

# EVALUATION OF CIRCULATING MICROPARTICLES IN HEALTHY MEDICAL WORKERS OCCUPATIONALLY EXPOSED TO IONIZING RADIATION: A PRELIMINARY STUDY

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## Abstract

**Objectives:** Ionizing radiation was known to cause disruption of cytoskeleton. However, the disorganization of the cytoskeleton leads to form microparticles (MP) that carry membrane and cytoplasmic constituents from their parent cells they are released from. Therefore, authors investigated the effect of the occupational exposure to low doses of ionizing radiation on MP levels. **Material and Methods:** The current study was conducted on 38 healthy medical workers occupationally exposed to low doses of ionizing radiation and 29 controls matched by gender, age, and smoking habits. The MP levels measured by flow cytometry were classified as positive or negative phosphatidylserine (PS<sup>+</sup> or PS<sup>-</sup>), and phenotyped according to their cellular origin. **Results:** Total MP (PS<sup>-</sup>/PS<sup>+</sup>) levels, regardless of phenotype, were significantly higher in workers occupationally exposed to ionizing radiation than in healthy individuals ( $p = 0.0004$ ). Negative phosphatidylserine microparticles were predominant in medical exposed workers and, to a lesser extent, in controls (68% and 57%, respectively). With regard to phenotypic characterization of cellular origin, MP derived from platelets (CD41a+), endothelial (CD146+), leucocytes (CD45+) and erythrocytes (CD235a+) MP were significantly enhanced in exposed workers compared with controls ( $p < 0.0001$ ). However, no significant difference was found in the proportion of the other blood elements in the peripheral circulation between the 2 groups. Serum levels of C-reactive protein were normal for all individuals. In addition, no association was observed between MP levels and the studied confounding factors. **Conclusions:** The results suggest that elevated circulating MP levels represent an indicator of cellular damage caused by medical exposure to low doses of ionizing radiation. By consequence, the quantification of MP seems to be a useful biomarker for assessing the negative effects of occupational exposure to ionizing radiation. *Int J Occup Med Environ Health* 2018;31(6):783–793

## Key words:

Biomarkers, Flow cytometry, Occupational exposure, Microparticles, Ionizing radiation, Cellular damage

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## INTRODUCTION

Human health risks induced by multiple circumstances of exposure including environmental, medical and ionizing radiation have been investigated. However, the risks resulting from the exposure to low doses of ionizing radiation remain ambiguous with intense debate. In fact, the relationship between chronic exposure to low doses of radiation and cardiovascular diseases is still currently scarce. It has been recently suggested that there is a probable association between cardiovascular diseases and exposure to low doses of ionizing radiation [1,2]. However, an increased incidence of such diseases has been well established after the exposure to high doses of ionizing radiation [3–5]. This increase maybe, in part, due to the deleterious effects of ionizing radiation on blood vessels [6]. Epidemiological studies are needed to clarify the possible relationship between exposure to ionizing radiation and vascular damage, which may contribute to understanding the risks on human health after chronic exposure to ionizing radiation. There are yet not truly satisfactory biological markers for evaluating the effects of low doses of ionizing radiation delivered over a long period of time [7]. Recently, it has been found that the incorporation of cellular biomarkers into epidemiological studies may be useful to develop new and more effective strategies for exposure monitoring, health surveillance and individual risk characterization. One of these relative recent advances in this field has been the discovery of the association between the circulating microparticles (MP) and cardiovascular diseases [8,9].

Microparticles (MP) are small fragments (0.1–1  $\mu\text{m}$ ) protruded from plasma membranes of virtually all cell types that are undergoing apoptosis or are being subjected to various types of stimulation or stress [10–12]. They contain cytosolic content surrounded by a phospholipid bilayer exposing several transmembrane proteins, which are specific for their cell origin. Previously, MP were identified by their positivity for phosphatidylserine (PS), but ample evi-

dence now exists that not all MP express PS on their surface and the percentage that do varies with the method used for PS detection and the source of stimulation [13–16].

The amount of MP in the peripheral blood varies according to the physiological state and the pathological condition [17,18]. An increase in their absolute number has been reported in cardiovascular diseases, where MP have been implicated in activation of coagulation, induction of inflammatory response, and vascular dysfunction [19–23]. *In vitro*, it has been previously demonstrated that the irradiation of cells with a high dose of  $\gamma$ -rays leads to externalization of PS on the external leaflet of the plasma membrane of cells resulting in the formation of membrane blebs [24–26]. However, these observations have still been qualitative and not quantitative. By contrast, it has been quantitatively demonstrated that the irradiation of human peripheral blood mononuclear cells leads to increase pro-coagulant cells-derived MP [27].

In this study, authors hypothesized that the medical exposure to low doses of ionizing radiation would contribute to cell injury or activation initiated by a loss of phospholipid asymmetry and MP vesiculation. The effect of long-term medical occupational exposure to low doses of ionizing radiation on MP levels has been therefore investigated.

## MATERIAL AND METHODS

### Study population

The study was performed on 38 medical workers occupationally exposed to low doses of ionizing radiation at Al-Bairouni University Hospital and 29 healthy individuals, who had never been occupationally exposed to ionizing radiation. The exposed workers were matched by gender, age, and smoking habits with controls. All the individuals (workers and healthy subjects) lived in the same city and worked in suitable ventilated conditions. Individuals with infection, medication intake or medical X-ray exposure during the last 12 months were excluded from the study. An informed consent form was signed by all the partici-

pants. Then, a structured questionnaire was conducted to collect data on sociodemographics, medical history, medication intake, and work-related factors. Socio-economic status (SES) information was not included in this study. The study protocol was approved by the local bioethical committee according to the guidelines prescribed by the Helsinki Declaration of the World Medical Association.

### Exposure monitoring

The occupational exposure to  $\gamma$ -rays is routinely monitored using the thermo-luminescence dosimeter (TLD) as a passive radiation detection device (Thermo Scientific™ Harshaw™ Model 8800, Thermo Fisher Scientific, Waltham, MA, USA). The measured doses for the exposed medical workers were quarterly recorded during the period of their work.

### Blood sampling

Peripheral blood samples were drawn at least 16 h after radiation exposure using a vacutainer tube containing 3.2% sodium citrate as an anticoagulant. The required criteria for handling the blood samples were applied in the study including transferring, freezing and thawing of the samples [28].

### C-reactive protein (CRP) assay

Measurements of CRP in the peripheral blood samples were done by the hospital's clinical pathology laboratory using a standard assay.

### Isolation and quantification of MP

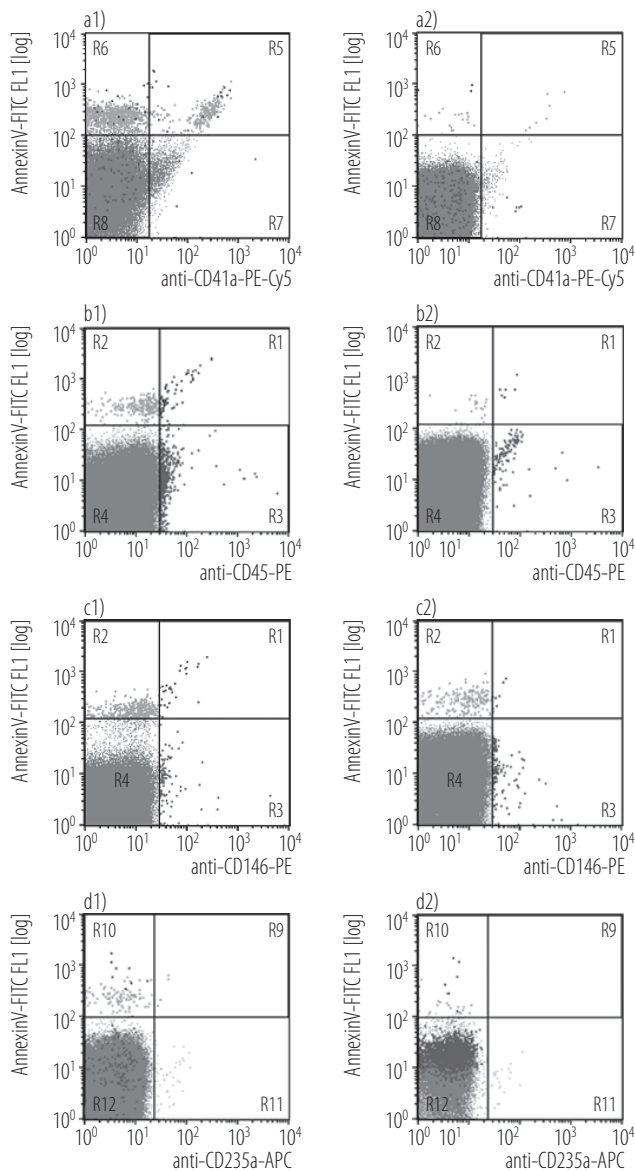
As previously described [29,30], platelet-poor plasma (PPP) had been obtained within 4 h of collection using 2 serial centrifugations (15 min at 1500 g, 2 min 13 000 g at 4°C). Plasma had then been divided into 500  $\mu$ l aliquots, and stored at -80°C until it was used. Samples were thawed at room temperature immediately before assay and were analyzed only after a single freeze-thaw cycle. Then 10  $\mu$ l of

re-suspended PPP was added to 490  $\mu$ l of Annexin V binding buffer in a round bottom sterile tube and according to the manufacturers' instructions, it was incubated with 1  $\mu$ l of fluorescein isothiocyanate conjugated Annexin V (BioVision, Milpitas, CA, USA), CD41-PE (platelet derived MP; PMP, BD Biosciences, San Jose, CA, USA), CD45-PE (leukocyte derived MP; LMP, Miltenyi Biotec, Bergisch Gladbach, Germany), CD146-PE (endothelial derived MP; EMP, Miltenyi Biotec, Bergisch Gladbach, Germany) and CD235a-PE-Cy5 (erythrocyte derived MP; RMP, BD Biosciences, San Jose, CA, USA) in the dark for 5 min at room temperature.

The samples were analyzed on FACSCalibur™ flow cytometer controlled by CellQuest Pro 4.0.2 software (BD Bioscience, San Jose, CA, USA). As previously prescribed [31], the lower and upper limits of the MP were defined using a blend of mono-dispersed fluorescent beads (0.5  $\mu$ m, 0.9  $\mu$ m and 3  $\mu$ m) (Megamix, BioCytex, Diagnostica Stago, France) for better determination of MP location. Thus, the analyzed events represent the main part of the total MP in human plasma. Microparticles were defined as particles < 1.0  $\mu$ m in size and positive or negative to Annexin V and their cell origin as described above (Figure 1).

### Statistical analysis

The population was divided into 2 groups according to the presence or absence of occupational exposure to ionizing radiation. Comparisons between groups were made using the Mann-Whitney U test and Chi<sup>2</sup> test for continuous and nominal data, respectively. Using the Pearson correlations, the significant associations between MP and the TLD record were analyzed. The logistic regression analysis was applied to identify the independent association between the measured doses and MP values. Difference between variables was considered statistically significant at p value < 0.05. Data analysis was performed using Statistical Package for Social Sciences (SPSS) soft-



Various markers were used for detecting MPs originating from various cells: platelet-derived MP (PMP) were detected by CD41a and Annexin V; leukocyte-derived MP (LMP) were identified by phycoerythrin-CD45 and Annexin V; endothelial-derived (EMP) were identified by phycoerythrin-CD146 markers and Annexin V and erythrocyte-derived (RMP) were identified by CD235a markers and Annexin V.

**Fig. 1.** Identification of microparticles (MP) and their subpopulations using flow cytometry in medical exposed workers (1) and healthy controls (2):

- a) platelet-derived MP (CD41a<sup>+</sup>PMP),
- b) leukocyte-derived MP (CD45<sup>+</sup>LMP),
- c) endothelial-derived (CD146<sup>+</sup>EMP),
- d) erythrocyte-derived (CD235a<sup>+</sup>RMP)

ware program for windows, version 15.0 (SPSS Inc., Chicago, IL, USA) and PRISM software (Graph Pad Inc., San Diego, CA, USA).

## RESULTS

### Demographic data

Thirty-eight workers (males: 34, females: 4) occupationally exposed to a low dose of ionizing radiation and 29 healthy individuals (males: 23, females: 6) were included in the study (Table 1). The mean age was 40 years (range 28–55 years) for the exposed medical workers and 37.24 years (range 25–51 years) for controls. The period of their employment was mean (M)  $\pm$  standard deviation (SD):  $9.5 \pm 5.18$  years with an average yearly dose measured by TLD of 7.43 mSv (range: 4.33–17.26 mSv). Controlled cardiovascular disease was reported for 2 (5.3%) workers and 2 (6.8%) healthy individuals. Furthermore, controlled diabetes and hypertension were observed in 2 (5.3%) and 5 (12.8%) exposed workers, respectively.

### Circulating microparticles characterization

The amount of total MP (PS<sup>-</sup>/PS<sup>+</sup>) was approximately 5 times greater in the blood of medical exposed workers than that in controls ( $p = 0.0004$ ) (Figure 2). Based on Annexin V binding, the population of MP was initially sub-grouped as particles positive for PS (PS<sup>+</sup>) or negative for PS (PS<sup>-</sup>). In medical exposed workers, a significant difference was found between the counts of PS<sup>-</sup>MP and PS<sup>+</sup>MP when analyzing total MP, PMP, EMP, LMP and RMP ( $p < 0.05$ ) (Figure 3). When expressed in terms of total MP, the percentage population of PS<sup>+</sup>MP represented  $31 \pm 4.5\%$  (M $\pm$ SD), and out of that  $13.9 \pm 2.9\%$  was derived from platelets,  $8 \pm 1.6\%$  – from leucocytes,  $6.4 \pm 1.18\%$  – from endothelial cells, and  $2.7 \pm 2.07\%$  – from erythrocytes.

In a further comparison of the levels of the different MP phenotypes with those of controls, the data analy-

**Table 1.** Demographic data and clinical characteristics of medical workers occupationally exposed to low doses of ionizing radiation and unexposed healthy individuals

Variable	Respondents	
	medical workers exposed to radiation (N = 38)	healthy individuals (N = 29)
Occupational exposure time [years] (Me (range))	9 (1–22)	0
Thermo-luminescence dosimeter (TLD) [mSv] (M (range))	7.43 (4.33–17.26)	n.a.
Age [years] (M±SEM)	40.00±1.04	37.24±1.49
Male [%]	89.50	79.31
Body mass index (BMI) (M±SEM)	27.47±0.63	26.39±0.75
Smoking [%]	60.5	58.0
Medical history		
cardiovascular disease	2	2
diabetic disease	2	0
hypertension	5	1
cancer	0	0
other	5	4
Treatment		
statin	0	0
antihypertensive	5	1

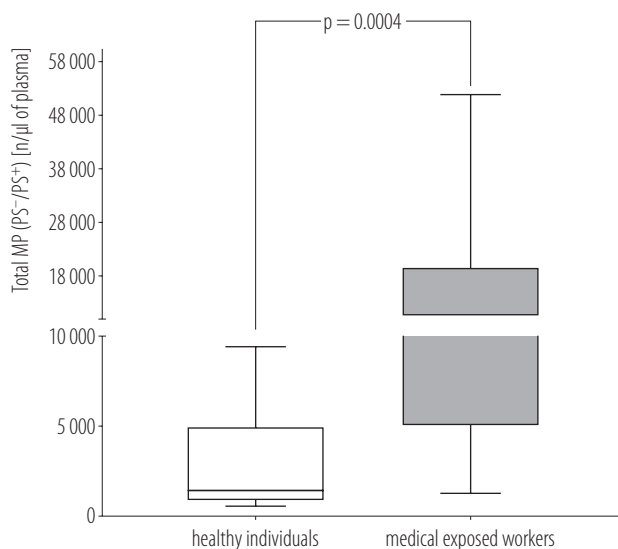
Me – median; M – mean; SEM – standard error of the mean; n.a. – not applicable.

sis indicated that populations of MP (PMP, EMP, RMP and LMP), regardless of PS surface marker expression, are more abundant in exposed workers compared to controls ( $p < 0.0001$ ). The relative abundance of PMP, LMP, EMP and RMP numbers in the case of the exposed workers was 56.6%, 20.1%, 17.2% and 6.1% of total MP, respectively.

However, there was no significant difference between the 2 groups in the mean values of basic hematological parameters including red blood cells ( $p = 0.76$ ), white blood cells ( $p = 0.94$ ), and platelets ( $p = 0.48$ ). C-reactive protein levels were within the normal range (up to 6 mg/l) for all individuals. Furthermore, no significant difference was found in the mean values of serum CRP levels

between exposed and unexposed workers ( $M \pm SD$ :  $2.15 \pm 1.12$  mg/l vs.  $1.97 \pm 0.88$ ,  $p = 0.63$ ).

The data analysis revealed that there was no significant change in total MP levels with gender, increasing age or body mass index (BMI) values. Similarly, no significant difference was obtained in the counts of MP between current smokers and non-smokers in the exposed group (mean  $\pm$  standard error of the mean (SEM):  $3695 \pm 597$  MP/ $\mu$ l vs.  $2381 \pm 546$ ,  $p = 0.14$ ). The multivariable regression analysis revealed that circulating MP levels were not significantly correlated to the other variables in the exposed group ( $p = 0.21$ ). Moreover, the period of occupational exposure to ionizing radiation was not significantly correlated with these variables.

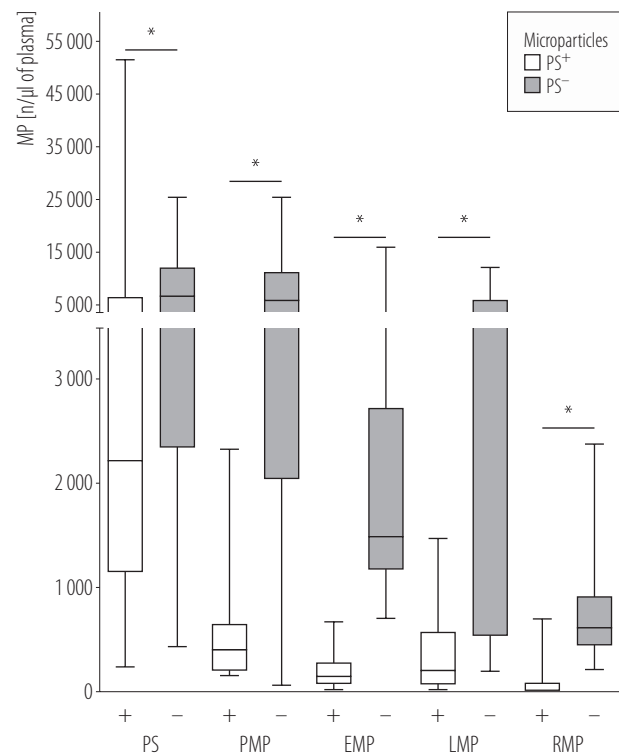


Mean values (M) of total MP  $\pm$  standard error of the mean (SEM) in medical workers occupationally exposed to low-level ionizing radiation (N = 38) compared with healthy individuals (N = 29). Results are expressed as M $\pm$ SEM (total PS<sup>+</sup>/PS<sup>-</sup> MP number in  $\mu$ l of blood). Total MP levels were significantly higher in medical workers occupationally exposed to ionizing radiation than those in healthy individuals (p = 0.0004).

**Fig. 2.** Levels of circulating total microparticles (MP) positive and negative for phosphatidylserine (PS<sup>-</sup>/PS<sup>+</sup>) in medical workers exposed to low doses of ionizing radiation and in healthy individuals

## DISCUSSION

Strong evidence suggests MP as a biomarker of vascular injury. It may also contribute to the initiation and the development of atherosclerosis. In fact, several biological mechanisms might be responsible for atherosclerosis caused by radiation exposure even at a low dose. Therefore, the risk of developing cardiovascular diseases for individuals occupationally exposed to ionizing radiation remains a health concern. To the best of the knowledge, this is the first study which investigates the effect of the occupational exposure to low doses of ionizing radiation on circulating MP levels *in vivo*. In the current study, authors tried to assess the cellular membrane damage resulting from occupational exposure to low doses of ionizing radiation by quantification MP levels. The authors originally



PS – phosphatidylserine.

Other abbreviations as in Figure 1.

Total MP (PS<sup>+</sup>/PS<sup>-</sup>) concentration and their subpopulations in medical workers (N = 38) exposed to low doses of ionizing radiation were detected using flow cytometry and defined as particles < 1  $\mu$ m in size and either positive or negative for Annexin V-FITC. Subpopulation were then phenotyped according to cellular origin. Each group (PS<sup>+</sup> vs. PS<sup>-</sup>) was compared with t-test.

\* p < 0.05.

**Fig. 3.** Distribution of phosphatidylserine negative (PS<sup>-</sup>) and phosphatidylserine positive (PS<sup>+</sup>) total microparticles (MP) and their subpopulations in medical workers exposed to low doses of ionizing radiation

found that populations of MP, regardless of their phenotypes and phosphatidylserine expression, were significantly higher in workers occupationally exposed to low doses of ionizing radiation than those in controls.

The study has shown that the levels of both PS<sup>+</sup> and PS<sup>-</sup> MP are significantly increased in the peripheral blood of medical exposed workers. The MP number was not proportional to PS exposure and the minority of the MP exposed PS. Approximately 14% of PMP were stained

with Annexin V-FITC, which is in agreement with results obtained in previous studies [13,14].

*In vivo*, the exact primary biological mechanisms, the factors and stimuli that regulate secretion of microparticles have not yet completely understood, but roles have been reported *in vitro* for ionizing radiation [27]. The recent study has demonstrated a significant increase in externalization of PS in irradiated cells [32]. It has also been demonstrated that PS externalization was associated with the release of cellular MP [26], which may in part explain the high circulating levels of PS<sup>+</sup> MP in workers occupationally exposed to low doses of ionizing radiation.

The externalization of PS probably represents an early sign of dying cells [33]. Therefore, higher levels of PS<sup>+</sup> MP may reflect cellular apoptosis induced by long-term exposure to low doses of ionizing radiation. However, the predominant PS<sup>-</sup> MP at high levels in the study suggest that these vesicles probably originate from blood cells through an activation caused by long-term exposure to low doses of ionizing radiation. Horstman et al. [34], have observed that MP from activated endothelial cells are rarely positive for PS, in contrast to MP from apoptotic endothelial cells. Nevertheless, the biological importance of PS<sup>-</sup> MP is not yet known, and the importance of this phenomenon should be assessed by future studies.

*In vitro* and *in vivo* studies have shown that several mechanisms may have a relevant role in radiation-induced cardiovascular effects [35]. These effects include inflammation, oxidative stress, alterations of coagulation and platelet activity, DNA damage, cell death and endothelial dysfunction [36]. As shown in the previous study [37], authors found that PMP occupied the greatest percentage of circulating MP in the exposed workers.

Indeed, it has been demonstrated that this phenotype of MP binds to the sub-endothelial matrix causing endothelial damage [38]. However, it has previously been reported that occupational radiation exposure leads to an increase in circulating endothelial cells (CEC) [39]. This

may be due, in part, to the endothelial dysfunction resulting from high PMP release after ionizing radiation.

Despite the heterogeneity of the measured doses of ionizing radiation, authors did not find an association between these measured doses and MP levels. This heterogeneity is probably due to the fact that these individuals had received a chronic radiation exposure during different periods of employment. However, the absence of such association could be explained by the small sample size, the lack of adjustment of some factors such as diet and SES information.

It should be noted that some biomarker responses appear to be linear with doses, while others are not [40]. Despite the elimination of MP from blood after 5.8 h (corresponding to their half-life) [41], the exposure to ionizing radiation may affect their levels. This suggestion could be supported by the fact that the lymphopenia (the decline in the lymphocytes count) following whole-body irradiation may persist for many years after exposure. However, in the absence of platelet activation, MP would need to be constantly shed from these platelets in order to produce MP in the plasma of healthy individuals. In pathological states including stress, the continuous production of MP overrides their rapid clearance. So elevated circulating MP levels could be expected. On the basis of these findings, increased MP levels appear to be a useful biomarker for radiation damage even for a long period after irradiation.

This study has demonstrated that blood cells counts in medical workers exposed to low doses of ionizing radiation remain within the normal range. Similar results have been reported in medical workers occupationally exposed to low doses of radiation [42]. However, previous studies suggest that the decline in blood cell counts after ionizing radiation significantly depends on the measured dose [43,44]. In fact, the measured doses in the medical workers were too low to cause a significant difference in the blood cells count between the 2 groups. However, it

seems that hematological parameters survey could not be a reliable test as the biological indicator of long-term exposure to a low dose of ionizing radiation. Therefore, the cellular damage induced by the exposure to low doses of radiation, which has not been revealed by a change in the blood cells, could be reflected by increased circulating MP derived from these cells.

It has been suggested that occupational exposure to low doses of ionizing radiation was not associated with inflammation. However, circulating markers of inflammation, such as CRP, is not specific to a particular disease or exposure to low doses of ionizing radiation. For instance, it is modulated in the case of cancer, rheumatoid arthritis, cardiovascular diseases, or upon high doses of ionizing radiation [45]. In agreement with a previous study [46], authors found that there was no significant change in CRP concentrations between exposed workers to low doses of ionizing radiation compared to controls. These findings suggest that occupational exposure to low doses of ionizing radiation is not associated with inflammation.

Taken together, it may be assumed that medical occupational exposure to low doses of ionizing radiation leads to high levels of circulating populations of MP, which may probably indicate a susceptibility to radiation injury.

## CONCLUSIONS

Results suggest circulating populations of MP as a useful biomarker for the detection of cellular membrane injury caused by long-term exposure to low doses of ionizing radiation. However, further prospective studies with a large cohort are warranted to investigate the possible correlation between total MP levels and the measured doses after the exposure to ionizing radiation.

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