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Original Contribution

Chromosomal Aberrations and Cancer Risk: Results of a Cohort Study from Central Europe

Paolo Boffetta[1], Olga van der Hel[1], Hannu Norppa[2], Eleonora Fabianova[3], Aleksandra Fucic[4], Sarolta Gundy[5], Juozas Lazutka[6], Antonina Cebulska-Wasilewska[7,8], Daniela Puskailerova[3], Ariana Znaor[9], Zsolt Kelecsenyi[5], Juozas Kurtinaitis[10], Jadwiga Rachtan[11], Alessandra Forni[12], Roel Vermeulen[13], and Stefano Bonassi[14]

[1] International Agency for Research on Cancer, Lyon, France.
[2] Finnish Institute of Occupational Health, Helsinki, Finland.
[3] Regional Public Health Authority, Banska Bystrica, Slovakia.
[4] Institute for Medical Research and Occupational Health, Zagreb, Croatia.
[5] National Institute of Oncology, Budapest, Hungary.
[6] Vilnius University, Vilnius, Lithuania.
[7] Epidemiology Department, Jagellonian University Medical College, Kraków, Poland.
[8] Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland.
[9] Croatian National Cancer Registry, Zagreb, Croatia.
[10] Lithuanian Cancer Register, Vilnius University Institute of Oncology, Vilnius, Lithuania.
[11] Cancer Epidemiology Department, Institute of Oncology, Kraków Branch, Kraków, Poland.
[12] Department of Occupational and Environmental Health, University of Milan, Milan, Italy.
[13] National Cancer Institute, Bethesda, MD.
[14] Unit of Molecular Epidemiology, National Cancer Research Institute, Genoa, Italy.

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A high level of chromosomal aberrations in peripheral blood lymphocytes may be an early marker of cancer risk, but data on risk of specific cancers and types of chromosomal aberrations (chromosome type and chromatid type) are limited. A total of 6,430 healthy individuals from nine laboratories in Croatia, Hungary, Lithuania, Poland, and Slovakia, included in chromosomal aberration surveys performed during 1978–2002, were followed up for cancer incidence or mortality for an average of 8.5 years; 200 cancer cases were observed. Compared with that for the low-tertile level of chromosomal aberrations, the relative risks of cancer for the medium and high tertiles were 1.78 (95% confidence interval: 1.19, 2.67) and 1.81 (95% confidence interval: 1.20, 2.73), respectively. The relative risk for chromosome-type aberrations above versus below the median was 1.50 (95% confidence interval: 1.12, 2.01), while that for chromatid-type aberrations was 0.97 (95% confidence interval: 0.72, 1.31). The analyses of risk of specific cancers were limited by small numbers, but the association was stronger for stomach cancer. This study confirms the previously reported association between level of chromosomal aberrations and cancer risk and provides novel information on the type of aberrations more strongly predictive of cancer risk and on the types of cancer more strongly predicted by chromosomal aberrations.

chromosome aberrations; cohort studies; cytogenetics; Europe; neoplasms; risk

Chromosomal aberrations in peripheral blood lymphocytes have been used for decades for the surveillance of healthy individuals exposed to known or potential mutagens and carcinogens (1). In addition, chromosome alterations

Correspondence to Dr. Paolo Boffetta, International Agency for Research on Cancer, 150 cours Albert Thomas, 69008 Lyon, France (e-mail: boffetta@iarc.fr).

are typical features of neoplastic cells, and for certain cancers specific chromosome abnormalities are commonly present (2, 3). Although specific chromosome alterations detected in neoplasms are generated during carcinogenesis, it has been hypothesized that the frequency of chromosomal aberrations in peripheral blood lymphocytes of healthy individuals represents a marker of susceptibility to cancer, on the basis of the concept that genetic damage in peripheral blood lymphocytes reflects similar damage in different target cells undergoing carcinogenesis (1, 4).

Four epidemiologic studies, from Northern Europe, Italy, the Czech Republic, and Taiwan, have reported an association between high frequency of chromosomal aberrations and increased cancer risk (5–8). Although broadly consistent, the results of these studies show some heterogeneity, with the cohorts from Nordic countries and Italy providing evidence of a stronger association between elevated frequency of chromosomal aberrations and cancer risk than does the Czech cohort.

Furthermore, the available data do not allow a full assessment of the predictive value of different types of chromosomal aberrations and of the risk of specific neoplasms, which would provide useful information on the mechanisms behind the cancer predictivity of chromosomal aberrations. The type of chromosomal aberrations occurring in peripheral blood lymphocytes may differ, depending upon the genotoxic agent or mixture of agents acting on the cell cycle as either S-phase-dependent or S-phase-independent agents. Thus, ionizing radiation produces mostly chromosome-type aberrations, and many chemical mutagens produce chromatid-type aberrations. Combined results from the Nordic and the Italian cohorts indicate that both subclasses of chromosomal aberrations have similar predictive value (9), whereas the Taiwanese study on a small cohort of arsenic-exposed subjects suggests that only chromosome-type aberrations are associated with cancer risk (7).

Chromosomal aberration-based surveillance programs have been implemented to a larger extent in countries of Central and Eastern Europe than in other countries. It is therefore possible to assemble and follow up for cancer occurrence large cohorts of individuals with historical chromosomal aberration measurements, in these countries, which are characterized by high incidence of cancer (10). Such a study has already been implemented in the Czech Republic (8). We report here the results of such a study based on cohorts from Croatia, Hungary, Lithuania, Poland, and Slovakia, with aims to provide an estimate of the association between a high level of chromosomal aberration and cancer risk based on a large independent population. Original aspects, not adequately considered by the existing literature, such as the different predictivity of subclasses of chromosomal aberration, that is, chromosome type and chromatid type, and the presence of association of chromosomal aberration with specific cancer type, are addressed.

MATERIALS AND METHODS

Cytogenetic laboratories from Croatia (one laboratory), Hungary (two laboratories), Lithuania (one laboratory), Poland (two laboratories), Russia (one laboratory), Serbia and Montenegro (one laboratory), and Slovakia (four laboratories) were contacted to assess the feasibility of a historical cohort study of subjects previously tested for chromosomal aberration in the framework of occupational or environmental cytogenetic surveys. From each laboratory we collected the following: 1) a detailed description of the procedures used for cytogenetic analysis; 2) a sample of 200 individual records abstracted from paper or electronic archives according to a common format; and 3) 10 slides randomly selected among those available. The records and slides were selected over the whole duration of activity of each laboratory. Details on procedures for cancer incidence or mortality follow-up were also obtained in each country from local epidemiologists.

An experienced cytogeneticist independent from the study laboratories blindly evaluated the slides and scored them in terms of quality. A set of 10 slides were collected from each country, and up to 50 metaphases per slide were rescored The quality of each slide was assessed according to the following parameters: 1) general aspect, 2) cellularity, 3) abundance of metaphases, 4) metaphase spreading, and 5) metaphase morphology. The five criteria were combined into a qualitative overall score. Each sample of 200 records underwent quality control procedures, including aspects such as completeness and logical checks, and the distribution of frequency of chromosomal aberrations was compared with published series.

The criteria for inclusion of laboratories in the full-scale study were as follows: 1) adherence to standard protocols for chromosomal aberration analysis (11, 12); 2) satisfactory quality of slides, based on the overall score described above; 3) concordance of chromosomal aberration frequency with published data; and 4) evidence of feasibility of follow-up. As a consequence of the feasibility study, three laboratories from Hungary, Russia, and Serbia and Montenegro were excluded, and data from nine laboratories were retained: one each from Croatia (13, 14), Hungary (15, 16), and Lithuania (17); two from Poland (18); and four from Slovakia (unpublished data).

The criteria for inclusion of subjects in the cohorts were as follows: 1) they had valid demographic data and were at least 15 years of age and without a previous cancer diagnosis at the time of the test, and 2) the cytogenetic analysis was based on a minimum of 100 metaphases. Table 1 reports selected characteristics of the cohorts. Overall, 6,430 subjects, from ad hoc investigation of specific occupational exposures or screened in the framework of a preventive program based on cytogenetic testing, were included in the study. All original investigations were of cross-sectional design. The largest cohorts were from Slovakia and Croatia, and the smallest was from Poland. The proportion of males varied from 45 percent to 100 percent among the cohorts and was 63 percent overall. Chromosomal aberration tests in the cohorts were performed in the years 1978–2002 (median: year 1993). Of the subjects included in the cohort, 13 percent had more than one chromosomal aberration test; for the purpose of this analysis, however, we considered only the result of the first test.

The follow-up period was defined at the time from the date of the first cytogenetic test until the date of death, cancer diagnosis, emigration, 85th birthday, or end of follow-up

TABLE 1. Selected characteristics of the study population and the results of chromosomal aberration tests, Central Europe, 1978–2002

Country	Laboratories (no.)	Subjects (no.)	Males (%)	Age at test (years)		Calendar year at test		Cancer cases (no.)	Follow-up time (years)		Chromosomal aberrations (no.)		Chromosome-type aberrations (median no.)	Chromatid-type aberrations (median no.)
				Median	Range	Median	Range		Median	Range	33rd percentile	67th percentile		
Croatia	1	1,320	56	35	15–67	1993	1982–2000	24 (1.8)*	7.5	0.8–19.5	2	4	1	2
Hungary	1	840	65	36	15–77	1997	1978–2001	54 (6.4)	6.0	0.1–25.7	1	3	1	1
Lithuania	1	812	78	39	18–78	1993	1981–2002	27 (3.3)	9.0	0.2–22.7	1.9	3	1	1
Poland	1	212	85	42	19–83	1997	1992–2001	7 (3.3)	5.7	1.7–10.7	0.5	1.4	0.9	0
	2	244	83	46	15–73	1987	1981–1991	16 (6.6)	15.7	0.2–21.7	0	0.9	0.6	0
Slovakia	1	1,244	62	38	17–69	1993	1988–2000	31 (2.5)	9.6	0.3–15.1	1	2	0	1
	2	1,240	45	40	17–73	1995	1985–2000	23 (1.9)	7.7	0.2–18.0	1	2	0	1
	3	254	53	37	18–63	1991	1989–2000	5 (2.0)	11.7	1.4–13.3	0	2	0	1
	4	264	100	39	22–56	1991	1987–2001	13 (4.9)	11.7	1.2–15.9	1	3	1	1
Total		6,430	62	38	15–83	1993	1978–2002	200 (3.1)	8.5	0.1–25.7	1	3	0.9	1

* Numbers in parentheses, cumulative incidence as percent.

(2000–2003, depending on the country), whichever occurred first. The median duration of the follow-up was 8.5 years. In Croatia, Lithuania, and Slovakia, information on cancer incidence was obtained through linkage with the respective nationwide cancer registry; in Hungary and Poland, an active system of follow-up for mortality and cancer incidence was set up via contacts with local population and cancer registries, municipalities of residence, employers, pension funds, and physicians. Because cases of nonmelanoma skin cancer were not systematically registered in the participating countries, this cancer was excluded from the analysis.

For each test, demographic data on the subject, information on exposure to genotoxic agents, smoking habit, and chromosomal aberration frequency were abstracted. Chromosomal aberration data included the date of the test, culture time, number of cells scored, and the number of chromatid breaks, dicentrics, chromosome breaks, chromatid exchanges, ring chromosomes, marker chromosomes, and aberrant cells. Total chromosomal aberrations were defined as the number of cells with aberrations, excluding gaps, per 100 cells. Chromosome-type aberrations included chromosome-type breaks, ring chromosomes, marker chromosomes, and dicentrics, and chromatid-type aberrations included chromatid-type breaks and chromatid exchanges. Culture time was 48 hours in all laboratories except in Lithuania, where it was 72 hours.

The results on total chromosomal aberration frequency were analyzed according to tertiles of the laboratory-specific distributions and categorized as low, medium, and high frequency of chromosomal aberrations. Chromosome-type and chromatid-type aberrations were classified in two groups according to the median value of the laboratory-specific distribution of each marker. The cutpoints for the distributions of chromosomal aberrations, chromosome-type aberrations, and chromatid-type aberrations are reported in table 1. In an analysis of combined chromosome-type and chromatid-type aberration results, the subjects were categorized in four groups defined according to the median value of each marker (i.e., low chromosome-type and chromatid-type aberrations, low for one and high for the other, and high for both).

Information on occupational exposure at the time of the chromosomal aberration test was available for most subjects, but the amount of detail varied among countries and laboratories. A common classification scheme was developed, on the basis of the following categories: exposed to reactive chemicals (e.g., vinyl chloride, acrylonitrile; 2,201 subjects; 71 percent from Slovakia and 10 percent each from Croatia and Poland, 7 percent from Lithuania, and 3 percent from Hungary); exposed to ionizing radiation (1,982 subjects, including 496 Chernobyl clean-up workers from Lithuania; out of the 1,486 other subjects, 65 percent were from Croatia, 18 percent from Poland, 10 percent from Hungary, and 7 percent from Lithuania); exposed to cytostatics (mainly nurses; 355 workers from Slovakia (72 percent) and Croatia (28 percent)); exposed to other or unknown agents (324 subjects; 65 percent from Slovakia, 22 percent from Poland, and 12 percent from Croatia); and unexposed (i.e., included in the test programs as controls; 1,568 subjects; 45 percent from Slovakia, 40 percent from

TABLE 2. Relative risk of cancer by frequency of total chromosomal aberrations, chromosome-type aberrations, and chromatid-type aberrations, Central Europe, 1978–2002

Chromosomal aberration frequency	Cancer cases (no.)	Subjects (no.)	Relative risk*	95% confidence interval	p_{trend}
Chromosomal aberrations					
Low tertile	40	2,004	1	Referent	
Medium tertile	83	2,436	1.78	1.19, 2.67	
High tertile	77	1,990	1.81	1.20, 2.73	0.01
Chromosome-type aberrations					
Low	99	3,886	1	Referent	
High	101	2,544	1.50	1.12, 2.01	
Chromatid-type aberrations					
Low	119	3,835	1	Referent	
High	81	2,595	0.97	0.72, 1.31	
Chromosome type low, chromatid type low	63	2,452	1	Referent	
Chromosome type low, chromatid type high	36	1,434	1.12	0.73, 1.71	
Chromosome type high, chromatid type low	56	1,383	1.66	1.14, 2.42	
Chromosome type high, chromatid type high	45	1,161	1.45	0.96, 2.18	

* Relative risk adjusted for age (continuous), sex, laboratory, year of test, occupational exposure, and smoking status at test. Relative risks for chromosome-type and chromatid-type aberration frequencies were also adjusted for each other.

Hungary, 10 percent from Poland, and 4 percent from Lithuania). Information on smoking status at the time of the test was available for 6,192 subjects (96.3 percent of the total), of whom 2,854 were smokers (46.1 percent). The amount of cigarettes smoked per day was available for a small proportion of subjects, and the quality of these data was not comparable among countries; therefore, it was not used.

The Kaplan-Meier method was used to analyze cancer occurrence during the follow-up: Kaplan-Meier curves of different chromosomal aberration categories were compared by a log-rank test (19). In addition, the association between cancer and the frequencies of chromosomal, chromosome-type, and chromatid-type aberrations was modeled according to Cox regression, adjusting for age at test, sex, year of test, laboratory, smoking, and occupational exposure. STATA computer software (StataCorp LP, College Station, Texas) was used for the statistical analysis. Additional analyses were performed on individual cancers and cancer groups with at least 12 observed cases and after stratification by time since test (based on the median time of 8.5 years), age at test, sex, country, ever smoking status, and exposure. Modification of the effect of chromosome damage frequency was examined by likelihood ratio testing, comparing the multivariate model without an interaction term with a model containing the relevant interaction term. The chromosomal aberration–cancer association was separately evaluated for countries in which the follow-up was based on linkage with a cancer registry and countries in which an active system of follow-up was implemented.

RESULTS

During the follow-up period, 200 subjects examined for chromosomal aberrations were diagnosed with (or died from) cancer. Exclusion of cases identified only via death certificate did not modify the results. The most common neoplasms were from the lung (26 cases), breast (23 cases), colon and rectum (20 cases), stomach (15 cases), lymphatic and hematopoietic organs (14 cases), and head and neck (oral cavity, pharynx, and larynx, 14 cases). The cumulative incidence of cancer was 3.1 percent in the whole cohort; it was lowest in Croatia (1.8 percent) and highest in Hungary (6.4 percent; for intercountry difference: $p < 0.05$). A detailed comparison of country-specific distributions of cancer types was hampered by small numbers, but the distribution of major groups of cancers across national cohorts was not significantly different ($p = 0.12$). The two most frequent cancers in each country were the following: Croatia: breast ($n = 5$) and stomach ($n = 3$); Hungary: breast ($n = 8$) and colon ($n = 7$); Lithuania: lung ($n = 5$) and stomach ($n = 4$); and Slovakia: lung ($n = 10$) and breast ($n = 8$).

The relative risk of cancer derived from Cox regression analysis was 1.78 (95 percent confidence interval: 1.19, 2.67) for the medium tertile and 1.81 (95 percent confidence interval: 1.20, 2.73) for the high tertile, as compared with the low tertile (table 2). The increase in cancer risk was seen for chromosome-type aberrations but not for chromatid-type aberrations. Similar conclusions were derived from analysis of the combined presence of high chromosome-type and

chromatid-type aberrations frequency. Levels of chromatid-type and chromosome-type aberrations were correlated ($r = 0.14$, $p < 0.01$). Cancer-free survival was significantly improved for individuals in the low tertile of chromosomal aberration frequency when compared with those in the medium and high tertiles (figure 1; $p = 0.01$).

No significant effect modification by sex ($p = 0.11$), age at test ($p = 0.81$), or time since test ($p = 0.72$) was observed. Specifically, stratification by time since test resulted in similar risk estimates before and after 8.5 years of follow-up (results not shown in detail). Although there was no significant evidence of effect modification by type of occupational exposure ($p = 0.55$), a stronger association was suggested among subjects exposed to ionizing radiation and to reactive chemicals than among unexposed subjects (table 3). Among workers exposed to ionizing radiation, an increased risk was present for both high chromosome-type and high chromatid-type aberrations, although it was statistically significant only for the former type of aberrations. A statistically significant increase in relative risk was seen in medium and high chromosomal aberration categories in smokers but not in nonsmokers (table 3); however, smoking did not have a significant modifying effect ($p = 0.79$). The increased risk among smokers was present for elevated chromosome-type aberrations (above the median: relative risk = 1.70, 95 percent confidence interval: 1.13, 2.55) but not for elevated chromatid-type aberrations.

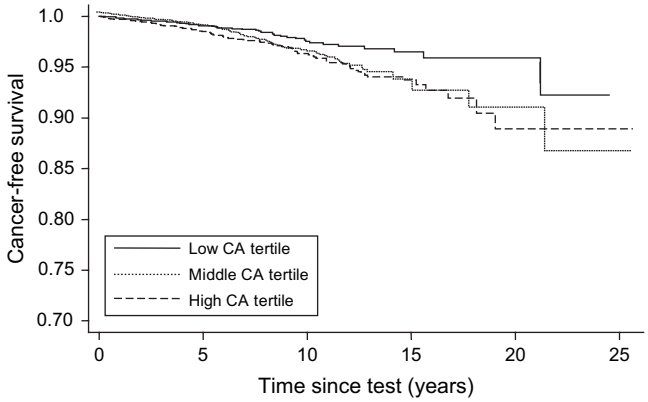

FIGURE 1. Kaplan-Meier curve for total cancer incidence by frequency of chromosomal aberrations, Central Europe, 1978–2002. CA, chromosomal aberrations.

Heterogeneity was observed among country-specific results (figure 2); the association between chromosomal

TABLE 3. Relative risk of cancer by frequency of chromosomal aberrations and tobacco smoking and occupational exposure group, Central Europe, 1978–2002

Chromosomal aberration category	Cancer cases (no.)	Subjects (no.)	Relative risk*	95% confidence interval	p_{trend}
Smokers					
Low tertile	18	792	1	Referent	
Medium tertile	49	1,075	2.00	1.16, 3.44	
High tertile	47	987	1.83	1.06, 3.18	0.06
Nonsmokers					
Low tertile	17	1,128	1	Referent	
Medium tertile	32	1,262	1.60	0.88, 2.92	
High tertile	28	948	1.70	0.91, 3.18	0.11
No occupational exposure					
Low tertile	16	599	1	Referent	
Medium tertile	21	632	1.03	0.50, 2.11	
High tertile	17	337	1.33	0.61, 2.94	0.46
Reactive chemicals					
Low tertile	10	518	1	Referent	
Medium tertile	22	792	1.61	0.73, 3.55	
High tertile	33	891	1.59	0.74, 3.41	0.31
Ionizing radiation					
Low tertile	13	647	1	Referent	
Medium tertile	36	736	2.43	1.20, 4.93	
High tertile	23	599	1.88	0.88, 4.03	0.18

* Relative risk adjusted for age (continuous), sex, laboratory, year of test, occupational exposure (analysis stratified by smoking), and smoking status at test (analysis stratified by occupation).

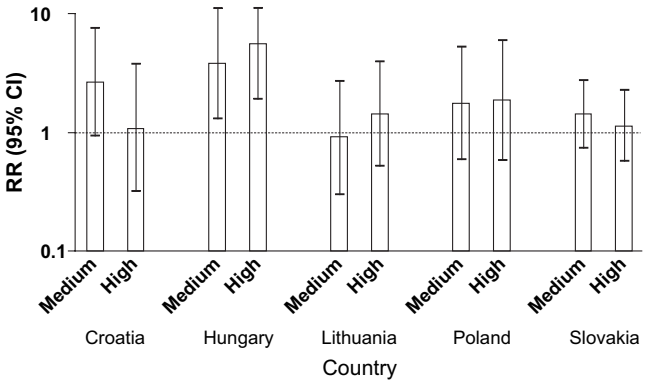

FIGURE 2. Country-specific relative risk of cancer by frequency of chromosomal aberrations, Central Europe, 1978–2002. RR, relative risk; CI, confidence interval; "medium," medium tertile; "high," high tertile. Reference category is the low tertile.

aberration level and cancer risk was stronger in countries where an active system of follow-up was used (Hungary and Poland) than in countries where the follow-up was based on linkage with cancer registry (Croatia, Lithuania, and Slovakia).

The analysis of specific types of cancer showed a relation between chromosomal aberration frequency and risk of cancers of the stomach and possibly of the colon and rectum, while for lung and breast cancer, the results were only suggestive of an association. Regarding the other cancer sites, no increase was detected (table 4). The analysis of an association between subclasses of chromosomal aberrations and specific cancers was hampered by small numbers.

DISCUSSION

The main rationale of using cytogenetic endpoints as a biomarker relevant for cancer risk is that genetic damage in a nontarget tissue, such as peripheral blood lymphocytes, reflects the occurrence of similar events in target tissues involved in carcinogenic processes (4). This approach is consistently substantiated by a large amount of evidence assessing the role of chromosome damage in the pathogenesis of cancer (3). Chromosomal aberrations are usually considered to derive from unrepaired or misrepaired DNA lesions induced by exogenous or endogenous exposure to DNA-damaging agents. An increase in chromosomal aberrations could also be due to genetic or acquired conditions conferring a higher susceptibility to genetic damage. Elevated levels of chromosomal aberrations in peripheral blood lymphocytes may be seen as an indicator of an early phase of carcinogenesis, where various genetic alterations are also generated in different tissues. In biologically susceptible target tissues, rare specific chromosomal aberrations and gene mutations may then pave the way for further steps in carcinogenesis. A comprehensive review of genetic rearrangements consequent to chromosome aberrations and their role in the pathogenesis of solid and hematologic cancers was recently reported (20).

The results of the present study provide support for the hypothesis that the occurrence of chromosomal aberrations in peripheral blood lymphocytes represents relevant events in carcinogenesis and may serve as a surrogate endpoint for cancer risk. We showed that a high frequency of chromosomal aberrations in peripheral blood lymphocytes, and in particular of chromosome-type aberrations, is associated with increased risk of cancer. The fact that this association is not dependent on the time elapsed since the test is consistent with the hypothesis that the level of chromosomal aberrations is predictive of cancer risk rather than being an early manifestation of a clinically undetected cancer.

A major support to the plausibility of our findings comes from the consistent results of cohort studies published so far evaluating chromosomal aberrations as a predictor of cancer risk, although with variable strength of the association (21). In particular, a recent report of the updated results of the Czech cohort failed to observe a significant association between cancer risk and chromosomal aberrations, although a significant increase of cancer was described in subjects with

TABLE 4. Relative risk of selected cancers and groups of cancer by frequency of chromosomal aberrations, Central Europe, 1978–2002

Cancer type	Chromosomal aberrations							p_{trend}
	Low tertile* (no. of cases)	Middle tertile ($n = 2,436$)			High tertile ($n = 1,990$)			
		No. of cases	Relative risk†	95% confidence interval	No. of cases	Relative risk	95% confidence interval	
Head and neck cancer	4	5	0.82	0.21, 3.18	5	0.96	0.24, 3.78	0.88
Stomach cancer	0	6	Not estimable	Not estimable	9	Not estimable	Not estimable	0.01
Colorectal cancer	5	8	2.20	0.57, 8.72	7	2.51	0.61, 10.4	0.22
Lung cancer	5	8	1.21	0.39, 3.73	13	1.68	0.58, 4.85	0.31
Breast cancer	8	5	0.83	0.25, 2.77	10	1.51	0.52, 4.44	0.42
Lymphatic and hematopoietic neoplasms	5	6	1.39	0.36, 5.35	3	0.93	0.19, 4.47	0.94

* Referent ($n = 2,004$).

† Relative risk adjusted for age (continuous), sex, laboratory, year of test, occupational exposure, and smoking status at test.

high chromosome-type aberrations (8), which is in line with the results of the present analysis.

Despite the biologic and epidemiologic evidence supporting chromosome-type aberrations as a better predictor of cancer, this evidence may not have a practical effect on the reliability of cancer prediction in healthy individuals. This is because mechanistic evidence developed for the early phases of the carcinogenic processes in the target organ hardly holds when evaluated in peripheral blood lymphocytes (or at least no data are available showing specific correlation between the two tissues). Furthermore, the presence of chromosome-type aberrations is a rarer event than that of other types of chromosomal aberrations, and therefore the increase of specificity is compensated by a loss of statistical power. This latter comment applies even more to specific types of chromosomal aberrations, such as dicentrics or marker chromosomes, which despite their higher relevance in the carcinogenic process are too rare to be singularly studied. The traditional method for chromosomal aberration analysis, on which the present data were based, does not give reliable frequency estimates for such important chromosomal aberration types as reciprocal translocations, inversions, insertions, or complex rearrangements, which are better detected by methods based on fluorescence in situ hybridization (22). Finally, the relative risk for subjects with a high frequency of chromosome-type aberrations is very similar to those estimated for total structural chromosomal aberrations, which remain by far the most suitable endpoint for application in human populations.

To clarify the association between chromosomal damage and risk of cancer, the presence of confounding or effect modification due to host factors or external genotoxic exposures has to be carefully evaluated. The available literature points toward the independence from exposure to carcinogens of the chromosomal aberration–cancer association; that is, the prediction of cancer risk associated with chromosomal aberration frequency is the same in exposed and unexposed subjects, despite earlier reports that the Czech cohort described a stronger association in a group of radon-exposed miners (23). Results from this study, although not substantiated by statistical significance, suggest a stronger chromosomal aberration–cancer association in subjects occupationally exposed to ionizing radiation and in smokers. These results provide an interesting issue for mechanistic modeling, since the possible involvement of ionizing radiation agrees very well with evidence of a higher relevance of chromosome-type aberrations in predicting cancer risk. In the present study, roughly one third of the subjects and cancer cases were classified as radiation exposed. The poor definition of exposure in these nonconcurrent cohort studies leaves open the issue that an effect modification might occur with certain exposures or at certain doses. From the public health standpoint, such modification would reduce emphasis on the role of individual susceptibility, stressing the need for a closer surveillance of subjects exposed to known genotoxic agents.

The relatively large size of this cohort allowed evaluation of the association between chromosomal aberrations in peripheral blood lymphocytes and the risk for specific cancer sites. Analysis of the data showed a clear association with the risk of stomach cancer. The biologic plausibility of this finding is reinforced by parallel evidence reported by the recent analysis of the large Czech cohort (8), which described stomach cancer as the specific cancer site most strongly predicted by chromosomal aberration frequency (for high vs. low tertile: relative risk = 7.79, 95 percent CI: 1.01, 60.0). As a biologic support to this finding, the presence of chromosome instability has been reported among the most relevant events determining susceptibility to both intestinal and diffuse stomach cancers (24), and cancer genes involved in the pathogenesis of stomach cancer have been reported to be rearranged as a consequence of balanced chromosomal alterations (20). Furthermore, there is growing evidence of a link between the metabolisms of agents relevant to stomach carcinogenesis, such as folic acid and vitamin B_{12}, and the maintenance of chromosome stability (25, 26).

Among the limitations of the current study is the poor quality of available data on relevant exposures such as cigarette smoking and occupational carcinogens. Information on other potential confounders, for example, dietary factors, was not available. Such limitations cannot properly be addressed, unless specific multicenter study designs are implemented, as in the case of the nested case-control study conducted by the Nordic-Italian study group (27). Additional limitations are the likely misclassification of chromosomal aberration categories due to the availability of a single measure of chromosomal aberrations and the undetermined extent of the correlation between the event measured in the surrogate tissue and that occurring in the target. An additional potential source of bias is the heterogeneity of results by country and, in particular, the different results between countries with follow-up based on linkage with cancer registries and countries with active follow-up. This heterogeneity would weaken our findings since the latter method of follow-up might be more prone to bias.

Differences among laboratories, including the difference in culture time between Lithuania and the other countries, may affect the frequency of chromosomal aberrations scored but have no major impact on the biologic meaning of the assay and on the ranking of the individuals. The classification of results in tertiles of the laboratory-specific distributions accounts for differences in laboratory protocols.

On the other hand, a number of strengths could be considered in evaluating the findings of this study. The prospective nature of the design provides a robust framework to validate the use of chromosomal aberrations in healthy subjects as a biomarker for predicting cancer risk in all conditions where a classic epidemiologic approach is not suitable. The large population under study, which represents a number of countries and laboratories, and the high follow-up rate, in particular in countries with nationwide cancer registration, are additional strengths of our investigation.

In conclusion, the findings of this study add to the large existing evidence that the frequency of chromosomal aberrations in peripheral blood lymphocytes of healthy individuals may be predictive of cancer risk. This evidence discloses a number of issues dealing with both ethics and public health policies. Despite the low absolute level of relative risk even in the high tertile of chromosomal aberration

distribution and the large interindividual variability of these results, an increased relative risk has been described in all studies published so far. Applicability at the individual level has still to come, but the use of chromosomal aberrations in evaluating cancer risk in population groups exposed to environmental or occupational carcinogens or characterized by a susceptible genetic profile should be planned now.

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