The value of D-dimer in lung transplant recipients with bronchiolitis obliterans syndrome

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Summary
Bronchiolitis obliterans syndrome (BOS) following lung transplantation is common and potentially devastating. Its exact cause is undefined, but multiple immune and nonimmune processes contribute to its pathogenesis. The diagnosis of BOS syndrome is based on clinical presentation of progressive decline in the lung functions together with appropriate pathological findings. Severe acute rejection and recurrent acute rejection have been shown to confer the greatest risk for obliterative bronchiolitis, signifying the central importance of alloimmunity in the disease process. BOS is associated with activation of the coagulation system, and is a major cause of lung allograft loss. The aim of the study was to determine if there is an association between D-dimer levels and functional exercise capacity in lung transplant recipients with BOS.

This prospective group comparison study was conducted at a tertiary-care, university-affiliated medical center. The sample included 46 patients (29%) who underwent lung transplantation between January 1997 and May 2006 and had positive findings on screening for BOS. Blood samples were collected for measurement of plasma D-dimer levels by the rapid MiniQuant assay. Correlational analysis was used to determine the association of D-dimer levels with demographic clinical data, pulmonary function, and functional exercise capacity parameters, including the 6-min walk test and cardiopulmonary exercise testing.

D-dimer levels were associated with FEV1 ($r = -0.43$, $p = 0.001$), 6-min walk test ($r = -0.53$, $p = 0.04$), and $V_{O2}$/kg/min ($r = -0.36$, $p = 0.04$). No correlations were noted between D-dimer levels and total lung capacity, diffusion capacity, and oxygen saturation.

On multivariate logistic regression, only FEV1 was a significant predictor of BOS (OR 0.885, CI: 0.812–0.965).

Abbreviations: BOS, bronchiolitis obliterans syndrome; FEV1, forced expiratory volume in 1 s; 6MWT, 6-min walk test; PAI-I, plasminogen activator inhibitor; TPA, tissue plasminogen activation; $V_{O2}$, oxygen consumption

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Introduction

Bronchiolitis obliterans syndrome (BOS) is a serious complication of lung transplantation, affecting about 40% of patients by 2 years after surgery.1–9 Multiple factors have been implicated in the development of BOS,6–8 including the number and severity of acute rejection episodes, cytomegalovirus infection, and HLA mismatching.9

Chronic allograft rejection is often associated with the presence of fibrin thrombi in the microcirculation.10 Persistent fibrin deposition has been reported in affected patients after kidney and cardiac transplantation.11,12 Moreover, Segal et al.12 observed that the presence of plasma coagulation markers predicted the occurrence of cardiac rejection. On the basis of these findings and the increasing use of D-dimer, a degradation product of fibrin, as a marker and prognostic factor in various thrombotic diseases,13,14 we hypothesized that the severity of BOS in lung transplant recipients is correlated with the activation of coagulation and fibrinolysis and, hence, higher D-dimer levels. The aim of the study was to determine if D-dimer levels can be used as a noninvasive and rapid marker of the severity of BOS, as measured by pulmonary exercise capacity parameters.

Patients and methods

Study subjects

All patients who underwent lung transplantation at Rabin Medical Center between January 1997 and May 2006 were screened for BOS. Forty-six patients (29%) had findings of deteriorating allograft function and were included in the study. All were clinically diagnosed with BOS stage 1a or higher on the basis of the criteria of the International Society for Heart and Lung Transplantation, as follows: a decline of at least 20% in forced expiratory volume in 1 s (FEV1) from their individual maximum post-transplant value, in the absence of acute infection or acute rejection.15 Acute infection was defined as a positive blood or sputum culture in the absence of acute infection or acute rejection.15 Acute rejection was diagnosed by asking the patient to breathe as fast and as deeply as possible for 12 s, and the result was multiplied by 5. Diffusion capacity of carbon monoxide was measured by a single-breath technique with a gas mixture containing air, 10% helium, and 0.3% carbon monoxide. Each measurement was adjusted to standard temperature and pressure. The predicted values of the parameters were obtained from the regression equations of the European Community for Coal and Steel.16

We conclude that in lung transplant recipients with BOS, D-dimer levels are highly associated with functional exercise capacity and may serve as a useful marker for noninvasive monitoring. Further coagulation assays are needed to complete our observations.

The Institutional Human Subjects Review Board approved the study protocol.

Sample collection and D-dimer assay

Following the diagnosis of BOS, blood samples were collected into test tubes containing 3.2% buffered sodium citrate. Platelet-poor plasma was separated by centrifugation within 4 h of collection and frozen at –70°C. Prior to analysis, all samples were rapidly thawed at 37°C and recentrifuged at 5000 rpm for 5 min. D-dimer levels were measured with the MiniQuant D-dimer assay (Biopool International, Venture, CA) according to the manufacturer’s instructions. All sample batches were assayed together with controls purchased from the manufacturer. Normal levels are below 150 mg/ml.

Pulmonary function test

The pulmonary function test included spirometry, lung volume, maximal voluntary ventilation, and diffusion capacity.16 Measurements were corrected for body temperature and pressure, saturated with water vapor. Testing was performed with the Medical Graphics Pulmonary Function System (1070-series 2, St. Paul, MN). Lung volumes were obtained by body plethysmography (model 1085, Medical Graphics, St. Paul, MN). Maximal voluntary ventilation was assessed by asking the patient to breathe as fast and as deeply as possible for 12 s, and the result was multiplied by 5. Diffusion capacity of carbon monoxide was measured by a single-breath technique with a gas mixture containing air, 10% helium, and 0.3% carbon monoxide. Each measurement was adjusted to standard temperature and pressure. The predicted values of the parameters were obtained from the regression equations of the European Community for Coal and Steel.16

Cardiopulmonary exercise testing protocol17

Each participant underwent an incremental cardiopulmonary exercise test according to the protocol of Wasserman et al.18 on an electrically braked cycle ergometer (Ergoline 800, Germany). Testing was conducted between 8:30 and 12:00 a.m. noon. On arrival at the exercise laboratory, patients were connected to a 12-lead electrocardiogram (Cardiofax, Nihon Kohden, Tokyo, Japan) with a single-lead (V5) monitor (VC-22, Nihon Kohden). Oxygen saturation (SaO2) was measured by pulse oximetry (Nellcor NPB-190, CA, USA) and blood pressure with a sphygmomanometer. Patients were then positioned on the ergometer. After a 3-min rest period, patients performed unloaded pedaling for 2 min at a rate of 60 rpm. The load was then progressively increased by 15 W/min (i.e., ramp protocol). The duration
of the test was symptom-limited; the end-point of the protocol was defined as the point in which the patient could not maintain a pedaling rate of more than 40 rpm.

Cardiopulmonary data were collected and analyzed by an exercise metabolic unit (CPX, Medical Graphics, St. Paul, MN, USA). Heart rate, minute ventilation, tidal volume, oxygen consumption, carbon dioxide production, oxygen pulse and oxygen saturation were recorded and calculated over 30-s intervals using standard formulas. Blood pressure was measured with a sphygmomanometer and every 2 min until peak exercise.

The 6-min walk test
The 6-min walk test (6MWT) protocol was performed according to the guidelines of the American Thoracic Society.19

Study protocol
The association of the D-dimer levels with the clinical and demographic variables, pulmonary function tests, 6MWT, and cardiopulmonary parameters was evaluated. All the technical assistants were blinded to the D-dimer levels.

Statistical analysis
Results are shown as mean±S.D. Pearson correlation coefficient (r) and the significance for it (p) were calculated between the variables.

To analyze differences in the distribution of categorical data, χ² test or Fisher exact test was used, as appropriate.

To predict BOS, a stepwise logistic regression was fitted to the data. Odds ratios and 95% confidence intervals were calculated from the model.

A p-value of 0.05 or less was considered statistically significant.

Results
Clinical characteristics
Table 1 summarizes the demographic characteristics of the lung transplant recipients with BOS. Most of the patients (n = 39, 85%) underwent lung transplantation because of chronic obstructive pulmonary disease and pulmonary fibrosis. Thirty-five patients (76%) underwent single-lung transplantation. All patients were treated with corticosteroids and 36 (78%) with a FK 506-based regimen.

Table 2 summarizes the clinical characteristics of the patients, including pulmonary lung function and results of the cardiopulmonary exercise test and 6MWT. Most of the patients had mild to moderate lung function abnormalities with preserved saturation and exercise tolerance.

Correlations between D-dimer and study parameters
Table 3 and Figure 1 show the Pearson correlation coefficient (r) and the significance for it (p) between D-dimer values and the study variables.

D-dimer levels were correlated with age (r = 0.34, p = 0.02), FEV₁ (r = −0.43, p = 0.001), 6MWT (r = −0.53, p = 0.04) and VO₂/kg/min (r = −0.36, p = 0.04).

No correlations were noted between D-dimer levels and total lung capacity, diffusion capacity and oxygen saturation. On multivariate logistic regression, only FEV₁ was a significant predictor of absence of BOS (OR 0.885, CI: 0.812−0.965).
6MWT and in lung transplant recipients with BOS, as measured by development of BOS. Studies have shown that BOS occurs in transplantation, long-term survival is limited by the development of BOS. In renal transplantation, and hypercoagulability continues to accumulate, especially in renal transplant recipients.10,22 In renal transplantation, transplantation.22

**Discussion**

Lung transplantation has emerged as an important therapeutic option for patients with end-stage pulmonary disease. Although short-term survival is improved with transplantation, long-term survival is limited by the development of BOS. Studies have shown that BOS occurs in 40–70% of recipients by 5 years after transplantation.15,21 For those who fail enhanced immunosuppressive regimens, other treatment options are rarely successful. Although most of the evidence suggests that BOS is immune-mediated, many transplant recipients experience activation of the coagulation and fibrinolytic systems that potentially worsens chronic rejection.

This study demonstrates that high D-dimer levels are associated with FEV1 as well as functional exercise capacity in lung transplant recipients with BOS, as measured by 6MWT and VO2/kg. These results have two major implications. First, the relationship offers possible evidence of a pathophysiological link between BOS and hypercoagulative state. Second, higher levels of D-dimer in lung transplant recipients may identify those at being at higher risk of BOS. Evidence of a link between allograft function and hypercoagulability continues to accumulate, especially in renal transplant recipients.10,22 In renal transplantation, endothelial cells form the interface between donor and recipient tissues. Antibody or thrombin-stimulated endothelium produces platelet-activating factor, and thrombin-stimulated endothelium releases both plasminogen activators and plasminogen activator inhibitors (PAI-I). Therefore, allograft recognition may initiate clotting and fibrinolytic phenomena which, in turn, cause monocytes and macrophages to produce tissue factor, a potent initiator of coagulation. Endothelial cells can also be stimulated to elaborate tissue factor by immune complexes, interleukin-1, or endotoxin. Christie et al.23 assessed the role of protein C type 1 PAI-I levels in plasma of lung transplant recipients with primary graft dysfunction (PGD). They found lower postoperative protein C and higher PAI-I plasma levels are associated with PGD after lung transplantation. Impaired fibrinolysis and enhanced coagulation may be important in PGD pathogenesis. These observations give cause to speculate that the link between immunity and coagulation could be products of allogenic recognition that activate factor VII and lead to fibrin deposition. This suggests new approaches of diagnosis and treatment of rejection reactions in organ transplantation.22

Segal et al.,12 in a study of plasma markers of coagulation in cardiac transplant recipients, found that p-selectin and prothrombin fragment levels significantly predicted organ rejection. The authors suggested that these markers may be useful for noninvasively monitoring patients for allograft rejection or response to treatment.

The influence of rejection on fibrinolytic regulators was also assessed by Perkowska et al.10 in 64 patients who underwent kidney transplantation from cadaveric allograft donors. They found significantly higher levels of tissue plasminogen activator (tPA) and lower levels of PAI-I than in healthy controls. Among the transplant recipients, PAI-I level was significantly higher in the patients with graft rejection than in the patients with stable graft function. The authors concluded that chronic allograft rejection is apparently associated with an increase PAI-I activity.10

An important immunocytochemical study of biopsy tissue from 68 transplanted hearts revealed that depleted arteriolar tPA was significantly associated with vascular and interstitial deposits of fibrin, plasmin, and endothelial tPA–PAI-I complexes.24 These findings indicate that hemostatic and fibrinolytic pathways are activated in failing allografts, and that they can supply evidence of depleted tPA even before clinical or histopathological signs of failure.

Our study assessed patients with BOS syndrome. This is common and potentially devastating. Its exact cause is undefined, but multiple immune and nonimmune processes contribute to its pathogenesis. Severe acute rejection and recurrent acute rejection have been shown to confer the greatest risk for the syndrome, signifying the central importance of alloimmunity in the disease process. As our understanding of the disease evolves, it is hoped that effective interventions targeted at specific pathogenetic steps will emerge. In the meantime, obliterative bronchiolitis remains the most important and sinister long-term complication of lung transplantation. Although the diagnosis of the syndrome based on the pathological stage, the clinical presentations are critical for the diagnosis.

Our study, though limited by the small sample size, the lack of serial D-dimer tests and other coagulation markers...
and data concerning the other lung transplant recipients has important advantages. First, the available data on the role of noninvasive markers in patients with allograft rejection\textsuperscript{25–27} are limited, and to the best of our knowledge, our study is the first to assess the role of D-dimer assay, a fibrinolytic marker, in lung transplantation. Second, the statistically significant correlation between D-dimer level and functional exercise capacity in lung transplant recipients with BOS, as measured by FEV\textsubscript{1}, 6MWT and VO\textsubscript{2}/kg/min, highlights the important involvement of the coagulation pathway in allograft rejection.

In summary, our findings suggest that the simple, noninvasive D-dimer test may have an important place in the evaluation of lung transplant recipients with BOS. Further coagulation assays are needed to complete our observations.

**Conflict of interest**

None of the authors have a conflict of interest to declare in relation to this work.

**References**