UDC 613.6; 616.24
DOI: 10.21668/health.risk/2022.1.16.eng

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Research article

NEOPTERIN AS A BIOMARKER SHOWING RISKS OF DEVELOPING PATHOLOGY IN BRONCHI AND LUNGS AMONG WORKERS WHO HAVE OCCUPATIONAL CONTACTS WITH INDUSTRIAL AEROSOLS

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Our research goal was to estimate neopterin level in blood serum of workers occupationally exposed to industrial aerosols with predominantly fibrogenic effects; to establish a relationship between this level and workers' age, working experience in hazardous working conditions, spirometric parameters and the level of C-reactive protein. We also aimed to assess neopterin as a possible biomarker showing risks of the developing inflammatory process in the bronchi and lungs at its early stage.

Our observation covered the following groups: workers employed at a metallurgic plant who had occupational contacts with industrial aerosols (exposure factors included welding and silicon-containing aerosols with predominantly fibrogenic effects in concentrations exceeding maximum permissible ones in workplace air); people suffering from chronic obstructive pulmonary disease of occupational etiology (COPD OE) in their post-exposure period; workers who didn't have any occupational contacts with industrial aerosols. We determined neopterin contents in blood serum with ELISA test using "Neopterin ELISA" reagent kit (IBL, Hamburg). Elevated neopterin levels were detected in blood serum of 56.1 % workers who were occupationally exposed to industrial aerosols and 53.3 % of patients with COPD OE; we also found a direct correlation between levels of neopterin and interferon gamma. Only 18.7 % workers without any occupational contacts with industrial aerosols had elevated neopterin levels in their blood serum and there were no authentic correlations between these levels and interferon gamma contents in this group. Workers who were occupationally exposed to industrial aerosols had a more apparent increase in the average level of neopterin at an age younger than 40 years and working experience shorter than 20 years in comparison with workers without any such exposure.

Neopterin can be used as a potential sensitive biomarker showing risks of an early inflammatory reaction in the lungs occurring in workers who are occupationally exposed to industrial aerosols. People with elevated neopterin levels in blood, especially those who are occupationally exposed to industrial aerosols, can be recommended to have their bronchi and lungs monitored in dynamics.

Key words: *neopterin, macrophages, industrial aerosols, pathology in the bronchi and lungs, risk factor.*

Diseases of the bronchi and lungs are among the most widely spread occupational pathologies. Pneumoconiosis, occupational bronchitis, and chronic obstructive pulmonary disease (COPD) often result from occupational exposure to industrial aerosols.

Industrial aerosols occur in workplace air when metal items are processed mechanically (when moldings are purified, polished, and ground), or due to thermal processes or sublimation of solid substances (melting, welding, etc.). Depending on their chemical structure,

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industrial aerosols can produce fibrogenic, irritating, toxic, allergenic, carcinogenic, and ionizing effects on the body[1]. Workers employed at metallurgic production or civil engineering enterprises are primarily exposed to aerosols with fibrogenic effects. Exposure to chemicals in contents not exceeding maximum permissible concentrations (MPC) doesn't exclude probable health disorders in excessively sensitive people[2]. Industrial aerosols can cause acute and, in case of long-term exposure, even chronic damage to the lungs that usually develops into fibrosis.

Health outcomes that may result from occupational exposure to industrial aerosols are given a lot of attention by experts in the field and studies usually concentrate on examining pathogenetic mechanisms of interaction between aerosol particles in workplace air and lung tissue cells [1]. Silicon-containing welding aerosols have significant capacity to activate the monocytic-macrophage system of the body.

Macrophages that circulate in blood and occur in lung tissues are the first barrier protecting the body from foreign substances. Alveolar macrophages (AMs) play a leading role in activating mucosal immunity mechanisms when various pathogenic substances penetrate airways mucosa. AMs generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), phagocytize actively and neutralize infectious agents; they are the key component that regulates inflammation [2, 3].

Macrophages are highly plastic and able to polarize. M1 phenotype macrophages produce apparent cytotoxic and antimicrobial effects. When activated classically, they maintain inflammation in the lung tissue by producing pro-inflammatory cytokines (interleukins-1β, -6, -12, -23, tumor necrosis factor alpha) and destructing an inflammation focus [4]. M2 phenotype macrophages, which are activated as per an alternative way, make for fibrogenesis, proliferative processes, and tissue regeneration [5, 6].

Chronic clinical course of lung diseases occurs obviously due to macrophages "reprogramming" themselves towards M2 profile [7]. We should note that lung macrophages phagocytize actively and neutralize infectious agents but they are unable to remove aerosol particles completely. Inert particles are not destroyed by macrophage lysosomal apparatus. Besides, aerosols activate the bactericide oxygen system of macrophages and thereby stimulate production of reactive oxygen species and make for oxidation stress development. Free radicals that occur in abundance destroy phospholipid membranes of phagosomes and, as a result, a macrophage dies, aerosol particles are released into the surrounding medium, are then captured by another macrophage, and the process repeats itself again and again [8]. When macrophages are activated and then destroyed, this results in release of proteases and chemokines that enhance inflammation and subsequently cause tissue lesions [9]. Besides, other factors are activated including those responsible for macrophage flow to a place where aerosols are deposited, for example, colony-stimulating factors, factors of granulocyte proliferation in the bone marrow, etc. Inflammation mediators are synthesized in greater quantities and neopterin is among them [10].

According to the latest concepts, neopterin is a non-specific highly sensitive marker showing activation of the monocytic section in the cellular immunity. Neopterin is a pteridine released by specific immune cells, primarily macrophages and monocytes, when a specific immune response involving T-cells is activated due to their stimulation by interferon gamma (IFNγ). Production of neopterin is usually directly linked to synthesis of IFNγ which can be released by inborn or adaptive immunity cells, in particular, so called "natural

[1] Professional'naya patologiya: natsional'noe rukovodstvo [Occupational pathology: the national guide]. In: N.F. Izmerov ed. Moscow, GEOTAR-Media Publ., 2011, 784 p. (in Russian).

[2] R 2.2.2006-05. 2.2. Occupational hygiene. Guide on Hygienic Assessment of Factors of Working Environment and Work Load. Criteria and Classification of Working Conditions: approved by G.G. Onishchenko, the RF Chief Sanitary Inspector on July 29, 2005. *KODEKS: an electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200040973 (November 18, 2021) (in Russian).

killers". Quantity of synthesized neopterin is directly proportionate to quantity of IFNγ [11]. A lot of research works focus on a role that belongs to neopterin in cardiovascular pathology developing and progressing as well as prognosis for it. Neopterin has been shown to be a predictor of clinical outcomes for chronic and acute ischemic heart disease (IHD). Coronary angiography has established that neopterin levels in blood serum depend on how IHD progresses in patients with stable angina pectoris. Research works by many authors confirm that neopterin is a significant component in estimating stability of atheromas in patients with IHD and in monitoring over health of patients after coronary stent implantation [12, 13]. Not so many works dwell on examining a role neopterin plays in lung diseases. Results produced by few studies indicate that monitoring over neopterin levels can have some diagnostic and prognostic value in case of diseases caused by exposure to silicon dioxide, for example, silicosis [14]. Some authors have considered neopterin to be an immunological biomarker eligible for assessing clinical course of pneumoconiosis in workers employed in coal mining [15]. Neopterin in blood serum has also been proved to be a significant indicator of inflammation and exacerbations in patients with COPD [16–18].

Our research goal was to estimate the neopterin level in blood serum of workers occupationally exposed to industrial aerosols with predominantly fibrogenic effects; to establish a relationship between this level and workers' age, working experience in hazardous working conditions, spirometric parameters and the level of C-reactive protein. We also aimed to assess neopterin as a possible biomarker showing risks of a developing inflammatory process in the bronchi and lungs at its early stage.

Materials and methods. Overall, 194 people took part in our research; they were divided into three groups:

– the 1st group (or the test group) included workers employed at a metallurgic plant located in Nizhniy Novgorod region (57 men aged 39.1 ± 9.5 years with their working experience being 13.8 ± 7.7 years) who were occupationally exposed to welding and silicon-containing aerosols with predominantly fibrogenic effects (electrogas welders, workers dealing with strops, metal cutters, rollers, and millers);

– the 2nd group (or the control group) was made up of patients with COPD OE with stable clinical course caused by long-term exposure to welding and silicon-containing aerosols with predominantly fibrogenic effects. They were all treated in the clinic of the Rospotrebnadzor's Nizhny Novgorod Scientific Research Institute for Hygiene and Occupational Pathology (30 patients overall (8 women and 22 men) aged 56.8 ± 7.8 years and with their working experience being 26.0 ± 8.0 years). The modified Tiffeneau-Pinelli index amounted to less than 70 % of its physiological standard in all of them. COPD was diagnosed based on criteria outlined in the Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease – GOLD, 2021, and clinical recommendations developed by the Russian respiratory society[3]. The disease was proven to have occupational etiology in the course of medical examinations according to the rules stipulated in the National guide on occupational respiratory diseases (sanitary-hygienic conditions at workplaces were analyzed, working experience under harmful and hazardous conditions was taken into account, and medical records were thoroughly examined)[4];

– the 3rd group (or the reference one) included people employed at various productions who were not exposed to industrial aerosols at their workplaces (107 people overall (49 men and 58 women) aged 40.8 ± 9.9 years and working experience being 13.9 ± 8.5 years).

[3] Khronicheskaya obstruktivnaya bolezn' legkikh: klinicheskie rekomendatsii [Chronic obstructive pulmonary disease: clinical recommendations]. *The RF Public Healthcare Ministry*. Moscow, the Russian Respiratory Society Publ., 2018. Available at: https://cr.minzdrav.gov.ru/schema/603_1 (May 27, 2021) (in Russian).

[4] Professional'nye zabolevaniya organov dykhaniya: natsional'noe rukovodstvo [Occupational respiratory diseases: the national guide]. In: N.F. Izmerov, A.G. Chuchalin eds. Moscow, GEOTAR-Media Publ., 2015, 806 p. (in Russian).

People from the 1st and the 3rd group had periodical medical examinations at the consultative polyclinic of the Rospotrebnadzor's Nizhny Novgorod Scientific Research Institute for Hygiene and Occupational Pathology.

We excluded people with acute communicable diseases, malignant neoplasms, diabetes mellitus, and exacerbated chronic diseases from our research.

Working conditions were estimated according to the Federal Law No. 426 issued on December 28, 2013 "On special assessment of working conditions"[5]. According to these estimates contents of particulate matter (dusts) were sometimes higher than maximum permissible concentrations in workplace air where people from the 1st group worked and working conditions there belonged to hazard category 3.1 (harmful, the 1st degree). Average shift concentrations of diiron trioxide in dusts varied from 0.65 to 7.2 mg/m^3 at different spots (MPC is 6.0 mg/m^3); silicon dioxide (accounting for 10 to 70 % in dusts), from 0.44 to 2.4 mg/m^3 (MPC is 2.0 mg/m^3); iron, from 1.65 to 2.6 mg/m^3 (MPC is 10.0 mg/m^3); electro-corundum (aluminum oxide), from 1.8 to 6.6 mg/m^3 (MPC is 6.0 mg/m^3); manganese (with its share in dusts being up to 20 %), from 0.25 to 0.72 mg/m^3 (MPC is 0.6 mg/m^3). Maximal concentrations of manganese, silicon dioxide, electro-corundum, and diiron trioxide in workplace air were by 1.1–1.2 times higher than MPC (working conditions belong to hazard category 3.1) at workplaces of metal cutters, millers and electrogas welders. When welding was performed, ozone contents in an area where a welder breathed were by 1.1 times higher than MPC. Occupational noise was higher than its maximum permissible level (more than 80 dBA) at the examined workplaces and reached 90–95 dBA at some of them (working conditions belonging to hazard category 3.2, ("harmful", the 2nd degree)). Overall assessment of working conditions assigned them into hazard categories 3.1–3.2 ("harmful conditions", the 1st and 2nd degree).

All participants gave their informed consent to take part in our research; the research work was approved by the local ethical committee at the Rospotrebnadzor's Nizhny Novgorod Scientific Research Institute for Hygiene and Occupational Pathology.

We examined external respiration in all patients using "SpirolabIII OXY" spirometer (Italy) and estimating the following spirometric parameters: forced vital capacity (FVC, $\%_{standard}$), forced expiratory volume over the 1st second (FEV_1, $\%_{standard}$), calculated ratio of these two indicators (FEV_1/FVC, %) or the modified Tiffeneau-Pinelli index (MTPI), and peak expiratory flow at 75 % FVC (PEF 75 %).

We determined neopterin and IFNγ contents in blood serum with ELISA tests using "Neopterin ELISA" reagent kit (IBL, Hamburg) and "gamma-Interferon-ELISA-BEST" ("Vector-Best" JSC, Russia). C-reactive protein (CRP) in blood serum was also detected with ELISA tests using highly sensitive "CRP-ELISA-BEST" reagent kit ("Vector-Best" JSC, Russia) with its detection limit being 0.05 IU/l.

To estimate age-specific neopterin contents in blood serum, we divided people in the 1st and 3rd groups into four age sub-groups: aged 25–30 years, 31–40 years, 41–50 years, 51–60 years and older.

To estimate neopterin contents in blood serum depending on working experience, people in the 1st and 3rd group were divided into three sub-groups: with working experience not exceeding 10 years, from 11 to 20 years, and longer than 20 years.

The results were statistically analyzed with variation statistic procedures using "Statistica 6.1" software package (StatSoft Inc., USA). We applied Shapiro – Wilk test to check if indicators were distributed normally and to analyze dispersion equality. In case indicators were distributed normally, we ana-

[5] O spetsial'noi otsenke uslovii truda: federal'nyi zakon FZ № 426 ot 28.12.2013 g. (prinyat Gos. Dumoi 23.12.2013, odobren Sovetom Federatsii 25.12.2013) [On special assessment of working conditions: The Federal Law No. 426 issued on December 28, 2013 (approved by the State Duma on December 23, 2013, approved by the Federation Council on December 25, 2013)]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_156555/ (November 27, 2021) (in Russian).

lyzed them using parametric statistic procedures. Distributions were estimated by calculating simple mean (*M*) and root-men-square deviation (δ). We estimated authenticity of differences between mean values obtained for different groups using parametric Student's t-test. We applied Pearson's correlation coefficient to determine whether there was a linear correlation between two quantitative indicators. In case indicators were not distributed normally, we applied non-parametric statistic procedures, for example, Mann – Whitney U test. The data were given as *Med* ± *IQR* (25–75 %). We applied non-parametric goodness-of-fit test χ^2 (Pearson's test) to estimate statistical significance of differences between several relative indicators (frequencies).

Critical level of significance was taken at $p < 0.05$. Values of p from 0.05 and up to 0.1 inclusively were considered to be trends.

Results and discussion. We didn't detect any sex-related differences in neopterin contents in blood serum and frequencies of its elevated levels (more than 10.0 nmol/l) in the exposed and non-exposed workers as well as patients with COPD OE ($p > 0.05$). Table 1 provides the data on neopterin contents in blood serum and frequencies of its elevated levels in the examined people from all three groups.

Neopterin concentrations in blood serum of the examined people varied from 2.8 to 21.9 nmol/l as we established by analyzing the obtained data. Its average concentration was the highest in blood serum of the workers exposed to industrial aerosols (Group 1) and patients with COPD (Group 2). Neopterin levels in blood serum of the patients with COPD OE were authentically higher that the same indicator in blood serum of the workers exposed to industrial aerosols ($p_{1–2} = 0.009$). The non-exposed workers had neopterin levels in their blood serum within reference values and these levels were authentically different from those detected in the first two groups ($p_{1–3} = 0.0001$; $p_{2–3} = 0.0001$). Elevated neopterin levels (more than 10.0 nmol/l) were detected in a half of the workers who were occupationally exposed to industrial aerosols and patients with COPD OE, 56.1 % and 53.3 % respectively. Such elevated levels were detected only in 18.7 % of the non-exposed workers, hence by 2.8–3 times less frequently against the first two groups.

Table 2 provides data on neopterin levels indifferent age sub-groups of the workers in the test group (Group 1) and reference group (Group 3). We detected the lowest neopterin levels among the workers exposed to industrial aerosols and non-exposed ones in the age sub-group from 25 to 30 years (9.1 ± 2.7 nmol/l and 6.3 ± 2.1 nmol/l respectively). Neopterin levels detected in this age sub-group were authentically different from those detected in people aged from 31 to 60 years ($p = 0.002$ for Group 1 and $p = 0.039$ for Group 3 (Student's t-test)). We should note that neopterin concentration in blood serum was authentically higher

Table 1

Neopterin concentration in blood serum and frequency of its elevated levels in workers and patients with COPD OE

Indicator	Examined groups		
	Group 1 (test) ($n = 57$)	Group 2 (control) ($n = 30$)	Group 3 (reference) ($n = 107$)
Neopterin concentration (nmol/l), $M \pm \delta$	10.4 ± 1.7	11.9 ± 2.3	7.5 ± 1.8
p (Student's t-test)	$p_{1–2} = 0.009$; $p_{1–3} = 0.0001$; $p_{2–3} = 0.0001$		
Frequency of elevated neopterin level (more than 10.0 nmol/l), abs. (%)	32 (56.1)	16 (53.3)	20 (18.7)
p^* (χ^2 test)	$\chi^2 = 0.528$, $p_{1–2} = 0.468$; $\chi^2 = 24.086$, $p_{1–3} < 0.001$; $\chi^2 = 14.514$, $p_{2–3} < 0.001$		

Note: p (Student's t-test) is authenticity of differences in neopterin contents between the groups; p^* (χ^2 test) is authenticity of differences in frequencies of elevated neopterin levels between the groups.

Table 2

Neopterin concentration in blood serum and frequency of its elevated levels in different age sub-groups

Age sub-groups							
Sub-group 1 (from 25 to 30 years)		Sub-group 2 (from 31 to 40 years)		Sub-group 3 (from 41 to 50 years)		Sub-group 4 (from 51 to 60 years)	
Group 1 test (n = 23)	Group 3 reference (n = 12)	Group 1 test (n = 11)	Group 3 reference (n = 35)	Group 1 test (n = 13)	Group 3 reference (n = 39)	Group 1 test (n = 10)	Group 3 reference (n = 21)
Age (years), $M \pm \delta$							
28.4 ± 1.6	27.5 ± 2.1	38.1 ± 1.19	37.8 ± 1.15	43.0 ± 2.44	43.3 ± 3.43	54.0 ± 2.8	55.0 ± 3.2
p_{1-3} = 0.19		p_{1-3} = 0.41		p_{1-3} = 0.91		p_{1-3} = 0.92	
Neopterin concentration (nmol/l), $M \pm \delta$							
9.1 ± 2.7	6.3 ± 2.1	12.0 ± 2.9	7.6 ± 2.5	11.7 ± 2.7	8.2 ± 2.6	10.7 ± 1.6	7.7 ± 2.5
p_{1-3}* = 0.007		p_{1-3}* = 0.0001		p_{1-3}* = 0.001		p_{1-3}* = 0.008	
Frequency of elevated neopterin levels (more than 10.0 nmol/l), abs. (%)							
9 (39.1)	0 (0)	6 (54.5)	4 (11.4)	9 (69.2)	10 (25.6)	8 (80.0)	6 (28.5)
χ^2 = 8.37 p_{1-3}** = 0.004		χ^2 = 12.538 p_{1-3}** < 0.001		χ^2 = 10.517 p_{1-3}** = 0.002		χ^2 = 10.608 p_{1-3}** = 0.002	

Note:

p (Student's t-test) is authenticity of differences in age in each age sub-group between Group 1 and Group 3;

p* (Student's t-test) is authenticity of differences in neopterin concentration each age sub-group between Group 1 and Group 3;

p** (χ^2 test) is authenticity of differences in frequencies of elevated neopterin levels in each age sub-group between Group 1 and Group 3.

in all age sub-groups of exposed workers (Group 1) against non-exposed ones (Group 3) (p_{1-3}* = 0.007 for the age sub-group from 25 to 30 years; p_{1-3}* = 0.0001, from 31 to 40 years; p_{1-3}* = 0.001, from 41 to 50 years; p_{1-3}* = 0.008, from 51 to 60 years).

Frequency of elevated neopterin levels (more than 10.0 nmol/l) grew with age in both groups (from 39.1 to 80.0 % in Group 1 and from 0 to 28.5 % in Group 3). This indicator was authentically higher in all age sub-groups of the workers who were occupationally exposed to industrial aerosols than in all age sub-groups of their non-exposed counterparts (p_{1-3}* = 0.004 for the age sub-group from 25 to 30 years; p_{1-3}* < 0.001, from 31 to 40 years; p_{1-3}* = 0.002, from 41 to 50 years; p_{1-3}* = 0.002, from 51 to 60 years).

Most patients with COPD OE were older than 50 years. Neopterin concentration in their blood serum didn't differ from those detected in age-sub-groups 2, 3 and 4 in Group 1 (p_{1-2} = = 0.42 from 31 to 40 years; p_{1-2} = 0.42 from 41 to 50 years; p_{1-2} = 0.41 from 51 to 60 years, Student's t-test). Frequency of elevated neopterin levels (more than 10.0 nmol/l) didn't differ from this indicator in Group 1 either in the age sub-groups 2, 3 and 4 (χ^2 = 0.005, p_{1-2} = 0.9 from 31 to 40 years; χ^2 = 0.94, p_{1-2} = 0.33 from 41 to 50 years; χ^2 = 2.222, p_{1-2} = 0.137 from 51 to 60 years).

Table 3 provides the data on neopterin concentration in the examined workers' blood serum and frequency of its elevated levels depending on working experience.

Having analyzed all the obtained data, we established that neopterin concentration in blood serum grew as working experience got longer in the sub-groups 1 and 2 in both test and reference group (p = 0.004 for Group 1; p = 0.01 for Group 3). Neopterin concentration in blood serum of the workers with their working experience being longer than 20 years (sub-group 3) didn't differ from levels detected in workers with their working experience being from 11 to 20 years and not longer than 10 years. Frequency of elevated neopterin levels grew as working experience got longer in both groups (from 33.3 % to 90.0 % in Group 1 and from 12.1 % to 27.3 %

in Group 3). This indicator was authentically higher in all sub-groups of the exposed workers with different working experience than in their non-exposed counterparts ($p_{1–3}$** = 0.024 for the workers with their experience not exceeding 10 years; $p_{1–3}$** = 0.014, from 11 to 20 years; $p_{1–3}$** < 0.001, longer than 20 years).

We analyzed correlations between neopterin concentration in the workers' blood serum and spirometric parameters. Table 4 provides the results.

Our research established that FEV_1 and PEF 75 % were authentically lower in the workers who were occupationally exposed to industrial aerosols than in their non-exposed counterparts ($p_{1–3}$ = 0.042 for FEV_1; $p_{1–3}$ = 0.015 for PEF 75 %). A trend for an inverse correlation was detected only between neopterin concentration and PEF 75 % (R = –0.26, p = 0.06).

Table 5 provides the results produced by analyzing CRP and IFNγ levels in blood serum of the examined people.

We analyzed CRP concentrations in blood serum of the examined people and established that they varied from 0.5 to 15.0 mg/l. Its average value was the highest in blood serum of patients with COPD OE and was authentically different from the values detected among

Table 3

Neopterin concentration in blood serum frequency of its elevated levels depending on working experience

Sub-groups as per working experience					
Sub-group 1 (not longer than 10 years)		Sub-group 2 (from 11 to 20 years)		Sub-group 3 (longer than 20 years)	
Group 1 test (n = 24)	Group 3 reference (n = 58)	Group 1 test (n = 23)	Group 3 reference (n = 16)	Group 1 test (n = 10)	Group 3 reference (n = 33)
Neopterin concentration (nmol/l), $M \pm \delta$					
9.2 ± 2.8	7.7 ± 2.1	11.5 ± 2.5	8.5 ± 2.6	10.9 ± 1.4	7.8 ± 2.9
$p_{1–3}$* = 0.009		$p_{1–3}$* = 0.001		$p_{1–3}$* = 0.002	
Frequency of elevated neopterin levels (more than 10.0 nmol/l), abs. (%)					
8 (33.3)	7 (12.1)	15 (65.2)	4 (25.0)	9 (90.0)	9 (27.3)
χ^2 = 5.136 $p_{1–3}$** = 0.024		χ^2 = 6.109 $p_{1–3}$** = 0.014		χ^2 = 12.4 $p_{1–3}$** < 0.001	

Note:

p* (Student's t-test) is authenticity of differences in neopterin concentration in each sub-group as per working experience between Group 1 and Group 3;

p** (χ^2 test) is authenticity of differences in frequencies of elevated neopterin levels in each sub-group as per working experience between Group 1 and Group 3.

Table 4

Spirometric parameters and correlations between them and neopterin concentrations in workers' blood serum

Parameter, $M \pm \delta$	Examined group			
	Group 1 (test) (n = 57)	Group 3 (reference) (n = 107)	$p_{1–3}$	R
FVC, $\%_{standard}$	105.0 ± 15.6	109 ± 14.7	0.12	–0.07, p = 0.57
FEV_1, $\%_{standard}$	97.4 ± 13.6	102 ± 13.1	0.042	–0.11, p = 0.38
MTPI, %	92.8 ± 7.7	94.3 ± 9.0	0.23	–0.03, p = 0.81
PEF 75 %	70.3 ± 20.8	80.4 ± 26.3	0.015	–0.26, p = 0.06

Note:

$p_{1–3}$ (Student's t-test) is authenticity of differences in spirometric parameters between Groups 1 and 3;

R is Pearson's correlation coefficient showing correlations between neopterin concentration in workers' blood serum (Groups 1 and 3) and spirometric parameters.

Table 5

CRP and IFNγ concentrations in blood serum and frequencies of their elevated levels in the workers and patients with COPD OE

Indicator	Examined groups		
	Group 1 (test) (n = 57)	Group 2 (control) (n = 30)	Group 3 (reference) (n = 107)
CRP concentration (mg/l), *Med* ± *IQR* (25–75 %)	4.9 (1.94–7.29)	9.25 (4.4–16.2)	3.57 (1.49–6.99)
p	$p_{1-2} = 0.001$; $p_{1-3} = 0.32$; $p_{2-3} = 0.0001$		
Frequency of elevated CRP level (more than 8.0 mg/l), abs. (%)	9 (15.7)	15 (50.0)	21 (19.7)
p*	$\chi^2 = 8.124$, $p_{1-2} = 0.005$; $\chi^2 = 3.547$, $p_{1-3} = 0.06$; $\chi^2 = 12.4$, $p_{2-3} < 0.001$		
IFNγ concentration (pg/l), $M \pm \delta$	1.24 ± 0.85	1.25 ± 0.91	0.88 ± 0.59
p**	$p_{1-2} = 0.82$; $p_{1-3} = 0.004$; $p_{2-3} = 0.005$		
Frequency of elevated IFNγ level (higher than 20.0 pg/ml), abs. (%)	0 (0)	0 (0)	0 (0)

Note:
p (Mann – Whitney test) is authenticity of differences in CRP concentration between Groups 1, 2 and 3;
p* (χ^2 test) is authenticity of differences in frequencies of elevated CRP concentrations between Groups 1, 2 and 3;
p** (Student's t-test) is authenticity of differences in IFNγ concentrations between Groups 1, 2 and 3.

workers from Group 1 and 3 (p_{1-2} = 0.001; p_{2-3} = 0,0001). We didn't detect any differences in CRP concentrations between the workers from Groups 1 and 3 (p_{1-3} = 0.32). Elevated CRP level (more than 8.0 mg/l) was detected in 15.7 % of the workers who were occupationally exposed to industrial aerosols; in 19.7 % of non-exposed workers; and in 50.0 % of the patients with COPD OE. We didn't detect any correlation between neopterin and CRP.

Our analysis of IFNγ concentrations in blood serum of the examined people established that they were within reference levels both in the workers and patients with COPD OE (Table 5). However, IFNγ concentration was authentically higher in the workers who were occupationally exposed to industrial aerosols and in the patients with COPD OE than in the non-exposed workers (p_{1-2} = 0.82; p_{1-3} = 0.004; p_{2-3} = 0.005).

The correlation analysis showed a direct correlation between IFNγ and neopterin concentrations in the exposed workers and patients with COPD OE (Groups 1 and 2), R = 0.35, p = 0.04 and R = 0.48, p = 0.01 respectively. We revealed only a trend for a correlation between neopterin and IFNγ concentrations for the non-exposed workers (Group 3) (R = 0.18, p = 0.08). However, we found an authentic direct correlation between neopterin and IFNγ levels (R = 0.51, p = 0.01) in the workers from this group who had high neopterin levels in their blood serum.

Therefore, the research results indicated that 56.1 % the workers who were occupationally exposed to industrial aerosols had elevated neopterin level in their blood serum which was also detected in the patients with COPD OE (53.3 %). This implies that industrial aerosols produced negative effects on the bronchi and lungs of the exposed workers. Elevated neopterin levels were by 3 times less frequently detected in the workers who didn't have any contacts with industrial aerosols at their workplaces. Neopterin synthesis is linked to IFNγ as it is confirmed by the detected direct correlations between neopterin and IFNγ concentrations. These correlations were more apparent in the workers who were occupationally exposed to industrial aerosols and in the patients with COPD OE in the post-exposure period. This fact shows that stimulated cellular immunity can possibly participate in developing and progressing bronchopulmonary diseases.

We established common regularities and differences in how neopterin levels changed and how frequently they were elevated in the workers who were occupationally exposed to industrial aerosols and in non-exposed ones. Average neopterin concentration grew in all workers who were younger than 40 years and was higher than the reference value (up to 10.0 nmol/l) in the workers exposed to industrial aerosols whereas it was within reference values in all age sub-groups of the non-exposed workers. Frequency of elevated neopterin levels also grew in all workers depending both on age and working experience; however, this growth was more apparent in the workers who were occupationally exposed to industrial aerosols. This growing neopterin level depending on working experience is probably due to only young workers (aged from 25 to 30) being included into the sub-group with working experience not exceeding 10 years. However, we can't exclude probable influence exerted on neopterin concentration in blood serum by longer contacts with industrial aerosols.

Research works performed by some authors indicate there is a correlation between neopterin and age. But their conclusions don't clarify whether higher neopterin levels occur due to normal immune ageing in a healthy body or they can be related to more patients with diseases which haven't been diagnosed so far but are accompanied with elevated neopterin levels. Some authors detected a weak linear growth in neopterin levels in older patients that started somewhere between the 3rd and 4th decades of their age. This allowed assuming that an increase in neopterin level could be considered a part of physiological immune ageing; however, we still can't exclude an alternative explanation, notably, occurrence of age-related diseases [19, 20].

Currently most authors consider neopterin to be a highly sensitive marker of inflammation [21]. Our comparative analysis of neopterin and CRP levels suggests that neopterin is a more specific factor reflecting inflammation in the bronchi and lungs. Elevated neopterin levels were detected in the workers who were occupationally exposed to industrial aerosols and in the patients with COPD OE in more than 50.0 % cases whereas CRP levels were detected with the same frequency among both exposed and non-exposed workers (15.7 % and 19.7 % respectively). We didn't detect any differences in CRP concentrations in blood serum between the workers from both groups (1 and 3) and there were authentic differences only between the workers and patients with COPD OE.

Since neopterin is closely connected with early stages in inflammatory processes in the lungs, it can provide an insight into early changes in the bronchial tubes and bronchioles of workers who are occupationally exposed to industrial aerosols. This is proven by a trend for an inverse correlation between neopterin level and PEF 75 % ($p = 0.06$).

Therefore, neopterin can be used as a diagnostic marker of developing inflammation in the bronchi and lungs at its early stage caused, among other things, by occupational exposure to industrial aerosols. On one hand, neopterin can be considered a biomarker of effect which is expressed by activated macrophages in the lungs as a result of exposure to a foreign substance, industrial aerosols being a good example here. On the other hand, neopterin reflects activation of macrophage section in the immunity that results from exposure to industrial aerosols. The latter seems to be an important pathogenetic mechanism of developing pathologies in the lungs caused by exposure to industrial aerosols.

Our research data indicate it is necessary not only to further investigate neopterin as a biomarker of effect and a risk of early developing pathologies in the bronchi and lungs but also to determine its clinical significance as a prognostic immunologic criterion regarding occupational lung pathologies. Our results can stimulate further clinical and experimental research aimed at examining immune pathogenesis of bronchopulmonary diseases and searching for new immunologic biomarkers which can be used in early diagnostics and prognosis. They can also be eligible for developing new therapeutic strategies for treatment of occupational lung diseases involving immunomodulatory drugs.

Funding. The research was not granted any financial support.

Competing interests. The authors declare no competing interests.

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Received: 22.12.2021
Approved: 16.02.2022
Accepted for publication: 11.03.2022