Exhaled NO may predict the decline in lung function in bronchiolitis obliterans syndrome

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ABSTRACT: **Bronchiolitis obliterans syndrome (BOS) remains the leading cause of morbidity/ mortality following lung transplantation. In recipients with BOS, markers predicting the decline in lung function are needed. The aim of this longitudinal study was to determine whether exhaled nitric oxide fraction (F_{eNO}) measurements provide useful information for discriminating patients with unstable BOS from those with stable BOS.**

During a 14-month period, 145 F_{eNO} measurements were performed in 50 lung transplant recipients. Among them, 16 recipients with BOS (32 F_{eNO} measurements) were analysed. For each F_{eNO} measurement, the patients were classified into three groups according to the decline in forced expiratory volume in one second (FEV1) within the following 6 months: 1) stable BOS free; 2) stable BOS (decline in FEV1 of <5%); and 3) unstable BOS (decline in FEV1 of $\geqslant$15%).

The mean F_{eNO} in patients with unstable BOS was significantly increased compared with that in stable BOS-free patients (18.4$\pm$5.7 versus 9.7$\pm$3.7 ppb) and that in patients with stable BOS (18.4$\pm$5.7 versus 9.7$\pm$3.3 ppb).

The present findings suggest that, in patients with bronchiolitis obliterans syndrome, a raised exhaled nitric oxide fraction may predict the development of worrisome functional impairment during long-term follow-up.

KEYWORDS: Bronchiolitis obliterans syndrome, exhaled nitric oxide, lung transplantation

Although lung transplantation is now an established treatment for patients with end-stage lung disease, the long-term functional status and survival of lung transplant recipients are still limited by the occurrence of bronchiolitis obliterans syndrome (BOS). BOS has been defined as a clinical syndrome of irreversible progressive airway obstruction in the pulmonary allograft caused by the presence of obliterative bronchiolitis. It is thought to represent a form of chronic rejection, which develops in >50% of lung transplant recipients 3 yrs after transplantation [1] and is the commonest cause of late graft failure. Early detection and treatment of BOS seem to be the most important factors that may permit stabilisation of lung function in patients with BOS, since better survival has been reported in patients with lower BOS stages [2]. In addition, once the BOS is established, another clinical challenge is to determine an adequate level of immunosuppression, allowing stability of lung function without excessive risk of infection due to overt immunosuppression. In this regard, there is no marker that can predict the different functional patterns of BOS over time. These patterns include either rapid loss of lung function, slow progression or intermittent loss of function, with a long plateau period during which pulmonary function is stable [3, 4]. Therefore, there is a need for new tools to monitor the lung allograft, especially for immunological complications.

Nitric oxide (NO) is implicated in the pathophysiology of airway diseases [5]. The upregulation of the inducible form of NO synthase (iNOS) in airway epithelial cells is associated with increased production and prolonged release of NO [6]. The fraction of NO in exhaled breath (F_{eNO}) has been shown to be correlated with the levels of NO found in the lower respiratory tract [7]. Compared with healthy subjects, F_{eNO} is increased in diseases associated with airway inflammation, such as asthma or respiratory infection [8, 9]. It has also been found that higher NO levels in patients with fibrosing lung disease were associated with increased disease activity [10] or subclinical inflammation [11], and that the same patients with more advanced fibrosing lung disease exhibited normal NO levels [11].

Accordingly, there is increasing interest in F_{eNO} as a noninvasive marker of airway inflammation after lung transplantation. In particular, it has recently been demonstrated that lung transplant recipients with BOS exhibit increased F_{eNO} compared with stable lung transplantation patients and healthy nonsmoking controls [12–14], and that F_{eNO} may be helpful in the early diagnosis of BOS [15].

As suggested for fibrosing lung disease [10, 11], it was hypothesised in the present study that the inflammatory activity in the airways of patients with unstable BOS would lead to increased iNOS expression and a subsequent increase in F_{eNO}, which would be downregulated once the inflammatory phase is replaced by fibrosis in patients with stable BOS. In this regard, the clinical utility of F_{eNO} measurements was evaluated in lung transplant recipients with BOS followed-up in the Beaujon Hospital (Clichy, France) over a 14-month period. The aim of the present clinical study was to determine whether F_{eNO} measurements provide information that is useful for discriminating patients with unstable BOS from those with stable BOS.

METHODS

Study population

Between November 2001 and December 2002, all lung transplant recipients surviving beyond 6 months following transplantation were eligible for recording of F_{eNO}. F_{eNO} measurement was performed: 1) in ambulatory recipients at each routine follow-up, and 2) in the case of respiratory complication.

During the study period, F_{eNO} was measured in 50 patients (11 double-lung (bilateral lung transplantation (BLT)) and 39 single-lung (single lung transplantation (SLT)) transplant recipients), at a median time after transplantation of 600 days (range 180–4,400 days). The median number of F_{eNO} measurements was three per patient (range 1–6). Each patient was followed-up for $\geqslant 6$ months after the last F_{eNO} measurement. The pre-transplant diagnoses were emphysema with or without α_1-antitrypsin deficiency (n=22), idiopathic pulmonary fibrosis (n=13), cystic fibrosis and other bronchiectatic diseases (n=5), and other respiratory diseases (n=10).

Immunosuppression

The immunosuppressive regimen consisted of cyclosporin A (trough level 200–300 ng·mL^{-1} for the first 3 months, and thereafter according to time after lung transplantation and renal function), azathioprine (1–2 mg·kg^{-1}·day^{-1}) and prednisone ($\geqslant 7.5$ mg·day^{-1}). Intravenous methylprednisolone was given at a dose of 500 mg pre-operatively and before reperfusion of the graft, 0.5 mg·kg^{-1}·day^{-1} on the following days, and thereafter replaced by oral prednisone, which was progressively tapered to 0.1 mg·kg^{-1}·day^{-1} after 12 weeks. Rabbit antithymocyte globulin induction therapy was given during the post-operative period (1.5 mg·kg^{-1}·day^{-1} for 3 days) only in patients without cytomegalovirus (CMV) mismatching.

Lung transplantation protocols

At each routine visit (at least every 3 months), or, in the case of pulmonary complication, pulmonary function testing (forced expiratory volume in one second (FEV1) and vital capacity) was performed in accordance with standardised American Thoracic Society guidelines $\geqslant 1$ h before the F_{eNO} measurement was recorded. Fibreoptic bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsy (TBB) was performed only for determination of clinical, physiological or radiographic changes. BAL specimens were cultured for determination of the presence of bacteria (including *Nocardia, Actinomyces, Legionella* and mycobacteria), viruses (with immunofluorescence testing for respiratory viruses and culture for CMV) and fungi (including *Aspergillus* spp.). All TBB specimens were interpreted by the same pathologist and acute rejection histology was graded according to the system proposed by YOUSEM *et al*. [16]. An episode of acute rejection was defined after histological confirmation through TBB. Acute rejection was treated with daily infusion of methylprednisolone (1 g intravenously) for 3 days, followed by prednisone (1 mg·kg^{-1}·day^{-1}), tapered to a maintenance dose over 3 weeks. Resistant acute rejection was treated with rabbit antithymocyte globulin (2.5 mg·kg^{-1}·day^{-1} for 5 days). BOS was defined according to the revised classification system of the International Society for Heart and Lung Transplantation [4]. According to this classification, potential BOS (BOS 0-p) was defined by a 10–19% decrease in FEV1 from baseline [4]. BOS status was ascertained with repeated pulmonary function testing at least every 3 months after transplantation.

Diagnostic categories

The clinical outcome of the patients was analysed within the 6 months following each F_{eNO} measurement. For each F_{eNO} measurement, the patients were allocated to the following categories by a physician blinded to the F_{eNO} results.

Group 1: stable patients free of BOS

Group 1 patients were those without any acute pulmonary complication within 1 month before or after F_{eNO} measurement, and without chronic pulmonary complication at the time of any F_{eNO} measurement during the study period.

Group 2: stable BOS

Group 2 patients were those with established BOS ($\geqslant$ BOS 1) at the time of F_{eNO} measurement, free from any acute complication (acute rejection, lymphocytic bronchiolitis and infection) within the previous month, and who retained stable pulmonary function for $\geqslant 6$ months following the date of F_{eNO} measurement. Pulmonary function was considered stable if the loss in FEV1 was <5% within the following 6 months.

Group 3: unstable BOS

Patients were classified as unstable in the case of: 1) an initial decline in pulmonary function corresponding to the first diagnosis of established BOS (BOS 1) at the date of F_{eNO} measurement, or 2) at least potential BOS (>BOS 0-p) at the time of F_{eNO} measurement with subsequent pulmonary functional decline within the following 3 months, leading to established BOS ($\geqslant$ BOS 1). Except for one patient (No. 2; table 1), patients classified as having unstable BOS showed a fractional decrease in FEV1 (expressed as the percentage decrease from the highest previous baseline FEV1 not attributed to other causes (exclusion of infection, acute rejection and anastomotic complication)) of $\geqslant 15\%$ during the 3–6-month period following F_{eNO} measurement. Patient No. 2 (table 1) had lacking functional data, but experienced a

TABLE 1	Clinical data from the nine patients with unstable bronchiolitis obliterans syndrome (BOS)									
Patient No.	TX	Initial disease	Sex	Age yrs	Treatment[#]	F_{eNO}[¶] ppb	First F_{eNO} days[+]	BOS status[#]	ΔFEV_1[§] % (mL)	BOS treatment[f]	Clinical outcome[##]
1	SL	Emp.	M	46	FK/MMF	20.0	191	BOS 0-p	-25 (-380)	Pulse steroids	Alive, BOS 1 (9 m)
2	SL	Emp.	F	47	CsA/AZA	26.0	4012	BOS 3	NA		Dead, BOS 3 (9 m)
3	DL	Emp.	M	48	CsA/MMF	23.8	288	BOS 0-p	-52 (-1550)	Pulse steroids; ATG	Dead, BOS 3 (8 m)
4	SL	IPF	F	42	FK/MMF	20.5	2342	BOS 1	-18 (-260)	Pulse steroids; ATG	Alive, BOS 2 (6 m)
5	SL	IPF	M	44	CsA/MMF	15.0	3896	BOS 1	-17 (-340)		Dead, BOS 2/infection (7 m)
6	DL	Bronch.	M	40	CsA/AZA	9.2	922	BOS 2	-48 (-1590)	Pulse steroids; ATG; switch to CsA/FK	Dead, BOS 3 (5 m)
7	SL	IPF	M	57	CsA/AZA	17.2	759	BOS 0-p	-16 (-370)	Pulse steroids; switch to CsA/FK	Alive, BOS 1 (8 m)
8	DL	IPF	F	31	CsA/AZA	14.1	2079	BOS 1	-29 (-480)	Pulse steroids; ATG/MTX	Alive, BOS 2 (14 m)
9	SL	Emp.	M	67	FK/AZA	29.0	3342	BOS 1	-16 (-310)	Pulse steroids; ATG	Alive, BOS 2 (15 m)

TX: transplantation; F_{eNO}: exhaled nitric oxide fraction; ΔFEV_1: change in forced expiratory volume in one second (FEV_1); SL: single lung; DL: double lung (bilateral); Emp.: emphysema; IPF: idiopathic pulmonary fibrosis; Bronch.: bronchiectasis; M: male; F: female; FK: tacrolimus; MMF: mycophenolate mofetil; CsA: cyclosporine A; AZA: azathioprine; BOS 0-p: potential BOS; ≥BOS 1: established BOS; NA: not available; ATG: antithymocyte globulin; MTX: methotrexate; m: months. [#]: on day of F_{eNO} measurement; [¶]: final value; [+]: post-transplantation; [§]: irreversible decline within 3 weeks before and 6 months after first F_{eNO} measurement expressed as percentage decrease from the highest previous baseline FEV_1 (absolute decline); [f]: administered within the 2 months following F_{eNO} measurement; [##]: at the last follow-up after first F_{eNO} measurement.

continuous functional decline attributed to the progression of BOS before death with confirmed obliterative bronchiolitis on autopsy and was, therefore, included in this group.

Analyses of F_{eNO} measurements from recipients who exhibited an active infection were excluded owing to the known effect of infections on F_{eNO} [9]. An active infection was diagnosed on the basis of the following: 1) clinical, radiological and/or physiological deterioration; 2) isolation of an organism from a clinically relevant BAL fluid culture; 3) antibiotic therapy resulting in a full recovery and return to pre-morbid lung function; and 4) other concurrent diagnoses excluded by TBB when appropriate. Simple colonisation was diagnosed on the basis of the following: 1) light growth of microorganisms on BAL fluid culture; 2) in the case of stable lung function, the patient did not receive any new antimicrobial therapy, and there were no adverse sequelae; and 3) in the case of deteriorating lung function, a concurrent diagnosis such as acute rejection, lymphocytic bronchiolitis or unstable BOS was established, and antibiotic therapy, if administered, had no effect on the deteriorating lung function.

Analysis of F_{eNO} measurements from patients with reversible graft dysfunction (acute rejection or lymphocytic bronchiolitis) at the date of measurement was also excluded. For those patients with active infection or reversible graft dysfunction, no F_{eNO} measurement was performed within the 3 months following the acute event.

For comparison, F_{eNO} measurements performed using the same standardised method were also recorded in 21 healthy nonsmoking control volunteers without a history of asthma or allergic rhinitis and free of upper and lower respiratory symptoms on the day of the test.

Exhaled nitric oxide fraction measurement
NO was measured using a rapid linear-response chemilumin-escent analyser (280B; Sievers, Boulder, CO, USA) with a lower limit of detection of ~1 ppb and a response time of 0.2 s (0–95% rise time). Before each measurement, a two-point calibration was performed using medical-grade NO (Air Liquide France, Limay, France) at concentrations of 0 ppb (Nitrogen High Purity Gas; Air Liquide France) and 45 ppm. For single-breath NO measurements, seated subjects were asked to exhale slowly from total lung capacity through a narrow Teflon-coated tube and recordings were made of exhaled NO levels in parts per billion. As NO concentrations are highly flow dependent, a constant flow rate (50 mL·s⁻¹) was maintained against a fixed resistance [17, 18]. The patients targeted a constant positive mouth pressure of 12–13 cmH$_2$O, which closed the velum to prevent nasal NO contamination [19]. The procedure was repeated until three technically acceptable measurements were recorded at the end of exhalation, i.e. three F_{eNO} that agreed within 10% of the mean value. F_{eNO} was calculated as the mean of these three values [17].

Statistical analysis
Continuous data are presented as mean±SD, and categorical data as count and proportion. The stable BOS-free group was analysed in order to establish normal values and the intraclass correlation coefficient. The confidence intervals of intraclass coefficients were determined using bootstrap resampling. Random-effect models were built to account for repeated F_{eNO} measurements in each patient. Adjusted p-values for multiple comparisons are reported using the Scheffé correction.

RESULTS
A total of 50 lung transplant recipients attended for review, and 145 F_{eNO} measurements were performed. Thirty-four patients (92 F_{eNO} measurements) did not experience irrevers-ible lung dysfunction throughout the study period (stable BOS-free patients). Sixteen patients showed irreversible lung

dysfunction and were diagnosed with BOS. Among these, seven patients with BOS retained stable lung function throughout the study, six patients exhibited unstable BOS and three other patients were successively classified in both groups (unstable and then stable BOS) during the study period. Thus, 10 patients were analysed in the stable BOS group (19 FeNO measurements), and nine in the unstable BOS group (13 FeNO measurements).

The remaining 21 FeNO measurements were excluded from analysis due to a diagnosis of active infection (17 FeNO measurements from 15 recipients) or reversible graft dysfunction (four FeNO measurements from two patients with A2 grade rejection and two patients with lymphocytic bronchiolitis) at the date of FeNO measurement.

BAL was performed in seven of the nine patients with unstable BOS at the time of FeNO measurement because of an initial decline in pulmonary function. In five cases, no organism was isolated. In the other two patients, simple colonisation with *Pseudomonas aeruginosa* (No. 6; table 1) and both *P. aeruginosa* and *Aspergillus fumigatus* (No. 1; table 1) was diagnosed.

The FeNO measurements for the different groups are shown in figure 1. The range of FeNO in the stable BOS-free patients (n=34) was 3.3–18.7 ppb (mean ± SD 9.7 ± 3.7 ppb). The reproducibility of FeNO measurements was good, with an intraclass correlation coefficient of 0.71 (95% confidence interval (CI) 0.45–0.86) in a subgroup of 27 stable BOS-free patients with two measurements, and an intraclass correlation coefficient of 0.64 (95% CI 0.39–0.78) in 14 stable BOS-free patients with three measurements. Mean FeNO in this stable BOS-free group was similar to that of the control group (9.7 ± 3.7 *versus* 9.6 ± 2.9 ppb, respectively; p=NS) and similar to that of the stable BOS group (9.7 ± 3.7 *versus* 9.7 ± 3.3 ppb; mean difference 0.02 (95% CI 3.10–3.19); p=0.99). Compared with stable BOS-free

patients, mean values of FeNO were significantly increased in patients with unstable BOS (9.7 ± 3.7 *versus* 18.4 ± 5.7 ppb, respectively; mean difference 8.7 (95% CI 6.4–11.1); p<0.001; fig. 1). Using an FeNO of 15 ppb as the cut-off value, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the mean FeNO in discriminating patients with unstable BOS from stable BOS-free patients were 77, 91, 70 and 93%, respectively. Three of the 34 stable BOS-free patients displayed a mean FeNO of >15 ppb and did not develop BOS during a follow-up of ⩾9 months.

In addition, in order to evaluate the potential bias due to the presence of a native lung on the accuracy of FeNO measurement, mean FeNO was also compared between patients who had undergone SLT and those who had undergone BLT in each diagnostic category (fig. 1). No significant difference was observed according to surgical procedure in each group (12.9 ± 0.67 *versus* 11.7 ± 1.10 ppb, respectively; p=0.31).

FeNO results from patients who had developed BOS, including patients with stable (n=10) and unstable BOS (n=9), are shown separately in figure 2. The mean FeNO of patients with unstable BOS was 18.4 ± 5.7 ppb, significantly higher than that of those with stable BOS (9.7 ± 3.3 ppb; mean difference 8.7 (95% CI 6.1–9.3); p<0.001). Each group (stable and unstable BOS) was subdivided according to BOS grade (BOS 1 and BOS 2/3) at the date of FeNO measurement. In both the stable and unstable BOS groups, no significant difference in FeNO was observed between patients with BOS 1 and those with BOS 2/3 (fig. 2).

The clinical data of the patients with unstable and stable BOS are detailed in tables 1 and 2. During the 6-month period following FeNO measurement, eight of the nine patients with unstable BOS displayed a fractional decrease in FEV1 of ⩾15% (expressed as the percentage decrease from the highest previous baseline FEV1), with an increase in BOS stage despite
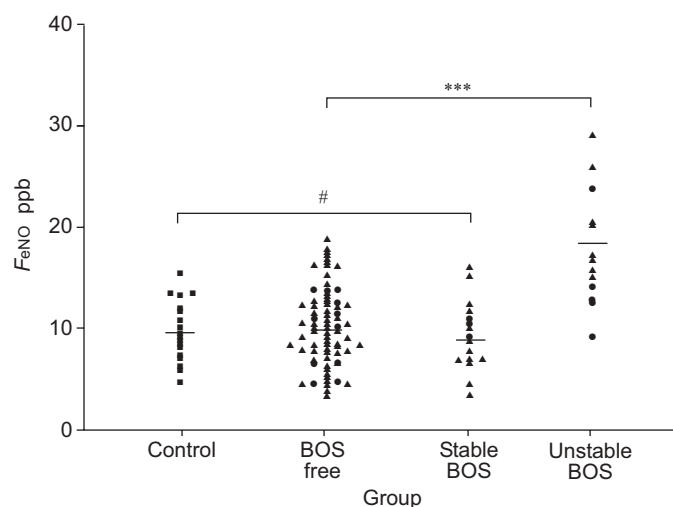
FIGURE 1. Mean exhaled nitric oxide fraction (FeNO) in lung transplant recipients with stable or unstable bronchiolitis obliterans syndrome (BOS), stable BOS-free patients and healthy controls (■: no transplantation; ▲: single lung transplantation; ●: bilateral lung transplantation). Horizontal bars represent means. ***: p<0.001, unstable *versus* stable patients; #: p=0.99 (NS), stable BOS *versus* BOS-free patients and controls.
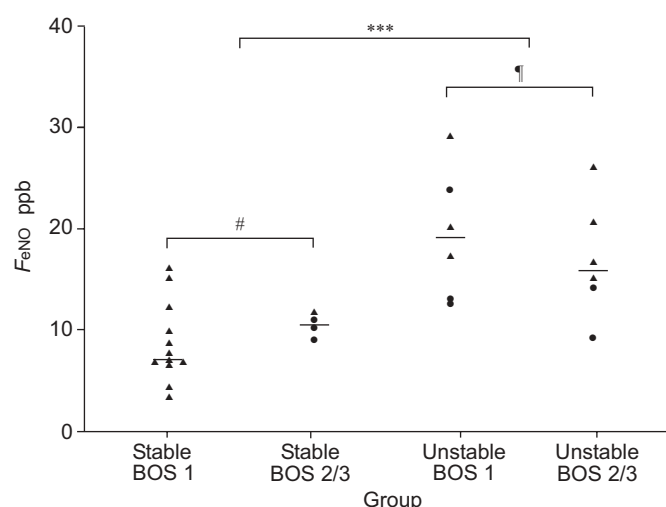
FIGURE 2. Mean exhaled nitric oxide fraction (FeNO) in lung transplant recipients with stable or unstable bronchiolitis obliterans syndrome (BOS) grade 1 or grades 2/3 (▲: single lung transplantation; ●: bilateral lung transplantation). Horizontal bars represent means. ***: p<0.001, unstable *versus* stable BOS; #: p=0.79 (NS), stable BOS 1 *versus* stable BOS 2/3; ¶: p=0.84 (NS), unstable BOS 1 *versus* unstable BOS 2/3.

TABLE 2 Clinical data from the 10 patients with stable bronchiolitis obliterans syndrome (BOS)

Patient No.	TX	Initial disease	Sex	Age yrs	Treatment#	F_{eNO}¶ ppb	First F_{eNO} days+	BOS status#	ΔFEV1§ %	Additional BOS treatment^f	Clinical outcome##
1	SL	Emp.	M	53	FK/MMF	6.6	1190	BOS 1	-3		Stable BOS 1 (14 m)
2	DL	Bronch.	M	44	CsA/MMF	10.9	4069	BOS 3	-3		Stable BOS 3 (6 m)
3	SL	HX	F	32	FK/MMF	12.2	2719	BOS 1	+4		Stable BOS 1 (12 m)
4	SL	Emp.	F	58	CsA/AZA	9.8	3045	BOS 2	+2		Stable BOS 2 (12 m)
5	SL	Emp.	F	53	FK	11.6	915	BOS 3	-3		Stable BOS 3 (7 m)
6	SL	Emp.	M	49	FK/MMF	6.8	529	BOS 1	-3		Stable BOS 1 (14 m)
7	SL	Emp.	F	54	FK/MMF	8.4	1211	BOS 1	+3		Stable BOS 1 (14 m)
8	SL	Emp.	M	67	FK/MMF	10.7	3522	BOS 2	-2		Stable BOS 2 (7 m)
9	SL	IPF	M	57	FK/MMF	4.3	939	BOS 1	-4		Stable BOS 1 (4 m)
10	DL	IPF	F	31	CsA/AZA	10.3	2259	BOS 2	-2		Stable BOS 2 (7 m)

TX: transplantation; F_{eNO}: exhaled nitric oxide fraction; ΔFEV1: change in forced expiratory volume in one second (FEV1); SL: single lung; DL: double lung (bilateral); Emp.: emphysema; Bronch.: bronchiectasis; HX: histiocytosis X; IPF: idiopathic pulmonary fibrosis; M: male; F: female; FK: tacrolimus; MMF: mycophenolate mofetil; CsA: cyclosporine A; AZA: azathioprine; ⩾BOS 1: established BOS; m: months. #: on day of F_{eNO} measurement; ¶: final value; +: post-transplantation; §: irreversible decline within 3 weeks before and 6 months after first F_{eNO} measurement expressed as percentage decrease from the highest previous baseline FEV1; ^{f}: administered within the study period following F_{eNO} measurement; ##: at the last follow-up after the first F_{eNO} measurement.

alteration of immunosuppression with pulse steroids, anti-thymocyte globulin and/or cyclosporin A/tacrolimus conversion in the vast majority of patients. In the final patient, already in BOS 3, progressive respiratory failure was observed, with ultimate death due to the progression of BOS. By contrast, patients classified as having stable BOS retained a stable FEV1 within the 6 months following F_{eNO} measurement and did not require alteration of the immunosuppressive regimen during this period.

Taking an F_{eNO} of 15 ppb as the cut-off value, the sensitivity, specificity, PPV and NPV of the mean F_{eNO} for detecting unstable BOS in each patient with BOS were 77, 100, 100 and 83%, respectively. Using the same cut-off value, the sensitivity, specificity, PPV and NPV of each F_{eNO} measurement taken independently for detecting unstable BOS were 69, 89, 81 and 80%, respectively (32 F_{eNO} measurements among 19 patients classified as having stable or unstable BOS).

There was no significant correlation between F_{eNO} and prednisone daily dose (r=-0.18; p=0.2), serum cyclosporin A concentration (r=-0.2; p=0.1) or tacrolimus trough level (r=-0.01; p=0.95). The mean daily dose of steroid in the patients of each group was not significantly different among the three groups (11.7±7.4 (group 1), 8.6±3.0 (group 2) and 9.3±5.9 (group 3) mg·day^{-1}; p=NS).

DISCUSSION
The clinical utility of F_{eNO} measurements in predicting functional outcome in the follow-up of lung transplant recipients with BOS was assessed. It was first observed that F_{eNO} measurements were reproducible in a BOS-free patient with stable pulmonary function over time, owing to a high intraclass correlation coefficient. Hence, measurements of F_{eNO} showed little day-to-day variability when subjects were clinically stable. Compared with stable BOS-free patients, elevated values of F_{eNO} were observed in patients with unstable

BOS. Using an F_{eNO} of 15 ppb as the cut-off value, the mean F_{eNO} showed relatively good specificity (91%) and NPV (93%) for discriminating patients with unstable BOS from stable BOS-free patients; however, there were still three false-positive patients among 34 stable BOS-free patients. These three patients did not develop BOS during follow-up (⩾9 months), were free of respiratory symptoms and had no history of asthma. The present authors can offer no clear explanation for the high F_{eNO} in these three stable BOS-free patients and can only consider that they represent overlap levels.

Among patients with BOS, elevated values of F_{eNO} were observed only in the case of unstable BOS, affecting patients who experienced a decline in lung function in the months following F_{eNO} measurement. By contrast, recipients with stable BOS showed similar F_{eNO} measurements to stable BOS-free recipients. This suggests that F_{eNO} measurements may provide information that is useful in discriminating patients with unstable BOS from those with stable BOS. In addition, the elevated F_{eNO} in patients with unstable BOS were found in both those with early disease (BOS 1) and those with more advanced disease (BOS 2/3). Conversely, the lack of elevated F_{eNO} in patients with stable BOS was observed independently of BOS stage (BOS 1 or BOS 2/3). In other clinical lung transplantation studies, raised F_{eNO} values were found in patients with BOS [12, 13, 15, 20], and, interestingly, F_{eNO} tended to be higher in those with recent clinical deterioration [13, 15, 20] or in patients with early disease, i.e. BOS 1 [12]. In prospective studies of lung transplant recipients free of BOS on inclusion, VERLEDEN and coworkers [15, 20] found increased F_{eNO} values in patients who developed BOS (BOS 0-p or BOS 1), with a decline in FEV1 during follow-up. In a cross-sectional study of lung transplant recipients, F_{eNO} values were increased in the BOS group as a whole, but there was a trend suggesting that the highest levels were found in patients showing recent clinical deterioration [13]. Another

cross-sectional study found an increase in FeNO in patients who had early disease (BOS 1), but this increase was lost in the more advanced stages of the disease, *i.e.* BOS 2/3 [12]. The present findings are in accordance with results from studies showing raised FeNO in lung transplant recipients at the time at which they experienced an irreversible decline in lung function, including patients who are developing BOS [15, 20] or with unstable BOS [13]. Conversely, the present data are in contrast with the study of FISHER *et al.* [12], since the raised FeNO in patients with unstable BOS were observed independently of BOS stage in the present study. In the study of FISHER *et al.* [12], patients were not classified as having stable or unstable BOS, and this fact could account for these conflicting results. In order to explain the observation of raised FeNO only in patients with unstable BOS among those with BOS, it can be hypothesised that successive periods of inflammatory activity occur over time in the airways of patients with BOS, leading to increased iNOS expression and a subsequent increase in exhaled NO levels, which may be downregulated when the inflammatory phase is replaced by fibrosis. This could reflect the different functional patterns already described in these patients [3]. Indeed, rapid decline in FEV1 can be followed by spontaneous or therapeutically mediated stabilisation of lung function during the course of BOS [3]. In this regard, there is no marker that can predict the different functional patterns of BOS over time. The present findings suggest that FeNO may be helpful in both precluding overt immunosuppression in patients with stable BOS and predicting early subsequent loss of lung function in those with unstable BOS, requiring early alteration of immunosuppression in order to hamper the progression of BOS. However, the question arises as to what a physician should do in clinical practice when a previously stable patient with BOS exhibits increased FeNO. Increased FeNO values are also observed in patients with respiratory infections [9], and conflicting data have been reported in lung transplant recipients showing acute rejection [12, 13, 21]. For these reasons, it seems prudent to first exclude such causes of reversible graft failure before attempting to appropriately interpret FeNO.

Measurement of FeNO in SLT recipients may have introduced some bias into the present results, since it can be hypothesised that the native lung has an impact on the measurement of FeNO after SLT. Nevertheless, it has recently been demonstrated that there is no significant difference in FeNO between SLT and BLT recipients, either in the stable condition or during chronic rejection [14]. This suggests that the impact of the native lung is negligible. The present results are concordant with these data, since the transplant procedure (SLT *versus* BLT) did not affect the FeNO, suggesting that, despite this potential concern, the present results are valid.

Since iNOS is very sensitive to corticosteroids, the daily dose of steroid is also a potential source of bias at the time of FeNO measurement. Nevertheless, no significant difference was found in the mean daily dose of steroid among the different groups, and no correlation between FeNO and prednisone dose was observed.

Although FeNO is also increased during infectious episodes, this seems irrelevant in the present study, due to the exclusion of every FeNO measurement from patients with an active infection at the time of measurement [9].

In summary, the present findings suggest that, in lung transplant recipients with established bronchiolitis obliterans syndrome or even potential bronchiolitis obliterans syndrome, raised exhaled nitric oxide fractions seem to indicate the development of worrisome functional impairment during follow-up. Although further studies are required, measurements of exhaled nitric oxide fraction, as a noninvasive marker, may represent a new tool for guiding immunosuppressant therapy in patients with bronchiolitis obliterans syndrome.

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