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**RESEARCH ARTICLE**

# Evaluation of radiation-induced chromosome instability in subjects with a family history of gastric cancer

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**Abstract**

Gastric cancer (GC) shows a familiar predisposition which is largely unexplained. In this study the hypothesis that radiation sensitivity is implicated in the familiar predisposition to GC was investigated by means of the cytokinesis-block micronucleus assay. Data indicate that a family history of GC is not associated with any of the biomarkers investigated and does not interact with the demographic variables considered. When study subjects were dichotomized around the median age, a significant prevalence of micronuclei was observed in older subjects. Age and both spontaneous and radiation-induced micronuclei were linearly correlated. The effect of age was not modified by gender or smoking habits.

**Keywords:** *Gastric cancer, first-degree relatives, micronuclei, ionizing radiation, aging*

## Introduction

Since the introduction of the cytochalasin-B protocol (Fenech & Morley 1986), the cytokinesis-block micronucleus (CBMN) assay has gained increasing popularity in investigations on genomic stability in human populations (Fenech et al. 1999). The scoring of micronuclei in binucleated cells allows a fast and reliable evaluation of both chromosome loss and breakage, which may be distinguished through the immunochemical labelling of kinetochores or the hybridization of micronuclei with centromeric probes (Norppa et al. 1993). The CBMN assay can also provide a comprehensive picture on chromosomal stability and cellular viability when integrated with the analysis of nucleoplasmatic bridges (NPBs), buds and biomarkers of cytotoxicity in a 'cytome assay' (Fenech 2006).

The CBMN assay has been widely applied in studies on the impact of dietary, occupational, environmental, lifestyle and genetic factors on chromosomal stability and mitotic function (Bonassi et al. 2005, Fenech et al. 2005, Iarmarcovai et al. 2008, Kirsch-Volders et al. 2006). A recent prospective analysis of data from an international database showed that an increased incidence of micronucleated blood cells may have a prognostic value for later cancer onset (Bonassi et al. 2007), supporting the use of this endpoint in human biomonitoring studies.

In addition to spontaneous micronucleus frequency, the number of micronuclei induced in blood cells by a chemical or physical challenge *ex vivo* is also considered to be a promising biomarker. In particular, the induction of micronuclei in peripheral lymphocytes following a challenge with ionizing radiation has been proposed as a biomarker of genetic susceptibility to cancer (El-Zein et al. 2006). In fact the response of irradiated lymphocytes in the CBMN assay, which reflects the number of non-repaired DNA breaks at the time of cell division (Bishay et al. 2001), has a high genetic component (Tedeschi et al.

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2004), and molecular epidemiology studies show that an augmented sensitivity to radiation – or to the radiomimetic agent bleomycin C – is a common trait of several cancer types (Berwick & Vineis 2000, Scott 2004).

An increased induction of micronuclei following irradiation was also observed in lymphocytes of healthy members of breast cancer families (Rothfub et al. 2000), highlighting an association between radiation sensitivity and familiar predisposition to the neoplasia (Stratton & Rahman 2008). It is conceivable that an altered response to radiation in the CBMN assay is also associated with the familiar predisposition displayed by other cancers. In this respect, gastric cancer (GC), the fourth most common neoplasia, has a well-recognized familiar predisposition (La Vecchia et al. 1992). A 2–3-fold increased risk has been observed in first-degree relatives of GC patients (Palli et al. 1994, Lissowska et al. 1999), and similar findings were provided by a Scandinavian Twin Study (Lichtenstein et al. 2000). The observed familiar predisposition for GC is only partially explained by the intrafamiliar clustering of *Helicobacter pylori* infection (Rocco et al. 2003), pointing to the possible involvement of hereditary susceptibility factors. Among these, the genetic polymorphisms in the inflammatory cytokines have received major consideration (Perez-Perez et al. 2005). However, it is also conceivable that genetic factors implicated in the processing of DNA damage, or controlling chromosome stability, might affect the individual susceptibility to GC. In fact, the loss of genomic stability is an early event in GC development, which leads to the progressive accumulation of genetic and epigenetic alterations (Ottini et al. 2006). Even though both microsatellite instability (MSI) and chromosome instability (CIN) are involved in the pathogenesis of GC, CIN is most commonly observed with about 60% of tumours characterized by changes in copy number and rearrangements in chromosome structure (Choi et al. 1998). As point mutations or loss of heterozygosity can lead to the acquisition of the CIN phenotype (Tamura 2006), it is expected that subjects less proficient in the processing of DNA damage or showing a decreased fidelity in chromosome segregation will be prone to genomic instability and thus to the development of neoplasia. This trait should reasonably affect the sensitivity to radiation of reporter cells, which can be assessed by evaluating the response in the CBMN test of blood cells irradiated *ex vivo* (Bishay et al. 2001).

Based on this premise, the implication of radiation sensitivity in the familiar predisposition to GC has been investigated in the present study. To this aim peripheral blood lymphocytes of 41 first-degree relatives of GC patients and 41 non-consanguineous family members were irradiated with γ-rays and analysed in a comprehensive CBMN assay.

## Materials and methods

### *Study population*

Subjects with a positive family history of GC (hereafter defined 'cases') were enrolled in three geographical areas of Tuscany (Florence, 14 subjects; Borgo San Lorenzo, 12 subjects; Casentino, 15 subjects), a high-risk region of central Italy (Buiatti et al. 1989), in the frame of other epidemiological studies carried out in the same areas. The inclusion criterion for the 41 cases was to have at least one first-degree relative (parents, siblings or children) affected by stomach cancer. The occurrence of GC in the first-degree relatives was confirmed by different sources (including the local Cancer Registry and Pathology Departments). Control subjects, matched to cases by age (± 2 years), area of residence and, whenever possible, by gender and smoking habits, were identified among non-consanguineous family members. Two standardized dietary and lifestyle questionnaires were filled in by each participant and provided detailed information on nutrient intake, smoking history, and alcohol and food consumption. The main demographic characteristics of the study population are summarized in Table 1. All subjects gave informed written consent for the participation in the study. The analyses were carried out on anonymous, coded samples.

### *Irradiation and processing of cell cultures*

Blood specimens were collected by venipuncture in heparinized tubes (BD, San Jose, CA, USA) from all study subjects. Within the next 24 h, whole blood samples were irradiated with 2 Gy γ-rays from a $^{137}$Cs source at a dose rate of 1 Gy min$^{-1}$. Lymphocyte cultures were established in RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 20% fetal calf serum (Hyclone, Logan, UT, USA), 2% phytohaemagglutinin (PHA, Remel Inc., Lenexa, KS, USA), 1% penicillin, streptomycin and incubated in a 5% $CO_2$ atmosphere at 37°C. Forty-four hours after PHA

**Table 1.** Distribution of study subjects according to age, gender and smoking habits.

| | Family history of gastric cancer | | |
|---|---|---|---|
| | Yes ($n=41$) | No ($n=41$) | All ($n=82$) |
| Age (years), mean ± SD | 58.0 ± 11.3 | 58.1 ± 11.7 | 58.06 ± 11.4 |
| Age groups, *n* | | | |
| ≤ 55 years[a] | 14 | 11 | 25 |
| > 55 years | 27 | 30 | 57 |
| Gender, *n* | | | |
| Male | 18 | 23 | 41 |
| Female | 23 | 18 | 41 |
| Smoking habits, *n* | | | |
| Current | 9 | 10 | 19 |
| Ex/never smokers | 32 | 31 | 63 |

[a]33rd percentile.

stimulation, cytokinesis was blocked with 6 μg ml$^{-1}$ of cytochalasin B (Sigma, Steinheim, Germany), and 22 h later lymphocytes were harvested and fixed as previously described (Carere et al. 1995). Cells were analysed in the comprehensive micronucleus test following the criteria set up by Fenech (2000). The frequencies of binucleated cells with micronuclei (MnBin), buds and NPBs were determined analysing 1000 binucleated lymphocytes with a well-preserved cytoplasm. Two scorers contributed with 500 cells each from coded slides; the correlation coefficient ($r$ = 0.960) of the two subsets of data was statistically significant ($p$ < 0.001). The Nuclear Division Index (NDI), a cell proliferation index, was determined in 1000 cells as follows: NDI = [mononucleated cells + (2 × binucleated cells) + (3 × trinucleated cells) + (4 × tetranucleated cells)]/1000 (Eastmond & Tucker 1989).

### *Determination of centromere content in micronuclei*

To identify micronuclei containing whole chromosomes, fluorescent *in situ* hybridization (FISH) was performed on binucleated cells with human pancentromeric probes (Appligene Oncor, Gaithersburg, MD, USA). Before hybridization slides were pretreated with 50 μg ml$^{-1}$ pepsin (Sigma), fixed in 1% formaldehyde, dehydrated with ethanol and denatured in 70% deionized formamide at 70°C for 2 min. The digoxigenin-conjugated alpha-satellite cocktail probe was applied and the slides were incubated overnight at 37°C in a humidified chamber. Detection of the labelled probe was performed using an anti-digoxigenin rhodamine-conjugated antibody (Roche Diagnostics, Mannheim, Germany). DNA was counterstained with 1 μg ml$^{-1}$ 4,6-diamidino-2-phenylindole (DAPI) in antifade solution (Vector Laboratories, Burlingame, CA, USA). For a reliable estimation of the relative proportion of centromere positive and negative micronuclei, at least 100 micronuclei were scored for each individual. This pilot analysis was performed on 10 subjects selected on the basis of age and frequency of radiation-induced MnBin (i.e. old individuals with a high frequency of MnBin and young individuals with a low frequency of MnBin) to amplify any difference. Selection criteria were also homogeneous smoking habits (no-smokers) and, whenever possible, gender.

### *Statistical analysis*

Arithmetic mean and standard deviation (SD) were used as descriptive measures of central tendency and variability. Data from different study groups were compared by the non-parametric two-tailed *U*-test; the *t*-test for matched samples was used to compare ln-transformed micronucleus data in cases with a positive family history of GC and a paired control. Odd ratios (OR) and their 95% confidence interval (CI) relating the prevalence of each variable (age, gender, smoking habits and family history of GC) with the biomarker score (dichotomized by the median value) were evaluated by unconditional logistic regression analysis. The frequency of radiation-induced MnBin was calculated by subtracting baseline incidence from observed MnBin after 2 Gy γ-rays, while the fold increase above baseline represented the ratio between MnBin observed after irradiation and spontaneous MnBin frequency in the same study subject. The interaction of family history of GC with the other study variables was evaluated by the Wald test of interaction coefficient in a multivariate random effect model to take into account the matching design. The same test was used to evaluate the interaction of gender and age. The influence of independent variables (age, gender, smoking habits and NDI) on radiation-induced micronuclei was estimated by multiple regression analysis. The correlation between the cytogenetic biomarkers investigated was evaluated by Pearson correlation analysis.

The Stata (StataCorp., 2005, Stata Statistical Software) and SPSS/PC statistical software packages were used for the analyses.

## Results

Data on the analyses of baseline and radiation-induced micronuclei, NPBs and buds in cytokinesis-blocked

**Table 2.** Results of cytokinesis-blocked micronucleus assays in study subjects stratified according to selected variables. Spontaneous values of biomarkers of DNA damage observed in cultured lymphocytes.

| | Biomarker (mean ± SD) | | | |
|---|---|---|---|---|
| Variable | MnBin (‰) | NPB (‰) | Buds (‰) | NDI |
| Family history of GC | | | | |
| No ($n$ = 41) | 16.2 ± 8.5 | 0.15 ± 0.4 | 1.15 ± 1.6 | 1.59 ± 0.14 |
| Yes ($n$ = 41) | 17.9 ± 10.1 | 0.19 ± 0.5 | 1.12 ± 1.6 | 1.60 ± 0.16 |
| Age group | | | | |
| ≤ 55 years ($n$ = 25) | 12.3 ± 7.1 | 0.18 ± 0.4 | 0.59 ± 0.8 | 1.68 ± 0.13 |
| > 55 years ($n$ = 57) | 19.3 ± 9.5** | 0.17 ± 0.4 | 1.40 ± 1.8 | 1.56 ± 0.14** |
| Gender | | | | |
| Male (n = 41) | 14.2 ± 6.44 | 0.12 ± 0.4 | 1.32 ± 1.9 | 1.58 ± 0.16 |
| Female ($n$ = 41) | 19.8 ± 10.8* | 0.22 ± 0.4 | 0.95 ± 1.1 | 1.61 ± 0.14 |
| Smoking habits | | | | |
| Smokers ($n$ = 19) | 15.0 ± 5.4 | 0.20 ± 0.4 | 0.70 ± 0.73 | 1.60 ± 0.11 |
| Ex/never smokers ($n$ = 63) | 17.7 ± 10.3 | 0.16 ± 0.4 | 1.27 ± 1.8 | 1.60 ± 0.16 |
| All (n = 82) | 17.1 ± 9.3 | 0.17 ± 0.4 | 1.13 ± 1.6 | 1.60 ± 0.15 |

GC, gastric cancer; MnBin, binucleated cells with micronuclei; NPB, nucleoplasmic bridges; NDI, Nuclear Division Index; *$p$ < 0.01; **$p$ < 0.001 ($t$-test).

lymphocytes of the study population are summarized in Tables 2 and 3. Pair-wise comparisons of biomarker values in subjects with and without a family history of GC did not show any statistically significant difference between the two study groups. Also cell proliferation rates, as measured by the NDI, were fairly similar in cases and controls, both in un-irradiated cells and after irradiation with γ-rays. The relative risk of higher net or relative increase of MnBin in response to irradiation was evaluated by logistic regression analysis. When the incidences of induced MnBin, subtracted from baseline values, were dichotomized around the median value of 211 induced MnBin/1000 cells, no significant association was observed between biomarker score and family history of GC (OR 1.4; 95% CI 0.6–3.6). Similarly, no association was observed between a family history of GC and a relative increase in MnBin when data were dichotomized around the median value of 14.8-fold (OR 1.3; 95% CI 0.5–3.5). No statistically significant interaction was observed between a family history of GC and the other study variables in the modulation of radiation-induced micronuclei (Table 4). A borderline interaction between gender and familiar predisposition for GC was only suggested by the lower response to irradiation of male subjects with a positive family history for GC compared with the controls; however such interaction was not statistically significant ($p = 0.079$).

Tables 2 and 3 also show data aggregated on the basis of the main demographic characteristics of the study population. In consideration of the prevalence of aged individuals, subjects were dichotomized around the 33rd percentile (55 years) instead of the median age (63 years). The comparison of data in the two classes of age highlighted a significantly greater frequency



**Table 3.** Results of cytokinesis-blocked micronucleus assays in study subjects stratified according to selected variables. Values of biomarkers of DNA damage measured after $G_0$ irradiation with 2 Gy γ-rays.

| | Biomarker (mean ± SD) | | | |
|---|---|---|---|---|
| Variable | MnBin (‰) | NPB (‰) | Buds (‰) | NDI |
| Family history of GC | | | | |
| No ($n = 41$) | 240.0 ± 65.7 | 3.92 ± 3.7 | 3.02 ± 2.3 | 1.51 ± 0.13 |
| Yes ($n = 41$) | 220.8 ± 71.9 | 3.95 ± 4.3 | 2.80 ± 2.6 | 1.49 ± 0.16 |
| Age (years) | | | | |
| ≤ 55 ($n = 25$) | 192.8 ± 73.4 | 3.41 ± 3.8 | 2.18 ± 2.0 | 1.57 ± 0.14 |
| > 55 ($n = 57$) | 250.8 ± 54.1** | 4.20 ± 4.1 | 3.27 ± 2.6 | 1.46 ± 0.13* |
| Gender | | | | |
| Male (n = 41) | 223.6 ± 50.5 | 4.02 ± 4.1 | 2.66 ± 2.3 | 1.48 ± 0.14 |
| Female ($n = 41$) | 237.2 ± 83.9 | 3.85 ± 3.9 | 3.17 ± 2.6 | 1.51 ± 0.14 |
| Smoking habits | | | | |
| Smokers ($n = 19$) | 221.5 ± 62.7 | 4.75 ± 4.7 | 2.65 ± 2.1 | 1.50 ± 0.16 |
| Ex/never smokers ($n = 63$) | 235.0 ± 68.0 | 3.68 ± 3.8 | 3.00 ± 2.6 | 1.50 ± 0.14 |
| All ($n = 82$) | 231.7 ± 66.6 | 3.94 ± 3.9 | 2.91 ± 2.4 | 1.50 ± 0.14 |

GC, gastric cancer; MnBin, binucleated cells with micronuclei; NPB, nucleoplasmic bridges; NDI, Nuclear Division Index; *$p < 0.01$; **$p < 0.001$ ($t$-test).

**Table 4.** Test for interaction between family history of gastric cancer and selected individual characteristics (dependent variable: frequency of micronucleated cells observed after irradiation with 2Gy γ-rays).

| | Family history of gastric cancer | | | | |
|---|---|---|---|---|---|
| | No | | Yes | | |
| Variable | MnBin (‰) (mean ± S.D.) | Unmatched $t$-test $p$-Value | MnBin (‰) (mean ± S.D.) | Unmatched $t$-test $p$-Value | Interaction coefficient Wald test $p$-Value[a] |
| Age (years) | | | | | |
| ≤ 55 | 209.1 ± 80.0 | | 177.6 ± 66.0 | | |
| > 55 | 254.3 ± 53.7 | 0.001 | 247.5 ± 67.3 | < 0.001 | 0.889 |
| Gender | | | | | |
| Male | 219.9 ± 53.6 | | 228.4 ± 47.2 | | |
| Female | 265.6 ± 72.1 | 0.058 | 219.6 ± 80.4 | 0.400 | 0.079 |
| Smoking habits | | | | | |
| Current | 223.1 ± 71.9 | | 220.0 ± 55.8 | | |
| Ex/never | 245.4 ± 63.9 | 0.267 | 224.6 ± 71.4 | 0.776 | 0.628 |

[a]Linear mixed model. MnBin, binucleated cells with micronuclei.

of both spontaneous and radiation-induced MnBin among older subjects ($p < 0.0001$). No statistically significant differences were observed in buds and NPBs. The characterization of radiation-induced micronuclei in a subset of study subjects of different age highlighted the overwhelming prevalence of acentric fragments in all cases, independently of the age of the subjects (Table 5). A plot with values of baseline and radiation-induced MnBin by age of subjects is shown in Figure 1. Data show a linear association between the frequency of MnBin and age ($p \leq 0.001$), with an absolute increase of approximately 3.5 spontaneous and 21 radiation-induced MnBin every 10 years of age.

A significantly higher ($p = 0.003$) incidence of spontaneous MnBin was also observed in female compared with male subjects (Table 2), while no gender differences were observed in response to radiation (Table 3). Gender did not modify the effect of age on baseline and radiation-induced MnBin (Table 6). Finally, no significant differences were observed when comparing active smokers with never-smokers and ex-smokers.

Proliferation of lymphocytes, expressed by the NDI, proved to be significantly depressed in older subjects both in irradiated and un-irradiated cells. As the rate of cell replication after irradiation may affect micronucleus formation, data on induced MnBin were further analysed by multivariate analysis in order to disclose the direct effect of age from that resulting from lower proliferation. Multiple regression analysis of ln-transformed data on radiation-induced MnBin with age, NDI, gender and smoking habits as independent variables, indicated that age was the only variable included in a statistical model, explaining about 14% of the total variance (Table 7).

Finally, possible relationships among the study biomarkers were assessed by the Pearson correlation analysis (Table 8). The correlation coefficients showed significant associations between baseline and radiation-induced micronuclei or NPBs, as well as between radiation-induced micronuclei and NPBs. Also buds proved

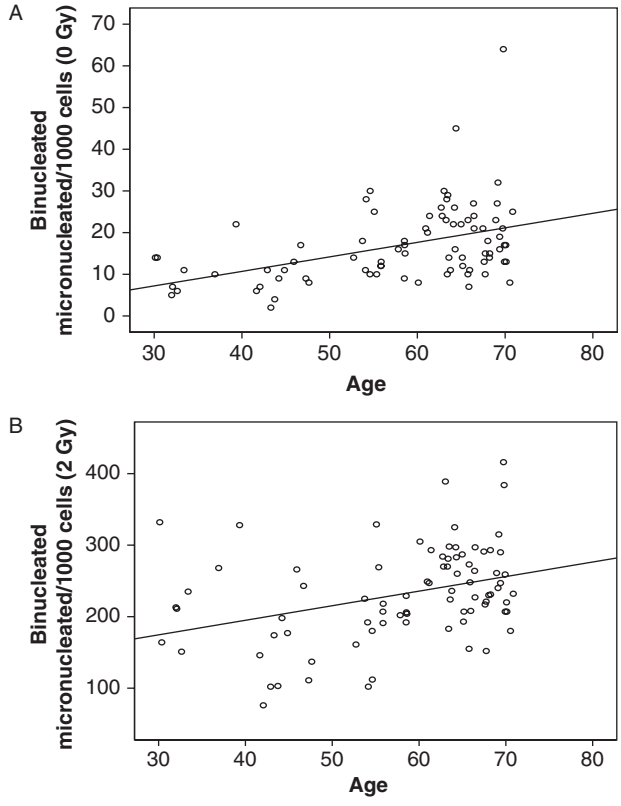


**Figure 1.** Plot of frequencies of binucleated lymphocytes with micronuclei in the study population by age of subjects. (A) Before irradiation and (B) after challenge with 2 Gy γ-rays.

**Table 5.** Characterization by fluorescence *in situ* hybridization of radiation-induced micronuclei in a subgroup of individuals selected by age.

| | | | 0 Gy | 2 Gy | 2 Gy[a] | |
|---|---|---|---|---|---|---|
| Subject | Age (years) | Gender | MnBin (‰) | MnBin (‰) | Cen−(%) | Cen+ (%) |
| 1 | 66 | F | 27 | 370 | 87 | 13 |
| 2 | 69 | F | 17 | 305 | 95 | 5 |
| 3 | 69 | F | 30 | 290 | 96 | 4 |
| 4 | 66 | F | 24 | 361 | 90 | 10 |
| 5 | 70 | F | 14 | 248 | 93 | 7 |
| 6 | 30 | F | 14 | 201 | 94 | 6 |
| 7 | 33 | M | 8 | 179 | 98 | 2 |
| 8 | 42 | F | 6 | 119 | 98 | 2 |
| 9 | 47 | F | 10 | 127 | 93 | 7 |
| 10 | 43 | M | 2 | 213 | 96 | 4 |

[a]Proportion of micronuclei displaying no centromeric signals (Cen−) or at least one centromeric signal (Cen+) in 100 analysed micronuclei per subject. MnBin, binucleated cells with micronuclei.

**Table 6.** Test for interaction between age and gender (dependent variable: frequency of micronucleated cells observed before and after γ-irradiation).

| | Gender | | | | |
|---|---|---|---|---|---|
| | Male | | Female | | Interaction coefficient |
| Treatment | MnBin (‰) (mean ± SD) | *p*-Value[a] | MnBin (‰) (mean ± SD) | *p*-Value[a] | Wald test *p*-Value |
| *0 Gy* | | | | | |
| Age (years) | | | | | |
| ≤ 55 | 7.9 ± 3.3 | | 14.5 ± 7.5 | | |
| > 55 | 16.0 ± 6.1 | < 0.0001 | 23.9 ± 11.5 | < 0.001 | 0.367 |
| *2 Gy* | | | | | |
| Age (years) | | | | | |
| ≤ 55 | 184.6 ± 61.3 | | 196.9 ± 80.1 | | |
| > 55 | 234.6 ± 41.8 | 0.001 | 273.3 ± 61.8 | < 0.001 | 0.514 |

[a]Linear mixed model. MnBin, binucleated cells with micronuclei.

**Table 7.** Association of radiation-induced micronuclei with study variables: multiple regression analysis.

| Variable | B[a] | SE[b] | *t* | *p*-Value |
|---|---|---|---|---|
| Constant[c] | 4.714 | 0.191 | 24.696 | < 0.001 |
| Age[d] | 0.012 | 0.003 | 3.587 | 0.001 |
| Excluded variables | | | | |
| Gender | - | - | 0.803 | 0.424 |
| Smoking habits | - | - | 0.237 | 0.813 |
| NDI[e] | - | - | 0.439 | 0.662 |

[a]Slope of the regression line; [b]standard error of the regression line; [c]estimated intercept value; [d]age of subjects in years (continuous variable); [e]nuclear division index of irradiated lymphocyte cultures. Significance of the model: $p < 0.001$, $F = 12.866$; total variance explained $R^2 = 0.139$. Dependent variable: ln MnBin (binucleated cells with micronuclei) after treatment with 2 Gy γ-rays.

**Table 8.** Pearson correlation analysis of cytogenetic biomarkers.

| | | MnBin 2 Gy | NPB 0 Gy | NPB 2 Gy | Bud 0 Gy | Bud 2 Gy |
|---|---|---|---|---|---|---|
| MnBin | *r* | 0.542 | 0.090 | 0.190 | 0.310 | 0.312 |
| 0 Gy | *p* | 0.000 | 0.427 | 0.087 | 0.005 | 0.004 |
| MnBin | *r* | | 0.163 | 0.413 | 0.136 | 0.473 |
| 2 Gy | *p* | | 0.146 | 0.000 | 0.222 | 0.000 |
| NPB | *r* | | | 0.331 | 0.282 | 0.035 |
| 0 Gy | *p* | | | 0.003 | 0.011 | 0.754 |
| NPB | *r* | | | | 0.167 | 0.442 |
| 2 Gy | *p* | | | | 0.133 | 0.000 |
| Bud | *r* | | | | | 0.182 |
| 0 Gy | *p* | | | | | 0.102 |

MnBin, binucleated cells with micronuclei; NPB, nucleoplasmic bridges; NDI, Nuclear Division Index; 0 Gy, not irradiated lymphocytes; 2 Gy, irradiated lymphocytes.

to be associated with other biomarkers in the Pearson correlation analysis.

## Discussion

Epidemiological studies indicate a 2–3-fold increased risk in first-degree relatives of patients with GC (Palli et al. 1994, Lissowska et al. 1999), pointing to the role of a genetic component in the individual susceptibility to the disease. In this study, the analysis of radiation sensitivity on peripheral lymphocytes from 41 first-degree relatives of patients with GC did not highlight significant differences in the response to radiation compared with matched non-consanguineous controls. Based on the group's size ($n = 41$), and the frequency of micronucleated cells in the control group, it was calculated that the study power allowed detection of a change in the incidence of MnBin greater than 0.8 SD with a probability ≥ 95% (β error ≤ 5%). A family history of GC did not interact to a significant extent with age, gender or smoking habits in determining individual response to radiation in the assay. Thus, although in principle subtle differences cannot be ruled out, the evidence provided herein is sufficient to conclude that healthy first-degree relatives of GC cases show no biologically significant increase in radiation-induced micronuclei compared with subjects with no affected relatives. This may indicate that the familiar predisposition for GC is not associated with chromosomal radiosensitivity, even though it should be acknowledged that other pathways leading to radiation sensitivity in $G_2$ cells may be not effective in the CBMN assay (Scott et al. 1999). Despite this reservation, the results obtained suggest that, although DNA damage is a distinctive trait of *H. pylori*-infected gastric mucosa (Ladeira et al. 2004), individual susceptibility to GC mainly relies on biological processes other than the processing of DNA damage. In this respect, individual variation in the inflammatory response to *H. pylori* infection, determined by genetic polymorphisms in the inflammatory cytokines, has been proposed to play a role in the modulation of GC risk especially in individuals harbouring high virulence genetic variants of *H. pylori* (Perez-Perez et al. 2005).

This study highlighted a positive association between age and radiation-induced micronuclei in peripheral blood lymphocytes irradiated *ex vivo*. Previous investigations with subjects in a lower range of age had provided contrasting indications suggesting a positive association in one study on 62 donors (age range 21–48 years) (Jocsić et al. 2004), and no effect in a more limited research on 32 subjects (age range 27–58 years) (Bishay et al. 2001). The results of the present study, which involved relatively older subjects (age range 30–71, median 63 years), provided compelling evidence on the influence of aging on radiation-induced micronuclei in human lymphocytes. It is well known that the higher spontaneous incidence of micronucleated lymphocytes in elderly individuals can mainly be attributed to increased chromosome loss rather than to chromosome breakage (Zijno et al. 1996); however, the mechanisms underlying the age-related increase in radiation-induced micronuclei have not yet been elucidated. To verify if chromosome loss was also responsible for the higher amount of radiation-induced micronuclei observed in elderly subjects, in the present study micronuclei were characterized for centromere content, highlighting the overwhelming prevalence of acentric fragments in all irradiated cultures independent of the age of the subjects. Thus, the age-related increase observed in the incidence of radiation-induced micronuclei resulted from a higher clastogenic effect of ionizing radiation in the lymphocytes of elderly donors. Aging negatively affects the rate of cell proliferation of mitogen-stimulated lymphocytes, as shown by the significantly lower NDI of older subjects. The statistical analysis of data on MnBin, however, indicated that

the increased formation of micronuclei in irradiated lymphocytes of aged donors was not secondary to the effect on NDI. Aging has also been related to a decline in the efficiency of rejoining of double-strand breaks in un-stimulated human lymphocytes (Mayer et al. 1989), which might result in increased micronucleus frequency. However, the consequences of such a decrease in DNA repair capacity cannot be anticipated, as the same study also indicated that DNA from older donors sustained fewer strand breaks than younger donors after irradiation. Beyond a decline in the efficiency of repair, changes in chromatin structure may also modulate the effect of aging on micronucleus induction. Interestingly, the age-related propensity of peripheral lymphocytes to micronucleus formation is lost after a few rounds of replication *in vitro* (A. Zijno, personal communication), pointing to the possible involvement of transient epigenetic modifications. Also alteration of the telomere structure may be involved in the observed age-related increase of MnBin in irradiated lymphocytes. In fact, telomeres shorten with age (Kruk et al. 1995, Blasco, 2007), and telomere length has recently been shown to modulate radiation sensitivity of peripheral blood lymphocytes *in vitro* (Castella et al. 2007).

Cell of older subjects also displayed a higher mean incidence of NPBs following irradiation. The difference between the two age groups did not attain statistical significance, possibly because of the low number of observations. However, in view of the strong association between induced MnBin and NPB ($p < 0.001$), it is most likely that aging similarly affected radiation-induced NBP. Interestingly, no correlation was observed between spontaneous MnBin and NPB ($p > 0.4$), nor any age-related trend in spontaneous NPB. This result may reflect the greater relative contribution of chromosome loss to spontaneous MnBin (Norppa et al. 1993), compared with radiation-induced MnBin (Sgura et al. 2001). In fact as NPB mainly visualize dicentric chromosomes, it is expected that NPB correlate more closely with radiation-induced MnBin which essentially result from clastogenic events.

Pearson correlation analysis highlighted a strong association between buds and MnBin, both in un-irradiated and irradiated cultures. Buds are generally interpreted as extrusion of amplified genetic material (Fenech 2006); however, according to a recent theory (Lindberg et al. 2007), buds may also be generated from anaphase laggards, anaphase bridges or any DNA left in the cytoplasm at mitosis and encapsulated in the nuclear membrane during the reconstitution of the nuclear envelope. According to the model these bodies may appear as buds or, if detached from the main nucleus, as micronuclei, thus resulting correlated.

Previous studies on irradiated human lymphocytes also pointed to possible gender differences in the induction and repair of double-strand breaks (Mayer et al. 1991). In the population investigated in this study, gender effectively modulated the spontaneous incidence of micronuclei, in agreement with the extensive data in the literature on spontaneous micronucleus incidence in humans (Bonassi et al. 2001), while no gender differences in radiation-induced micronuclei were observed. Similar results were obtained in a previous investigation on younger subjects (Jocsić et al. 2004), suggesting that the balance between lower induction and slow repair of strand breaks observed in irradiated lymphocytes of female donors (Mayer et al. 1991) does not result in increased formation of micronucleated cells. No significant effect of gender on the other study variables was observed.

The effect of smoking on spontaneous micronucleus incidence in the CBMN assay has been evaluated within an international collaborative project (Bonassi et al. 2003). The pooled re-analysis of databases performed in the HUMN project highlighted a small decrease of spontaneous MnBin in current smokers; the results presented here are basically in agreement with such findings. On the other hand, an association between smoking habits and more proficient DNA repair capacity was reported from studies on mutagen sensitivity of peripheral lymphocytes (Spitz et al. 2003). This result, which implies a stimulation of DNA repair capacity in response to DNA damage caused by tobacco carcinogens, is also supported by a previous study from this laboratory, in which a significantly lower incidence of chromosomal aberrations was observed in irradiated lymphocytes of heavy smokers compared with non-smokers (Marcon et al. 2003). A similar trend was observed in this work, even though data did not attain statistical significance. Due to the numerous possible confounders, it is conceivable that the effect of smoking on radiation sensitivity could only be established in properly designed studies, taking into account, *inter alia*, genetic susceptibility factors. In this respect robust indications are expected from a study on identical twins with discordant smoking habits which is ongoing in this laboratory.

In conclusion, the present results indicate that the familiar predisposition for GC is not associated with increased radiosensitivity in the CBMN assay. On the other hand, data suggest that aging plays an important role in modulating the sensitivity to radiation in both study groups, providing a clue with practical implications for the design of molecular epidemiology studies.

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