Research on correlations between specific genomic lesions and occupation-related environmental factors

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Introduction

The research theme is: exploring 'exposure-specific genomic lesions'. Indications of a relationship between external exposure and clonal chromosomal anomalies have been observed for a number of years. The idea derives from a paper by Van den Berghe H. et al published in 1974 in 'Nature', entitled: "Distinct haematological disorder with deletion of the long arm of No. 5 chromosome." This acquired structural genomic disorder found in myelodysplasia/leukaemia opened up the whole research field: myeloid leukaemia as an environmentally induced process.

This research moves between two questions, the first of which has been answered partly and the second hardly explored. The first question is: can the presence of specific clonal chromosomal anomalies be an indication that external exposure is the major explanation for the appearance of leukaemia? The second question is: can specific chromosomal anomalies found in malignancies be directly attributed to specific exposure factors?

The first question was answered in the affirmative through experience with so-called secondary leukaemia characterised by specific chromosomal anomalies following the administration of drugs for cancer chemotherapy. In seeking an answer to the second question a major difficulty arises: how can one distinguish a clonal manifestation characterised by a specific chromosomal aberration resulting directly from a specific –be it direct or indirect- effect on the genome, from a clonal manifestation resulting from selection of randomly occurring, i.e. non-specific, chromosomal aberrations? This question applies, for instance, to the established associations between alkylating (chemotherapeutic)

agents and aberrations of chromosomes 5 and 7, and between epidophyllotoxins and aberrations of chromosome 11, respectively. Why different loci are affected remains unexplained.

The aim of this project was therefore to study the relationship between possible environmental causes of leukaemia/MDS and specific genomic lesions in the cells, and the possible relevance of such knowledge for prevention, identification, and forensic recognition of occupational diseases. As an additional objective and extension, it was decided during the investigation to elaborate a research proposal to explore the possible role of genetic susceptibility factors, in particular of polymorphic genes possibly involved in metabolising xenobiotics.

The teams in Leuven, Namur, and Antwerp combined their efforts and concentrated their research mainly on three core activities (two studies and a new common research proposal) considered necessary or useful to approach key questions related to the research topic. Myelodysplastic syndrome was at the heart of the research interest.

Analysis of a historical MDS database

Materials and methods

A historical database of cases of haematological malignancies together with the ascertained clonal chromosomal anomalies was explored. This unique data collection at the Centre for Human Genetics in Leuven was used for the very first time, in this research, for large-scale analysis of chromosomal anomalies in MDS. Due to the lack of standardisation, it was not possible to use the data directly for such an investigation. Through a systematic process that often meant checking the original files, the research teams succeeded in compiling a more restricted but still very large and reliable series of data for the study of clonal chromosomal anomalies in MDS. It was initially planned to relate the data to social security data revealing previous occupational fields, but this, finally, was not done because the expected relevance of the output and the administrative burden of such an exercise did not justify making it a priority.

Results

The analysis of these data has resulted in a new finding: patients with karyotype anomalies are significantly younger than ones with a normal karyotype, especially in the case of women. Chromosomes 11 and 12p in particular seem to have a role in the pathogenesis of chomosome-5- and chromosome-7-independent MDS. These findings must be studied further through epidemiological and genetic research that could increase knowledge on this research topic.

Epidemiological study of MDS

Materials and methods

The study of exposure-specific genomic lesions poses specific epidemiological challenges. Because only small numbers of cases and controls are available, and since for each pathology studied only a

limited number of cases carry the considered chromosomal anomaly, the challenge was to develop a method enabling the teams to demonstrate effects or correlations with an acceptable significance level. In addition, studying the relationship between clonal chromosomal anomalies and exposure factors requires an extremely accurate exposure assessment.

Therefore, we tried to increase the sensitivity of the epidemiological method by developing a new questioning strategy and a new method of analysis. These were elaborated and applied in a case-control study of myelodysplastic syndrome.

The essential and inseparable elements of the new questioning method are: a conversational questioning approach enabling nuances in the answers to be captured; reconstruction of the exposure histories on a time scale; the interviewers must have medical expertise and expertise in environmental exposure issues; one must strive for a complete assessment of all kinds of exposure situations and factors; one must strive to construct answers as correctly as possible by seeking a balance between two approaches: direct questioning about precise exposure factors on the one hand, and on the other hand describing exposure situations, these being subjected to expert evaluation for the identification of exposure factors and levels. In processing the data, a new approach was used for subdivision into exposure categories, based on a ranking of the exposure histories as described by the interviewees, rather than on pre-determined 'theoretical' subdivisions.

Results

This highly labour-intensive method was elaborated and applied successfully. In a matched study with thirty cases and thirty controls –which is likely to mean a very low statistical power- a statistically significant association was demonstrated for a series of exposure factors. One of these factors gives a direct validation of the method. It is the statistical significance of taking medication known to cause haematolymphopoietic disorders. A remarkable result is that following the demonstration of a positive association of MDS with a history of exposure to pesticides, a significant association was revealed with chromosomal anomalies considered signatures of environmental exposure.

One may assume that this new epidemiological approach could be of considerable help in further exploring the research theme.

Elaboration of a project to study the influence of genetic susceptibility

Materials and methods

A third core activity was the development of a proposal for research that should increase our understanding of the possible role of genetic susceptibility factors - in this case affecting polymorphic genes involved in metabolising xenobiotics - in the causation/development of MDS and other haematolymphopoietic disorders. If genetic susceptibility factors can considerably affect the response to genotoxic agents, their identification may be important in studies of exposure-specific genomic lesions.

As a starting point we used a database compiled during an earlier research project (funded by the OSCT). The database contains approximately thirteen thousand peripheral blood cell counts of a few hundred employees with documented moderate exposure to benzene. The blood cell counts were

collected over a period of 20 years. The global effects of exposure to benzene on the peripheral blood cell count are well known in this population. In this context a method was developed for classifying the individuals in the population according to some typical 'patterns' in the peripheral blood cell counts. Rewarding contacts were made with all concerned parties and with a specialised Finnish laboratory. The haematological centres participating in the epidemiological MDS study were contacted as to the possibility of conducting the same genetic tests on patients suffering from MDS or possibly from benzene-related leukaemia.

Results

A research protocol was prepared and the necessary contacts established to conduct the research. The research should make it possible to compare groups of persons suffering from possible benzene-induced malignant disorders with groups of benzene-exposed employees with particular peripheral blood cell evolutions as regards the distribution frequency of genetic polymorphisms. This may increase our insight into the meaning of some changes in peripheral blood cell counts found during periodical medical examinations in occupational health. Such knowledge could at the same time contribute to better insight into the causation of haematological malignancies.

Conclusions

We have developed an epidemiological research method for case-control studies, aimed at maximising the exactness of the recorded information, thus enabling studies with smaller sample size to achieve sufficient sensitivity. Such a method should make it possible to study more reliably the relationship between exposures and specific genomic lesions. The preliminary results of the MDS study indirectly illustrate how a more sensitive method of this kind can contribute to better knowledge of, and insight into, the relationship between specific exposure factors and clonal chromosomal anomalies found in malignancies. In time, this research method could thus contribute to refining forensic criteria for the recognition of several occupation-related malignancies.

Experience with epidemiological research on MDS and the analysis of the historical karyotype database has shown how a specific MDS register containing complete data on Belgian residents can contribute to further exploration of both MDS and the general research topic. This has been the subject of consultation with foreign research teams which both share this concern and have occupational diseases as a major subject of study. Identical registration procedures could indeed constitute considerable progress for this research.

The project on genetic susceptibility may help to solve unanswered but necessary questions as to the possible relationship between specific genetic polymorphisms and the risk for haematological malignancies related to specific exposures. Insights in this area may or may not be a stimulus for introducing additional variables into epidemiological research on exposure-specific genomic lesions. Moreover, this research may lead to better insight into the health surveillance of workers exposed to benzene and analogous agents.