Cardiac catheterization and long-term chromosomal damage in children with congenital heart disease

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Introduction

According to the best available evidences stemming from estimates of the International Commission of Radiological Protection, medical radiological exposure is thought to be associated with a higher risk of oncogenesis.1,2 Oncogenic risk is magnified in children when compared with adults.3–5 Children with repaired congenital heart disease (CHD) are theoretically at a relatively greater cancer risk as the radiological exposure can be intensive in these patients.6

The biological effect of the received radiation dose is normally expressed in milliSievert (mSv), with 1 mSv corresponding to the radiation dose of 50 chest X-rays. Recently, a median effective dose of 4.6 mSv has been reported for diagnostic cardiac catheterizations.7 Therapeutic procedures resulted in a higher median effective dose of 6.0 mSv because of the prolonged use of fluoroscopy and the larger number of cine runs.7 According to the data provided by the International Commission on Radiological Protection, this exposure dose corresponds to an additional risk of fatal cancer in 1 of 700 patients exposed at less than 1 year of age.3–5

In addition, most of the cumulative radiological exposure takes place in the early periods of life, when the genotoxic effect of radiations on rapidly proliferating tissues is higher.3–5 However, only a few follow-up studies of children subjected to cardiac catheterization have been performed, yielding inconsistent results.8–10

Cytogenetic biomarkers in peripheral lymphocytes are currently employed in order to study the human exposure to environmental carcinogens. In particular, chromosomal aberrations (CA) and micronuclei (MN) in peripheral blood lymphocytes are validated biomarkers of somatic chromosomal damage and intermediate endpoints in carcinogenesis.11,12 In fact, recent findings from European cohort studies have shown a significant increase for cancer in subjects with high frequency of CA.11–13 Both CA and MN are now accepted as being the most reliable ‘biological dosimeters’ for estimating the exposure of ionizing radiation.14,15

Accordingly, we designed the present study in order to assess the frequency of CA and MN in patients with CHD and a positive history of diagnostic procedures employing ionizing radiation.

Methods

Patients

Between May 2003 and December 2004, we recruited a group of operated children with CHD who lived in the area and attended...
regular follow-up visits in one of the major and oldest Italian medical paediatric cardiac surgery centre in Massa, Italy. Patients were considered eligible for inclusion if they underwent cardiac ionizing procedures (with at least one cardiac catheterization) at less than 1 year of age, between 1965 and 1990.

Exclusion criteria included: (i) surgery at other institutions; (ii) inability to obtain consent from the child’s parents, and (iii) a history of cancer, therapeutic radiation, chemotherapy, or active viral infection. A total of 38 patients who met these criteria have been enrolled in the present study. Of these eligible patients, four were excluded because of the inability to obtain a sufficient blood sample for the chromosomal analysis, as well as two additional patients for the impossibility to reconstruct an accurate history for both the type and number of radiological procedure. The final cohort (Group I) comprised 32 exposed patients: 17 male and 15 female subjects with a mean age of 15.5 ± 8.3 years (range: 5 months–39 years).

An age- and gender-matched control group of 32 healthy subjects (17 males) with a mean age of 14.1 ± 12.3 years (range: newborn to 43 years) and with negative, or almost negative, radiological history (Group II) has been enrolled.

The control group consisted of peripheral blood samples from 19 healthy volunteers, recruited by local contacts within our health department community and by word of mouth. A method of matched samples, in the selection of a subject of similar age and gender for each examined patient, has been used in the selection of our control group.

Their overall health conditions were assessed by a questionnaire that included information on their date of birth, past health status, and medical treatments involving radiation exposure. Cord blood samples from 13 healthy full-term neonates were also obtained.

In order to exclude the hypothetical direct effect of CHD on CA, a group (Group III) of 10 newborns (7 males) with similar heart defects was also included in the study in the form of explorative data analysis. The specific CHD for Groups I and III are reported in Table 1. The Local Ethics Committee approved the study protocol. All the patients (via the parents) gave their informed consent before entering the study. In all patients, a detailed radiological history has been collected from the patient interview and the retrieval of hospital records in order to build a radiological risk score.

Demographic characteristics of the studied patients have been reported in Table 2. Venous blood samples were collected at the study entry and they were coded and read in a blinded fashion for cytogenetic analysis.

**Radiological risk score**

The radiological Risk Score was derived as follows. For each examination, the average mSv exposure has been considered according to the European Commission 2001 medical imaging guidelines: for instance, a chest X-ray (one projection) is 0.02 mSv; a pulmonary scintigraphy is 1 mSv; a cardiac catheterization is 5 mSv; a chest Computed Tomography (CT) is 8 mSv. This method is subject to approximation, as these values are average estimations. In addition, more recent procedures—such as electrophysiological study or electrophysiological ablation—are not listed in the European guidelines, and therefore, the data has been derived from the latest literature.16,17

**Lymphocyte preparation**

Peripheral blood was collected using heparin as an anticoagulant; two duplicate cultures from each sample have been set up by mixing 0.3 mL of whole blood with 4.7 mL of Ham’s F12 medium (ICN, Irvine, USA), supplemented with 10% fetal calf serum (ICN), 1.5% phytohaemagglutinin (Wellcome, UK) and antibiotics (penicillin 100 IU/mL and streptomycin 100 μg/mL, Sigma, St Louis, MO, USA). All cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 and 95% O2. For the CA analysis, the cultures were fixed after 48 h of incubation, following a terminal 2 h treatment with colcemid (final concentration 0.1 μg/mL; Sigma), in order to arrest the lymphocytes in metaphase, according to our standard protocols;18 fixed cells were dropped onto clean microscopic slides, air dried and stained by the Giemsa technique. The blood samples were blindly coded, and the slides were read by the same two investigators. For each sample, 50-100 metaphases were scored. Lesions were classified according to the International System of Cytogenesis Nomenclature (ISCN) for acquired chromosome aberrations.19

The analysis of CA included the appearance of chromatid and chromosome types aberrations. A chromatid or chromosome break was identified as a discontinuity of the chromatin region that was equal to or greater than the width of the chromatid/ chromosome.19 Acentrics are non-centromere-containing fragments that result from the generation of several aberration types (e.g., dicentrics).

Total CAs without gaps were expressed as the percentage of aberrant cells.

For MN assay, cytochalasin B (6 μg/mL) was added 44 h after culture initiation. Cells were successively harvested at 72 h and fixed according to the standard method used in our laboratory.20

A MN may derive from acentric fragments (clastogenic event) or from the whole chromosome (aneugenic event leading to chromosomal loss), not correctly incorporated in one of the two daughter cells at the end of the mitotic division. Therefore, the frequencies of binucleated cells with MN provide an indication of the chromosome damage accumulated in human lymphocytes. For each sample, 2000 binucleated cells were blindly scored under optical microscope (final magnification 400×) for MN analysis, following the criteria for MN acceptance.15 The micronucleated–binucleated cells frequency was evaluated as the number of micronucleated cells per 1000 cells. Two microscopists scored two slides, each from two different cultures, for a total of 50 metaphases and 1000 binucleated cells, respectively. This method was previously shown to be reproducible between expert observers. Variation on replicate counts by same scorer or between scorers was 15 and 16%, respectively.18,20

**Table 1** CHD in Groups I and III

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Group I</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular septal defect</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Transposition of great arteries</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Double outlet right ventricle</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Aortic coarctation</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary atresia</td>
<td>3</td>
<td>/</td>
</tr>
</tbody>
</table>

**Table 2** Demographic characteristics of the study patients population

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>32</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>Age, years</td>
<td>15.5 ± 8.3 (range: 5 months–39 years)</td>
<td>14.1 ± 12.3 (range: newborn to 43 years)</td>
<td>Newborn</td>
</tr>
<tr>
<td>Gender, males</td>
<td>17</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Radiological score, mSv</td>
<td>19.4 ± 8.9 (range: 5.2–38.9)</td>
<td>0.006 ± 0.022 (range: 0–0.120)</td>
<td>0</td>
</tr>
</tbody>
</table>
Statistical analysis

The sample size requirement was based on the primary aim of the study: to compare cytogenetic data between two groups of the same size (Groups I and II).

Assuming a baseline frequency of aberrant cells, 1.0%; SD, 0.5 and of 5.2‰ cells with MN; SD,5.0 in healthy subjects aged 0–19,21,22 sample size of 32 subjects in each group was required to detect a 70% increased frequency of CA with the study of 80% power, at a 5% significance level.

Qualitative and quantitative comparisons in demographic characteristics between Groups I and II were evaluated by χ²-analysis or the Student’s t-test, respectively. Statistical differences in cytogenetic data between the two groups were determined with the non-parametric Mann–Whitney U test. The Kruskal–Wallis test was also used to compare the differences in cytogenetic values among three different groups. The Spearman test was used to analyse the correlations. Data are reported as mean (±SD). A two-tailed P-value < 0.05 was chosen as the level of significance.

Results

Exposed patients of Group I underwent at least one cardiac catheterization with a mean value of 2.9 ± 1.4 cardiac catheterization (range 1–5) procedures per person. All the patients had ≥4 chest X-ray with a mean of 26.8 ± 15.9 chest X-ray per patient (range 4–53). Nine patients had at least one pulmonary scintigraphy and 10 patients had at least one chest CT. The mean radiological risk score was 19.4 ± 8.9 mSv (range 5.2–38.9). The mean CA frequencies were significantly higher in exposed patients (Group I) than in control subjects (Group II) (2.8 ± 1.9% vs. 0.7 ± 0.7%, P < 0.0001). Mean values of cells with MN were also significantly higher in Group I than in Group II (12.3 ± 5.1% vs. 6.0 ± 3.8%, P < 0.0001). The mean values of CA and MN in newborn patients (Group III) were 0.8 ± 0.8% and 4.0 ± 1.4%, respectively. No significant differences were observed between Groups II and III for CA (P = 0.70) and MN (P = 0.14) frequencies. The median values of CA and MN are presented as box plot format in Figure 1. Typical examples of cytogenetical alterations observed in the exposed patients are reported in Figures 2 and 3. Chromosome-type aberrations identified were dicentrics, breaks, and acentric fragments; chromatid-type aberrations identified in this study were only break. The results indicated a significantly higher chromatid breaks and acentric fragments in exposed patients than in control subjects (Table 3). Interestingly, eight dicentric chromosomes were found in the Group I and none in the Group II, but the mean frequency were not significantly different.

A positive correlation has been observed between total individual values of CA and MN (P = 0.003). A positive correlation was also observed between total individual radiological score and both CA (P < 0.0001) and MN (P < 0.0001) frequency in the whole population, but no significant relationship has been found between CA (P = 0.60) or MN (P = 0.18) and radiological score considering only Group I. To evaluate radiation-dose effect, patients were categorized into three groups according to the tertiles of radiological score (< 10 mSv; 11–16 mSv; > 16 mSv). However, no significant differences were observed for both CA (P = 0.60) and MN frequency (P = 0.14).

Discussion

Children with CHD subjected to diagnostic procedures employing ionizing radiation develop a significant increase in circulating lymphocytes of CA and MN, which are the biomarkers of genetic damage for carcinogenesis.
The analysis of CA and MN is the gold standard endpoint for radiation biological dosimetry. The majority of the aberrations in our study were of the chromosome-type, as expected after irradiation of the lymphocytes in G0 phase. Moreover, we recorded an increased level of dicentrics in the exposed patients, an excellent indicator of radiation exposure and have been widely used as a key marker in the radiation dosimetry. Our observation of an increased level of dicentrics at relatively long times after exposure allows us to suppose that this asymmetrical exchanges may arise from blood stem cells or long-life lymphocytes.

**Comparison with previous studies**

Low-dose ionizing radiation of medical use is one of the definite risk factors for cancer development—as acknowledged by official statements of International Commission of Radiological Protection, United States Nations Scientific Committee on the Effects of Atomic Radiation, International Agency for Research on Cancer, and National Toxicology Program. Children exposed to low-dose ionizing radiation are theoretically at a relatively greater cancer risk as they have more rapidly dividing cells than adults and have longer life expectancy. It is important to examine the lowest levels of dose at which a significantly elevated level of radiation-induced cancer has been observed in human populations. Relevant information from epidemiological studies is available from the studies of the risk of cancer in children following radiation exposure both in utero and after birth. Cancer risk estimates are now available from individuals exposed over 50 years ago to doses comparable to those currently involved in diagnostic–therapeutic procedures, such as helical CT or interventional cardiology. There is a small but statistically significant excess incidence of cancer at doses down to 50 mSv. As stated by Hall, 'no theories, no extrapolations, no models are involved' to accept the link between radiation exposure and cancer above this threshold. For cumulative doses below 50 mSv, direct epidemiological evidence supporting the increased risk of cancer is lacking, although current radiation standards and practices are based on the premise that any radiation dose, no matter how small, can result in detrimental health effects. In the specific model of CHD, only a few follow-up studies of children subjected to cardiac catheterization have been performed, yielding inconsistent results. A retrospective cohort study conducted by Spengler et al. and McLaughlin et al. in Canada, on the basis of 4891 children with CHD who underwent cardiac catheterization between 1946 and 1968, did not demonstrate a significant increase in leukaemia during an average follow-up period of 13 years. Modan et al. have evaluated 674 children following cardiac catheterization. The mean age at the study entry was 8.9 years, and the mean age at follow-up was 37 years. They observed a 2.3 excess cancer risk in children exposed to radiations, with an excess mainly due to the higher incidence of lymphoma and melanoma.

These studies are meritorious and important, but they do have inherent limits of being statistically underpowered. There were only seven cancer deaths and 13 cancer cases in the ‘negative’ study by McLaughlin et al. and 11 cancer cases in the ‘positive’ study by Modan et al. It has been estimated that it would require an epidemiological study of more than 5 million people in order to be able to directly quantify the risk of cancer from exposure to doses of radiation of 10 mSv or less i.e. the typical doses delivered by diagnostic X-rays.

To overcome the severe practical limitations of the epidemiological approach, in the present study, we decided to look for surrogate endpoints of radiation-induced carcinogenesis. The underlying assumption is that changes in the surrogate somewhat mirror the long-term clinical effect. The link between the surrogate and the true clinical event is obviously critical, and indeed, there is now solid evidence from epidemiological data that high number of chromosome aberrations predict long-term incidence of cancer. Interestingly, in a pioneering study conducted in 1978 by Adams et al., CAs were evaluated before and after cardiac catheterization in children and showed chromosome damage in all. The obtained findings supported the hypothesis of a persistence of damage in the surviving population of radiation-induced genetically aberrant cells (long-life lymphocytes and/or blood stem cells). The estimated average exposure of the observed population was around 20 mSv at age less than 1 year. Therefore, low-dose radiation exposure associated with cardiac catheterization in children is

**Table 3** Total number of cells with different types of CAs and MN

<table>
<thead>
<tr>
<th>Types of CAs</th>
<th>Groups</th>
<th>P-value</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromatid breaks</td>
<td>1.1 ± 1.1</td>
<td>0.4 ± 0.6</td>
<td>0.01</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Chromosome breaks</td>
<td>0.4 ± 0.7</td>
<td>0.06 ± 0.2</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acentric fragment</td>
<td>1.1 ± 1.2</td>
<td>0.2 ± 0.4</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicentric chromosome</td>
<td>0.4 ± 0.7</td>
<td>0</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aberrant cells</td>
<td>2.8 ± 1.9</td>
<td>0.7 ± 0.7</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>MN</td>
<td>12.3 ± 5.1</td>
<td>6.0 ± 3.8</td>
<td>&lt;0.0001</td>
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</table>
associated with sufficient somatic DNA damage to be detectable—acutely and in the long-term—as increased CAs in circulating lymphocytes that represent an intermediate endpoint of cancer.

**Study limitations**

No direct radiation dose estimates were available in the observed patients. The radiation history for all patients was derived from hospital records and was accurate for both the type and number of radiological procedure. Our estimation was based on an average estimation of dose exposure according to the available estimation of European Commission of imaging guidelines and International Commission of Radiological Protection. However, the exact dose exposure may vary according to the type of equipment, proper training of the operator, constant awareness, and several other variables related to patient, operator, and technology.

In addition, the absence of a dose–effect relationship might be explained by other factors, such as the elimination of aberrant cells and different lifespan of lymphocytes. Moreover, genetic susceptibility may account for the inter-individual differences to radiation sensitivity. In contrast, the biological variability is also intrinsic to the very definition of stochastic effect of low-dose radiation, where only some patients will receive a meaningful damage—and we cannot predict in advance the patient who will suffer from radiation.

**Clinical implications**

Adult, grown-up patients with surgically repaired CHD—who were exposed as children to significant ionizing radiation for cardiac catheterization—are a large and growing population, estimated to be around 1 million in the US in the year 2000, compared with an estimated 300 000 in 1980, and 1.4 million are anticipated in 2020. Numbers are likely similar in EU, although no hard figures are available.

Radiological procedures involve a small risk which must be balanced against the potential for a significant benefit. Unnecessary radiation dose levels must be eliminated. When possible, non-ionizing techniques should be the preferred choice whenever the provided diagnostic information is comparable. When a radiological examination has to be performed, great care should be given in both minimizing and optimizing radiation exposure—as this might allow to substantially reduce the radiation burden. For the present time, an increased radiological awareness among the physicians and the public is warranted in order to minimize the long-term oncogenic damage of radiations, which remains a fundamental and irreplaceable tool in contemporary medical practice. The long-term effects of paediatric exposure to ionizing radiation remain unknown, but possibly they might become apparent in the exposed individuals only after decades of follow-up. As a matter of fact, the radiation exposure in these diseased children is even increasing now, with the development of high radiation load techniques, such as therapeutic interventional cardiology and diagnostic CT. At present, these techniques are largely employed in the paediatric population, with limited attention paid to the possible long-term consequences. Our observations appear to be especially important to quantify the biological risk of ionizing medical testing during infancy, as strongly encouraged by the latest Biological Effects of Ionizing Radiation VII report that underlines "the need of studies of infants who are exposed to diagnostic radiation because catheters have been placed in their hearts, as well as infants who receive multiple X-rays to monitor pulmonary development. CT scans, often referred to as whole body scans, result in higher doses of radiation than typically experienced with conventional X-ray."

Our data suggest that even relatively low-dose diagnostic exposure may leave a long-lasting mark in the somatic DNA of exposed children.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

**Acknowledgement**

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**Conflict of interest** none declared.

**References**


