SERUM NEOPTERIN IN SILICOSIS PATIENTS AND WORKERS EXPOSED TO INORGANIC DUST

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Reviewed by: Accos. Prof. B. Kavaldjieva

ABSTRACT

After stimulation with interferon- γ , human macrophages produce neopterin which serves as a marker of activated cell-mediated immune response. **Aim:** Assessment of serum neopterin levels in silicosis patients, workers exposed to inorganic dust, and a control group. **Methods:** Serum neopterin levels were measured using ELISA in 60 patients with silicosis, according to conventional X-ray observation (ILO, 2002), 92 male workers exposed to dust containing free crystalline silica, and 43 controls. **Results:** Serum neopterin levels in silicosis patients (2,74 ng/ml) and in exposed workers (3,22 ng/ml) were significantly higher ($p < 0.0005$) compared to the control group (1,6 ng/ml). Significant difference was also found between silicosis patients and exposed workers ($p < 0.005$). **Conclusions:** Increased serum neopterin levels could be used as a marker for effect of exposure to inorganic dust containing more than 2% of free crystalline silica.

Key words: silicosis, neopterin

INTRODUCTION

The term silicosis was introduced by Visconti in 1883 and is derived from the latin word *silex*, meaning silicon. Silicosis is the most common pneumoconiosis. The relatively high count of cases, chronic and progressive development as well as the severe disability determines the disease as the most significant professional pulmonary disease in Bulgaria (1,2). Branches of the economy linked with high risk of developing silicosis are underground ore and coal mining, metallurgy, machine building, glass and ceramics industry and many others. According to P. Cherneva, R. Lukanova and Z. Mecheva from the years 1998, 2000 and 2003, dust analysis in the working environment shows high levels of total dust in underground mines for extracting brown (Bobov dol), anthracite (Antra - Svoge) and black coal (Balkan - Tvarditsa). exists An actual risk for developing of occupational dust induced pulmonary diseases exists in all underground, as well as ground coal mining facilities. The causes of the fibrogenic effect of the free crystalline silica are not fully clarified (3,4,11). The inhaled respirable dust particles in the alveoles are subject to phagocytosis; a lysis of the protein membrane by the proteolytic enzymes in the damaged phagosomes occurs and the dust particles are released, only to be taken up by other phagocytes. The activated macrophages release superoxide radicals, cytokines (IL-1, TNF- α , leukotriens and INF- γ) and profibrotic factors (PDGF and IGF-1) (5,8,9,15).

Neopterin is regarded as an early biomarker of the cellular immune response. It is a low-molecular-mass compound belonging to the class of pteridines and is produced by the activated macrophages after stimulation with χ -interferon (12,13,14). G. Gulumian et al., 2001 acknowledge serum neopterin as a possible marker of the effect of exposition to silica (6).

The purpose of this study is to determine the levels of neopterin in silicosis patients and in workers exposed to inorganic dust aerosols containing different amount of free crystalline silica.

MATERIALS AND METHODS

The study was conducted among 60 silicosis patients aged 38 to 76 years ($63,90 \pm 9,57$), 92 male workers aged 25 to 55 years ($43,48 \pm 6,63$), exposed to inorganic dust containing different amount of free crystalline SiO_2 , and 43 healthy workers - controls ($55,62 \pm 10,65$ years), without exposure to dust aerosols or history for autoimmune diseases.

The workers exposed to inorganic dust were divided into two groups depending on workplace (group A and B) and the measured levels of inorganic dust in the working environment.

The subjects' presence of pulmonary and autoimmune diseases, smoking habits and their duration, etc. was determined by method of inquiry.

A blood sample was taken from every studied person after signing a consent form for participation in the study. Serum neopterin levels were determined by ELISA, kit DRG Diagnostics, Germany in ng/mL.

The obtained data was processed using Basic Statistic and Table for Windows.

RESULTS AND DISCUSSION

Figure 1. shows the levels of serum neopterin in the examined subject groups. Serum neopterin is significantly higher in the silicosis patients ($p < 0.0005$) as well as the workers exposed to inorganic dust ($p < 0.0005$) compared to the control group. These results show activation of the cellular immunity during the time of exposure, as well as after variously long latent period and development of silicosis.

A significant difference was found between neopterin levels of silicosis patients and of those exposed to inorganic dust ($p < 0.005$). This fact could be explained with the present exposure to dust, containing over 2% of free crystalline silica, and activation of the cell mediated immune responses. The important role of macrophages in those reactions, demonstrated in publications by other authors, is also confirmed in our results. This outlines the possible use of macrophage-released neopterin as biomarker of effect for exposure to inorganic dust, containing over 2% of FCS in the respirable fraction.

Fig. 1. Serum neopterin levels in silicosis patients, workers exposed to inorganic dust and control group.

The following facts present special interest for the present study. The studied workers are from the coal mining industry (ground coal mines). According to the Regulation for protecting the workers from risks connected to exposure to occupational chemical agents, no special effects are given for coal dust containing over 2% of FCS. Meanwhile irritating and fibrogenic effects are cited for mixed dust, containing over 2% of FCS in the respirable fraction. The results of our study on patients with silicosis and on workers exposed to inorganic dust show that the possible effects of coal dust containing over 2% of FCS are similar to those of mixed inorganic dust.

The average group level of neopterin in all three groups of silicosis patients was significantly higher than in the control group, but there was no significant difference in neopterin

levels between the groups with different X-ray and morphological characteristics of silicosis (Table 1.). This indicates that at this point neopterin cannot be used for diagnostics and determining the stage of silicosis.

Significantly higher neopterin levels are found in workers exposed to dust, compared to the control group (3.22 ng/ml and 1.60 ng/ml respectively), $p < 0.05$. Neopterin levels in workers from group A and B are 3.24 ng/ml and 3.21 ng/ml respectively and also are significantly higher than the controls, $p < 0.05$. No significant difference in the serum neopterin levels were found in the analyzed samples from both of the exposed workers' groups (Fig. 2). Correlation analysis between neopterin levels and length of service (duration of exposure) shows linear correlation with coefficient $r = 0.423$; $p > 0.05$.

Table 1. Serum neopterin in silicosis patients with different radiomorphological stage of the disease.

Groups	Age (years)	Exposition (years)	Latent period (years)	Serum neopterin ng/mL
I-st group n=21	59,19 ±10,05	16,5 ±8,68	9,17 ±4,82	2,53 ±0,99 *(p<0.001)
II-nd group n=23	61,91 ±8,42	16,86 ±7,47	8,62 ±7,76	2,66 ±1,42 *(p<0.006)
III-rd group n=16	72,25 ±3,83 **(p<0.02)	12,31 ±7,67	8,50 ±6,40	3,07 ±1,08 *(p<0.0001)
Control group n=43	55,62 ±10,65	-	-	1,60 ±0,41

* - statistically significant difference to the control group ($p < 0.05$)

** - statistically significant difference to other groups ($p < 0.05$)

We believe that the lack of significant difference in the mean neopterin levels in both groups is related to the almost equal amount of FCS in the respirable fraction. Free crystalline silica varies from 4,6 to 7,7 % for group A and from 3,7 to 7,9 % for group B.

Fig. 2. Neopterin levels in workers exposed to inorganic dust and in control group.

These results offer the possible explanation that activation of the cellular immune response in the pulmonary tissue de-

depends on the amount of FCS in the respirable dust fraction, and not on the specific type of dust.

Fig. 3. Neopterin levels in workers exposed to inorganic dust depending on age.

No significant difference is found in neopterin levels for workers of different age gaps (Fig. 3). Correlation coefficient between neopterin and age is linear ($r=0,679$ and $p>0,05$). Similar results were reported by S. Harald et al., 2002 (7).

No significant differences were found in neopterin levels in smokers and non-smokers, as well as in the groups divided according to duration of smoking in packet years (Fig. 4).

Fig. 4. Neopterin levels in workers exposed to inorganic dust with different duration of smoking in packet years.

The close values of serum neopterin levels in silicosis patients and in exposed workers, as well as the absence of significant differences between the groups with different stages of silicosis and between the groups with different workplaces supports the possible use of serum neopterin as a biomarker for effect. This effect is revealed through exposure to dust with FCS above 2% in the respirable fraction. The lack of significant correlation coefficients between age, length of exposure and risk factors like smoking on one hand and serum neopterin on the other, shows that neopterin levels in both examined groups (silicosis patients and workers exposed to dust) are not influenced by individual features but mainly by exposure to FCS. This further confirms the fact that neopterin can be used as a biomarker during exposure to inorganic dust aerosols containing over 2% of FCS in the respirable fraction.

CONCLUSIONS

The high serum neopterin levels in silicosis patients and in workers exposed to inorganic dust confirm activation of the cell-mediated immune response.

The significantly higher neopterin levels in the exposed workers indicate higher immune activity during continuous exposure to the dust.

The current study shows activation of macrophages under the effect of dust containing over 2% of FCS in the respirable fraction. This confirms the necessity for a quality analysis of inorganic dust during health hazard evaluation.

Future studies could confirm or reject the possible use of serum neopterin as an additional method for prognosis in the development of pulmonary fibrosis.

Acknowledgements

This study was supported by Grants N:4/2004 and N:10/2006 from the Fund for Scientific and Mobile project, Faculty of Medicine, Trakia University, Stara Zagora.

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