

Original

Comparison of biomarkers in serum and induced sputum of patients with occupational asthma and chronic obstructive pulmonary disease

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Abstract: Objectives: Occupational asthma and chronic obstructive pulmonary disease (COPD) are associated with the airway inflammatory process. The aim of this study was to compare the sputum and serum markers of inflammation in patients with occupational asthma and COPD. **Methods:** The study group included 20 patients with stable COPD, 24 patients with asthma, and 22 healthy subjects. Interleukin (IL)-6, IL-1 β , tumor necrosis factor (TNF)- α , matrix metalloproteinase (MMP)-9 levels in serum and induced sputum as well as fibrinogen and CRP in serum were determined in all the subjects. **Results:** Higher concentrations of IL-1 β , IL-6, TNF- α , and MMP-9 in induced sputum and an increased concentration of acute-phase proteins in serum were observed in COPD patients compared with healthy subjects. Higher concentrations of IL-1 β and MMP-9 in induced sputum and a higher concentration of C-reactive protein (CRP) were detected in COPD patients than in asthmatic subjects. Never smokers with COPD had significantly higher levels of IL-1 β and MMP-9 in induced sputum than never smoker controls. There was no significant difference between the serum and sputum levels of cytokines and MMP-9 of never smokers and smokers with COPD. **Conclusions:** Higher concentrations of IL-1 β and MMP-9 in induced sputum and a higher concentration of CRP in serum allow distinguishing between biomarker profiles of COPD patients and asthmatic patients. Occupational exposure induces a systemic proinflammatory state with increased levels of acute-phase proteins in stable COPD patients. MMP-9 and IL-1 β concentrations are increased

in induced sputum of never smokers with COPD, which is associated with occupational exposure. (J Occup Health 2016; 58: 333-339)
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Key words: Pathogenesis, Chronic obstructive pulmonary disease, Occupational exposure, Asthma, Biomarkers

Introduction

Chronic obstructive pulmonary disease (COPD) and asthma may be associated with work. Although they are different conditions, both are associated with the airway inflammatory process.

Although a number of previous studies have examined the concentration of biomarkers in asthma or COPD, to our knowledge, there has been no direct comparison of such a wide panel of local and systemic biomarkers between patients with occupational asthma and those suffering from COPD. Furthermore, in the present study, we focused on a novel aspect of COPD pathogenesis, i.e., occupational risk factors. Although mechanisms of COPD pathogenesis have been constantly studied, there is still a lack of holistic understanding of COPD development particularly in the context of occupational exposure.

In general, asthma and COPD are associated with an inflammatory process^{1,3}. Matrix metalloproteinase-9 (MMP-9), interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α and acute-phase protein such as fibrinogen, and C-reactive protein (CRP) may all be involved in their pathogenesis^{1,4}.

In the current study, our primary objective was to compare the sputum markers of local inflammation and serum markers of systemic inflammation in a cohort of people

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with COPD and asthma. Specifically, we hypothesized that finding biomarker profiles, which would enable the distinction between asthma and COPD, may be helpful in differential diagnosis in problematic cases. As a secondary aim, we compared biomarkers in induced sputum and serum of COPD patients (never smokers) exposed at work to vapors, gases, dusts, and fumes (VGDF) with that of COPD patients (smokers) and healthy never smokers (control group) to investigate the role of occupational exposure in COPD pathogenesis.

Materials and Methods

The study group included 44 subjects (24 individuals with occupational asthma and 20 with COPD diagnosed at the Department of Occupational Diseases). COPD patients were exposed at work to VGDF. The control group consisted of 22 healthy subjects. All of the study subjects underwent a questionnaire, a clinical examination, spirometry (VicatestP2A, Mijnhardt, Holanda), and arterialized capillary blood gasometry. Induced sputum and serum were collected. The whole process of induced sputum collecting has been described elsewhere⁵. IL-6, IL-1 β , TNF- α , and MMP-9 (Human TNF-alpha Quantikine-ELISA kit, Human IL-1beta/IL-1F2 QuantikineELISA kit, Human IL-6 QuantikineELISA kit, Human MMP-9 QuantikineELISA kit) in serum and induced sputum using ELISA (Quantikine™ assay, R&D Systems, Minneapolis, USA), fibrinogen and CRP in serum were evaluated in all the subjects.

The Regional Bioethical Committee approved the study protocol. Before commencement of the study all of the participants gave their informed consent to participate in it.

Statistical analyses were performed using Statistica 8. Continuous variables were expressed as mean values of standard deviations, while nominal variables were expressed as numbers and percentages. The Mann Whitney *U* test was used to compare concentrations of IL-6, IL-1 β , TNF- α , and MMP-9 in induced sputum and serum, as well as CRP and fibrinogen in serum of the asthmatic subjects, healthy controls, and patients with suspicion of occupational COPD.

Furthermore, a comparison of concentrations of IL-6, IL-1 β , TNF- α , and MMP-9 in induced sputum and serum as well as CRP and fibrinogen in serum of the COPD subjects (never smokers), COPD subjects (smokers), and healthy controls (never smokers) was also performed.

A *p*-value of <0.05 was considered as significant.

Only patients with stable chronic asthma and COPD participated in the study. Study participants with asthma and COPD did not receive any systemic or local medication. Inhaled short-acting β_2 -agonists were stopped at least 8 h before the test; inhaled long-acting β_2 -agonists were stopped at least 48 h before the test, inhaled steroids

were stopped at least 5 days before the test, systemic steroids were stopped at least 14 days before the test, and antihistamine medications were stopped at least 7-42 days before test depending on their duration of action. The patient's oral statement determined compliance with such recommendations. The healthy subjects did not receive any medications.

Inclusion and exclusion criteria

1. Asthmatic subjects

a. inclusion criteria:

- positive methacholine challenge test (PC20<8),
- an improvement of at least 12% and 200 ml in either the FEV1 or FVC following inhalation of a β -adrenergic agonist,
- diagnosis of occupational asthma (confirmed by SICT),
- presence of work-related symptoms of airflow obstruction (cough, wheezing, and dyspnea).

b. exclusion criteria:

- respiratory tract infection or asthma exacerbation within 8 weeks before the study,
- symptoms of bronchospasm on physical examination.

2) Subjects with COPD

a. inclusion criteria:

- recognition of COPD in accordance with GOLD guidelines on the basis of the presence of a post-bronchodilator FEV1/FVC <0.70,
- confirmed occupational exposure to VGDF,
- occupational exposure of at least 10 years.

b. exclusion criteria:

- respiratory tract infection or COPD exacerbation within 8 weeks before the study.

3) Control group

a. inclusion criteria:

- lack of symptoms of airflow obstruction (cough, wheezing, and dyspnea),
- negative personal and family history of atopy,
- normal spirometry,
- negative methacholine challenge test.

b. exclusion criteria:

- respiratory tract infection within 8 weeks before the study.

Results

Characteristics of the study groups are summarized in Table 1. The analysis of occupational exposure of COPD patients indicated that in the study population, five subjects (25%) were exposed to dust containing free crystalline silica, five (25%) were exposed to metallic dusts, two (10%) were exposed to organic dusts, one (5%) was exposed to paints, one (5%) was exposed to wood dust, and six (30%) were exposed to mixed industrial dust. Medium values of pulmonary function tests and gasometry results

Table 1. Characteristics of the study groups

	COPD (N=20)	Asthma (N=24)	Control (N=22)
Age (years) (mean±SD)	59,8±6,7 (min: 50,0; max. 75,0)	43,0±11,3 (min: 25; max. 61)	43,7±14,4 (min: 24; max. 65)
Sex (male/female)	20 (100%)/0 (0%)	20 (83,3%)/ 4 (16,7%)	15 (68,2%)/ 7 (31,8%)
Duration of symptoms [years] (mean±SD) range [min; max]	12,8±11,0 (min: 2,0; max. 38,0)	7,3±5,2 (min: 1,0; max. 20,0)	-
Latency period - duration of exposure be- fore the occurrence of symptoms[years] (mean±SD) range [min; max]	24,5±8,6 (min: 10,0; max. 38,0)	16,2±8,7 (min: 1,0; max. 34,0)	-
Duration of exposure [years] (mean±SD) range [min; max]	26,6±10,5 (min: 1,0; max. 42,0)	21,5±9,4 (min: 1,0; max. 37,0)	-
Smoking status			
Active smokers	5 (25%)	3 (12,5%)	3 (13,6%)
Ex smokers	10 (50%)	9 (37,5%)	9 (40,9%)
Never smokers	5 (25%)	12 (50%)	10 (45,5%)
Durtion of smoking [years] (mean±SD) range [min; max]	23,0±5,9 (min: 15,0; max. 38,0)	12,5±7,1 (min: 1,0; max. 20,0)	23,6±13,7 (min: 3; max. 40)
The prevalence of reported symptoms in the study population			
At least one symptom	20 (100%)	24 (100%)	0
Cough	17 (85%)	23 (95,8%)	0
dry	3 (15%)	17 (70,8%)	0
productive	17 (85%)	6 (25%)	0
Dyspnoea	20 (100%)	23 (95,8%)	0

Table 2. Medium values of pulmonary function tests and gasometry in the study groups

	COPD (N=20)	Asthma (N=24)	Control (N=22)
Spirometry			
FEV ₁ %	70,6±14,6 (min: 46,6; max. 93,2)	94,1±12,0 (min: 65,0; max. 115,0)	106,0±16,7 (min: 77; max. 136,5)
FVC %	89,8±17,2 (min: 71,4; max. 98,2)	107,4±12,9 (min: 85,5; max. 130,5)	112,2±18,3 (min: 83,5; max. 156,4)
FEV ₁ /FVC %	62,93±7,88 (min: 42,7; max. 70,0)	73,4±10,1 (min: 56,8; max. 98,7)	79,7±6,7 (min: 75; max. 93,1)
Gasometry			
pO ₂ (mmHg)	65,0±8,3 (min: 56,3; max. 86,8)	70,7±10,9 (min: 56,5; max. 90,3)	71,4±8,4 (min: 61,4; max. 92,8)
pCO ₂ (mmHg)	37,5±4,8 (min: 27,7; max. 46,9)	37,3±3,2 (min: 31,4; max. 43,1)	38,3±2,9 (min: 34,1; max. 43,7)
pH	7,42±0,03 (min: 7,36; max. 7,53)	7,42±0,02 (min: 7,39; max. 7,5)	7,4±0,02 (min: 7,37; max. 7,46)
BE (mEq/l)	-0,79±1,32 (min: -2,8; max. 2,3)	-0,46±1,01 (min: -2,1; max. 1,3)	-0,95±1,4 (min: -3,2; max. 1,8)
HCO ₃ (mmol/l)	23,4±1,86 (min: 20,9; max. 27,7)	23,7±1,26 (min: 22,3; max. 25,7)	23,5±1,6 (min: 21,2; max. 26,7)

in the study groups are shown in Table 2. Asthmatic subjects had significantly higher eosinophils percentages in induced sputum in comparison to COPD patients ($p=0.004$). COPD subjects had significantly higher macro-

phages ($p=0.001$), neutrophils ($p=0.030$), and eosinophils ($p=0.009$) percentages in induced sputum in comparison to healthy controls (Table 3).

The subjects with COPD had significantly higher levels

Table 3. Induced sputum results in the study groups

	COPD (N=20)	Asthma (N=24)	Control (N=22)	COPD vs Asthma	COPD vs Control
Macrophages	32.92±13.7 (min: 14.0; max. 59.0)	36.6±18.0 (min: 13.0; max. 72.0)	56.81±22.01 (min: 14.0; max. 101.0)	p=0.532	p=0.001*
Epithelia	18.1±9.2 (min: 1.0; max. 33.0)	25.9±14.4 (min: 4; max. 49)	22.0±14.6 (min: 2; max. 49)	p=0.091	p=0.389
Neutrophils	58.2±17.2 (min: 21.0; max. 83.0)	51.2±19.5 (min: 17; max. 82)	43.0±19.8 (min: 3; max. 85)	p=0.302	p=0.030*
Eosinophils	1.92±1.04 (min: 0; max. 4.0)	7.95±8.9 (min: 1.0; max. 36.0)	0.95±0.86 (min: 0; max. 3)	p=0.004*	p=0.009*
Lymphocytes	1.1±1.1 (min: 0; max. 3.0)	1.33±1.11 (min: 0.0; max. 4.0)	1.71±1.76 (min: 0; max. 7)	p=0.567	p=0.254
Monocytes	0.15±0.38 (min: 0; max. 1.0)	0	0.19±0.4 (min: 0; max. 1)	p=0.075	p=0.809

of cytokines: IL-6 ($p<0.001$), IL-1 β ($p<0.001$), TNF- α ($p=0.010$), and MMP-9 ($p<0.001$) in induced sputum in comparison with the healthy subjects. Additionally, increased concentrations of acute-phase proteins such as CRP ($p=0.004$) and fibrinogen ($p=0.009$) in the serum of COPD patients were found in comparison to the control group. There was no significant difference between the serum levels of IL-6, IL-1 β , TNF- α , and MMP-9 of COPD patients and healthy subjects.

Never smokers with COPD had significantly higher levels of IL-1 β ($p=0.025$) and MMP-9 ($p=0.0194$) in induced sputum in comparison with the healthy never smoker subjects. However, there was no significant difference between the serum and sputum levels of IL-6, IL-1 β , TNF- α , and MMP-9 of the never smokers with COPD and smokers with COPD (Fig. 1).

Determination of IL-1 β , IL-6, TNF- α , and MMP-9 in induced sputum of COPD patients in comparison with the asthmatic subjects revealed significantly higher concentrations of IL-1 β ($p=0.021$) and MMP-9 ($p=0.005$) (Fig. 2). Moreover, concentration of CRP protein in serum of patients with occupational COPD in comparison with the asthmatic subjects was significantly higher ($p=0.044$).

Discussion

Currently, the role of biomarkers in respiratory diseases constitutes the focus of much research³⁻⁵. However, there is little data concerning their usefulness among patients who suffer from occupational respiratory diseases.

In the present study we used a panel of biomarkers to compare patients with occupational asthma and those with COPD as well as investigate the role of occupational exposure in COPD pathogenesis.

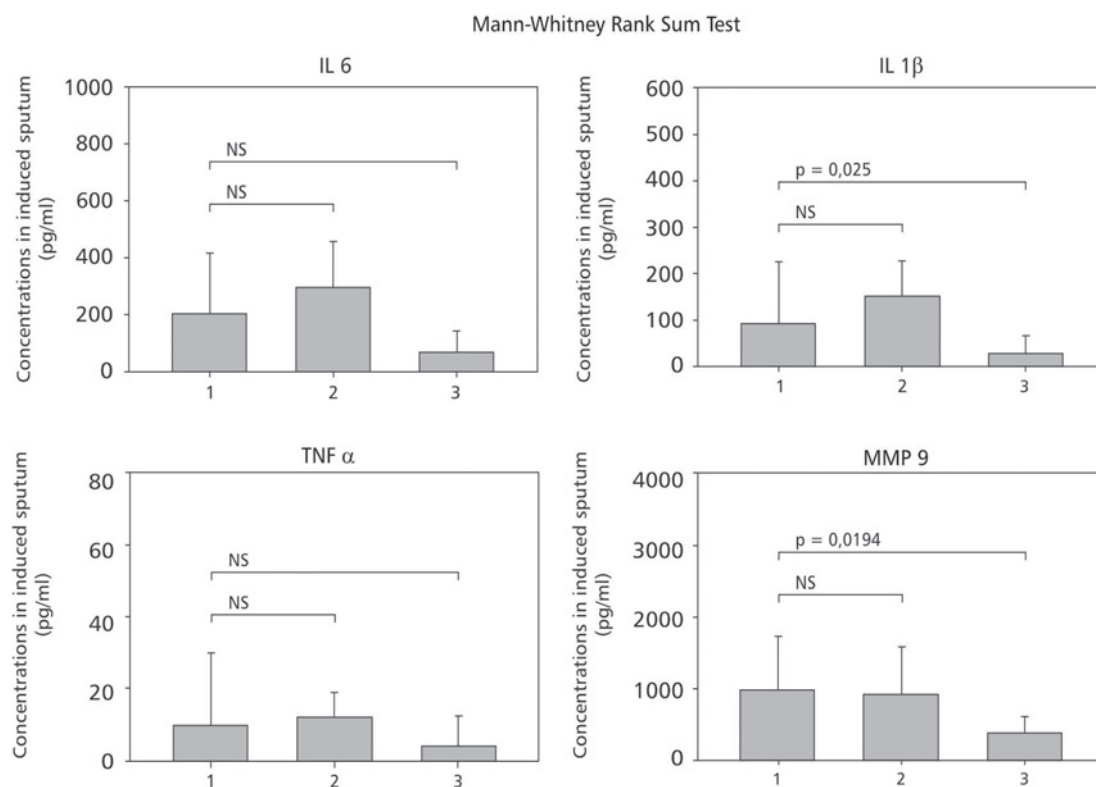
The results of our study revealed that biomarker profiles of COPD patients differ from those seen in asthmatics.

It is known that many patients with asthma or COPD have overlapping characteristics of both the diseases. Overlap syndrome accounts approximately for 15%-25% of obstructive airway diseases^{6,7}. In some cases, recognition of occupational asthma or COPD may be uncertain, e.g. in patients with low spirometric values who have contraindications against a specific inhalation challenge test. Therefore, the distinction between asthma and COPD based solely on spirometry may be difficult. However, in our opinion, combination of lung function testing with newly developed biological techniques, which allow for the assessment of biomarker profiles, can simplify the diagnostic process.

A few recent studies have carried out comparisons of biomarker profiles in COPD patients and asthmatic subjects and have had conflicting results. Dima et al. found that COPD patients had increased sputum neutrophils, IL-8, and TNF- α levels compared to smoking asthmatics⁸. In another study, induced sputum of COPD patients displayed a significantly higher IL-6 level than that of asthmatic subjects⁹. However, in our study, determination of IL-1 β and MMP-9 in induced sputum and CRP in serum of COPD patients was higher in comparison to subjects suffering from occupational asthma. The reason for this difference is uncertain at this moment. On the basis of our results, we can only conclude that the cytokine profile seen in COPD caused by workplace exposure is different from that observed in asthma, which has been also confirmed by other authors¹⁰.

Furthermore, in the present study we also focused on the novel aspects of COPD pathogenesis, i.e., occupational risk factors.

It has been proven that occupational exposure to VGDF can initiate a local inflammatory process in the airways¹¹. In our study, the assessment of induced sputum revealed that IL-1 β , IL-6, TNF- α , and MMP-9 are involved in a local inflammatory process in the airways of



STUDY GROUPS:

1 COPD never smokers 2 COPD smokers 3 Control never smokers

Fig. 1. The determinations of IL-1 β IL-6, TNF- α , and MMP-9 in induced sputum of never smokers with COPD, smokers with COPD, and control group (never smokers)

COPD patients although their serum concentrations were not increased. Contrary to our findings, in a study by Montano et al., an increase in plasma levels of MMPs (MMP-1, MMP-7, and MMP-9) in COPD patients in comparison to healthy controls has been found¹². Similarly, in a study by He et al., a positive correlation between sputum and blood IL-6 levels has been observed¹³. This can be explained by the fact that the increased cytokine levels in the airway may occur earlier than in the blood and can reflect the degree of airflow limitation better than the peripheral blood measurements. Therefore, determination of IL-1 β , IL-6, TNF- α , and MMP-9 in induced sputum seems to be more useful than the analogous assay in serum in a local inflammatory process assessment of stable COPD patients exposed in their workplace to VGDF.

Furthermore, it has been pointed out that COPD is characterized not only by a local but also by a low-grade systemic inflammation^{14,15}. Similar to previous studies, in our study, serum biomarkers assessments revealed an increased concentration of acute-phase proteins, i.e., CRP and fibrinogen, in COPD patients, whereas the levels of cytokines and MMP-9 were not raised. Our findings are consistent with those of Karadag et al. who have shown

that serum CRP is significantly higher in COPD patients than in control subjects, whereas TNF- α and IL-6 concentrations are not statistically different¹⁶. Gan et al. have also found that individuals with chronic airflow limitation had significantly raised levels of CRP and fibrinogen¹⁷. Two other studies have revealed that stable COPD patients have a pro-inflammatory state within increased levels of acute-phase proteins^{18,19}. This confirms that CRP and fibrinogen are important systemic biomarkers of an inflammatory process in COPD.

Several studies have established that occupational exposure to VGDF is nowadays considered an important risk factor for COPD. However, little is known about the role of these molecules in the pathogenesis of COPD. To further investigate this issue, we divided COPD patients into smokers and never smokers, and then, we compared test results in these groups with control subjects (healthy never smokers). The main finding of our study is that MMP-9 and IL-1 β concentrations are increased in the induced sputum of never smokers with COPD associated with occupational exposure. Although the study group was small (15 smokers and five never smoking patients), those study suggest that people exposed at work to VGDF develop a local inflammatory process in the airways simi-

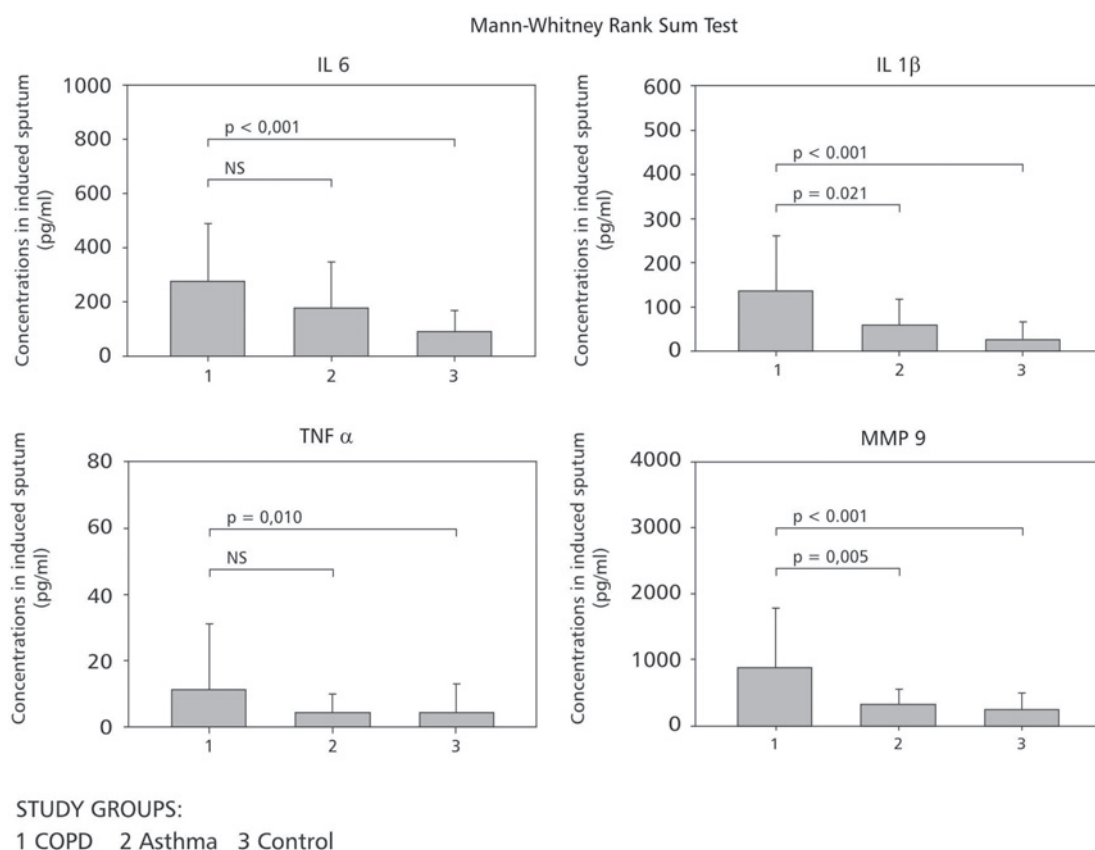


Fig. 2. The determinations of IL-1 β , IL-6, TNF- α , and MMP-9 in induced sputum of COPD patients, asthmatic subjects, and healthy controls

lar to that observed in smokers.

Therefore, we propose that in the future, biomarker assessment can expand our knowledge about the role of occupational exposure in COPD pathogenesis in workers exposed to VGDF, and consequently, contribute to early detection of COPD and the simplification of the distinction between asthma and COPD.

To summarize, the biomarker profiles of COPD patients differ from those seen in asthmatics, which confirms the different pathogenesis of asthma and COPD. Local and systemic inflammations are present in patients with stable COPD caused by workplace exposure. CRP and fibrinogen are important systemic biomarkers while IL-1 β , IL-6, TNF- α , and MMP-9 appear to be promising as local biomarkers of an inflammatory process in the airways of COPD patients. However, further studies are required to establish the role of various biomarkers in occupational respiratory diseases.

Conflicts of interest: Authors deny any potential conflicts of interest and/or funding.

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