Examination of miners’ immune response to coal dust and their quality of life

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Introduction
As one of the most important energy resources in the world, coal makes a great contribution to the world economy. Coal mining and processing involves multiple dust generation processes including coal cutting, transportation, crushing and grinding, etc. Coal dust is one of the main sources of danger to the health of coal workers. Exposure to coal dust can be prevented through

Summary
Introduction. Inhalation of coal dust during blasting in brown coal mines has been shown to lead to a lung disease called pneumoconiosis. There is very little data in the literature on the direct impact of coal on the quality of life of people who work in coal mines as well as the body’s immune response to the effects of coal dust. The aim was to examine the immune response to exposure to coal dust in miners in a brown coal mine and whether mine workers have poorer quality of life compared to those not exposed to coal dust.

Methods. This is a cross-sectional study among 100 employees in the Brown Coal Mine in Ugljevik, of which 50 of them are exposed to coal dust on a daily basis. Blood samples were taken from all subjects to test for the presence of cytokines IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22, IFN-γ and TNF-α. The quality of life of employees was measured using a questionnaire for self-assessment of physical and mental health (36-item Short-Form Health Survey, SF-36).

Results. Group of miners had a significantly (p<0.05) higher concentrations of pro-inflammatory cytokines IL-6, IFN-γ, IL-17A and IL-22 when compared to the control group. Subjects from the control group had significantly (p<0.05) higher concentrations of anti-inflammatory cytokines IL-4 and IL-10 when compared to the group of miners. The quality of life was significantly (p<0.05) better in the control group when compared to the group of miners.

Conclusion. Physical functioning, general health, mental health and Physical component summary were significantly poorer in the group of miners. Exposition to coal dust led to a significant increase in the production of pro-inflammatory cytokines and a decrease in the production of anti-inflammatory cytokines.

Keywords: immune response, cytokines, coal dust
administrative and engineering controls. Ineffective control of exposure to coal dust can harm the health of workers in coal mines [1]. Given that there has been an unexpected increase in miners’ pneumoconiosis (CWP) in the Appalachian Basin (USA) in recent years, Ting Liu has reviewed the literature on the importance of coal dust to miners’ health. They pointed out that the impact of coal dust on the health of miners is not yet well understood and that further improvements are needed [1]. There is a large number of conducted researches related to environmental problems caused by mining in coal mines, processing, combustion and similar problems such as acid rain, smog occurrence, gas emissions, etc. In contrast, there is very little data in the literature on the direct impact of coal on the health of people who work in coal mines and who use coal on a daily basis [2].

Inhalation of coal dust during blasting in brown coal mines leads to the development of lung dust diseases caused by coal dust (CMDLD). The most common manifestation of CMDLD is pneumoconiosis of the lungs. Coal worker’s pneumoconiosis (CWP) is a parenchymal lung disease caused by the accumulation of coal dust in the lungs and the consequent reaction of lung tissue to the formation of fibrous nodular lesions [3]. Other CMDLD diseases include progressive pulmonary fibrosis, chronic bronchitis, pulmonary emphysema, and diffuse pulmonary fibrosis associated with coal dust [4]. Workers in coal mines can also get chronic obstructive pulmonary disease, especially workers who are smokers [5]. The risk of developing lung diseases increases in underground mines [6].

The pathophysiological mechanisms of pneumoconiosis in miners have not been fully elucidated, but it is known that cytokines produced by alveolar macrophages also play a significant role in pathogenesis [7]. The role of IL-1, TNF-α, IL-6, TGF-β in patients with pneumoconiosis was investigated [7,8,9,10,11]. However, there are no data in the literature on the concentration of these and other important cytokines, such as IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22 and IFN-γ, measured in the serum of patients with CWP. In their study, Attfield and co-workers concluded that there is a negative effect of exposure to mine dust on pulmonary function that occurs even in the absence of radiographically detected pneumoconiosis [12]. In this sense, it is assumed that among the miners exposed to coal dust there are those who have triggered the pathophysiological mechanisms of pneumoconiosis and other health disorders associated with coal dust, but it has not been proven radiologically. Therefore, a cytokine immune response is expected in the exposed. The combination of a number of risk factors in brown coal mines can lead to a reduced quality of life for these workers due to exposure to a large amount of occupational hazards, including silica dust, coal dust, noise, vibration and heat [13]. The aim of the study was to examine the immune response to coal dust by testing the concentration of cytokines IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22, IFN-γ and TNF-α, as well as to examine the quality of life of miners in the Brown coal mine in Ugljevik.

**Methods**

A cross-sectional study was conducted between two groups of employees in the Brown Coal Mine and Thermal Power Plant in Ugljevik, the Republic of Srpska, Bosnia and Herzegovina from April 2018 to December 2020. The research included 100 respondents in the Brown Coal Mine in Ugljevik, of which 50 were exposed to coal dust and 50 were not exposed. The first group was represented by employees who were exposed to coal dust, and the second by employees who were not exposed. Respondents were selected by random selection with prior informed consent. All respondents completed a questionnaire, which contained demographic and health data.
To examine the quality of life of workers in the brown coal mine, we used a standard questionnaire for self-assessment of physical and mental health (36-item Short-Form Health Survey, SF-36), which is widely used to assess the quality of life of people in many fields and studies.

Cytokine concentrations were determined in the serum of subjects using fluorescent beads labeled with anti-cytokine antibodies (Biolegend, San Diego, CA, USA) on a flow cytometer (Attune Acoustic Focusing Cytometer, Applied Biosystems, Thermo Fisher, USA) in a Center for biomedical research at the Faculty of Medicine in Foca, University of East Sarajevo.

The methods of descriptive and analytical statistics were used in the paper. Among the methods of descriptive statistics, measures of central tendency and measures of variability were used, namely arithmetic mean with standard deviation and relative numbers for categorical variables. Among the methods of analytical statistics, Student’s t-test and non-parametric alternative, Mann-Whitney U test were used for bound samples. The chi-squared test and Fisher’s test, non-parametric tests, were also used to assess the difference between the groups. The usual value of p<0.05 was taken as the level of statistical significance of differences, while the values of p<0.01 were considered highly statistically significant. Results were statistically analyzed in GraphPad Prism software (GraphPad, La Jolla, CA, USA) and SPSS software package version 21.0 (Statistical Package for Social Sciences SPSS 21.0 Inc, USA).

**Results**

The study involved 100 respondents, half of them were miners working in the brown coal mine, while the remaining 50% of respondents were a control healthy group, which was not exposed to coal dust. Table 1 shows that a high statistically significant difference in relation to gender was observed between the two groups of respondents (p=0.001), with a significantly higher percentage of men working in the brown coal mine. Subjects belonging to the group of miners have breathing difficulties in a statistically significantly (p=0.017) higher percentage (26%) compared to the control group (8%), and also, the group of miners (28%) significantly more often (p=0.046) has cough compared to the control group of subjects (12%). Differences between groups of subjects in relation to the smoking status, occurrence of expectoration of dark sputum, present lung diseases, hospitalization due to pneumonia, heart disease, rheumatic diseases and hypertension were not observed (Table 1).

### Table 1. Socio-demographic and clinical characteristics of the respondents

| Variables                          | Group of miners, % or M ± SD | Control group, % or M ± SD | P      |
|-----------------------------------|------------------------------|---------------------------|--------|
| Gender, male                      | 88                           | 24                        | 0.001* |
| Age, years                        | 44.5±10.6                    | 43.0±13.72                | 0.543# |
| Dyspnea                           | 26                           | 8                         | 0.017* |
| Cough                             | 28                           | 12                        | 0.046* |
| Smoking                           | 17                           | 12                        | 0.342* |
| Coughing up dark sputum           | 8                            | 0                         | 0.117**|
| Lung diseases                     | 18                           | 6                         | 0.065**|
| Hospitalization for pneumonia     | 6                            | 0                         | 0.242**|
| Heart diseases                    | 2                            | 8                         | 0.362**|
| Rheumatic diseases                | 16                           | 10                        | 0.371* |
| Hypertension                      | 8                            | 16                        | 0.371* |
| Total, number (%)                 | 50 (50%)                     | 50 (50%)                  |        |

*Chi-square test, **Fisher’s test; *t test for independent samples; #M – mean, SD – standard deviation
Respondents who work in the brown coal mine have significantly lower average values of the domains of physical functioning (p = 0.005), general health (p = 0.001) and mental health (p = 0.041) compared to the control group of subjects. Also, the average values of the common physical component of quality of life were significantly (p = 0.007) lower in the group of miners compared to the control group of respondents (Table 2). Differences in other domains of the SF-36 questionnaire between groups of respondents were not observed (Table 2).

There was no statistically significant difference between the groups of respondents in the average values of the pro-inflammatory cytokines IL-2, IL-5, IL-9, IL-21 and TNF-α in serum (Figure 1).

Subjects from the miners’ group had statistically significantly (p < 0.05) higher average values of the pro-inflammatory cytokines IL-6, IFN-γ, IL-17A and IL-22 in serum compared to the subjects from the control group. There was no statistically significant difference in the average values of the pro-inflammatory cytokine IL-17F in serum between the groups of subjects (Figure 2).

Subjects from the miners’ group had statistically significantly (p < 0.05) lower values of the anti-inflammatory cytokines IL-4 and IL-10 in serum, compared to the subjects from the control group, while a statistically significant difference in the average values of the anti-inflammatory cytokine IL-13 in serum was not observed between the groups of subjects (Figure 3).

Figure 1. Average values of pro-inflammatory cytokines IL-2, IL-5, IL-9, IL-21 and TNF-α in serum of coal miners and control group of respondents. M – mean, SD – standard deviation; Mann-Whitney U test
Table 2. Respondents’ quality of life measured by SF-36 questionnaire

| Variables                  | Group of miners, M ± SD | Control group, M ± SD | P       |
|----------------------------|-------------------------|-----------------------|---------|
| Physical functioning       | 66.4±35.9               | 82.7±18.6             | 0.005** |
| Role physical              | 66.8±40.6               | 76.12±34.4            | 0.199** |
| Bodily pain                | 72.8±33.4               | 75.8±29.6             | 0.640*  |
| General health             | 43.6±22.3               | 56.8±17.2             | 0.001*  |
| Vitality                   | 47.4±19.9               | 47.8±10.5             | 0.900*  |
| Social functioning         | 80.8±54.8               | 81.4±20.1             | 0.944** |
| Role emotional             | 84.6±59.9               | 76.3±38.2             | 0.406** |
| Mental health              | 54.3±20.1               | 69.1±12.9             | 0.041*  |
| Physical component summary| 54.3±30.7               | 69.1±21.9             | 0.007** |
| Mental component summary   | 62.4±23.7               | 66.8±12.5             | 0.245*  |

Total, number (%) 50 (50%) 50 (50%)

*i test for independent samples; **Mann-Whitney test; #M – mean, SD – standard deviation

Figure 2. Average values of pro-inflammatory cytokines IL-6, IFN-γ, IL-17A, IL17F and IL-22 in serum of coal miners and control group of respondents. M – mean, SD – standard deviation; Mann-Whitney U test
Discussion

Research on the quality of life and the immune response to coal dust was conducted at the Ugljevik mine and thermal power plant. The exploitation of brown coal in this mine is superficial. It is a mine in the eastern part of the Republic of Srpska, Bosnia and Herzegovina. The mine produces about 1.8 million tons of coal, of which about 95% ends up in the furnace of the thermal power plant, and the rest goes to commercial sale. The reserves of the coal basin amount to about 430 million tons of coal. The company has a special service for safety and health at work, which takes care of the personal protection of workers exposed to coal dust and which regularly organizes preventive health examinations [14].

The workers in the Ugljevik mine and thermal power plant are mostly male. In our sample, men were in the majority in the group of exposed workers (88%), and women were in the majority in the group of non-exposed workers (76%), which was statistically significant (p = 0.001). The group exposed to coal dust more often had symptoms of respiratory diseases, such as dyspnea (p = 0.017) and cough (p = 0.046).

When it comes to indicators of quality of life of employees in the mine and thermal power plant, our groups differed statistically in the following parameters: Physical functioning (p = 0.005), General health (p = 0.001), Mental health (p = 0.041) and Physical component Summary (p = 0.007). Quality of life is a multidimensional concept, as an important indicator of medical care. A study conducted by Han et al. [15] in China found that there is a difference in the domain of physical health problems between surface miners and underground miners (p = 0.005). Compared to the normal population, their subjects had lower scores in both the physical health and mental health components, and many factors contributed to this, including the duration of work due to dust exposure, chronic illness, health

Figure 3. Average values of anti-inflammatory cytokines IL-4, IL-10 and IL-13 in serum of coal miners and control group of respondents; M – mean, SD – standard deviation, t test for independent samples
insurance, and so on. Differences in the components of physical and mental health were also found in our study by comparing workers who were exposed to coal dust and those who were not exposed [15].

In order to study the impact of social factors on the quality of life of workers in the coal industry, Petrov [16] devised a new method for assessing the individual quality of life of miners, based on the WHO quality of life assessment. According to the research, the value of quality of life on average was 27.7%. According to the scale shown, the average quality of life of miners is low. The variability in the quality of life of miners is mainly determined by the index of social satisfaction and the index of satisfaction with health [16]. Concerns about health and the quality of social protection are the main factors in the low quality of life of workers in the coal industry. Ivoilov and co-workers [17] examined the quality of life in the Kamelovo region of Russia. It turned out that the parameters of the quality of life of workers decreased with the age of 20 to 64 years. The parameters of quality of life on the scales of pain, physical functioning and general health are inversely correlated with the age and length of service in dangerous working conditions for workers in a coal mine [17].

Yu and co-workers [18] conducted a cross-sectional study in China among 305 workers who are exposed to coal dust and 200 workers exposed to coal dust from five coal mines in Shanxi Province with the aim of examining the quality of their life. They used a short version of the Quality of Life Questionnaire of the Chinese Health Organization (WHOQOL-BREF). All functional domains of the Chinese WHOQOL-BREF were significantly worse in workers who are exposed to coal dust compared to workers who are not exposed to coal dust, except for psychological health. They pointed out that appropriate health policies should be developed to improve quality of life of coal dust workers [18].

It is known that components from coal dust react with cells in the lungs causing cell membrane damage which is accompanied by lipid peroxidation. The pathogenetic mechanism of CWP takes place in three phases. Initially, there is an accumulation and activation of inflammatory cells in the lungs. Damaged cells release intracellular enzymes, which provoke further tissue damage, resulting in scarring or destruction of alveolar septa. Coal dust phagocytized by alveolar macrophages stimulates the formation of reactive oxygen species which then stimulate the secretion of cytokines and chemokines. These inflammatory cytokines act as chemoattractants that attract polymorphonuclear leukocytes and macrophages from the pulmonary capillaries to the alveoli, resulting in chronic inflammation [19]. Current concepts of CWP pathogenesis suggest that alveolar macrophages play a key role because of their ability to release various mediators such as proteolytic enzymes and growth and differentiation factors. In the chronic phase of CWP leading to pulmonary fibrosis in pneumoconiotic lungs, it is clear that cytokines produced by alveolar macrophages play a significant role in the pathogenesis of CWP [7].

In our study, we did not find statistically significant differences in values of pro-inflammatory cytokines IL-2, IL-5, IL-9, IL-21 and TNF-α in serum between the groups of respondents. According to a study by Vanhee et al., after in vitro exposure of alveolar macrophages to coal dust, coal dust particles caused significant secretion of TNF-α and IL-6 [7]. Accordingly, in our study, we found a statistically significantly higher level of IL-6 in serum of the workers exposed to coal dust. Even though it has been shown that coal dust exposure stimulates pro-inflammatory response leading to increased release of cytokines from monocytes such as TNF-α [20, 21]. However, in our study we found no differences in serum TNF-α concentration between the groups of respondents.
In a study by Yao et al. [22] to examine the expression of type 1 and type 2 (Th1 and Th2) cytokines from serum of coal miners, 630 coal miners were studied. Authors concluded that the median level of pro-inflammatory cytokines IL-1β, IL-8, IFN-γ and IL-6 in cases group (groups suspected with CWP or with diagnosed CWP) were significantly higher (p<0.05) than that of non-cases group (control group of healthy miners) [22]. Our results coincides with this study, where IFN-γ concentration was significantly higher in a group of miners when compared to the control group (p<0.05). In our study we did not measured concentrations of IL-1β and IL-8, but we measured other pro-inflammatory cytokines such as IL-17A and IL-22 and we determined that both of them were significantly higher in the group of miners when compared to the control group. The IL-17A has been linked to chronic inflammation and the genetic association between single nucleotide polymorphisms of IL-17A and CWP in Chinese population is proven. In a case-control study by Han et al. [22] conducted among 1,391 subjects logistic regression analysis showed that the genotypes of rs3748067 AA (OR = 0.43, 95 % CI = 0.23-0.83) and rs8193036 TT (OR = 0.59, 95 % CI = 0.40-0.86) were associated with a decreased risk of CWP [23].

Yao et al. [21] also determined that the level of anti-inflammatory cytokine IL-10 (654.08 pg/ml) was significantly lower in CWP than that of control group (596.64 pg/ml) [19], which corroborates our results, where anti-inflammatory response was significantly higher in the control group of our respondents (higher concentrations of IL-10 and IL-4) when compared to the group of miners.

The limitation of our study is the relatively small number of cases. Therefore, further randomized and double-blind studies, involving larger numbers of miners, may contribute to better understanding of the additional role of inflammatory and immunomodulatory pathway in mine workers exposed to coal dust. Also, further limitation is the significant difference of respondents by gender distribution. However, this difference does not affect the results because gender is not expected to play a significant role in the results of this study.

Respiratory symptoms, such as dyspnea and cough were significantly more often present in the group of miners when compared to the control group of respondents. Physical functioning, general health, mental health and physical component summary were significantly poorer in the group of miners. Exposition to coal dust led to a significant increase in the production of pro-inflammatory cytokines (IL-6, IFN-γ, IL-17A and IL-22), and to decrease in the production of anti-inflammatory cytokines (IL-4 and IL-10). These results suggest that exposition to coal dust induce pro-inflammatory and decrease anti-inflammatory immune response.

**Conclusion**

Physical functioning, general health, mental health and physical component summary were significantly poorer in the group of miners. Exposition to coal dust led to a significant increase in the production of pro-inflammatory cytokines and a decrease in the production of anti-inflammatory cytokines.

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**Ethical approval.** The Ethics Committee of the Faculty of Medicine Foca approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

**Conflicts of interest.** The authors declare no conflict of interest.
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Ispitivanje imunološkog odgovora rudara na ugljenu prašinu

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Kratak sadržaj

Uvod. Pokazalo se da udisanje ugljene prašine tokom miniranja u rudnicima mrkog uglja dovodi do bolesti pluća koja se zove pneumokonioza. U literaturi postoji vrlo malo podataka o direktnom utjecaju uglja na kvalitet života ljudi koji rade u rudnicima uglja, kao i o imunskom odgovoru organizma na dejstvo ugljene prašine. Cilj je bio da se ispita imunski odgovor na izloženost ugljenoj prašini kod rudara u rudniku mrkog uglja i da li radnici u rudniku imaju lošiji kvalitet života u poređenju sa onima koji nisu izloženi ugljenoj prašini.

Metode. Ova studija presjeka je obuhvatila 100 zaposlenih u Rudniku mrkog uglja u Ugljeviku, od kojih je 50 svakodnevno izloženo ugljenoj prašini. Svim ispitanicima su uzeti uzorci krvi radi ispitivanja prisustva citokina IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22, IFN-γ i TNF-α. Kvalitet života zaposlenih mjeren je upitnikom za samoprocjenu fizičkog i mentalnog zdravlja (Short-Form Health Survey, 36 stavki, SF-36).

Rezultati. Grupa rudara je imala značajno (p<0,05) veće koncentracije proinflamatornih citokina IL-6, IFN-γ, IL-17A i IL-22 u poređenju sa kontrolnom grupom. Ispitanici iz kontrolne grupe imali su značajno (p<0,05) veće koncentracije antiinflamatornih citokina IL-4 i IL-10 u poređenju sa grupom rudara. Kvalitet života bio je značajno bolji (p<0,05) u kontrolnoj grupi u poređenju sa grupom rudara.

Zaključak. Fizičko funkcionisanje, opšte zdravlje, mentalno zdravlje i zajednička fizička komponenta zdravlja bili su značajno lošiji u grupi rudara. Izlaganje ugljenoj prašini dovelo je do značajnog povećanja proizvodnje proinflamatornih citokina i smanjenja proizvodnje antiinflamatornih citokina.

Ključne riječi: imunološka reakcija, citokini, ugljena prašina