

Serial monitoring of exhaled nitric oxide in lung transplant recipients



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KEYWORDS:

exhaled nitric oxide;
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infection;
bronchiolitis obliterans
syndrome

BACKGROUND: Exhaled nitric oxide (FeNO), a marker of airway inflammation, is often elevated in lung transplant recipients (LTxRs) with acute rejection or infection. Isolated measurements in the setting of bronchiolitis obliterans syndrome have been variable. We sought to assess the utility of serial FeNO in predicting chronic allograft dysfunction or the presence of acute rejection or infection.

METHODS: Eighty-six LTxRs underwent 325 serial FeNO measurements at an expiratory flow rate of 50 ml/s. The change in FeNO (Δ FeNO) between two measurements obtained during a stable state (Δ FeNO-SS) was compared with Δ FeNO, where the first measurement was taken during a stable state and the second during an unstable state (defined as a subsequent decline in FEV₁ > 10% over 3 months [Δ FeNO-SU]) or an acute complication (acute rejection, lymphocytic bronchiolitis or acute infection [Δ FeNO-SAC]). The median follow-up time after the baseline FeNO was 10 (range 3 to 25) months.

RESULTS: Δ FeNO-SS in 117 FeNO pairs was similar to Δ FeNO-SU in 26 pairs (2.1 ± 3 ppb vs 2.3 ± 4 ppb; $p = 0.2$). Δ FeNO-SAC in 17 pairs was markedly increased (27 ± 20 ppb; $p < 0.001$ vs Δ FeNO-SS). The area under the receiver-operating characteristic curve for Δ FeNO in detecting an acute complication was 0.93 ($p < 0.001$). By applying a cut-off of > 10 ppb, the sensitivity and specificity was 82% and 100%, respectively, with positive and negative predictive values of 100% and 97.5%.

CONCLUSIONS: Changes in FeNO may serve as a useful adjunct in the detection of acute complications after lung transplantation. In this limited analysis, Δ FeNO was not predictive of a subsequent decline in allograft function.

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Lung transplantation (LTx) is an important therapeutic option for patients with end-stage pulmonary disorders.¹ However, chronic lung allograft dysfunction manifesting as bronchiolitis obliterans syndrome (BOS) continues to be highly prevalent and is the leading cause of long-term

mortality after LTx.² Although the pathogenesis of BOS is not fully understood, airway injury is thought to induce an initial inflammatory process that eventually leads to fibrosis and small airway obliteration.^{3,4} Detection of airway inflammation that could precede the development of BOS offers an opportunity for early intervention, such as introduction of azithromycin or other potential treatment strategies.^{5,6} In addition, acute rejection (AR), lymphocytic bronchiolitis (LB) and respiratory infections have been implicated as risk factors for BOS.^{5,7–11} Early diagnosis and treatment of these

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acute events may reduce the incidence of BOS.⁸ Unfortunately, the current diagnostic modalities for early detection of both BOS and its risk factors have some limitations.¹² Thus, a reliable, non-invasive diagnostic biomarker is needed.

Exhaled nitric oxide (the fractional expired concentration, or FeNO) is a standardized and validated technique for assessing airway inflammation and the response to pharmacologic treatment in patients with asthma.^{13–15} In LTx, previous reports suggested elevated FeNO in the setting of acute infections, LB and AR.^{16–18} FeNO appears to be highly variable in subjects with BOS.¹⁹ Most of these studies have been cross-sectional in design with a single measurement. Two longitudinal studies have assessed changes in FeNO and subsequent development of BOS.^{20,21} van Muylem et al found limited diagnostic utility using a high expiratory flow rate for FeNO of 200 ml/s, compared with the American Thoracic Society (ATS) recommended rate of 50 ml/s.²² Neurohr et al reported excellent negative predictive value for the subsequent development of BOS, but limited positive predictive value for a single FeNO value of >20 ppb. Because normal individuals demonstrate a fairly wide range of FeNO,²³ the magnitude of increase may be more informative than an absolute threshold value. Moreover, studies of longitudinal FeNO changes among recipients developing acute complications have been limited. In this study, we hypothesized that the change in FeNO measured serially in lung transplant recipients would predict a subsequent decline in lung function and acute complications.

Methods

Study subjects and FeNO measurements

This study included 86 consecutive bilateral or combined heart–lung LTxRs seen in the outpatient clinic of the Johns Hopkins transplant program during the period from June 2010 to March 2012. The study protocol was approved by the local institutional review board and all subjects gave written informed consent. Basiliximab or daclizimab were given for induction, and immunosuppression was maintained with triple therapy consisting of tacrolimus or cyclosporine, mycophenolate mofetil or azathioprine and corticosteroids.

FeNO was obtained during outpatient visits and/or before surveillance or clinically indicated bronchoscopies according to ATS guidelines using an analyzer (NIOX Flex; Aerocrine), at an expiratory flow rate of 50 ml/s.²² Active smokers, those using inhaled steroids within 2 weeks of the study, and those with bronchial stenosis or symptoms of allergic rhinitis at the time of testing were not included.^{24–27} Sixty-six healthy subjects (non-smokers and without history of asthma) served as a normal control group, providing a single measurement.

Definitions and groups

The clinical status at each FeNO measurement was categorized into 5 different groups, as modified from Neurohr et al²⁰:

Stable non-BOS: BOS Stage 0/0-p,⁴ without any acute complications (AR, LB or infection) within the previous month and <10% decline in forced expiratory volume in 1 second (FEV₁) during the 3 months after FeNO measurement.

Unstable non-BOS: BOS Stage 0/0-p, without any acute complications within the previous month, but a decline of ≥10% in FEV₁ during the next 3 months that was not attributable to an acute complication or other process.

Stable BOS: BOS Stage ≥1, without any acute complications within previous month and <10% decline in FEV₁ during the 3 months after FeNO measurement.

Unstable BOS: BOS Stage ≥1, without any acute complications within the previous month, but an otherwise unexplained decline of ≥10% in FEV₁ during the 3 months after FeNO measurement.

Acute complications group: Diagnosis with AR, LB or an acute respiratory infection within 1 month of the FeNO measurement. AR and LB diagnoses based on transbronchial biopsy findings. Respiratory infection was defined as signs and symptoms, such as new cough, sputum, radiographic abnormality or positive cultures and the addition of a new anti-microbial agent by the treating physician.

Serial FeNO

The change in FeNO between two consecutive measurements (Δ FeNO), where both were taken during a stable state (Δ FeNO-SS), was compared with Δ FeNO in which the first measurement was taken during a stable state and the second during an unstable state (Δ FeNO-SU). Similarly, Δ FeNO-SS was compared with FeNO pairs where the first value was obtained during a stable state and the second taken during an acute complication (Δ FeNO-SAC). A receiver-operating characteristic (ROC) curve was constructed to assess the diagnostic performance of Δ FeNO for the detection of an acute complication.

Statistical analysis

Data are expressed as mean \pm standard deviation, unless otherwise indicated. Comparisons between groups were made with unpaired or paired *t*-tests, as appropriate. Non-normally distributed variables were analyzed by the Mann–Whitney *U*-test. Differences between multiple groups were analyzed by the Kruskal–Wallis test. The coefficient of variation (CV) for FeNO was computed in 36 stable non-BOS subjects who had three FeNO measurements at least 1 month apart.

Results

Subjects' characteristics

A total of 325 FeNO measurements were obtained from 86 patients. Subjects' characteristics are summarized in Table 1. The mean follow-up time after the baseline FeNO measurement was 10.6 \pm 6.2 months (median 10, range 3 to 25 months). Ninety percent (291) of the measurements were eligible to be included under one of the study groups. Thirty-four (10%) met the exclusion criteria. Of these, 24 measurements were excluded because of bronchial stenosis (new/unstable), 6 were excluded because of inhaled steroids use 2 weeks before FeNO measurement, and others were excluded because of allergic rhinitis symptoms at time of measurement. At the beginning of the study, 11 patients were in BOS Stage ≥1, and 2 more recipients, who were BOS-free at baseline, developed BOS by the end of the

Table 1 Baseline Characteristics of Study Subjects

Total number of LTxRs (males/females)	86 (34/52)
Race (black/white/other)	20/62/4
LTx type (bilateral lung/heart–lung)	80/6
LTx reason (COPD/CF/IPF/other)	25/21/9/31
Age (years)	51.2 ± 13.1
Post-operative months (min/max)	39.4 ± 46.9 (1/263)
Best post-operative FEV ₁ (liters)	2.0 ± 0.6
Best post-operative FEV ₁ (% predicted)	73 ± 21.1
Number of measurements/patient	3.8 ± 2.3

CF, cystic fibrosis; COPD, chronic obstructive lung disease; FEV₁, forced expiratory volume in 1 second; IPF, idiopathic pulmonary fibrosis; LTxR, lung transplant recipient.

study. These patients had an overwhelmingly obstructive pattern of chronic allograft dysfunction with only 1 having an FEV₁/forced vital capacity ratio of >0.7, with all ratios being <0.8.²⁸ The remaining patients (73) were BOS-free during the entire follow-up period.

Single FeNO measurements in recipients without an acute complication

FeNO measurements in stable non-BOS subjects were comparable to healthy controls (16 ± 8.2 vs 17.6 ± 7.5 ppb). No significant differences were found between BOS vs non-BOS or stable vs unstable LTxRs without acute complications (Figure 1). Based on these data, we calculated a 78% power to detect a 10-ppb difference between stable and unstable subjects at an alpha level of 0.05. The CV of FeNO among stable non-BOS subjects was 25.5 ± 11.3%.

ΔFeNO in stable and unstable LTxRs

To determine whether a change in FeNO could predict a subsequent decline in lung function, 117 ΔFeNO-SS pairs in 51 patients (46 stable non-BOS and 5 stable BOS) were compared with 26 ΔFeNO-SU pairs in 16 patients with the first measurement taken during a stable state and the second in a non-stable state (12 unstable non-BOS and 4 unstable BOS). No difference was observed (2.1 ± 2.5 ppb vs 2.3 ± 4.3 ppb; *p* = 0.2). Based on these data, we had a >99% power to detect a change in FeNO of 10 ppb in the unstable subjects and >90% power to detect a change of 5 ppb. The mean fall in FEV₁ in the unstable subjects was 16.6 ± 5%. Subsequent pulmonary function was available for review beyond the initial 3-month follow-up window in 14 patients. In 11 of these, FEV₁ had returned to within 10% of the baseline value. For the 2 patients who developed BOS during the course of the study, ΔFeNO was +2.9 ppb and −2.9 ppb, respectively.

FeNO in LTxRs with acute complications

Forty-two FeNO measurements were taken from 27 LTxRs with an acute complication (AR = 9, LB = 5, acute infection = 28). FeNO was measured before any treatment

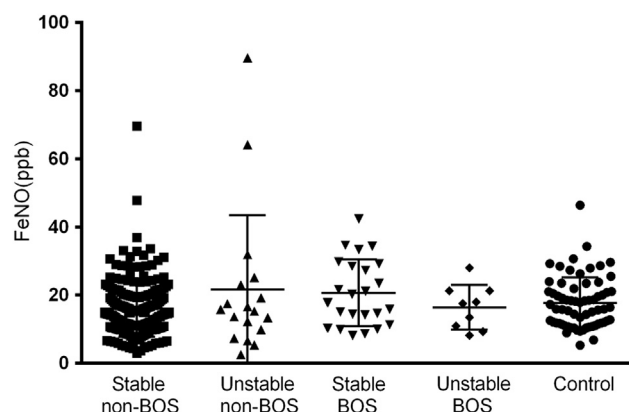


Figure 1 FeNO levels in lung transplant recipients and healthy control subjects. Stable non-BOS: *n* = 198; unstable non-BOS: *n* = 18; stable BOS: *n* = 12; unstable BOS: *n* = 9; control subjects: *n* = 66. Data presented as mean and standard deviation. *p* > 0.05.

in 21 of these episodes in 16 subjects. In all these cases, testing occurred within 2 days of the diagnosis. FeNO during untreated acute complications increased significantly compared with the stable non-BOS group (40.9 ± 28.7 vs 16 ± 8.2 ppb; *p* < 0.001). Those patients studied after therapy for an acute complication had levels comparable (16.7 ± 10.5 ppb; *p* = 0.8) to those of stable non-BOS patients.

ΔFeNO and acute complications

Seventeen FeNO pairs were obtained from 13 subjects, where the first measurement was during a stable state and the second during an untreated acute complication (AR = 2, LB = 3, acute infection = 12). As shown in Figure 2, ΔFeNO-SAC (26.9 ± 19.6 ppb) was dramatically increased in this group compared with essentially no change observed in ΔFeNO-SS (*p* < 0.001). Details of these 17 acute complication episodes are presented in Table 2. FeNO was repeated after therapy for 9 episodes. As demonstrated in Figure 3, values fell considerably after treatment (50 ± 24 vs 22 ± 7 ppb; *p* = 0.01). Accompanying the ΔFeNO reduction was an improvement in FEV₁ after therapy of 0.2 ± 0.2 liters (*p* = 0.02).

Diagnostic utility of change in FeNO for detection of acute complication

The area under the ROC curve (Figure 4) for ΔFeNO in detecting an acute complication was quite high at 0.93 (*p* < 0.001). By applying the optimal cut-off of ≥10 ppb, we calculated a sensitivity of 82.3% and a specificity of 100%. The positive and negative predictive values were 100% and 97.5%, respectively.

Discussion

The main finding of this study is the robust rise in serial FeNO during acute complications (acute rejection, lymphocytic bronchiolitis and acute infection). Although previous

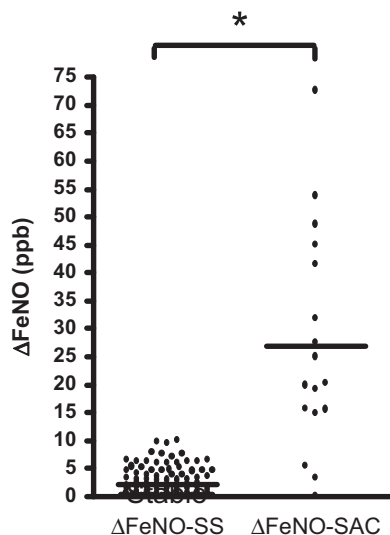


Figure 2 Δ FeNO in stable LTxRs and untreated acute complications. Dot plots show the change in FeNO between two consecutive measurements taken during a stable state (Δ FeNO-SS) and where the first measure was during a stable state and second during an untreated acute complication (Δ FeNO-SAC). * $p < 0.001$.

reports have shown elevated values during these episodes compared with stable LTxR, this is the first longitudinal study to describe the operating characteristics of a change in FeNO for detection of these episodes. Given that such events are established risk factors for BOS, exhaled NO may be a useful adjunct in the monitoring of LTxR.

Several cross-sectional studies have demonstrated increased FeNO in LTxRs with lymphocytic bronchiolitis and acute infection, suggesting a role in identifying airway inflammation.^{17,18} De Soyza et al showed an increase in FeNO (at an expiratory flow rate of 250 ml/s) in 14 LTxRs who developed LB, and a subsequent return to baseline after inhaled steroid treatment.²⁴ FeNO values during AR have been varied.^{17,18} We observed only 2 cases of AR, 1 with a B grade that could not be ascertained. A longitudinal study by Antus et al showed increased levels of FeNO in LTxRs during acute infection. However, they investigated a relatively small number of LTxRs and included both upper and lower respiratory infection cases. FeNO has been correlated with bronchoalveolar lavage neutrophilia and epithelial inducible nitric oxide synthase (iNOS) expression in LTxRs.²⁹ Moreover, FeNO is an established marker of airway inflammation in asthma. Interestingly, the optimal cut-point of ≥ 10 ppb for a change

Table 2 Characteristics of Recipients With Serial FeNO During Acute Complications

LTxR no.	Reason for LTx	POD (months)	BOS stage	Baseline FEV ₁ (%)	FEV ₁ (%) at Dx	Acute complication	Radiologic findings	BAL Biopsy	culture	Rx	Δ FeNO	Δ FeNO after Rx
1	A1AD	9.6	0	4.4 (93.4)	3.2 (84.2)	AR	None	A2B0	None	ST	15.45	-9.8
2	CF	14.7	0-p	3.6 (76.7)	2.9 (74.7)	AR	None	A2Bx	None	ST	19.00	-5
3	PH	44.4	0	1.7 (58.8)	1.5 (53.6)	Infection, Abx treated	None	N/A	N/A	Abx	20.20	-18.2
4	COPD	31.4	0-p	2 (69.1)	1 (42.5)	Infection, Abx treated	None	N/A	N/A	Abx	27.55	-22.6
5	CF	148.9	2	3.1 (105)	1.6 (67)	Infection, Abx treated	None	N/A	N/A	Abx	31.80	-21.1
6	CF	10.6	0-p	3.6 (77.7)	2.9 (75.9)	Infection, Abx treated	New infiltrate	A0B0	PA	Abx	53.70	-55.8
7	CF	13.1	0-p	3.6 (77.7)	2.9 (75.9)	Infection, Abx treated	New infiltrate	A0B0	PA	Abx	72.55	-74.6
8	CHD	48.4	0	3.7 (84.8)	2.9 (78.4)	Infection, Abx treated	None	N/A	N/A	Abx	48.60	N/A
9	PH	72.2	0	3.5 (85.2)	2.5 (75.5)	Infection, Abx treated	None	N/A	N/A	Abx	3.15	N/A
10	PH	31.1	1	1.9 (72.6)	1.3 (59)	Infection, Abx treated	None	A0Bx	None	Abx	5.40	N/A
11	IPF	12.6	1	3.5 (103)	1.9 (75.2)	Infection, other Rx	None	A0B0	MPV	IVIg	19.75	-19
12	CF	143.8	0-p	3 (103)	1.6 (65.6)	Infection, other Rx	None	A0B0	RSV	IVIg	45.00	-54.5
13	PH	96.1	0-p	3.2 (94.9)	2.1 (72.5)	Infection, other Rx	None	A0B0	ASP	AF	41.35	N/A
14	IPF	3.2	0	2.2 (69.5)	1.8 (61)	Infection, other Rx	New infiltrate	A0B0	RSV	IVIg	0.00	N/A
15	COPD	48.9	0	2.5 (79)	1.2 (51.6)	LB	None	LB	None	ST	14.85	N/A
16	CF	15.1	0	3.3 (95.9)	1.9 (65.6)	LB	None	LB	None	ST	24.90	N/A
17	CF	17.2	0	3.3 (95.9)	1.9 (65.6)	LB	None	LB	None	ST	15.50	N/A

A1AD, α_1 -anti-trypsin deficiency; Abx, antibiotics; AF, anti-fungal; ASP, aspergillosis; BAL, bronchoalveolar lavage; BOS, bronchiolitis obliterans syndrome; CF, cystic fibrosis; CHD, congenital heart disease; COPD, chronic obstructive pulmonary disease; Dx, diagnosis; FEV₁, forced expiratory volume in 1 second (liters/% predicted); IPF, idiopathic pulmonary fibrosis; IVIg, intravenous immunoglobulin; MPV, metapneumovirus; N/A, not applicable; PA, *Pseudomonas aeruginosa*; PH, pulmonary hypertension; POD, post-operative day; RSV, respiratory syncytial virus; Rx, therapy; ST, steroids.

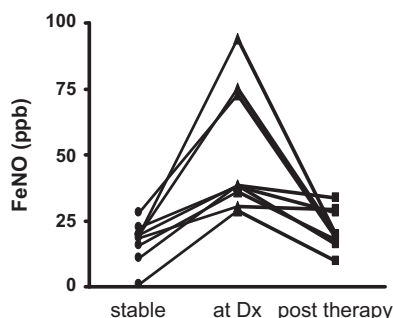


Figure 3 Serial individual FeNO values in LTxRs with an acute complication during stable state, at the time of diagnosis (Dx), and after therapy. $p = 0.01$.

in FeNO to detect an acute complication in our study is identical to the increase suggested as a significant change in asthma in a recent consensus statement.¹³

If airway inflammation precedes BOS, FeNO could be a useful predictor. Previous studies showed higher FeNO in early¹⁷ and unstable BOS,¹⁹ but not advanced stages of disease without an active decline in lung function, suggesting that occult airway inflammation precedes fibrous obliteration. Normal individuals have wide-ranging FeNO levels.²³ Hence, serial measurements are likely to provide more information compared with a single test. In a longitudinal study of 65 patients, van Muyhem et al found a small rise in FeNO as LTxR progressed from BOS Stage 0 to 0-p, but no change between Stages 0 and 1.²¹ Similarly, Verleden et al demonstrated an increase in FeNO at early stages of BOS.³⁰ However, both studies measured FeNO at an expiratory flow rate of 200 ml/s. FeNO falls exponentially with higher flow rates, potentially diluting changes induced by airway inflammation. We measured FeNO at the ATS recommended rate of 50 ml/s.²² Neurohr et al,²⁰ using a rate of 50 ml/s, demonstrated higher FeNO values among LTxRs who subsequently developed new or worsening BOS (defined as unstable). Furthermore, a single FeNO measurement of ≥ 20 ppb had a positive and negative predictive value of 69% and 97%, respectively, for a subsequent unstable course. With serial testing, unstable patients had greater variability in FeNO compared with stable subjects. However, the diagnostic performance characteristics of a change in FeNO were not reported.

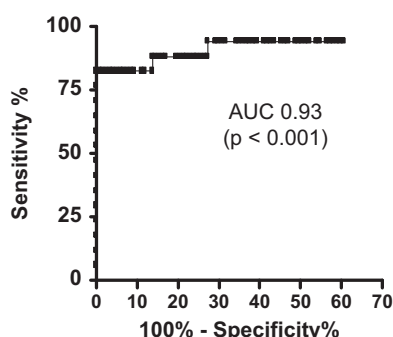


Figure 4 Receiver-operating characteristic (ROC) curve for Δ FeNO to detect an acute complication.

We did not detect a difference in single FeNO values between non-BOS and BOS, consistent with previous data,³¹ although the number of BOS patients was small. We also could not detect a difference in FeNO between stable vs unstable LTxRs, defined as a subsequent 10% fall in FEV₁. Thus, we were unable to duplicate the results of Neurohr et al.²⁰ Although our study had about half the number of subjects, and was somewhat underpowered to detect a difference in single FeNO values, others described a similar experience.³¹ Moreover, we did not detect a change in serial measurements between stable and unstable clinical states and did have sufficient power to detect such a change. It is possible that a large proportion of patients develop fibroproliferative airways disease without a preceding inflammatory phase. Alternatively, inflammation may be transient and not detected during periodic FeNO testing. A major limitation to this observation is the subsequent recovery of lung function in many of the unstable patients.

Although the number of acute complication episodes in this study is small, the Δ FeNO data suggest that this simple, non-invasive, inexpensive test could serve as a useful adjunct in the monitoring of LTxRs. An increase in 10 ppb was both highly sensitive and specific for acute complication. The high negative predictive value suggests that patients with a decline in lung function, but with a <10 -ppb rise in FeNO, could be safely observed, whereas those with an increase of ≥ 10 ppb would require additional investigation, including bronchoscopy. Additional studies are required to confirm these findings and determine whether isolated acute vascular rejection is also associated with a rise in FeNO.

Small, portable nitric oxide analyzers are being utilized more frequently in the clinical outpatient setting.³² Hand-held devices suitable for home use are in development, making daily monitoring potentially feasible.^{33,34} Using such devices, future studies could determine whether FeNO does indeed predict development of BOS and its utility in detecting acute complications.

Disclosure statement

The authors have no conflicts of interest to disclose.

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