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Serial monitoring of exhaled nitric oxide in lung transplant recipients

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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 (ΔFeNO) between two measurements obtained during a stable state (ΔFeNO-SS) was compared with ΔFeNO, where the first measurement was taken during a stable state and the second during an unstable state (defined as a subsequent decline in $FEV_1 > 10\%$ over 3 months [ΔFeNO-SU]) or an acute complication (acute rejection, lymphocytic bronchiolitis or acute infection [ΔFeNO-SAC]). The median follow-up time after the baseline FeNO was 10 (range 3 to 25) months.

RESULTS: ΔFeNO-SS in 117 FeNO pairs was similar to ΔFeNO-SU in 26 pairs (2.1 ± 3 ppb vs 2.3 ± 4 ppb; $p = 0.2$). ΔFeNO-SAC in 17 pairs was markedly increased (27 ± 20 ppb; $p < 0.001$ vs ΔFeNO-SS). The area under the receiver-operating characteristic curve for ΔFeNO 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, ΔFeNO 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 dacluzimab 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 al[20]:

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 (FEV_1) 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 FEV_1 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 FEV_1 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 FEV_1 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 (ΔFeNO), where both were taken during a stable state (ΔFeNO-SS), was compared with ΔFeNO in which the first measurement was taken during a stable state and the second during an unstable state (ΔFeNO-SU). Similarly, ΔFeNO-SS was compared with FeNO pairs where the first value was obtained during a stable state and the second taken during an acute complication (ΔFeNO-SAC). A receiver-operating characteristic (ROC) curve was constructed to assess the diagnostic performance of ΔFeNO for the detection of an acute complication.

Statistical analysis

Data are expressed as mean ± 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 ± 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