Detection of Occult Lung Impairment in Welders by Induced Sputum Particles and Breath Oxidation

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Background We evaluated particulate matter in combined induced sputum (IS) and oxidation in exhaled breath condensate (EBC) to test whether underlying inflammatory changes are present in asymptomatic welders.

Methods Thirty welders from the Israel Defense Forces exposed to aluminum/iron (Group 1) or to cadmium/chromium/iron/lead/nickel (Group 2, N = 16) and 27 non-exposed administrators were studied. IS was recovered, particle size distribution, hydrogen peroxide and pH were measured, and exhaled breath condensate was collected.

Results Group 2 had a higher % neutrophils than all other participants (P = 0.0001) and a higher % particles >2 μm in diameter (P = 0.0017). Percent particles and years of exposure highly correlated (P = 0.051). All welders EBC samples had higher concentrations of hydrogen peroxide than controls (P = 0.0001). pH was lower only for Group 2 (P = 0.0001).

Conclusions Combined IS and EBC measurements detect underlying inflammation in airways of asymptomatic welders. It emerged that airway inflammation is present in asymptomatic welders, and that the particle burden, inflammatory cells, and level of oxidative stress are a function of the type and the duration of welding. Am. J. Ind. Med. 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: welding; exposure; undetected; pulmonary inflammation; noninvasive

INTRODUCTION

Welding, a fabrication process that joins metals or thermoplastics by causing coalescence, is indispensable in modern society and ubiquitous in industry, with ~1,000,000 full-time welders employed worldwide [Sundin, 1988; Antonini, 2003]. Welding generates fumes that contain several metals (e.g., cadmium [Cd], chromium [Cr], iron [Fe], lead [Pb], manganese [Mn], and nickel [Ni]) and toxic gases (e.g., carbon monoxide, ozone, and nitrogen oxides) [Antonini, 2003]. Metals and gases comprise fine and ultrafine particles [Glinsmann and Rosenthal, 1985] with the potential to adversely affect health.

Epidemiological studies have shown that exposure to welding fumes is associated with metal fume fever [Mueller and Seger, 1985; El-Zein et al., 2003a] and increased respiratory symptoms [Mueller and Seger, 1985; Beckett
et al., 1996; El-Zein et al., 2003a), including asthma and chronic bronchitis [Wang et al., 1994; Özdemir et al., 1995; Bradshaw et al., 1998; El-Zein et al., 2003b], all associated with reduction in lung function [Wang et al., 1994; Erkinjuntti-Pekkanen et al., 1999]. Although epidemiological studies have demonstrated an increase in pulmonary illness after exposure to welding fumes, there is little information on the pathophysiologic mechanisms involved in pathology subsequent to the inhalation of welding fumes.

Systemic parameters in asymptomatic shipyard welders indicated that exposure-induced changes in biomarkers of oxidative stress may be valuable in monitoring disease [Han et al., 2005]. Moreover, exposure to copper, zinc, lead and iron induced alterations in erythrocytic superoxide dismutase activity and serum malondialdehyde [Li et al., 2004]. It was also found that Mn fumes suppresses secretion of anti-inflammatory and immunosuppressive Clara cell proteins into the respiratory tract, enhancing the absorption of particles and increasing the incidence of subclinical neurotoxic symptoms related to airborne Mn levels [Halatek et al., 2005]. Therefore, an understanding of possible adverse health effects of exposure to welding fumes is essential to risk assessment and to the development of prevention strategies relevant to large populations of workers. To this end, a cohort of asymptomatic welders exposed to variable levels of fumes and a group of non-exposed administrators (controls) were recruited from the Israeli Defense Force (IDF) in order to evaluate particulate matter in the tracheobronchial airways by means of combined induced sputum (IS) and markers of oxidation in exhaled breath condensate (EBC) approaches for detecting occult pulmonary disease.

METHODS

Welders were recruited from the IDF and divided into two groups based on the metals to which they were exposed: Group 1 was exposed to aluminum and iron in the workplace of an air force base and Group 2 was exposed to Cd, Cr, Fe, Pb, Mn, and Ni in another military workplace. The control group was comprised of individuals working in offices in the same workplaces.

Ethical approval was granted by the Israeli Defense Force (IDF) and the Tel-Aviv Sourasky Medical Center Institutional Ethics Committee. All the subjects gave written informed consent and the clinical assessment, pulmonary function testing (PFT), induction of sputum and EBC were carried out during the same visit.

PFTs

PFTs were performed by a Masterlab spirometer (Masterlab E. Jaeger, Wurzburg, Germany). Measurements were made according to standard protocols of American Thoracic Society guidelines [American Thoracic Society 1987].

Sputum Induction and Processing

Sputum induction and processing were performed using a slightly modified method of Pin et al. [1992] and Popov et al. [1994] as previously described in detail [Fireman et al., 1999].

Evaluation of the Phenotype of Lavage and Sputum Cells

Flow cytometric analysis was performed on a dual FACS 440 equipped with an Ar+ and Kr laser (Becton-Dickinson, Franklin Lakes, NJ). Data were collected and analyzed using the Consort VAX and Disp4 and Disp2D programs (Becton-Dickinson). The data were expressed on a logarithmic scale. The selection of lymphocyte population was based on a side-scatter and the expression of CD 45. Lymphocytic subsets were identified by monoclonal antibodies as follows: CD3 = total T cells, CD4 = T helper cells, and CD8 = T suppressor-cytotoxic cells. Monoclonal antibodies were directly conjugated to either phycoerythrin (RD1) or fluorescein isothiocyanate (FITC). Cells were incubated for 10 min with Epics Coulter Q-Prep and read either immediately or after 24 hr.

EBC Collection

The apparatus and methods for collecting EBC have been described in detail elsewhere [Mutlu et al., 2001]. Briefly, subjects were asked to breathe tidally for 15 min into a perfluoroalkoxy Teflon tubing Nalgene Dupont, Rochester, NY) with a 0.5 cm internal diameter immersed in an ice-filled bucket of a specially designed double-wall plastic condenser system. They breathed tidally for 20 min under supervised conditions according to European Respiratory Society recommendations [Horvath et al., 2005]. The EBC was collected into a 10-ml polypropylene tube with no preservatives, emptied into an Eppendorf vial, and the volume was recorded. The sample was kept on ice until analysis within 30 min of collection.

H2O2 Determination

H2O2 was measured by a simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture [Pick and Keisari, 1980] based on oxidation of phenolsulfophthalein (phenol red) mediated by horseradish peroxidases (HRPO). Briefly, 2.5 μl of EBC samples was incubated with 247.5 μl phenol red solution (PRS) containing phenol red (Biological Industries, Beit Ha’Emek, Israel) and
HRPO (Sigma, St. Louis, MO) in 0.5 M potassium phosphate buffer. The results were plotted against a standard curve made by using the same batch of PRS and H$_2$O$_2$ 30\% (Sigma) to result in final concentrations of 0.1, 0.3, 1.0, 3.0, 6.25, and 12.0 $\mu$M. The lower detection range of the assay is 0.1 $\mu$M. The reaction was stopped after 5 min by 1 N NaOH. Samples were read at 610 nm against a blank of PRS to which 10 $\mu$l NaOH 1 N was added.

**pH Measurement**

The pH was measured using a pH meter (Hanna Instruments, Leighton, UK) after argon gas was passed over the sample at 2 L/min for 10 min with a sensitive electrode (Hamilton, Reno, NV).

**Quantitative Particle Analysis**

Particle size distribution analyses were performed by a laser technique based on the time of transition theory using a Cis 1 Analyzer (Ankersmid YOHAKNEAM-ISRAEL) on all IS samples. Intracellular particles were measured by adding two drops of a suspension of sputum-rich cell fraction of the processed plugs into a quartz cuvette containing stirred water as previously described [Fireman et al., 1999].

**Scanning Electron Microscopy**

Equal volumes of formalin were added to the remaining sputum samples and kept refrigerated at 4°C until examination. The samples were then treated and examined by scanning electron microscopy as previously described [Fireman et al., 1999].

**Other Parameters**

Relevant data on personal or familial medical history, smoking habits, self-reported diseases and symptoms and years of exposure were elicited via a standard questionnaire prior to the participants undergoing PFTs and IS and EBC collection. All welders were actively involved in their trade during the month preceding study entry. Relevant data on personal or familial medical history, smoking habits, self-reported diseases and symptoms and years of exposure were elicited via a standard questionnaire prior to the participants undergoing PFTs and IS and EBC collection. All welders were actively involved in their trade during the month preceding study entry.

**Statistical Methods**

Since most of the parameters were not normally distributed, statistical tests were performed on the ranks of the original parameters. Analyses of demographic and clinical parameters were performed by a one-way analysis of variance (ANOVA) and Chi-square tests. Multiple comparisons between each pair of groups were done using the Gabriel and Games-Howell tests. One-way ANOVA was performed as above with smoking and age as covariates.

Pearson and Spearman correlation coefficients were calculated to study the relationship between all continuous parameters in each group separately and for the entire sample.

Significance was set at 0.05 and the SPSS (Chicago, IL) for Windows software, Version 13.0 was used for the analysis.

**RESULTS**

A total of 46 welders exposed to variable levels of fumes were recruited from the IDF and divided into 30 soldiers working as welders (all males, mean age 36 ± 11 years) who were exposed to aluminum and iron in the workplace of an air force base (Group 1) and 16 civilian welders (all males, mean age 50 ± 9 years) who were exposed to cadmium chromium, iron, lead and nickel in another military workplace (Group 2). The control group was comprised of 27 males (mean age 35 ± 10 years) working in administrative facilities of both work complexes and who had no history of any occupational exposure. The demographic, clinical parameters and self-reported diseases and symptoms in all participants are listed in Tables I and II. The exposed welders did not work in one specific job or work site on a regular basis but were involved in various welding-, cutting-, and fitting-related processes under various conditions (e.g., semi-enclosed or open areas) for 8–10 hr daily. They were protected by MTM ClearVisor with Adflo System 3M Air-Respirators.

Group 2 reported more common colds and wheezing episodes than both Group 1 and the controls ($P = 0.01$ and $P = 0.035$, respectively, Table II). PFT spirometry and lung volumes were normal in all participants, with the only significant difference noted in diffusing capacity of the

**TABLE I.** Demographic and Clinical Parameters of all Participants (N = 73)

<table>
<thead>
<tr>
<th>Study groups</th>
<th>n (%)</th>
<th>Age (years)</th>
<th>Smoking (no)*</th>
<th>Education (years)</th>
<th>Exposure (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>30 (41%)</td>
<td>36 ± 11</td>
<td>23/30 (77%)</td>
<td>&gt;12–14 (32%)</td>
<td>10.3 ± 10.8</td>
</tr>
<tr>
<td>Group 2</td>
<td>16 (22%)</td>
<td>50 ± 9*</td>
<td>14/16 (87%)</td>
<td>12 (56%)</td>
<td>21 ± 7.6</td>
</tr>
<tr>
<td>Control</td>
<td>27 (37%)</td>
<td>35 ± 10</td>
<td>26/27 (93%)</td>
<td>&gt;14 (44%)</td>
<td>—</td>
</tr>
<tr>
<td>$P$-value*</td>
<td>0.0001</td>
<td>0.063</td>
<td>0.0003</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

*Analysis of variance (ANOVA) = a $P$-value < 0.05 was considered significant.

*Code of smoking status = no: never; yes: past + ex-smoker.
studied in EBC collected from all subjects. H2O2 was fumes.

These differences were probably due to exposure to welding age, years of exposure and smoking status, indicating that all these differences remain unchanged after adjustment for

\(20%\) and 23\(\%\) respectively) \(P\)-value 0.4 0.035 0.7 0.14 0.01 1.0

\(\text{Group } 1\) 1.5 ± 0.2 3.7% 63% 78% 19 ± 1.5 3.7%

\(\text{Group } 2\) 1.17 ± 0.3 18.8%* 56% 62% 0.63 ± 0.81* 1%

\(\text{Control}\) 1.2 ± 0.2 0% 52% 52% 1.6 ± 2.0 7.4%

\(P\)-value 0.4 0.035 0.7 0.14 0.01 1.0

aRespir. symptoms = average of four symptoms (cough, mucus, wheezing, shortness of breath): 0 no symptoms; 1 = only one symptom; 2 = 2 symptoms; 3 = 3 symptoms; 4 = 4 symptoms.

bSympt and colds = self-report on feeling less well because of cold symptoms.

Differential counts were performed from samples recovered from all participants (n = 76). Epithelial cells were for all samples <25%. Samples from Group 2 contained a significantly higher level of % neutrophils \((P = 0.001)\), and a significantly lower percentage of macrophages \((P = 0.004)\) and lymphocytes \((P = 0.004)\) than those from Group 1 and from the controls. The CD4/CD8 ratio was similar in all groups, but Group 1 contained a higher percentage of CD4 and CD8 than Group 2 and the controls \((42 ± 19%\) vs. 25 ± 20% and 23 ± 15%, respectively, \(P = 0.012\); 19 ± 11% vs. 10 ± 5% and 9 ± 7%, respectively \(P = 0.001)\) (Table IV). All these differences remain unchanged after adjustment for age, years of exposure and smoking status, indicating that these differences were probably due to exposure to welding fumes.

Other markers of inflammation and oxidative stress were studied in EBC collected from all subjects. H2O2 was significantly higher in Groups 1 and 2 compared to controls \((0.19 ± 0.11\) and 0.19 ± 0.16 \(\mu\)M vs. 0.03 ± 0.04 \(\mu\)M, \(P = 0.0001)\) while the pH of the samples were significantly alkaline in Group 1 than in Group 2 and the controls \((8.0 ± 0.64\) vs. 7.52 ± 0.48 and 7.7 ± 0.74, \(P = 0.001)\) (Table V).

Quantitative and qualitative analyses of the IS particles revealed that the aerodynamic diameter of 92.9 ± 3.51\% of the particles was <1 \(\mu\)m (Fig. 1). The major differences of particle size distribution in sputum were for those in Group 2: there were fewer smaller particles (<2 \(\mu\)m) than those in the IS of Group 1 and of the controls \((97 ± 1.4%\) vs. 99 ± 0.7\% and 98 ± 1.1\%, respectively, \(P = 0.019)\). Instead, Group 2 had more larger particles (>5\(\mu\)) \((0.4 ± 0.3%\) vs. 0.18 ± 0.17\% and 0.26 ± 0.26\%, respectively, \(P = 0.04)\) (Table VI).

The parameters of age \((P = 0.018)\) and years of exposure \((P = 0.009)\) significantly influenced the particle size distribution of all sputum samples. A good correlation was found between the years of exposure and the number of particles >2 \(\mu\)m and those between 2 and 5 \(\mu\)m in the welders of Group 2 \((P = 0.512\), \(r = 0.051\) and \(P = 0.061\), \(r = 0.496\), respectively) (Fig. 2A,B). Smoking status \((P = 0.27)\) was not a confounding factor.

Scanning electron microscopic analysis was done arbitrarily in two samples of welders from each group showing high load particulate matter in Giemsa-stained slides compared to two control samples. The Group 2 samples did not show traces of Ni and Cr as expected (Table VII).

**TABLE II.** Self-Reported Diseases and Symptoms in all Participants

<table>
<thead>
<tr>
<th>Study group</th>
<th>Respir. symptoms*</th>
<th>Whistle (yes)</th>
<th>Cough (yes)</th>
<th>Mucus (yes)</th>
<th>Cold symptoms b</th>
<th>Shortness of breath (yes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1.5 ± 0.2</td>
<td>3.7%</td>
<td>63%</td>
<td>78%</td>
<td>19 ± 1.5</td>
<td>3.7%</td>
</tr>
<tr>
<td>Group 2</td>
<td>1.17 ± 0.3</td>
<td>18.8%*</td>
<td>56%</td>
<td>62%</td>
<td>0.63 ± 0.81*</td>
<td>1%</td>
</tr>
<tr>
<td>Control</td>
<td>1.2 ± 0.2</td>
<td>0%</td>
<td>52%</td>
<td>52%</td>
<td>1.6 ± 2.0</td>
<td>7.4%</td>
</tr>
<tr>
<td>(P)-value</td>
<td>0.4</td>
<td>0.035</td>
<td>0.7</td>
<td>0.14</td>
<td>0.01</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Analysis of variance (ANOVA) \(a P\)-value < 0.05 was considered significant.

**TABLE III.** Pulmonary Function Tests (PFTs), all Participants (\(N = 73)\)

<table>
<thead>
<tr>
<th>Study group</th>
<th>FVC (%)</th>
<th>FEV1/FVC</th>
<th>TLC (%)</th>
<th>DLCO-SB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>94 ± 11</td>
<td>85 ± 6</td>
<td>91 ± 10</td>
<td>92 ± 15*</td>
</tr>
<tr>
<td>Group 2</td>
<td>95 ± 16</td>
<td>82 ± 6</td>
<td>96 ± 17</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>Control</td>
<td>95 ± 14</td>
<td>85 ± 7.7</td>
<td>97 ± 14</td>
<td>84 ± 9</td>
</tr>
<tr>
<td>(P)-value*</td>
<td>0.87</td>
<td>0.23</td>
<td>0.12</td>
<td>0.032</td>
</tr>
</tbody>
</table>

PFTs were performed by a Masterlab spirometer.

FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion lung CO; L, liters.

*Analysis of variance (ANOVA) \(a P\)-value < 0.05 was considered significant.
TABLE IV. Differential Cells Counts (DCC) From Induced Sputum Samples of all Participants (N = 73)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Neutro (%)</th>
<th>Eos (%)</th>
<th>Lymp (%)</th>
<th>Macro (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>42 ± 28</td>
<td>17 ± 3.6*</td>
<td>15 ± 8.7</td>
<td>42 ± 26</td>
<td>42 ± 19*</td>
<td>19 ± 11*</td>
<td>2.7 ± 16</td>
</tr>
<tr>
<td>Group 2</td>
<td>71 ± 14*</td>
<td>0.73 ± 2.2</td>
<td>93 ± 5.3*</td>
<td>18 ± 12*</td>
<td>25 ± 20</td>
<td>10 ± 5</td>
<td>2.4 ± 12</td>
</tr>
<tr>
<td>Control</td>
<td>47 ± 21</td>
<td>0.49 ± 1.0</td>
<td>14 ± 7.1</td>
<td>38 ± 20</td>
<td>23 ± 15</td>
<td>9 ± 7</td>
<td>2.6 ± 12</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001</td>
<td>0.06</td>
<td>0.043</td>
<td>0.004</td>
<td>0.012</td>
<td>0.001</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Differential cell counts were performed by counting 300 cells in Giemsa-stained cytopreps.

Neutro, neutrophils; Eos, eosinophils; Lymph, lymphocytes; Macroph, macrophages; CD4, cluster of differentiation 4 helper T cells; CD8, cluster of differentiation 8 suppressor T cells.

*Analysis of variance (ANOVA) = a P-value < 0.05 was considered significant.

DISCUSSION

We used a noninvasive approach to establish the exposure profile and the inflammatory status of airways in asymptomatic welders. IS samples can demonstrate the environmental particulate burden of inhaled material. Our aim was to characterize particle size distribution pattern in airways of workers exposed to welding fumes in correlation to inflammatory cells in sputum and oxidative stress biomarkers in condensate in order to demonstrate early inflammatory changes in asymptomatic welders without clinical and functional abnormalities as parameters for biological monitoring. We a priori recruited asymptomatic workers in order to isolate the effect of welding on subclinical inflammatory status associated with inhaled particulate matter.

We demonstrate here, for the first time, the crucial factor of the length of exposure over time, dividing our study population into those exposed short and long period of times. In contrast, a very recent study on the inflammatory responses to the occupational inhalation of metal fume [Palmer et al., 2006] failed to show neutrophilic changes in IS since they combined welders who had been exposed for as little as 6 months with those whose exposure history extended to 40 years.

Materials inhaled in the workplace can lead to all the major chronic lung diseases associated with exposure to welding fumes which are well documented and include bronchitis, occupational asthma, lung function changes, pneumoconiosis, and an increase in lung cancer incidence [Beckett, 2000]. Several studies [Wang et al., 1994; Erkinjuntti-Pekkanen et al., 1999; Luo et al., 2006] showed restrictive and obstructive lung abnormalities which are associated with spot arc and welding exposures. The “healthy-worker survivor” effect that is well characterized in occupational epidemiologic studies [Arrighi and Herz-Picciotto, 1994] needs to be taken into consideration in this setting. It has been suggested that when exposed to various contaminants, susceptible individuals will have symptoms that may result in their leaving the industry, resulting in a working population that appears to be healthier than the general population. This health-based selection process has been shown to occur in swine-confinement facilities [Chenard et al., 2007]. Exposure may lead to respiratory symptoms and potential occupational asthma or asthma-like syndrome in some individuals within a short time of commencing work, necessitating withdrawal from the toxic facilities [Chenard et al., 2007]. We found that age and years of exposure significantly affected self-reporting of respiratory symptoms: older welders with more years of experience subjectively reported more respiratory symptoms than those with fewer years on the job, even though their PFT results were normal. The appearance of respiratory symptoms may be due to abnormalities in oxidant status and airways neutrophils infiltration without functional impairment. This disconnection between oxidant and inflammatory parameters

TABLE V. pH+ and H2O2+ in Exhaled Breath Condensate (EBC) in all Samples (N = 73)

<table>
<thead>
<tr>
<th>Study group</th>
<th>pH (before argon)*</th>
<th>pH (after argon)*</th>
<th>H2O2 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>6.4 ± 0.54</td>
<td>8.0 ± 0.64*</td>
<td>0.19 ± 0.11</td>
</tr>
<tr>
<td>Group 2</td>
<td>6.4 ± 0.42</td>
<td>7.52 ± 0.48</td>
<td>0.19 ± 0.16</td>
</tr>
<tr>
<td>Control</td>
<td>6.5 ± 0.5</td>
<td>7.7 ± 0.74</td>
<td>0.03 ± 0.04*</td>
</tr>
<tr>
<td>P-value*</td>
<td>0.85</td>
<td>0.001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

pH and H2O2 assessments were performed as described in Methods Section.

*Analysis of variance (ANOVA) = a P-value < 0.05 was considered significant.

The de-aeration of the EBC samples with argon was done to normalize carbon dioxide and sits atop the sample keeping atmospheric carbon dioxide (CO2) from re-entering the sample during pH measurement.
from functional ones was already shown by others [Thomas et al., 2004]. Moreover, as noted by Blomberg et al. [1999] the acidity in EBC seen in Group 2 may be another reason for the presence of cough [Blomberg et al., 1999]. The differential cell counting was significantly different between groups. Study Group 2 had significantly higher levels of % neutrophils than both Group 1 and the controls. Although it was reported that there was higher % neutrophils in the sputum samples of older normal volunteers [Hunt et al., 2006], we adjusted our results to age and they showed that the main differences may be due to exposure time. Evidence of correlation between other occupational exposures and neutrophilic inflammation was seen in popcorn production workers exposed to flavoring agents [Akpinar-Elci et al., 2005]. It was shown that the most common pattern in severe asthma is non-eosinophilic, associated with a neutrophil influx and mediated by IL-8 secretion: this indicates that there are differing mechanisms in operation that may impact on treatment responses [Gibson et al., 2001]. Importantly, neutrophils in IS samples were found to be a good predictor for clinical deterioration and the absence of response to treatment [Papi et al., 2006].

Although the CD4/CD8 ratio was normal for all participants, there was a differential distribution of each of

### TABLE VI. Particle Size Distribution$^a$ in Induced Sputum Samples From all Participants (N = 73)

<table>
<thead>
<tr>
<th>Individuals</th>
<th>% particles $&lt; 2 \mu m$ diameter</th>
<th>% particles $&gt; 2 \mu m$ diameter</th>
<th>% particles $&gt; 5 \mu m$ diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>99 ± 0.7</td>
<td>12 ± 0.7</td>
<td>0.18 ± 0.17</td>
</tr>
<tr>
<td>Group 2</td>
<td>97 ± 14*</td>
<td>2.5 ± 14*</td>
<td>0.4 ± 0.3*</td>
</tr>
<tr>
<td>Control</td>
<td>98 ± 11</td>
<td>13 ± 0.8</td>
<td>0.26 ± 0.26</td>
</tr>
<tr>
<td>P-value</td>
<td>0.019</td>
<td>0.017</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$^a$Assessed as described in Methods Section.

$^*$Analysis of variance (ANOVA) — a $P$-value < 0.05 was considered significant.
the subsets between the three groups. Tanigawa et al. [2004] found that these cells are very sensitive to the type of exposure, and this was demonstrated by Nakata et al. [2006] in welders exposed to manganese and by Park et al. [2000] in fluorescent-lamp makers exposed to mercury. We showed similar findings in our two study groups, that is, one exposed to iron and aluminum and the other to chromium and nickel.

The EBC samples from the study Group 2 showed lower pH values than the study Group 1 and controls. This is in concordance with the neutrophilic infiltration of airways in this group of welders, similar to COPD patients.
Six samples were selected according to their high burden of particles as detected on Cr in the samples of study Group 2 and so this may be in the welders working for long period of time. Thus, explaining retention of bigger particles showed that the retention of particles in the airways depends on particle size. Finally, Ally et al.'s [2006] experimental model performed on exposed firefighters 10 months after the collapse of World Trade Center: the particles in their airways had been retained over time and there were significant differences between the particle size distributions in their IS samples compared to those of a control group [Fireman et al., 2004].

In conclusion, the findings of our study demonstrated that exposure to welding significantly alters inflammatory and oxidative status in asymptomatic welders. Induced sputum particle size distribution clearly pointed to particle accumulation over time, and we consider that particle size distribution in IS may serve as a time-dependent marker for biological monitoring and future pulmonary injury.

**TABLE VII.** Scanning Electron Microscope (SEM) Analysis in Welders (N = 4) and Controls (2)

<table>
<thead>
<tr>
<th>N</th>
<th>Minerals in induced sputum (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welder 1</td>
<td>Welder 2</td>
</tr>
<tr>
<td>Welder 1</td>
<td>Si, Al, Fe</td>
</tr>
<tr>
<td>Welder 2</td>
<td>Si, Al, Fe, Ca</td>
</tr>
<tr>
<td>Control</td>
<td>Soil particles</td>
</tr>
<tr>
<td>Control</td>
<td>Soil particles</td>
</tr>
</tbody>
</table>

SEM was performed as described in Methods Section. Six samples were selected according to their high burden of particles as detected on Giemsa cytoreps. [Repine et al., 1997]. It is speculated that the main reason for the fall in the pH values is neutrophil myeloperoxidase that catalyzes the reaction between hydrogen peroxide and a chloride to form hypochlorous acid (HOCl) [Kostikas et al., 2002].

Our results of studying the oxidative stress status by measuring hydrogen peroxide in condensate clearly demonstrated that welding increased the presence of this metabolite in both groups and did so without any correlation to age, smoking status and type of exposure. This is in concordance with the elevation of oxidative systemic mediators that was found in asymptomatic shipyard welders [Han et al., 2005]. Matczak and Chmielnicka [1993] observed that the acid-base balance is also disturbed and that differences between groups of welders may be associated to the type of welding. In contrast with our previous studies [Fireman et al., 2007], we did not found any correlation between hydrogen peroxide secretion and neutrophils. A possible explanation for this difference is that welding damages epithelial a cells, which are also involved in oxidative burst [Moraes et al., 2006].

The novelty of the present work is the attempt to perform biological monitoring in the airways of asymptomatic welders using the accumulation of various-sized particles in IS, an entirely noninvasive manner. We demonstrated a higher percentage of particles >2 μm in the airways of welders that was in correlation to the years of exposure to the noxious air-borne substances.

We had previously reported our findings on a study we performed on exposed firefighters 10 months after the collapse of World Trade Center: the particles in their airways had been retained over time and there were significant differences between the particle size distributions in their IS samples compared to those of a control group [Fireman et al., 2004]. Finally, Ally et al.’s [2006] experimental model showed that the retention of particles in the airways depends on particle size. Thus, explaining retention of bigger particles in the welders working for long period of time.

In terms of SEM analysis, we did not find any Ni and Cr in the samples of study Group 2 and so this may be a false negative result due to lack of sensitivity in our detection method. The neutrophilic pattern shown in the samples of those workers may also be attributed to the different metal constituents used during welding [Antonini et al., 2004].

**Study Limitations**

We recognize that the particle size distribution index in IS is not the optimal parameter to biologically monitor workers exposed to hazardous dust since it does not express concentration or absolute number of particles in airways. To the best of our knowledge, however, no other attempts have been made to determine whether other well-established parameters can be used in order to find any kind of threshold for risk assessments alone or in combination with environmental monitoring.

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