SERUM CLARA CELL PROTEIN AS AN INDICATOR OF PULMONARY IMPAIRMENT IN OCCUPATIONAL EXPOSURE AT ALUMINUM FOUNDRY

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Abstract

Objectives: Although some of the exposures in aluminum (Al) smelting have been well characterized, and respiratory disorders in aluminum production workers are well known, the relationship between internal aluminum loads and appropriate lung biomarkers have not been elucidated. The aim of our work was to carry out a comprehensive investigation in workers employed in the Aluminum Foundry Casting Department with special reference to currently existing hygiene standards, known as threshold limit values (TLV) based on aluminum effects on the respiratory system. The measurement of serum anti-inflammatory Clara cell protein (CC16) was employed as a peripheral marker of the lung epithelium function.

Materials and Methods: A group of 50 casting smelters, 5 locksmiths, 11 sawyers and auxiliary workers exposed to dust containing 14% of aluminum, and a group of 42 controls were examined. Respiratory function tests were performed and forced volume capacity (FVC), forced expiratory volume in 1 s (FEV1), forced expiratory volume in the first percent (FEV1%), forced expiratory flows in 50% VC (FEV50), and markers of foundry workers’ exposure and body burden, Al concentration in the breathing zone, blood and urine, biomarkers of the effects of exposure, concentration of CC16 and hyaluronic acid (HA) in serum were determined in all examined workers. Additional measurements comprised determinations of serum iron (Fe) levels, myeloperoxidase (MPO), eosinophil cationic protein (ECP), immunoglobulin E (IgE), glutathione S-transferase (GST), and superoxide dismutase (SOD) activity in erythrocytes.

Results: The group of casting smelters was characterized by the highest levels of aluminum in urine (Al-U) (43.7 mg L−1), high levels of MPO, ECP and IgE, high SOD activity, low CC16 levels, and low activity of GST. Lower Al-U excretion was observed in locksmiths (35.2 mg L−1) and sawyers (21.7 mg L−1). Serum CC16 proved to be the most sensitive biomarker, showing high inverse relationship with serum Al (Al-S) concentrations in casting smelters (p = 0.006).

Conclusions: The study showed that in conditions of occupational exposure, dusts containing Al2O3 < 1 mg m−3 cause changes in the respiratory system and biomarkers in serum, especially in CC16, connected with altered functioning of this system. Changes in concentrations of the examined biomarkers and also in respiratory parameters of the study subjects were observed when Al-U concentration was > 40 µg L−1.

Key words: Clara cell protein, Aluminum, Respiratory system, Foundry, Occupational exposure

INTRODUCTION

Aluminum (Al) production is evidently associated with decreasing pulmonary function parameters [1] and the development of occupational asthma and fibrosis [2–9]. Several clinical and spirometric effects were ascertained in the exposed people [10,11]. At present, there is no clear consensus on appropriate methodology for the assessment of the risk of occupational Al exposure. To protect workers from detrimental effects of Al exposure, values of hygiene standards have been established in many countries;
unfortunately, they considerably differ between individual countries. Referring to the effect at low dose levels, the health base exposure limits are assumed to workplace occupational limit values (TLV) in the range from 2.0 to 10 mg m\(^{-3}\), depending on the chemical form of Al [12]. Also the application of biomarkers linked to toxic mechanisms in the process of risk assessment is very important [13]. Airway inflammation is a central feature of exposure to Al potroom emissions and induces pathological alterations, such as potroom asthma, similar to those described in other types of asthma. This kind of exposure affects the bronchiole-alveolar epithelium as the primary target site, causing lung injury. As a cell-specific marker for non-ciliated bronchiolar epithelial cells, which play a part in immunomodulatory and/or anti-inflammatory processes, assessment of Clara cell protein in serum (CC16) was proposed by various authors [14–17]. Recent studies on humans and rats have shown that CC16 is a sensitive biomarker of respiratory epithelial injury in exposures to welder fumes, glutaraldehyde, nitric oxides, or Al foundry dusts [18–23]. Clara cell proteins are involved in the pathogenic mechanisms leading to fibrosis [15,24], chronic obstructive pulmonary disease (COPD) [25,26], and asthma in humans [27–29] and in murine asthma model [30,31]. Low level of CC16 was observed in serum and bronchoalveolar lavage fluid (BAL) of active smokers [32]. A number of works described changes in the respiratory system without reference to the impact of Al concentration in the ambient air [9,33–38]. Rare studies reported changes in the respiratory system related to Al concentration in serum (Al-S) or urine (Al-U) [39,40], however, without any reference to biomarkers. A few authors give values of Al concentrations in urine and serum with reference to concentrations in the ambient air [1,41–43]. The aim of our study was to perform: 1) spirometric assessment of lung function, including determinations of forced volume capacity (FVC), forced expiratory volume in 1 s (FEV\(_1\)), FEV\(_1\)% (Tiffenau index), forced expiratory flow in 50% VC (FEF\(_{50}\)); 2) determination of biomarkers in serum (CC16, serum iron (Fe-S), hyaluronic acid (HA), myeloperoxidase (MPO), eosinophil cationic protein (ECP) and immunoglobulin E (IgE) and in erythrocytes (activity levels in superoxide dismutase (SOD) and glutathione S-transferase (GST)) of the exposed people, with reference to changes in the respiratory system; and 3) determination of the relationships between Al biomarkers and concentrations in the workplace atmosphere and Al body burden, Al-U and Al-S. The study design was approved by the Regional Bioethical Committee of the Nofer Institute of Occupational Medicine in Łódź and complied with the current law in Poland. The examined subjects were informed of the scope and purpose of the examinations and their informed consent was obtained.

MATERIALS AND METHODS

Subjects and study design

The study groups included 66 male workers, including 50 casting smelters, 5 locksmiths, 11 sawyers and auxiliary workers, and the control group included 42 non-exposed people. All groups were matched for age, period of employment and smoking habit. Markers of exposure of the foundry workers (Al concentrations in air, blood and urine), biomarkers of the effect of exposure (concentration of CC16 and HA in serum), and respiratory functional tests were determined in all examined workers.

Sampling and metal determination

Personal air sampling at the breathing zone was applied (Casella AFC-123, flow rate 2 l/min). Total dust was collected (over a period of about a 7-h shift) on membrane filter (Sartorius 111304, 0.8 \(\mu\)m, \(\varnothing 32\) mm). In the sample collected on the filter, total dust was determined gravimetrically and Al concentration measured by flame atomic absorption spectrometry (using a Varian SpectrAA 250 atomic spectrometer).

Blood was drawn into red-top Vacutainers (Becton-Dickinson, NJ) and allowed to stand at room temperature for 3 h to facilitate clotting. It was then refrigerated at 4°C until centrifugation, almost always within a few hours. Serum was transferred into two polyethylene 4.5 ml Cryotubes and stored at -20°C until determination. For erythrocytes isolation green-top Vacutainers Lith/Heparin were used.
After centrifugation supernatants containing plasma and leukocytes were removed and red blood cells (RBC) were washed three times with saline, RBC were suspended in saline and stored at -20°C until determination.

Aluminum in serum was determined by graphite furnace atomic absorption spectrophotometry using a Perkin-Elmer model 4100 ZL Zeeman unit with an autosampler AS-70 and computer data station PE 6200. The precision (coefficient of variation, CV) of the method for serum at 10–100 mg L⁻¹ and 25–200 mg L⁻¹ was 7.0 and 7.9%, respectively, and for urine at 25–150 mg L⁻¹ the corresponding value was 4.5%. The accuracy of the method was verified by analyzing serum and urine reference material (Seronorm™ Trace Elements, No. 704121). The German Interlaboratory Comparison Program was used for the validation of the reliability of the analytical results [44].

Iron in serum was determined by spectrophotometry by measuring pink complexes of ferrous ion with a FeroZine unit, Alpha Diagnostic, Warsaw [45].

Biochemical determinations

Clara cell protein was determined by latex immunoassay (LIA) [46,47]. Specific rabbit antibody against CC16/Protein 1 from Dako A/S, Denmark was used. To eliminate possible interference (from complement, chylomicrons), the serum samples for CC16 estimation were pre-treated by heating at 56°C for 30 min and by the addition of polyethylene glycol 600 (16%, v/v 1/1) and trichloroacetic acid (10%, v/v 1/40). After overnight precipitation, the samples were centrifuged and CC16 was determined in supernatants. Serum HA was measured by enzymatic-immunoassay (ELISA), which uses a capture molecule, hyaluronic acid binding protein (HABP) from Chugai Diagnostics Science Co. Ltd. (Tokyo, Japan). GST (U mg⁻¹ Hb) in RBC was determined according to the Habig method with CDNB as a substrate [48]. SOD activity (U mg⁻¹ Hb) in RBC was assayed according to Beauchamp and Fridovich [49] with xantine oxidase and nitroblue tetrasolium salt. Mieloperoxidase quantitative radioimmunoassay was done in human serum with use of Pharmacia MPO RIA set (Pharmacia and Upjohn, Diagnostics, Uppsala, Sweden) according to Hoffstein [50]. Fluoroenzymatic immunoassay (FEIA) tests were performed to estimate eosinophil cationic protein (ECP) and IgE in serum with Pharmacia UniCAP ECP and Pharmacia UniCAP IgE (Pharmacia and Upjohn, Diagnostics, Uppsala, Sweden).

Spirometry

After medical follow-up examination, workers were subjected to respiratory function tests. FVC in standing position, FEV₁, and FEF₅₀ were measured in each exposed and non-exposed worker with use of a Satellite DPU-411 Type II (USA) electronic apparatus with digital readout. Spirometric measurements were taken after an 8-h work shift prior to collection of urine and blood samples. Of the three consecutive measurements, the best results for a given person were selected for further analysis. In addition, FEV₁% (Tiffeneau index) was calculated. For each worker, the observed values of ventilation parameters were expressed as a percentage of the predicted values calculated according to the subject’s gender, age, weight, and height.

Statistical analysis

The characteristic parameters of the foundry workers by workplace and of controls were shown as means and its SD. Smoking habit among workers was presented as the percentage of the whole study group. Correlations between values were analyzed by Spearman’s partial rank-order analysis. Significance was assigned at p < 0.05. The differences between means for the groups selected were tested by the analysis of variance and multiple comparison procedure. We used Dunn’s test for this purpose. The parameters with a skew distortion were transformed logarithmically. The relationship between continuous variables was determined by multiple linear regression. All variables were entered into the regression model (enter method). STATA 8 software was used for the statistical analysis.

RESULTS

Table 1 summarizes characteristics of Al foundry male workers and the level of exposure to Al₂O₃ at the breathing zone by workposts. The smelters had the shortest
time of employment and the lowest levels of alumina in
the breathing zone. In all groups, the percentage of ac-
tive smokers did not exceed 50%. Figure 1 presents
the categories of workers exposed in the Aluminum Foundry
Casting Department and smelters classified according to
the smoking habit.

Figure 2 shows the correlation between total dust and
Al₂O₃ in the workers’ breathing zones. Results of spiro-
metric examinations show slight changes, such as lower
levels of spirometric indices in foundry workers as com-
pared to controls (Table 2). Those changes were depen-
dent on the age and employment duration. Low levels of
FEV₁, FEV₁% (Tiffenau index), and FEF₅₀ were found
in locksmiths, the oldest group of workers employed in
the foundry. Evident detrimental effects of the air pol-
lutants on the respiratory function, expressed by spiro-
metric FVC, FEV₁ and FEF₅₀ values, could be noted in
smelters as compared to sawyers group with comparable
age and employment duration and percent values of ac-
tive smokers. The relation between the results of spiro-
meter digital automatic pattern of pulmonary functional
changes, enabling determination of the extent of obstruc-
tion, shows the highest degree of obstruction related to
the increased Al concentration (expressed as Al₂O₃) in
the ambient air (Fig. 3). After having arbitrarily divid-

ted smelters into three groups according to the level of
Al₂O₃ in the air with mean values x̄₁ = 0.2, x̄₂ = 0.3, x̄₃ =
0.45 mg/m³, results of spirometric examinations (normal
– very slight obstruction – mild to moderate obstruction)
were specified separately for each of the three exposure

Table 1. Characteristics of the aluminum foundry workers and levels
of exposure to Al₂O₃ in the workplace breathing zones

<table>
<thead>
<tr>
<th>Variables</th>
<th>Aluminum foundry workers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smelters (n = 50)</td>
<td>Locksmiths (n = 5)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.9 ± 5.9</td>
<td>51.0 ± 1.9</td>
</tr>
<tr>
<td>Employment duration (years)</td>
<td>14.8 ± 8.0</td>
<td>28.4 ± 2.0</td>
</tr>
<tr>
<td>Exposure to Al₂O₃ (mg m⁻³)</td>
<td>0.32 ± 0.18</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>Cigarette smoking (pack/years)</td>
<td>16.5 ± 10.8</td>
<td>18.0 ± 4.0</td>
</tr>
<tr>
<td>Smoking habits (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>30.6</td>
<td>40.0</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>14.3</td>
<td>20.0</td>
</tr>
<tr>
<td>Passive smokers</td>
<td>10.2</td>
<td>0</td>
</tr>
<tr>
<td>Current smokers</td>
<td>44.9</td>
<td>40.0</td>
</tr>
</tbody>
</table>

Table 2. Spirometric characteristics of aluminum foundry workers,
% of predicted values

<table>
<thead>
<tr>
<th>Variables</th>
<th>Aluminum foundry workers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smelters (n = 50)</td>
<td>Locksmiths (n = 5)</td>
</tr>
<tr>
<td>FVC</td>
<td>98.0 ± 15.5</td>
<td>112.7 ± 22.0</td>
</tr>
<tr>
<td>FEV₁</td>
<td>89.6 ± 15.7</td>
<td>82.7 ± 9.5</td>
</tr>
<tr>
<td>FEV₁%</td>
<td>91.2 ± 9.1</td>
<td>78.7 ± 16.1</td>
</tr>
<tr>
<td>FEF₅₀</td>
<td>80.4 ± 29.5</td>
<td>69.0 ± 17.3</td>
</tr>
</tbody>
</table>

Fig. 1. Flowchart of spirometric and biochemical examinations of the
aluminum foundry workers.

Fig. 2. Correlation between quantity of total dust and alumina (Al₂O₃)
levels in the breathing zone of all foundry workers (collected during
an 8-h shift).
Mild or moderate obstruction was most frequent in the 0.45 Al2O3 mg m−3 group.

Table 3 shows the studied biomarkers: CC16, HA serum levels, Al-S and Al-U in casting smelters, locksmiths and sawyers in relation to control subjects. The group of smelters was characterized by the highest Al-U levels (43.7 μg L−1) and the lowest serum CC16 levels. Locksmiths and sawyers showed Al-U levels respectively three and two times higher than controls.

Figure 4 represents a schematic comparison of changes of all study biomarkers in the casting smelter, locksmith and sawyer groups. The figure shows serum levels of CC16, Al-S, Fe-S, HA, MPO, ECP and IgE and erythrocyte SOD and GST activity in different exposed groups in terms of percent values in respective controls. The group of smelters was characterized by statistically significantly elevated SOD activity, high MPO level, the lowest CC16 levels, the highest, although statistically insignificant, IgE levels, and the lowest GST activity. In the locksmiths, the oldest group of workers, most characteristic changes were: high HA level, a marker of inflammatory condition; however, the MPO marker of neutrophil activity was not changed.

The sawyers exposed to high Al concentrations (expressed as Al2O3) in the air presented low CC16 levels, while the levels of other biomarkers were comparable to controls (Table 1).

It is worth noting that the smelter group presented high Spearman correlation coefficients between different biomarkers both in smoking and non-smoking smelters (Table 4). The set of biomarkers selected for this study confirmed their importance for studying the respiratory system in casting smelters. In Spearman analysis of smokers, serum CC16 correlated negatively with Al-S, and IgE. Al-S in smokers correlated negatively also with HA and Fe.

Non-smokers showed high values of the correlation coefficient between Al-U and Fe-S concentrations and spirometric indicators. In non-smoking casting smelters, low Fe levels correlated with decreased pulmonary function and higher MPO and IgE levels.

To eliminate the effects of confounding parameters, such as smoking and age of the smelters, linear regression analysis was applied for markers of exposure – AlO2 in air, and Al-U and Al-S concentrations in relation to spirometric indices (FVC, FEV1) and serum CC16 (Table 5). Al-U levels, a marker of body burden and toxicity, showed a significant inverse correlation with FVC and FEV1. Strong negative correlation was found to occur between Al-S and CC16 in serum, the most characteristic change in casting smelters (p = 0.006).

![Fig. 3. The effects of Al2O3 in the breathing zone atmosphere on the pulmonary function. Changes in the lung function were determined separately for each of the three [low, x̄ = 0.2 Al2O3 mg m−3 (N = 15); medium, x̄ = 0.3 Al2O3 mg m−3 (N = 17); high, x̄ = 0.45 (N = 18) Al2O3, mg m−3] exposure levels in arbitrarily determined smelter groups. The values specified in the figure represent the proportion of workers (%) who experienced one of three specified modes of lung function obstruction [normal (LFN-D), very slight obstruction (VOBS-D), and mild or moderate obstruction (MOBS-D)] in each group of the exposed smelters (with low, medium and high Al2O3 levels in breath zone during an 8-h shift). D – digital automatic pattern of pulmonary functional changes.](image-url)
* p < 0.05.

**Fig. 4.** Biomarker levels in serum of the smelters (n = 50), locksmiths (n = 5), sawyers and auxiliary workers (n = 11) in terms of percent values for respective controls (n = 42).

**Table 4.** Spearman analysis between biomarkers in serum and urine and spirometric indices in smoking (n = 23) and non-smoking (*) smelters (n = 15)

<table>
<thead>
<tr>
<th>Correlation coefficients (p)</th>
<th>CC16 (µg L⁻¹)</th>
<th>HA (µg L⁻¹)</th>
<th>Fe (mg dL⁻¹)</th>
<th>IgE (kU L⁻¹)</th>
<th>MPO (µg L⁻¹)</th>
<th>FVC (%)</th>
<th>FEV₁ (%)</th>
<th>FEF₅₀ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC16</td>
<td>-</td>
<td>0.51</td>
<td>-0.58</td>
<td>-0.54</td>
<td>0.60*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-S</td>
<td>-0.54</td>
<td>-0.47</td>
<td>-0.54</td>
<td>0.69*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-U</td>
<td>-0.54</td>
<td>-0.47</td>
<td>-0.54</td>
<td>0.69*</td>
<td>0.76*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>-0.56*</td>
<td>-0.61*</td>
<td>0.83*</td>
<td>0.76*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>0.51</td>
<td>0.70*</td>
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</table>
DISCUSSION

According to WHO Environmental Health Criteria 210 [51] for considerably toxic effects at various dose levels, the dose range of interest is generally the lower dose range, since it usually reflects the human exposure situations. Several authors report respiratory Al health effects in workers [34,35,37,38]. Although some of the exposures in Al smelting have been well characterized, particularly in potrooms, little has been published regarding exposures in other Al production departments [52]. In several studies, attention was focused on polycyclic aromatic hydrocarbon (PAH) biomarkers in the Al industry [53–55].

The differences in the results of spirometric lung assessment are connected with concentration of dust and Al$_2$O$_3$ in relation to particular jobs. In our study in the Aluminum Foundry Casting Department, it was shown that Al$_2$O$_3$ concentrations observed at workplaces can cause various functional respiratory impairments (Tables 1 and 2), depending on the obstruction severity pattern (Fig. 3) and variation of biomarker levels referred to Al-S and Al-U in relation to particular jobs (Table 3, Fig. 4). In the groups exposed to foundry dust and fumes, the prevalence of Al-U levels exceeding 40 μg L$^{-1}$ was markedly higher in smelters than in the control group (Table 3). Although not well-defined, Al-S and Al-U concentrations are presumed to be related to the total body burden. In study performed by Rihimaki [56], daily fluctuation of Al-S was observed, where the urinary excretion of current Al uptake was more protracted. In the study by Elinder [57] for low-level Al exposure (below 0.5 mg/m$^3$), these indicators (especially Al-U) make it possible to distinguish the exposed groups from the general population without, however, any clear relationship with various environmental Al concentrations. It was also seen that Al-U increased with increasing work seniority and was more marked in certain processes, such as casting, and in the first few months or years of employment [42].

A relevant reduction of the spirometric indices of respiratory lung function (Table 2) accompanied by loss of serum CC16 and Al-S between Clara cell protein in serum of aluminum smelters (n = 50)

Table 5. Multiple linear regression of Al$_2$O$_3$, Al-U and spirometric parameters (FVC and FEV$_1$) and Al-S between Clara cell protein in serum of aluminum smelters (n = 50)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Log Al$_2$O$_3$ (FVC)</td>
<td>10.004</td>
<td>-1.779</td>
<td>0.447</td>
</tr>
<tr>
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<td>0.004</td>
<td>-1.779</td>
<td>0.447</td>
</tr>
<tr>
<td>Log Al-U (FVC)</td>
<td>-6.772</td>
<td>-1.779</td>
<td>0.447</td>
</tr>
<tr>
<td>Log Al-U (FVC)</td>
<td>-2.295</td>
<td>-4.204</td>
<td>0.386</td>
</tr>
<tr>
<td>Log Al-U (FEV$_1$)</td>
<td>-8.772</td>
<td>-1.779</td>
<td>0.447</td>
</tr>
<tr>
<td>Log Al-U (FEV$_1$)</td>
<td>-2.295</td>
<td>-4.204</td>
<td>0.386</td>
</tr>
<tr>
<td>Log Al-S (CC16)</td>
<td>-1.113</td>
<td>-1.779</td>
<td>0.447</td>
</tr>
<tr>
<td>Non-smoking Age</td>
<td>0.055</td>
<td>-0.031</td>
<td>0.141</td>
</tr>
<tr>
<td>Non-smoking Non-smoking</td>
<td>0.142</td>
<td>-1.769</td>
<td>2.054</td>
</tr>
<tr>
<td>Smoking Non-smoking</td>
<td>2.295</td>
<td>-4.204</td>
<td>0.386</td>
</tr>
</tbody>
</table>

* Statistically significant.
the activation of Th2 cells is sufficient for the induction of inflammation and chronic pathological changes associated with asthma [64]. The decrease in CC16 and increase in MPO and SOD were statistically valid, when related to controls, in the group of smelters only (Fig. 4). In this group of workers, IgE was 150% higher than the levels found in controls. Increase in IgE and ECP is a characteristic parameter in diagnosing asthma [63]. The study by Monteserin et al. [65] showed that IgE-dependent mechanism might promote MPO released by neutrophils at allergic sites. In atopic asthmatic children, higher levels of IgE, MPO and ECP were observed [66]. In our study, statistically significant inverse correlation was found between CC16 and IgE in smoking smelters (Table 4). Statistically significant increase in IgE was found in smokers and former smokers in the group of smelters as compared to controls (data not shown), confirming adjuvant effects of smoking [67]. Animal studies showed that IgE were induced by Al hydroxide inhalation [68], or intratracheal instillation of Al silicate [69]. Shijubo et al. [27] showed significantly decreased proportions of CC16-positive epithelial cells in small airways of asthmatics. Increased MPO and ECP confirm the role of neutrophils and eosinophils in airway inflammation of potroom workers [70] and asthmatic patients [71]. Moreover, it was documented that eosinophilia might be an early subclinical marker indicating the risk of developing Al-induced lung disease [72]. Indeed, in other pulmonary diseases, eosinophilia has been shown to indicate rapid development of disease and impend fibrosis formation [73–75]. Figure 4 shows that serum HA marker of inflammatory condition or remodeling of lung tissues was increased in smelters together with the increased activity of neutrophils and eosinophils. Eklund [76] showed that BAL of potroom workers contained more fibronec-tin, albumin, and HA than the fluid of those non-exposed. Increased HA, MPO and ECP levels were found in the patients with chronic bronchitis [77]. HA was the only biomarker showing a distinct, although statistically insignificant, increase in the oldest locksmith workers (Table 3, Fig. 4), confirming marked age-dependence [78].

The decrease in CC16 and increase in MPO and SOD were statistically valid, when related to controls, in the group of smelters only (Fig. 4). In this group of workers, IgE was 150% higher than the levels found in controls. Increase in IgE and ECP is a characteristic parameter in diagnosing asthma [63]. The study by Monteserin et al. [65] showed that IgE-dependent mechanism might promote MPO released by neutrophils at allergic sites. In atopic asthmatic children, higher levels of IgE, MPO and ECP were observed [66]. In our study, statistically significant inverse correlation was found between CC16 and IgE in smoking smelters (Table 4). Statistically significant increase in IgE was found in smokers and former smokers in the group of smelters as compared to controls (data not shown), confirming adjuvant effects of smoking [67]. Animal studies showed that IgE were induced by Al hydroxide inhalation [68], or intratracheal instillation of Al silicate [69]. Shijubo et al. [27] showed significantly decreased proportions of CC16-positive epithelial cells in small airways of asthmatics. Increased MPO and ECP confirm the role of neutrophils and eosinophils in airway inflammation of potroom workers [70] and asthmatic patients [71]. Moreover, it was documented that eosinophilia might be an early subclinical marker indicating the risk of developing Al-induced lung disease [72]. Indeed, in other pulmonary diseases, eosinophilia has been shown to indicate rapid development of disease and impend fibrosis formation [73–75]. Figure 4 shows that serum HA marker of inflammatory condition or remodeling of lung tissues was increased in smelters together with the increased activity of neutrophils and eosinophils. Eklund [76] showed that BAL of potroom workers contained more fibronec-tin, albumin, and HA than the fluid of those non-exposed. Increased HA, MPO and ECP levels were found in the patients with chronic bronchitis [77]. HA was the only biomarker showing a distinct, although statistically insignificant, increase in the oldest locksmith workers (Table 3, Fig. 4), confirming marked age-dependence [78].

Lower iron levels, not statistically significant, were detected in serum of the smelters and sawyers (Fig. 4). Both those groups of workers had low levels of serum CC16. In the group of sawyers, signs of inflammations measured by the employed markers were not observed (Fig. 4). In Spearman’s partial rank-order analysis (Table 4), Fe level correlated significantly (negatively) with Al-S of smelters (in active smokers) and positively with Al-U (in non-smokers), confirming the effect of Al and Fe competition with transferrin (a transfer ligand) in serum [79,80]. In our study, we noted a negative correlation between IgE, MPO and Fe levels in non-smoking smelters, while dislocation of iron in chronic inflammation may be an additional factor affecting serum transferrin iron level [81] (Table 4). Al affects various stages of heme biosynthesis by changing the activity of some enzymes [82–85], iron metabolism [86–89] or disrupting erythrocyte membrane [90].

Linear regression analysis (Table 5), eliminating the effects of respective confounding factors (e.g., age, tobacco smoking) shows that CC16 and Al-S correlation was very strong (p = 0.006). It should be noted that this close relation supports the opinion that both markers (Al-S and CC16) are related, at least in part, to the daily fluctuation as already observed in cross-week CC16 evaluation in blood of shipyard welders and workers of a chemical plant exposed to nitric oxide [21,22]. Thus, inhibition of Clara cell protein secretion in smelters heavily exposed to Al can probably enhance Al-S levels by suppressing anti-inflammatory effect in the respiratory tract. Although the exact mechanism of the lung-blood transfer of CC16 remains to be clarified, it appears to be governed by a number of factors, among which the permeability of the tight bronchoalveolar epithelium seems to be most essential. The molecular features of the lung proteins and their exchangeable pool appear to be critical. Bearing all this in mind, it seems reasonable to believe that the aluminum indirectly interacts in the CC16 decrease via metal-related changes in the lipid matrix of the membrane [91–95].

In our study group (foundry department workers), only casting smelters showed Al-U levels significantly higher than in controls. In the study by Alesso [96], the mean Al-U levels in a group of 227 subjects occupationally exposed to aluminum at 0.1–10.0 mg/m³ were higher than those of the reference population; however, Al-U levels of
the workers were generally lower than 20 \( \mu g \) L\(^{-1}\), which is the upper limit for the reference population.

The lowest dose which can cause impairment of human health is used in setting the hygiene standards; thus it is essential to determine experimentally the dose/effect parameters.

Current hygiene standards in the USA [12] allow several TLV levels, depending on the chemical form of Al. For alumina dust, TLV is 10 mg m\(^{-3}\), for welder fumes (as Al) 5 mg m\(^{-3}\), and for dissolved salts (as Al) 2 mg m\(^{-3}\). In Germany, for metallic Al and alumina fumes, the level of MAK is 1.5 mg m\(^{-3}\) (respirable fraction) [97]. The Polish OEL value for Al\(_2O_3\) has been set at 2.5 mg m\(^{-3}\) (as Al) and 1.2 mg m\(^{-3}\) (as Al) for respirable fraction [98]. The American Conference of Governmental Industrial Hygienists (ACGIH) has not proposed any level of BEI for Al in biological materials. In Germany, those levels are set at 200 \( \mu g \) L\(^{-1}\) in samples of urine collected after the end of work shift [97]. In Finland, BEI has been set at 160 \( \mu g \) L\(^{-1}\) measured on Monday before work [99].

The most important observation in this study was reduction of spirometric indices and loss of CC16 levels in serum with statistically significant increase in MPO and SOD levels in smelters exposed to Al\(_2O_3\) within the range 0.1–1.0 mg m\(^{-3}\) (the mean levels \( \bar{\chi} = 0.3 \) mg m\(^{-3}\)). Our results and those of several other authors [43,96,100,101] show that an extensive study is needed to determine a more correct TLV and health-based permissible concentration for occupational exposure to Al. Changes in and adverse effects on the central nervous system, for example, which can be observed at levels below the actual German and Finnish BEI standard for Al-U concentration in Al welders should be taken into account [56,95]. Future studies on respiratory morbidity should explore the interaction of mixtures, which may show a synergistic activity.

**CONCLUSIONS**

1. In conditions of occupational exposure, dusts containing Al\(_2O_3\) < 1 mg m\(^{-3}\) cause deterioration of the respiratory system and also changes of biomarkers in serum, associated with altered functioning of this system.

2. Clara cell protein (CC16) proved to be the most sensitive biomarker, showing high correlation with Al concentrations in serum of the smelters.

3. Changes in concentrations of the examined biomarkers and also in respiratory parameters of the study subjects were observed when the urinary Al concentration was > 40 \( \mu g \) L\(^{-1}\).

**ACKNOWLEDGEMENTS**

Thanks are due to G. Raźniewska, MSc. for Al determination in urine and serum, and B. Kur, MSc. for the measurement of IgE, MPO and ECP in serum. Special thanks are due to Ms. A. Kubiak for her participation in technical procedures and to Dr. W. Sobala for the statistical analysis of the results.

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