Chromosomal Aberrations in Lymphocytes Predict Human Cancer: A Report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH)

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ABSTRACT

Chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and micronuclei (MN) in peripheral blood lymphocytes have for decades been used as cytogenetic biomarkers to survey genotoxic risks in the work environment. The conceptual basis for this application has been the idea that increased cytogenetic damage reflects an enhanced cancer risk. Nordic and Italian cohorts have been established to evaluate this hypothesis, and analyses presented previously have shown a positive trend between CA frequency and increased cancer risk. We now report on a pooled analysis of updated data for 3541 subjects examined for CAs, 2703 for SCEs, and 1496 for MN. To standardize for interlaboratory variation, the results for the various cytogenetic end points were trichotomized on the basis of the absolute value distribution within each laboratory as "low" (1-33 percentile), "medium" (34-66 percentile), or "high" (67-100 percentile). In the Nordic cohort, there was an elevated standardized mortality ratio (SMR) for all cancer among subjects with high CA frequency [1.53; 95% confidence interval (CI), 1.13-2.05] but not for those with medium or low CA frequency. In the Italian cohort, a SMR in cancer of 2.01 (95% CI, 1.35-2.89) was obtained for those with a high CA frequency level, whereas the SMRs for those with medium or low did not noticeably differ from unity. Cox's proportional hazards models gave no evidence that the effect of CAs on total cancer incidence/mortality was modified by gender, age at test, or time since test. No association was seen between the SCEs or the MN frequencies and subsequent cancer incidence/mortality. The present study further supports our previous observation on the cancer predictive value of CAs. Since the 1960s, CAs in PBLs have been used in occupational health surveillance programs to assess genotoxic risks. The conceptual basis for this biomarker has been the hypothesis that the extent of genetic damage in PBLs reflects similar events in the precursor cells for carcinogenic processes in the target tissues. Besides CAs, two other cytogenetic end points in PBLs have been used as indicators of chromosomal damage: SCEs and MN. SCEs represent symmetrical exchanges between sister chromatids; generally, they do not result in alteration of the chromosome morphology (4). MN in PBLs represent small, additional nuclei formed by the exclusion of chromosome fragments or whole chromosomes lagging at mitosis. MN rates therefore indirectly reflect chromosome breakage or impairment of the mitotic apparatus. The health significance of increased levels of SCEs and MN is poorly understood (3, 5).

To evaluate whether a high frequency of CAs, SCEs, or MN in PBLs from healthy subjects has any predictive value for cancer risk, Nordic and Italian cohorts of subjects examined with cytogenetic tests were established (6-11). A positive trend between CA frequency and increased cancer risk was observed in both studies. In contrast, the preliminary results did not support any such predictive value for SCEs and were inconclusive for MN.

We now report on a pooled analysis of updated data from these two cohorts, which has enabled a more detailed assessment, including the potentially modifying effects of gender, age at cytogenetic testing, and time since cytogenetic testing on the cancer predictive value of CAs.

INTRODUCTION

Numerous chemicals, some of which are carcinogenic and mutagenic, are used or introduced in the occupational setting. Toxicological data from animal experiments can only be used to a limited extent for human risk assessment due to, e.g., species differences. It is therefore important to survey exposed workers by using biomarkers for early detection of cancer risks. It is generally accepted that chromosomal mutations are causal events in the development of neoplasia (1), and it has been postulated, but hitherto not proven, that increased cytogenetic damage may reflect an enhanced cancer risk (2, 3). This ought to be clarified, because a firm knowledge of the predictive value of cytogenetic biomarkers may become an important tool when implementing preventive measures for increasing the safety and health of workers.

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SUBJECTS AND METHODS

Cohorts and Cytogenetic Biomarkers. The present study base comprises 3184 individuals examined 1970-1988 for at least one cytogenetic biomarker in 10 Swedish, Finnish, Norwegian, and Danish laboratories and 2087 individuals examined 1965-1988 in 10 Italian laboratories. All subjects were at least 15 years of age at the date of cytogenetic testing, i.e., when the follow-up period started. The subjects were originally selected for cytogenetic studies because of various, mainly occupational, exposures to mutagens or carcinogens or as unexposed referents. Subjects with cancer diagnosed before the cytogenetic analysis were not included in the cohort. Information on gender, age at test, and follow-up time are given for the specific cohorts of 3541 subjects examined for CAs, 2703 for SCEs, and 1496 for MN (Table 1).
Previous analyses of the cohorts from the Nordic countries and a part of the Italian cohort have been reported (8, 9).

For each subject, a personal identification code and the time and result of the cytogenetic analysis were registered. When a subject had been analyzed for a specific cytogenetic end point more than once, the first examination was chosen. Eight Swedish, 5 Norwegian, 3 Danish, and 44 Italian subjects, whose personal identification codes could not be retrieved, were not included in the cohorts.

To standardize for the interlaboratory variation, the results for the various cytogenetic end points were trichotomized within each laboratory as follows. The 33rd and 67th percentiles were determined for each end point, and each subject was classified as “low” (1–33 percentile), “medium” (34–66 percentile), or “high” (67–100 percentile) by comparing each individual end point value with the relevant percentile values, as has been described in detail earlier (8, 9). Fifteen hundred fifty-three subjects had been examined for both CAs and SCEs. There was poor agreement between the trichotomizations for these two end points (kappa value, 0.03). The corresponding figures for those 773 subjects who were examined for both CAs and MN and those 526 examined for both SCEs and MN were similarly low (kappa values = 0.07, and 0.02, respectively).

For CAs, classified according to the recommendations of International System for Human Cytogenetic Nomenclature (12), at least 100 metaphases were scored from each individual. Gaps were not included. The culture time had been 48 or 72 h. The CA data based on 48-h culture time were considered. Modification of the effect of CAs at test was examined by likelihood ratio testing, comparing the multivariate model without interaction term to a model containing the relevant interaction term (15). The factor age at test was divided into five categories (≤45, 46–50, 51–55, 56–60, and 61 + years).

Cancer Incidence and Mortality. In the Nordic cohorts, information on malignant tumors diagnosed from the date of cytogenetic testing until the end of 1993 (Sweden, Denmark), 1994 (Norway), or 1995 (Finland), respectively, was obtained from the National Cancer Registries. In the Italian cohorts, specific causes of death until April 30, 1996 were obtained from the municipality of residence. These dates constituted the end of the overall follow-up period. The number of subjects in each cohort who had died during the follow-up period is given in Table 1. The fraction of subjects who had emigrated during the follow-up period ranged between 0.3 and 0.6%.

Expected cancer morbidities for the Nordic cohorts were calculated by calendar year-, gender-, and five-year age group-specific incidences for each country, obtained from the National Cancer Registries. Individual follow-up was continued until the date of death, tumor diagnosis, emigration, or a person’s 85th birthday. Similarly, the expected cancer mortality in the Italian cohorts was calculated by calendar year-, gender-, and five-year age group-specific death rates for the Italian population.

Statistical Methods. The 95% CIs for SIRs and SMRs were calculated by treating the observed number as a Poisson variable or a normal variable if the observed value was >1.5. The Kaplan-Meier method was used to describe the total cancer incidence/mortality during the follow-up of the cohorts. The associations between total cancer incidence and mortality, respectively, and CAs at test, gender, age at test, time since test, and country (only for cancer incidence) were modeled by means of Cox’s regression (15), using time since test as the time variable. The purpose was to estimate the effect of CAs at test on total cancer incidence/mortality (where the effect measure is an incidence ratio or a mortality ratio), based on the internal data from the cohorts (excluding the Danish cohort because only two cases were observed in that cohort) and also to examine whether the effect was modified by each of the other factors considered. Modification of the effect of CAs at test was examined by likelihood ratio testing, comparing the multivariate model without interaction with a model containing the relevant interaction term (15). The factor age at test was divided into five categories (≤45, 46–50, 51–55, 56–60, and 61 + years).

RESULTS

During the follow-up, 91 Nordic subjects examined for CAs were diagnosed with cancer (Table 2), and 64 Italian subjects examined for CAs died from a malignant tumor (Table 3). There was in the total

![Table 1 Distribution of gender, age at test, and length of follow-up time in the cohorts of subjects cytogenetically tested previously for CAs, SCEs, and MN](link)
Table 4. In the Nordic cohorts, the incidence ratio between the high and low CA groups was estimated to be 2.08 (95% CI, 1.26-3.40) when the additional factors of age at test, gender, and country were included in the model. The corresponding incidence ratio between the high and medium groups was 1.69 (1.01-2.81; not in the table). In the Italian cohort, the age- and sex-adjusted mortality ratio between the high and low groups was 2.56 (1.35-4.86). The corresponding mortality ratio between the high and medium groups was 1.86 (1.04-3.31; not in the table). There was no significant evidence that the incidence and mortality ratios between the CA groups were modified by country (only considered for cancer incidence), sex, age at test, or time since test (all Ps > 0.08; see Table 4).

Seventy-three cancer cases in the Nordic cohort had been monitored for SCE before their diagnosis, but no association was seen between the SCE frequency and subsequent cancer incidence (Table 5). The Italian data on SCEs and cancer mortality were very sparse and did not indicate any association.

Eighteen of the cancer cases in the Swedish cohort had been monitored for MN before their diagnosis (Table 6). Moreover, nine Italian subjects examined for MN had died from cancer during the follow-up period. Neither cancer incidence nor cancer mortality was associated with the outcome of the MN test.

### DISCUSSION

Using biomarkers of early biological effect as outcome variables in occupational cancer epidemiological studies would allow for identi-
fication of risk factors at a time when control measures still could be implemented. Thus, there is a need for an adequate validation of such candidate biomarkers (16, 17). The most important aspect of this validation is to investigate, in prospective cohort studies, whether the biomarkers will predict cancer risk. Based on the analysis of a joint Nordic and Italian study population, we can now report that an elevated CA frequency in PBLs from healthy subjects predicted increased cancer risk, whereas no such association was observed for SCEs. The concordance between the results for the country-specific cohorts strengthen our finding. No tendency of a cancer predictive value of high MN frequencies was observed, but because only relatively few cancer cases have been diagnosed among those tested for MN, we have to wait for future follow-ups before any firm conclusion can be drawn with respect to this cytogenetic end point. In general, when interpreting a “negative” finding, the 95% CI for the SIR/SMR should be taken into account. If such a CI is considerably wide, the finding should be inferred to be inconclusive rather than “negative.” The present results corroborate the findings from earlier analyses of the Nordic and Italian cohorts (8, 9). The Italian cohort is, however, only partly similar to the one reported previously.

Considering that there was no intra-individual association between classifications as low, medium, and high for the other two cytogenetic end points and CAs, it is not surprising that no cancer predictive was found for SCEs and MN. It could, however, be discussed whether there are also methodological and mechanistic arguments in favor of the CA biomarker. The baseline levels appear to fluctuate more for SCEs and MN than for CAs because of culture techniques and interscorer variability (7). This might result in a higher degree of misclassification for SCEs and MN when adding up the results from different studies and performing the trichotomization on a laboratory basis. Unlike CA, SCEs are detected after replication of a DNA template containing bromodeoxyuridine, an agent that by itself induces SCE (18). Moreover, the active process of SCE formation is taking place during the S-phase of cells replicating in vitro, whereas at least part of the damage scored as chromosome and chromatid breaks are true in vivo events. Thus, the SCE frequency scored in PBLs might be more severely influenced by factors operating during in vitro cultivation than the CA frequency and, therefore, be less predictive of in vivo damage.

Because chromosome breakage results in MN formation, MN analysis would have been expected to show an association with cancer. Besides technical variation, the possible association may be distorted by the fact that a high proportion of MN harbors whole chromosomes (19, 20), which may be uninformative in this context. Our results on MN were mainly based on cancer cases observed in subjects studied using the conventional MN analysis, which has been suggested to be sensitive to variation in lymphocyte proliferation rate (13). No conclusions could be drawn on the cytokinesis-block technique, considered to overcome this problem.

One could presume that a genotoxic occupational exposure at the time of the cytogenetic test, but not later on, might result in the strongest cancer predictivity for the CA biomarker during time interval after the test, depending on the relevant induction-latency period assumption. Such a significant modifying effect was, however, not found in the present analysis. There are at least three possibilities that may explain this finding: (a) the occupational exposures may in most cases have continued for a long period after the cytogenetic testing. This will be evaluated further in an ongoing, cohort-based case-referent study, where time since first exposure, period of exposure, and time since test (i.e., follow-up time in the present study) will be taken into account; (b) because the relevant induction-latency period may vary substantially between different cancer diagnoses, there will be no apparent modifying effect of time since test; and (c) a high CA frequency will not only reflect genotoxic exposure but may also indicate a proneness to develop cancer due to individual susceptibility factors. There are a number of studies indicating an overrepresentation of some genotypes or phenotypes for polymorphic metabolizing enzymes in various forms of cancer (21–23). Moreover, the limited data available suggest that some of these polymorphisms influence baseline or induced level of CAs in lymphocytes (24). Besides xenobiotic metabolizing genes, individual differences in DNA repair capacity may also contribute to a proneness for tumors (25).

The Cox regression models used showed no significant modifying effect of age or gender on the cancer predictivity of the CA biomarker. Thus, there is no evidence that the cancer predictivity of CAs was limited to only one of the genders or to a certain age stratum.

A statistical association between the CA frequency and an increased cancer risk does not necessarily mean that there is a mechanistic link between these events. For example, both smoking and occupational exposure could cause CAs in PBLs as well as a genetic damage in the target cells directly associated with the cancer process, without these outcomes being involved in the same chain of events. If it can be shown that the CA frequency in PBLs predicts cancer risk irrespective of occupational exposure and smoking, it will increase the usefulness of the CA biomarker. This cannot yet be evaluated due to a lack of accurate and precise data on these factors, but it is the primary objective of an ongoing case-referent study within the joint study base. Moreover, irrespective of the mechanistic background, the cancer predictivity of the CA frequency would still be useful as a mean of prevention.

The cancer predictivity of CAs in PBLs might very well differ substantially in magnitude between different types of malignancies. The limited number of diagnosed cancer cases in the study base unable to perform diagnosis-specific risk analyses at the present time. This will be an important task for future follow-ups of the study base. Whether new advances in analysis of CAs, using e.g., fluorescence in situ hybridization, will result in an even stronger association with future cancer risk should also be evaluated.

In conclusion, the present study has given further support for our previous observation on the cancer predictivity of the CA biomarker, which seems to be independent of age at test, gender, and time since test. The findings suggest that CAs are a relevant early biological effect biomarker for cancer risk in humans. The observed lack of predictivity for SCE stresses the importance to assess the validity of also other candidate biomarkers.

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**Table 6 Relationship in the Swedish and Italian cohorts between the frequency of MN in PBLs and cancer incidence and mortality, respectively.**

The calculations were based on 686 subjects and 6739 person-years in the Swedish cohort and 810 subjects and 4543 person-years in the Italian cohort.

<table>
<thead>
<tr>
<th>Frequency of MN*</th>
<th>Swedish cohort</th>
<th>Italian cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obs SIR 95% CI</td>
<td>Obs SMR 95% CI</td>
</tr>
<tr>
<td>Low</td>
<td>3 0.57 0.12–1.67</td>
<td>2 0.56 0.07–2.03</td>
</tr>
<tr>
<td>Medium</td>
<td>10 1.63 0.78–3.00</td>
<td>5 1.68 0.55–3.93</td>
</tr>
<tr>
<td>High</td>
<td>5 0.56 0.18–1.31</td>
<td>2 0.49 0.06–1.79</td>
</tr>
<tr>
<td>All</td>
<td>18 0.89 0.54–1.43</td>
<td>9 0.85 0.39–1.61</td>
</tr>
</tbody>
</table>

*Low, 1–33 percentiles; Medium, 34–66 percentiles; High, 67–100 percentiles.

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Angelo Abbondandolo, Marcella De Ferrari, and Claudia Bolognesi contributed data for the Italian cohort.

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