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Multiplex Cytokine Profiling in whole blood from individuals occupationally exposed to particulate coal species

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Abstract

Adverse health effects can result from occupational exposure to dust, fine particles, or toxic substances. Accumulating evidence demonstrates that particulate air pollutants can cause both pulmonary and airway inflammation.

The aim of the paper was to perform an exploratory study on peripheral whole-blood using Luminex® 200™ xMAP (multi-analyte profiling) technology to analyze early effects of occupational exposure in coal fired power plants, taking into account that the studied groups contained 10 years, respectively 20 years exposed persons. The altered expression of cytokine profiling in response to coal particulate matter (PM) exposure compared with control subjects has been related to inflammatory response.

The results indicate that exposure to coal dustand ash at work can be considered potential etiological factor for respiratory diseases of the subjects investigated and may aggravate the existing dysfunctions of the biochemical and functional parameters of the exposed persons, arterial blood pressure, dyslipidemia, excess of serum creatinin, urea and uric acid, associated with mixed and restrictive ventilator dysfunction.

The changes of the in vivo and ex vivo inflammatory cytokine profile analyzed using xMAP technology in whole blood system exhibit early statistically significant differences in exposed groups compared to control.

The test tool assaying whole blood parameters is a noninvasive, rapid methodology and can be an useful prerequisite in clinical monitoring of individuals occupationally exposed to coal particulate pollutants in power plants.

Keywords: multiplex cytokine profiling, inflammation, whole-blood, occupational exposure, coal ash and dust, power plants

1. Introduction

The working environment from coal fired power plants contains high levels of various particulate matter (PM) derived from coal combustion, that are dependently on the coal sources and the combustion process. Coal fly ashes resulted from coal combustion process are a mixture of particles, including nanoparticles composed from elemental and organic carbon, metals and inorganic compounds, mainly silica. The detrimental effects on the exposed subjects depend both on the physical characteristics of PM, their chemical composition, the exposure time and on the health state of the people exposed. Understanding the connection between ambient aerosols and their impact on human health requires consideration that

occupational exposure, virtually identical in inorganic dust results in the quantitative differences in the release of mediators due to factors in host susceptibility [1-7].

Accumulating evidences demonstrate that particulate air pollutants can cause both pulmonary and airway inflammation. At the site of inflammation, structural cells, as well as immune effector cells, produce and secrete cytokines, proteins that transmit signals between the cells. It is now clear that cytokine production is not limited to lymphoid and myeloid cells, and that cytokines produced by epithelial and mesenchymal cells amplify inflammatory responses in the lungs and other organs. Each cytokine is capable of modulating more than one cellular function [8-11].

Given that tissues and cells are exposed to complex cytokine mixtures rather than to individual cytokines, recent attention has turned to understanding how cytokines interact. Cytokines are produced in "cascades" in which the initial cytokine signals are amplified many-fold by target cells, such as epithelial cells, fibroblasts, and endothelial cells. Cytokines function in "networks" in which feedback occurs at many points to coordinate and regulate cytokine and cellular responses [12, 13].

It is recognized that a balance of pro-inflammatory and anti-inflammatory factors influences the inflammatory response to pollutant exposure. The structural cells as well as immune effector cells of the lung are capable of cytokine production and release. The initial activation of inflammatory cells in relation to pulmonary diseases including coal workers pneumoconiosis is associated with secretion of interleukin-1 beta (IL-1β), transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), interleukin-8 (IL-8), and interleukin-6 (IL-6) [14-16].

The measurement of soluble cytokines and other analytes *in vivo* and *in vitro* is becoming increasingly important in the study and management of pulmonary and many other diseases including cancer. During the past decade, several major scientific and technical developments have greatly increased the efficiency of measurement of whole blood cytokines by a multiplexed immunoassay system based on fluorescent bead arrays using Luminex® xMAP technology [17, 18].

Compared with ELISA (enzyme-linked immunosorbent assays) that is performed for each separate analyte, multiplex arrays have the ability to detect large numbers (up to 100) of analytes simultaneously in a single sample and therefore provides a powerful tool for profiling multiple cytokines in a volume required to test a single cytokine by ELISA [17, 19, 20].

Several studies have been focused to validate the multiplex cytokine assay (xMAP technology) for detection of the cytokines in serum or in supernatants of whole blood culture. The results show that the multiplex assay is comparable in sensitivity, accuracy, and reproducibility to the "gold standard" ELISA [20, 21, 22].

Biomarker research has been rapidly expanded by the progressive development of the multiplex assay research tools. The relationship between changes in cytokine levels and the development of toxicity functional manifestations, recommends the cytokine profiling as candidate for novel biomarker in preclinical, clinical and potentially in epidemiological studies of risk assessment [5, 23, 24, 25, 26, 27].

The aim of this study was to investigate the changes in expression of proinflammatory cytokines using Luminex® xMAP technology on whole-blood from occupationally exposed population in coal fired power plants. From the knowledge we have, this study seems to be among the first to investigate comparatively *in vivo* and *in vitro* whole-blood inflammatory cytokine levels by using xMAP technology.

2. Materials and Methods

Three target groups have been established: the first group is formed from subjects (male, mean age 40 years, range 27-51 years) with about 10 years of work exposure within the power plant (E10), the second group with around 20 years of work exposure within the power plant (E20) and the control group consisting in healthy subjects (nonsmokers). Blood samples were collected into vacutainer for serum preparation and, respectively, for *in vitro* whole blood culture, from each subject included in E10, E20 and control group. Experimental protocol was approved by the institutional ethical committee of the SC Electrocentrale Deva SA.

Blood sampling for performing serum and peripheral whole blood cultures

Serum samples 5mL of peripheral blood were drawn from each of the subjects using standardized procedures. Handling and processing was identically for all patients. Blood samples were collected without anticoagulant into red top vacutainers and allowed to coagulate for 20 to 30 min at room temperature. Serum was separated by centrifugation, and the samples maintained at 2-8°C while handling, or all specimens immediately aliquoted, frozen, and stored at −80°C. No more than two freeze-thaw cycles were allowed before testing for each sample.

Whole blood culture Heparinized venous blood collected from exposed and healthy subjects was diluted 1:20 with endotoxin free RPMI 1640 supplemented with penicillin, streptomycin and glutamine (2 mM). Samples 200μL were distributed in 96 well microplate and cultured 48h at 37°C stimulated with 50ng/mL LPS (*Escherichia coli* lypopolisaccharide from Sigma-Aldrich, St Louis, USA) and 10μg/mL SiO_2 , suspension of fine structured crystalline silica powders, sterilized by autoclaving (sample of 92% fine SiO_2, with average size of 234 nm, provided from Metallurgic Plant Complex for Silica-Alloys, Tulcea, Romania). Cell-free supernatants were collected and stored at -80°C until cytokine analysis has been simultaneously performed from each sample (well) by xMAP immunoassay.

Blood measurements

The usual hematological parameters were carried out on Horiba ABX Micros 60 Hematology Analyzers (ABX Diagnostics, Montpellier, France) using volume impedance and spectrophotometrical methods. Total WBC count with differential, red blood cell count, platelet count, hemoglobin, hematocrit, and erythrocyte indices: mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width were tested.

Serum biochemical parameters were measured spectrophotometrically with the Hitachi 912 Automatic Analyzer (Roche Diagnostics Co., Mannheim, Germany). The tests included usual metabolic indicators of renal and liver function : urea, uric acid, creatinine, total protein, bilirubin, transaminases (AST, ALT), γ-glutamyl-transpeptidase (GGT), glycemia, lactic dehydrogenase (LDH) .

xMAP technology was performed on Luminex® 200™ platform - a multiplexed immunoassay system based on fluorescent bead arrays using precise ratios of two fluorophores for creating 100 different bead sets. Each set is distinguished based on its internal dye ratio and can therefore carry a unique biological reagent. Six specific bead sets were chosen and antibodies were bound to the bead surface to serve as capture reagents for targets. Serum and whole blood culture supernatants were evaluated using Human Fluorokine MAP Base Kit Panel A (R&D Systems, USA) with the following analyte-specific bead sets: IL-1β (interleukin -1beta), TNF-α (tumor necrosis factor-alpha), IL-6 (interleukin-6), IL-8 (interleukin-8), GM-CSF (granulocyte-macrophage colony-stimulating factor), MCP-1 (monocyte chemoattractant protein-1), according to the manufacturer protocols. Data

acquisition and analysis was achieved using the StarStationTM software. The standard curve was generated by a 5-parameters logistic fit.

Statistical analysis – All values are expressed as the mean ± standard deviation (SD). The statistical significance between the different groups was analyzed by Student's t-test. The values of $p<0.05$ were considered to represent statistically significant differences for a two-tailed distribution.

3.Results

3.1. Hematological and biochemical serum parameters

The occupational exposure risk was evaluated by comparatively assessing the impact on the health state of two target groups: E10 and E20 compared to a control / healthy group.

The hematological parameters: white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCHC), mean corpuscular hemoglobin (MCH) concentration are shown in table 1.

The results indicates a variability of hematological parameters in both exposed groups, but there was no significant difference between the two groups compared to control, excepting the platelet values that exceed biological values in E20 group.

Total WBCs expressed an insignificant exposure time dependent rise, while MCH exhibits a decreasing exposure time dependent trend.

Table 1. Hematological parameters in the target groups compared to control

	Control	E10	E20
WBC $10^3/mm^3$ (3.5 – 10)	7.02±0.85	7.84±2.47 P=0.51	8.8±2.31 P=0.09
RBC $10^6/mm^3$ (3.8 – 5.8)	4.51 ± 0.39	4.64± 0.39 P=0.57	4.52±0.28 P=0.99
HB g/dL (11.0 –16.5)	12.67± 1.35	13.42±1.40 P=0.58	11.68±1.44 P=0.17
HCT % (35 – 50)	38.57 ±2.9	41.86±3.36 P=0.10	36.86±3.22 P=0.27
MCV μm^3 (80- 97)	85.9 ± 9.0	90.6± 6.65 P=0.28	82.0 ±9.82 P=0.40
MCH pg (26.5 -33.5)	28.33±4.31	27.06±2.26 P=0.72	**25.98±4.16 P=0.33**
MCHC g/dl (31.5 - 35)	32.79±2.17	32.06±1.88 P=0.51	31.54±1.01 P=0.21
PLT $10^3/mm^3$ (150 - 390)	326.9± 34.46	261± 34.46 P=0.19	**434.4±163.77 P=0.15**

Control group (healthy subjects); E10 - subjects occupationally exposed 10 years and E20- subjects occupationally exposed 20 years within the power plant. All values are expressed as the mean ± standard deviation (SD). The statistical significance between the different groups was analyzed by Student's t-test. $p<0.05$ was considered statistically significant

Biochemical parameters of exposed and control groups are presented in Table 2.

Serum levels of urea, uric acid and creatinine, as markers of renal function, showed statistically significant differences compared to control beginning from the E10 group ($p<0.05$) and amplified in E20 group ($p<0.01$).

Table 2. Biochemical serum parameters in the target groups compared to control

Target groups	Control	E10	E20
Urea (10-50 mg/dL)	30.1 ±4.70	34±7.78	**51±3.87***
Uric Acid (2.4-7.0 g/dL)	4.43±1.21	**7.148±1.07***	6.96±0.56
Creatinine (0.5-1.2 g/dL)	1.017±0.09	**1.33±0.15***	**1.24±0.06****
Serum protein (6.6-8.7g/dL)	7.59±0.29	8.42±0.24	8.21±1.12
Glycemia (70-115 mg/dL)	96.6±1.58	107±25.08	110±22.73
AST (5-37 U/L)	22.38±5.84	18.2±1.53	22.7±8.44
ALT (5-41 U/L)	19.33±7.26	16.92±1.41	23.18±3.62
Cholesterol (50-200 mg/dL)	191.4±2.67	**239±62.75***	**220.4±43.79***
HDL Cholesterol (45-90 mg/dL)	53.55±0.98	**34.2±10.27***	45.96±10.94

Control group (healthy subjects); E10, subjects occupationally exposed 10 years and E20 subjects occupationally exposed 20 years within the power plant; all values are expressed as the mean ± standard deviation (SD). The statistical significance between the different groups was analyzed by Student's t-test. Statistically significant differences *$p<0.05$; **$p<0.01$

All the liver function parameters involving metabolism of carbohydrate, lipid and protein, enzymes, tended to vary in the biological ranges, or on their close proximity; only total cholesterol ($p<0.05$) and HDL cholesterol ($p<0.05$) increased with statistically significant differences compared to control.

The functional parameters of the exposed subjects to dust from power plant and epidemiological data may indicate the following aspects:

- mixed ventilatory dysfunction was associated with dyslipidemia and arterial hypertension in 60% of the investigated subjects, predominantly from E20 group;
- 50% of exposed non-smoking subjects exhibited restrictive ventilatory dysfunction and dyslipidemia

3.2. Serum cytokine levels

The pro-inflammatory cytokines IL-1β, IL-6, TNF-α, GM-CSF, chemokines MCP-1, IL-8, were measured in serum from E10 and E20 target groups compared to control group using xMAP multiplexed technology performed on Luminex® 200™ platform. Results are shown as mean ± SD; $p<0.05$ was considered statistically significant.

The results are presented in figures 1 and 2.

IL-1β showed an exposure time dependent trend with very high difference between values of the two target groups: values of E20 subjects were about 3.84 fold higher compared to values of E10 group. A similar trend expressed serum IL-6, mean of value E20 subjects being 3.73 fold higher than the mean of E10 group. TNF-α values increased dependently on the exposure time, arising on a relative high level for the control group, slowly increased in E10 group and statistically significant increased ($p<0.001$) in long time exposure (E20) subjects compared to controls. The inflammatory serum cytokines IL-1β, IL-6, TNF-α increased starting from E10 exposed group and exacerbating in (E20) subjects group, where all cytokine levels were statistically significant increased ($p<0.01$) compared to control.

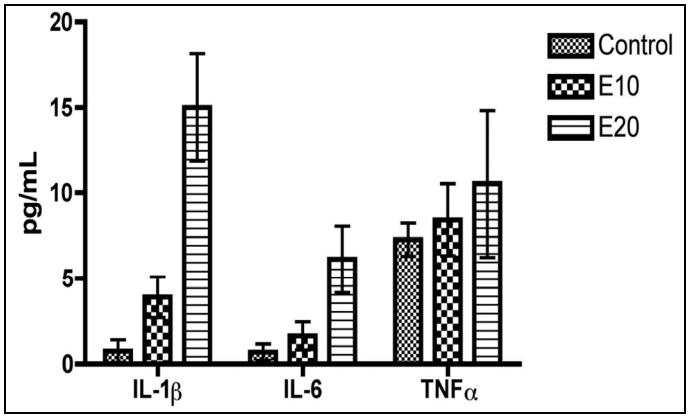

Fig. 1. The inflammatory serum cytokines IL-1β, IL-6, TNF-α

Serum levels of GM-CSF and chemokines IL-8, MCP-1 response to coal ash and dust exposure times presented two aspects: firstly, an appreciable decrease in E10 group compared to control followed by a restoring trend in E20 for GM-CSF and IL-8; secondly, an appreciable increase in E10 group compared to control, continuing to increase slowly in E20 group for MCP-1 (Fig 2).

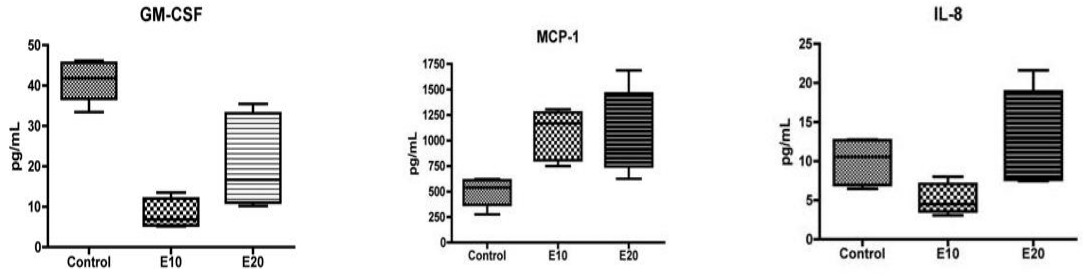

Fig. 2. The serum levels of GM-CSF and chemokines IL-8, MCP-1.

Coal ash and dust exposure had significantly diminished cytokine responses in E10 group compared to control ($p<0.01$), followed by a restoring trend in E20 for GM-CSF. Serum IL-8 response followed a similarly typed curve - diminished level in E10 group compared to control followed by an increasing trend in E20.

MCP-1 increased appreciably in E10 and in E20 groups, with statistically significant difference for E20 group ($p<0.05$) compared to control.

3.3. *Ex vivo* cytokine release

Cytokine levels have been analyzed after activating *ex vivo* – peripheral whole blood cultures from the target groups using the physiologic stimulant lipopolysaccharide (LPS) comparatively with SiO2, as the main particulate component of coal dust.

The basal/unstimulated values for the control unexposed subjects were ranged between 0.1- 0.21pg/mL and all samples were detectable for the cytokines investigated: IL-1β, IL-6, IL-8, TNF-α, MCP-1, GM-CSF.

The basal peripheral whole blood culture performed from both E10 and E20 subjects, expressed also low levels for all cytokines: between 0.01-0.5 pg/mL, undetectable in up to half of the supernatant samples for IL-1β, TNF-α and in all values for IL-6, GM-CSF. Higher basal values were expressed by IL-8: between 0.82 -1.65pg/mL in E10 and 1.37-9.48 pg/mL in E20 subjects.

Ex vivo exposure to LPS and SiO_2 of the peripheral blood cells from the E10, E20 and control subjects induced significant higher expression for pro-inflammatory cytokines IL-1β, IL-6 and TNF-α when comparing the two exposed subject groups with the control group for both stimulation conditions; except for TNF-α level in E10 group stimulated with SiO_2.

Relative changes of the IL-1β values across two time exposure groups E10 and E20 were statistically significant compared to controls both in the LPS and SiO_2 post-exposure measurements: LPS stimulation induces increased IL-1β values in E10 group ($p<0.05$) and in E20 group ($p<0.01$) and SiO_2 stimulation increased their level in E10 group ($p<0.01$) and in E20 group ($p<0.001$).

Il-6 and TNF-α response in E10 and E20 were also significantly increased compared to controls in the LPS and SiO_2 *ex vivo* exposure measurements (Fig.3).

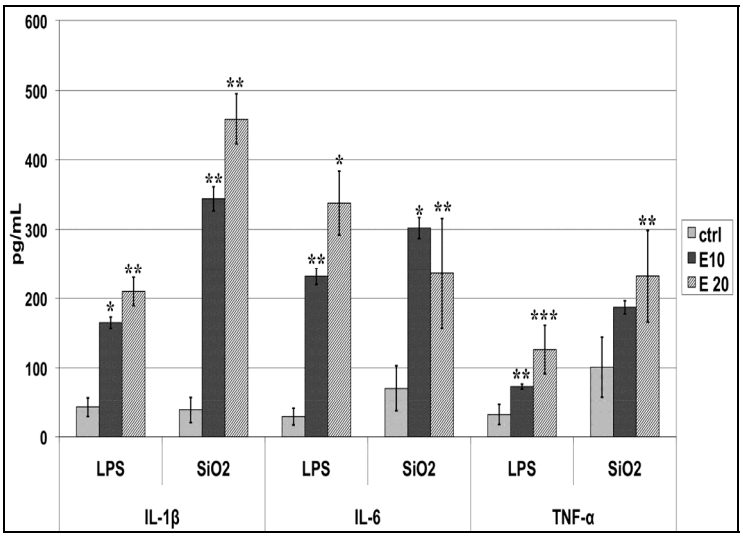

Fig. 3. *Ex vivo* response to LPS and SiO_2 stimulation.

Significantly higher expression levels was found for IL-1β, IL-6 and TNF-α when comparing the two exposed groups with the control group for both stimulation conditions. LPS stimulation increased cytokine secretion in E10 ($p<0.05$) and in E20 group ($p<0.01$), while SiO2 stimulation induced in E10 group ($p<0.01$) and in E20 group ($p<0.001$). SiO2 induced higher values compared to LPS for IL-1β and TNF-α and closely for IL-6 in E10 and E20.

After ex vivo LPS, respectively, SiO2 stimulation, chemokine IL-8 was the most abundant released. Notably, a substantial variation of IL-8 levels is observed in our population individuals: the E10 individuals presented the most elevated levels followed by an unexpected decrease in the E20 (Fig 4).

The statistically significant variation of IL-8 levels is observed in the exposed groups compared to control: the individuals from E10 presented the most elevated levels, statistically different compared to control ($p<0.05$), while the E20 were decreased in a statistically different manner ($p<0.001$) (Fig 4).

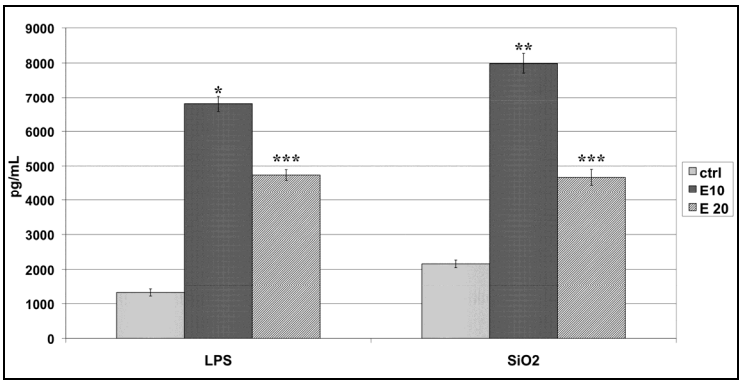

Fig. 4. Variation of IL-8 levels in *ex vivo* exposure conditions.

The profiles of GM-CSF levels after LPS and SiO_2 post-exposure conditions are presented in figure 5.

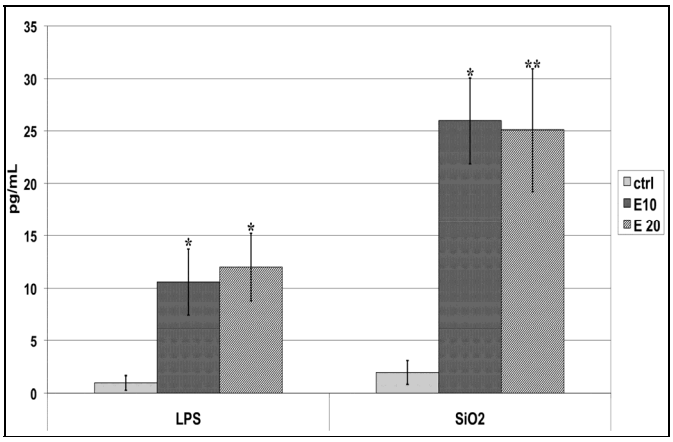

Fig 5. GM-CSF levels in two target groups compared to control.

The profile of GM-CSF were similar, with close values for E10 and E20 both in LPS and SiO2 post-exposure, but with 2 fold higher values in presence of SiO2 compared to LPS stimulation. The values induced by LPS and SiO2 stimulation were statistically different in E10 group ($p<0.01$) and in E20 group ($p<0.001$) compared to control.

4. Discussion

The results indicate that the investigated ambiental exposure conditions did not significantly alter hematologic markers of the exposed groups compared to control. PM induced an insignificant dose-dependent increase in WBCs, no change was seen in RBCs. Platelet results were variable.

The most of the biochemical parameters vary in the biological ranges, or on their close proximity in the two target groups, excepting the significant increase of renal indicators and total cholesterol, HDL cholesterol.

Significant increases of serum renal indicators - urea, uric acid, creatinin - beginning from the E10 group ($p<0.05$) and amplified in E20 group ($p<0.01$) may express the disturbance degree induced by toxic aggression in the renal clearance activity, before arising pathologic processes of hepatic function, or hematologic changes.

A correlation of the functional parameters of the exposed subjects to dust from power plant and these presented data may indicate the following aspects:

- mixed ventilatory dysfunction was associated with dyslipidemia and arterial hypertension in 60% of the investigated subjects, predominantly from E20 group;
- 50% of exposed non-smoking subjects exhibited restrictive ventilatory dysfunction and dyslipidemia.

The occupational exposure to PM mixture from coal fly ash and coal dust resulted in power plant environment induced high levels of serum pro-inflammatory cytokines IL-1β, IL-6, TNF-α starting on in subjects from 10 years exposed group (E10) and exacerbating in long time exposed group (E20), where the differences were statistically significant ($p<0.01$) compared to control.

Interest in the roles of TNF-α and IL-1β, as early response cytokines was stimulated by the recognition of its ability to stimulate production of other cytokine by lung epithelial and mesenchymal cells that do not respond directly to bacteria and /or toxics. TNF-α and IL-6 expression was associated with the presence of coal mine dust particles, suggesting a direct role of mineral particles in the cytokine production and development of pneumoconiotic lesions in coal workers' pneumoconiosis [8].

There are data indicating that significant increase of TNF-α and IL-1β was present in lung fluids of patients with early stage of pulmonary diseases, suggesting that these cytokines are spontaneously released by activated macrophages in the alveolar spaces. Other cells of the alveolar environment produce α- and β-chemokines in response to the pro-inflammatory cytokines TNF-α and IL-1β, and not directly in response to bacterial products such as LPS [9, 13, 16].

IL-6 is an important mediator in inflammatory processes, due to its ability to induce cellular adhesion molecules on monocytes, which facilitates their infiltration into the lung. IL-6 production is partially induced by TNF-α and by IL-1β, and it has been proposed that IL-6 "integrates" signals early produced in the inflammatory response. In vivo, IL-6 seems to be implicated in autoimmune processes in association with TNF-α and IL-1β [13, 28, 29].

Activation and possible alteration of the structural cells results in release of growth factors (GM-CSF) and accumulation of blood derived inflammatory cells and cytokine cascades could account for the chronic nature of the inflammation. GM-CSF a cytokine that functions as a WBC growth factor, acts at early differentiation processes at myeloid progenitors or resting monocytes [30].

GM-CSF and chemokine MCP-1 (monocyte chemotactic peptide) are important mediators in the production and mobilization of monocytes from the bone marrow. Some side effects are probably not due to direct actions of GM-CSF but are caused by the GM-CSF induced secretion of other cytokines such as TNF-α, IL-1β and IL-6.

Both IL-6 and GM-CSF stimulate the marrow to produce and release monocytes while the acute response cytokines, IL-1β and TNF-α, secreted in response to PM stimulation induce the production of monocytic chemoattractants such as MCP-1.

Significant increase of MCP-1 may contribute to platelet activation, either by their chemoattractant properties or by their effect on endothelial permeability and is detectable at the beginning and persists in the lungs of patients with sustained respiratory diseases [31].

Interleukin-8 (IL-8) is a potent chemoattractant (α-chemokine) and activator of neutrophil granulocytes released by alveolar macrophages and other cell types that have chemoattractant effects for PMN. There are evidences indicating that free oxygen radicals may act as intracellular second messengers for the induction of IL-8 gene expression and IL-8 production. Although other potent leukocyte chemoattractants exist, in acute lung injury the neutrophil chemotactic activity is due predominantly to IL-8 [15, 32, 33].

The behaviour of IL-8 in dust exposure resulted in inflammatory response attenuated over time, suggesting that a number of anti-inflammatory mechanisms may counteract the alterations but repetitive exposures can result in chronic respiratory diseases. Repetitive organic dust exposure significantly decreases markers of antigen presentation and host defense function. Mechanisms underlying this modulated response are not clear and current efforts are directed at defining the cytokine balance that exists in the exposed subjects and how this balance changes over time. [34].

Significantly high levels were registered for proinflammatory cytokines in *ex vivo* response to LPS and SiO_2 stimulation, when comparing peripheral whole blood cultures from the two exposed groups with the peripheral whole blood cultures from control, for both stimulation conditions. SiO_2 induced higher values compared to LPS for IL-1β and TNF-α and closely for IL-6 response in E10 and E20 groups.

In *ex vivo* stimulation of the peripheral blood cells with LPS and SiO_2 , IL-8 was the most abundant released the E10 individuals presented the most elevated levels followed by an unexpected decrease in the E20.

The profile of GM-CSF were similar, with close values for E10 and E20 both in LPS and SiO_2 post-exposure, but with 2 fold higher values in presence of SiO2 compared to LPS stimulation. The values induced by LPS and SiO_2 stimulation were statistically different in E10 group ($p<0.01$) and in E20 group ($p<0.001$) compared to control.

Significant increase of GM-CSF, IL-6, IL-1β, TNF-α, IL-8 released in the *ex vivo* stimulation with LPS and SiO2 of the individuals from the two target groups, may be an indicator of the response / defense capacity of the occupationally exposed subjects to new infectious or toxic aggressions.

Subsequent *ex vivo* exposure to LPS and of the peripheral blood cells from the E10, E20 and control subjects, indicated higher susceptibility to SiO_2 for all samples, suggesting that persistent PM exposure (over)stimulates inflammatory cytokine production.

The analysis of the changes in profile of the *in vivo* and *ex vivo* inflammatory cytokine profile using xMAP® technology in whole blood system indicates early statistically significant differences started from E10 and exacerbating in E20 exposed group compared to control.

The results support previous reports indicating the cytokine release as an early detectable and/or predictable indicator correlated with the exposure effects in different toxic aggression [17, 21, 23, 27].

The new multiplex immunoassays using Luminex-bead based microarray technology facilitates the simultaneous evaluation of multiple immune mediators with advantages of higher throughput, smaller sample volume, and lower cost.

Applying microarray technology on peripheral blood cytokine production may provide new insights of variations in global cytokine expression specifically associated with normal metabolism and disease status.

In the last decade peripheral whole-blood cytokine release have attracted increasing interest, and are broadly used for pharmacological *in vitro* and *ex vivo* studies, as methods for immunotoxicology testing the potency of immunostimulants and immunosuppressants.

Peripheral blood is an essential tissue type for biomedical and clinical research because of the simplicity of collection and considering that the whole blood culture system may represent more closely the natural environment with the presence of various immunomodulating and pro- and anti-inflammatory mediators. Furthermore, peripheral blood is considered essential for discovery of novel biomarkers or surrogate markers of a wide range of pathologies [17, 25, 26].

We have applied previously the microarray technology on peripheral blood cytokine production in our studies on *in vivo* and *ex vivo* cytokine profile as a novel developmental

immunotoxicology monitoring risk assessment for human populations of individuals exposed to environmental PM mixtures and food contaminants exposure [23, 24, 27].

Multiplexed cytokine immunoassays are easily applied to evaluate the levels of one given inflammatory molecule in the context of multiple others, their application having ability to perform repeated measures of the same cytokine panels in the same subjects under the same experimental assay conditions to measure *in vivo* and *in vitro* blood cytokines.

5. Conclusions

Exposure to dust at workplaces can be considered potential etiological factor for respiratory diseases and may aggravate the existing perturbation of biochemical and functional parameters: excess of serum creatinine, urea and uric acid, dyslipidemia, associated arterial blood pressure and mixed restrictive ventilatory dysfunction of the exposed persons.

Significantly increase of the serum pro-inflammatory cytokines exhibit a pattern highly dependent on exposure time – slowly increased in E10 group and exacerbated in E20 long time exposed group.

The changes of the cytokine profile analyzed using xMAP® technology in whole blood system *in vitro / ex vivo* exhibit earlier statistically significant differences in exposed groups compared to control and may evaluate the defense capacity of the occupational exposed subjects to new infectious or toxic aggressions.

The test tool assaying whole blood parameters is a noninvasive, rapid methodology and can be usefully for identifying early occupational health hazard before it may come clinically apparent and for monitoring of individuals occupationally exposed to particulate coal pollutants in power plants.

The data may provide information for the development of preventive measures to minimize the adverse effects of the exposures and offers the opportunity to improve worker health and reduces health-related costs.

Acknowledgement

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