

EXHALED NITRIC OXIDE IN SARCOIDOSIS

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ABSTRACT

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 elevated in sarcoidosis and correlate with morphologic extent and functional severity of disease. Fifty-two patients with sarcoidosis (29 male, mean age 42) underwent thin section computed tomography (CT), pulmonary function tests, and measurement of exhaled NO. 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. These results demonstrate that exhaled NO levels are not increased in pulmonary sarcoidosis.

INTRODUCTION

Sarcoidosis is a multi-system granulomatous disorder characterised by the accumulation of activated T lymphocytes releasing the TH1 cytokines IL-2 and IFN- γ at sites of inflammation and granuloma formation (1). The disease is believed to result from IL-12 dysregulation, with perpetuation of a TH1 response to an irrelevant antigen, although another cytokine, IL-18, is also implicated in pathogenesis (2).

Nitric oxide (NO) is a regulator of many biological functions, including T helper 1 (TH1) and T helper 2 (TH2) balance (3), and has many roles in cell signalling and host defence. It is involved in the killing or inhibition of pathogens associated with TH1 type immune responses, such as *Mycobacterium tuberculosis* (4). Exhaled NO levels, a marker of NO production in the respiratory tract, correlate with airway inflammation, especially in asthma (5,6). As NO may have anti-inflammatory and pro-inflammatory effects and sarcoidosis is characterised by granulomatous airway inflammation, we hypothesised that eNO levels would be elevated in this disease and would correlate with disease severity, as judged by lung function tests and HRCT.

Exhaled NO levels have been reported in only two studies of patients with sarcoidosis and these have shown conflicting results. O'Donnell *et al* showed that eNO was not elevated in 10 patients with sarcoidosis (7), but in contrast Moodley *et al* reported the opposite in a cohort of 12 patients(8). Neither study correlated the eNO levels with HRCT appearances or detailed lung function testing although Moody *et al* did use FEV₁. In this study we have examined eNO in a cohort of 52 patients with sarcoidosis, correlating levels with detailed lung function testing and HRCT.

METHODS

Subjects

The study subjects comprised 52 patients (29 male, mean age 42 (range 23-66), with sarcoidosis diagnosed on the basis of typical histology or unequivocal HRCT findings in association with typical clinical features. All patients were from the Auckland region and the ethnicity was as follows: Caucasian; 41, Indian; 5, Polynesian; 4, Asian; 1, African; 1. Seven patients were current smokers and there were five ex-smokers. No patient was on treatment (oral or inhaled) at the time of study or in the preceding 3 months. Fifteen patients had the exhaled NO measurements made at initial presentation and 37 during follow-up as part of routine lung function testing. CXR abnormalities were as follows: stage 0 Stillbach; 3, stage I; 13, stage II; 21, stage III; 14, stage IV; 1. Fifteen patients (29%) had extrapulmonary manifestations of their disease at some stage during their illness. Thirty-one patients also had assessment of atopy, defined as one or more positive serum RAST responses to 4 common aeroallergens, as part of another study. The data were compared with that from 44 normal non-smoking volunteers recruited as part of an earlier study (9).

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 on-line recording of exhaled NO concentration. In addition to NO, the analyser also measured CO₂ (resolution of 0.1%, CO₂ response time of 200 milliseconds). When not in use the system sampled charcoal scrubbed air which was free of nitric oxide and the machine was not exposed to ambient air. Calibration was checked weekly using a standard gas at 100ppb NO. All measurements were undertaken at ambient NO levels of < 20ppb.

Subjects were studied in the sitting position. Exhaled NO was measured during slow exhalation from total lung capacity to residual volume. Exhalation flow rate was 15L/min and was kept as constant as possible by using a biofeedback visual display. Expired air was sampled via a side arm tube (at 250ml.min⁻¹) directly into the analyser for NO measurement. The plateau level of the last part of exhalation, when CO₂ was 70-80% of maximum was taken as the expired NO concentration. To measure nasal NO, a wide bore Teflon coated catheter was placed in the nares (front of nose) and a sample aspirated at 500ml.min⁻¹ 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 has shown a co-efficient of variation of 12.7% derived from the single determination standard deviation of measurements taken on separate days (9).

Lung Function Measurement

The following lung function measurements (lung volumes and flow measurements) were under-taken in the Lung Function Laboratory at Green Lane Hospital according to American Thoracic Society (ATS) standards (10) by a qualified technician using a 6200 Autobox DL (SensorMedics, Yorba Linda, USA): FEV₁, FVC, FRC, RV, TLC, MEF₂₅₋₇₅ and DLCO. The European Community Coal and Steel Recommendations (11) were used as reference normal values. All measurements were undertaken the same day as exhaled NO measurement and within a month of the CT scan.

CT

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 lung function tests. The extent of individual CT patterns was estimated in each lobe as follows: grade 1 = $\leq 25\%$ 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: (a) mosaic attenuation (scored using expiratory images); (b) ground glass opacification; (c) nodules up to 8mm in diameter; (d) consolidation; and (e) a reticular pattern (i.e. 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 was also quantified, using a four point scale (0-3) in each lobe, and thus a 19 point scale (0-18) (12). The summed scores of the two observers were used for the analysis.

Statistical Analysis

Group data were expressed as mean values (SDs) or median values with ranges for non-normally distributed variables. Univariate correlations between functional indices and eNO levels, and CT patterns and eNO levels were examined using Spearman rank correlation test (STATA data analysis software; Computing Resource Center, Santa Monica, Calif). Comparisons of paired data were made using the Wilcoxon's rank sum test. Multivariate relationships between a) individual lung function indices and CT scores: and b) 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 8 patients). All models satisfied the parametric assumptions of multiple linear regression, as judged by testing for heteroscedasticity.

RESULTS

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

Pulmonary function abnormalities, expressed as 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 ± 25.0 ; TLC 97.7 ± 13.3 ; RV 91.7 ± 26.1 ; DLco 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*.



ENO Table 1.doc

Before examination of the relationship between eNO levels and disease severity, key functional morphologic relationships were identified. The strongest univariate associations, all involving FEV1 or FEF25-75, are shown in *Table 2*.



ENO Table 2.doc

On stepwise regression, the percentage predicted FEF25-75 ($R^2 = 0.62$) and FEV1 ($R^2 = 0.56$) were much more closely related to morphologic abnormalities than other functional variables (DLco, $R^2 = 0.29$; FVC, $R^2 = 0.27$; other variables, $R^2 < 0.20$). Reductions in FEF25-75 were independently associated with increasing reticular abnormalities (regression coefficient (RC) = -1.09; 95% confidence intervals (CI) = -1.81, -0.36; p=0.003), consolidation (RC = -2.10; CI = -3.22, -0.97; p<0.001), mosaic attenuation (RC = -1.39; CI = -2.16, -0.63; p<0.001) and bronchial wall thickness (RC = -2.09; CI = -3.33, -0.84; p<0.001). Reductions in FEV1 were independently associated with increasing reticular abnormalities (RC = -0.90; CI = -1.46, -0.33; p=0.002) consolidation (RC = -1.25; CI = -2.06, -0.43; p=0.002) and mosaic attenuation (RC = -0.89; CI = -1.49, -0.28; p=0.004).

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

DISCUSSION

This study has not demonstrated an increase in eNO levels in sarcoidosis, nor any relationship between eNO and the morphologic extent or functional severity of disease. The data suggest that, as in other contexts, eNO levels may be higher in a sub-set with atopy (13), but there is no evidence that sarcoidosis per se contributes to elevation of eNO. Thus, our findings confirm the observations of O'Donnell *et al*, but in a much larger patient cohort and using our own population normal values for eNO. Our study did confirm our earlier observation that in sarcoidosis FEF25-75 and FEV1 correlate better with morphologic abnormalities than other functional variables (14). However no particular morphologic pattern correlated with eNO levels.

Our results are at odds with the report from Moodley *et al*. 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 co-existent atopy confounded the results in Moodley's study and in this regard the decrease in eNO in 8 of their patients who were treated with corticosteroids may reflect modification of atopy rather than sarcoidosis.

Exhaled NO levels are variably elevated in other interstitial diseases. ENO has been reported to be higher in 47 patients with scleroderma particularly with associated interstitial lung disease (15). In a smaller study ENO was likewise higher in patients with scleroderma associated fibrosing alveolitis (n=17) and lone CFA (n=11) compared with 13 non-smoking controls (16). In this study the eNO levels were higher in the untreated patients and correlated with BAL lymphocyte counts. The authors postulated that the initial activation of lung inflammatory cells stimulated the production of iNOS which in turn increased production of peroxynitrite thus perpetuating the inflammatory response, citing the work of Saleh *et al* who demonstrated such *ex vivo* in CFA (17). A further study has shown that eNO is increased in patients with scleroderma without interstitial lung disease but with increased broncho-alveolar lavage cellularity (18). In that study however the patients with interstitial lung disease had similar eNO levels to normal controls. The expression of inducible nitric oxide synthase (iNOS) is reportedly increased in inflammatory but not fibrotic lesions of a variety of ILDs most prominently in the granulomas of extrinsic allergic alveolitis and sarcoidosis (18). 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 than those under long term follow-up. The data would however be in keeping with Moodley's observations as all of their patients had acute constitutional symptoms associated with acute sarcoidosis (8). Relatively small numbers and confounding by co-existent 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 elevated 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.

ACKNOWLEDGEMENTS

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ETHICS

The determination of ENO levels in normal volunteers and the measurement of serum RAST in all subjects was done with the approval of the Northern Regional Ethics Committee, Auckland, New Zealand.

CONFLICT OF INTEREST

The authors have no conflict of interests to declare in relation to this study.

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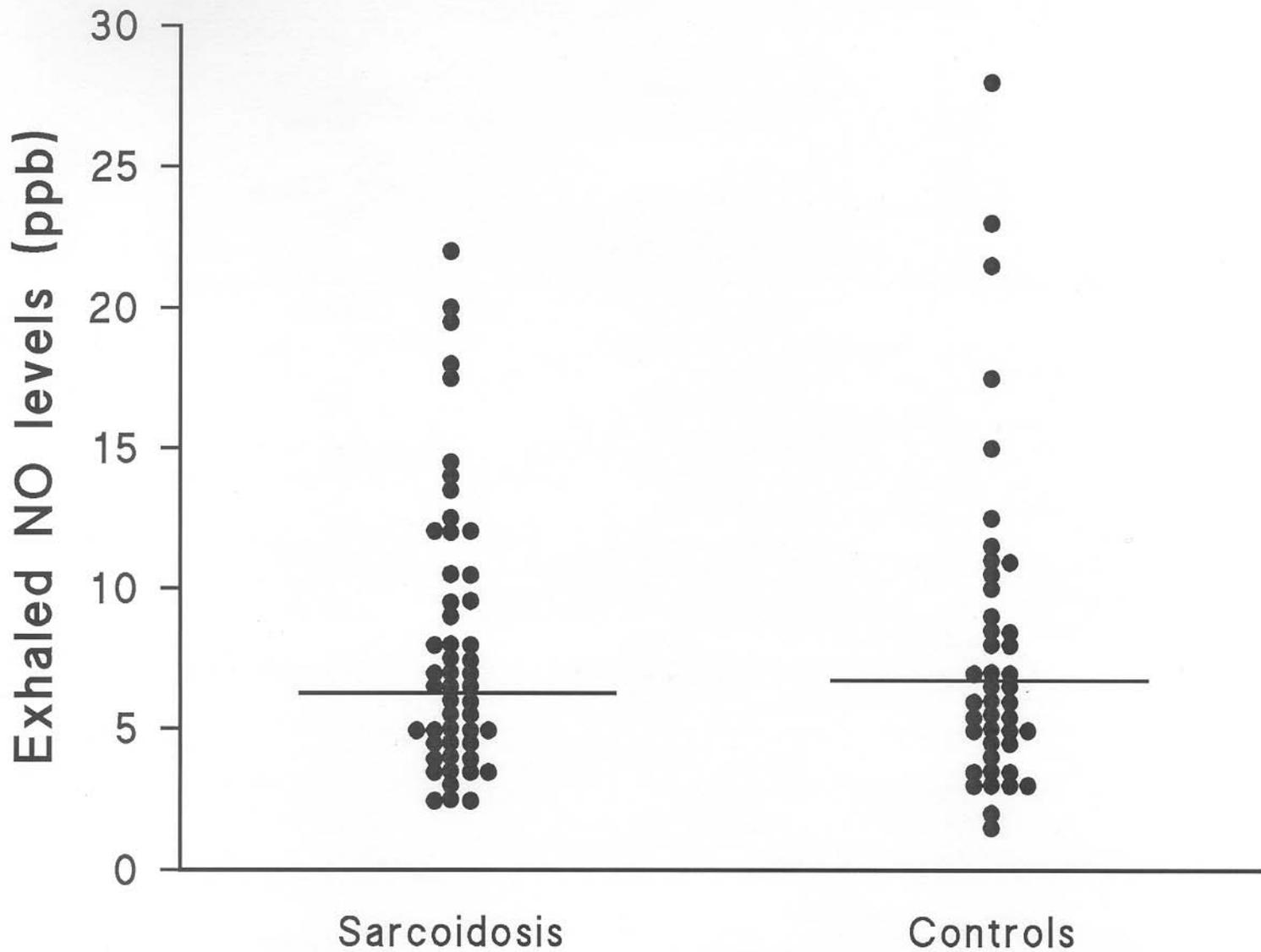
LEGENDS FOR FIGURES

Figure 1

Exhaled nitric oxide levels in 52 patients with sarcoidosis and 44 normal nonsmoking controls.

Figure 2

Exhaled nitric oxide levels in atopic (12) and non atopic (19) patients with sarcoidosis.



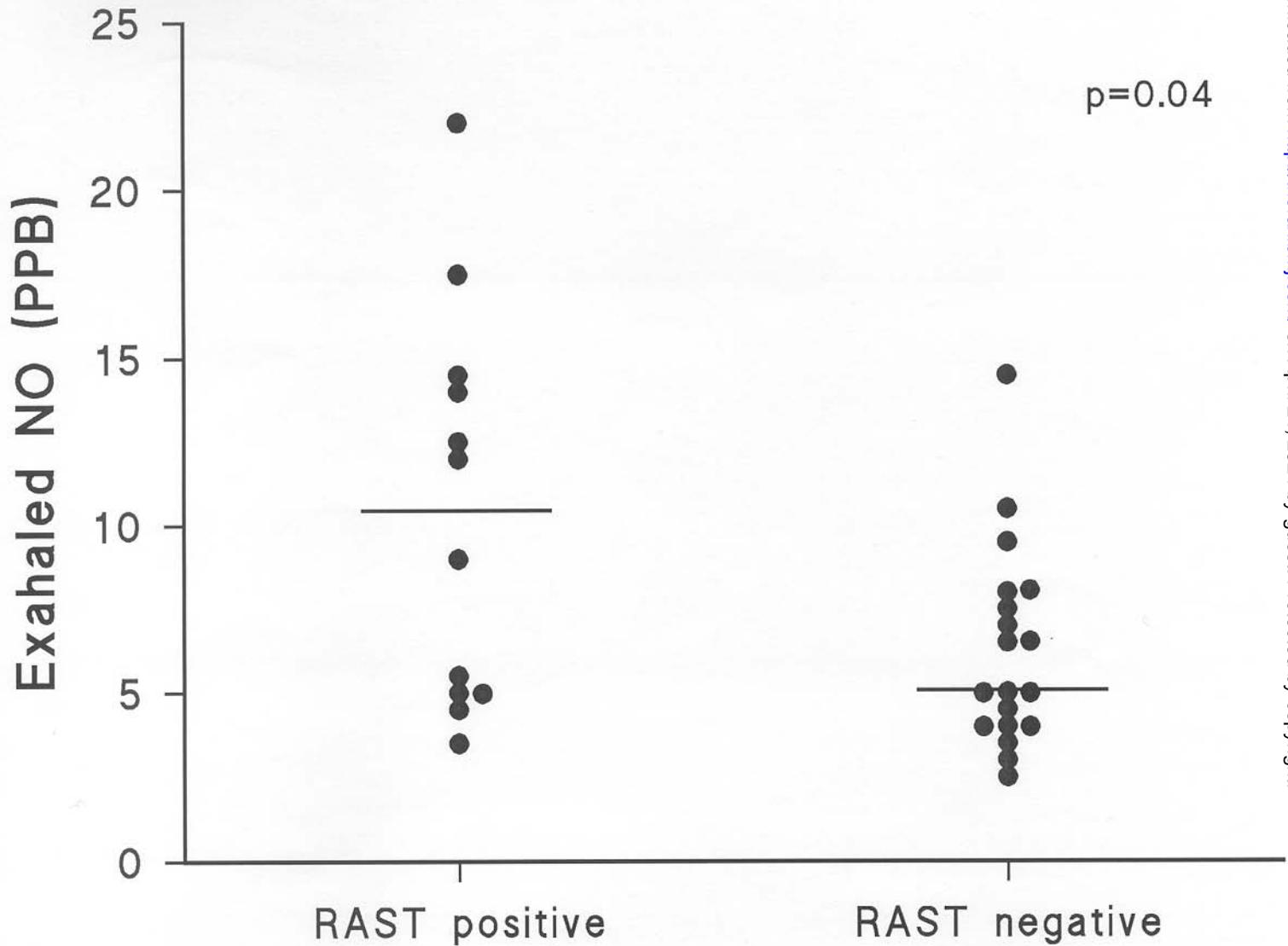


Table 1

	Median CT score (range)
Nodules (n=42)	10 (2-27)
Reticular abnormalities (n=37)	6 (2-30)
Consolidation (n=30)	6 (2-18)
Ground-glass attenuation (n=25)	2 (2-38)
Mosaic attenuation (n=45)	10 (2-24)
Bronchial wall thickness (n=29)	6 (2-14)
Bronchiectasis (n=8)	3 (2-11)

Legend: The prevalence of CT abnormalities in 52 patients with sarcoidosis, with median CT scores (with ranges).

Table 2

	Reticular score	Consolidation Score	Mosaic attenuation Score
FEV1 (% predicted)	R = - 0.59 p<0.0005	R = - 0.61 p<0.0005	R = - 0.37 p<0.01
FEF 25-75 (% predicted)	R = - 0.61 p<0.0005	R = - 0.58 p<0.0005	R = - 0.47 p<0.0005

Legend: The most powerful functional-morphologic univariate relationships (expressed as Spearman rank correlation coefficients) between pulmonary function indices and CT scores. Increasing reticular abnormalities, consolidation and mosaic attenuation were all associated with reductions in FEV1 and FEF 25-75.