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Non-invasive techniques to assess restrictive lung disease in workers exposed to free crystalline silica

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Summary

Objectives: *To compare the reliability of spirometry and body plethysmography in detecting restrictive lung disease in clay excavation workers exposed to free crystalline silica (FCS). The exhaled breath condensate (EBC) biomarkers of oxidative stress were also assessed in order to evaluate early lung damage.* **Methods**: *The study involved 62 workers (58 males and 4 females) at a company that extracts and processes clay.* **Results**: *Body plethysmography (total lung capacity below the lower normal limit) and spirometry respectively indicated restrictive pattern prevalence rates of 22.6% and 1.6%. EBC 4-hydroxynonenale levels were not sufficiently sensitive to highlight a restrictive deficit, but did distinguish low and high rates of occupational exposure. There was no correlation between plethysmography values and the intensity or duration of exposure.* **Conclusions:** *Only one out of 14 cases of restrictive deficit diagnosed on the basis of body plethysmography values was also identified by means of spirometry. This finding supports the need to use body plethysmography in the health surveillance of clay workers exposed to FCS.*

Riassunto

«Tecniche non invasive per la valutazione della patologia polmonare restrittiva in lavoratori esposti a silice libera cristallina». **Obiettivi**: *Investigare in lavoratori impegnati nell'estrazione e lavorazione dell'argilla, esposti a silice libera cristallina, la capacità della spirometria di rilevare la presenza di un pattern restrittivo.* **Metodi**: *Sono stati reclutati 62 lavoratori (58 maschi e 4 femmine) da una ditta, che estrae e produce argilla.* **Risultati**: *Dalla pletismografia corporea, la prevalenza di deficit restrittivo (TLC <LLN) era 22.6% vs 1.6% della spirometria. Il 4-idrossinonenale (4-HNE) nel CAE non è un biomarcatore sufficientemente sensibile per evidenziare deficit restrittivo, ma è in grado di discriminare tra bassa ed alta esposizione. Correlazioni non sono state trovate tra valori pletismografici e periodi e grado di esposizione.* **Conclusioni**: *La ricerca evidenzia che per 14 casi di restrizione diagnosticati dalla pletismografia corporea su un totale di 62 lavoratori, la spirometria pone il sospetto solo per un lavoratore. Questo evidenzia l'importanza di effettuare la pletismografia corporea nella sorveglianza sanitaria per lavoratori esposti a FCS impiegati nell'estrazione dell'argilla.*

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Introduction

Spirometry is the traditional means of evaluating respiratory function in workers exposed to inhaled toxic substances and is considered the gold standard for diagnosing obstructive airway dysfunctions (30, 36). An obstructive respiratory deficit is defined as a reduction in the ratio between forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) to below the fifth percentile of the predicted value (the lower limit of normal, LLN; FEV_1/FVC <LLN) and FEV_1 <LLN. However, although spirometry can be used to exclude restrictive respiratory diseases because of its high negative predictive value (NPV) of >98% (35), it cannot provide complete information concerning restrictive patterns because it has a low positive predictive value (PPV) of <60% (1) and a high false positive rate. This means that restrictive lung impairment ((FVC reduced (FVC <LLN) and FEV_1/FVC normal (FEV_1/FVC ≥LLN)) can only suspected by spirometry (6).

Suspected pulmonary restriction must be confirmed by second-level body plethysmography (12), which allows an assessment of functional residual capacity (FRC), residual volume (RV) and total lung capacity (TLC) (49). According to the American Thoracic Society/European Respiratory (ATS/ERS) guidelines, a restrictive deficit is definitely proven only by a decrease in TLC below the fifth percentile of the predicted value (TLC <LLN) (1, 11). Over the years, various algorithms have been proposed that use spirometric data alone to identify subjects requiring a second-level examination. De Matteis et al. (13) have recently proposed a model to improve the PPV of spirometry in diagnosing restriction: this was developed using the spirometry data of a general population aged <65 years and comparing the findings with those of body plethysmography while considering a low probability of lung restriction (1-10%). However, this algorithm has not yet been used for workers with occupational exposure.

It is known that excavating clay may lead to exposure to free crystalline silica (FCS), whose effects on the respiratory tract include lung restriction (7, 44), but no assessment has been made of the validity of using spirometric data to rule out restriction in workers engaged in this type of niche processing. This is important because spirometry alone is widely used in occupational medicine to evaluate respiratory function in workers exposed to FCS (45).

FCS, which is sclerogenic for the lung, is characterised by a regular spatial structure and a high degree of cellular toxicity (17). It is recognised that quartz, tridymite and cristobalite cause silicosis (14), with tridymite and cristobalite being more toxic than quartz (24). Exposure to inhaled FCS (32) can lead to changes in respiratory test results (25), which mainly have restrictive patterns, although mixed obstructive-restrictive patterns may also be detected, not always associated with radiologically visible nodular fibrosis (43). It is also possible to observe a reduction in the diffusing capacity of the lung for carbon monoxide (DLCO) especially when marked nodular dissemination leads to a reduction in pulmonary tissue.

The aim of this study was to investigate the validity of using spirometric parameters to formulate a suspicion of restrictive lung disease in clay excavation workers exposed to FCS and compare the findings with body plethysmographic data. An exploratory endpoint was to evaluate whether the biomarkers of oxidative stress in the non-invasive biological matrix of exhaled breath condensate (EBC) (9, 10) correlate with the parameters of respiratory function as Sakhvidi et al. (42) found that they do correlate with ambient silicon dioxide (SiO_2) data in ceramic production workers.

Methods

Study design and population

This cross-sectional study involved 62 workers (58 men and four women with a mean age of 46.5±9.3 years) from a company that extracts and processes clay. Forty-six were engaged in manufacturing or handling clay, and sixteen were laboratory technicians. The manufactured goods of the company are mainly used in geotechnical, prefabricated, concrete and green building construction. All subjects gave their written informed consent to participate in the study, which was approved by the Institutional Review Board (IRB) of the University of Parma (Prot. No. 0007/2017). The biological samples were collected in accordance with the principles of the Declaration of Helsinki.

The workers completed a clinical anamnestic questionnaire covering their smoking habits, the characteristics and frequency of workplace exposure, work-related respiratory symptoms and conditions, including cough, wheezing, bronchial catarrh, physician-diagnosed restriction or obstruction, and allergic diseases. Reports concerning any previous radiographic investigations (chest X-rays) and health surveillance protocol were provided by the company's occupational physician, who had also trained the workers to use personal protective equipment (PPE).

Lung function tests

The workers underwent spirometry and body plethysmography with DLco (Flow Spirometer and Body Plethysmography Vmax 22 and 6200; Sensor Medics, Yorba Linda, CA, USA) in accordance with the 2005 ATS/ERS guideline (49), and the results were compared with Global Lung Initiative (GLI 2012) reference values (30, 36, 39) and reference values for lung volumes and DLCO (40, 49).

Collection and analysis of EBC biomarkers

EBC samples were collected using a transportable TURBO-DECCS condenser (Medivac, Parma, Italy) (21) inside the company at the end of the last working shift of a working week in order to ensure that they were the most representative of cumulative exposure. The workers were asked to breath tidally for 15 minutes through a mouthpiece with a two-way non-rebreathing valve in order separate inspiratory and expiratory air and trap saliva. The condenser respected all of the recommended practical standards and anti-contamination principles for EBC collection published by the ATS/ERS (26), and its temperature was set at -5°C. The collected samples (1-2 mL) were subsequently labelled and kept frozen at -80°C until analysis.

The assayed EBC biomarkers were hydrogen peroxide, (H_2O_2), 8-isoprostane, malondhyaldeide (MDA), and 4-hydroxynonenale (4-HNE). H_2O_2 was measured as previously described (8) using a commercial kit (Amplex Red Hydrogen Peroxide/ Peroxidase assay kit, Molecular Probes, Eugene, OR, USA), with the H_2O_2 calibration curve consisting of seven concentration levels (range 0-5.0 μM); 8-isoprostane was measured using a specific enzyme immunoassay (EIA) kit (Cayman Chemical Milan, Italy) as described elsewhere (4), with the method being modified to lower the detection limit to 1 pg/mL; MDA and 4-HNE were determined by means of tandem liquid chromatography-mass spectrometry (LC-MS-MS) after derivatisation by 2,4-dinitro-phenylhydrazine (DNPH) as previously described (3), with some modifications. The EBC samples were derivatised with an equal volume of DNPH (1.26 mM) and stored at room temperature for two hours, after which 10 μL were injected into the LC-MS-MS system, which consisted of an Agilent HP 1100 series binary pump (Palo Alto, CA) coupled to a AB Sciex API 4000 triple-quadruple mass spectrometer (AB SCIEX, Framingham, MA, USA) equipped with a TurboIonSpray (TISP) interface. MDA was ionised in positive-ion mode and 4-HNE in negative-ion mode, and the quantification limits were respectively 0.1 and 0.04 nM.

Exposure assessment

The FCS air monitoring data were provided by the company's occupational physician. The UNI 10568 method was used to quantify FCS in airborne powders by means of fixed and personal samplers. In both cases, the aspirated air was filtered through a PVC membrane, and the line was equipped with a stabiliser and flow control dumper. The weighted white filter was prepared at known reference concentrations (silicon dioxide, SiO_2) and FCS was determined gravimetrically after X-ray diffraction by subtracting the absolute weight. The volume-related curve indicates the concentration in the sample. Monitoring continued throughout the duration of the working shift.

The sampling could be considered totally representative of normal working conditions. The survey was conducted by isolating the individual operations and verifying that the workers worked on the investigated task throughout the duration of the sampling. The workers were divided into those that were not exposed (FCS exposure <1/10 of the TLV ACGIH value, <0.0025 mg/m^3), those with low exposure (FCS exposure between 1/10 of the TLV ACGIH value and the TLV ACGIH value, 0.0025-0.025 mg/m^3), and those with high exposure (FCS exposure above the TLV ACGIH value, >0.025 mg/m^3).

Statistical analysis and sample size calculation

The normality of the data was evaluated using Kolmogorov-Smirnov and Shapiro-Wilk tests and, when the results of these were statistically significant, the data are given as median values and interquartile ranges (IQRs). Mann-Whitney or Kruskal-Wallis non-parametric tests were used to make between-group comparisons; when the Kruskal-Wallis test was significant, Dunn's multiple comparison test was applied. The prevalence of the restrictive spirometry pattern was compared with the prevalence of the restrictive body plethysmography pattern. Correlations between the continuous variables were assessed using Spearman's rank correlation coefficient (ρ). A two-sided p value of <0.05 considered statistically significant. Logistic binary regression was used with restriction as the outcome and various predictors.

The prevalence of the restrictive respiratory pattern was ~20% (2, 20); the sample size was in line with the expected prevalence (12-31%) with a power of 80% and an α of 0.05. The data were statistically analysed using IBM SPSS v. 25 (IBM, Armonk, NY, USA) and Graphpad Prism v. 5.0 software (Graphpad, La Jolla, CA, USA).

Results

Table 1 shows the main anthropometric and clinical data of workers without restriction (48 subjects) and the same data relating to the 14 subjects with respiratory restriction (TLC<LLN) and normal FVC.

The spirometry and body plethysmography findings respectively indicated restrictive deficit prevalence rates of 1.6% and 22.6% (table 2), thus indicat-

Table 1 - Main characteristic of workers exposed to free crystalline silica (FCS) without respiratory restriction (No 48) and with respiratory restriction (No 14)

	Without respiratory restriction	With respiratory restriction
No. of workers	48	14
Males/Females	44/4	14/0
Age, years	46.2±9.6	47.4±8.2
Smokers, yes/no/ex-	24/20/4	6/4/4
Body mass index (BMI), kg/m^2	25.0 (22.3-28.0)	26.5 (24.0-30.3)
Duration of exposure, years	15.5±10.6	16.7±8.6
Degree of exposure[a], no/low/high exposure	11/30/7	3/10/1
Work-related respiratory diseases, yes/no	5/43	4/10
DLCo/Va(mL/mmHg/min/L	4.54 (3.98-4.93)	4.73 (4.11-5.12)
DLCo/Va, percentage of predicted (DLCo/Va %)	100.0 (88.0-109.0)	106.5 (93.5-116.3)
RV (L)	1.38 (1.16-1.57)	0.98 (0.82-1.16)
RV, percentage of predicted (RV %)	65.0 (54.0-80.0)	46.0 (38.0-53.5)
FRC (L)	3.03 (2.57-3.65)	2.38 (2.06-2.71)
FRC, percentage of predicted (FRC%)	92.1 (75.8-105.5)	68.0 (59.8.-73.5)

Age and duration of exposure: mean value±SD; BMI, D_{LCO}/Va, D_{LCO}/Va%, RV, RV%, FRC and FRC%: median values and interquartile ranges (IQRs)

[a]The degree of exposure is based on air monitoring data provided by the company, with the workers being divided into: those with no exposure (exposure to FCS <1/10 of TLV ACGIH, <0.0025 mg/m^3), those with little exposure (exposure to FCS between 1/10 of TLV ACGIH and TLV ACGIH, 0.0025-0.025 mg/m^3) those with much exposure (exposure to FCS >TLV ACGIH, >0.025 mg/m^3)

ing the poor sensitivity (7%) and accuracy (79%) of spirometry in highlighting restriction or suspected restriction. Only one case of spirometric suspicion was confirmed by body plethysmography.

Workers with and without restriction were not distinguished by the degree and duration of exposure, number of cigarette pack-years, BMI and age.

Ten of the 14 workers with restrictive patterns were classified as being at low risk of exposure to FCS, three were not exposed, and only one was considered at high risk.

On the basis of the air monitoring data provided by the company's occupational physician, it was estimated that the cumulative level of FCS exposure was 0.03 mg/m^3-year.

Table 3 shows the anthropometric and body plethysmography data of the workers by level of exposure. In comparison with the predicted values, DLCO values were low in five workers (three exposed to FCS and two not exposed) in the absence of any other respiratory function abnormalities.

A statistically significant association between exposure to FCS and the occurrence of restrictive patterns did not emerge (p=0.74). Comparing TLC values with the exposure levels to FCS (no exposure, low and high exposure), statistically significant differences were not found between these three groups (table 3). Most of the workers with a restrictive deficit were classified as being subject to a low level of exposure. DLCO/Va values also did not seem to be influenced by exposure levels (table 3). Moreover, there was no statistically significant difference between the considered ranges of exposure duration (1-16 years and 17-39 years) and TLC values (p=0.852, Mann-Whitney U test).

None of the workers were obese (all had a body mass index [BMI] of <30 kg/m^2): the median BMI of the workers with a restrictive pattern was 26.5

Table 2 - Spirometry *vs* body plethysmography

	No. of workers with a restrictive pattern/ total number	No. of workers with an obstructive pattern/ total number	No. of workers with a normal pattern/ total number
Spirometry	1/62 (1.6%)	3/62 (4.8%)	58/62 (93.5%)
Body plethysmography	14/62 (22.6%)	3/62 (4.8%)	45/62 (72.6%)

Frequencies shown in brackets

Table 3 - Anthropometric and plethysmographic data by degree of exposure

(No. of workers)	No exposure (14)	Low exposure (40)	High exposure (8)	Kruskal-Wallis test
Age, years	45.0 (39.5-54.8)	44.5 (40.0-55.0)	50.5 (40.5-56.3)	p=0.926
BMI, kg/m^2	25.0 (23.8-27.8)	27.0 (23.0-29.8)	24.5 (22.5-25.0)	p=0.648
Pack-years	0.0 (0.0-12.5)	10.5 (0.0-26.5)	0.0 (0.0-16.5)	p=0.101
Duration of exposure, years	13.5 (6.0-26.0)	15.5 (9.0-21.0)	19.5 (5.3-35.8)	p=1.00
FVC (L)	5.0 (4.4-5.2)	5.0 (4.5-5.4)	4.7 (3.9-5.0)	p=0.671
FVC, percentage of predicted, FVC%	111.5 (96.5-120.5)	108.0 (99.3-114.0)	115.0 (99.3-132.0)	p=0.686
FEV_1 (L)	3.9 (3.1-4.2)	3.8 (3.4-4.3)	3.6 (3.0-4.1)	p=0.993
FEV_1, percentage of predicted, FEV_1%	97.0 (91.5-116.0)	99.0 (91.3-110.0)	109.5 (99.3-118.3)	p=0.807
TLC (L)	6.3 (5.6-6.9)	6.4 (5.8-7.0)	6.3 (4.6-7.0)	p=0.827
TLC, percentage of predicted, TLC%	94.0 (83.8-103.3)	92.0 (83.0-101.0)	94.0 (86.0-110.3)	p=0.717
D_{LCO}/Va (mL/mmHg/min/L)	4.29 (3.92-5.02)	4.65 (4.13-4.99)	4.69 (3.87-5.20)	p=0.713
D_{LCO}/Va, percentage of predicted, DLCO/Va%	97.0 (88.0-107.5)	102.5 (90.3-109.8)	110.0 (77.5-119.8)	p=0.817

Median values and interquartile ranges (IQRs)

(IQR 24.0-30.3) kg/m^2, and the median BMI of those without restriction was 25 (IQR 22.3-28.0) kg/m^2 ($p=0.09$).

DLCO and DLCO/Va values inversely correlated with smoking habits expressed in terms of pack-years (DLCO: $\rho=-0.3$ and $p=0.03$; DLCO/Va: $\rho=-0.51$ and $p<0.001$), with the DLCO/Va values being statistically different between smokers and non-smokers ($p=0.001$).

There were no statistically significant differences in terms of the respiratory function parameters of FVC, FEV_1, TLC, DLCO and EBC 4-HNE levels between smokers and non-smokers (including ex-smokers) (Mann-Whitney U test).

The spirometry and body plethysmography data were also compared using three of the most widely used diagnostic algorithms in order to identify which of these was most likely to predict restriction on the basis of spirometry data alone. Using the algorithm suggested by Glady (18) (FVC <85% and FEV_1/FVC ≥55%), an FVC of <85% was detected in only two workers, and the model proposed by the ATS/ERS (31) (FVC <LLN and FEV_1/FVC ≥LLN) identified only one worker with suspected restriction, as did the latest model proposed by De Matteis et al. (13) (FVC <70% and FEV_1/FVC ≥70%).

EBC biomarkers did not correlate with a restrictive deficit (table 4). When the data were stratified by the degree of exposure (none, low and high), there were no statistically significant differences in the biomarkers of oxidative stress (H_2O_2, 8-isoprostane, MDA) (table 5). However, EBC H_2O_2 values distinguished smokers and non-smokers ($p=0.01$); 8-isoprostane significantly correlated with MDA ($\rho=0.3$, $p=0.02$) and 4-HNE ($\rho=0.4$, $p=0.002$); and 4-HNE distinguished the workers who were low exposed from those who were high exposed ($p=0.0046$) (table 5), but was not sufficiently sensitive to diagnose a restrictive deficit. Cigarette smoking did not affect EBC 4-HNE concentrations.

Discussion

This study evaluated the capacity of spirometry (primary endpoint) and oxidative stress biomarkers (at exploratory level) to detect functional restrictive lung changes in workers engaged in extracting

Table 4 - EBC biomarkers of oxidative stress

EBC biomarkers	Restrictive pattern (14 workers)	Non-restrictive pattern (48 workers)	Mann-Whitney U test
Hydrogen peroxide (H_2O_2), µM	0.28 (0.20-0.38)	0.33 (0.22-0.38)	p=0.506
Malondialdehyde (MDA), nM	2.20 (1.51-2.71)	1.61 (1.28-2.30)	p=0.114
8-isoprostane (8-ISO), pg/ml	4.13 (3.13-5.83)	3.22 (1.35-5.42)	p=0.227
4-hydroxynonenale (4-HNE), nM	0.43 (0.35-0.53)	0.43 (0.35-0.53)	p=0.987

Median values and interquartile ranges (IQRs)

Table 5 - EBC biomarkers of oxidative stress

EBC biomarkers	No exposure (14 workers)	Low exposure (40 workers)	High exposure (8 workers)	Kruskal-Wallis test
Hydrogen peroxide (H_2O_2), µM	0.31 (0.24-0.42)	0.33 (0.21-0.37)	0.25 (0.19-0.38)	p=0.592
Malondialdehyde (MDA), nM	1.49 (1.27-2.43)	1.61 (1.29-2.37)	2.23 (1.72-2.58)	p=0.096
8-isoprostane (8-ISO), pg/ml	3.28 (2.41-6.03)	3.61 (1.35-4.83)	3.38 (2.94-6.92)	p=0.259
4-Hydroxynonenale (4-HNE), nM	0.44 (0.37-0.49)	0.39 (0.31-0.47)	0.54 (0.50-0.57)	p=0.0046*

Median values and interquartile ranges (IQRs).

*When the Kruskal-Wallis test was statistically significant, Dunn's multiple comparison test was applied: the statistically significant difference was between 4-HNE little exposure and 4-HNE much exposure ($p<0.05$).

and processing clay, and therefore exposed to FCS, which is known to cause lung cancer (24, 27) but can also cause benign lung diseases (7, 41).

The lung function data showed that the prevalence of restrictive and obstructive disease was respectively 22.6% and 4.8%. Other studies carried out in the West indicate a stable general population prevalence of restrictive disease of 10-15% (20), but many of them also indicate a comparable prevalence of obstructive disease, whereas we found that the prevalence of restrictive disease was almost five times as high as that of obstructive disease. This could have been due to the risk factor of occupational exposure to FCS, but it is worth noting that, although 44% of the workers with work-related respiratory symptoms showed a restrictive pattern, most of them were classified as being low exposed (table 1).

Studies have found that the cumulative exposure level associated with the incidence of lung cancer or mortality varies from 0.026 mg/m^3 a year to 5 mg/m^3 a year (29), but there are no data specifically related to restrictive disease. Three workers with a restrictive deficit were classified as not exposed (table 1), but they had previously worked in the production department where the levels of FCS were probably higher.

There was no statistically significant difference in TLC values between the workers exposed for 1-16 years and those exposed for 17-39 years (p=0.852, Mann-Whitney U test), which suggests that even the workers who had been working for almost forty years were not at greater risk of developing restrictive respiratory disorders.

Smoking plays an important role in inflammation and the maintenance of airway inflammatory processes. We found that DLCO and DLCO/Va inversely correlated with smoking habits expressed as the number of pack-years. This is in line with the findings of Gläser et al. (19) who showed that smoking is related to impaired DLCO in a general population of subjects aged 25-85 years.

It is known that DLCO is the parameter that changes earliest in the presence of an inflammatory process (19) but, in order to assess the integrity and efficiency of gas transfer, it must be standardised in relation to alveolar volume. The DLCO/Va ratio of all 62 workers within the normal range but, although smoking was not a statistically significant covariate in relation to respiratory function parameters (FVC, FEV_1, TLC and DLCO), it did distinguish smokers and non-smokers.

Table 1 shows the 14 workers with respiratory restriction and a normal FVC. They had reduced RV and FRC. Six were smokers and four ex-smokers, but smoking did not distinguish the workers with and without restriction.

Clay et al. have recently found that patients with complex restriction (a pattern in which the predicted percentage FVC is disproportionately reduced in relation to TLC) may have a body mass index (BMI) of >40 kg/m^2) (5). BMI is an important confounder when considering TLC (16, 22). In line with the general population findings of Wan et al. (48), most of our workers with restriction who were exposed to FCS as a result of handling clay had a high BMI, but BMI did not distinguish those with and without restriction, and none of our workers were obese because their BMI was lower than 30 kg/m^2. The question is complex because the majority of obese subjects in the Third National Health and Nutrition Examination Survey (33) and the Burden of Lung Disease studies (34) had normal lung function. Wan et al. (48) have reported that the prevalence of a restrictive pattern in the United States has remained relatively stable despite the increasing prevalence of obesity, and so restriction is unlikely to be a simple "epiphenomenon" of the condition (20). However, our findings confirm that BMI is an important covariate in subjects with a work-related restrictive pattern.

Only one of the 14 cases diagnosed on the basis of body plethysmography results had a spirometry pattern that raised the suspicion of restriction (table 2). The published diagnostic algorithms based on spirometry data do not seem to be as useful as body plethysmography as their sensitivity is poor (7%) and they are significantly less accurate (79%) in highlighting restriction or a suspected restrictive pattern (1, 18, 28, 47).

Subjects with a restrictive lung deficit are at highest risk of developing respiratory symptoms (23) and chronic diseases such as diabetes (46) and the metabolic syndrome (15), all of which may be related to increased mortality (23). These aspects should therefore also be investigated in the case of work-related restrictive respiratory impairment.

Among the EBC biomarkers of oxidative stress, 4-HNE seemed to be the only one capable of discriminating low and high exposure (table 5), but it did not seem to be sufficiently sensitive to diagnose restrictive deficit (table 4). Oxidative stress in lung lining fluid is probably the first of the initial changes in respiratory function in workers exposed to pneumotoxic compounds, particularly FCS (38), and its presence was confirmed by the ability of EBC hydrogen peroxide concentrations to distinguish smokers from non-smokers.

Specific studies of a larger sample of workers exposed to FCS are therefore required to establish whether 4-HNE can be used as an early and non-invasive biomarker of restriction.

Although there were no significant differences in 8-isoprostane levels between workers with or without restriction or between those exposed to a higher or lower extent, there was a significant correlation with MDA (Spearman's ρ=0.3, p=0.02) and HNE levels (ρ=0.4, p=0.002), thus demonstrating the involvement of these mediators in oxidative damage (37).

None of the EBC biomarkers was particularly specific in identifying subjects who already had restrictive changes (table 3), but it would be worth further investigating this biological matrix, which is in direct contact with the lung, in subjects exposed to FCS and other breathable powders whose main absorption pathway is respiratory.

Despite the limited size of the study population (it was not possible to increase recruitment because of the niche nature of clay processing in our region), it was sufficient in terms of the first study endpoint comparing the efficacy of spirometry and plethysmography in diagnosing restriction.

Conclusions

Spirometry was not sufficiently sensitive to diagnose respiratory restriction in workers exposed to FCS. Furthermore, although it did not seem to be sufficiently sensitive to detect a restrictive deficit, EBC 4-HNE may be a useful marker of the extent of FCS exposure. The early detection of even minimally restrictive respiratory changes is a key preventive measure because such changes can still be treated by increasing personal and environmental prevention, and because it is very important in ensuring timely management in occupational health settings.

Ethical approval: The study was approved by the Institutional Review Board (IRB) of the University of Parma (Prot. No. 0007/2017)

No potential conflict of interest relevant to this article was reported by the authors

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