

Acute chromosomal DNA damage in human lymphocytes after radiation exposure in invasive cardiovascular procedures

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Aims We evaluated whether radiation exposure during interventional cardiovascular procedures can induce damage to deoxyribonucleic acid (DNA).

Methods and results Micronucleus assay (MN) was performed as biomarker of chromosomal damage and intermediate endpoint in carcinogenesis. Seventy-two patients (54 males, age = 63.8 ± 10.5 years) undergoing a wide range of radiation exposure during invasive cardiovascular procedures (coronary angiography, $n = 9$; percutaneous coronary intervention, $n = 9$; peripheral transluminal angioplasty, $n = 37$; and cardiac resynchronization therapy, $n = 17$) were enrolled. MN frequency was evaluated before, 2, and 24 h after the radiation exposure. Dose–area product (DAP; Gy cm²) was assessed as physical measure of radiation load. DAP value was 96.0 ± 63.9 Gy cm². MN frequency was $15.1 \pm 7.1\%$ at baseline and showed a significant rise at 2 h ($17.5 \pm 6.5\%$, $P = 0.03$) and 24 h ($18.5 \pm 7.3\%$, $P = 0.004$) after procedures.

Conclusion Our results corroborate the current radioprotection assumption that even modest radiation load can damage the DNA of the cell and induce chromosome alterations which are early predictors of increased cancer risk.

Introduction

Invasive cardiovascular procedures are an essential tool for diagnosis and treatment of cardiovascular disease, but they also significantly contribute to the radiation exposure of patients and personnel.^{1–4} The radiation exposure to the patient may range anywhere in between a few hundreds (for a coronary angiography) to several thousands of chest X-rays (for multiple coronary stent placement).^{1–4} Various advisory bodies use the conservative assumption that no level of radiation is without excess risk, that is, the zero threshold hypothesis.^{5–7} Currently, a linear relationship between dose and long-term risk of cancer is used in the risk model for low-level exposures.^{5–7} At low dose, the assessment of health risk has always been a focus of controversy and the estimate is essentially derived by extrapolation of the dose–effect curve obtained from high doses.⁸ Although such estimates summarize the best available evidence, they are mainly thought to be applied in large existing populations.^{5–7} In the individual patient, the oncogenic risk is so low to be practically immeasurable with clinical endpoints. The difficulties of low-dose exposure assessment could be overcome with biocellular markers of radiation-induced DNA damage.⁷ Moreover, exploring the radiation

effects of exposure to patients can provide specific information about health risk of common diagnostic procedures.⁷ Therefore, the purpose of this research was to assess the potential risk due to very low radiation exposure in circulating lymphocytes with micronucleus (MN) assay, which is a sensitive biomarker of chromosomal damage and intermediate endpoint in carcinogenesis.^{9–11}

MN are small, extranuclear bodies that arise in dividing cells from acentric chromosome/chromatid fragments or whole chromosomes/chromatids that lag behind in anaphase and are not included in the daughter nuclei in telophase. Compared with the analysis of chromosomal aberrations in metaphases, the MN assay is easier, more rapid, less expensive, and ideally suited to assess serial genotoxic changes in exposed patients and personnel.^{12–15} The frequency of MN was assessed in patients referred to the invasive cardiovascular procedures involving radiation immediately before, at 2, and 24 h after the radiation exposure.

Methods

Patients

The study population consisted of 127 consecutive patients undergoing invasive cardiovascular procedures involving radiation. Of the set of patients initially considered for inclusion, 27 patients were excluded from the study as they had acute or chronic inflammatory disease, immunological disease, and neoplastic disease; 19 patients

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were not included for an incomplete blood collection and nine patients did not give their consent for participation in the study. The final study population comprised 72 adult patients (54 males, SD in mean age = 63.8 ± 0.5 years) undergoing coronary angiography (CA; $n = 9$), percutaneous coronary intervention (PCI; $n = 9$), peripheral transluminal angioplasty (PTA; $n = 37$), and cardiac resynchronization therapy (CRT; $n = 17$). The X-ray equipments used in this study were Philips Integris H5000C Monoplane and Integris Allura Monoplane with the X-ray tube for both Systems: MRC 200 0508 ROT GS 1001. The dose-area product (DAP; Gy cm²) metre attached on the X-ray unit has been used for the estimation of the radiation dose received by the patient. The DAP value is a quantitative measure of the radiography dose emitted by the radiography tube per centimetre area.^{16–21} These data are obtained from a circular ionization chamber built into the output of the radiography tube. Most newer radiography systems in the cardiac catheterization lab have this device built-in. In interventional procedures, the majority of the DAP value (on average, about 60%) is caused by cineangiography, whereas cineangiography occupies only a minority (on average, about 15%) of the total exposure time.^{16–21}

Fluoroscopic time (FT) has been also assessed for each cath-lab procedure. Using the conversion factor derived from the literature,¹⁶ the effective dose [in millisievert (mSv)] was also calculated. The cumulated DAP for a procedure is a surrogate measurement for the total amount of X-ray energy delivered to the patient, and is considered a valid indicator of a patient's dose and consequent risk for radiation-induced effects.²¹ All patients gave their informed consent before entering the study. The Ethical Committee approved the study. Samples were collected from each subject and the laboratory analyses were performed in a random order. The samples were coded and laboratory analyses were performed without knowledge of patient identity and exposure status.

Collection of blood samples and cytokinesis-block micronucleus test

Blood samples were collected at baseline, 2 h following the procedure, and 24 h after end of the catheterization procedures for MN assay (Figure 1). Two separate cultures from each sample were set-up by mixing 0.3 mL of whole blood with 4.7 RPMI 1640 medium: the cultures were incubated at 37°C for 72 h. Cytochalasin B (6 µg/mL) was added 44 h after culture initiation. Cells were then harvested and fixed according to the standard methods.^{14,15} For each sample, 1000 binucleated cells were scored under optical microscope (final magnification 400×) for MN analysis, following the criteria for MN acceptance. We evaluated the MN frequency

as the number of micronucleated cells per 1000 cells (%). MN frequency was evaluated by the same three microscopists blinded to patient identity.

Statistical analysis

Statistical analyses of the data were conducted with the Statview statistical package, version 5.0.1 (Abacus Concepts, Berkeley, CA, USA). Data are expressed as mean (\pm SD). The size of the sample has been defined for detecting a significant ($\alpha = 0.05$) 15% increased levels of MN, with a minimum power of 90% ($\alpha = 0.05$), calculated on the average baseline levels previously described.¹⁵

Because of the skewness of the distributions of MN values, analyses have been performed using the logarithmic transformation of data. Differences between the means of the two continuous variables were evaluated by the Student's *t*-test. Differences in non-continuous variables were tested by χ^2 analysis. The data for three different types of MN analysis for each patient were analysed by ANOVA, analysis and significant differences among pairs of means were tested by the Scheffe's test. A two-tailed *P*-value < 0.05 was chosen as the level of significance.

Results

Patients' characteristics according to cardiac catheterization procedures are summarized in Table 1. The prevalence of hypertension and dyslipidaemia was significantly different in the patient group undergoing different cardiac catheterization procedures. Prior to the catheterization procedures, there was no significant difference between MN basal frequencies in patients undergoing different cardiac catheterization procedures ($P = 0.1$). However, baseline levels of MN was slightly skewed among the study participants. It ranged from 4 to 42 with a median of 13‰ with a 25th–75th interquartile range of 11–18.5‰. Higher baseline levels of MN frequency were associated with female gender (19.2 ± 8.9 vs. 13.7 ± 5.8 ‰, $P = 0.004$) and presence of diabetes (18.8 ± 11.0 vs. 14.2 ± 5.7 ‰, $P = 0.03$). A history of previous invasive radiation cardiovascular procedures was also an independent significant predictor of baseline MN levels ($P = 0.01$) (Figure 2).

The mean DAP value was 96.0 ± 63.9 Gy cm² with a median of 77 Gy cm² with a 25th–75th interquartile range of 48–133.2 Gy cm². Dosimetric parameters according to

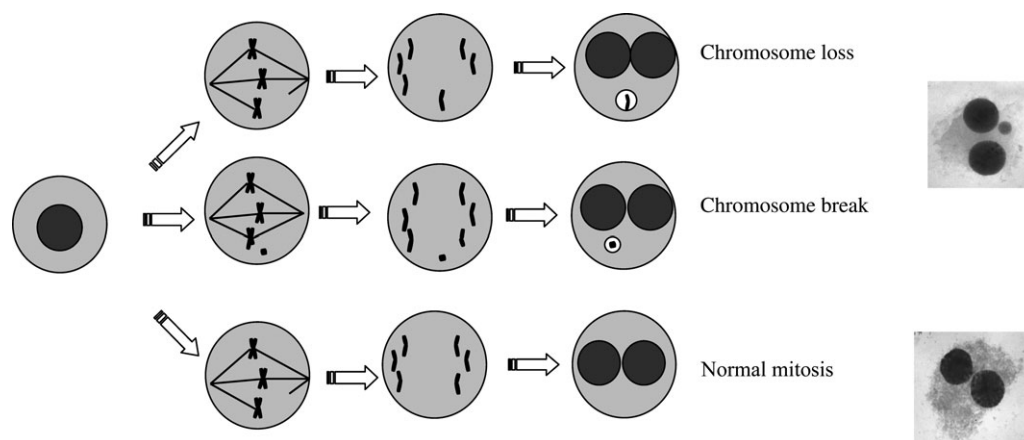


Figure 1 Schematic representation of the micronucleus formation. Micronuclei are small particles in the cytoplasm consisting of acentric fragments of chromosomes or entire chromosomes, which do not integrate in the daughter nuclei during the cell division. See online supplementary material for a colour version of this figure.

Table 1 Clinical and demographic characteristics of study patients

	CA (n = 9)	PCI (n = 9)	PTA (n = 37)	CRT (n = 17)	P-value
Mean age (\pm SD)	58.0 \pm 10.8	62.2 \pm 9.8	66.2 \pm 10.8	66.9 \pm 9.0	0.1
Male sex, n (%)	5 (55.5)	7 (77.8)	29 (78.3)	13 (76.5)	0.5
Hypertension, n (%)	6 (66.7)	6 (66.7)	11 (29.7)	14 (82.4)	0.002
Diabetes, n (%)	0	1 (11.1)	6 (16.2)	6 (35.3)	0.1
Dyslipidaemia, n (%)	3 (33.3)	8 (88.9)	15 (40.5)	7 (41.2)	0.05
Smoking, n (%)					0.3
Never smokers	6 (66.7)	5 (55.5)	15 (40.5)	3 (17.6)	
Former smokers	3 (33.3)	3 (33.3)	18 (48.6)	11 (64.7)	
Smokers	0	1 (11.1)	4 (10.8)	3 (17.6)	
MN frequency (%)	14.3 \pm 4.5	12.0 \pm 3.1	14.3 \pm 6.8	18.9 \pm 9.0	0.1

CA, coronary angiography; PCI, percutaneous coronary intervention; PTA, peripheral transluminal angioplasty; CRT, cardiac resynchronization therapy.

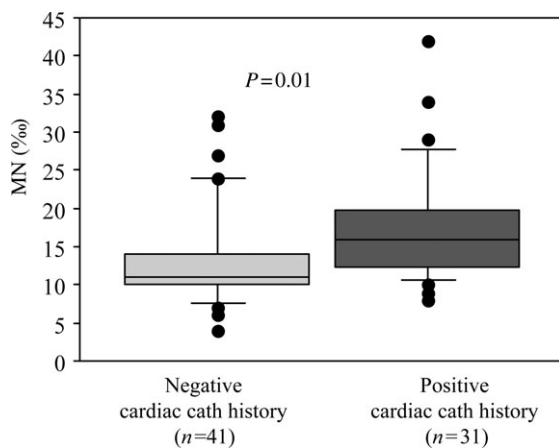


Figure 2 Micronucleus (MN) levels in patients with a previous history of ionizing radiation due to invasive cardiovascular procedures. The results are expressed as boxes with five horizontal lines, displaying the 10th, 25th, 50th (median), 75th, and 90th percentiles of the MN. All values above the 90th and below the 10th are plotted separately as circles.

different cardiac catheterization procedures are given in Table 2. PCIs presented the highest values of DAP. MN frequency was $15.1 \pm 7.1\%$ at baseline and showed a significant rise at 2 h ($17.5 \pm 6.5\%$, $P = 0.03$) and 24 h ($18.5 \pm 7.3\%$, $P = 0.004$) after procedures. Separate statistical analysis also showed that there was a significant difference after PCI, PTA, and CRT (Figure 3).

Discussion

In patients undergoing invasive cardiovascular procedures involving radiation, a significant somatic DNA damage develops following the procedure mirrored by an acute increase in MN of circulating lymphocytes, which are cellular biomarkers of chromosome damage and early predictors of increased cancer risk.^{10,11} For the purpose of radiation protection, the dose-response curve for radiation-induced cancer is assumed to have no minimum threshold. The cytogenetic data of our study are also broadly consistent with the 'no-threshold' model endorsed recently by the most updated statements of Biological Effects of Ionizing Radiation (BEIR) (2005) and International Commission on Radiological Protection (ICRP) (2007),^{5,7} suggesting that any

ionizing radiation dose, no matter how small, can result in detrimental biological effects.

Comparison with previous studies

The European DIMOND approach to defining dose reference levels for CA and PCI has proposed 45 and 75 Gy cm² for DAP, and 7.5 and 17 min for fluoroscopy time.¹⁸ Our recorded values of DAP and FT for these catheterization procedures compare with the reference levels recommended by DIMOND, with somewhat higher values likely linked to two reasons. First, diagnostic coronary angiography was often integrated with left ventriculography and aortography as needed. Secondly, PCI procedures were often complex and multiples.¹⁹ The estimated effective doses received by patients were 12 and 22 mSv for CA and PCI, respectively. According to data provided by the ICRP,⁵ patient risk of developing fatal cancer is 0.04% for CA and 0.1% for PCI. The most recent update of the risk of low-dose radiation-induced carcinogenesis comes from the National Research Council Committee on the BEIR (VII) of the National Academy of Sciences. The BEIR VII states that a single adult population effective dose of 10 mSv results in a 1 in 1000 lifetime risk of developing radiation-induced solid cancer or leukaemia.⁷ This 'average' risk varies as a function of both gender and age.⁷ For women, the risks of developing cancer after exposure to radiation were 37.5% higher than men. According to these estimates, the risk of cancer for a 10 mSv exposure is 1 in 1000 for an adult male, and 1 in 700 for a woman and 1 in 2000 for an elderly patient. Children are especially vulnerable. The same dose confers an extra-risk of 1 in 250 to a male child (<1 year), and 1 in 125 in female child (<1 year).⁷

Furthermore, each diagnostic ionizing radiation procedures exposes patients to a certain amount of radiation and risk. Our data also showed that a previous history of ionizing radiation due to invasive cardiovascular procedures is associated with an increased chromosomal damage.

The reported increase in MN is in agreement with previous studies describing that acute medical X-ray exposure in the range of 5–15 mSv determines an increase in chromosome aberrations of circulating lymphocytes. This finding has been observed in children submitted to cardiac catheterization,²² days or decades²³ after radiation exposure, as well as in adults undergoing coronary angiography and angioplasty,¹⁵ or other radiology procedures associated with

Table 2 DAP, fluoroscopic time, and effective dose

Procedure	DAP (Gy cm ²)		FT (min)		Effective dose (mSv)
	Median	25th–75th range	Median	25th–75th range	
CA	68	48–80.2	5	2.4–6.2	12
PCI	124	96.5–155	19.5	13–19.5	22
PTA	87	55–162	25.1	20.5–35.3	21
CRT	45	21.5–55.8	42.5	25.6–60	8

CA, coronary angiography; PCI, percutaneous coronary intervention; PTA, peripheral transluminal angioplasty; CRT, cardiac resynchronization therapy; DAP: dose–area product; FT: fluoroscopic time; mSv: millisievert.

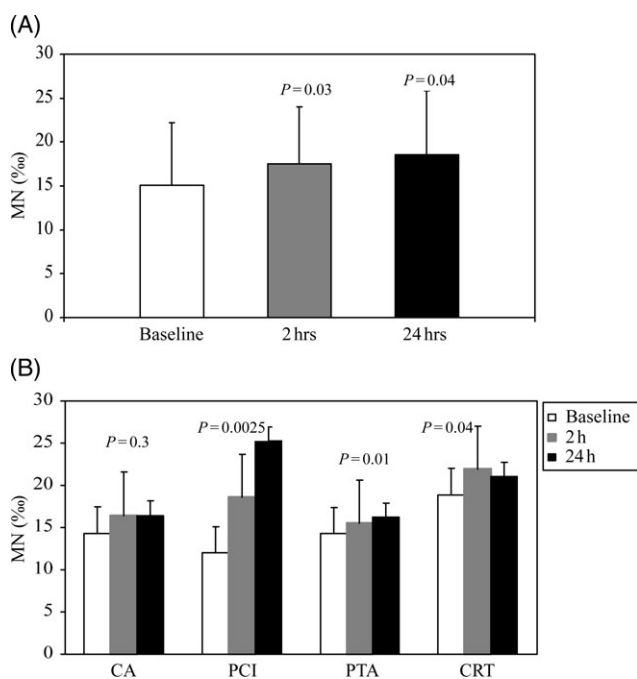


Figure 3 Frequency of MN at baseline, 2, and 24 h after cardiac catheterization procedures in the overall population (A) and in each group of cath-lab procedures (B).

contrast injection.²⁴ Our findings corroborate, from a cytogenetic perspective, the clinical concept that good radiography technique optimizing radiation exposure is all part of good patient care and management in the cardiac catheterization laboratory.^{2,4,21,25} As stated in most recent clinical competence statement on interventional cardiovascular procedures, it is the responsibility of all physicians to minimize radiation injury hazard to their patients, to their professional staff, and to themselves.²¹

Biomarkers to estimate cancer risk

The present data also support the usefulness of biomarkers that measure true cellular injury resulting from exposure of low-dose radiation. According to the latest ICRP recommendation,⁵ there is a general agreement that epidemiological methods used for estimation of cancer risk do not have the power to directly reveal cancer risks in the

dose range between a few millisieverts and a few tens of millisieverts'. Among key recommended research needs, the BEIR VII report also identified the importance of further research in the biomarker area.⁷ Such studies could have the greatest potential for providing an understanding of relationship between radiation exposure and cancer.^{5,7} There are two major cytogenetic endpoints applied for genotoxicity studies and biomonitoring purposes: chromosome aberrations and MN in peripheral lymphocytes. Peripheral lymphocytes are advantageous for exposure analysis because they circulate throughout the body and are continuously exchanged with lymphocytes in tissue. This means that lymphocytes with chromosome aberrations that have been induced anywhere in the body will eventually be present in the peripheral blood.^{9,12} The key advantages of the MN assay lie in its ability to detect both clastogenic and aneugenic events and to identify cells which divided once in culture.¹⁰ Chromosome alterations mirrored by increased MN frequency are early predictors of increased cancer risk.^{10,11} This is the reason why IAEA presently recommends biological dosimetry as a more appropriate marker of risk than physical dosimetry for exposed population.⁹ Cytogenetic studies can provide useful information for exploring the health effects of low-dose radiation better.

Study limitations

No direct radiation dose estimates with dosimeters placed on patients were available in the observed population. We relied on DAP, which is a quantitative measure of the radiography dose emitted and is considered a valid indicator of a patient's dose and consequent risk for radiation-induced effects.²¹ Furthermore, MN assay has also some limitations that needs to be acknowledged. The value of MN frequency as a long-term predictor of cancer was recently established,¹¹ but more confirmatory data from multicentre, prospective studies are certainly needed at this point.¹¹ We did not have a control group with serial testing for MN in the absence of radiation exposure. Our study was underpowered to assess any relationship between DAP and MN. Finally, we did not investigate the role of genetic polymorphism of genes involved in DNA damage and repair which are known to modulate the vulnerability to radiation exposure in the very low dose range.²⁶

Conclusion

This study clearly showed that a significant somatic DNA damage develops after invasive cardiovascular procedures involving radiation exposure, which falls in the dose range of commonly employed non-invasive cardiovascular imaging examinations.^{27–29} The direct evidence on DNA damage connected with the use of invasive ionizing cardiological procedures may stimulate the awareness of dose and risk, and the development of strategies for reducing the radiation burden, as encouraged by European Commission medical imaging guidelines.³⁰

Supplementary material is available at *European Heart Journal* online.

Conflict of interest: none declared.

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