High alveolar nitric oxide is associated with steeper lung function decline in foundry workers

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ABSTRACT

Background: Occupational dust exposure induces inflammatory responses that often precede the onset of clinical disease. Inflammation in the peripheral part of the lung can be demonstrated by measuring the alveolar NO concentration (C_ANO) in exhaled breath.

Objective: The aim of the study was to assess whether cumulative dust exposure affects the change in C_ANO during follow-up and whether baseline C_ANO can predict an impairment in lung function during follow-up in foundry workers.

Methods: We examined 74 dust-exposed and 42 nonexposed foundry workers and measured C_ANO and lung function at baseline and after 7 years of follow-up.

Results: An increase in C_ANO during the follow-up period was positively associated with cumulative dust exposure in foundry work ($p=0.035$). Furthermore, a higher baseline C_ANO was associated with an accelerated decline in the forced vital capacity (FVC) during the follow-up period (absolute decrease in FVC $p=0.021$, relative decrease in FVC $p=0.017$).

Conclusion: Higher cumulative dust exposure in foundry work is associated with a greater increase in C_ANO during follow-up, suggesting ongoing pulmonary inflammation in these subjects. Importantly, a high baseline C_ANO is associated with an accelerated decline in lung function, suggesting that C_ANO measurements might serve as a screening tool for high-risk workers.

INTRODUCTION

Measuring the exhaled nitric oxide concentration (F_ENO) at a single exhalation flow rate is a standard method for assessing central airway inflammation in asthma[1]. Measuring F_ENO at several different flow rates allows the alveolar NO concentration (C_ANO) to be calculated, which reflects NO dynamics in the lung periphery[2]. C_ANO can be increased via many pathological processes, such as inflammation of the lung parenchyma or impaired gas diffusion, which are features often associated with interstitial lung diseases. C_ANO is increased in alveolitis and inversely correlates with pulmonary diffusing capacity and pulmonary volume.[3] C_ANO is also increased in idiopathic pulmonary fibrosis (IPF) and interstitial lung disease associated with systemic sclerosis (SSC-ILD). In both of these diseases, higher C_ANO is associated with impaired lung function and worse prognosis[4,5]. In SSC-ILD, higher C_ANO is a good predictor of the treatment response to cyclophosphamide[6].

Foundry work is associated with exposure to diverse inhalable substances, including dusts (including silica), fumes, gases and other air impurities. Traditionally, long-term occupational exposure to silica in foundry work has resulted in silicosis[7]. We have previously shown that C_ANO is slightly increased in subjects exposed to dust during foundry work, even in the absence of any clinical lung diseases[8]. Additionally, occupational exposure to other substances, such as asbestos and silica dust, may increase C_ANO even in subjects who do not yet have pneumoconiosis associated with their occupational exposure[9,10]. C_ANO might therefore serve as a marker of damage to the pulmonary parenchyma in subjects exposed to harmful agents, even before they develop pulmonary disease, and C_ANO might predict a decline in lung function in exposed workers.

The aim of this study was to assess whether cumulative dust exposure in foundry workers affects the change in C_ANO and whether C_ANO at baseline predicts impairment of lung function during follow-up.

METHODS

Participants and study setup

We previously reported a cross-sectional study assessing the relationship between occupational exposure and markers of pulmonary and systemic inflammation in workers at two Finnish foundries during 2004-2005[8]. A follow-up study was conducted in one of these two foundries in the steel cast foundry in Tampere during 2011-2013. The follow-up period was 7 years on average and varied between 6 and 8 years. A total of 249 persons from this foundry (either exposed workers from the foundry (n=174) or unexposed controls working at the same enterprise (n=75)) participated in the baseline study. At the time of the follow-up, 77 subjects had left the factory (retired, laid off or changed workplace), two subjects had died, and 172 subjects were still working at the same company (either exposed or controls). The flow chart describing the structure of our follow-up foundry study is presented in Figure 1.

A total of 135 subjects participated in the follow-up study, and the participation rate was 55 %. The inclusion criteria for the exposed group were current or previous occupational exposure to airborne impurities in the foundry. The control group consisted of male assembly workers from the same workplace without occupational exposure to dusts, gases or fumes. The exclusion criteria were asthma diagnosed by a physician (n=9) or possible confounding occupational exposure at the foundry or their new workplace (n=8) based on an evaluation of data at the follow-up. We also excluded female workers (n=2) given that gender might affect inflammatory markers or lung function decline. The final cohort included 116 participants: 74 exposed and 42 unexposed workers.

Participation was voluntary, and all the participants provided written informed consent. Ethical approval was obtained from the Coordinating Ethics Committee of the Helsinki University Hospital.

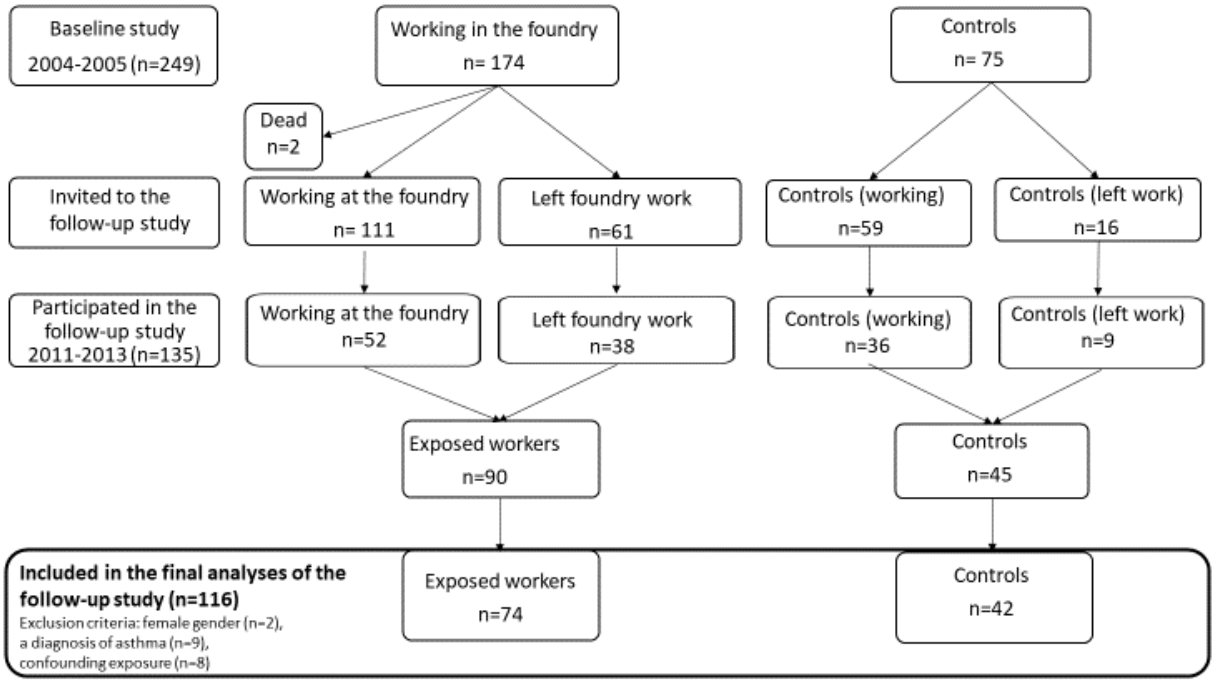

Figure 1. Flow chart of the follow-up study.

Assessment of exposure

The assessment of cumulative exposure to dust and respirable quartz, which was reported as milligram-years (mg-y), during the working history until 2005 was performed as part of the baseline study as previously described[8]. During the baseline study, the exposed participants were divided into two cumulative dust exposure categories (low, ≤ 53 mg-y, and high, > 53 mg-y) using the median value of the cumulative dust exposure as the cut-off. We estimated that the exposure level of exposed participants had remained similar or slightly decreased as a result of hygienic improvements in the workplace during the follow-up period. Based on the questionnaire, no exposed workers had changed to tasks without occupational dust exposure, or vice versa, in the workplace during the follow-up period. The occupational exposure of retired persons ceased during the follow-up period.

Spirometry

All participants underwent spirometry (Spiromaster, Medikro Oy, Finland, and Vmax, CareFusion, USA)[11]. The forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1) and FEV_1/FVC were measured and interpreted using Finnish reference values[12]. The two spirometers were compared and found to provide similar results. A bronchodilation test (400 µg of inhaled salbutamol) was performed on persons who had FVC, FEV_1 or FEV_1/FVC values below the lower limit of normal (LLN) and if there were no contraindications for giving salbutamol.

Exhaled NO measurements

Exhaled NO was measured using a Sievers NOA 280i analyser (Sievers Instruments, Boulder, Colorado, USA) with a Nofla-flow regulator (designed at Tampere University). Exhaled NO measurements were performed at eight exhalation flows between 10 and 400 ml/s, including standard F_ENO_{50} without nose clips. The participants exhaled through a mass flow metre connected to a computer-controlled adjustable flow restrictor to achieve the desired exhalation flow rate[13]. Exhaled NO at flow rates of 100, 200 and 300 ml/s were used to calculate C_ANO and $J_{aw}NO$ according to Tsoukias and George[14]. Altogether, 23 (20 %) participants (17 at baseline and 6 at follow-up) had failed C_ANO measurements at one or other timepoints and were discarded from longitudinal analyses.

Statistical methods

SPSS 25 (IBM Corp. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) software was used for statistical analysis. The normality of the distributions was evaluated using a histogram and Q-Q-Plot. Age, body mass index (BMI) and pack-years of smoking at baseline in exposed subjects and unexposed control subjects were compared using the Mann-Whitney U-test. Smoking status (current, ex-smoker and nonsmoker) at baseline in exposed and unexposed subjects was compared using the chi-square test.

We defined impaired lung function as at least one lung function variable (FVC, FEV_1 or FEV_1/FVC) below a Z-score of -1.65. The baseline and follow-up distributions of impaired lung function in exposed subjects

or control subjects were compared with the related samples McNemar test. The baseline and follow-up distributions of impaired lung function between unexposed controls and exposed subjects were compared using the chi-square test.

The baseline and follow-up values of lung function variables as well as C_ANO, $J_{aw}NO$ and F_ENO_{50} in unexposed controls and exposed subjects were compared using the paired samples T-test or the related samples Wilcoxon signed rank test depending on the normality of the variables. The changes in lung function variables or in C_ANO, $J_{aw}NO$ and F_ENO_{50} during the follow-up period between unexposed controls and exposed subjects were compared using T-tests and Mann-Whitney U-tests.

To analyse the effect of cumulative dust exposure on the change in C_ANO during the follow-up, we used repeated measures ANOVA with explanatory variables (cumulative dust exposure in mg-y, pack-years of smoking and age) as covariates. Three models were created: 1) cumulative dust exposure as a covariate, 2) cumulative dust exposure and pack-years of smoking at baseline as covariates and 3) cumulative dust exposure, pack-years of smoking and age at baseline as covariates. The model with the lowest error mean square (BS) was chosen.

The correlation between baseline C_ANO and the change in lung function test variables was first assessed with Spearman's rho. To study whether baseline C_ANO predicted a change in lung function during the follow-up period, we used the general linear model (GLM) with the change in each lung function variable (absolute change, relative change, change in % predicted and change in Z-score of FVC, FEV_1 and FEV_1/FVC) as outcome variables and the baseline values of C_ANO, pack-years of smoking and BMI as explanatory variables.

Our main focus was on C_ANO, but for comparison, we performed the same statistical analyses for F_ENO_{50} and $J_{aw}NO$ to analyse the effect of cumulative dust exposure on the change in F_ENO_{50} and $J_{aw}NO$ during follow-up as well as the correlation between baseline values of F_ENO_{50} and $J_{aw}NO$ and the change in lung function variables during the follow-up period.

In GLM, we performed variable transformation of the outcome variables that were not normally distributed. After variable transformation, the model residuals were normally distributed.

The characteristics of subjects with successful or failed C_ANO measurements were compared using the Mann-Whitney U-test and the chi-square test.

A P-value of < 0.05 was considered statistically significant.

RESULTS

Subject characteristics

Table 1 shows the characteristics of the study population at baseline. The mean age was greater in exposed subjects than in unexposed control subjects. BMI, smoking status and smoking history in pack-years were similar between the subgroups. None of the participants had a diagnosis of silicosis. The median cumulative dust exposure among exposed subjects was 55 mg-y (range 3-441 mg-y).

Table 1. Characteristics of the study participants at baseline.

	Unexposed control subjects (n=42)	Exposed subjects (n=74)	p-value
Age at baseline, years	44.9 (9.6)	49.7 (10.4)	0.003 *
BMI at baseline, kg/m^2	26.5 (2.7)	27.8 (4.1)	0.138*
Pack-years of smoking among current and ex-smokers at baseline	10.3 (11.3)	9.7 (12.2)	0.572 *
Smoking status at baseline Current smoker Ex-smoker Never smoker	 15 (36 %) 16 (38 %) 11 (26 %)	 19 (26 %) 32 (43 %) 23 (31 %)	0.519 #

The results are presented as the mean (SD) or number (%); * Mann-Whitney U-test; # Chi-square test

Changes in lung function and C_ANO during the follow-up

Table 2 shows the lung function variables, the proportion of subjects with impaired lung function, and C_ANO, $J_{aw}NO$ and $FENO_{50}$ at baseline and at follow-up in unexposed controls and exposed subjects.

As expected, absolute lung function decreased significantly at the follow-up in both unexposed and exposed subjects (Table 2), but there was no statistically significant difference between the two groups (p=0.592 for the absolute decrease in FVC (l), p=0.483 for the absolute decrease in FEV_1 (l) and p=0.073 for the decrease in FEV_1/FVC). However, there was no significant change in FVC % predicted or FVC Z-score in unexposed controls or exposed subjects during the follow-up period, and no statistically significant differences were noted between the two groups (p=0.673 for the FVC % predicted and p=0.915 for the FVC Z-score). On the other hand, FEV_1 % predicted, FEV_1 Z-score, FEV_1/FVC % predicted and FEV_1/FVC Z-score decreased statistically significantly during the follow-up in both unexposed controls and exposed subjects. The differences between unexposed controls and exposed subjects were not statistically significant (p=0.398 for FEV_1 % predicted, p=0.705 for FEV_1 Z-score, p=0.086 for FEV_1/FVC % predicted and p=0.081 for FEV_1/FVC Z-score).

During the follow-up period, the proportion of subjects with impaired lung function did not change statistically significantly in unexposed controls or exposed subjects (at baseline 14 subjects and at follow-up 22 subjects altogether). The baseline and follow-up distributions of impaired lung function between unexposed controls and exposed subjects did not statistically significantly vary (p=0.526 for baseline and p=0.634 for follow-up).

C_ANO, $J_{aw}NO$ and F_ENO_{50} increased statistically significantly during the follow-up period in exposed subjects but not in unexposed controls.

Table 2. Lung function variables, proportions of subjects with impaired lung function, and C_ANO, $J_{aw}NO$ and F_ENO_{50} at baseline and follow-up in unexposed controls and exposed subjects.

	Unexposed control subjects			Exposed subjects		
	Baseline	Follow-up	Baseline vs. follow-up p-value	Baseline	Follow-up	Baseline vs. follow-up p-value
FVC (l)	5.37 (0.90)	5.18 (1.00)	0.012 §	4.93 (0.83)	4.78 (0.86)	0.002 §
FVC % predicted	98.10 (11.16)	97.55 (13.63)	0.681 §	92.43 (12.10)	92.55 (13.05)	0.990 *
FVC Z-score	-0.12 (0.93)	-0.15 (1.06)	0.821 §	-0.59 (0.98)	-0.53 (0.93)	0.574 *
FEV_1 (l)	4.43 (0.81)	4.07 (0.80)	<0.001 §	4.02 (0.71)	3.67 (0.72)	<0.001 §
FEV_1 % predicted	103.19 (14.07)	99.67 (14.08)	0.008 §	97.22 (13.62)	93.31 (14.38)	<0.001 *
FEV_1 Z-score	0.30 (1.11)	0.02 (1.03)	0.008 §	-0.21 (1.05)	-0.46 (0.98)	0.001 *
FEV_1/FVC	0.82 (0.06)	0.79 (0.06)	0.0001§	0.81 (0.05)	0.77 (0.07)	<0.001 *
FEV_1/FVC % predicted	104.88 (7.23)	102.31 (8.36)	<0.001 §	105.12 (7.14)	100.69 (9.67)	<0.001 §
FEV_1/FVC Z-score	0.69 (1.02)	0.33 (1.17)	<0.001 §	0.72 (1.00)	0.10 (1.34)	<0.001 *
Impaired lung function FVC Z-score, FEV_1 Z-score or FEV_1/FVC Z-score < -1.65	4 (10 %)	7 (17 %)	0.375 #	10 (14 %)	15 (20 %)	0.125 #
C_ANO (ppb)	1.63 (0.62)	1.82 (0.88)	0.338 *	1.73 (0.68)	2.28 (0.87)	<0.001 *
$J_{aw}NO$ (nl/s)	0.68 (0.45)	0.94 (0.64)	0.016 *	0.060 (0.39)	0.80 (0.45)	<0.001 *

F_ENO_{50} (ml/s)	15.37 (8.37)	21.18 (15.06)	0.010 *	14.08 (9.22)	18.46 (10.25)	<0.001 *

The results are presented as the mean (SD); # Related samples McNemar test; § Paired samples T-test; * Related samples Wilcoxon signed rank test

The effect of dust exposure on the change in C_ANO

To analyse the effect of cumulative dust exposure on C_ANO during the follow-up period, we used repeated measures ANOVA. Model 2 with cumulative exposure and pack-years of smoking as explanatory variables was chosen for C_ANO because adding age to the model did not improve it, and age was not associated with C_ANO, not even in controls (p=0.795).

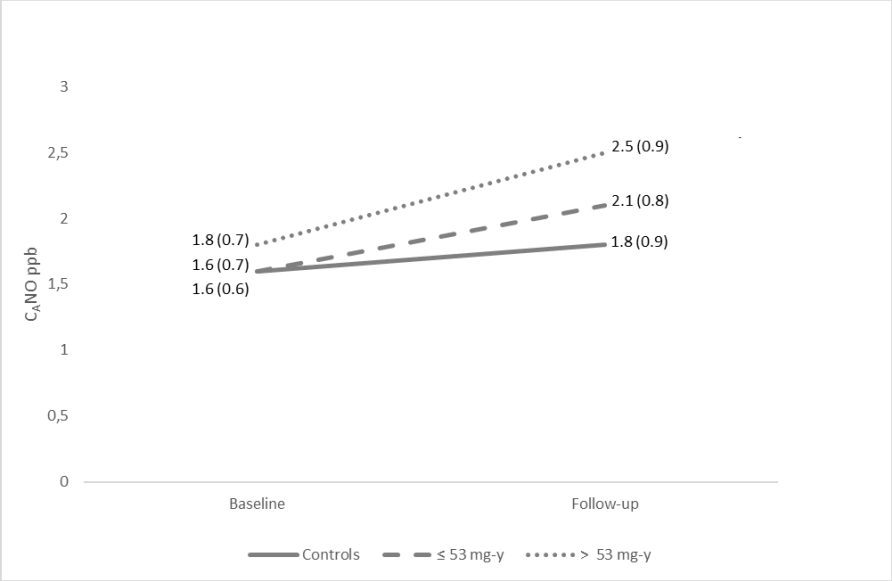

Figure 2. Mean C_ANO (SD) at baseline and follow-up in three different subgroups: unexposed controls and exposed foundry workers with either low (≤ 53 mg-y) or high (> 53 mg-y) cumulative dust exposure.

We found that cumulative dust exposure and the interaction between cumulative dust exposure and the time course were each significantly associated with C_ANO (p=0.003 and 0.031, respectively), whereas pack-years of smoking was not significantly associated with C_ANO. The change in C_ANO at the follow-up was positively associated with the amount of cumulative dust exposure. To illustrate these findings,

Figure 2 shows C_ANO at baseline and follow-up in unexposed controls and exposed foundry workers with either low (≤ 53 mg-y) or high (> 53 mg-y) cumulative dust exposure.

Neither cumulative dust exposure nor the interaction between cumulative dust exposure and time was associated with $J_{aw}NO$ or F_ENO_{50} (p=0.991 and 0.738, respectively, for cumulative dust exposure and p=0.790 and 0.963 for the interaction between cumulative dust exposure and time).

C_ANO at baseline predicts changes in lung function at follow-up in exposed foundry workers

We found that baseline C_ANO in exposed foundry workers correlated statistically significantly with the absolute decrease in FVC (Spearman's rho ρ -0.350, p=0.004), the relative decrease in FVC (FVC difference divided by baseline FVC (ρ -0.354, p=0.003), the decrease in FVC % predicted (ρ -0.342, p=0.005) and the decrease in FVC Z-score (ρ -0.327, p=0.007) during the follow-up period. Baseline C_ANO did not correlate statistically significantly with the absolute or relative decrease in FEV_1 or with the decreases in FEV_1 % predicted, FEV_1 Z-score, FEV_1/FVC, FEV_1/FVC % predicted or FEV_1/FVC Z-score.

Baseline JawNO or F_ENO_{50} in exposed foundry workers did not correlate statistically significantly with any of the changes in the lung function variables.

Additionally, in GLM analysis, we found that a higher baseline C_ANO in exposed workers was associated with accelerated absolute and relative decreases in FVC, the decrease in FVC % predicted and FVC Z-score both without adjusting (p=0.021, 0.017, 0.033 and 0.033, respectively) and after adjusting for pack-years of smoking and BMI at baseline (p=0.024, 0.021, 0.035 and 0.043, respectively). Pack-years of smoking at baseline was not significantly associated with absolute or relative decreases in FVC, the decrease in FVC % predicted or FVC Z-score.

According to correlation analysis, a higher baseline C_ANO was not associated with an absolute or a relative decrease in FEV_1 or with decreases in FEV_1 % predicted, FEV_1 Z-score, FEV_1/FVC, FEV_1/FVC % predicted or FEV_1/FVC Z-score.

For comparison, we also performed statistical analyses with pack-years of smoking at follow-up. The results remained the same. We further compared subjects with successful or failed C_ANO measurements

and found no statistically significant differences between these groups in terms of age, exposure category, pack-years of smoking or smoking habits.

DISCUSSION

To our knowledge, this is the first longitudinal study assessing the effect of occupational dust exposure on C_ANO and the ability of C_ANO to predict later lung function decline in previously healthy workers. We found that the increase in C_ANO during the follow-up period was greater in foundry workers with greater cumulative dust exposure. Furthermore, a greater baseline C_ANO was associated with a greater decline in FVC during the follow-up period.

In our previous cross-sectional study, we demonstrated that higher cumulative dust exposure in foundry work is associated with increased C_ANO[8]. In this follow-up study, we found that the C_ANO of exposed subjects increased significantly during the follow-up period, but this was not observed in unexposed control subjects. We also found that the increase in C_ANO during the follow-up period was greater in exposed subjects with greater cumulative dust exposure. Previously, Sauni et al. reported elevated C_ANO in heavily quartz-exposed persons in prefabrication factories, quarries and stone-cutting companies, although only 20 subjects (21 %) had decreased lung function[9]. Elevated C_ANO has also been reported in patients with asbestosis[13]. In addition, Lehtimäki et al. reported increased C_ANO in moderately or heavily asbestos-exposed persons with borderline parenchymal changes in HRCT without proper asbestosis[10]. It was concluded that the increase in C_ANO was due to the asbestos-induced process in pulmonary tissue[10], which is consistent with the fact that the pathogenesis of asbestos-associated diseases is associated with a persistent inflammatory response[15]. Interestingly, even short-term experimental exposure to wood smoke has been reported to increase C_ANO[16].

Our findings are consistent with previously published studies showing that C_ANO increases as a result of inhalation exposure to foundry pollutants. Foundry workers are exposed to a mixture of different substances, such as respirable inorganic dust, including respirable silica, metal fumes, polycyclic aromatic hydrocarbons (PAHs) and binder compounds, which may have adverse effects on the respiratory system. Traditionally, occupational exposure to silica has been the major health concern in foundries since exposure to silica occurs in many phases of production[17]. Silica exposure can cause silicosis, a pneumoconiosis typically characterised by nodular changes[18].

The increase in C_ANO may be derived from an inflammatory reaction, which induces increased production of NO in alveolar tissue. Another explanation for increased C_ANO is that damage in pulmonary tissue leads to the deterioration of diffusion capacity, which increases C_ANO by inhibiting the diffusion of NO from alveolar air to pulmonary capillaries.[14] Unfortunately, measurements of pulmonary diffusing capacity were not available, as this information would have facilitated differentiation between these two mechanisms leading to increased C_ANO. C_ANO has been reported to be increased, for example, in subjects with IPF, nonspecific interstitial pneumonia (NSIP), SSC-ILD and alveolitis[3,19,20] , all of which are associated with both parenchyma inflammation and impaired diffusion between alveolar air and pulmonary capillaries.

In this follow-up study, we also found that higher baseline C_ANO was associated with a greater decline in FVC during the follow-up period in exposed subjects, suggesting that C_ANO may serve as an indicator for early parenchymal changes. A correlation between C_ANO and impaired lung function has been previously reported in IPF[4]. Similarly, C_ANO has been reported to have diagnostic value in detecting SSC-ILD[21].

The strength of our study is that we were able to investigate changes in C_ANO and lung function in both exposed and unexposed subjects over a follow-up period of approximately 7 years. To minimise confounding effects, we excluded all persons with asthma diagnosed by a physician at the time of follow-up. Impaired lung function was found in 14 subjects at baseline and in 22 subjects at follow-up. However, none of the participants had a significant reversibility suggesting asthma, and none of the participants were diagnosed with silicosis. Two of the participants had a history of sarcoidosis, but the results of the statistical analyses remained the same after exclusion of these cases (data not shown). We were also able to adjust the results for pack-years of smoking and BMI in the statistical analyses. Given that cumulative dust exposure and ageing were multicollinear (Spearman's rho $\rho = 0.482$, $p<0.001$), we performed a separate analysis on the effect of age on C_ANO in control subjects, and we found that age was not associated with C_ANO in controls.

In our previous cross-sectional foundry study, we explored inflammatory markers in exhaled breath and serum in workers at two foundries (iron and steel foundries)[8]. A weakness of our study is that we were only able to perform a follow-up study at the steel foundry due to financial and logistical reasons. The

modest participation rate of the follow-up study can be explained by the concurrent negotiation to decrease the workforce in the corporation, which may have influenced the willingness of foundry workers to participate in our study. Nevertheless, the size of the study population (n=116) was quite large, including both exposed subjects with variable cumulative dust exposure levels and unexposed controls, which should enable reliable statistical conclusions.

The cumulative exposure (mg-y) of every participant was estimated on the basis of their working history and objective measurements at the workplace by occupational hygienists as a part of the baseline study[8]. A limitation of our study is that the exposure levels during the follow-up period were based on expert evaluations. However, the stability of the exposure levels was also supported by information from the questionnaire, which showed that none of the exposed workers had changed to tasks without occupational dust exposure, and none of the unexposed subjects had changed to tasks with occupational dust exposure. Healthy worker selection bias may have influenced our results. Foundry workers undergo a pre-employment health examination before starting work at a foundry, and it is unlikely that persons with a previous respiratory disease will be able to start foundry work. Foundry workers also undergo health examinations regularly. It is possible that this healthy worker selection bias attenuated the results, reducing the differences between unexposed and exposed workers.

Despite this potential bias, we were able to demonstrate distinct results regarding the change in C_ANO during the follow-up period in exposed workers and the association between higher baseline C_ANO and greater decline in FVC during the follow-up period.

The change in FVC was modest in our sample of exposed subjects, and this is a limitation of our study. The relation between NO output and changes in lung function could be studied in more detail in a sample with a high proportion of subjects developing pulmonary disease during the follow-up. However, in terms of occupational health, the finding that the degree of impairment in lung function was modest among the workers of the foundry under investigation is reassuring.

In conclusion, high cumulative dust exposure in foundry work was associated with a steeper increase in C_ANO, whereas high C_ANO at baseline was associated with a greater decline in FVC during the 7 year follow-up. These results suggest that C_ANO is a valuable noninvasive marker of subclinical pulmonary inflammation triggered by intense long-term dust exposure, leading to pulmonary damage and a decline in lung function. Large, long-term follow-up studies are needed to confirm whether C_ANO measurements

represent a useful screening method for identifying vulnerable workers and whether high levels of C_ANO can predict the occurrence of a clinical lung disease caused by occupational dust exposure.

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Ethical statement

Participation was voluntary, and all the participants provided written informed consent. Ethical approval was obtained from the Coordinating Ethics Committee of the Helsinki University Hospital.

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