Biological effects of low-dose ionizing radiation exposure on interventional cardiologists

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Background
Interventional cardiologists (ICs) are likely to receive high radiation exposure as a result of procedures they undertake.

Aims
To assess the effects of low-dose X-ray radiation exposure on chromosomal damage and on selected indices of cellular and humoral immunity in ICs.

Methods
The study population consisted of 37 ICs and 37 clinical physicians as the control group with similar age, sex and duration of employment, without any work-related exposure to ionizing radiation. Cytogenetic studies were performed by chromosome aberration analysis and immunological studies by flow cytometry, enzyme-linked immunosorbent assay and immunodiffusion techniques.

Results
The frequencies of aberrant cells, chromosome breaks and dicentrics plus centric rings were significantly higher in the exposed group compared to the control group (P < 0.05; P < 0.01; P < 0.001, respectively), without positive correlation between the frequency of dicentric and centric ring aberrations and the cumulative doses of the ICs (r = 0.24, not significant). A significant increase was observed in the expression of activation marker CD69 on TCD4+ stimulated cells in serum immunoglobulin G and interleukin (IL)-2 (P < 0.05) and a significant decrease in serum IL-10 (P < 0.05) in the ICs compared with that of the control group. There was no statistical difference between the two groups in terms of number of white blood cells and lymphocytes, CD3+, CD4+ and CD8+ T cells, CD19+ and CD16+ 56+ cells and concentrations of interferon (IFN)-γ, IL-4, IL-6 and IL-8 cytokines.

Conclusions
While cytogenetic results show higher chromosomal damage, some immune responses are stimulated or modulated immunologically in ICs.

Key words
cardiology; cytogenetics; ionizing radiation; occupational exposure; X-ray.

Introduction
It is known that occupational doses of radiation in interventional procedures guided by fluoroscopy are the highest doses registered among medical staff using X-rays [1].

Ionizing radiation accounts for risk-dose-dependent stochastic effects (no threshold dose) and dose-dependent deterministic effects (threshold dose). Deterministic effects, such as erythema or cataract, have a threshold dose below which the biological response is not observed [2]. Some interventional procedures with long screening times and multiple image acquisition may give rise to deterministic effects in both staff and patients [3]. Stochastic effects such as induction of cancer and genetic defects are probabilistic events and may differ among individuals. The linear no threshold hypothesis emphasizes the stochastic nature of DNA damage caused by ionizing radiation [2].

Ionizing radiation can induce various forms of DNA damage, including the possibility of increasing the incidence of chromosomal aberrations (CAs) and micronuclei (MN). CAs are the most fully developed biological indicator of ionizing radiation exposure. The analysis of dicentric and centric ring CAs has for many
years been the most sensitive biological method for radiation dose assessment. Recently, the results of a cohort study provide support for the hypothesis that the occurrence of CAs in peripheral blood lymphocytes (PBLs) represents relevant events in carcinogenesis and may serve as a surrogate end point for cancer risk [4].

Cytotoxic effects of X-rays in occupationally exposed workers were recorded in several earlier studies. High incidence of dicentrics, rings and acentric fragments were observed in the PBLs of medical staff that were occupationally exposed to X-rays [5]. Maffei et al. [6] reported that the frequency of chromosome breaks in physicians and technicians in the units of radiology, radiotherapy and cardiology (both subgroups with whole-body equivalent doses of ≤50 mSv and >50 mSv) were significantly higher than in controls. The occurrence of higher levels of somatic DNA damage [7,8], cataracts [3] and the debate about a higher incidence of brain cancer in interventional cardiologists (ICs) has led to intense research in this field in recent years.

Apart from cytogenetic changes, there is a growing body of evidence regarding immunological changes induced by low-dose radiation (LDR). There is in vivo evidence for both enhancement and depression of immune responses after exposure to LDR. Levels of CD4+ T lymphocytes and humoral immune response were found to be weaker in exposed radiology workers compared with controls [9].

Depression or dysfunction of the highly radiosensitive cellular components of the immune system, such as the CD4+ T cells, can lead to serious consequences, including increased risk for cancer. However, there are reports that LDR exposure can result in radio adaptation that can be beneficial. Liu [10] has reported that the stimulation of immunity by LDR concerns most anticancer parameters, including antibody formation, natural killer (NK) and macrophage activity, secretion of cytokines as well as other cellular changes. Although proposed mechanisms include more efficient DNA repair and stimulated immunity, the underlying mechanisms remain unclear.

In our previous study, we found that ICs had higher rates of CAs and MN frequencies than nuclear medicine physicians and conventional radiologists [11]. However, there is a lack of studies concerning the influence of LDR on immune parameters in ICs. Therefore, the aim of this study was to assess the effects of low-dose X-ray exposure on chromosomal damage and on selected indices of cellular and humoral immunity in ICs.

Methods

All the study population were male. Their overall health status was assessed by a questionnaire. Their occupational exposure to ionizing radiation was routinely monitored by film badges that read every 2 months. The International Commission on Radiological Protection (ICRP) recommends a limit on effective dose of 20 mSv/year for application in occupational exposure, averaged over 5 years (100 mSv in 5 years), with the further provision that the effective dose should not exceed 50 mSv in any single year [12]. The study was approved by the national ethical committee. Informed consent was obtained from each person and the study protocol conformed to the ethical guidelines of the World Medical Association (Declaration of Helsinki).

Fifteen millilitres of blood was collected from each subject by venipuncture. Total white blood cells (WBC) and lymphocytes were counted using a haematology auto analyser.

Cytogenetic analysis performed basically following the improved method of chromosome preparation for low-dose study [13].

The coded chromosome slides were stained with Giemsa’s solution. At least 500 first-cycle well-spread metaphases were analysed from each individual.

The concentration of different serum immunoglobulins (Igs) of IgM, IgG, IgA and components of the complement system (C3, C4, C1 inactivator) were investigated by Single Radial ImmunoDiffusion of Mansoni and the concentration of total serum IgE and cytokine content investigated by enzyme-linked immunosorbent assay using commercially available kits according to the manufacturer’s protocol.

Lymphocyte subpopulations were studied by Becton-Dickinson (BD, Franklin Lakes, NJ, USA) FACScan flow cytometry in PBLs employing BD monoclonal CD3+, CD4+, CD8+, CD19+, CD3+ and CD1656+ according to the method previously described [14].

In order to study the activation marker of CD69 expression, Fast immune Bundle Kit for three colours staining of lymphocytes was used (BD, USA). The lymphocytes were gated based on ssc/CD3 expression and the percentage of either CD4+CD69+ or CD8+CD69+ was determined for unstimulated and stimulated T cells. The processing of samples was done based on manufacturer’s instruction.

Statistical data analysis and comparison of the two groups was conducted using non-parametric Wilcoxon test and correlation between the parameters was assessed by Pearson’s correlation coefficient, in S-PLUS 2000 software. Differences with a confidence level >95% were considered to be statistically significant (P < 0.05).

Results

The study group consisted of 37 ICs with mean age of 45.1 years and with at least 5 years employment in high-caseload cardiac catheterization laboratories (average 12.1 years). The control group comprised 37 clinical physicians working in the same hospitals without any work-related exposure to ionizing radiation. No difference was observed between the two groups in terms of years of employment. None of the study population were current
smokers or had special dietary habits or consumed prescribed medication. According to dosimetry results, none of the subjects exceeded the ICRP-specified dose limits. ICs were exposed to $8.14 \pm 7.81$ mSv/year and $30.5 \pm 24.3$ during the last 5 years.

Table 1 summarizes the demographic characteristics and the frequency of aberrant cells and CAs in lymphocytes of the studied groups. The results showed that the mean frequencies of aberrant cells and of chromosome breaks and dicentrics plus centric rings were significantly higher in the ICs compared to the control group ($P < 0.05; P < 0.01; P < 0.001$, respectively). No positive correlation was found between the frequency of dicentric plus centric ring aberrations and the cumulative dose of the ICs group ($r = 0.24$, NS).

Data on humoral immunity parameters of the study groups are summarized in Table 2. There were no significant differences across exposure in serum Ig levels and components of complement system between the study groups, except significant increase in serum IgG in ICs compared to the controls ($P < 0.05$) with no correlation with employment period and exposure rates ($r = 0.21, r = 0.34$, NS). Significant increase in IL-2 ($P < 0.05$) and significant decrease in serum IL-10 ($P < 0.05$) concentrations were observed in the ICs as compared to the controls (Figure 1a and b). No correlation was found between IL-2 with employment period ($r = 0.14$) and exposure rates ($r = 0.17$) and similarly for IL-10 ($r = 0.18, r = 0.22$, NS). Higher mean levels of IFN-$\gamma$, IL-6 and IL-8 and lower mean level of IL-4 have been found in the ICs in comparison with the control group, which were not statistically significant.

Table 3 gives the haematology results, the PBL subpopulations and expression of CD69 on stimulated lymphocytes in the ICs and the controls. There was no statistical difference between study groups in terms of number of WBC, absolute number and percentage of lymphocytes, Values were within the population reference range, although moderate increase of WBC was observed in the ICs. No significant differences were found in the percentage of basic lymphocyte subsets of TCD4$^+$, TCD8$^+$, CD19$^+$ and CD16$^+$ cells.

A significant increase of CD69 expression on CD4$^+$ stimulated T cells was found in the ICs compared to the control group ($P < 0.01$).

Overall, no correlation was observed between significant immune changes and CAs and between each one with employment period and exposure doses (all correlation coefficients were between 0.1 and 0.3).

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### Table 1. Demographic characteristics and the frequencies of CAs in the studied groups

<table>
<thead>
<tr>
<th></th>
<th>ICs ($n = 37$)</th>
<th>Clinical physicians ($n = 37$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.1 ± 8.2 (range = 36–59)</td>
<td>43.9 ± 6.80 (range = 33–56)</td>
</tr>
<tr>
<td>Gender</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Working period (year)</td>
<td>12.1 ± 6.6 (range = 5–30)</td>
<td>11.8 ± 6.29 (range = 5–28)</td>
</tr>
<tr>
<td>Dose (mSv/year)</td>
<td>8.14 ± 7.81 (range = 1.2–27.8)</td>
<td>–</td>
</tr>
<tr>
<td>Accumulated dose of last 5 years (mSv)</td>
<td>30.5 ± 24.3 (range = 2.45–74.5)</td>
<td>–</td>
</tr>
<tr>
<td>Total no. of cells scored</td>
<td>23 300</td>
<td>22 500</td>
</tr>
<tr>
<td>Aberrant cells (%)</td>
<td>2.78 ± 1.63*</td>
<td>1.27 ± 1.07</td>
</tr>
<tr>
<td>Chromatid breaks (%)</td>
<td>1.32 ± 1.30</td>
<td>0.96 ± 0.88</td>
</tr>
<tr>
<td>Chromosome breaks (%)</td>
<td>1.72 ± 1.98**</td>
<td>0.42 ± 0.75</td>
</tr>
<tr>
<td>Gaps (%)</td>
<td>1.38 ± 1.41</td>
<td>0.85 ± 0.70</td>
</tr>
<tr>
<td>Dicentrics plus centric rings (%)</td>
<td>0.25 ± 0.16***</td>
<td>0.04 ± 0.03</td>
</tr>
</tbody>
</table>

Data are expressed as mean values with standard deviation.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

### Table 2. The average concentration of different classes of serum Igs, components of complement system in the study groups

<table>
<thead>
<tr>
<th>Humoral factors</th>
<th>Intervventional cardiologists ($n = 37$)</th>
<th>Clinical physicians ($n = 37$)</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM, mg% (95% CI)</td>
<td>123 (93–153)</td>
<td>14765* (1108–1842)</td>
<td>80–320</td>
</tr>
<tr>
<td>IgG, mg% (95% CI)</td>
<td>14765* (1108–1842)</td>
<td>123 (93–153)</td>
<td>70–2100</td>
</tr>
<tr>
<td>IgA, mg% (95% CI)</td>
<td>258 (160–356)</td>
<td>77 (64–90)</td>
<td>100–430</td>
</tr>
<tr>
<td>IgE, IU/l (95% CI)</td>
<td>77 (64–90)</td>
<td>103 (81–125)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>C3, mg% (95% CI)</td>
<td>103 (81–125)</td>
<td>23 (15–31)</td>
<td>70–170</td>
</tr>
<tr>
<td>C4, mg% (95% CI)</td>
<td>23 (15–31)</td>
<td>19 (16–22)</td>
<td>15–55</td>
</tr>
<tr>
<td>C1-inactivator, mg% (95% CI)</td>
<td>19 (16–22)</td>
<td>23 (17–29)</td>
<td>10–30</td>
</tr>
</tbody>
</table>

* $P < 0.05$. 
Our study found that the overall frequency of aberrant cells, chromosome breaks and dicentrics plus centric rings was significantly higher in ICs compared to a control group. The present observations agree with many cytogenetic studies carried out in workers exposed to LDR [5–7]. These results are also in agreement with those who showed a higher significant MN values in ICs when compared with clinical cardiologists working outside the catheterization laboratory [8].

Despite the increase in unstable-type aberrations, no positive correlation was found between the frequency of dicentric plus centric ring aberrations and the cumulative doses in the IC group. The dicentric chromosomes are unstable aberrations that are destined to disappear by cell division and therefore decrease over time. In cases of chronic radiation exposure, it is therefore difficult to correlate dicentric frequencies with lifetime dose [15].

This result also confirms earlier studies [16] that reported a higher frequency of dicentrics and acentrics in people occupationally exposed to X-rays. There was no dose-dependent increase in the yield as a function of duration of exposure. Similarly, Leonard et al. [17] have reported a higher frequency of dicentrics in nuclear power plant workers than in controls. However, there was no dose-dependent increase when aberration yield was adjusted for lifespan of lymphocytes and duration of exposure.

Translocation is a stable-type aberration that accumulates in the body and therefore may increase the risk of causing malignant disease. Thus, further study of stable aberrations in ICs by FISH chromosome painting method is needed. A number of studies have identified an increased rate of unstable CAs such as dicentrics and rings in flight crew members and related these to cosmic radiation exposure [15,18]. The mean number of

![Figure 1](image_url). Cytokine levels (pg/ml) in the ICs group compared to the control group. IL-2 and IL-4 concentrations were determined in the supernatants of cultured isolated lymphocytes in the presence of phytohaemagglutinin (PHA) mitogen (a) and concentrations of IL-6, IL-8, IL-10 and IFN-γ were determined in serum samples (b). Cytokine levels were determined using ELISA kits according to the manufacturer’s protocol. The increase of IL-2 and decrease of IL-10 concentrations in the ICs group were significantly higher than that of control group (P < 0.05).

**Table 3.** Haematologic results, the percentage of basic lymphocyte subsets, the average number of CD4+CD69+ and CD8+CD69+ T cells in 10 000 CD3+ cells, in stimulated with PHA and unstimulated lymphocytes of study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (95% CI)</th>
<th>No. of lymphocytes (95% CI)</th>
<th>Lymphocyte CD3+ (%), CD4+ (%), CD8+ (%), CD4+/CD8+ ratio, CD4+CD16+ (%), Unstimulated CD19+ (%), Unstimulated CD16+ (%), Stimulation by PHA (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICs (n = 37)</td>
<td>6932 (5187–8686)</td>
<td>2201 (2150–2252)</td>
<td>56 (51–61), 68 (59–77), 41 (37–46), 27 (24–30), 1.75 (1.5–2.0), 17 (14–19), 254 (225–283), 294 (265–323), 2148 (1800–2500), 1920 (1500–2300)</td>
</tr>
</tbody>
</table>

**Discussion**

Our study found that the overall frequency of aberrant cells, chromosome breaks and dicentrics plus centric rings was significantly higher in ICs compared to a control group. The present observations agree with many cytogenetic studies carried out in workers exposed to LDR [5–7]. These results are also in agreement with those who showed a higher significant MN values in ICs when compared with clinical cardiologists working outside the catheterization laboratory [8].

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stable translocations per cell was also significantly higher among the airline pilots than among the controls [19].

Our findings are in agreement with Hrycek et al. [20] who reported no statistically significant differences in the number of T CD4\(^+\), CD8\(^-\) and CD4\(^+\)/CD8\(^+\) lymphocyte ratio in persons operating X-ray equipment compared with controls. However, it is inconsistent with Godekmerdan et al. [9] who studied effects of LDR on X-ray workers and found lower number of CD4\(^+\) T cells and weaker humoral immune responses of total Igs (IgG, IgA and IgM) and complements (C3 and C4). This inconsistency probably is due to the exposure rates, duration of the exposure and variable immune status of individuals under test and the different sample sizes.

A significant serum increase of IL-2 and decrease of IL-10 in the ICs group compared with the control group was observed. Xu et al. [14] showed that LDR, in the range commonly received by atomic radiation workers or as a result of minor medical diagnostic procedures (0.25–10 mGy), stimulate the expression of IL-2 receptors on the surface of PBLs taken from normal human donors. Activation of the T lymphocytes, especially the T helper, with increased production of IL-2, might be a critical step in the whole process of immunoenhancement. Shieh et al. [21] showed that a single low-dose irradiation in mice could increase the ability of IgG synthesis by stimulating IL-2 secretion. Although the model of a single low-dose irradiation in mice is different from the long-term radiation effects, these indicate a positive biological effect in immune system and it might help to understand the mechanisms of radiation hormesis.

Although some functions of CD4\(^+\) cells are resistant to radiation, other functions, particularly those that depend on the production of IL-4 and IL-5, are greatly diminished by ionizing radiation [22]. This is partially in agreement with our previous results on medical personnel of the cardiovascular laboratories with significant lower production of IL-5 and IL-10 compared with the control group [23]. In the present study, we found lower production of IL-4 in the ICs group compared with control group, but it was not statistically significant.

In our study, ICs were exposed to 8.14 ± 7.81 mSv/year. The literature provides effective doses to the cardiologist per procedure ranging from 0.2 to 18.8 μSv. Even if we assume a rather high caseload of 1000 angiographies per year, the annual threshold level of 20 mSv will be hardly exceeded. However, higher exposure levels and deterministic effects to physicians or staff performing interventional procedures have been observed as a consequence of the lack of radiological awareness and irregular use of protective devices.

In a recent study, the lifetime attributable risk of cancer was estimated using the approach of Biological Effects of Ionizing Radiations (BEIR) [2] for workers of the cardiovascular catheterization laboratory with the median individual effective dose of 46 mSv (interquartile range = 24–64). The median risk of (fatal and non-fatal) cancer was 1 in 192 [24].

In view of the importance of immune surveillance in cancer control, the effect of radiation on the immune system has been one of the chief research fields in radiation biology and radiation protection. However, not many studies examine dose–response relationships of radiation-induced immune changes. Most studies have focused on the changes in immunity after exposure to doses >0.2 Gy and irregular patterns are often observed. This is due, in part, to the complexity of the immune system. In our study, the exposure doses were much lower and we could not find a dose–response relationship with significant immune changes.

While cytogenetic results show higher chromosomal damage in ICs, humoral immunity seems to be more resistant to effects of such a LDR exposure although some cellular responses are stimulated and some cytokine productions are immunomodulated.

It has been reported that risk estimation in the low-dose range should be strictly qualitative accentuating a range of hypothetical health outcomes with a likely possibility of zero adverse health effects [25].

Since ICs have the highest radiation exposure among health professionals, they should be aware of the ICRP’s recommendations and international Basic Safety Standards requirement for radiation protection and local rules. They must comply with the ionizing radiation regulations and other relevant legislation. The main document from ICRP containing recommendations for improving radiation safety in interventional radiology is Publication 85 [12]. Recommendations and practical advice to improve staff radiation protection are also summarized by Vano [26]. The most successful means of reducing occupational exposures has been training in radiation protection. The use of ceiling-suspended protective screens and lead shields in a systematic way by ICs and the programme of patient dose reduction are important complementary actions. Evaluation and follow-up of occupational doses should be considered an important part of quality assurance programmes.

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Key points

- The frequencies of chromosomal aberrations were significantly higher in the interventional cardiologists compared to the clinical physicians.
- Immune responses seem to be more resistant to effects of such a low-dose ionizing radiation exposure although some cellular responses are stimulated and some cytokine productions are modulated.
- Since interventional cardiologists have the highest radiation exposure among health professionals, major awareness of radiation safety and training in radiological protection are crucial for them.
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Conflicts of interest
None declared.

References