



Artificial stone dust-induced functional and inflammatory abnormalities in exposed workers monitored quantitatively by biometrics

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ABSTRACT The manufacture of kitchen and bath countertops in Israel is based mainly on artificial stone that contains 93% silica as natural quartz, and ~3500 workers are involved in cutting and processing it. Artificial stone produces high concentrations of silica dust. Exposure to crystalline silica may cause silicosis, an irreversible lung disease. Our aim was to screen exposed workers by quantitative biometric monitoring of functional and inflammatory parameters.

68 exposed artificial stone workers were compared to 48 nonexposed individuals (controls). Exposed workers filled in questionnaires, and all participants underwent pulmonary function tests and induced sputum analyses. Silica was quantitated by a Niton XL3 X-ray fluorescence spectrometer.

Pulmonary function test results of exposed workers were significantly lower and induced sputa showed significantly higher neutrophilic inflammation compared to controls; both processes were slowed down by the use of protective measures in the workplace. Particle size distribution in induced sputum samples of exposed workers was similar to that of artificial stone dust, which contained aluminium, zirconium and titanium in addition to silica.

In conclusion, the quantitation of biometric parameters is useful for monitoring workers exposed to artificial stone in order to avoid deterioration over time.



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Introduction

Silicosis is a fibrotic lung disease initiated by prolonged and extensive exposure to respirable free crystalline silica. Occupations traditionally associated with increased risk of silicosis include glass and pottery making, mining and quarrying, sandblasting, and any construction trades that generate silica dust through stone or concrete work. A high prevalence of advanced, life-threatening silicosis linked to extensive use of artificial stone with high silica content in the marble industry was reported in Israel [1]. Similar outbreaks of occupational silicosis have been reported in Spain, Italy and, more recently, in Australia and the USA [2–6]. The artificial stone is a composite material made of quartz as the major filler, with the addition of coloured glass, shells, metals or mirrors bound together by a polymer resin. Environmental exposure metrics defined the permissible exposure limits for respirable silica as varying between 0.025 and 0.35 mg·m⁻³ [7], but these standards have not been confirmed as being fully protective by epidemiology studies. Environmental monitoring is carried out by measuring the number of toxic particles in the air of a given location. As such, it ignores the pollutant's internal dose (body burden) at the personal level, the route of exposure (e.g. inhalation) and the markers of early adverse health effects (e.g. inflammatory responses). The effect of particle deposition in the lungs and its toxicity by means of the inflammatory effect calls for biometric monitoring in order to determine its impact on human health. An easily performed, noninvasive biological monitoring system would be of enormous value for the early detection of pulmonary injury and the screening of large populations of individuals exposed to noxious particulate matter in the work environment.

Induced sputum is a noninvasive method that has demonstrated the heterogeneity of airway inflammation in asthma, chronic obstructive pulmonary disease (COPD) and chronic cough [8]. In addition, several occupational and environmental studies have shown that quantitative and qualitative analysis of particles recovered by induced sputum can serve as a biological monitoring method in addition to the traditional sources of exposure history and environmental measurements [9–12].

The current gold standard for diagnosing silicosis is by chest radiography and pulmonary function tests (PFTs) [13]. Lung biopsies can also be used. However, a review that explored the relationship between PFTs and silicosis indicated that reduced lung function was more likely to be associated with higher International Labor Office radiographic categories of silicosis of the than with lower ones. No consistent pattern of association was observed with the latter, suggesting that PFTs are not sensitive enough to detect early lung injury among exposed populations [14].

We studied a population of individuals employed in the manufacture of kitchen and bathroom countertops made of an artificial raw material containing >90% free crystalline silica. In Israel, according to the Israeli Institute for Occupational Safety and Hygiene, the maximum exposure rate to SiO₂ dust is 0.1 mg·m⁻³ for respirable dust (≤7 μm) and 0.3 mg·m⁻³ for floating dust. Importantly, these standards refer to environmental exposure and ignore cumulative internal exposure.

We investigated here novel particulate deposition in airways and inflammatory biomarkers (*i.e.* particle size distribution and percentage of neutrophils in induced sputum samples) retrieved by noninvasive procedures to screen a large population of workers exposed to artificial stone. Our aim was to apply sensitive biological markers for the early identification of workers at high risk of developing advanced stages of silicosis.

Methods

Study design and study population

116 individuals working mainly with artificial stone dust up to the last 20 years were recruited from small enterprises throughout the country. Recruitment was by physician referral, direct contact with marble factories and self-referral. Over one-third (36.8%) of the exposed workers had no previous diagnosis of silicosis and 63.2% were known to have silicosis on the day of evaluation. Ethical approval was granted by the Institutional Ethics Committee in Tel Aviv Sourasky Medical Center. All participants gave written informed consent, and paid one visit for clinical assessment by PFT and sputum induction. The controls were healthy, nonexposed individuals from the community who volunteered to undergo PFTs and sputum induction.

Occupational questionnaire and exposure assessment

A self-reported occupational questionnaire was completed by all exposed workers. The questionnaire was validated by the Epidemiological and Preventive Medicine Dept in the Tel Aviv University School of Public Health. It included demographic and occupational parameters, smoking and alcohol habits, a general health profile, respiratory protective measures applied in the workshop (water cutting machine and dust ventilation system), and personal protective equipment (mask, goggles, gloves, special footwear and cloths).

Pulmonary function tests

PFTs were performed using a Masterlab spirometer (Masterlab E; Jaeger, Wurzburg, Germany). Measurements were carried out according to standard protocols of the American Thoracic Society guidelines [15].

Sputum induction and processing

Sputum induction and processing were performed with an aerosol of hypertonic saline generated by an Ultrasonic Nebulizer, model Omron U1 (Omron Health Care, Lake Forest, IL, USA), that has an output of $0.5 \text{ mL}\cdot\text{min}^{-1}$ and particles with $<5 \mu\text{m}$ aerodynamic mass median diameters [16, 17].

Particle analyses: size and shape

Particle size analyses were performed according to a laser technique based on the time of transition theory using a DIPA 2000 Analyzer (Donner Technologies Ltd, Israel) [18, 19]. The experiment was repeated three times in three different tubes (a total of nine measurements). The particle measurement was performed long enough to reach 95% confidence intervals. The results of the particle size distribution were the average of three sequential measurements. The results were expressed as cumulative values for each fraction of particles.

The curve of the particulate matter retrieved from induced sputum samples that represented the raw material (*i.e.* the artificial stone collected from one of the factories) to which the stone workers were exposed was superimposed over the curve of the artificial stone dust analysis.

Quantification of mineral content using an X-ray fluorescence spectrometer analyser

Induced sputum samples were obtained from three exposed workers randomly chosen from high-quality sputum specimens and three nonexposed individuals from the control group. After separation of the plugs and viscous materials, all fractions of induced sputum were preserved in 10% formalin and stored at 4°C until analysis of mineral particles. We used the samples containing both extracellular and intracellular particles for the X-ray fluorescence (XRF) analysis. The samples were treated with 14% formamide solution and filtered onto a $0.8\text{-}\mu\text{m}$ carbon-coated Nuclepore filter (Millipore Filter Corp., Bedford, MA, USA). Each filter was scanned twice in different areas by a Niton XL3 XRF analyser (Thermo Fischer Scientific, Munich, Germany) and the average of two repeated measurements of three subjects for each group was calculated. Artificial and natural stone dust samples were obtained at two plants, each involved exclusively in one type of stone work.

Statistical analysis

Comparisons between groups were performed by an independent t-test, the Kruskal–Wallis test and the Chi-square test. Correlation between particles $<5 \mu\text{m}$ and continuous variables was assessed by Spearman correlation. The percentages of particles ≤ 5 and $\leq 10 \mu\text{m}$ were divided into dichotomy parameters by a receiver operating characteristic (ROC) curve at the point with the highest sensitivity and specificity that differentiates between subjects with or without neutrophilic inflammation. Associations between the use of protective methods (yes or no) and various covariates were tested by the Fisher exact test for categorical variables. All statistical analyses were performed using the SPSS software version 22.0 for Windows (SPSS, Chicago, IL, USA). All p-values are two-sided and a p-value <0.05 was considered significant.

Results

The study population included 116 males (68 artificial stone-exposed workers and 48 individuals with no occupational exposure history). 32 were past smokers and none was a current smoker. The exposed workers were older (mean \pm SD 48.6 ± 11.4 versus 38.0 ± 17.1 years, $p<0.01$) and had a higher body mass index (27.0 ± 4.0 versus 23.7 ± 2.8) than the nonexposed controls (table 1).

The PFT scores of the exposed workers were significantly lower than those of the controls (table 2). The exposed workers had a higher percentage of neutrophils in their induced sputum samples ($68.6\pm 22.6\%$ versus $46.7\pm 18.8\%$, respectively; $p<0.001$), and a lower percentage of macrophages and eosinophils ($16.8\pm 18.9\%$ versus $30.4\pm 23.1\%$ and $1.3\pm 3.0\%$ versus $8.5\pm 3.6\%$, respectively; $p<0.001$) (table 2).

TABLE 1 Demographic and clinical parameters of the study population (n=116)

	Males/females	Age years	Exposure years	Body mass index
Exposed workers	68/0	48.3 ± 11.1	20.5 ± 10.0	27.0 ± 4.0
Nonexposed controls	32/16	38.0 ± 17.1	0	23.7 ± 2.8
p-value*	<0.01	<0.01		<0.01

Data are presented as mean \pm SD unless otherwise stated. *: $p<0.05$ for age and body mass index (t-test) and sex (Pearson Chi-squared test).

TABLE 2 Pulmonary function tests and differential cells counts in induced sputum

	Exposed workers	Nonexposed controls	p-value*
VC % pred	77.4±18.6 (n=68)	97.8±14.1 (n=35)	<0.01
TLC % pred	93.9±15.4 (n=68)	106.5±10.7 (n=35)	<0.01
FVC % pred	79.0±19.8 (n=68)	98.3±13.7 (n=44)	<0.01
FEV ₁ % pred	74.6±22.5 (n=68)	97.9±14.4 (n=45)	<0.01
FEV ₁ /FVC	76.0±12.1 (n=68)	85.5±8.9 (n=45)	<0.01
D _{LCO_{sb}} % pred	75.8±17.6 (n=66)	91.4±11.0 (n=36)	<0.01
Neutrophils %	68.6±22.6 (n=59)	46.7±18.8 (n=41)	<0.01
Macrophages %	16.8±18.9 (n=59)	30.4±23.1 (n=42)	<0.01
Eosinophils %	1.3±3.0 (n=59)	8.5±13.6 (n=42)	<0.01
Lymphocytes %	12.5±9.1 (n=59)	12.8±6.9 (n=42)	0.859

Differential cell counts were adjusted for sex and age. VC: vital capacity; TLC: total lung capacity; FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 s; D_{LCO_{sb}}: single-breath diffusing capacity of the lung for carbon monoxide. *: p<0.05 (independent t-test).

By superimposing the curves of the particle size distribution of raw material and the induced sputum samples, we demonstrated that they both had a similar distribution pattern. The particles derived from the exposed group's samples contained a significantly higher fraction of small particles (<5 µm) and a lower percentage of particles between 5 and 10 µm than the control group (94.0±5.6% versus 89.4±4.7% (p<0.01) and 4.3±3.7% versus 6.2±4.6% (p=0.025), respectively) (tables 3 and 4, and figure 1).

The use of dust protection measures (masks, water cutting machines, ventilation and cloths) decreased the percentage of accumulated particles in the airways for all sizes tested (61.6±11.8% versus 69.80±10.7%, 92.2±5.0% versus 95.5±5.8% and 97.8±1.5% versus 98.7±2.5% for <2 µm, <5 µm and <10 µm in protected versus unprotected workers, respectively (p<0.01)) (table 3). The particles retrieved from induced sputa of the exposed group were significantly smaller, less spherical and less smooth, more concave and more opaque than those retrieved from the unexposed group (table 4). Moreover, they were significantly changed by the use of personal protective precautions.

We performed a quantitative analysis using the XRF instrument of the particles retrieved from the induced sputa of three exposed workers and three nonexposed controls, all randomly chosen, and compared the results to the raw artificial and the natural stone dust. The analysis showed that both the artificial stone and the induced sputum samples of exposed workers contained significantly higher concentrations of silica (5739.6 ppb versus below the limit of detection in induced sputum and 647 603.4 versus 4041.9 ppb in the raw material). Other metals were also identified (table 5).

The accumulation of particles <5 µm negatively correlated with single-breath diffusing capacity of the lung for carbon monoxide (D_{LCO_{sb}}), forced expiratory volume in 1 s and forced vital capacity (FVC), and positively correlated with neutrophil counts in the induced sputum specimens (figure 2).

Having demonstrated that neutrophils in the induced sputa of exposed workers were significantly more numerous than those from the controls and that their percentage was positively correlated with particle

TABLE 3 Comparison of size of particulate matter in the study population according to use of personal protective precautions

Particle size µm	Exposed workers (n=58)	Nonexposed controls (n=44)	p-value	Protection*		p-value
				Yes (n=27)	No (n=30)	
<2	66.0±11.8	58.0±21.5	<0.01	61.6±11.8	69.80±10.7	0.01
<5	94.0±5.6	89.4±4.7	<0.01	92.2±5.0	95.5±5.8	<0.01
<10	98.3±2.1	95.4±4.3	<0.01	97.8±1.5	98.7±2.5	<0.01
5-10	4.3±3.7	6.2±4.6	0.025	5.6±3.7	3.2±3.4	0.013

Data are presented as mean±SD % unless otherwise stated. Protective measures applied in the workshop and personal protective equipment are described in the Methods section. Sizes measured by image analysis as described in the Methods section. The use of protection means was evaluated by the questionnaire. *: p<0.05 (t-test) for dust exposure and Kruskal-Wallis for protection method.

TABLE 4 Comparison of shape of particulate matter in the study population according to use of personal protective precautions

	Exposed workers (n=29)	Nonexposed controls (n=40)	Protection*		p-value
			Yes (n=13)	No (n=16)	
Grey level	85.0±1.0	114.8±29.9	85.3±1.0	84.8±1.0	0.254
Average Ferret diameter μm	3.6±0.7	4.4±1.7	3.15±0.32	3.90±0.84	0.014
Circularity	0.46±0.02	0.53±0.1	0.47±0.02	0.45±0.01	0.084
Convexity	0.85±0.009	0.87±0.03	0.853±0.01	0.847±0.008	0.066
Concavity	0.84±0.006	0.86±0.03	0.841±0.006	0.836±0.005	0.031

Data are presented as mean±sd unless otherwise stated. Protective measures applied in the workshop and personal protective equipment are described in the Methods section. Sizes measured by image analysis as described in the Methods section. The use of protection means was evaluated by the questionnaire. *: p<0.05 (t-test) for dust exposure and Kruskal-Wallis for protection method.

accumulation results, we analysed which threshold of accumulated particles had the best specificity and sensitivity to predict neutrophilic inflammation (>65%). The ROC curve showed a sensitivity of 60% and a specificity of 63% for the accumulation of 91.93% particles <5 μm , and a sensitivity of 88% and a specificity of 38% for the accumulation of 95.17% particles <10 μm . These thresholds represented a significant difference in neutrophil counts among the two groups, with a trend towards neutrophilic inflammation for the group with more particles <5 μm and <10 μm in induced sputum (64.4±24.9% and 65.7±25.2%, respectively; p<0.05) (table 6).

Notably, the unprotected workers also had an increased risk of having more particles <5 μm and <10 μm in induced sputum samples, as well as neutrophilic inflammation and DLCO_{sb} impairment, compared to the protected group (table 7). The accumulated amounts of particles and neutrophils in induced sputum samples over time was higher in the exposed workers who reported not having used any kind of protection compared to those who did so regularly. Their DLCO_{sb} also declined more rapidly (Chi-squared test $r=3.976$, $p=0.046$) (figure 3).

Discussion

The diagnosis of silicosis is traditionally based on clinical history and imaging findings [7, 13, 20]. Although they are not used as diagnostic tools, PFTs are widely employed in the assessment of individuals with silicosis in order to evaluate functional parameters of the lung [21]. However, none of these methods is sensitive enough to detect changes at an early stage that might point to the onset of tissue damage. We had previously shown that abnormal radiological findings of lung pathology were present without any comparable evidence in functional parameters in a group of dental technicians [12]. Similar findings were

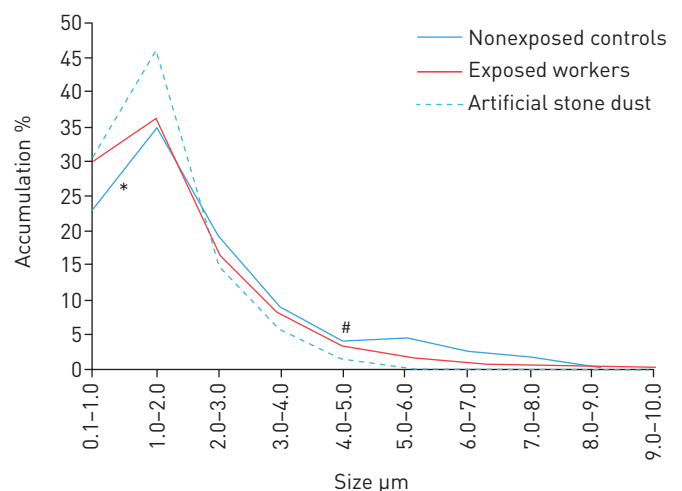


FIGURE 1 Accumulation of particulate matter in induced sputum in the exposed and nonexposed (control) workers, and in the raw material. Particle size was assessed by a DIPA analyser, as described in the Methods section. *: p<0.05 (independent t-test) for accumulated particles 5–6 μm ; #: p<0.05 (independent t-test) for accumulated particles 0.1–1 μm .

TABLE 5 Chemical analysis by X-ray fluorescence in induced sputum particles retrieved from exposed workers and from raw material dust

	Nonexposed controls	Exposed workers	p-value	Natural stone	Artificial stone
Zinc	<LOD	160.2±97.8	<0.01	<LOD	1233.7±24.78
Copper	178.4±16.6	216.8±58.1	0.217	<LOD	57.8±13.92
Iron	293.4±59.6	1479.2±760.1	0.012	507.260.34	3574.0±80.8
Titanium	<LOD	603.6±268	0.064	<LOD	41412.2±255.7
Calcium	4522±811.2	19321.2±10151	0.016	546368.6±309	9058.2±245.9
Aluminium	<LOD	3192.7±2052	<0.01	899.1±170.3	20146.9±430.2
Silica	<LOD	5739.6±1577	<0.01	4041.9±102	647603.4±1198
Chloride	517548.7±136374	18634.9±7005	<0.01	<LOD	13167.5±96.7
Sulfur	153316.3±57024	434674.1±213233	0.022	794.8±41.7	3350.4±89.7

Data are presented as the mean±SD of two measurements in three subjects for each group. LOD: limit of detection. p<0.05 (independent t-test).

reported in 105 dental technicians in Paris, France, whose PFT values (normal) did not correlate to the duration of exposure [21], and in silicotic stonemasons in Brazil who had lower (but normal) values of FVC and total lung capacity compared to co-workers without evidence of silicosis [22]. Consistent with those results, we currently found lower PFT parameters among the exposed workers compared to controls but within normal ranges for all tested parameters except $DLCO_{sb}$.

These functional parameters are clearly not sensitive enough to alert to impending lung injury. We demonstrate here what we believe to be novel biomarkers capable of identifying populations at high risk of advanced inflammatory and functional deterioration as the result of continuing exposure to artificial stone dust. Induced sputum samples in exposed workers showed neutrophilic inflammation compared to nonexposed controls. The biomarkers also provided evidence of the benefit of personal protection in potentially hazardous workplaces.

Our findings are in accordance with those from previous studies performed in our laboratory in which the neutrophil counts in induced sputa were shown to correlate to exposure duration and disease severity in welders [9], dental technicians [12] and firefighters that were on duty during the World Trade Center

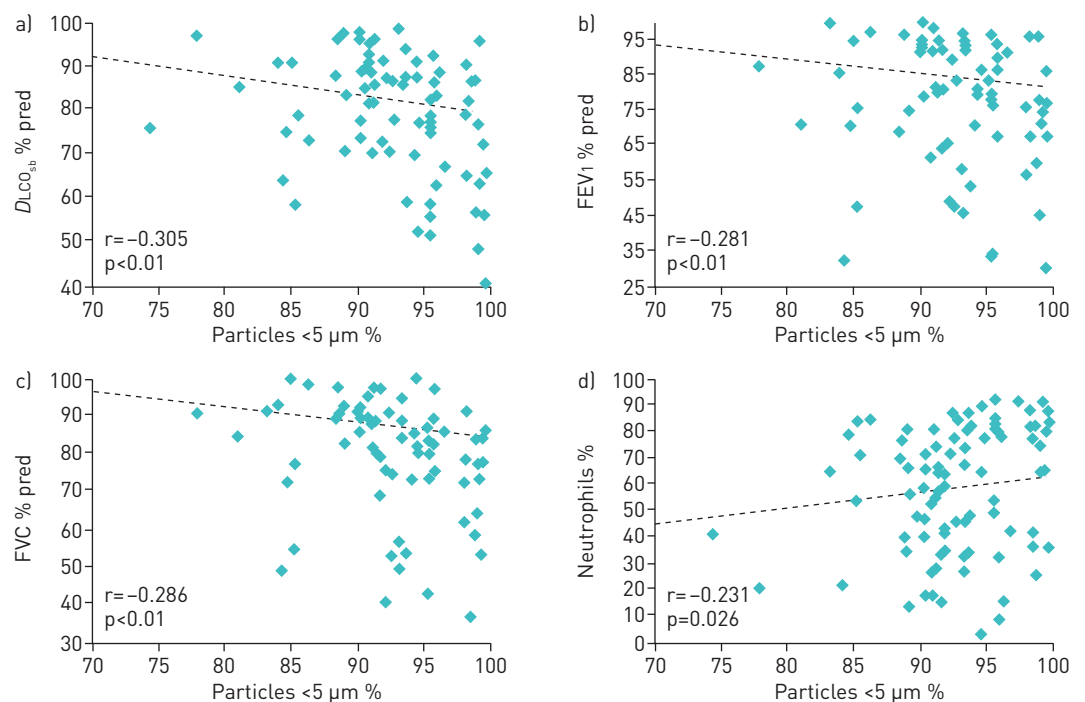


FIGURE 2 Correlation between particles in induced sputum <5 μm and a) single-breath diffusing capacity of the lung for carbon monoxide ($DLCO_{sb}$), b) forced expiratory volume in 1 s (FEV_1), c) forced vital capacity (FVC) and d) induced sputum neutrophil count. p<0.01 (Spearman correlation).

TABLE 6 Correlation between induced sputum neutrophils and particles <5 µm and <10 µm

	Neutrophils % mean±sd
Particles <5 µm[#]	
≥91.93%	64.4±24.9 (n=48)
<91.93%	52.4±21.1 (n=45)
Particles <10 µm[¶]	
≥95.17%	65.7±25.2 (n=64)
<95.17%	55.5±22.6 (n=29)

[#]: p=0.014 (independent t-test); [¶]: p=0.055 (independent t-test).

collapse [10]. The utility of neutrophil counts in induced sputum samples was demonstrated in other pulmonary disorders as well. For example, sputum neutrophil counts were associated with more severe asthma phenotypes using cluster analysis [23]. Additionally, eosinophilic and neutrophilic counts in induced sputum samples could differentiate between asthma, COPD and asthma-COPD overlap syndrome [24].

The ability of particles to penetrate the respiratory system may be one of several factors relevant to the development of silicosis. Although many studies have found associations between ambient particulate matter concentrations and health effects in occupational exposure to crystalline silica [7, 13], none estimated the internal personal-level burden by means of biological monitoring. We now demonstrated that most of the particles in the airways showed a distribution pattern similar to that of the raw artificial stone material, and that there was a significantly higher fraction of small particles <5 µm and a lower fraction of particles between 5 and 10 µm in the exposed group compared to the nonexposed control group. It is well known that penetration of inhaled particles into the thorax is dependent on their deposition in the upper respiratory tract during inspiration, which varies with particle size, flow rate and tidal volume, as well as *in vivo* airway dimensions [25]. The fraction of small particles was shown to be a more dangerous one, with intratracheal instillation of ultrafine particles leading to a significantly greater pulmonary inflammatory response than larger-sized particles [26]. Fine particles that penetrate into the interstitium will make contact with interstitial macrophages and other sensitive cell populations, and this is likely to have a powerful inflammogenic effect that underlies the development of subsequent disease [27].

Furthermore, we found that the shape of the particle in the airways of exposed workers was significantly different compared to the nonexposed controls. In a recent review dealing with the potential biological effects of very small particles, the authors highly recommended that the shape, crystal phase, dispersion, surface coating and chemical composition should also be well characterised [28]. Our data indicated that a cut-off at 91.93% of particles <5 µm in size is associated with the risk of having neutrophilic inflammation (neutrophils >65%) and $DLCO_{sb}$ impairment (<80%). The association between particle size, decreasing $DLCO_{sb}$ and high neutrophil counts may reflect the highly sensitive ability of these novel biomarker parameters to show early pathological changes in the lung in these exposed workers.

We had earlier reported that the use of masks and hoods and the type of exposure yielded differences in the shape of induced sputum particles of dental technicians working with any kind of protection compared to those without protection [12]. We show here that unprotected workers had an increased risk of having more particles <5 µm and <10 µm in induced sputum samples, neutrophilic inflammation and $DLCO_{sb}$ impairment compared to the group who regularly used personal protection. Direct impairments of PFT have been shown to correlated with efficient use of personal protection equipment in iron foundry

TABLE 7 Odds ratios for associations between the use of personal dust protection and neutrophil accumulation and single-breath diffusing capacity of the lung for carbon monoxide ($DLCO_{sb}$) decline

	OR (95% CI)	p-value [#]
Particles <5 µm >91.93%	7.0 (1.3–36.2)	0.017
Particles <10 µm >95.17%	7.8 (2.4–25.5)	0.001
Neutrophils >65%	1.382 (0.4–4.2)	0.777
$DLCO_{sb}$ <80% pred	1.471 (0.5–4.0)	0.613

[#]: significance was set at p<0.05.

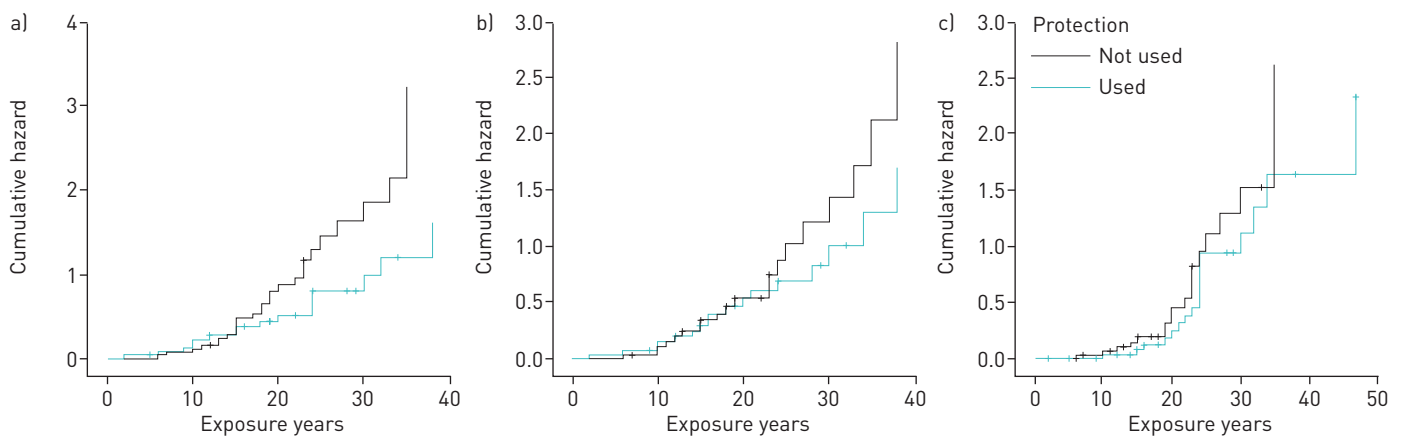


FIGURE 3 Hazard ratio for a) accumulation of particulate matter (particles $<5\ \mu\text{m}$), b) neutrophilic inflammation, and c) decreased single-breath diffusing capacity of the lung for carbon monoxide following years of exposure according to the use of personal protective measures.

workers [29] and in agate grinding workers exposed to silica [30]. To the best of our knowledge, this is the first study to document a direct effect of personal protection on internal exposure metrics in correlation to inflammatory parameters.

Our examination of the composition of the artificial stone material yielded interesting findings. Although it does contain mainly silica, we found that metals such as zirconium, titanium and aluminium were present in significantly higher percentages than their content in natural stone. Those metals are well known to induce sarcoid-like granulomatosis [31]. Importantly, the pattern of a high content of silica and metals contained in the material was reflected in the content of these metals in the induced sputum samples. This also goes along with the reported radiological presentation of many of the artificial dust-induced silicosis cases that mimic sarcoidosis [32].

This report also describes the first application of XRF technology for quantitating elements in biological samples. Previously used methods, such as scanning electron microscopy, only detect the chemical elements qualitatively [33].

There are some limitations to this study that need to be addressed. Our study population was relatively small, but they were all exposed to the same dust and so we believe that they are representative of workers in comparable workplaces. Another limitation was the lack of data on environmental monitoring, since these small enterprises are not regularly monitored; instead, we examined the settled dust, which turned out to have the same composition as the airborne particles. Another practical limitation is that sputum induction is a labour-intensive technique mainly performed in specialised centres by trained technicians. It is, however, a relatively inexpensive and noninvasive method that can be applied in high-risk, exposed populations.

We can conclude, however, that the novel functional and inflammatory biomarkers in induced sputum samples can effectively detect and monitor lung injury associated with artificial dust-induced silicosis. Finally, although the implementation of protective measures is highly recommended, they may only slow down the development of disease.

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