



SERUM NEOPTERIN IN WORKERS EXPOSED TO ASBESTOS AND COAL DUST AND IN PATIENTS WITH SILICOSIS

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ABSTRACT

The main *purpose* of this study was to investigate serum neopterin levels in workers exposed to coal and asbestos dust and to compare it to the levels found in silicosis patients and in a control group.

Methods: Serum neopterin was measured in 190 workers exposed to inorganic dust, in 95 patients with silicosis and in 16 control subjects. Depending on characteristics of the dust the surveyed workers were divided into three groups: Group I (44) – exposed to dust containing less than 2% free crystalline silica, Group II (94) - exposed to dust containing more than 2% FCS and Group III (52) - exposed to asbestos dust. Neopterin was determined by ELISA method, kit DRG Diagnostics, Germany in ng/mL. *Results:* We found higher levels of neopterin in workers exposed to dust ($3,26 \pm 0,57$ ng/mL) compared to the control group ($1,56 \pm 0,38$ ng/mL) and to the patients with silicosis ($2,84 \pm 1,19$ ng/mL). Serum neopterin was significantly higher in workers from Group II ($3,32 \pm 0,77$ ng/mL) and Group III ($3,20 \pm 0,48$ ng/mL) compared to those from Group I and the controls. *Conclusions:* These results confirm the involvement of macrophages and cell-mediated immune reactions in the pathogenesis of pulmonary fibrosis in the development of pneumoconioses. Levels of neopterin in workers exposed to asbestos dust were examined for the first time and were found to be significantly higher than those of the control group.

Keywords: neopterin, coal dust, asbestos dust, silicosis

INTRODUCTION

Silicosis, coal workers' pneumoconiosis and asbestosis are the most important occupational lung diseases which are still being diagnosed, despite the fact that they are preventable by continuous control of airborne dust in the working environment. The norms of inorganic dust under Regulation №13/2003 by the Ministry of Labour and Social Policies and the Ministry of Health depend largely on the content of free crystalline silica (FCS) in the respirable fraction (1).

It is known that silica represents 28% of the

earth's crust and occurs in combination with many minerals and metals. All five polymorphic forms of SiO₂ were proven to possess fibrogenic properties and the most dangerous is quartz, followed by tridymite and cristobalite. The permissible exposure level (PEL) of free crystalline silica dust (quartz, cristobalite and tridymite) is 0.07 mg/m³ in the respirable fraction, while for mixed coal dust permissible levels depend on the percentage of FCS. PEL of the respirable fraction of coal dust containing over 2% FCS is determined by a specific formula: $0.07 \times 100 / \% \text{ of FCS}$ in the fine dust. For anthracite PEL is 2 mg/m³ and for lignite and brown coal - 4 mg/m³. PEL for the inhalable fraction of coal dust containing over 2% FCS is 5 mg/m³.

According to Regulation № 9/2006 by the Ministry of Labour and Social Policies and the

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Ministry of Health the PEL of asbestos dust is determined by the number of fibers per cm^3 and is 0.1 fibers/ cm^3 in the respirable fraction (2).

Significant progress in clarifying the pathogenic mechanisms of development and progression of pneumoconioses notwithstanding, at this time it still cannot be said with certainty that the achievements made have finally solved the problems with these occupational diseases. In this regard innovative research on identifying risk groups of workers, early diagnosis and developing strategies for treatment are of particular importance.

Interstitial lung diseases caused by exposure to silica and/or coal dust are due to damage to lung cells and activation of fibrotic processes in the lung parenchyma. Castranova, V., 1998; Lapp, N.L. et al., 1993, proposed several mechanisms of injury: 1. direct cytotoxicity; 2. activation of oxidative stress by alveolar macrophages; 3. secretion of inflammatory cytokines, chemokines and fibrogenic factors from alveolar macrophages and/or alveolar epithelial cells (3, 4).

The presence of phagocytic cells in the alveolar spaces is a response to particulate matter induced production of chemotactic cytokines and chemokines from alveolar macrophages and alveolar epithelial cells type II (5). Neopterin, a low molecular weight type of pteridine, produced by activated macrophages after stimulation with interferon γ is indicated as an early biomarker of cellular immune response (6-10). Gulumian, G. et al., 2001 indicate serum neopterin as a possible

marker for effect during exposure to silica (11).

The main *objective* of this study was to examine the contents of neopterin in serum of workers exposed to asbestos and coal dust and in patients with silicosis.

MATERIALS AND METHODS

Serum neopterin in 190 workers exposed to dust, 95 silicosis patients and 16 controls was investigated. Depending on the quantity and quality characteristics of the inorganic dust, workers surveyed were divided into three groups: Group I - exposed to dust containing less than 2% FCS (n = 44), Group II - exposed to coal dust containing more than 2% FCS (n = 94) and Group III - exposed to asbestos dust (n = 52). Blood samples were taken from the tested subjects via venipuncture after signing an informed consent for participation in the study. Inquiry method was used for determining the presence of lung and autoimmune diseases and smoking habits.

Serum neopterin was identified by ELISA method, kit DRG Diagnostics, Germany in ng/mL. The data obtained was processed using Basic Statistic and Table for Windows.

RESULTS

Figure 1 presents the levels of neopterin in the studied groups. Significantly higher mean levels of neopterin were established in silicosis patients ($2,84 \pm 1,19$ ng/mL, $p < 0,05$) and in workers exposed to inorganic dust ($3,26 \pm 0,57$ ng/mL, $p < 0,05$) compared to the control group ($1,56 \pm 0,38$ ng/mL).

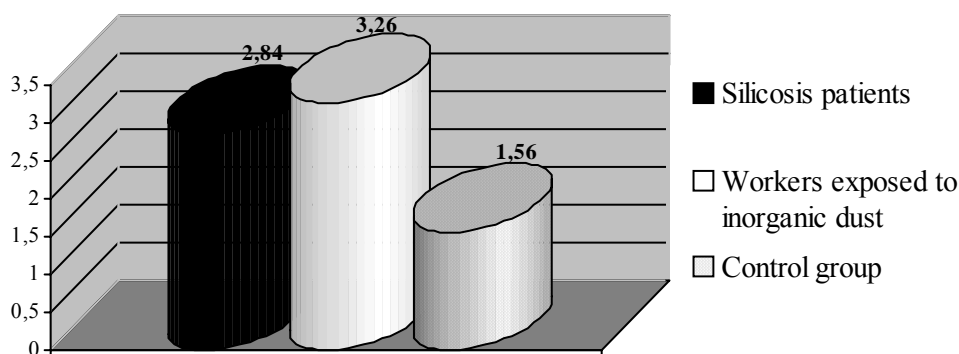


Figure 1. Serum neopterin in patients with silicosis in workers exposed to inorganic dust and in control group

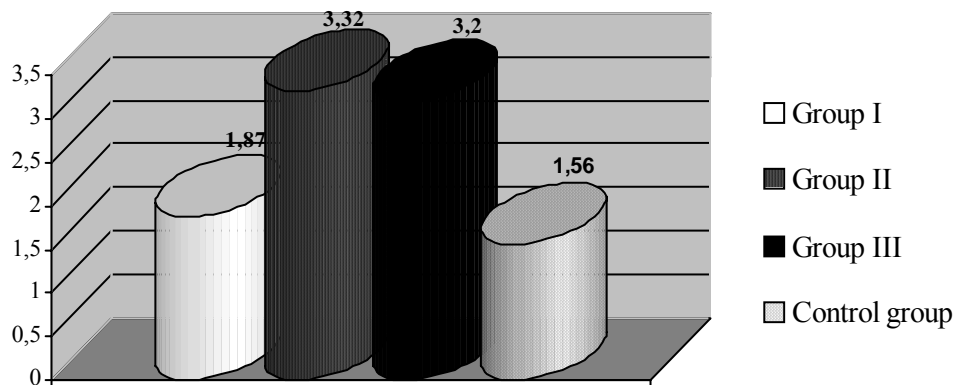


Figure 2. Serum neopterin in workers exposed to inorganic dust containing FCS and in control group

Figure 2 shows the values of neopterin in workers exposed to inorganic dust and in control subjects. No statistically significant difference was found between mean neopterin levels of workers (Group I) exposed to inorganic dust containing less than 2% FCS ($1,87 \pm 0,42$ ng/mL; $p = 0,53$) and the control group ($1,56 \pm 0,38$ ng/mL). Neopterin concentrations in subjects of Group II (exposed to coal dust) and Group III (exposed to asbestos dust), $3,32 \pm 0,77$ ng/mL and $3,20 \pm 0,48$ ng/mL respectively, were significantly higher than those of the controls and Group I ($p < 0,05$). No significant difference was found between neopterin levels of subjects from Group II and III ($p = 0,68$).

Of some interest are the quantity and quality characteristics of the inorganic dust, to which the examined workers were exposed. Group I workers were exposed to inorganic dust containing less than 2% FCS. According to Regulation №13 on protecting workers from risks related to exposure to chemical agents at work (1), the permissible exposure level of the inhalable fraction of this type of dust is set at 10 mg/m^3 and of the respirable fraction – at 4 mg/m^3 . The measured levels of inorganic dust in the working environment of Group I subjects were between $7,1 \text{ mg/m}^3$ and $9,9 \text{ mg/m}^3$ for the inhalable and $0,13 \pm 0,20 \text{ mg/m}^3$ for the respirable fraction (**Figure 3**).

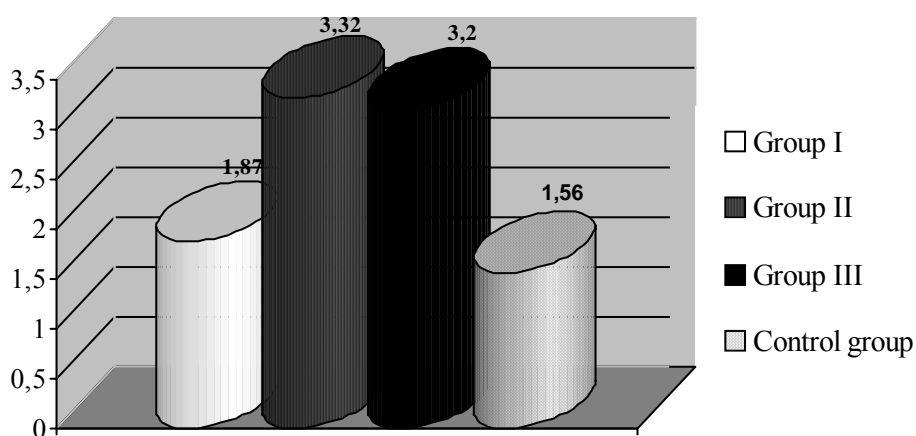


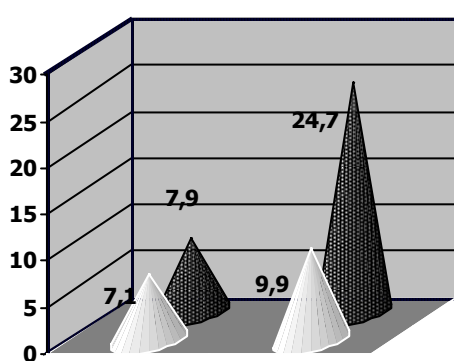
Figure 3. Inhalable and respirable fractions of inorganic dust found in the working environment of Group I and Group II subjects

The values of the inhalable fraction of Group II workers (exposed to coal dust) were in the

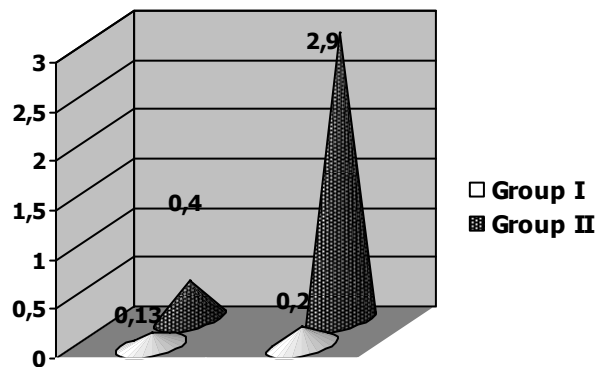
range of $7,9$ to $24,7 \text{ mg/m}^3$ and $0,4$ to $2,9 \text{ mg/m}^3$ for the respirable fraction (**Figure 3**).

On **Figure 4** asbestos levels from three workplaces are presented, where Group III subjects usually resided. In most cases the

established values exceeded the limit of 0,1 fibers/cm³.



Inhalable fraction



Respirable fraction

Figure 4. Concentrations of asbestos dust in the working environment of Group III (in fibers/cm³)

DISCUSSION

Our results confirmed the activation of cell-mediated immune responses, and of the macrophages in particular, in workers exposed to inorganic dust and in patients with silicosis. Similar conclusions made Saito, M. et al., 1996, who established fairly high levels of neopterin in supernatants of alveolar macrophage cultures from patients with interstitial lung disease as marker for activation of alveolar macrophages. The authors found that macrophage colony-stimulating factor and interleukin-1 (IL-1) increased production of neopterin by the alveolar macrophages (7). Ulker, O.C. et al., 2007, assuming the idea of activation and increased levels of neopterin in patients with immune-related diseases, examined the possible correlation between these levels and the progression and severity of coal workers' pneumoconiosis (12). The authors established significantly higher levels of neopterin in serum, urine and bronchoalveolar lavage (BAL) in patients with progressive massive fibrosis and pneumoconiosis compared to a control group. The presence of significantly higher levels of neopterin in BAL in patients with massive fibrosis compared to those with ordinary fibrosis warranted the authors to propose that measuring of neopterin in BAL be used as a suitable biomarker for determining that particular type of pneumoconiosis.

There was a higher mean neopterin level in the exposed workers compared to the silicosis patients, which may be explained by destruction of macrophages and release of neopterin during continuous dust exposure. The absence of significant difference between neopterin levels in workers from Group II and Group III showed participation of activated alveolar macrophages during exposure to both types of inorganic dust – containing free crystalline silica and containing asbestos fibers.

Several studies have shown that in rats exposed to silica the concentration of polynuclear leucocytes (PNLs) from pulmonary capillaries into alveolar spaces is a distinctive mark of the onset and progression of silica-induced pulmonary fibrosis (13-16). Castranova V et al., 1996; Vallyathan V et al., 1995, found that the degree of lung inflammation is determined by the ability of the silica particles to form radicals (14, 17). Crowding of PNLs is also proven to be a distinctive mark of acute silicosis in humans (18).

Exposure of animals to coal dust also causes inflammation characterized by increasing numbers of macrophages and PNLs in the alveolar spaces (19, 20). Blackford, J.A., 1997, and Bowden, D.H., 1978, indicated that the degree of inflammatory response to coal dust is less potent compared to the response to silica dust and is less dominated by crowding of PNLs (19, 21). Increased number of alveolar

macrophages was found in coal miners and the number of those cells in BAL correlated to the severity of the coal workers' pneumoconiosis (22).

Cytokines produced by alveolar macrophages have a regulatory effect on fibroblast growth and/or collagen synthesis. When the balance between fibrogenic and anti-fibrogenic mediators is disturbed pulmonary fibrosis may develop. It was found that interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF- α), platelet-derived growth factor (PDGF), fibronectin, alveolar macrophage-derived growth factor and insulin-like growth factor type 1 increased fibroblast proliferation (23-27). Alveolar macrophage-derived growth factor is the major growth factor responsible for the proliferation of fibroblasts. There are a number of studies on the effects of silica and coal dust exposure on the alveolar macrophage-produced cytokines that regulate fibrogenesis. Inorganic dust exposure stimulates growth factor release and expression of fibrogenic and anti-fibrogenic processes, with significant prevalence of the fibrogenic ones. It has been proven that TNF- α , insulin-like growth factor type 1 and PDGF cause aggravation of coal workers' pneumoconiosis into progressive massive fibrosis.

In the available database we found no reference on possible changes in serum neopterin levels caused by exposure to asbestos dust. Our results on Group III workers were probably linked to presence of FCS in the fine dust fraction. This find confirms the need for quality characterization of the content of free crystalline silica in the respirable fraction of inorganic dusts.

The results of this study allowed for the following conclusions:

1. Serum neopterin was significantly higher in workers exposed to inorganic dust containing over 2% FCS, in workers exposed to asbestos dust and in patients with silicosis compared to the control group and the group exposed to dust containing less than 2% FCS in the fine fraction;
2. Serum neopterin levels in workers exposed to dust containing over 2% FCS in the respirable fraction were significantly higher than neopterin levels in silicosis patients;
3. Involvement of cell-mediated immunity and macrophages followed by elevated levels of

serum neopterin in the pathogenesis of pneumoconiosis was confirmed;

4. Macrophage activity was higher in workers exposed to coal and asbestos dust compared to silicosis patients;
5. Neopterin levels in workers exposed to asbestos dust were examined for the first time and were found to be significantly higher than those of the control group.

Abbreviations

- FCS - free crystalline silica
 PEL - permissible exposure level
 BAL - bronchoalveolar lavage
 PNLs - polynuclear leucocytes
 IL-1 - interleukin-1
 TNF- α - tumor necrosis factor-alpha
 PDGF - platelet-derived growth factor

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