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# Chromosomal damage in peripheral blood lymphocytes from nurses occupationally exposed to chemicals

A Santovito, P Cervella and M Delpero

## Abstract

In the present study, we evaluated the induced genome damage in peripheral blood lymphocytes from a sample of nurses occupationally exposed to low doses of different chemicals. A comprehensive multi-biomarker approach using cytogenetic endpoints was employed for analyzing chromosomal aberrations (CAs) and sister chromatid exchange (SCE) assay. The study included 20 nurses and 20 control subjects matched in age, gender and smoking habits. Nurses were exposed to different chemicals, such as cytostatic drugs, anaesthetics, formaldehyde and other sterilizing gases. Significant differences were found between exposure group and control group in terms of SCEs frequency ( $p < 0.001$ ) but not in terms of replication index value ( $p = 0.845$ ) and CAs ( $p = 0.236$ ). Regression analyses indicated that the age and the exposure years did not influence the amount of the chromosomal damage among nurses. Vice versa, among controls, a positive correlation was found between the number of SCEs and age. In conclusion, our results suggest that a continuous long-term exposure to low doses of chemicals could result in increased levels of SCEs among nurses. This data emphasize the importance of biomonitoring nurses and other hospital workers handling drugs.

## Keywords

Nurses, SCE assay, chromosomal aberrations, occupational exposure

## Introduction

At the workplace, nurses are exposed to a wide spectrum of different drugs in sub-therapeutic concentrations, with unknown biological consequences. In general, occupational exposure to cytostatic drugs, anaesthetic and sterilizing gases with potential mutagenic and carcinogenic capacity is a major hazard for the health care personnel.

For example, ethylene oxide and formaldehyde, used for sterilization, are well-known human carcinogens and are related to an increase of both chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) among exposed subjects.<sup>1,2</sup> Moreover, some of antineoplastic drugs (including alkylating agents, antimetabolites, antibiotics and hormones) used for the treatment of various types of cancer and immunologic diseases have been classified to be carcinogenic to humans according to their mutagenic and clastogenic properties.<sup>3,4</sup>

It is known that formaldehyde and antineoplastic drugs induce reactive oxygen species (ROS) that can

cause DNA strand breaks, alteration in bases and chromosomal rearrangements. Moreover, ROS can affect cell function by acting directly not only on DNA but also on lipids and proteins, thereby destroying the cellular structure.<sup>5</sup> Antineoplastic drugs are a heterogeneous group of compounds (such as alkylating agents, metabolic antagonists, antibiotics, mitotic spindle inhibitors, hormones etc) able to inhibit tumour growth by disrupting cell division and actively killing growing cells. These compounds interact with DNA inhibiting the activities of topoisomerase I and II<sup>6,7</sup> and inducing double- and single-strand breaks, cross-links and alkylations.<sup>8</sup>

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**Table 1.** Demographic characteristics of the studied groups.

Groups	N	Age		Exposure years	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Nurses	20	37.350 $\pm$ 11.775	21–58	11.850 $\pm$ 7.184	1–28
Department 1	10	33.900 $\pm$ 11.775	21–50	10.300 $\pm$ 7.103	1–22
Department 2	10	40.800 $\pm$ 12.309	23–58	13.400 $\pm$ 7.291	1–28
Controls	20	39.650 $\pm$ 5.344	32–53	11.200 $\pm$ 3.205	7–20
Department 1	10	39.200 $\pm$ 6.512	32–53	11.300 $\pm$ 3.802	7–20
Department 2	10	40.100 $\pm$ 4.175	34–47	11.100 $\pm$ 2.685	7–15

SCEs occur as a consequence of interchanges between DNA replication products at apparently homologous chromosomal loci and these exchanges involve DNA breakage and reunion.<sup>9</sup> SCEs are induced by those agents forming covalent adducts to DNA or otherwise interfere with DNA metabolism and repair. CAs reflect damage occurred during the  $G_1$  phase in regions that have not undergone repair or have evolved to a rearranged element. CAs are breaks, acentric fragments, rings, dicentric and inter-chromosomal exchanges, which are often unstable aberrations and will lead to cell death during proliferation.<sup>10</sup> Generally, SCE analysis represents a more sensitive test, particularly for  $S$  phase-dependent agents (e.g. alkylating agents), allowing to detect genotoxic effects at much lower concentrations than those required to induce CAs.<sup>11</sup>

Hospital workers might be exposed to drugs throughout their use in health care environments. This occupational exposure may occur in different ways, such as inhalation of airborne agents, absorption through skin contact, ingestion during drug preparation and/or contact with the patient's body fluids.<sup>12–14</sup>

To minimize the risk of occupational exposure, several guidelines and safety recommendations for the handling of different drugs were issued.<sup>15,16</sup> Nevertheless, despite the adoption of these guidelines in health care institutions, published reports suggest that some health care workers do not follow the standards established by their employers, putting themselves at risk for mutagenicity.<sup>17</sup>

In this scenario, the use of biomonitoring processes among personnel with potential worksite exposure is of primary interest in biological safety. Some cytogenetic studies have proven an increased number of CAs, SCEs and gene mutations among nurses and other hospital workers.<sup>6,18–21</sup> Nevertheless, other studies resulted to be ambiguous, probably because of different exposure conditions and because some

confounder factors, such as smoking habits and/or alcohol abuse, were not always properly taken into account.<sup>22,23</sup>

In the present study, we evaluated the incidence of CAs and SCEs in a sample of hospital nurses that used complete protective equipment and that have neither smoked nor consumed alcohol or drugs at least 2 years before analysis. The aim of the study was to evaluate the eventual genotoxic damage of nurses chronically exposed to low doses of different drugs. As expected, the results of this study might be potentially useful in the implementation of intervention measures aimed to minimize genotoxic risks and eliminate or significantly reduce worker exposure.

## Methods

### Study population

The study included 20 female nurses from 2 analogous departments of 2 different hospitals and 20 female control subjects belonging to the administrative staff and working at the same hospitals without any work-related exposure to hazardous agents. Demographic characteristics of the studied groups are reported in Table 1.

The nurses were exposed to different chemicals: mainly antibiotics and sporadically cytostatic drugs (cyclophosphamide, ifosfamide, 5-fluoro-uracil etc), anaesthetic and sterilizing gases such as ethylene oxide and formaldehyde. All nurses used complete protective equipment, according to the Italian guidelines and were routinely tested for urinary and blood drugs concentrations. In our sample, we exclusively considered individuals who have not smoked nor consumed drugs and have not been subjected to diagnostic examinations for a period of at least 2 years prior to the analysis. All the subjects were healthy volunteers, received information about the study and were extensively interviewed by a specialized physician with a

detailed questionnaire in order to provide important information for the study. The procedures followed in this work were in agreement with the ethical standards of the local responsible committee on human experimentation and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

### Blood sample collection and cell cultures

Blood samples were obtained by venipuncture (5–10 ml) and collected into heparinized tubes for genotoxicity testing. All blood samples were coded, cooled (4°C) and processed within 2 h after collection.

Heparinized venous blood (0.3 ml) was cultured in 25 cm<sup>2</sup> flasks in 6 ml RPMI-1640 (Biological Industries, Israel) supplemented with 20% fetal calf serum, 2% of the mitogenic agent phytohemagglutinin-M (Difco) (0.2 ml), L-glutamine (2 mM) and antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin). The cultures were incubated for 48 h for CAs assay and 72 h for SCEs assay, at 37°C in an atmosphere of 5% carbon dioxide in air. To arrest cells in mitosis, colchicine (0.25 µg/ml; Sigma, St Louis, Missouri, USA) was added at a concentration of 0.06 µg/ml during the last 2 h of culture. Chromosome preparation was carried out following standard procedures. Cells were centrifuged at 1000 r/min, slowly resuspended in 10 ml of pre-warmed hypotonic solution (0.075 M potassium chloride, pre-warmed to 37°C), and incubated for 15 min in a 37°C water bath. The cells were centrifuged at 1000 r/min again and fixed in cold methanol:acetic acid (3:1) for 20 min at room temperature. The treatment with the fixative was repeated three times. Finally, the supernatant was discarded; and the pellet, dissolved in a minimal volume of fixative, was seeded on the slides.

### CAs assay

Air-dried slides were stained for 20 min with 5% Giemsa stain (pH 6.8) prepared in a Sörensen buffer. For each subject, a total of 200 well-spread metaphases were analysed for the following categories of CAs: chromatid breaks (B'), chromosome breaks (B''), dicentrics (Dic), acentric fragments (AF), rings (R) and tri- or tetra-radials (TR). Gaps (a-chromatid lesions) were not scored as CAs. Cells containing any type of CAs were scored as cells with aberrations (CAB).

### SCEs assay

To measure SCEs in second-division metaphases, bromodeoxyuridine (BrdU, 5 µg/ml) was added at 24 h. BrdU closely resembles thymidine and is efficiently incorporated into the elongating DNA strands during replication. After two cell cycles in BrdU medium, the two sister chromatids differ in the amount of BrdU present and the chromatid with more BrdU is lighter in appearance ('bleaching' effect).

For sister chromatid differentiation, the cells were stained with fluorescence dye Hoechst 33258 (Sigma, 10 µg/ml, 20 min, at room temperature in the dark) and subsequently irradiated with an 8-W ultraviolet lamp (254 nm) at a distance of about 20 cm for 30 min. Subsequently, the slides were incubated in 2×standard saline concentration for 1 h at 60°C and then stained with 5% Giemsa (Sigma) in the Sörensen buffer for 10 min. Microscopic analyses were performed at 1000×magnification on a light microscope (CX40, Olympus, Tokyo, Japan).

In order to determine the number of SCE/cell for each subject, we scored 50 well-spread second-division metaphases containing 46 chromosomes. A total of 100 cells from each donor were scored for the determination of the replication index (RI) and calculated according to the following formula:  $RI = (M_1 + 2M_2 + 3M_3)/N$ , where  $M_1$ ,  $M_2$  and  $M_3$  represent the number of cells undergoing first, second and third mitosis, and  $N$  is the total number of scored metaphases (NSM).

### Statistical analysis

Statistical analysis was assessed using the SYSTAT software statistical package programme (version 10.0, Chicago, Illinois, USA). A non-parametric Wilcoxon test was used to compare the mean frequencies of SCEs and CAs between nurses and controls. Multiple regression analysis was used to evaluate the influence of age and exposure years on SCEs and CAs frequencies of both groups. All  $p$  values were two-tailed; and the level of statistical significance was set at  $p < 0.05$  for all tests.

## Results

No significant differences were found between groups in terms of mean age ( $p = 0.501$ ) and exposure years ( $p = 0.825$ ; Table 1).

Results of the SCE analysis are summarized in Table 2. Significant differences were found between

**Table 2.** SCEs frequency and RI values in metaphases of lymphocytes from nurses and controls.<sup>a</sup>

Groups	N	NSM	SCEs	SCEs/NSM $\pm$ SE	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	RI $\pm$ SE
Nurses	20	1000	6545	6.545 $\pm$ 0.325 <sup>b</sup>	686	735	579	1.946 $\pm$ 0.055
Department 1	10	500	3338	6.676 $\pm$ 0.508	335	372	293	1.958 $\pm$ 0.082
Department 2	10	500	3207	6.414 $\pm$ 0.431	351	363	286	1.935 $\pm$ 0.077
Controls	20	1000	4101	4.101 $\pm$ 0.371 <sup>b</sup>	651	829	505	1.945 $\pm$ 0.044
Department 1	10	500	1991	3.982 $\pm$ 0.566	349	420	218	1.965 $\pm$ 0.062
Department 2	10	500	2110	4.220 $\pm$ 0.507	302	409	287	1.925 $\pm$ 0.066

N: number of individuals sampled; NSM: number of scored metaphases; SCEs: sister chromatid exchanges; SE: standard error; RI: replication index

<sup>a</sup>RI = (M<sub>1</sub> + 2M<sub>2</sub> + 3M<sub>3</sub>)/N, where M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> represent the number of cells undergoing first second and third mitosis and N is the total number of metaphase scored.

<sup>b</sup>p < 0.001.

**Table 3.** CAs frequency in lymphocytes from nurses and controls.

Groups	N	NSM	Chromosome Aberrations								CAs/NSM (mean $\pm$ SE)	CAB/NSM (mean $\pm$ SE)
			B'	B''	Dic	AF	R	TR	Total CAs	Total CABs		
Nurses	20	4000	43	31	2	19	2	4	101	99	0.0252 $\pm$ 0.0030	0.0247 $\pm$ 0.0030
Department 1	10	2000	24	15	1	10	0	2	52	50	0.0260 $\pm$ 0.0050	0.0250 $\pm$ 0.0045
Department 2	10	2000	19	16	1	9	2	2	49	49	0.0245 $\pm$ 0.0040	0.0245 $\pm$ 0.0040
Controls	20	4000	47	9	5	18	1	0	80	78	0.0200 $\pm$ 0.0030	0.0195 $\pm$ 0.0030
Department 1	10	2000	23	5	2	9	0	0	39	39	0.0195 $\pm$ 0.0050	0.0195 $\pm$ 0.0050
Department 2	10	2000	24	4	3	9	1	0	41	39	0.0205 $\pm$ 0.0050	0.0195 $\pm$ 0.0050

N: number of individuals sampled; NSM: number of scored metaphases; B': chromatid breaks; B'': chromosome breaks; Dic: dicentric chromosome; AF: acentric fragments; R: ring; TR: tri- or tetra-radials; CAs: chromosomal aberrations; CAB: cells with aberrations; SE: standard error.

exposed and controls in terms of SCEs/NSM frequency ( $p < 0.001$ ) but not in terms of RI value ( $p = 0.845$ ). In either group, no statistical significant differences were found between departments in terms of SCEs/NSM ( $p = 0.721$  among nurses and  $p = 0.508$  among controls) and RI ( $p = 0.799$  among nurses and  $p = 0.646$  among controls). Among nurses, regression analyses indicated that the age and the exposure years did not influence the amount of SCEs ( $p = 0.609$  and  $p = 0.831$ , respectively). Vice versa, in the control group, a positive correlation was found between SCEs/NSM and age ( $p = 0.002$ ; Table 4).

Results of the CA analysis are summarized in Table 3. No statistically significant differences were found between exposed and control subjects in terms of CAs/NSM ( $p = 0.236$ ) and CAB/NSM ( $p = 0.266$ ). Similar to SCEs results, in either group, no statistical significant differences were found between departments in terms of CAs/NSM ( $p = 0.725$  among nurses and  $p = 0.858$  among controls) and CAB/NSM ( $p = 0.779$  among nurses and  $p = 0.858$  among controls).

Regression analyses indicated that the age and the exposure years did not influence the level of the CAs among both exposed ( $p = 0.829$  and  $p = 0.821$ , respectively) and control ( $p = 0.708$  and  $p = 0.572$ , respectively) groups (Table 4). Finally, no significant differences were found between the two departments in terms of SCEs/NSM and CAs/NSM among both nurses and controls (Tables 2 and 3).

## Discussion

Several published studies were focused on the occupational risks of nurses and other hospital workers due to the handling of several types of drugs. Despite the improvement of safety protection measures, the contamination via inhalation of drug aerosols and/or accidents during the preparation of potentially genotoxic/mutagenic drugs cannot be completely excluded among nurses.<sup>24–26</sup> Indeed, transient increases of SCEs and micronuclei (MNs) in cases of accidental contamination,<sup>23</sup> as well as significantly increased

**Table 4.** Multiple regression analysis of confounding factors on SCEs and CAs frequencies in peripheral lymphocytes of the study groups.

CF	SCEs frequency			CAs frequency		
	$\beta$ -co	p-value	95% CI Lower–Upper	$\beta$ -co	p-Value	95% CI lower–upper limit
<b>Nurses</b>						
Age	1.680	0.609	–5.140 to 8.499	0.029	0.829	–0.251 to 0.308
EY	1.117	0.831	–9.816 to 12.050	–0.112	0.821	–0.497 to 0.399
Department	28.153	0.430	–45.512 to 101.817	0.349	0.810	–2.671 to 3.368
<b>Controls</b>						
Age	12.261	0.002	5.054 to 19.467	–0.068	0.708	–0.443 to 0.308
EY	–4.117	0.477	–16.093 to 7.859	0.170	0.572	–0.454 to 0.794
Department	–0.042	0.999	–60.955 to 60.871	–0.295	0.846	–3.468 to 2.879

CF: confounding factor;  $\beta$ -co:  $\beta$ -coefficient; EY: exposure years; CAs: chromosomal aberrations.

rates of SCEs, CAs and MNs in occupationally exposed nurses, were observed.<sup>24,27,28</sup>

Although previous published studies have demonstrated a significant increase of CAs among hospital workers occupationally exposed to drugs,<sup>24,27,29,30</sup> in the present study, no chromosomal damage, in terms of increase of the CAs and CAB frequencies, was observed among our nurses sample.

Vice versa, we found the frequency of SCEs has significantly increased. This finding confirms the results of previous investigations<sup>31,32</sup> about occupationally exposed nurses, whereas for other hospital workers, such as pharmacy personnel, this pattern was not observed.<sup>23</sup>

Increased frequency of CAs is recognized as a potential predictor of cancer,<sup>33,34</sup> whereas no clear association has been observed between high SCE frequencies and cancer risk.<sup>35,36</sup> Increased levels of DNA damage are not necessarily associated with the onset of cancer since the damage actually measured is a consequence of the equilibrium between damage infliction and repair. In this scenario, the higher SCEs rate recorded among occupationally exposed subjects could be considered as a signal suggesting potential defects in DNA repair processes.<sup>10</sup> Defects in cellular DNA repair have been linked to genome instability, heritable cancers, premature ageing syndromes and neurological diseases.<sup>37</sup> Moreover, accumulation of DNA lesions in repair-defective individuals may cause cell death, either by progressively depriving the cell of vital transcripts or through apoptosis.<sup>38</sup>

Results obtained in this study indicate that age appears to influence the SCEs rate but not the CAs frequency among control subjects. This finding is in agreement with previously reported data on the age-

related incidence of chromosomal damage among control populations. Indeed, while some authors did not find an increase of CAs with age,<sup>39,40</sup> others reported a significant correlation between age and SCEs frequency in peripheral blood lymphocytes.<sup>41–43</sup> The lack of a similar pattern among the professionally exposed nurses could be due to a greater incidence of chromosomal damage among younger individuals.

Finally, we did not find a correlation between chromosomal damage and duration of exposure, which may reflect the fact that, during chronic exposure, part of the chromosomal damage is not detectable in vivo because of the death of lymphocytes.

## Conclusion

Our results suggest that a continuous long-term exposure to low doses of chemicals could result in increased levels of SCEs among nurses. This data emphasize the importance of biomonitoring of nurses and other hospital workers handling drugs.

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## Conflict of interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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