society for Heart and Lung Transplantation Society guidelines[15]: stage 0, $FEV_1$ of 90% or more and forced expiratory flow at 25% to 75% of the vital capacity of 75% or more; stage 0-p, $FEV_1$ of 81% to 89%; stage 1, $FEV_1$ of 66% to 80%; stage 2, $FEV_1$ 51% to 65%; and stage 3, $FEV_1$ of less than 50%. Other causes of a decline in the $FEV_1$ were excluded by bronchoscopy with TBB and BAL and high-resolution computed tomography.

### Diagnosis and Treatment of Rejection and Infection

Rejection was diagnosed histologically in the TBB samples and graded according to the International Society for Heart and Lung Transplantation scoring. Rejection was suspected clinically if patients had new radiographic pulmonary opacification and deterioration of the blood gases and pulmonary function without evidence of infection despite a TBB score of A0 or B0 to B1. These patients underwent steroid pulse therapy at 1 g/d for 3 days. If the patient was resistant to the steroid pulse therapy, muromonab-CD3 (OKT-3) was administered at 5 mg/d for 7 days. An episode of infection was defined clinically and approved using microbiologic, serologic, or histologic tests. CMV screening was performed by measurement of CMV serology (quantitative polymerase chain reaction [PCR]) and the detection of CMV early antigen in blood, urine, throat smear, and BAL. Bacterial and fungal cultures and PCR for toxoplasmosis and pneumocystis carinii were performed in each BAL. The TBB specimens were also histologically screened for CMV, pneumocystis carinii, toxoplasmosis, and bacterial or invasive fungal infection. Colonization was considered present if the BAL microbiology was positive without histologic or clinical evidence of infection.[16]

### Immunosuppression

The immunosuppression protocol has been previously described.[16] In brief, the patients received 1 g methylprednisolone intraoperatively, followed by 125 mg at 8, 16, and 24 hours postoperatively. Thereafter, prednisolone was administered at 1 mg/kg/d and tapered to 0.25 mg/kg within 3 months. All patients received rabbit-antithymocyte globulin (Thymoglobulin; Sero-Merieux, Lyon, France) 2.5 mg/kg intravenously for the first 4 postoperative days. Mycophenolate mofetil (CellCept; Hoffmann-La Roche, Basel, Switzerland) at 3 g/d was given intravenously on the first postoperative day and then orally. Cyclosporine A (target level 350 ng/mL) or tacrolimus (target level $15 \pm 3$ ng/mL) was administered intravenously immediately after surgery and subsequently switched to oral administration.

### BOS Management

The treatment of patients with BOS was initiated after the diagnosis of BOS from the pulmonary function test results when other causes of $FEV_1$ decline had been excluded. Recipients with BOS received steroid bolus therapy at 1000 mg/d for 3 days. Next, the steroid was reduced to 1 mg/kg within 2 weeks. The recipients then underwent pulmonary function testing and bronchoscopy every 2 weeks until stabilization. In the case of BOS resistance, the immunosuppression regimen was changed as follows. Cyclosporine A was switched to tacrolimus with a target serum level of $18 \pm 2$ ng/mL, depending on kidney function. If the switch in immunosuppression was not effective, the steroid treatment was repeated. If this therapy failed, the patient received clarithromycin, rabbit antithymocyte globulin (Thymoglobulin) or muromonab-CD3 (OKT-3), as described in the ''Diagnosis and treatment of rejection and infection'' section.

### Enzyme-Linked Immunosorbent Assay

An enzyme-linked immunosorbent assay for ET-1 (TiterZyme EIA, Catalogue No. 900–020; Assay Designs, Minneapolis, Minn) was performed according to the manufacturer's protocol. Standard or serum samples were added to each polyclonal ET-1 antibody precoated well and incubated at 4°C overnight. The substrate reaction was quantified spectrophotometrically using a 96-well, automated microplate reader (Anthos, Salzburg, Austria) at 450 nm. The cross reactivity of the assay with other endothelin isotypes was less than 0.1.[13]

### Quantitative Real-Time Reverse Transcriptase PCR

Total RNA was isolated from the TBB specimens with TRIzol (Invitrogen, Carlsbad, Calif), using MagNA Lyser (Roche, Mannheim, Germany). Real-time reverse transcriptase PCR was performed on a LightCycler instrument (Roche), as previously described.[13] The primer sequences were sense/antisense: ET-1: 5′-GGAAAAGACTGTTCCAAGC-3′/5′-GGTTG TGGGTCACATAACG-3′; and $\beta_2$-microglobulin: 5′-GATGAGTATGCCT GCCGTGTG-3′/5′-CAATCCAAATGCGGCATCT-3′. The expression of the target gene was determined by relative quantification (ie, normalization to the expression of the housekeeping gene, $\beta_2$-microglobulin[13]) and