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Functional, inflammatory and interstitial impairment due to artificial stone dust ultrafine particles exposure

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ABSTRACT

Objective Artificial stone dust (ASD) contains high levels of ultrafine particles (UFP <1 µm) which penetrate deeply into the lungs. This study aimed to demonstrate the direct effect of UFP in the lungs of ASD-exposed workers on functional inflammatory and imaging parameters.

Methods 68 workers with up to 20 years of ASD exposure at the workplace were recruited from small enterprises throughout the country and compared with 48 non-exposed individuals. Pulmonary function test (PFT), CT, induced sputum (IS) and cytokine analyses were performed by conventional methods. The CT scans were evaluated for features indicative of silicosis in three zones of each lung. UFP were quantitated by the NanoSight LM20 system (NanoSight, Salisbury) using the Nanoparticle Tracking Analysis. Interleukin (IL)-6, IL-8 and tumour necrosis factor alpha (TNF-α) levels were measured by Luminex (R&D Systems).

Results Thirty-four patients had CT scores between 0 and 42, and 29 of them were diagnosed with silicosis. Content of the UFP retrieved from IS supernatants correlated negatively with the PFT results (total lung capacity r=−0.347, p=0.011; forced expiratory volume in 1 s r=−0.299, p=0.046; diffusion lung carbon monoxide in a single breath r=−0.425, p=0.004) and with the CT score (r=0.378, p=0.023), and with the inflammatory cytokines IL-8 (r=0.336, p=0.024), IL-6 (r=0.294, p=0.065) and TNF-α (r=0.409, p=0.007). Raw material of ASD was left to sedimentate in water for <15 min, and 50% of the floating particles were UFP. A cut-off of 8×10^6 UFP/mL in IS samples had a sensitivity of 77% to predict pulmonary disease.

Conclusions This is the first demonstration of an association between UFP-related decreased PFT results, worsening of CT findings and elevation of inflammatory cytokines, which may be attributed to high-dose inhalation of UFP of ASD at the workplace.

Key messages

What is already known about this subject?
- Advanced life-threatening silicosis linked to artificial stone dust (ASD) with high silica content was first reported in Israel in 2006.
- Exposure to this dust induced deleterious effects on the respiratory airways of exposed workers.

What are the new findings?
- This is the first report of workers exposed to ultrafine particles (UFP) that showed a possible association between UFP and poorer pulmonary function test results, worsening of findings on CT and elevated inflammatory cytokines.

How might this impact on policy or clinical practice in the foreseeable future?
- Our results stress the need for urgent action and for measures to control primary dust exposure together with health surveillance in order to decrease internal doses of UFP in the airways of the exposed population.
- These findings contribute to the development of a biological marker for UFP concentrations in the airways of workers exposed to ASD to serve as an index of a high risk to develop lung disease.

INTRODUCTION

Silicosis is a form of occupational pneumoconiosis caused by inhalation of crystalline silicon dioxide. This disease is one of several well-described pulmonary complications associated with toxic exposures in the workplace, along with asbestosis, berylliosis, coal miner's lung, hard metal pneumoconiosis and others.[1] Given that crystalline silica may be present in many workplaces and industries, silicosis emerges as a major work-related interstitial lung disease.[2] Natural marble, an artificial stone substitute, became commercially available in 1986, and the first retrospective series describing 25 silicosis cases was published in 2012 on patients who were admitted to the Israeli National Lung Transplantation Center between 1997 and 2010.[3] Similar outbreaks of occupational silicosis have since been described in Spain, Italy, and more recently in Australia, USA and Belgium.[4–10] A recently published systematic review summarised different epidemiological and clinical characteristics of this new emerging disease.[11] Mild respiratory symptoms of cough and shortness of breath on exertion were reported in most of the patients. Workers were rarely asymptomatic, and the duration of symptoms before diagnosis ranged from 6 months to 3 years, with evidence of a positive correlation between silica exposure and autoimmune diseases. Radiological diagnoses were in line with clinical silicosis manifestations that mostly showed a bilateral diffuse micronodular pattern as well as a restrictive pattern, together with a reduced diffusing capacity for carbon monoxide (DLCO).[11] We studied this

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population of individuals employed in the manufacture of kitchen and bathroom countertops made of an artificial raw material containing more than 90% free crystalline silica as the major filler, with the addition of coloured glass, shells, metals or mirrors bound together by a resin-containing polymer.[12 13] We had earlier demonstrated that most of the particles in the airways of exposed workers showed a pattern similar to that of the raw artificial stone material, and that there was a significantly higher fraction of particles <5 µm compared with a non-exposed control group.[14] Moreover, we directly measured ultrafine particles (UFP) in the induced sputum (IS) and exhaled breath condensate samples from the airways of individuals exposed to occupational dust while executing their work compared with non-exposed individuals. The results showed that UFP were accumulated in the airways of the exposed workers and that they had mainly sustained neutrophilic inflammation.[15]

In view of our former results, we focused our current study on the nanoscale fraction of the particles in artificial stone dust (ASD) in correlation with inflammatory and imaging parameters.

METHODS

Study design and population

Sixty-eight non-smoker individuals working mainly with ASD for up to the last 20 years were recruited from small enterprises throughout the country. The nature of their work involved cutting and grinding slabs of the raw material in the production of kitchen countertops and bathtubs and sinks. Recruitment into the study was by physician referral, direct contact with artificial stone factories and self-referral. Each participant paid one visit for clinical assessment by pulmonary function test (PFT) and sputum induction. The 48 non-smoker controls were healthy non-exposed individuals from the community who volunteered to undergo PFT and IS.

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 Department at the Tel Aviv University School of Public Health. It included demographic and occupational parameters, smoking and alcohol habits, general health profile, specific respiratory, protective measures applied in the workshop (water cutting machine, dust ventilation system), and personal protective equipment (mask, goggles, gloves, special footwear and clothes).

Pulmonary function test

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 (ATS) guidelines.[16]

Sputum induction and processing

Sputum induction and processing were performed with an aerosol of hypertonic saline generated by an ultrasonic nebuliser, Model Omron U1 (Omron Healthcare, USA), that has an output of 0.5 mL/min and particles with <5 µm aerodynamic mass median diameters.[17 18]

Sedimentation of the ASD

ASD was collected from an artificial stone factory directly from the settled material around the working surface of a countertop. Since the chemical composition of ASD contains 93% silica,[5] a saturated solution was determined by a saturation index of silicon dioxide at a pH of 7.0 and at 25°C[19] by adding ASD until the point at which it was deposited at the bottom of the tube. The saturated solution had a weight of 4.4 mg in 10 mL double-distilled water, a level that is considered to represent the presence of a low soluble material. The upper fraction was transferred to a new tube after 15 min of rest. The mixture was stirred by vortex for 10 s, and 50 µL was extracted from the middle of the tube (around the 5 mL line) and measured using the NanoSight LM20 instrument (NanoSight, London, UK). We then let the solution rest in the tube and took additional samples after 5, 15, 60 and 1440 min.

UFP measurement

The particle size distribution of $PM_{0.1}$ was assessed in the IS samples using the NanoSight LM20 instrument equipped with the Nanoparticle Tracking Analysis V.2.3 analytical software for visualising and analysing particles in liquids that relates the rate of Brownian motion to particle size. The rate of movement is related only to the viscosity of the liquid, the temperature and the size of the particle, and is not influenced by particle density or refractive index. The particles contained in the sample can be visualised by virtue of the light they scatter when illuminated by a laser light source. Approximately 0.3 mL of sputum supernatant was introduced into the viewing unit by means of a disposable syringe. Three videos of 60 s duration were recorded and analysed for each sample. The total particle count, the per cent of particles that were in the nanosize range and the total nanosized particle counts were recorded.

Imaging score

Chest CT scans of the study subjects were evaluated for features indicative of silicosis, including nodular distribution and progressive massive fibrosis in three zones (upper, middle and lower) of each lung, using a system for scaling severity described earlier by several groups. Each lung zone was graded between 0 and 7 according to the severity of findings. The sum of all the grades from the six lung zones yielded the total grade for the subject (ranging from 0 to 42).[20]

Cytokine multiplex method

The Human HS Cytokine A Premixed Mag Luminex Performance Assay (4 PLEX) was used to measure interleukin-1 beta (IL-1β), IL-6, IL-8 and tumour necrosis factor alpha (TNF-α) (FCSTM09-08, R&D Systems, Wiesbaden-Nordenstadt, Germany). The Human IL-18 Platinum ELISA Kit (BMS267/2, eBioscience) was used to quantitate IL-18. Assays were run according to the manufacturer's protocols with a detection limit of 0.3–0.95 pg/mL for IL-1β, IL-6, IL-8 and TNF-α, and 9 pg/mL for IL-18. The IS supernatants and serum from exposed artificial stone workers were thawed and analysed according to the manufacturer's instructions, and the analyses were carried out in the presence of a representative from the respective manufacturer. All data were analysed as recommended by the manufacturers.

Statistical analysis

Comparisons between groups were performed by an independent t-test, Kruskal-Wallis test and χ^2 test. Correlations between particles <5 µm and continuous variables were done using Spearman's correlation. To determine the optimal cut-off values of each independent variable for abnormal CT findings, receiver operating characteristic (ROC) curves were obtained and the area under the curve (AUC) was calculated with 95% CI. All statistical analyses were performed using the SPSS V.24.0 software for

Table 1 Demographic, clinical parameters, pulmonary function tests and differential cell counts in induced sputum of the study population (n=116)[18]

	Exposed workers	Non-exposed control	P value*
Gender (male/female)	68/0	32/16	<0.01*
Age (years) (±SD)	48.3 (11.1)	38.0 (17.1)	<0.01*
Exposure (years) (±SD)	20.5 (10.0)	0	
Body mass index (±SD)	27.0 (4.0)	23.7 (2.8)	<0.01
VC (%)	77.4 (18.6)†, n=68	97.8 (14.1), n=35	<0.01
TLC (%)	93.9 (15.4), n=68	106.5 (10.7), n=35	<0.01
FVC (%)	79.0 (19.8), n=68	98.3 (13.7), n=44	<0.01
FEV_1 (%)	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
DLCOsb (%)	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

The differential cell counts were adjusted to gender and age.
With permission.[19]
*P<0.05 (for age and body mass index (t-test) and gender (Pearson's χ^2)).
†Values are mean (±SD).
DLCOsb, diffusion lung carbon monoxide in a single breath; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; VC, vital capacity.

Windows. All p values were two-sided, and a p value <0.05 was considered significant.

RESULTS

The exposed workers (study group) differed from the control group in gender (68 men vs 32 men and 16 women, respectively, p<0.01), age (48.3±11.1 vs 38±17.1 years, respectively, p<0.01) and body mass index (27.0±4.0 vs 23.7±2.8, respectively, p<0.01; table 1). All of the PFT parameters of the study group were significantly lower than those of the control group, and the differential cell counting in the IS samples clearly revealed the presence of neutrophilic inflammation (68.6±22.6 vs 46.7±18.8, respectively, p<0.01; table 1). Thirty-four patients had CT scores between 0 and 42, and 29 of them were diagnosed with silicosis.

Analysis of the particle size distribution in the IS samples of the study group revealed that the smallest fraction of the particles was 32.08 nm (ranging between 32.08 nm and 108.9 nm), compared with the smallest fraction of the control group which ranged between 61.4 nm and 110.6 nm. A sample of the raw material source of the ASD collected from one of the factories was tested by allowing it to settle for 15 min in a mixture of water. The distribution of the particles was almost identical to the curve of the distribution in the samples from the exposed workers (range 28.1–110.1 nm; figure 1A). The percentage of the nano-ranged particles in the artificial raw material was 14.93±0.72%, similar to the percentage measured in the IS samples (12.12±0.86%). Both fractions were significantly higher than the nano-ranged particles in the control group (1.56±0.11%, p<0.05; figure 1B).

In order to determine whether the ASD is similar or different from natural stone dust from our area (Hebron), we tested the particle size distribution from both types of dust. The differential particle size distribution emerged as being completely different for each fraction tested (figure 1C).

The PFT results correlated with the concentration of nanoscale particles in the IS samples. Specifically, there was a negative correlation for total lung capacity (TLC), forced expiratory

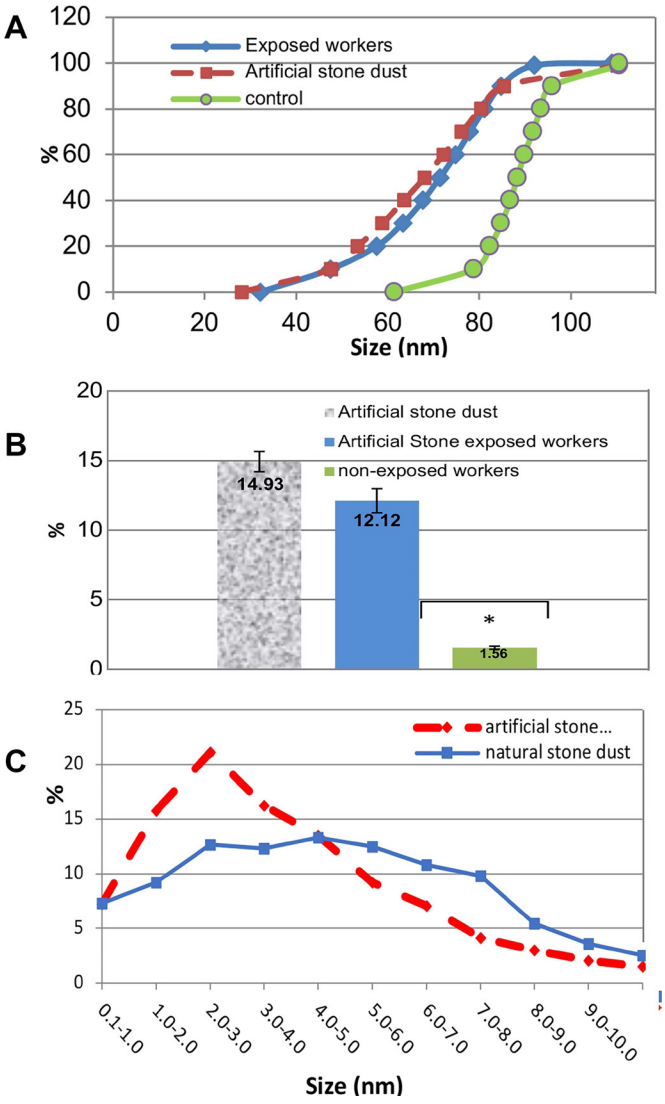

Figure 1 (A) Percentile of ultrafine particles (UFP) in induced sputum (IS) samples and artificial stone dust (ASD) in exposed workers and non-exposed subjects. (B) Particle size distribution (PSD) of ASD compared with natural stone. (C) Percentile of UFP in IS samples of exposed workers versus non-exposed subjects. PSD was evaluated by a DonnerTech Innovative Particle Analyzer (DIPA) analyser, and UFP were evaluated by a NanoSight LM20 in the sputum specimens and in the dust collected from an artificial stone factory. The y-axis represents the frequency for each size. *P<0.05 was considered significant by Mann-Whitney test. The results are ±SE of at least six independent experiments.

volume in 1 s (FEV_1) and DLCO: TLC Spearman's r=−0.347, p=0.011, n=45 (table 2); FEV_1 Spearman's r=−0.299, p=0.046, n=45 (figure 2B); and diffusion lung carbon monoxide in a single breath Spearman's r=−0.425, p=0.004, n=45 (table 2). The CT score correlated positively with the accumulation of nanoparticles <1 µm in the sputum (Spearman's r=0.378, p=0.023, n=36; table 2). The UFP measured in the serum of the study group was negatively correlated with the CT score (Spearman's r=0.369, p=0.021, n=39; table 2).

Cytokines were analysed in the supernatant of the IS samples to determine whether the nanoscale particles induced an inflammatory reaction. The results showed a positive correlation between the accumulation of nanoparticles <1 µm in IS and inflammatory cytokines in those samples: IL-8 (Spearman's r=0.336,

Table 2 Correlation between ultrafine particle concentration and respiratory symptoms, and inflammatory parameters, pulmonary function tests and CT score

	UFP concentration		Respiratory symptoms	
Parameter	r	P value	r	P value
IS IL-8	0.336	0.024	0.284	0.069
Serum IL-8	0.664	0.01	0.542	0.021
IS IL-6	0.294	0.065	0.369	0.021
IS TNF-α	0.409	0.007	ND	ND
IS NLRP3	0.272	0.09	ND	ND
TLC (%)	−0.374	0.011	ND	ND
FEV_1	−0.299	0.046	ND	ND
DLCOsb (%)	−0.425	0.004		
CT score	0.378	0.023		
CT score	0.369*	0.021		

UFP were evaluated by NanoSight LM20 in the IS specimens.
P values <0.05 were considered significant for Spearman's correlation.
*Correlation with serum UFP concentration.
DLCOsb, diffusion lung carbon monoxide in a single breath; FEV_1, forced expiratory volume in 1 s; IL-6, interleukin 6; IL-8, interleukin 8; IS, induced sputum; ND, Not Done; NLRP3, nucleotide-binding domain leucine-rich repeat containing pyrin 3; TLC, total lung capacity; TNF-α, tumour necrosis factor alpha; UFP, ultrafine particles.

p=0.024, n=45), IL-6 (Spearman's r=0.294, p=0.065, n=40), TNF-α (Spearman's r=0.409, p=0.007, n=42) and nucleotide-binding domain leucine-rich repeat containing pyrin 3 (r=0.272, p=0.09). IL-8 in the serum also showed a positive correlation (r=0.664, p=0.01; table 2). Respiratory symptoms had a positive and significant correlation with sputum IL-8 (r=0.284, p=0.069), serum IL-8 (r=0.369, p=0.021) and sputum IL-6 (r=0.369, p=0.021; table 2).

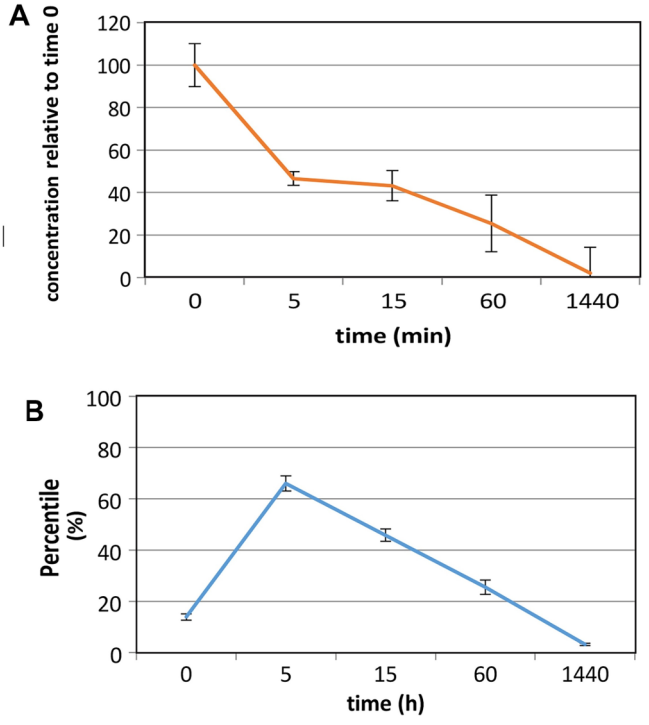

Figure 2 Concentration (A) and Percentile of ultrafine particles (UFP) (B) of artificial stone dust in the water phase after raw material sedimentation over time. UFP were evaluated by NanoSight LM20 in artificial stone dust collected from an artificial stone factory. The results are expressed relative to time 0±SE of six independent experiments.

A stone cutter fitted with a jet water spray attachment has been used for the past few years to reduce the number of aerosolised dust particles by combining them with drops of water which settle on the floor. To mimic this phenomenon, we mixed ASD with water and measured the content of nanoscale particles in the upper water fraction of the mixture throughout a period of 24 hours. The results showed a consistent concentration of very small nano-ranged particles in the upper water fraction during the entire period of time. Measurements after 5, 15 and 60 min of sedimentation in this fraction showed 46.65±3.21, 43.26±7.1 and 25.5±13.3 10^8 particles/L, respectively, which represent 66±2.95, 45.83±2.39 and 25.5%±2.8%, respectively, of the original dust (figure 2A,B).

We then performed an ROC curve for UFP in IS to determine the best cut-off for detecting workers with abnormalities in their CT scans (ie, a CT score >0) among the non-smokers in the study group (n=50). The AUC was 0.723 (n=43; 95% CI 0.561 to 0.885, p<0.05; online supplementary figure 1). The optimal cut-off value was a sputum UFP level at 8.1×10^8 particles/mL, which had a sensitivity of 71% and a specificity of 58%. The AUC for serum UFP was 0.769 (95% CI 0.630 to 0.909, p<0.001; figure 3b), and the optimal cut-off value for serum UFP was 6.65×10^8 particles/mL, with a sensitivity of 77% and a specificity of 64% for the presence of abnormal findings on CT imaging.

DISCUSSION

In the present study, we show the deleterious effect of UFP derived from ASD on exposed workers' functional, inflammatory and imaging parameters. Our results are based on UFP measurements done in biological samples retrieved from workers exposed to ASD. To the best of our knowledge, this is the first report on the biomonitoring of workers exposed to ASD particles in the nano-range scale. The current approaches used to derive realistic exposure metrics values of nanoparticles in a nanotechnology workplace are generally based on workplace measurements or on simulations in laboratories.[21] None of them takes into account the actual quantities of sedimentation in the lungs.

To measure the fraction of ASD present in the air of the workplace, we resuspended the raw material of the artificial stone and let it settle for 24 hours, after which we measured the fraction of UFP that remained in the upper water fraction. After 60 min, 20% of the UFP were still present, indicating that the exposed workers were continuously exposed to at least a small fraction of ASD. Those inhaled UFP and nanoparticles are more toxic compared with larger particles because of their increased reactivity, surface area particle number mass, deposition potential and ability to translocate to other organ systems, such as the cardiovascular and/or neuronal system, where they elicit adverse effects.[22 23] Moreover, these particles can either avoid immune system recognition or they can specifically inhibit or enhance the immune responses, thereby promoting inflammatory or autoimmune disorders,[24] as we had shown in detail elsewhere.[25] The levels of inflammatory cytokines in the IS and peripheral blood samples of the current study population were positively correlated with UFP concentration, indicating a causative correlation between UFP and a high state of inflammation in the airways. This supported our findings[26] and those of others,[27] both in animal models, that UFP reach the lower airways and gain access to the most permeable barrier in the human body

(measuring only 0.1–0.2 µm), with the enlargement of gap junction channels caused by inflammation.[28]

There are several limitations to this study. We had demonstrated that IS is a non-invasive surrogate to alveolar findings by means of Bronchoalveolar Lavage (BAL),[29] and later found that the particles in IS and the mineral content of lung biopsies are comparable.[30] The advantage of non-invasive IS over more invasive procedures such as BAL and biopsies is clear, but IS sampling and processing are currently confined to specialised laboratories and are not available in some parts of the world. Second, nanomaterials of the same chemical composition can have many different physicochemical properties (eg, size, shape, charge and so on). Since the variations among different nanoforms are much larger compared with non-nanomaterials, the data we present herein cannot be extrapolated to other types of silica dusts.

In conclusion, the results of this study demonstrate, for the first time, an association between UFP, decreased PFT results, worsening of findings on CT and elevated inflammatory cytokines, and exposure to ASD at the workplace. Our results highlight the need for personal protective equipment with the aim of decreasing the internal doses of UFP and thereby mitigating respiratory pathophysiology in exposed populations. These novel data contribute to the development of a biological marker for UFP concentrations in the airways of workers exposed to ASD as an index of risk to develop lung disease.

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Competing interests None declared.

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