MEDICAL AND BIOLOGICAL ASPECTS RELATED TO ASSESSMENT OF IMPACTS EXERTED BY RISK FACTORS

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Research article

GLUTATHIONE AS A PROGNOSTIC FACTOR OF HEALTH RISK IN WORKING POPULATION

T.V. Blinova, L.A. Strakhova, V.V. Troshin, S.A. Kolesov, I.A. Umnyagina, J.V. Ivanova

Nizhny Novgorod Scientific Research Institute for Hygiene and Occupational Pathology, 20 Semashko Str., Nizhny Novgorod, 603105, Russian Federation

Redox balance plays the key role in maintaining health. Optimizing glutathione levels has been proposed as a strategy for health promotion and disease prevention, although cause-effect relationships between glutathione status and disease risk or treatment have not been fully clarified. This study aims to estimate glutathione as a non-specific prognostic risk factor of health disorders in people exposed to industrial aerosols at their workplaces. Our observation covered the following occupational groups: workers employed at a metallurgic plant who contacted industrial aerosols (welding and silicon-containing aerosols with predominantly fibrogenic effects); patients with non-obstructive chronic industrial bronchitis (NCIB) without exacerbation; patients suffering from occupational chronic obstructive pulmonary disease (oCOPD) who were in a post-exposure period; workers who were not exposed to industrial aerosols at their workplaces. Total glutathione (TG), reduced glutathione (GSH) and oxidized glutathione (GSSG) were identified in whole blood by the Ellman method.

Elevated GSSG levels (higher than 100 μmol/l) and low values of the GSH/GSSG ratio (less than 10 units) were identified in more than 50 % of the workers exposed to industrial aerosols. These markers were established to have diagnostic sensitivity of more than 50 %, diagnostic specificity of more than 85 % and prognostic significance of more than 80 % for the examined groups. The GSSG level and GSH/GSSG ratio can be used as a prognostic indicator of health disorders in workers exposed to industrial aerosols and a possibility of chronic bronchopulmonary pathology developing in future.

Keywords: *reduced glutathione, oxidized glutathione, ratio of glutathione fractions, industrial aerosols, bronchopulmonary pathology, oxidative stress, risk factor, working population.*

Health protection is the most vital task within the state policy. It is closely connected with the quality of public healthcare in the country and development of effective programs aimed at protecting health of all population groups regardless of their social status, occupation or welfare[1]. Great

Tatyana V. Blinova – Doctor of Medical Sciences, Leading Researcher at the Clinical Department (e-mail: btvdn@yandex.ru; tel.: +7 (915) 944-38-75; ORCID: http://orcid.org/0000-0001-5254-9378).

Larisa A. Strakhova – Researcher at the Clinical Department (e-mail: strahova.laris2019@yandex.ru; tel.: +7 (910) 381-72-47; ORCID: http://orcid.org/0000-0003-0672-6622).

Vyacheslav V. Troshin – Candidate of Medical Sciences, Head of the Clinical Department (e-mail: recept@nniigp.ru; tel.: +7 (831) 419-61-94; ORCID: http://orcid.org/0000-0002-7077-0014).

Sergei A. Kolesov – Candidate of Biological Sciences, Senior Researcher at the Clinical Department (e-mail: recept@nniigp.ru; tel.: +7 (831) 419-61-94; ORCID: http://orcid.org/0000-0003-4379-0228).

Irina A. Umnyagina – Candidate of Medical Sciences, director (e-mail: recept@nniigp.ru; tel.: +7 (831) 419-61-94; ORCID: http://orcid.org/0000-0002-9276-7043).

Julija V. Ivanova – Candidate of Medical Sciences, Researcher at the Clinical Department (e-mail: iul.999@yandex.ru; ORCID: http://orcid.org/0000-0002-1251-4610).

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attention is paid to health of working age population exposed to harmful occupational factors at their workplaces [1]. In this case, the key role belongs to preliminary and periodical medical examinations; among other things, their aim is to detect early signs of occupational and work-related diseases. Early diagnostics of diseases relies on biomarkers, or quantitative health indicators. A biomarker can be an indicator of a risk and progression of a disease; it can be used to diagnose a disease or estimate whether treatment is effective.

Glutathione as a biochemical marker is a good example. Although structural and functional relationships within the glutathione system have been investigated for decades, many issues regarding glutathione functions in health and in a pathological state still require profound examination.

Glutathione is a major intracellular antioxidant responsible for removing reactive oxygen species by an enzymatic or non-enzymatic way. Intracellular glutathione exists as a monomer in its reduced form (GSH) and as a disulfide dimer in its oxidized form (GSSG), which is formed due to GSH oxidation. Reduced and oxidized glutathione forms are the main cellular redox buffer. In physiologically normal conditions, GSH usually appears in higher concentrations than GSSG. Some authors consider the GHS / GSSG ratio to be a marker of oxidative stress (OS) [2]. GSH deficiency or lower GSH / GSSG ratios largely indicate OS is developing and cell antioxidant properties are weakened; elevated GSH levels are associated with enhanced antioxidant capabilities and resistance to OS [3]. Studies with their focus on glutathione fractions established that the GSH / GSSG ratio was approximately 10:1 in healthy people whereas any decrease in this ratio was a marker of oxidative stress [4].

Glutathione has many various functions. It protects cells against oxidative stress, supports the immune system functioning, participates in post-translation protein modification and also takes some part in DNA synthesis and recovery, cell proliferation and differentiation; it regulates cell death, apoptosis included. Glutathione plays a significant role in non-enzymatic protein glutathionylation thereby regulating a structure and functions of a protein, changing forms, charges and sizes of target proteins; it also protects proteins against further irreversible peroxidation [5].

Disorders in the glutathione system have been detected in many diseases. Lower levels of reduced glutathione and higher levels of oxidized one were identified in patients with type II diabetes, stroke, hypertension, after cardiac surgery, neurologic diseases, schizophrenia and Alzheimer disease [6–9]. Lower GSH and higher GSSG levels were observed in patients with many lung diseases including chronic obstructive pulmonary disease, bronchial asthma, idiopathic pulmonary fibrosis, cystic fibrosis, and acute respiratory distress syndrome [10, 11]. Redox balance was established to be crucial for maintaining health. Given that, optimizing glutathione levels has been proposed as a strategy for health promotion and disease prevention, although cause-effect relationships between glutathione status and disease risk or treatment have not been fully clarified [12]. Bearing multiple roles of glutathione in mind, we believe it is really difficult to establish a cause-effect relation between changes in GSH levels and progression of a disease [13].

The glutathione system includes several enzymes with vital antioxidant functions. Glutathione peroxidases neutralize hydrogen peroxide and reduce oxidized lipids [14]. Glutathione reductase reduces oxidized glutathione (GSSG) and maintains the permanent level of reduced glutathione (GSH) in cells [15]. Glutathione-S-transferases protect cells against environmental exposures due to detoxification acting as catalysts in GSH conjugation [16].

Under unfavorable conditions, the glutathione system aims to maintain homeostasis in the body by stimulating its enzyme systems responsible for keeping balance between specific fractions. That is, oxidized glutathione is rapidly transformed into reduced one; the glutathione system again recovers and performs its antioxidant functions. Exposure to harmful

environmental and occupational factors (ambient air pollution, tobacco smoke, radiation, chemical exposures at a workplace, industrial aerosols, noise exposure, chemical intake with food, etc.) stimulates excessive production of free radicals [17, 18]. According to results obtained by some researchers, harmful occupational factors disrupt well-balanced functioning of oxidant and antioxidant systems [6, 7]. In case free radicals are produced in excessive quantities and the system of free radical oxidation does not function properly, glutathione antioxidant functions might be impaired as well; ultimately, this leads to excessive formation of oxidized glutathione and a decline in levels of reduced one. Having generalized data of multiple research works on the subject, we can state that any failure in the glutathione system produces negative effects on the clinical course and forecast of various diseases already diagnosed in a patient and can also facilitate occurrence of new pathologies with varied genesis.

In this study, our aim was to estimate glutathione as a non-specific prognostic risk factor of health disorders in people exposed to industrial aerosols at their workplaces.

Materials and methods. Within this study, 245 people were observed; they were divided into five groups.

The 1st group (control) was made of practically healthy people employed in various branches who were free of any exposure to industrial aerosols at their workplaces (advertising agency staff, managers, office clerks and accountants); overall, there were 44 men in this group, their average age being 57 years (53–59), average work records, 13.9 ± 8.5 years.

The 2nd group included practically healthy people employed at a metallurgic plant in the Nizhniy Novgorod region (55 men aged 52 (47–54) years with work records being 13.8 ± 7.7 years) who were exposed to welding and silicon-containing aerosols with predominantly fibrogenic effects (electric gas welders, slingers, metal cutters, milling and rolling machine operators) and did not have any functional signs of impaired lung ventilation.

The 3rd group was made of practically healthy people employed at a metallurgic plant in the Nizhniy Novgorod region (39 men aged 51 (45–55) years, average work records being 13.3 ± 7.5 years) who were exposed to welding and silicon-containing aerosols with predominantly fibrogenic effects (electric gas welders, slingers, metal cutters, milling and rolling machine operators) and already had some functional signs of impaired lung ventilation.

The 4th group included workers with long-term work records employed at car production in Nizhniy Novgorod. They suffered from non-obstructive chronic industrial bronchitis (NCIB) caused by long-term exposure to welding and silicon-containing aerosols with predominantly fibrogenic effects. The workers in this group did not have exacerbation and were in a post-exposure period when this study was being accomplished. They were all being treated in the therapeutic clinic of the Nizhniy Novgorod Research Institute for Hygiene and Occupational Pathology of Rospotrebnadzor. Overall, the group included 29 people (14 men and 15 women) aged 59 (55–60) years with average work records under harmful exposure being equal to 27.8 ± 8.0 years.

The 5th group was made of workers with long-term work records employed at car production in Nizhniy Novgorod who suffered from occupational chronic obstructive pulmonary disease (oCOPD) with a stable clinical course. The disease had been caused by long-term exposure to welding and silicon-containing aerosols with predominantly fibrogenic effects. The workers in this group were also being treated in the therapeutic clinics of the Nizhny Novgorod Research Institute for Hygiene and Occupational Pathology of Rospotrebnadzor. Overall, there were 78 people in this group (12 women and 66 men) aged 59 (58–63) years with average work records under harmful exposure being equal to 26.0 ± 8.0 years.

People with acute communicable diseases, malignant neoplasms, diabetes mellitus or a chronic disease in exacerbation were excluded from the study.

All the observed patients gave their informed consent to participating in the study that was then approved by the Local Commit-

tee of Ethics of the Nizhniy Novgorod Research Institute for Hygiene and Occupational Pathology of Rospotrebnadzor.

Data on working conditions at workplaces of the workers from the 2nd and 3rd group were provided by their employer in accordance with the Federal Law No. 426 issued on December 28, 2013 On Special Assessment of Working Conditions[2]. According to this assessment, average shift levels of dusts with diiron trioxide varied between 0.65 to 7.2 mg/m^3 in different spots (MPC is 6.0 mg/m^3); silicon dioxide (its share in dusts varying between 10 and 70 %), between 0.44 and 2.4 mg/m^3 (MPC is 2.0 mg/m^3); electrocorundum, between 1.8 and 6.6 mg/m^3 (MPC is 6.0 mg/м^3). Average shift concentrations of silicon dioxide, electrocorundum and diiron trioxide levels in workplace air were by 1.1–1.2 times higher than MPC. The working conditions were assigned into the hazard category 3.1 (harmful conditions, hazard category 1).

COPD was diagnosed based on the criteria provided by the Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease – GOLD, 2021 [19] and the Federal Clinical Recommendations of the Russian Respiratory Society [20]. NCIB was diagnosed based on the criteria fixed in the National Guide[3]. The disease was diagnosed as 'occupational' in accordance with the Provisions on Investigating and Accounting of Occupational Diseases (The RF Government Order issued on December 15, 2000 No. 967) [4] and the Order by the RF Ministry for Public Healthcare and Social Development issued on April 27, 2012 No. 417n On Approval of the List of Occupational Diseases[5].

All the participating workers had their breathing examined with a Spirolab III OXY spirometer (Italy), the following indicators being estimated in the process: forced vital capacity (FVC, %$_{standard}$), forced exhalation volume in 1 sec (FEV_1, %$_{standard}$), a calculated ratio of these two indicators (FEV_1 / FVC, %) or modified Tiffeneau-Pinelli index (MTPI) and maximum forced expiratory flow at FCV 75 % (FEF 75 %).

Levels of total (TG), reduced (GSH) and oxidized glutathione (GSSG) were identified in whole blood of all the examined workers by the Ellman method [21]. As sampling was completed, the blood samples were placed in ice and frozen under 70–80 °C below zero. At all stages in analyzing, the samples were centrifuged under 4 °C in a preliminary cooled centrifuged at 10,000 rpm for 10 minutes. The GSH / GSSG ratio was calculated and its value lower than 10 was estimated as critical; it indicated functional failure of the antioxidant system [4].

The results were statistically analyzed with variation statistics methods in Statistica 6.1 software package (StatSoft Inc, USA). We applied the Shapiro – Wilk test to examine how close the data were to normal distribution and to analyze equality of dispersions. In case data deviated from normality, non-parametric Mann – Whitney U-test was applied. The data

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were given as Med ± IQR (25–75 %). Chi-square test (χ^2) with Yates correction was applied to determine whether differences between qualitative indicators were statistically significant. In case a value of an expected fact was lower than 10, the exact Fisher's test was applied (Fisher's F-test). We calculated prognostic significance of glutathione fractions[6] as well as a risk of imbalance between its fractions in people exposed to industrial aerosols at their workplaces. To compare probabilities of outcomes depending on a risk factor, we created a fourfold contingency table, calculated a relative risk (*RR*) and its 95 % confidence interval (95 % CI). The indicator was considered positive if its value was > 1. The differences were considered valid if the confidence interval for this indicator did not include 1. We calculated odds ratio (*OR*) and 95 % confidence interval (95 % CI) to determine influence of a risk factor on a probability of an outcome. Critical significance of the study results was taken as $p < 0.05$. Values of p between 0.05 and 0.1 inclusively were estimated as a trend.

Results and discussion. Table 1 presents spirometry data of the examined workers.

The study revealed that the workers from the 2nd and 3rd group mostly did not have any health complaints although some functional disorders of lung ventilations were identified in the 3rd group (FEF 75 % varied between 37 and 68 %). Five workers in this group had initial NCIB signs including periodical coughing and minor shortness of breath; the workers did not make much of it. The average FEF 75 % was authentically lower in the 3rd group than in the 2nd one where it varied between 79 and 98 % ($p_{2,3} = 0.002$, the Mann – Whitney test) and was authentically by 20–29 % higher than FEF 75 % in the 4th and 5th group ($p_{3,4} = 0.02$; $p_{3,5} = 0.012$, the Mann – Whitney test).

Table 2 provides the results of identifying glutathione and its fractions in blood of the examined workers.

The analysis of the obtained data revealed valid differences in quantitative levels of glutathione and its fractions between the control group and the 3rd, 4th and 5th group. Authentic differences between the 1st and 2nd group were identified only as regards GSSG levels and GSH / GSSG ratios ($p^{GSSG}_{1,2} = 0.023$; $p^{GSH/GSSG}_{1,2} = 0.01$, the Mann – Whitney test). It is worth noting that authentic differences were also established between the workers with functional disorders of lung ventilation (the 3rd group) and the workers without such disorders (the 2nd group) as regards quantitative levels of glutathione and its fractions: ($p^{TG}_{2,3} = 0.015$; $p^{GSSG}_{2,3} = 0.01$; $p^{GSH/GSSG}_{2,3} = 0.019$; $p^{\%GSSG}_{2,3} = 0.021$, the Mann – Whitney test).

To calculate diagnostic sensitivity, diagnostic specificity and prognostic significance of glutathione fractions, we took the indicators with their values being authentically different in all the experiment groups of exposed workers and patients (groups 2–5) against the same values in the control group. GSSG and GSH / GSSG were selected relying on the data provided in Table 2. Diagnostic specificity identified for these two indicators equaled 93.2 % and 88.6 % accordingly. Table 3 provides data on diagnostic sensitivity and prognostic significance of GSSG fraction and the GSH / GSSG ratio in the examined workers exposed to industrial aerosols and patients with bronchopulmonary pathologies.

The analysis of the obtained data revealed that elevated GSSG levels (higher than 100 μmol/l) were identified in the groups 2–4 with frequency higher than 50 % (diagnostic sensitivity). Diagnostic sensitivity was the same in both groups of the workers exposed to industrial aerosols at their workplaces ($\chi^2 = 2.045$, $p_{2,3} = 0.153$) and was authentically by 7–8 times higher in them against the control ($F = 0.00007$, $p_{1,2} < 0.05$; $F = 0.00000$, $p_{1,3} < 0.05$). Similar results were obtained for the groups of patients with NCIB and oCOPD: diagnostic sensitivity was the same in both groups ($\chi^2 = 0.534$, $p_{4,5} = 0.466$) and was authentically higher than in the control ($F = 0.00001$, $p_{1,4} < 0.05$; $F = 0.00001$, $p_{1,5} < 0.05$).

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Table 1

Spirometry indicators in the examined workers, Med ± IQR (25–75 %)

Groups	FVC, %	FEV_1, %	MTPI	FEF 75 %
Group 1 (control) (n = 44)	100.6 (95–113)	98.5 (94–106)	0.86 (0.80–0.98)	82 (80.1–85.2)
Group 2 (FEF 75 % equal to 70 % and above) (n = 55)	105.8 (96–117)	95.4 (91–101.2)	0.92 (0.83–0.98)	89 (81.0–96.0)
Group 3 (FEF 75 % below 70 %) (n = 39)	98.4 (88.7–102.5)	100 (96–109)	0.89 (0.81–0.95)	58 (46.5–64.0)
Group 4 Patients with NCIB (n = 29)	65.5 (58–74)	59 (54–71.5)	0.78 (0.73–0.85)	46 (41.8–49)
Group 5 Patients with oCOPD (n = 78)	62 (61–75)	50 (52–63)	0.65 (0.65–0.69)	41 (38.7–43)

Note: FVC is forced vital capacity, % of the standard value; FEV_1 is forced expiration volume in 1 sec, % of the standard value; MTPI is modified Tiffeneau – Pinelli index; FEF 75 % is the maximum forced expiratory flow with an expiration being 75 % of FVC.

Table 2

Quantitative indicators of glutathione and its fractions in workers exposed to industrial aerosols, patients with NCIB and oCOPD, (Med ± IQR (25–75 %))

Indicator	The experiment groups				
	Group 1 (control) (n = 44)	Group 2 (FEF 75 % equal to 70 % and above) (n = 55)	Group 3 (FEF 75 % Lower than 70 %) (n = 39)	Group 4 Patients with NCIB (n = 29)	Group 5 Patients with oCOPD (n = 78)
Glutathione fractions (reference levels)	Levels of glutathione and its fractions (Med ± IQR (25–75 %)				
TG (900–1500 μmol/l)	1270.8 (1145.8–1370.5)	1269.5 (1128.5–1401.3)	993.9 (856.1–1121.5)	1000.1 (891.3–1101.1)	968.2 (820.1–1060.2)
GSH (750–1300 μmol/l)	1072.5 (1002.5–1272.8)	1035.6 (910.0–1144.5)	990.5 (933.3–1077.6)	806.7 (632.5–869.9)	783.4 (584.2–929.4)
GSSG (45–100 μmol/l)	62.6 (28.8–109.6)	96.0* (71.5–123.4)	110.8* (87.5–164.5)	109.4* (71.7–127.4)	99.7* (49.1–129.8)
GSH / GSSG (equal to 10 and above)	19.6 (9.9–40.9)	11.1* (6.8–13.3)	8.7* (5.9–11.9)	6.7* (4.7–11.5)	8.7* (5.8–14.6)
% GSSG of TG (less than 10 %)	4.0 (2.6–7.2)	4.1 (2.9–8.8)	9.0 (7.9–13.7)	11.4 (7.1–14.4)	9.3 (6.0–12.9)

Note: * means p (the Mann – Whitney test) indicating a statistically significant difference in GSSG levels and GSH / GSSG ratios against the control ($p < 0.05$).

Table 3

Diagnostic sensitivity and prognostic significance of GSSG and GSH / GSSG in workers exposed to industrial aerosols and patients with bronchopulmonary pathology, %

Indicator (reference values)	The experiment groups				
	Group 1 (control) (n = 44)	Group 2 (FEF 75 % equal to 70 % and above) (n = 55)	Group 3 (FEF 75 % Lower than 70 %) (n = 39)	Group 4 Patients with NCIB (n = 29)	Group 5 Patients with oCOPD (n = 78)
	Diagnostic sensitivity (frequency of identified elevated (↑) and low (↓) levels, %)				
GSSG (45–100 μmol/l)	6.8 (↑)	50.9 (↑)	58.9 (↑)	55.2 (↑)	44.9 (↑)
GSH / GSSG (equal to 10 and above)	11.4 (↓)	52.7 (↓)	53.8 (↓)	62.1 (↓)	61.5 (↓)
	Prognostic significance (%)				
GSSG (45–100 μmol/l)	-	88.2	89.6	89	86.8
GSH / GSSG (equal to10 and above)	-	82.2	82.5	84.5	84.4

Lower GSH / GSSG ratios (less than 10 units) were identified with 50–60 % frequency in all the examined groups. In the groups of the exposed workers, diagnostic sensitivity of the GSH / GSSG ratio was the same (χ^2 = 0.588, p = 0.444) and was authentically by 4–5 times higher against the control (χ^2 = 10.783, $p_{1,2}$ = 0.002; χ^2 = 15.426, $p_{1,3}$ < 0.001). In the groups of the patients with NCIB and oCOPD, diagnostic sensitivity of the GSH / GSSG ratio was also the same (χ^2 = 0.30, p = 0.863) and was authentically higher than in the control group (χ^2 = 18.542, $p_{1,4}$ < 0.001; χ^2 = 26.818, $p_{1,5}$ < 0.001).

We identified high prognostic significance (80 % and above) for the GSH / GSSG ratio and GSSG levels for the workers exposed to industrial aerosols. We established a significant risk of improper GSH / GSSG ratios for the workers exposed to industrial aerosols at their workplaces (*RR* = 3.208, 95 % CI (1.143–9.002), p < 0.05). This relative risk level indicates that industrial aerosols with fibrogenic effects have certain influence on impairments of the glutathione system functioning. We established that a risk of impairments in the glutathione system functioning was by 12 times higher under exposure to industrial aerosols (*OR* = 11.632, 95 % CI (2.369–57.099)); by 11 times higher for the patients with NCIB (*OR* = 10.632, 95 % CI (2.008–56.334)); by 10 times higher for the patients with oCOPD (*OR* = 10.400, 95 % CI (2.192–49.346)) against the control group.

Therefore, our study results indicate that more than a half of the examined workers exposed to industrial aerosols had negative changes in the glutathione system. This indicated that the redox balance was impaired, oxidative stress was developing and antioxidant protection with glutathione participation was weakened. Similar changes were identified in the patients with bronchopulmonary pathologies, NCIB and oCOPD, who were in a post-exposure period. Although any contacts with industrial aerosols had long ceased, the glutathione system remained impaired in the patients with bronchopulmonary pathology regardless of a therapy; the mechanism of this impairment requires further investigation. It is noteworthy that glutathione is a non-specific marker that describes antioxidant protection of the body and its prognostic role in development of a particular disease will obviously depend on exogenous risk factors influencing workers. In occupational pathology, it is extremely difficult to find highly specific informative tests for a particular occupational disease since its development is highly influenced by harmful occupational factors. These factors, apart from their direct influence on organs and systems, can affect a biomarker and its metabolism. Given that, a possibility to use tests with their sensitivity exceeding 50 % and prognostic significance being not less than 80 % is quite justified in occupational pathology.

In this study, we made an attempt to analyze informative value of glutathione fractions. The analysis established that reduced glutathione had low sensitivity (7.5 % in the workers exposed to industrial aerosols and 42 % in the patients with chronic bronchopulmonary pathology) despite high specificity (its lower values were not detected in any workers form the control group). It can be applied only as an indicator of a progressing disease and developing complications. Oxidized glutathione levels and reduced to oxidized glutathione ratio are much more informative. Both indicators have sufficient diagnostic specificity (more than 80 %) and sensitivity (more than 50 %) in all the examined groups. They can be applied both to create risk groups among people exposed to industrial aerosols at workplaces for more profound monitoring of their health and as indicators pointing at a risk that development of a bronchopulmonary pathology is quite probable. In patients with NCIB and oCOPD caused by exposure to industrial aerosols, these indicators can be a signal that a disease might progress unfavorably and a current treatment can hardly be considered effective.

Undoubtedly, it is hard to identify cause-effect relations between development of a particular pathology and impairments of the glutathione system. To achieve this, more profound investigations are required. Our study results do not provide clear evidence of a role

glutathione might play in development of bronchopulmonary pathology, in particular NCIB and oCOPD, since its functions are too diverse. Still, we can't fail to note that weaker antioxidant protection in which glutathione plays the key role leads to elevated oxidative stress, an important component in COPD progression. However, too little attention is paid by clinicians, occupational pathologists included, to accomplishing treatment and prevention activities aimed at reducing excessive quantities of free radicals and strengthening antioxidant protection of the body including the glutathione system. It is necessary to perform more profound examination of effects produced by environmental and occupational factors on free radical oxidation and antioxidant protection.

Many studies show that 'anti-oxidant approaches', including proper diets, vitamin therapy, neuroprotectors and anti-inflammatory drugs that neutralize reactive oxygen species, can have certain therapeutic effectiveness when they are used to treat various diseases. Issues of applying glutathione and its metabolites in therapy are being discussed actively [22–24].

Based on the accomplished study, oxidized glutathione and reduced to oxidized glutathione ratio were selected as the most informative prognostic indicators of health risks for workers exposed to industrial aerosols at their workplaces and a probability that a bronchopulmonary pathology would develop in them in future.

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