Exhaled nitric oxide in sarcoidosis

M L Wilsher, W Fergusson, D Milne, A U Wells

Background: Increased production of nitric oxide (NO) by the lower respiratory tract is viewed as a marker of airway inflammation in asthma and bronchiectasis. NO is a potentially important immune modulator, inhibiting the release of several key pro-inflammatory cytokines. As sarcoidosis is characterised by granulomatous airway inflammation, we hypothesised that exhaled NO levels might be raised in sarcoidosis and correlate with the morphological extent and functional severity of disease.

Methods: Fifty two patients with sarcoidosis (29 men) of mean age 42 years underwent thin section computed tomography (CT), pulmonary function tests, and measurement of exhaled NO.

Results: Exhaled NO levels (median 6.8 ppb, range 2.4–21.8) did not differ significantly from values in 44 control subjects, and were not related to the extent of individual CT abnormalities or the level of pulmonary function impairment.

Conclusion: Exhaled NO levels are not increased in pulmonary sarcoidosis.

Exhaled nitric oxide measurements

Nitric oxide was measured with a modified chemiluminescence analyser (model LR2000; Logan Research, Rochester, UK) sensitive to NO from 1 to 5000 ppb (by volume), and a resolution of 1 ppb which had been designed for online recording of the eNO concentration. In addition to NO, the analyser also measured CO2 (resolution of 0.1%, CO2 response time of 200 ms). When not in use the system sampled charcoal scrubbed air which was free of NO and the machine was not exposed to ambient air. Calibration was checked weekly using a standard gas at 100 ppb NO. All measurements were undertaken at ambient NO levels of <20 ppb.

Subjects were studied in the sitting position. Exhaled NO was measured during slow exhalation from total lung capacity to residual volume. The exhalation flow rate was 15 l/min and was kept as constant as possible by using a biofeedback visual display. Expired air was sampled via a side arm tube (at 250 ml/min) directly into the analyser for NO measurement. The plateau level of the last part of exhalation was measured during slow exhalation from total lung capacity to residual volume. The exhalation flow rate was 15 l/min and was kept as constant as possible by using a biofeedback visual display. Expired air was sampled via a side arm tube (at 250 ml/min) directly into the analyser for NO measurement.
To measure nasal NO a wide bore Teflon coated catheter was placed in the nares (front of nose) and a sample aspirated at 500 ml/min during breath holding at the end of inspiration. The plateau NO level was taken as the level of nasal NO. Continued soft palate closure was confirmed by the absence of an increase in CO₂ during sampling. Measurements of NO were performed with no technician awareness of previous recordings.

Data from our laboratory have shown a coefficient of variation of 12.7% derived from the single determination standard deviation of measurements taken on separate days.¹

**Lung function measurement**

The following lung function measurements (lung volumes and flow measurements) were undertaken in the Lung Function Laboratory at Green Lane Hospital, according to American Thoracic Society (ATS) standards¹⁰ by a qualified technician using a 6200 Autobox DL (Sensormedics, Yorba Linda, USA): FEV₁, forced vital capacity (FVC), functional residual capacity (FRC), residual volume (RV), total lung capacity (TLC), mean forced expiratory flow during the residual capacity (FRC), residual volume (RV), total lung capacity (TLC), mean forced expiratory flow during the middle half of the FVC (FEF₂₅₋₇₅) and carbon monoxide lung transfer factor (TLCO). The European Community Coal and Steel recommendations¹¹ were used as reference normal values. All measurements were undertaken on the same day as the eNO measurement and within a month of the CT scan.

**CT scanning**

Thin section CT studies were performed using a General Electric Prospeed Advantage CT scanner. One millimetre collimation scans were performed at 10 mm intervals at full inspiration. Limited further interspaced sections were performed at end expiration. The CT scans were assessed in random order by two independent experienced observers (DM and AUW) without knowledge of clinical findings or awareness of previous recordings. The extent of individual CT patterns was estimated in each lobe as follows: grade 1 = 0–25% of the lobe; grade 2 = 26–50% of the lobe; grade 3 = 51–75% of the lobe; grade 4 = >75% of the lobe. CT patterns quantified in this way were: (1) mosaic attenuation (scored using inspiratory images); (2) ground glass opacification; (3) nodules up to 8 mm in diameter; (4) consolidation; and (5) a reticular pattern (interlacing linear opacities, including thickened interlobular septa) or honeycombing. The total extent of each pattern in each patient was determined by adding the scores in the six lobes, providing a 25 point scale (0–24). Bronchial wall thickening and bronchiectasis were also quantified using a four point scale (0–3) in each lobe, and thus a 19 point scale (0–18)². The summed scores of the two observers were used for the analysis.

**Statistical analysis**

Group data were expressed as mean (SD) values or median (range) values for non-normally distributed variables. Univariate correlations between functional indices and eNO levels and between CT patterns and eNO levels were examined using Spearman rank correlation test (STATA data analysis software; Computing Resource Center, Santa Monica, CA, USA). Comparisons of paired data were made using the Wilcoxon’s rank sum test. Multivariate relationships between (1) individual lung function indices and CT scores and (2) eNO levels and CT scores were examined using stepwise linear regression. Individual functional indices and eNO were evaluated as dependent variables in separate models. Covariates consisted of the CT scores listed in table 1 (with the exception of bronchiectasis scores, positive in only eight patients). All models satisfied the parametric assumptions of multiple linear regression as judged by testing for heteroscedasticity.

**RESULTS**

As shown in fig 1, eNO levels (median 6.8 ppb, range 2.4–21.8) did not differ significantly from eNO levels in 44 normal subjects (median 6.3 ppb, range 1.6–28.0). There was no correlation between eNO levels and the duration of disease (time since diagnosis). There was a trend to higher eNO levels in the 12 atopic patients (median eNO 10.7 ppb (range 3.4–21.8), fig 2). There was no correlation between eNO levels and total serum IgE (r = −0.12, p = 0.53).

Pulmonary function abnormalities, expressed as mean (SD) percentages of normal predicted values, varied from severe obstruction to restriction, with neither predominating overall (FEV₁ 87.9 (18.5); FVC 93.7 (16.1); FEF₂₅₋₇₅ 64.1

**Table 1 Prevalence of CT abnormalities in 52 patients with sarcoidosis**

<table>
<thead>
<tr>
<th>CT score</th>
<th>Median (range)</th>
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<tbody>
<tr>
<td>Nodules (n = 42)</td>
<td>10 (2–27)</td>
</tr>
<tr>
<td>Reticular abnormalities (n = 37)</td>
<td>6 (2–30)</td>
</tr>
<tr>
<td>Consolidation (n = 30)</td>
<td>6 (2–18)</td>
</tr>
<tr>
<td>Ground glass attenuation (n = 25)</td>
<td>2 (2–8)</td>
</tr>
<tr>
<td>Mosaic attenuation (n = 45)</td>
<td>10 (2–24)</td>
</tr>
<tr>
<td>Bronchial wall thickness (n = 29)</td>
<td>6 (2–14)</td>
</tr>
<tr>
<td>Bronchiectasis (n = 8)</td>
<td>3 (2–11)</td>
</tr>
</tbody>
</table>

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*Figure 1* Exhaled nitric oxide levels in 52 patients with sarcoidosis and 44 normal non-smoking controls.

*Figure 2* Exhaled nitric oxide levels in 12 atopic and 19 non-atopic patients with sarcoidosis.
(25.0); TLC 97.7 (13.3); RV 91.7 (26.1); TLC 85.5 (18.9); Kco 104.5 (18.4)). The prevalence of individual CT abnormalities and the median CT scores with ranges are shown in table 1.

Before examination of the relationship between eNO levels and disease severity, key functional morphological relationships were identified. The strongest univariate associations, all involving FEV\(_1\) or FEV\(_{25–75}\), are shown in table 2.

On stepwise regression, the percentage predicted FEV\(_{25–75}\) (R\(^2\) = 0.62) and FEV\(_1\) (R\(^2\) = 0.56) were much more closely related to morphological abnormalities than other functional variables (TLC, R\(^2\) = 0.29; FVC, R\(^2\) = 0.27; other variables, R\(^2\) < 0.20). Reductions in FEV\(_{25–75}\) were independently associated with increasing reticular abnormalities (regression coefficient (RC) = −1.09; 95% confidence interval CI) −1.81 to −0.36; p = 0.003), consolidation (RC = −2.10; 95% CI −3.22 to −0.97; p < 0.001), mosaic attenuation (RC = −1.39; 95% CI −2.16 to −0.63; p = 0.001), and bronchial wall thickness (RC = −2.09; 95% CI −3.33 to −0.84; p < 0.001). Reductions in FEV\(_1\) were independently associated with increasing reticular abnormalities (RC = −0.90; 95% CI −1.46 to −0.33; p = 0.002), consolidation (RC = −1.25; 95% CI −2.06 to −0.43; p = 0.002), and mosaic attenuation (RC = −0.89; 95% CI −1.49 to −0.28; p = 0.004).

On univariate analysis, eNO levels were not linked to individual pulmonary function variables or CT scores. On stepwise regression, no independent relationships were found between eNO levels and individual CT scores.

**DISCUSSION**

This study did not find an increase in eNO levels in sarcoidosis nor any relationship between eNO and the morphological extent or functional severity of disease. The data suggest that, as in other contexts, eNO levels may be higher in a subset of patients with atopy,\(^\text{13}\) but there is no evidence that sarcoidosis per se contributes to an increase in eNO. Our study did confirm our earlier observation that, in sarcoidosis, FEV\(_{25–75}\) and FEV\(_1\) correlate better with morphological abnormalities than other functional variables.\(^\text{14}\) However, no particular morphological pattern correlated with eNO levels.

Our results are at odds with the findings of Moodley et al\(^\text{8}\) who reported that a cohort of 12 patients with newly diagnosed sarcoidosis had higher eNO levels than 21 normal subjects. There was, in fact, no difference in eNO levels between newly diagnosed and follow up patients in our cohort. It is possible that coexistent atopy confounded the results in the study by Moodley et al and, in this regard, the decrease in eNO in eight of their patients who were treated with corticosteroids may reflect modification of atopy rather than sarcoidosis.

Exhaled NO levels are variably raised in other interstitial diseases. Higher eNO levels were reported in 47 patients with scleroderma, particularly with associated interstitial lung disease.\(^\text{15}\) In a smaller study by Paredi et al,\(^\text{16}\) eNO levels were likewise higher in patients with scleroderma-associated fibrosing alveolitis (n = 17) and lone cryptogenic fibrosing alveolitis (CFA, n = 11) compared with 13 non-smoking controls. In this study the eNO levels were higher in the untreated patients and correlated with bronchoalveolar lavage (BAL) fluid lymphocyte counts. The authors postulated that the initial activation of lung inflammatory cells stimulated the production of inducible nitric oxide synthase (iNOS) which, in turn, increased production of peroxynitrite thus perpetuating the inflammatory response, citing the work of Saleh et al who demonstrated such in CFA.\(^\text{17}\)

A study by Lakari et al\(^\text{18}\) has shown that eNO is increased in patients with scleroderma without interstitial lung disease but with increased BAL fluid cellularity. In that study, however, the patients with interstitial lung disease had similar eNO levels to normal controls. The expression of iNOS is reportedly increased in inflammatory but not fibrotic lesions in a number of interstitial lung diseases, most prominently in the granulomas of extrinsic allergic alveolitis and sarcoidosis.\(^\text{19}\) This suggests that active granulomatous inflammation may result in increased production of NO. However, in our study, patients with newly diagnosed disease were no more likely to have increased eNO levels than those under long term follow up. The data would, however, be in keeping with the observations of Moodley et al as all of their patients had acute constitutional symptoms associated with acute sarcoidosis.\(^\text{4}\) Relatively small numbers and confounding by coexistent atopy may in part explain the discrepant findings of these studies.

In conclusion, we have shown that eNO is not increased in patients with sarcoidosis although atopic patients may have raised levels. These data do not support the use of eNO to monitor disease progression in sarcoidosis as the levels do not correlate with either the HRCT appearances or the lung function tests.

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Competing interests: none declared

**REFERENCES**


**Table 2** Most powerful functional morphological univariate relationships (expressed as Spearman rank correlation coefficients) between pulmonary function indices and CT scores

<table>
<thead>
<tr>
<th>FEV(_1) [% predicted]</th>
<th>Reticular score</th>
<th>Consolidation score</th>
<th>Mosaic attenuation score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.59 (&lt;0.0005)</td>
<td>R = −0.59</td>
<td>R = −0.61</td>
<td>R = −0.37 (&lt;0.01)</td>
</tr>
<tr>
<td>0.56 (&lt;0.0005)</td>
<td>R = −0.61</td>
<td>R = −0.58</td>
<td>R = −0.47 (&lt;0.0005)</td>
</tr>
</tbody>
</table>

Increasing reticular abnormalities, consolidation, and mosaic attenuation were all associated with reductions in FEV\(_1\) and FEV\(_{25–75}\).
Does antibiotic treatment increase bacterial virulence in cystic fibrosis?

Biofilm formation is an important bacterial protective mechanism that appears in the environment and clinical practice. The cystic fibrosis (CF) lung is a common model used to investigate biofilms. Recent data suggest that treatments used in CF may in part contribute to the phenotype.

This study reports that very low concentrations of aminoglycosides (including tobramycin, amikacin and streptomycin) induce biofilm formation in Pseudomonas strains, with tobramycin the most active. Thirteen of 14 CF isolates tested formed tobramycin induced biofilms at low concentrations of this antibiotic. Biofilms were not induced by the cationic antimicrobial peptide polymyxin B, although polymyxin E (colistin) was not assessed. The induction of biofilm was not dependent on the presence of bacterial outer membrane properties such as flagellin, pili, or the alginate gene responsible for “mucoidy”.

A transposon induced bank of mutant Pseudomonas strains allowed the identification of the gene responsible for tobramycin induced biofilms. The arr gene (aminoglycoside response regulator) was found to be conserved and had cyclic di-GMP (c-di-GMP) phosphodiesterase activity. Exogenous GTP (a c-di-GMP antagonist) inhibited aminoglycoside induced biofilm formation. Notably, biofilms of arr mutants were 100 times more sensitive to tobramycin in vitro. Given recent data that nebulised aminoglycoside treatment in the short term improves lung function, these new findings need to be interpreted with caution. The availability of a number of PDE inhibitors including dipyridamole and sildenafil have yet to be tested against the arr gene product and may offer a new therapeutic target.

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