

Chronic lymphatic leukaemia and engine exhausts, fresh wood, and DDT: a case-referent study

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ABSTRACT The effect of potential risk factors for chronic lymphatic leukaemia was evaluated in a case-referent study encompassing 111 cases and 431 randomised referents, all alive. Information on exposure was obtained by questionnaires posted to the subjects. Crude rate ratios were increased for occupational exposure to solvents, DDT, engine exhausts, fresh wood (lumberjacks, paper pulp workers, and sawmill workers, for example) and also in farming. Further analysis of the material by means of the Miettinen confounder score technique reduced the number of rate ratios significantly exceeding unity to encompass only occupational exposure to engine exhaust, fresh wood, DDT, and contact with horses.

Chronic lymphatic leukaemia has been epidemiologically associated with farming^{1,2} and exposure to benzene is an established risk factor for development of myeloid leukaemia.³ So far benzene has not been definitely linked with other malignancies; it is unclear whether or not benzene may induce chronic lymphatic leukaemia but two studies give some indication that this may be the case,^{4,5} as discussed below. Some other agents, however, have been associated with chronic lymphatic leukaemia—namely, carbon tetrachloride and carbon disulphide.⁶ Unspecified solvent exposure in the rubber industry has also been connected with lymphatic leukaemia, the crude rate ratio for high grade exposure amounting to about seven.⁴ In the Swedish Cancer-Environment Registry (the linkage of census data with the Cancer Registry) shoemakers were at excess risk for lymphatic leukaemia. They have probably been exposed to benzene and other solvents but no accurate information is given about exposure.⁷

Benzene produced from coal tar and xylene may be more leukaemogenic than petroleum based solvents according to the results of Arp *et al.*⁸ In two register studies, linking occupations by death certificates, car mechanics, gas station personnel, lumber and plywood millworkers, school teachers, and health professionals had an increased risk from lymphatic leukaemia as had carpenters.⁹

By contrast with myeloid leukaemia, no relation has been observed between ionising radiation and chronic lymphatic leukaemia.

The aim of the present case-referent study was to determine whether or not an effect could be seen in relation to reported risk factors and if some other relation could also be observed. Furthermore, it was important to see whether or not the relation with background radiation found in our previous studies for myeloid leukaemia would appear also for this disorder^{10,11}; if so the relation found in these earlier studies would have to be looked on as likely to be due to some sort of bias.

Materials and methods

CASES

Cases of chronic lymphatic leukaemia (International Classification of Diseases 1965, ICD 204.15) were obtained from the registers of the cytological departments at the hospitals of Linköping, Norrköping, and Jönköping and from the medical clinics at the hospitals of Kalmar and Örebro. These hospitals are in the middle and south east of Sweden in provinces with a total population of about 1.5 million. The diagnoses of the cases had to be confirmed both by cytologists and clinical physicians. Most cases were diagnosed between 1975 and 1984 but some had been diagnosed as early as 1964, surviving into the period 1981-3 when all cases fulfilling certain criteria were contacted. Hence, the age when answering the questionnaire should not be above 80, and they should be mentally capable of answering a questionnaire. They had to be Swedish born and live in the catchment areas of the particular hospitals at the time of the diagnosis. Eleven cases were not willing to participate but 111 cases joined the study.

REFERENCES

Referents were randomly drawn from the population registers corresponding to the catchment area of the particular hospitals. Since we also conducted a parallel study on multiple myeloma, two referents were drawn for each case of either multiple myeloma or chronic lymphatic leukaemia which resulted in enrolling 434 referents. Of these, 17% (n = 83) were not able or willing to reply and were replaced by other, similarly drawn, subjects. In the analyses the age span for the referents was restricted to 40-80, since the cases were in that age range, thereby reducing the primarily eligible referents by 53 so that the material finally consists of 431 randomly drawn referents.

ASSESSMENT OF EXPOSURE

Information about various types of exposure among the cases and referents was obtained by means of a nine page questionnaire, preceded by an introductory letter and posted to the subjects. The questionnaire contained 17 main questions, of which 10 concerned occupational exposures, some of which were further subdivided with regard to certain details. Five questions were devoted to medical care, particularly the use of drugs, x ray treatment, and x ray examinations.

A minimum exposure time of one year was required and in general a five year latency time requirement was applied—that is, the last five years of exposure were disregarded both for cases and referents. Since the effect of low dose radiation was one of the considerations in our series of studies of haematological malignancies^{10,11} (U Flodin, unpublished observations), dose estimates for the red bone marrow with regard to xray examinations were considered and based on available estimates of radiation doses to patients.¹² The exposures were divided into three categories, light, medium, and heavy. In that part of the referent series that was directly tied to chronic lymphatic leukaemia only those examinations that took place between five and 25 years before the diagnosis were accounted for in view of latency considerations which have been discussed in one of our previous studies on myeloid leukaemia.¹⁰ For the referents originally chosen for the multiple myeloma study the corresponding "time window" was 10-30 years before diagnosis; a slight inconsistency had to be accepted for cost related technical reasons, since the exposure of the latter referents were primarily coded as optimal for multiple myeloma.

X Ray treatment for joint pain, skin disorders, and tumours, for example, also results in a dose to the red bone marrow. Such treatments were considered without any quantification, however, because the few subjects affected did not permit a useful subclassification into different exposure categories.

Exposure was considered in the same way as in a previous study on acute myeloid leukaemia¹³; estimated exposure to background radiation from the building material in the houses was categorised according to a radiation index as explained below. Only exposure that had taken place between five and 25 years before diagnosis was taken into account as with the exposure from x ray examination.

The use of a time window relates to the fact that the incidence of (myeloid) leukaemia among the Japanese A-bomb survivors seems to have increased at about five years after exposure.¹³ Further exposure considerations were based on an earlier Swedish investigation showing that the gamma radiation in wooden houses is similar in magnitude to outdoor background radiation¹⁴ (the average absorbed dose being about 1mGy or 0.1 rad/year, cosmic radiation included), whereas concrete houses seem to provide about double that exposure; plastered houses and brick houses have exposure levels in between these two extremes.

These various aspects were accounted for by a gamma radiation index that was created to make the study as sensitive as possible with regard to background radiation exposure. Hence, for every year of residence in a concrete house a person got two thirds of a point in terms of this index, whereas working in a concrete building gave one third of a point a year; this exposure was time weighted over the day. Furthermore, one "concrete point" could be obtained for every year and be cumulated from a minimum of zero up to a maximum of 20 within the time window. Three categories of the index were then chosen for analyses, a first category of 0-6.99 points, a second category of 7-13.99 points, and a third category of 14-20 points. The detailed gamma radiation classification was assessed blindly for the subjects with regard to their status as cases or referents.

Information about solvent exposure in qualitative terms was directly obtained from the questionnaires whereas the quantitative classification was referred to five categories or intensity on a judgmental basis, similarly to a classification used by Ranskov *et al.*¹⁵

Information about exposure to engine exhaust was obtained from the questionnaire as was information about smoking habits and various environmental aspects and leisure time.

STATISTICAL METHODS

The statistical analyses of the data were based on stratification and application of the Mantel-Haenszel procedures.¹⁶ The Miettinen confounder technique¹⁷ involving multiple linear regression was also used as an alternative or an additional method in the analyses. The method applied for calculating approximate confidence intervals of the rate ratios has been out-

Table 1 Status or exposure (risk indicators found for chronic lymphatic leukaemia were selected for this table) among the cases and referents with estimates of the crude rate ratio

Status or exposure	No of individual exposed or with status			
	Cases (n = 111)	Referents (n = 431)	Crude rate ratio	95% Confidence interval
Solvent exposure (intensity category 2-5 v 0-1)	18	41	1.8	1.0-3.4
Horses	19	41	2.0	1.1-3.5
DDT	6	4	6.1	1.9-19.0*
Farmers	25	59	1.8	1.0-3.1
Fresh wood (sawmill workers, lumberjacks, paper pulp workers)	13	17	3.2	1.5-6.6*
Painters	4	4	4.0	1.0-14.7
Engine exhaust, occupational	31	58	2.5	1.5-4.0*
Male sex	71	200	2.0	1.3-3.1

*Remaining risk factors ($p < 0.05$) after multiple regression analysis (cf text and equation in table 3).

lined by Miettinen.¹⁸ Multiple linear regression was also applied and based on the SPSS-X package.¹⁹ Since the study is a combination of an incidence density and a prevalence type, the estimates are referred to as incidence rate ratios rather than odds ratios although numerically the same.^{20,21} Fisher's exact test was applied in crude analyses when the numbers concerned were unsatisfactorily small for the Mantel-Haenszel chi-square test.

Results

Of the 111 cases, 71 were men, whereas of the 431 referents, only 200 were men, which reflects the well known relative excess of women in higher age groups. This gives a crude rate ratio for male gender of 2.05 (95% confidence interval, 1.34-3.14).

Several analyses were undertaken with regard to various exposures for orientation and identification of confounding factors. This was done by calculating crude rate ratios as a first step. Slightly deviating risk ratios would not, even after more sensitive analyses,

indicate significant risk factors (or preventive factors) and were therefore not further considered.

Crude rate ratios were significantly raised for those exposed to solvents in higher exposure categories, 2-5, as compared with those with low grade or no exposure. Occupational contacts with horses, DDT, fresh wood (lumberjacks, paper pulp workers, sawmill workers) were found to be associated with chronic lymphatic leukaemia as were the occupations of farmers and painters. Professional exposure to engine exhaust also appeared as a determinant of the disease (table 1).

Dwelling in a concrete building (gamma radiation category III) was not found to be a determinant for chronic lymphatic leukaemia; nor was radiological work or radiological treatment or heavy x-ray examination associated with any excess risk. Working in the plastics or rubber industries or welding did not appear to be risk factors, nor were the occupations of electrician or chemical worker associated with any significantly raised risk (cf discussion in reference to findings for myeloid leukaemia). Long term drug consumption was not seen more often among the cases

Table 2 Status or exposure (examples of non-risk indicators of some interest were selected for this table) among the cases and referents with estimates of the crude rate ratio

Status or exposure	No of individual exposed or with status			
	Cases (n = 111)	Referents (n = 431)	Crude rate ratio	95% Confidence interval
Gamma radiation category III v I	11	54	0.75	0.3-1.5
Gamma radiation category II v I	20	81	0.95	0.5-1.6
Radiological work	1	7	0.35	0.1-4.4
x-Ray therapy	4	15	1.0	0.3-3.2
Plastic/rubber/chemicals	4	11	1.4	0.4-4.5
Electricians	2	15	0.5	0.1-2.2
Chemical work	0	2	0	
Smoking	21	107	0.71	0.4-1.2

than among the referents and then was no association with smoking (table 2).

Since crude analysis might be affected by confounding effects such as the fact that the cases were older than the referents, and the sex distribution was unequal between cases and referents, and because various exposures might confound the effect of each other, further analyses were necessary. The needs for an evaluation with regard to confounding in the data between the different risk factors was based on judgments after the calculations of the crude rate ratio as shown in table 1. The subsequent use of evaluation by multiple linear regression indicated age > 60, engine exhaust, DDT, working in contact with fresh wood (paper pulp industry, lumberjacks, sawmill workers) to be pertinent risk factors ($p < 0.05$). Application of the Miettinen confounder (core technique, based on the linear multiple regression function to account for the stronger determinants of risk in this material (those factors with a lower than 95% confidence limit for crude rate ratios exceeding unity (exposure to fresh wood, age over 60, engine exhausts, DDT, solvent, fresh wood, horses, farmers, —&)) gave estimates of the Mantel-Haenszel rate ratios with a lower limit of the 95% confidence interval exceeding unity only for age over 60, engine exhausts, DDT, and contacts with horses. For fresh wood the 90% but not the 95% confidence interval exceeded unity (table 5).

Discussion

The incidence for chronic lymphatic leukaemia rises with increasing age, presumably due to some inherent factor in aging itself rather than to any particular exposure.

Wood workers in contact with fresh wood as in sawmills and paper pulp factories and lumberjacks were at a raised risk for chronic lymphatic leukaemia in this study, a finding corresponding to results reported by other authors.⁸ Milham has reported a non-significant excess of lymphatic leukaemia among paper pulp workers.²²

Soft wood is handled to a considerable extent in Sweden. Naturally occurring compounds in the wood may exert a cytotoxic effect on man, and it is known that different types of terpenes may cause irritation of the mucous membranes according to studies on workers in sawmills.²³ Nothing seems to have been described so far about the mutagenic effects from pure wood, however, and it should be emphasised in this context that according to the analyses made, the effect of creosote or wood preservatives do not confound the excess risk seen after contact with fresh wood. Working with wood that had been stored for drying was not associated with any significantly raised risk in carpenters, for example. The reason

could perhaps be that the responsible compounds in fresh wood may change their character when the wood is stored.

In this context it is interesting that an effect of soft wood bedding on the liver enzymes of rodents is known from laboratory experiments.^{24,25} This effect is strongest for red cedar but other types of soft woods also increase the activity of liver enzymes more than hard wood bedding.^{24,25} Furthermore, in some susceptible strains of mice the use of red cedar bedding significantly increased the incidence of spontaneous tumours of the liver and mammary glands.²⁶ Hence, animal studies also seem to suggest some carcinogenic activity from soft wood; further studies in this respect are certainly warranted before any more definite conclusions can be drawn.

As could be expected from the experiences of the atomic bomb survivors and other existing information no relation was found between chronic lymphatic leukaemia and x-ray therapy or heavy x-ray examination or dwelling in concrete buildings for a long period (gamma radiation category 3).¹³ To our knowledge chronic lymphatic leukaemia has never been described as being induced by ionising radiation. The negative finding in this respect seems to validate the observation of a relation between background radiation and myeloid leukaemia in our previous studies as the methodology in those studies was similar or identical to that of the present study.

Exhaust both from diesel and gasoline engines and from two stroke cycle engines appeared to be associated with an excess risk from chronic lymphatic leukaemia. Lumberjacks with exposure to two stroke engines are often farmers and therefore often exposed to the exhausts from tractors, so that some indistinguishable confounding might be involved in this respect.⁹ The observed effect from engine exhaust might well be biologically valid, however, since both gasoline and diesel exhaust contain mutagenic agents.⁹ No information about the possible mutagenicity of two stroke engine exhausts seems to have been published so far. Earlier studies dealing with the effect of engine exhaust has focused on lung cancer²⁷; no study on chronic lymphatic leukaemia as related to engine exhaust has been found.

Measurements of benzene concentrations within car cabins in city traffic have shown levels of 40 $\mu\text{g}/\text{m}^3$ of benzene in diesel car when driving, and when queuing, benzene concentrations are even higher, up to 110 $\mu\text{g}/\text{m}^3$. During forestry work with a motor saw, benzene concentrations seem to be much higher, levels up to 700 $\mu\text{g}/\text{m}^3$ (Swedish TLV = 16000 $\mu\text{g}/\text{m}^3$) have been measured.²⁸ Benzene concentrations of around 1 ppm (3200 $\mu\text{g}/\text{m}^3$) may be leukaemogenic,³⁰ but no certain effects have been seen from benzene at lower levels, so that other agents should be thought of

Status or exposure	No of individual exposed or with status			
	Cases (n = 111)	Referents (n = 431)	Mantel-Haenszel rate ratio	95% Confidence interval
Engine exhaust M	31	53	2.2	1.2-4.2
DDT M + F	4	6	6.0	1.5-23
Horses M	16	22	2.5	1.2-5.1
Fresh wood M (sawmill, paper pulp mill workers, lumberjacks)	12	16	2.4	0.97-5.0 (1.1-5.0 90% confidence interval)
Solvents M	18	34	1.7	0.8-3.6
Farmers M	25	53	1.0	0.5-2.0
Male sex	71	200	1.5	0.8-2.5

*Y_{corrected} = 0.11993 + 0.14282 (fresh wood) + 0.0694 (solvents) - 0.04494 (farmers) + 0.20652 (age) + 0.11538 (exhausts) + 0.31224 (DDT) - 0.04830 (gender) + 0.09934 (horses).

as responsible for the leukaemogenic effects of engine exhaust.

Solvents of different types have been discussed as risk factors for chronic lymphatic leukaemia. Benzene is established as a risk factor for myeloid leukaemia and there are some reports also indicating an association with lymphatic leukaemia.^{4,5} In our study two cases and two referents were clearly exposed to benzene, resulting in a crude rate ratio of 3.3, but the numbers are obviously too small to permit any conclusions.

In the cohort studies mentioned in the introduction^{4,5,7} positive correlations are described between different types of solvents and chronic lymphatic leukaemia, but no analyses have been undertaken to elucidate the effect of confounding from other possible risk factors. The lack of an elaborate analysis in these studies may therefore explain the findings of solvents in general as associated with chronic lymphatic leukaemia.

DDT is leukaemogenic in mice,³¹ and oral administration has been reported to cause lymphomas and lung neoplasms.³² Among people there is also an observation of an association between DDT and malignant lymphoma.³³

Analyses regarding associations between different types of domestic animals and chronic lymphatic leukaemia were undertaken but resulted in a positive finding with respect only to the horse. Gallo *et al* have described a viral aetiology for leukaemias among wild mice, domestic cats, cattle, and gibbon monkeys.³⁴ So far there is no definite information about the viral aetiology of leukaemia in man, however. Exposures to chemicals should not be forgotten in this context, as they occur in the maintenance of harness for honer.

Farmers have been reported to be at an excess risk from chronic lymphatic leukaemia.^{1,2} In our study contact with horses, engine exhausts, and DDT seems

to explain the excess seen in farmers. This is also reflected in the regression equation (table 3) where farmers were a negative coefficient.

In summary, this study has shown a rather complex picture with regard to various exposures which may be potentially important for the aetiology of chronic lymphatic leukaemia. Exposure to engine exhausts and contact with fresh wood appear to be convincing risk factors in view of the relatively large number of individuals reporting these exposures and there is also a relatively good biological explanation for the means by which these exposures exert their effect, even if the specific agents involved remain unidentified.

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