Childhood leukaemia in The Netherlands.

A register based epidemiologic study

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In dit proefschrift zijn gegevens bewerkt van de morbiditeitsregistratie van de Stichting Nederlandse Werkgroep Leukemie bij Kinderen. Het onderzoek is gesubsidieerd door het Ministerie van Volksgezondheid en Milieuhygiëne.
In de aanmaak en drukkosten van het proefschrift werd bijgedragen door Eli Lilly Nederland.

CHILDHOOD LEUKAEMIA IN THE NETHERLANDS

A REGISTER BASED EPIDEMIOLOGIC STUDY

(LEUKEMIE BIJ KINDEREN IN NEDERLAND. EEN – OP EEN REGISTRATIE GEBASEERD – EPIDEMIOLOGISCH ONDERZOEK).

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. J. SPERNA WEILAND
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 21 SEPTEMBER 1983 TE 15.45 UUR.

DOOR

HENRIETTE ALBERTINE VAN STEENSEL-MOLL

GEBOREN TE VLAARDINGEN

PROMOTOR: PROF. DR. H.A. VALKENBURG

to my mother

Contents

Chapter 1

Introduction

1.1 *General*

Leukaemia, literally "white blood", is the most common type of malignancy in childhood (Birch et al., 1980). The clinical features are caused by abnormal proliferation of one or more of the blood-forming cellular elements. The immature malignant cells usually disturb normal haematopoiesis and often invade other organs or tissues.

Since the 'seventies the survival of children with leukaemia, especially acute lymphocytic leukaemia (ALL), has improved and cure can be obtained in approximately 50% of the paediatric patients with ALL (Pinkel, 1976; van der Does-van den Berg, 1980). Therefore morbidity rather than mortality data are needed for epidemiological studies on aetiological factors of childhood leukaemia.

The impetus for this study was a suggestion originating in Germany, that childhood leukaemia occurs more frequently in children who lived near nuclear plants. In the Dutch Parliament questions were asked about the possible relation between proximity to nuclear plants and the development of leukaemia. In The Netherlands there are three such nuclear plants. The Dutch Childhood Leukaemia Study Group (DCLSG), in a letter to the Minister of Public Health and Environmental Hygiene, offered to initiate a study on environmental factors in childhood leukaemia, since this group maintains a morbidity register of childhood leukaemia from 1972 onwards. Subsequently the Ministry established a grant for this study (Persbericht Ministerie van Volksgezondheid en Milieuhygiene, 1979).

In view of the specific nature of the study, the Institute of Epidemiology of the Erasmus University Rotterdam, was asked to cooperate.

1.2 *Aims of the study*

The aim of the study is to investigate the incidence of childhood leukaemia in The Netherlands and to detect factors which might be of importance in the aetiology of childhood leukaemia:

1) To compare type, age and sex specific leukaemia incidence rates in The Netherlands with those in other countries and further to analyse these incidence rates according to year of diagnosis and year of birth.
2) To study the geographical distribution of childhood leukaemia in The Netherlands by calculating the incidence rates for specific geographical regions, as well as for areas with different degrees of urbanization
3) To study the seasonal incidence of childhood leukaemia by relating the incidence to the month of birth and month of diagnosis, and to search an interaction between place of diagnosis and time of diagnosis (time space clustering).
4) To study potential risk factors of childhood leukaemia by means of a case-control study.
   To this end the parental occupations during pregnancy and one year before the leukaemia was diagnosed, were analysed. Furthermore, the medical histories of both parents were evaluated one year before the relevant pregnancy.
   In addition an analysis was carried out about the mother's use of oral contraceptives, eventual miscarriages and still births, and her medical history, alcohol consumption and smoking habits during the pregnancy. Infectious diseases in the first year of life of the child and factors which might influence the infection risk profile: birth order, family size, social class and type of housing, were also evaluated.

Chapter 2

Insights into the origin of human cancer

Different human populations tend to suffer from different types of cancer: among inhabitants of third world countries the incidence of cancer of the liver is high while Burkitt's lymphoma is the most common type of malignancy in childhood. Those of the Western nations are more likely to have cancer of the breast, lung and colon. The Japanese exhibit an excess of stomach cancer and a low incidence of breast cancer. Leukaemia is the most common childhood malignancy in these developed countries (Cairns, 1981; Doll & Peto, 1981). The leukaemia-lymphoma ratio for children in Uganda was found to be 1:7, whereas for infants in the United Kingdom and the United States it is 3:1 (Olisa et al., 1975).

That this variation must be related to differences in diet, customs, environmental and socio-economical factors is based on the following observations. Migrant populations tend to take on the pattern of cancer that is characteristic of their new environment (Reif, 1981). Among black Americans, for example, the cancer incidence rates generally resemble those of white Americans much more than those of the black population in West Africa. For every type of cancer (except that of the lung), the rates for Japanese migrants in Hawaii are closer to those for the Caucasian residents than those for the inhabitants of Japan. Support for the influence of some environmental factors on childhood malignancies might be provided by the change noted in the leukaemia-lymphoma ratio for a single ethnic group subjected to profound socio-economical improvements (increase in leukaemia incidence and decrease in lymphoma incidence; Ramot & Magrath, 1982).

On the other hand, the rare occurrence of Ewing sarcoma in (American and African) black children compared to the incidence among whites might indicate, that genetics influence susceptibility to this special type of malignancy (Miller, 1977).

Further evidence for the role of environmental factors is the change

in cancer incidence from one generation to the next. Furthermore, members of religious groups with a specific life style, e.g. Mormons and Seventh Day Adventists, exhibit lower incidence rates for cancer of the respiratory, gastro-intestinal and genital systems (Doll & Peto, 1981).

Experimental carcinogenesis as well as genetic research has provided some insights into the mechanism of cancer development. Various agents have been shown to be carcinogenic in experimental animal studies:

- Physical or chemical agents that might damage DNA (Della Porta et al., 1979; Terracini, 1980).
- Retroviruses, which have been identified as a factor in the pathogenesis of many naturally occurring leukaemias and lymphomas in several animal species (Gallo & Wong-Staal, 1982).

Observations in the past had already suggested a relation between human cancers and chromosomes. Chronic myeloid leukaemia was the first malignant disease discovered to be associated with a consistent chromosome marker, the Philadelphia chromosome (Nowell & Hungerford, 1960).

For the childhood malignancies, Wilms' tumour and retinoblastoma, a hereditary and a non-hereditary form have been suggested. The hereditary form occurs in younger children and is usually bilateral. As a rule, an autosomal dominant inheritance pattern is observed (Knudson, 1978; Breslow & Beckwith, 1982). Knudson (1976) described a two-step mutation hypothesis for these childhood malignancies.

Bloom's syndrome, Fanconi's anaemia and ataxia-teleangiectasia — three so-called chromosomal-breakage syndromes — represent genetic conditions in which an increased risk of cancer (especially leukaemia and lymphoma) as well as increased chromosomal instability exists (Fraumeni & Miller, 1967; Ray & German, 1981).

Increasing knowledge of molecular genetics, together with the arrival of new techniques, led to rapid advances in the understanding of the role of chromosomes in cancer (Logan & Cairns, 1982).

That the oncogenic viruses of animals bear relevant information about carcinogenesis in man, has recently become true. Some RNA tumour viruses contain specific genes, responsible for the tumourigenic properties (viral oncogenes), which have been shown to

be homologous to DNA sequences in normal vertebrate cells (Bishop, 1983).

Other research groups have isolated oncogenes from human tumour cell lines with unknown or unsuspected viral aetiologies. They found that DNA fragments from human tumour cells were able to transform some non-neoplastic cultured mouse cells, giving rise to tumours. These malignant cells contained an oncogene which proved to be the cellular oncogene identified by tumour virologists (Weinberg, 1982).

Further research revealed that oncogenes are present in normal cells and regulate cell differentiation and proliferation. Any disturbance of this system might cause abnormal cell growth (Bishop, 1983).

The function of oncogenes can be modified by three mechanisms which might lead to neoplastic cell transformation.

1. Retroviruses:

Acutely transforming retroviruses contain in the genome a cellular oncogene which, because it is under the control of the viral promotor of transcription, is inevitably transcribed at too high a level.

However, chronically transforming retroviruses, which do not have such an oncogene integrated in their own genome, appear to exploit cellular oncogenes. They provide a strong promotor when the virus becomes integrated next to one of the cell's own oncogenes. This is a much less efficient route to oncogenesis and usually succeeds only after a long time interval. The latter possibility is suggested for human T-cell leukaemia-lymphoma and some other T-cell malignancies (Gallo & Wong-Staal, 1982; Bishop, 1983).

2. chromosomal abnormalities (deletions or translocations):

A chromosomal deletion has among others been found in subgroups of patients with retinoblastoma or Wilms' tumour.

In Burkitt's lymphoma and chronic myeloid leukaemia, a tumour-specific translocation between two chromosomes is found. Chromosomal breakage is on the side of an oncogene (Forman & Rowley, 1982; de Klein et al., 1982). For Burkitt's lymphoma it is suggested that the level of expression of the transforming oncogene is affected by the neighbouring active immunoglobulin genes.

In general it is assumed that by translocation the oncogene is "activated" to contribute to the neoplastic change in the cell.

3. Point mutations:
Cells derived from bladder carcinoma, have been found to contain an oncogene which only differs from the naturally occurring gene in the replacement of one specific nucleotide in the DNA sequence by another (Tabin et al., 1982).

Recent developments in genetics provide some insights in the mechanism of neoplastic cell change.

These new insights have made it possible to visualize carcinogenesis by mutagens and viruses in the same basic conceptual framework. The role of oncogenes in malignant transformation might be based on either increased production of certain gene products or production of abnormal gene products.

However, the provision of an active oncogene presumably corresponds to only one of a sequence of steps (Gilbert, 1983).

In an empirical way, epidemiologic studies have given insights in some determinants of cancer. Incidence and analytical studies have established that environmental factors are associated with the occurrence of some malignancies.

It must be kept in mind that natural human carcinogenesis is a multistage process. It is not known what the rate-limiting steps are, nor which of them are most sensitive to environmental influences (Logan & Cairns, 1982).

Chapter 3

An epidemiologic approach to childhood leukaemia in The Netherlands

3.1 *Description of childhood leukaemia in The Netherlands*

3.1.1 *Childhood leukaemia register*

The rarity of childhood leukaemia may constitute a major obstacle to a study of this disease by means of classical epidemiological methods. Large population samples are required to investigate changes in incidence over time and geographical location. Follow-up studies, which establish the incidence of the disease among exposed and non-exposed persons, require a very large group of subjects under study or a long period of follow-up to detect the occurrence of low-incidence diseases, such as childhood leukaemia (incidence rate 3.1 per 100,000 person years).

Furthermore a large childhood population is needed to select a

sufficient number of patients with leukaemia for a case-control study. In this type of study the occurrence of potential risk factors among patients and controls is evaluated.

The development of cooperative treatment trials for childhood leukaemia led to the establishment of a national register of this disease in The Netherlands (Dutch Childhood Leukaemia Study Group). This provided the opportunity to carry out a nation-wide descriptive and analytical epidemiological study. In the descriptive part of this investigation the occurrence and distribution of childhood leukaemia within the population of The Netherlands is studied. The analytical part focuses on possible determinants of the disease.

The register of the Dutch Childhood Leukaemia Study Group (DCLSG) laid the basis for this study. It is unique because it is a nation-wide morbidity register, with an over 95% coverage of patients. Furthermore, the cytomorphological diagnosis of all patients recorded in the register is based on histological examination of bone marrow slides by two independent experts who applied previously determined criteria. Nearly 160 paediatricians in The Netherlands collaborate in the DCLSG in an effort to optimize the treatment of children with leukaemia. They routinely send the blood and bone marrow slides of each child with leukaemia or under suspicion of this disease to the DCLSG.

The leukaemia patients who were accepted for this study were all < 15 years at the time of diagnosis, i.e. between January 1st, 1973 and January 1st, 1980 and the diagnosis was confirmed by histological examination of bone marrow slides at the DCLSG laboratory. In different studies on the incidence of childhood neoplasms, histological confirmation of the malignancies (all types) has been reported to vary between 86% and 95% (Teppo et al., 1975; Ericsson et al., 1978; Pastore et al., 1981; McWhirter & Bacon, 1981). However, the percentage of available diagnostic bone marrow smears among leukaemia patients may have been lower. A review of the histological material is presented only in the report on the Manchester Children's Tumour Register (94% of all types of malignancies) and by the Swedish Childhood Leukaemia Group (Birch et al., 1980; Gustafsson & Kreuger, 1982). However, for the Manchester Register bone marrow reports by hospital haematologists were also accepted as proof of the diagnosis of leukaemia.

The completeness of the Dutch register was checked by a questionnaire mailed to all paediatricians in The Netherlands; the

response rate was 92.6%. Seventeen children were reported who had leukaemia but were not listed in the register. The years, in which the diagnosis was established for these children, were nearly equally distributed over the 7 years of the study. For the various childhood cancer registers a coverage varying between 80% and 98% is reported. This was often checked, although not for all studies on the incidence of leukaemia (Pastore et al., 1981; Young & Miller, 1975).

Childhood cancer registers in Finland, the United Kingdom (Manchester Children's Tumour Register) and Australia were checked for completeness either by comparing the number of deaths recorded per year in the register with the corresponding figures from a (national) mortality register (Teppo et al., 1975; McWhirter & Bacon, 1981; Stiller & Draper, 1982) or by comparing the data from the register with those from hospitals and other regional cancer registers (Leck et al., 1976).

In conclusion, the morbidity register of the DCLSG forms an unique basis for an epidemiologic study.

3.1.2. *Incidence (addendum 1)*

In The Netherlands no incidence rates for childhood leukaemia were available.

Differences in the incidence of childhood leukaemia among different countries might provide some indication of aetiological factors. Trends in incidence might be associated with trends in environmental or socio-economical factors.

The overall incidence of childhood leukaemia in The Netherlands (3.11 per 10^5) is comparable to that reported for the Manchester Region. Higher rates, however, were observed in the United States, Finland, Sweden and Australia (Young & Miller, 1975; Teppo et al., 1975; Ericsson et al., 1978; McWhirter & Bacon, 1981). These differences may reflect the use of stricter criteria in The Netherlands and in Manchester.

In the present study the incidence of acute lymphocytic leukaemia (ALL) was calculated to be 2.56 per 10^5 while for the Manchester Region a rate of 2.61 per 10^5 was found. ALL is the most common type of leukaemia in childhood (82.4%), while acute non-lymphocytic leukaemia and chronic myeloid leukaemia account for 13.6% and 2.9%, respectively.

In accordance with other studies, the overall male/female ratio for ALL in the study period (1973 – 1980) was 1.2. For the years 1979 and

1980, however, a reversal of the sex ratio was found: 0.8 and 0.9, respectively. Since this ratio varied between 1.1 and 1.6 in the preceding years and proved to be 1.2 for 1981, the reversal of the sex ratio for 1979 and 1980 remained an unexplained transient finding.

The occurrence of a peak incidence of ALL in the age group 3 – 4 years was also observed in this study. This might indicate that the prenatal period or early infancy is an important period in the aetiology.

The overall incidence of childhood leukaemia in The Netherlands during the period of study did not change with time. However, the study covered only seven years. Time trend analyses were also performed for subgroups of the patients based on type of leukaemia, age and sex. Only the incidence of ALL for girls showed a borderline significant trend due to a single high value in 1979 (3.56 per 10^5). In contrast, the incidences in 1980 (3.05 per 10^5) and 1981 (2.57 per 10^5) were lower than in 1979.

In the Manchester Region and in the U.K. as a whole an increase in childhood leukaemia, especially in boys aged 1 – 4 years, was observed (Birch et al., 1981; Stiller & Draper, 1982). A recent report from Sweden described a higher overall incidence of leukaemia in the period 1975 – 1980 (4.5 per 10^5) compared with the period 1958 – 1974 (3.9 per 10^5; Gustafsson & Kreuger, 1982). The authors observed a slightly lower male/female ratio for the more recent period (1.1 for all leukaemias). Thus in contrast to the British data, their finding might be due to an increase in the incidence of leukaemia among girls.

In The Netherlands no trend in incidence with respect to the year of birth was found. This analysis, which was based on very small groups of patients per year of birth (1958 – 1979), therefore does not reflect the increasing incidence of leukaemia established for British children born after 1964. The increase in the U.K. was most pronounced for boys aged 0 – 4 years, although the same trend was observed for girls.

The difference in the trend for childhood leukaemia observed in the U.K. (1968 – 1978) and Sweden (1958 – 1980) may reflect changes in the registration process itself: improved standards of diagnosis, improvement in the readiness of physicians in a given area to provide data for the cancer register or changes in the definition of what constitutes leukaemia (Doll & Peto, 1981). On the other hand the trend may be real. Arguments for the first possibility might be the lack

of homogeneity in diagnostic criteria and in the completeness of the register over the whole period. Furthermore, diagnostic improvements in this period may have influenced the quality of the register. Therefore, it is important to follow the incidence of childhood leukaemia in The Netherlands, since the existing register was set up according to standardized administrative and diagnostic procedures.

3.1.3 *Geographical distribution (addendum 2)*

The geographical distribution of childhood leukaemia was evaluated to detect those areas with a high or low incidence of leukaemia which could possibly be associated with environmental factors.

The classification into geographical areas was based on administrative units: provinces (n = 11) and the smaller COROP-areas (n = 40). Provinces are comparable with counties. The division into COROP-areas is based on sociological, administrative and geographical factors. These units are not necessarily homogeneous as far as demographic or environmental factors are concerned. Furthermore, we were compelled to analyse rather large areas, since the nominators for leukaemia are small.

The distribution of leukaemia (all types and ALL) according to residence at the time of diagnosis as well as birthplace was evaluated. In the province of Drente (in the North) significantly fewer leukaemic patients (all types) were born when compared with the national incidence, while significantly more patients with ALL lived in the province of Overijssel (a mid-eastern province) at the time of diagnosis.

As already mentioned, provinces are large areas that are heterogeneous as far as environmental factors are concerned. The patients from Overijssel or Drente did not differ from the other children with leukaemia with respect to age at diagnosis or sex distribution. The patients from the province of Drente did not differ from the other patients with regard to the type of leukaemia.

The ALL incidence for the different COROP-areas at the time of diagnosis or at birth varied between 0.89 and 4.82 per 10^5 and between 0.89 and 4.26 per 10^5 child-years, respectively. Only in the COROP-area east-Groningen was found a significant increased ALL incidence (4.82 per 10^5). In this area 12 patients with leukaemia were diagnosed; they did not differ with regard to age and sex from the other ALL patients.

In Sweden two of the 24 counties showed an increased morbidity

rate for leukaemia. In one of these counties a cluster in a small community was noted, which remained unexplained (Gustafsson & Kreuger, 1982). A leukaemia mortality study in the U.S.A. revealed high incidence counties for children (< 20 years) and adults. When compared with all other U.S.A. counties, the high-rate counties (all ages) tended to be more urban and to contain higher socio-economic levels and more subjects who worked in the food-processing, printing, metal products and machinery industries. This geographical pattern of leukaemia may be explained by variations in both diagnostic modes and the quality of medical care (Blair et al., 1980). The geographical variation in the mortality rate for leukaemia (all ages) in the U.S.A., however, is more limited than that seen for several other malignancies (Blot & Fraumeni, 1982).

We also analysed the incidence of leukaemia in relation to the degree of urbanization, since it is assumed that urban and rural environments might be associated with different potential hazards. For this analysis a classification into three degrees of urbanization (rural, intermediate urban, strictly urban) was used. The municipalities with the same degree of urbanization were combined, so that a substantial number of patients per category was available for analysis.

An increasing incidence for the heterogeneous category of all of the leukaemias with increasing degree of urbanization was found. For ALL no trend was seen, although the incidence in urban areas with more than 100,000 inhabitants (3.2 per 10^5) was slightly higher than the rate for rural areas (2.8 per 10^5).

A higher incidence of leukaemia in urbanized areas is in accordance with some studies (McWhirter & Bacon, 1980; Blair et al., 1980). A mortality study of leukaemia according to type in the U.S.A., however, revealed no urban versus rural difference in mortality for children with leukaemia (Selvin et al., 1983).

If high and low incidence areas are determined, it is of interest to evaluate geographically related factors by means of a case-control study. However, in our study the cases were matched with the controls according to geography for practical reasons.

3.1.4 *Time space clustering (addendum 3)*

The occurrence of time space clusters of childhood leukaemia might indicate that the disease of these patients has the same origin.

Studies of time space clustering of lympho-proliferative

malignancies were particularly popular in the 'sixties after animal studies had established that leukaemias and solid tumours in mice could be induced by viruses (Caldwell & Heath, 1976). At that time statistical techniques were developed to analyse time space clustering (Knox, 1964; Mantel, 1967; Ederer et al., 1964). Most of the cluster analyses of childhood leukaemia were based on mortality data (Till et al., 1967; Gunz & Spears, 1968; Stark & Mantel, 1967; Smith et al., 1976; Klauber & Mustacchi, 1970) and a few made use of data taken from cancer registers and/or hospital records (Knox, 1964; Lock & Merrington, 1967; Ederer et al., 1964). Since leukaemia was almost always fatal in that period, mortality data could be considered in theory to reflect the incidence of the disease fairly well. It should be noted that the validity of mortality data depends on the diagnostic procedures preceding death and eventual post-mortem examination. Some of the data in the above mentioned literature stem from the 'forties and early'fifties. It could be true that in this period infectious diseases masked the leukaemia in some cases.

Since morbidity data were available in The Netherlands, a search for time space clustering was undertaken. Furthermore, recent virological, serological and epidemiological findings suggest that some adult T-cell malignancies are associated with a RNA tumour virus (human T-cell leukaemia/lymphoma virus; Catovsky et al., 1982).

In general two approaches for the detection of clustering are available: the "cell occupation method" and the "interval method". In the first method units of space function as "cells", in which a random distribution of the cases over time, is tested. The second method uses time and distance intervals between all possible pairs of cases. The hypothesis that pairs of patients who are close in time are also close in space is tested. The major method employing this latter approach is that developed by Knox and modified by Mantel. The interval methods have proven to be more useful and reliable than the "cell occupation method", primarily because they are not as strongly influenced by population shifts and socio-economic differences that might be associated with time or space units and might lead to spurious patterns of case clustering. Therefore, our data for patients with childhood leukaemia in the Western part of The Netherlands were analysed for clustering in time (date of diagnosis) and space (place of diagnosis) using both Knox's and Mantel's method.

The following subgroups of patients were analysed: all leukaemias, ALL, ALL in boys and girls separately, ALL in children under 6 years

of age at the time of diagnosis, ALL $\geqslant$ 50,000 leucocytes/mm^3 initially and acute non-lymphocytic leukaemia. With the method of Knox we only found clustering for children with ALL and those with a high initial leucocyte count ($\geqslant$ 50,000/mm^3). This subgroup was analysed separately since the prognosis and history of these children with a high tumour load differs from that of children with a lower initial leucocyte count (Simone et al., 1975). This might suggest that this group of children constitutes a separate entity. However, with Mantel's method (a time versus space regression analysis) no clustering was found. Knox's analysis is an exploratory statistical method, which applies varying critical time and distance intervals. These time and distance intervals are arbitrary chosen. With this method a total of 360 analyses was conducted, so our finding could be due to a chance event. For that reason, Mantel's cluster analysis is to be prefered over Knox's method.

The studies of time space clustering in childhood leukaemia have either produced negative findings or weak positive findings (Caldwell & Heath, 1976). However, long and varying latency periods could mask clustering.

In the present study no seasonal incidence of leukaemia, classified according to type, age, sex and tumour load (initial leucocyte count), was found. This is in accordance with most previous studies (Gustafsson & Kreuger, 1982; Till et al., 1967; Gunz & Spears, 1968). However, interpretation of seasonal analyses of the months of diagnosis is rather difficult since the moment of diagnosis (in this study: the date the diagnostic bone marrow smear was taken) is not the exact moment of disease onset.

Finally, studies on the incidence and geographical distribution of childhood leukaemia can only suggest correlations between environmental factors and the occurrence of leukaemia. Furthermore, as to the precise factor, this is all a matter of speculation since individual exposures are unknown. For that reason, descriptive epidemiological studies can only provide suggestions for future analytical studies. Lastly, multicausality or multistaging of the disease constitutes another problem in tracing the role of aetiological factors.

In the present study neither a clear trend in incidence, nor pronounced geographical differences in incidence that correlated with environmental or demographical factors, nor time space interactions were found.

3.2 *Case-control study*

3.2.1 *General aspects of the case-control study*

Case-control studies are frequently termed retrospective studies because one is looking back from disease to exposure. In such studies, persons with the disease of interest are selected as "cases" and persons without the disease as "controls". A number of exposures in relation to the disease are generally evaluated.

This study was designed as an exploratory investigation into possible causes of childhood leukaemia. For that reason an extensive questionnaire was designed to collect information on exposure to potential risk factors as well as data on potential confounding variables. Two objectives were covered by this study design; firstly quantification of the factors, both new and those already known from the literature, which may play a role in the development of leukaemia in children and secondly more specific leads to the causal pathways in childhood leukaemia and to the design of future studies.

The patients were drawn from the complete nation-wide morbidity register of the DCLSG. Therefore, selection bias seems unlikely. It should be noted that in a small number of cases, patients with mediastinal enlargement and leukaemic infiltration of the bone marrow were diagnosed as having a lymphoma in one paediatric centre, while all other paediatrician-oncologists diagnosed leukaemia and reported these patients to the register.

Since the patients were derived from the total Dutch childhood population, we decided to select the controls from the same population. It would have been preferable to draw a random sample of all Dutch children. However, in The Netherlands no general register for the whole country exists: citizens are obligated to register in the place of residence. Therefore, the controls were matched with the cases according to residence (municipality) at the time of diagnosis. Age and sex were also used as criteria for matching, since a predominance of leukaemia in boys is known and nearly 50% of the children with leukaemia are younger than 5 years at the time of diagnosis. Consequently, the controls were randomly drawn from the available group of children with the same date of birth (within 2

months) and sex who lived in the same municipality as the patients at diagnosis.

Self-administered questionnaires were mailed to the parents. Advantages of this approach are the elimination of interviewer bias, the lower costs and reduction in time, and the possibility of more conscientious answers from the respondent, who could complete the questionnaire at leisure. Most of the questions were close-ended (yes or no answers) for greater precision, uniformity and easier coding and tabulation of the responses. Open-ended questions require recalling and explanation, whereas close-ended questions demand only recognition (Stolley & Schlesselman, 1982). The open-ended questions in this study nearly always concerned part of a close-ended question (example: "were you hospitalized during pregnancy?" yes/no. "If yes, could you give the reason for hospitalization?"). Of course questions concerning date of birth, birth order, family size, etc. were open-ended questions. The questions were formulated in simple phrases and the different time periods for the respective questions were clearly indicated.

Since the pilot study did not reveal insurmountable difficulties the investigation was conducted in the following way.

The attending paediatricians were given the opportunity to inform the parents of their patients about the study. They received a list of the parents, who were to be approached one month later. Over 50% of the paediatricians contacted the parents. This might have introduced response bias; however, the response rates for informed and non-informed parents were similar (addendum 7).

The covering letters (addendum 8D & 8F), which accompanied the questionnaire, were slightly different for cases and controls. In the letter to the parents of the patients the objective of the study was described, i.e. to investigate possible causes of childhood leukaemia. For the controls the objective of the study was stated in a more general way, namely a study into the possible causes of serious chronic illnesses in childhood. Otherwise, the letters to the parents of patients and controls were similar. The questionnaire for the case child covered some general variables as well as the first year of life and the period before diagnosis; for the controls the questions covered a comparable period (= age of the patient at diagnosis). We found that among the controls the questions concerning this latter period were more frequently answered in the affirmative. This phenomenon was noted for all kinds of questions concerning the control child and their sibs

(childhood diseases, traumas, schooling, hospitalization, etc.) Exposure misclassification is a likely explanation for this finding: for the control child exposure to a risk factor extended over a longer period, i.e. until the moment of this study. Therefore, this section of the questionnaire was not evaluated.

Since much information was collected, emphasis on specific hypothetical questions was minimized (Monson, 1980).

A method for checking on the consistency of response is to repeat the questions in a slightly different manner at different places in the questionnaire (Stolley & Schlesselman, 1982). For example, in the questionnaires for both the mother and the father we asked about pets in the year preceding pregnancy. In more than 90% an overlap in the answers was found. The answers to the questions about whether the parents were related given by the mothers and fathers agreed. The answers to the open-ended question "did you use drugs during pregnancy" and the close-ended question "did you use drugs for threatened abortion" were in accordance with one another. Only once there was a discrepancy between the answers to the open-ended and close-ended question.

Response bias can influence the results of the study. The response rates for the parents of patients and the parents of controls were 88% and 70%, respectively (addendum 7). Differences in response rates between cases and controls are a common finding of studies using population controls. In most studies, as in ours, second controls are included to enlarge the reference group. The reasons generally given for the lower response among population controls are that they are less interested in the investigation and not as involved in the study (Cole, 1979). However, it should always be realized that we do not in fact know the reason for non-response. Furthermore, it is still unknown whether the response is related to differential exposure. The response of the parents of deceased children was slightly lower (84%) compared to that of parents of children who were off treatment (91%). The reaction of the parents of the patients to our study was overwhelmingly positive. This was expressed in the high response rate, the remarks on the questionnaire, the telephone calls and the letters (addendum 7).

From data of case-control studies an association between disease and exposure can be expressed in the relative risk (RR, rate ratio). For the cases one can calculate the exposure odds: exposed cases/ non-exposed cases; likewise one can calculate the exposure odds for the controls. The exposure odds ratio ((exposed cases/non-exposed

cases)/ (exposed controls/non-exposed controls)) is an approximation of the relative risk. Since the cases and controls were matched for age and sex, the RR was computed over sex and three age strata by means of the method of Mantel-Haenszel. In order to adjust for the possible confounding effects of several factors simultaneously, multiple logistic regression analysis was applied to some of the risk factors (Breslow & Day, 1980).

3.2.2 *Parental factors (addendum 4 & 5)*

Since transplacental carcinogenesis is observed in animals and an increased risk of malignancies was found in humans after in utero exposure to diethylstilboestrol or diagnostic radiation, exposure to various potential risk factors during pregnancy was evaluated in this case-control study. In addition, the period one year before pregnancy was studied because it covers the period of maturation of the germ cells and the conception.

Since it has been suggested that children whose parents work with toxic materials may suffer adverse health effects when exposed to their parents' soiled clothing (Chrisolm, 1978), the occupations of both parents in the arbitrary chosen period "one year before diagnosis" were evaluated.

One study suggested a relation between "hydrocarbon-related" occupations of the father at the time of birth and childhood leukaemia (Fabia & Thuy, 1974). However, other studies could not support this finding (Sanders et al., 1981). The occupation of the mother during pregnancy has rarely been evaluated.

In our study the parents listed all occupations as well as the periods in which they worked in those fields. The jobs during pregnancy and one year before diagnosis were coded according to a generally accepted code book by trained personnel. The "hydrocarbon-related" occupations were those defined by Zack et al. (1980).

During pregnancy more mothers of patients had "hydrocarbon-related" occupations (relative risk (RR) = 2.5; 95% confidence interval (CI) 0.7 − 9.4). This association was not found for maternal jobs one year before diagnosis nor for paternal jobs in either period. The standardized close-ended questions concerning different occupational exposures to potential risk factors supported our former finding: significantly more mothers of patients reported occupational exposure to chemicals (paint, petrol products and other unspecified chemicals). The RR was 2.4(95% CI 1.2 − 4.6). For the fathers this association was

not found, which is an argument against systematic overreporting by the parents of children with leukaemia. Another check on bias was a comparison of the job distribution between cases and controls within the category of exposure to chemicals. Only nurses were more frequently reported among the case mothers in the category with chemical exposure, while the opposite was found for the category without chemical exposure. We have no ready explanation for this finding, except a bias of these women who are more knowledgeable about medical affairs. No other job categories exhibit such a phenomenon. Since more mothers of patients worked outside the home during pregnancy, the RR for "hydrocarbon-related" occupations was calculated relative to the other jobs outside the home. The RR was 2.0; 95% CI 0.5 – 7.6. The RR for exposure to chemicals relative to the job-category without chemical exposure was 2.0 (95% CI 1.0 – 4.2).

In this study the RR for oral contraceptive (OC) use in the entire period preceding pregnancy was 1.2 (95% CI 0.9 – 1.6). The duration of use exhibited no correlation. Of the mothers of patients and controls who had ever used oral contraceptives, 74% and 70% respectively, reported OC use in the year before pregnancy. The period between discontinuation of OC and pregnancy was slightly longer for the mothers of patients. This might suggest decreased fertility. However, the use of other methods of contraception in this period was not studied.

A history of two or more miscarriages was reported significantly more frequently by mothers of patients (RR = 1.6; 95% CI 1.0 – 2.7). Since the number of deliveries, maternal age and social class can influence the number of miscarriages, we adjusted for these variables in the logistic model (RR = 1.6; 0.9 – 2.8). An increased RR (2.0; 95% CI 0.8 – 5.2), although based on small numbers, was also found for a history of two or more miscarriages in the restricted period preceding the pregnancy. This finding supports the fact that the mothers' answers were consistent.

One year before pregnancy nearly three times as many case mothers as control mothers used "hormones" for non-contraceptive purposes. The difference is due to the use of clomiphene and "preparations to induce pregnancy" - probably clomiphene (5 cases versus 0 controls). Hospitalization or specialist consultation for fertility problems (RR = 6.0; 95% CI 0.9 – 38.2) or miscarriages and curettages (RR = 1.8; 95% CI 0.7 – 5.0) in this period were also

reported more often by the mothers of patients. However, these findings are based on low exposure frequencies.

During the relevant pregnancy "drugs to maintain pregnancy" and threatened abortion showed a significant RR of 1.9 (95% CI 1.0 – 3.5) and 1.6 (1.0 – 2.6), respectively. Consistent with these findings is the increased RR for complications of pregnancy which required hospitalization or specialist consultation.

Hormonal treatment before and during pregnancy was adjusted for maternal age and number of preceding miscarriages. For the former oral contraceptive use and for the latter hormonal treatment before pregnancy were also included as indicator variables in the logistic model. The RR for hormonal treatment before pregnancy decreased slightly to 2.6 (95% CI 0.7 – 9.7) after adjustment. The RR for hormonal treatment during pregnancy increased to 2.6 (95% CI 1.2 – 5.5) when hormonal treatment one year before pregnancy was included in the model in addition to maternal age and preceding miscarriages.

Since neither all diseases which required hospitalization or a specialist consultation nor all types of drugs were reported more frequently by the mothers of patients, systematic overreporting only seems an unlikely explanation for our findings.

Studies on chromosomal abnormalities in the embryos of mice and rabbits after superovulation have yielded conflicting results. The same applies for investigation into the correlation between subfertility in women and congenital defects in the offspring (IARC, 1979).

It has been reported that the use of hormones during pregnancy might be associated with childhood malignancies in general (Kinnier Wilson et al., 1981). However, another case-control study did not find this association (Teppo et al., 1975). In accordance with our study, the Oxford Childhood Cancer Survey (OCCS) found that threatened abortion during the relevant pregnancy was related to case-control status (Stewart et al., 1958).

A leukaemogenic effect of low-dose radiation has been confirmed in animal studies and in studies of radiologists, adults irradiated for ankylosing spondylitis and children prenatally exposed to X-rays (Miller, 1972). In our study a significantly increased relative risk for prenatal X-ray exposure (RR = 2.2; 95% CI 1.2 – 3.8) was found. This confirmation of a previously established relation between childhood leukaemia and prenatal irradiation might be considered as an indication of the validity of our study and its comparability with other

investigations. The suggestion that the increased RR for diagnostic radiation is based on biased reporting was rejected by the retrospective OCCS, since a check on the X-ray exposures listed in the medical records still revealed an increased risk (Hewitt et al., 1966). Evidence for a possible causal association is the dose-response relation (Bithell & Stewart, 1975) and confirmation of the increased risk in a large prospective study (MacMahon, 1962). In a recent report the increased RR for prenatal X-ray exposure seems to be associated specifically with leukaemia and not with other childhood malignancies (Miller, 1982).

Our study revealed no correlation for the frequency of alcohol consumption, smoking habits, quantity of cigarettes and viral infections in the year before the pregnancy for either the mother or the father. Exposure to these risk factors during pregnancy also showed no relation with the case-control status of the mother.

3.2.3 *Infectious diseases in the first year of life of the child (addendum 6)*

We studied the occurrence of infections in the child since studies in adults have suggested a relation between neoplasms of the lymphoproliferative system and immunosuppression (Kinlen et al., 1980). Furthermore, patients with primary immunodeficiency diseases are at increased risk for leukaemia and lymphoma (Kersey et al., 1973). This latter observation has also been reported for children with ataxia teleangiectasia, Bloom's syndrome and Fanconi's anaemia. In these children increased chromosomal breakages have been observed. Furthermore, dysfunction of the immune system is nearly always present (Ray & German, 1981).

The first year of life of the child may be relevant since in this period a transition from passive to active immunity takes place and the first contacts with many antigens occur.

In the present study an increased incidence of infections, such as common colds, periods of fever and primary childhood infections as well as infections which required hospitalization or specialist consultation, was reported for the controls. The most frequent reasons for specialist care were pneumonia, otitis media, meningitis, urinary tract infections and sepsis. The general infection risk profile (described by birth order, family size, social class, number of rooms in the house) also differs between cases and controls and is compatible with the former. The lower frequency of infections found for patients

contradicts our expectations, namely a higher infection rate if a general immunodeficiency plays a role in the development of leukaemia. We considered the possibility of bias in the information on infectious diseases. A systematic overreporting of all diseases by the parents of controls due to misclassification over time or an underreporting of diseases for the child with leukaemia seems unlikely since other disorders, such as exchange transfusions (3 cases/0 controls), congenital defects (26/19) and seizures (4/0), were reported more frequently for the cases. Furthermore, from another source, the incidence of urinary tract infections and sepsis in the neonatal period (28 days old) was calculated to be $10-13$ and $1-5$ per 1000 life births, respectively (Glasgow & Overall, 1979). For the 500 controls in this study the incidence was 5 and 1, respectively, which is an argument against overreporting due to misclassification.

We assume that the accuracy of the mothers' memory with respect to infections might be supported by the fact that in The Netherlands routine vaccination of the infant is delayed when (mild) infections are present.

In reviewing the literature as related to our findings, three lines of thought emerge:

1. Children that are likely to develop leukaemia die in their first year of life due to infections. This could be caused by the underlying leukaemia or by an impairment of the immune system. Considering the improvement in antibiotic treatment over the past 20 years, this explanation seems unlikely.
2. Decreased stimulation of the immune system or postponement of infections until a later age - possibly due to better infant care - might play a role in the development of leukaemia. Evidence - although poor - for this suggestion is the finding of a low incidence of infections and a high incidence of leukaemia in the developed countries in contrast to the high mortality rate for infections and the low mortality rate for leukaemia in the underdeveloped countries. However, the low incidence of leukaemia for young adults compared to that for childhood does not corroborate this suggestion.
3. Infections might play a modulating role in the development of malignancies of the haematopoietic and lymphatic system. The OCCS reported a higher frequency of infections in the four or more years before death for children with lymphoma than for children with leukaemia (Kneale & Stewart, submitted for

publication). Furthermore, Burkitt's lymphoma occurs mostly in areas with holoendemic malaria; in these same areas leukaemia is very rare. The massive antigenic load of malaria might suppress the (auto) immune system (Greenwood et al., 1972) and might influence the development of Burkitt's lymphoma.

3.3 *Weighing the results of this case-control study and suggestions for future studies*

The increased relative risks found in this study of leukaemia are based on very low exposure frequencies and might therefore possibly offer an explanation for the occurrence of leukaemia in a few patients only. It should be realized, however, that the overall incidence of a disease in a population is a function of the number of causal pathways leading to that disease, as well as the rates at which they operate. The ability to detect a risk factor that is a component of one causal pathway to disease, is diminished by the presence of other causal pathways in which that particular risk factor plays no role (Weiss & Liff, 1983). The apparently homogeneous disease ALL might be rather heterogeneous when clinical manifestations, prognosis and the cytomorphological, chromosomal and immunological characteristics of the leukaemic blasts are considered. This might be a reflection of the actions of different causal pathways.

These new insights into the heterogeneity of the disease leukaemia are being developed rapidly, but for this study only the morphological categories ALL, acute non-lymphocytic leukaemia and chronic myeloid leukaemia could be considered separately. For this reason, the results presented are restricted to the still rather "heterogeneous" category ALL, which accounts for 82% of all leukaemias. Furthermore, environmental factors might be one of a sequence of steps which play a role in cancer development. Considering the restrictions of our case-control study, the following suggestions could be made.

- Radiation and occupational exposure to chemicals in the prenatal period give an increased relative risk for leukaemia in the child concerned. In animal experiments the mutagenicity of radiation and certain chemicals has been demonstrated. Since exposure to these agents does not always lead to the development of leukaemia,

host factors, promotor or other factors probably also play a role. As already established from other epidemiological studies and laboratory experiments, natural carcinogenesis is a multistage process (Logan & Cairns, 1982).
Analysis of the interaction of variables, which is an evaluation of the effect of the simultaneous occurrence of two risk factors, revealed a not-significant doubling of the risk of leukaemia only for the combination of a history of two or more miscarriages and prenatal radiation (RR = 4.0; 95% CI 0.5 – 30.7).

- The more frequent occurrence of habitual abortion, subfertility, clomiphene treatment, use of "drugs to maintain pregnancy" and threatened abortion among the mothers of children with leukaemia seem to indicate a similar causal pathway.
  This finding might suggest a role for chromosomal or gene abnormalities. In 50% of the spontaneous abortions, the foetus exhibited chromosomal abnormalities (Jacobs, 1977). Subfertility of the mother might be a state of repeated loss of the conceptus, unknown to the mother, due to chromosomal abnormalities. However, our findings could also be explained by hormonal imbalances. This could form a joint explanation for habitual abortion, subfertility, threatened abortion and the related use of hormones during pregnancy. The role of hormones in association with leukaemia could be causal, based on the underlying "hormonal imbalance" or a related risk-determining factor. Since the data of this study, unfortunately, do not enable us to discriminate between possible causal pathways, it all remains a matter of speculation.
- We have no clear explanation for the lower incidence of infections found for children with leukaemia in their first year of life.

Future case-control studies should be based on newly diagnosed cases of childhood leukaemia. In the present study the parents were approached 2 to 9 years after the diagnosis of their child's disease. This might have introduced recall difficulties as well as risk factor misclassification since the parents of the controls included events occurring over a longer period of time.

In general, controls may be selected from either the hospitals or the community. The decision to choose a particular control group will depend on the source of the patients, practical reasons (cost, efficiency) and the following reflections. The response rate for population controls is always less than that for patients. Therefore, response bias could influence the results. For hospital controls the response rate is

comparable to that of the cases. However, hospital controls might have a disease related to the risk factor under consideration. Therefore, hospital controls are often selected from various diagnostic groups that are believed to be free of any association with the risk factor(s) under study.

Since knowledge of the disease and recall bias could influence the answers about different risk factors, a prospective study is to be preferred. A real prospective cohort study, in which the occurrence of a disease in a group of exposed and non-exposed subjects is evaluated, is difficult to perform since childhood leukaemia is a very rare disease and therefore a very large population group has to be followed.

A prospective case-control study, in which more than one potential risk factor can be evaluated, could concentrate on children who have undergone a bone marrow aspiration. Before the parents know the result of the histological examination, they might be asked to complete a short questionnaire. Some of the children will turn out to have leukaemia (case group), while the others will have other diseases such as idiopathic thrombocytopenic purpura (I.T.P.), joint complaints or others (control group).

However, it cannot be excluded that the controls in such a study might have a causal pathway in common with the cases. Moreover the infections experienced by the child cannot be studied since I.T.P. is also related to this risk factor. On the other hand, the prenatal period and fertility problems of the mother could be evaluated in such a study. Although problems in interpretation will emerge because of the small number of patients per subgroup, it would be interesting in the future to study the leukaemias in smaller subgroups, based on morphological, immunological or chromosomal characteristics.

Subjects of interest will be the prenatal and preconceptive factors suggested in this study and infections of the child in both the first year of life and the total period preceding diagnosis. Future studies should be designed with more detailed questions concerning one specific hypothesis.Furthermore serological confirmation of infections or verification of the answers by medical records might validate the results. However, the way in which cancer research is planned may change. In recent decades there have been research groups which argue that the prime objective should be to understand the phenomenon of malignancy. Others hold that it would be better to concentrate on identifying (empirically) the causes of cancer. These different points of view may merge together since the technology for

studying genes and their pattern of expression in cancer is now advancing so fast, that the molecular biologist may soon be able to indicate to the epidemiologist what to look for and which genetic subgroup to consider as a separate aetiological entity.

On the other hand the combination of genetic and epidemiologic research may lead to the discovery of a molecular basis of carcinogenic factors.

Chapter 4

Summary

In chapter 1 the impetus for this investigation and the possibility to conduct an epidemiologic study based on a nation-wide register of children with leukaemia in The Netherlands (Dutch Childhood Leukaemia Study Group) are discussed. The aims of the study are described: to determine the incidence of childhood leukaemia in time and/or space and to identify potential risk factors of childhood leukaemia.

In chapter 2 recent insights into the origin of human cancer are given. Epidemiological studies have provided evidence that environmental factors play a role in the occurrence of malignancies: differences in cancer incidence among different communities, differences between migrants from a community and those who remain behind, and variation with time in the incidence of cancer within particular communities. Molecular biological research led to the discovery of oncogenes. The presence of an active oncogene, whether by activation of a cellular gene through rearrangement of the chromosome (translocation or deletion) or mutation or by viral infections, plays a role in neoplastic cellular change. Natural carcinogenesis seems to be a multistage process.

In chapter 3 the methodological aspects as well as the results of the study are discussed. The nation-wide morbidity register of childhood leukaemia forms the basis of this study. This register has an over 95% completeness and the diagnosis leukaemia is based on central review of bone marrow aspirates. The incidence of childhood leukaemia (0 – 15 years) in The Netherlands between 1973 and 1980 was 3.11 per 10^5 person years.

Acute lymphocytic leukaemia (ALL) accounted for 82.4% of the patients. ALL occurs more frequently in boys (sex ratio 1.2) and shows a peak incidence at 3 – 4 years. In The Netherlands no increase in incidence rate per year of diagnosis during the study period was found, nor was a trend in incidence per year of birth detected.

Some variations in incidence rates in COROP areas and provinces were found. The incidence calculated according to province of birth was lower in Drente (1.89 per 100,000, all leukaemias). The ALL incidence calculated according to residence at the time of diagnosis was higher in the province Overijssel. The ALL incidence rates in COROP areas calculated according to place of birth and residence at the time of diagnosis varied from 0.89 to 4.82 and 0.89 to 4.26 per 100,000, respectively. However, these changes could not be related to environmental factors. The problems arising from a study of the incidence of a rare disease per geographical area are described. For all leukaemias, an increasing incidence rate with increasing degree of urbanization was found.

Time space cluster analyses of the data for 293 Dutch children with leukaemia in the Western part of The Netherlands (North Holland, South Holland and Utrecht) were performed. The methods of Knox and Mantel were both applied. No time space clustering of date and place of diagnosis was found for all types of leukaemia, ALL, ALL in boys and in girls, ALL in children under 6 years old at diagnosis and for acute non-lymphocytic leukaemia. Only Knox's method showed clustering for children with ALL and a high initial leucocyte count ($\geqslant 50{,}000/mm^3$). A seasonal variation in month of birth or month of diagnosis was not observed.

In addition to the descriptive study an analytical - case-control - study was conducted. The exploratory character of the study, the selection of patients and matched controls, the mailed questionnaires, the covering letter and the consistency of the answers are discussed. The response of parents of patients and controls was 88% and 70%, respectively. The responses as related to first approach via the paediatrician or treatment result are described. The reaction of the parents of the patients was overwhelmingly positive. This was expressed in the remarks on the questionnaire, the telephone calls and the letters from the parents.

The job categories for both parents are described. More mothers of patients worked in "hydrocarbon-related" occupations during pregnancy (relative risk (RR) = 2.5; 95% confidence interval (CI) 0.7 − 9.4). In addition more case mothers reported occupational exposure to chemicals (RR = 2.4; 95% CI 1.2 − 4.8).

No association between leukaemia and paternal occupation was found. The possibility of bias is discussed. More mothers of leukaemic patients reported two or more miscarriages (RR = 1.6; 1.0 − 2.7),

fertility problems (RR = 6.0; 0.9 − 38.2) and hormonal treatment in the year before pregnancy (RR = 2.8; 0.7 − 10.5). During the relevant pregnancy they experienced threatened abortion (RR = 1.6; 1.0 − 2.6), took "drugs to maintain pregnancy" (RR = 1.9; 1.0 − 3.5) and used sedatives and sleeping pills (RR = 2.9; 1.2 − 2.7) significantly more often. The RR for diagnostic radiation during pregnancy was 2.2 (95% CI 1.2 − 3.8). No association between leukaemia and viral infections, smoking habits or alcohol consumption in the periods described was found. Alcohol consumption, smoking habits and the occurrence of viral infections did not differ between the fathers of cases and controls in the year before the relevant pregnancy. Recall bias and systematic overreporting by the parents of patients are discussed. Interactions between two potential risk factors were evaluated. Two or more miscarriages and exposure to radiation doubled the relative risk (4.0; 95% CI 0.5 − 30.7), although this was not statistically significant.

The lower frequency of common infections as well as serious infections requiring hospitalization or specialist consultation (RR = 0.7; 95% CI 0.4 − 1.0) among ALL patients in their first year of life is described.

The general risk profile for infections also differed between cases and controls. It is compatible with the following findings: among the leukaemic patients there were more first-born children from small families of higher social class, living in large houses. Exposure misclassification is discussed, but seems unlikely. Three hypothetical explanations for our findings in the first year of life are discussed.

Finally some remarks upon the study design and suggestions for future epidemiologic studies are made.

Chapter 5

Samenvatting

In hoofdstuk 1 wordt de direkte aanleiding tot dit onderzoek en de mogelijkheid om in Nederland epidemiologisch onderzoek te verrichten gebaseerd op een landelijke morbiditeitsregistratie van leukemie bij kinderen (Stichting Nederlandse Werkgroep Leukemie bij Kinderen) beschreven. De doelstelling van deze studie is het beschrijven van vóórkomen van leukemie bij kinderen in tijd en/of plaats en het vaststellen van factoren die mogelijk verbandhouden met het ontstaan van leukemie.

In hoofdstuk 2 worden huidige inzichten in het ontstaan van maligniteiten besproken. Uit epidemiologische studies kan geconcludeerd worden dat omgevingsfactoren een rol spelen bij het vóórkomen van maligniteiten: verschillen in kanker-incidentie tussen verschillende landen, verschillen tussen migranten van een bepaald land en zij die achter blijven en de variatie in incidentie in de tijd. Moleculair biologisch onderzoek leidde tot de ontdekking van oncogenen. Deze oncogenen spelen een rol in de neoplastische verandering van de cel indien ze geaktiveerd worden door herordening van het chromosoom (deletie, translocatie), puntmutatie of virusinfectie. Natuurlijke carcinogenese lijkt een proces dat in meerdere stappen verloopt.

In hoofdstuk 3 worden zowel de methodologische aspecten als de uitkomsten van het onderzoek besproken. De basis voor deze studie wordt gevormd door de landelijke morbiditeitsregistratie van kinderleukemie, die voor meer dan 95% volledig is en waarbij de diagnose leukemie berust op centrale beoordeling van beenmergpreparaten.

De incidentie van kinderleukemie (0 – 15 jaar) in Nederland in de periode 1973 tot 1980 bedraagt voor alle typen leukemie te zamen 3,11 per 100.000 kinderjaren. Acute lymfatische leukemie (ALL) is het meest vóórkomende type (82,4%). ALL komt vaker voor bij jongens (sex ratio 1,2) en toont een piek in incidentie bij 3 – 4-jarigen. In

Nederland werd geen toename van de incidentie per diagnosejaar noch per geboortejaar vastgesteld.

Enige geografische verschillen in incidentiecijfers op het niveau van COROP gebieden (40) en provincies (11) werden vastgesteld. De incidentie per provincie van geboorte was verlaagd in Drente (1,89 per 100.000, alle typen leukemie). De ALL-incidentie was verhoogd in de provincie Overijssel op het tijdstip van diagnose. In de COROP gebieden van diagnose en geboorte werden respectievelijk ALL-incidenties van 0,89 tot 4,82 en van 0,89 tot 4,26 per 100.000 kinderjaren vastgesteld. Voor deze verschillen kan geen duidelijke aan omgevingsfactoren gerelateerde verklaring worden gegeven. De problemen bij het beschrijven van de incidentie van een zeldzame ziekte per geografische gebiedseenheid worden besproken. De incidenties naar urbanisatiegraad laten voor alle typen leukemie te zamen een toename met de mate van verstedelijking zien.

Clusteranalyses naar tijd en plaats van diagnose van 293 nederlandse kinderen met leukemie uit de Randstad (Noord-Holland, Zuid-Holland en Utrecht) werden volgens de methode van Knox en Mantel uitgevoerd. Geen clustering werd vastgesteld voor alle typen leukemie, acute lymfatische leukemie (ALL), ALL bij jongens en meisjes, ALL bij kinderen jonger dan 6 jaar bij diagnose en acute niet lymfatische leukemie. Voor kinderen met ALL en initieel $\geqslant 50.000$ leucocyten per mm^3 werd alleen met de methode van Knox clustering vastgesteld. Een seizoensinvloed op de geboorte of diagnosemaanden van de patienten werd niet waargenomen.

Naast het descriptieve onderzoek werd een analytisch - case-control - onderzoek uitgevoerd. Het exploratieve karakter van de case-control studie, de selectie van patienten en matched controles, de post-enquetes, de begeleidende brief, het contact met de kinderartsen en de consistentie van de antwoorden worden besproken. De response van de ouders van patienten en controles bedroeg respectievelijk 88% en 70%. De response zowel in relatie tot het wel of niet geinformeerd zijn door de kinderarts als tot de behandelingsresultaten wordt besproken. Aan de hand van telefonische reacties, opmerkingen bij de vragenlijsten en brieven, wordt een indruk gegeven over de positieve houding van de ouders ten opzichte van deze studie.

De beroepen van de ouders tijdens de zwangerschap en één jaar voor diagnose werden geëvalueerd. Moeders van patienten bleken vaker gedurende de zwangerschap werkzaam te zijn in met "koolwaterstof-gerelateerde" beroepen (relatieve risico (RR) = 2,5;

95% betrouwbaarheidsgrens (BG) 0,7 – 9,4). Zij gaven significant vaker blootstelling in het beroep aan chemische stoffen op (RR = 2,4; 95% BG 1,2 – 4,8). Geen associatie tussen leukemie en beroep van de vader werd vastgesteld. De mogelijkheid van bias wordt besproken.

Mogelijke andere risicofactoren bleken fertiliteitsproblemen (RR = 6,0; 95% BG 0,9 – 38,2) en hormonale behandeling (RR = 2,8; 95% BG 0,7 – 10,5) één jaar voorafgaand aan de betreffende zwangerschap te zijn. Twee of meer miskramen werden significant vaker opgegeven door moeders van patienten (RR = 1,6; 95% BG 1,0 – 2,7), terwijl gedurende de betreffende zwangerschap significant vaker sprake was van een dreigende miskraam (RR = 1,6; 95% BG 1,0 – 2,6), gebruik van medicijnen om de zwangerschap te behouden (RR = 1,9; 95% BG 1,0 – 3,5) en gebruik van sedativa en slaapmiddelen (RR = 2,9; 95% BG 1,2 – 2,7). Het RR voor röntgenonderzoek was tweevoudig verhoogd (RR = 2,2; 95% BG 1,2 – 3,8). Geen relaties tussen leukemie en virusinfecties, roken of alcoholgebruik van de moeder in de genoemde perioden werden vastgesteld. Het vóórkomen van virale infecties, het alcohol gebruik en rookgewoonten verschilden niet duidelijk tussen de vaders van beide groepen in de periode één jaar voor de zwangerschap. De mogelijkheden van systematische overrapportage door ouders van patienten en recall bias worden besproken.

Interacties tussen twee mogelijke risicofactoren werden beschouwd. Twee of meer miskramen en expositie aan röntgenfoto's tijdens de zwangerschap verdubbelde het RR (4,0; 95% BG 0,5 – 30,7), dit was echter statistisch niet significant.

In het eerste levensjaar van kinderen met ALL werd het minder frequent vóórkomen van zowel infectieziekten zoals verkoudheden, koortsperioden en kinderziekten, als ernstige infecties die (poli-) klinisch behandeld werden (RR = 0,7; 95% BG 0,4 – 1,0) vastgesteld.

De algemene variabelen die het infectierisico kunnen beinvloeden passen hierbij: leukemie patienten zijn vaker eerstgeborenen uit kleine gezinnen met een hogere sociale klasse, wonend in relatief grote huizen. De mogelijkheid van misclassificatie wordt besproken maar lijkt onwaarschijnlijk. Drie hypothetische verklaringen voor deze bevindingen in het eerste levensjaar worden besproken.

Tenslotte worden enkele kanttekeningen bij de onderzoeksopzet gemaakt en suggesties voor toekomstig epidemiologisch onderzoek gegeven.

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Addendum 1

Incidence of childhood leukaemia in The Netherlands (1973 – 1980)

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Abstract

The childhood leukaemia incidence rate for The Netherlands was estimated at 3.11 per 100,000 children (aged 0 – 15 year) per year, based on a complete nation-wide childhood leukaemia registry comprising the years 1973 – 1980. Acute lymphocytic leukaemia (ALL) accounted for 82.4% of the patients, acute non-lymphocytic leukaemia for 13.6% and chronic myeloid leukaemia for 2.9%. ALL occurred more frequently in boys (sex ratio 1.2). The highest ALL rate was observed in the 3 – 4 age group. These figures corresponded with the data of the Manchester Children's Tumour Registry. Neither the incidence rates according to year of diagnosis nor the incidence rates according to year of birth showed a significant trend with time. The total leukaemia incidence rate in urban areas was somewhat higher than in rural areas. While the direct comparison of the incidence rate between these areas is not significant, the trend over the three categories of urbanization is significant.

Introduction

Childhood leukaemia incidence varies across different countries. In some an increase in incidence rates has been noted in recent years (Birch et al., 1981; Ericsson et al., 1978; Stiller & Draper, 1982) and in others no time trend was found (Teppo et al., 1975; Young & Miller, 1975).

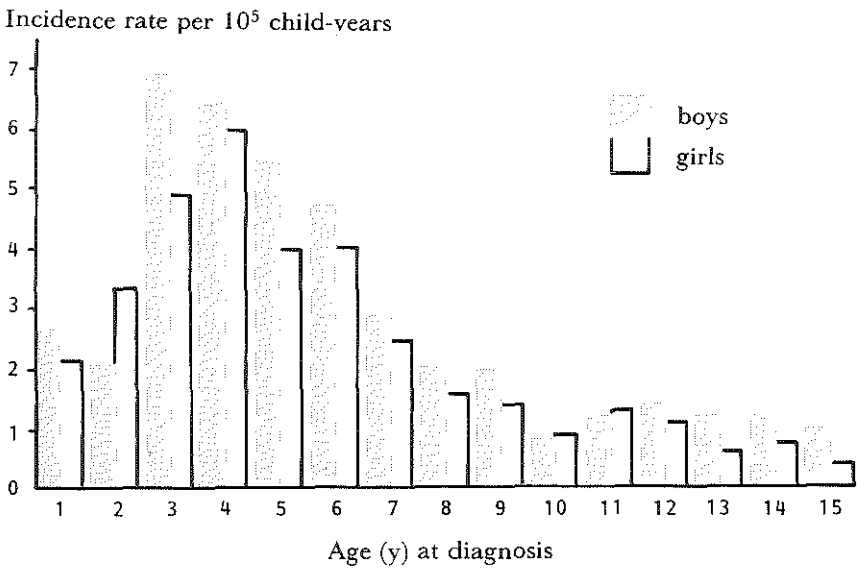
The existence of a complete nation wide children's leukaemia

Table 1. Total number, incidence rates and frequency of different morphological types of leukaemia

Type	Number of patients	Incidence rate per 100,000 person years	Total %
ALL	624	2.56	82.4
ANLL	103	0.42	13.6
CML*	22	0.09	2.9
Unclassified	8	–	1.1
Total	757	3.11	100

* Including juvenile and adult type

Fig. 1 Incidence rate of acute lymphocytic leukaemia (ALL) per 10^5 person years, by age and sex, 1973 – 1980 (n = 624)



Age

The age-specific incidence curve of ALL (Fig.1) showed a peak at the age of 3 – 4 years for boys as well as for girls.

Incidence trends according to year of diagnosis

The annual incidence rates of all types of leukaemia, for ALL and for acute non-lymphocytic leukaemia (ANLL) are shown in Fig.2. In 1979 the incidence rate of ALL was higher than the rates in the preceding years. However, logit analysis of the annual incidence rates in the period of study did not show any significant time trend in incidence neither for all cases, nor for any morphological subgroup (all p-values for analysis >0.10). Moreover, a preliminary calculation of the incidence rate in 1980 revealed an overall leukaemia incidence rate of 3.85 per 10^5 children, similar to the one for 1979.

The incidence rate for ALL in 1980 was 2.98 per 10^5 and therefore lower than in 1979. The logit analysis for both sexes separately showed a borderline significant trend with time for girls. On inspection of the date this seemed to be due to the high 1979 figure. Presumably, 1979 had an exceptionally high incidence of leukaemia which is unexplained.

The annual incidence rates of ALL for the three age groups 0 – 4, 5 – 9 and 10 – 14 separately, are shown in Fig.3.
The trend in incidence with time was not significant for any of the three age groups (all p-values for analysis >0.10). For these age groups the annual incidence rates were also analysed according to sex. However, no time trend was found.

Incidence trend according to year of birth

The incidence rates for all types of leukaemia as well as for ALL per annual birth cohort (1959 – 1978) showed no trend with time ($p>0.10$).

Urbanization

Leukaemia incidence rates in urban and rural areas (i.e. residence of patients at time of diagnosis) are presented in Table 2, which shows somewhat higher rates for urban than for rural areas. For all leukaemia types the rate difference (RD) between urban and rural areas was not statistically significant at the 5% level (RD = 0.49, 95% confidence

Fig.2 Incidence rate of childhood leukaemia per 10^5 person years, 1973 – 1980

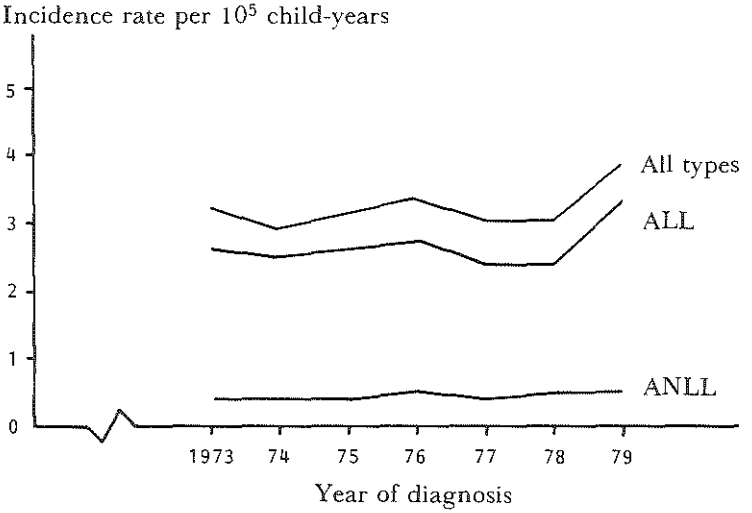
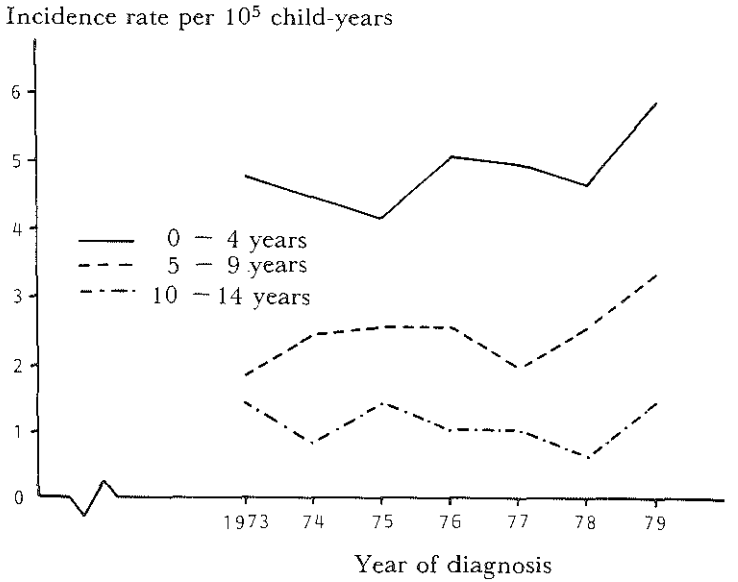


Fig.3 Incidence rate of acute lymphocytic leukaemia per 10^5 person years by age group, 1973 – 1980



interval −0.39 and 1.39). The trend over the three categories of urbanization was significant (p = 0.013).

Table 2. Incidence rates of leukaemia in urban and rural areas per 100,000 person years

	All leukaemia types		ALL	
Urbanization	Nr.	Incidence rate (SE)	Nr.	Incidence rate (SE)
Rural[1]	90	3.11(0.33)	80	2.77(0.31)
Intermediate[2] urban	506	3.42(0.15)	411	2.78(0.14)
Urban[3]	161	3.88(0.31)	133	3.21(0.28)

[1] Rural areas, more than 20% of the adult population working in agriculture

[2] Small cities and villages with a population of less than 100,000

[3] Cities with a population of more than 100,000

For ALL neither direct comparison of incidence rates between urban and rural areas, nor the trend in incidence over these areas was statistically significant (all p-values for analysis > 0.10).

Discussion

The national leukaemia morbidity registry of the DCLSG meets all three criteria mentioned by Young & Miller (1975) to determine the incidence rate of childhood leukaemia, i.e. a population-based registry with accurate denominators for every year of study, nearly complete ascertainment of all cases and confirmation of the diagnosis on bone marrow samples of all patients. The cytomorphological diagnosis is based on previously determined criteria and made by two independent experts. Therefore the diagnostic homogeneity of the Dutch childhood leukaemia morbidity registry is unique and the data presented reflect the true incidence of childhood leukaemia in The Netherlands.

Type

The leukaemia incidence rates in different countries are presented in Table 3. The Netherlands total leukaemia incidence rate corresponds with the one in the Manchester region. The Netherlands incidence rate of acute lymphocytic leukaemia (ALL) is 2.56 per 10^5 compared to 2.61 per 10^5 in the Manchester region, U.K. (Birch et al., 1980). Higher total leukaemia incidence rates were observed in Australia, Finland, Sweden and the U.S.A. These differences might reflect stricter registry criteria in The Netherlands and Manchester.

In all studies ALL is the most common type of leukaemia in childhood. In the Manchester region (Birch et al., 1980) and in The Netherlands it accounts for 79% and 82.4% respectively.

Sex

In previous studies a predominance of ALL in boys is found (Birch et al., 1980; Ericsson et al., 1978; McWhirter & Bacon, 1981; Teppo et al., 1975; Young & Miller, 1975). According to this finding the male/female ratio for ALL is 1.2 in The Netherlands.

Age

The usual age-specific incidence curve of ALL, with a peak at age 3 – 4 years is also found in this study. This might suggest that factors in the prenatal period or the first years of life are of importance in the aetiology of childhood leukaemia (Birch et al., 1980).

Table 3. Leukaemia incidence rates in different countries

Country	Authors	Leukaemia incidence rate per 100,000 person years
The Netherlands		3.11
Manchester region, U.K.	Birch et al. (1980)	3.31
Queensland, Australia	McWhirter & Bacon (1981)	3.60
Finland	Teppo et al. (1975)	3.93
Sweden	Ericsson et al. (1978)	3.90
U.S.A. (whites)	Young & Miller (1975)	4.21

Incidence trends according to year of diagnosis

In the Manchester region an increase in ALL incidence rates in children since 1970 has been detected. The increases especially concerned the youngest age-group of 1 – 4 years (Birch et al., 1981). For acute myeloid leukaemia no change in incidence rate was detected. In the U.K. as a whole, an increase in annual registry rates

of leukaemia in boys aged 0 – 4 years was found (Stiller & Draper, 1982). In Sweden and Finland no increase in the overall leukaemia incidence rate was established, though in Sweden significant increases in leukaemia incidence rates in girls aged 0 – 4 years and in boys aged 5 – 9 years were observed. In Finland the three age groups were not analysed separately. Neither the annual Dutch incidence rates of all types of leukaemia, nor the ALL and ANLL subgroups, showed any significant trend with the time during the period 1973 – 1980. Only one borderline significant trend appeared in the ALL incidence rates for girls, presumably due to a single high figure. The uniformity of incidence rates might indicate that environmental factors are of no importance in the aetiology of leukaemia in children or, alternatively, that these factors are evenly distributed in time, which suggests that newly introduced environmental factors do not influence leukaemia incidence rates in children (Birch et al., 1980).

Incidence trends according to year of birth
Recent analyses of leukaemia registry data in the U.K. revealed an increased incidence of ALL in childhood for the birth cohorts after 1964 (Stiller & Draper, 1982). The increase was only significant for boys < 5 years: The Dutch incidence rates according to year of birth (1958 – 1978) did not show any significant trend with time. However, the analysis was based on small numbers of patients per year of birth. For this reason boys and girls were not analysed separately.

Urbanization
A study in Australia suggested a higher childhood leukaemia incidence in Brisbane city than its rural environment (McWhirter & Bacon, 1980). However, the difference was small. In the U.S.A. leukaemia mortality was higher in counties with at least 75% of the population in urban areas, but this may have been caused by differences in diagnostic possibilities and quality of medical care (Blair et al., 1980). In The Netherlands the total leukaemia incidence rate is also higher in urban than in rural areas, although the difference is small. We hesitate to offer an explanation for this finding.

In summary, The Netherlands childhood leukaemia incidence rate corresponds well to the figure in the Manchester region. In contrast with Manchester and the U.K. as a whole, the incidence rates per year of diagnosis do not show a trend with time. The increase of incidence rates according to year of birth in the U.K. is not found in The

Netherlands. Lastly, in this study there is some urban-rural gradient too.

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Addendum 2

The geographical distribution of children with leukaemia in The Netherlands

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Abstract

This study of the geographical distribution of childhood leukaemia (0 – 15 years) in The Netherlands is based on data from the national morbidity register of the Stichting Nederlandse Werkgroep Leukemie bij Kinderen (Dutch Childhood Leukaemia Study Group) covering the period 1973 – 1980. An evaluation of the completeness of this register showed that 95.4 – 99.9% of all cases were registered. Some differences in the incidence of leukaemia were found among the provinces and so-called COROP-areas. The incidence appeared to be slightly higher in the major cities than in rural areas. As yet no clear-cut geography-dependent causes can be demonstrated for these differences in incidence.

Introduction

A study of the geographical distribution of childhood leukaemia might yield some indications of possible aetiological factors of the disease. For this purpose the possibility of a relation between characteristics of certain areas and the incidence of leukaemia was investigated.

The direct incentive to this study was the news report that in another country childhood leukaemia seemed to occur more often in the neighbourhood of nuclear reactors than in other regions (Persbericht Ministerie van Volksgezondheid en Milieuhygiene, August 1979). Since there is a complete national morbidity register of childhood leukaemia in The Netherlands, it was possible to determine

and compare the incidence of leukaemia in various geographical areas.

Patients and methods

The morbidity register of the Dutch Childhood Leukaemia Study Group (DCLSG), started in 1972, lists all 0 – 15-year-children with leukaemia in The Netherlands (population in 1976: 13.9 million, with approximately 3.4 million children under 15 years of age). The DCLSG includes 160 participating paediatricians who, through cooperative effort, intend to improve the treatment of children with leukaemia. For this purpose they routinely send peripheral blood smears and bone marrow biopsies of all children with leukaemia as well as those suspected of having this disease to the DCLSG laboratory. These preparations are independently reviewed by two experts, using previously established criteria. Until 1975 the DCLSG had its own criteria based on morphology and cytochemistry (Sudan black and periodic acid-Schiff (PAS)). As of 1975 the French-American-British (FAB) classification is used (van Wering & Vissers-Praalder, 1979). Treatment of the children is co-ordinated by the study group and registration of the clinical data on all patients has been standardized (van der Does-van den Berg, 1980).

To obtain an insight into the degree of completeness of the register of the DCLSG, a questionnaire was sent to all paediatricians in The Netherlands in 1980. They were asked to report the date of birth, date of diagnosis and sex of all leukaemia patients younger than 15 years old whom they had seen for treatment or consultation in the period 1973 – 1980. The participating paediatricians of the DCLSG received in addition a list of all of their patients included in the register. In total 92.6% of the 462 paediatricians returned this questionnaire. In this manner 17 children with leukaemia were traced who were not yet registered with the DCLSG. Bone marrow biopsies from 12 of these patients were still available so that the diagnosis of leukaemia could be confirmed in the DCLSG laboratory. On the basis of these findings the minimum and maximum completeness of the register could be estimated. If the paediatricians who did not return the questionnaire see on the average the same number of leukaemia patients as the participating paediatricians of the DCLSG (excluding those participating in oncological centres), then the completeness of the register is 95.4%. If they see the same number as the non-participating

paediatricians who returned the questionnaire, then the completeness is 99.9%.

The patients were eligible for the study of the geographical distribution of leukaemia if:

- age at diagnosis was 0 – 15 years;
- diagnosis established in the period January 1st, 1973 and January 1st, 1980 (date of diagnosis = date the diagnostic bone marrow biopsy was taken);
- diagnosis established or confirmed in the DCLSG laboratory.

The choice of geographical units was determined by both the division of The Netherlands into geographical areas by the Central Bureau of Statistics (CBS) and the size of these areas (it is essential that the number of patients per unit is not too small). As a result a division was chosen which gave the largest possible areas: 11 provinces and 40 so-called COROP-areas. The Coordinating Committee for Regional Study Programmes (Coordinatie Commissie Regionaal Onderzoeksprogramma, COROP) was closely involved in the choice of the 40 functional regions based on local living conditions, job opportunities, social services and social interrelationships (CBS).

The incidence was calculated both per province and COROP region in which the patients lived at the time of diagnosis and per province and COROP region where the child was born. The incidence per degree of urbanization was calculated only for the place of residence at the time of diagnosis. The denominator was the total number of children in the relevant area in the middle of the period under study, i.e. 1976 (CBS, 1976). Direct comparison of the incidence in the various regions can only be carried out if the values are independent of age distribution. Correction for differences in age distribution was obtained by direct standardization. For this purpose the age-specific incidences of a region were applied to the age distribution of a standard population. The region with the smallest population was always used as standard population, which caused the smallest variance in the comparisons.

The standard error of the incidence, which reflects the range of this value, was also calculated (Armitage, 1971). The incidence for each province was compared with that for the remaining provinces together. In contrast the incidence for each COROP region was compared with the total overall incidence, including the relevant COROP region, since the absolute number of patients per COROP

region was small (maximum 7.8% of the total number of children with leukaemia).

Another way to gain insight into the geographical distribution of leukaemia might be to combine the municipalities according to degree of urbanization and then compare the incidence in the various urbanized regions. For these calculations, use was made of the categorization of municipalities according to degree of urbanization provided by the CBS. The incidences for (1) rural districts in which more than 20% of the population are farmers, (2) non-rural districts with less than 5000 inhabitants; small and medium-sized towns with 5000 – 100,000 inhabitants (intermediate urban), and (3) major cities with more than 100,000 inhabitants (urban) were compared.

Differences in incidence were tested under the nulhypothesis: there is no difference in incidence. If this difference equals zero or the value 0 lies within the 95% confidence interval, then the nulhypothesis is accepted. There is, however, a significant difference at the 5% level if the value 0 does not lie within the 95% confidence interval (Rothman & Boice, 1979). In addition a trend analysis was performed over the three categories of urbanization by (weighted) linear regression analysis using the inverse of the variances of the incidence rates as weights (Snedecor & Cochran, 1980).

Results

In the period 1973 – 1980 757 children in The Netherlands were found to have leukaemia: this is more than 100 patients per year, range 97 to 124. Specifically 82.4% of the children had acute lymphocytic leukaemia (ALL), 13.6% had acute non-lymphocytic leukaemia (ANLL) and 2.9% had chronic myeloid leukaemia (CML). The type of leukaemia could not be established in only 1.1% of the cases. The overall incidence of leukaemia is 3.11 per 100,000 child-years (all types of leukaemia); for ALL the incidence is 2.56 per 100,000 child-years.

Incidence according to province

The geographical distribution of all types of leukaemia together was calculated per province at diagnosis and province of birth. The incidence for each of the 11 provinces at diagnosis does not differ significantly from the mean incidence. Analysis of the incidence per province of birth yielded only a significantly lower incidence in the

Tabel 1. Incidence of acute lymphocytic leukaemia per 100,000 child-years per province of birth and province at the time of diagnosis

	Province of birth			Province at diagnosis		
Province	Nr.	Incid. rate	SE	Nr.	Incid. rate	SE
Groningen	29	3.22	0.60	25	2.78	0.55
Friesland	29	2.72	0.50	31	2.90	0.55
Drente	11	1.48	0.44	14	1.88	0.50
Overijssel	55	2.96	0.40	62	3.34*	0.42
Gelderland	85	2.90	0.31	85	2.89	0.31
Utrecht	42	2.85	0.43	46	3.13	0.45
North-Holl.	89	2.52	0.26	88	2.50	0.26
South-Holl.	118	2.41	0.22	114	2.33	0.22
Zeeland	15	2.61	0.67	17	2.96	0.72
North-Brab.	86	2.38	0.25	90	2.49	0.26
Limburg	48	2.71	0.38	51	2.92	0.39
N.E.Polder	1			1		
Foreign country	14					
Unknown	2					
Total	624	2.67	0.11			

* significantly higher ($p < 0.05$)

SE = standard error

Tabel 3. Incidence of leukaemia per 100,000 child-years according to degree of urbanization

Degree of urbanization	All types of leukaemia		ALL	
	Nr.	Incidence (SE)	Nr.	Incidence (SE)
Rural[1]	90	3.11(0.33)	80	2.77(0.31)
Small & medium-sized towns[2]	506	3.42(0.15)	411	2.78(0.14)
Major cities[3]	161	3.88(0.31)	133	3.21(0.28)

1 Rural areas = more than 20% of the population are farmers

2 Non-rural areas (< 5000 inhabitants), small & medium-sized towns (5000-100,000 inhabitants)

3 Major cities (100,000 or more inhabitants)

SE = standard error

Discussion

Studies of the geographical distribution of malignancies are usually based on mortality data, the reliability and completeness of which are often questionable. (Moens, 1982; Blair et al., 1980; CBS, 1980). In The Netherlands the incidence of childhood leukaemia per geographical region was calculated from morbidity data provided by the DCLSG. This register of leukaemia patients is highly complete and in all cases the diagnosis was established or confirmed on bone marrow samples.

In general it can be stated that studies of the geographical distribution of diseases make use of geographical units that are in fact administrative units. This division is not necessarily related to epidemiological factors. Moreover for rare diseases both the size and the population density of the geographical area influence the validity of the calculated incidence. If the areas are large, such as provinces, a more accurate estimation of the incidence is obtained with a relatively small standard error. On the other hand the incidence calculated for small regions, such as the COROP regions, probably provides more insight into aetiological factors because such a small region may be characterized by certain environmental factors. The small number of patients per region however, gives rise to a fairly large standard error for the calculated incidence so that interpretation becomes difficult.

The problem of the small number of patients per region can be solved by combining areas with similar characteristics. One example of this in our study is the combination of municipalities according to the degree of urbanization.

For a correct interpretation of the geographical distribution of a disease the period evaluated must be relatively short so that it can be assumed that characteristics of specific regions will not have changed.

Another factor that must be taken into account is migration. In view of the fact that leukaemia develops in the very young (53% of the ALL patients were below 5 years of age), this factor probably does not play a role here (Davies & Chilvers, 1980).

Incidence according to province
In The Netherlands several differences in the incidence of childhood leukaemia were demonstrated among the provinces. Thus it appeared that significantly fewer patients with leukaemia were born in Drente than in the other provinces. Relatively more ALL patients lived in the province Overijssel at the time of diagnosis than in the rest of The Netherlands. However, conclusions based on these data should be approached with care since provinces are political units with heterogeneous environmental factors.

Incidence according to COROP region
Analysis of the incidence per COROP region in which the patient lived at the time of diagnosis, revealed one region with a significantly higher incidence of leukaemia and three regions with significantly lower incidences. In the first region one finds both agriculture and industry. The latter three regions, Amsterdam, east South Holland and 's-Hertogenbosch plus north-east North Brabant are partly industrial (especially Amsterdam) and partly agricultural. Using the Atlas of The Netherlands, the water table, geological configurations, presence of specific minerals, strenght of the magnetic field, composition of the soil, quantity of surface water, water pollution and source of the drinking water in these regions were compared: no clear similarities could be found (Stichting Wetenschappelijke Atlas van Nederland, 1963 – 1977).

If the incidence of leukaemia is calculated for the COROP region in which the child was born, then lower incidences are found for 's-Hertogenbosch plus north-east North Brabant, east South Holland, Rijnmond and Amsterdam. As is known, Rijnmond is one of the most highly industrialized regions in The Netherlands. The first two regions, on the other hand, have little industry. Moreover no similarities could be found for the geological characteristics mentioned above.

The incidence for the COROP regions exhibit a fairly broad range (standard error) and, as already mentioned, they are administrative units. Interpretation of these results is therefore difficult. In addition the differences found could be a chance event. In The Netherlands there are 40 regions and according to the laws of probability, 5% (1 out of every 20) may deviate significantly from the mean.

We evaluated the incidence of leukaemia in the municipalities

Borssele, Dodewaard and Petten together, since there is a nuclear or high flux reactor in each of these municipalities. The incidence in these three municipalities is some lower than the overall Dutch leukaemia incidence; this difference is however not significant.

Incidence according to degree of urbanization
As far as the differences in incidences found between cities and rural areas are concerned, an American study based on mortality data indicated that the incidence of childhood leukaemia is higher in urbanized areas than in rural areas. The difference might be explained by differences in available diagnostic procedures and the quality of medical care (Blair et al., 1980). In Olmsted, Minnesota, no difference in the incidence of leukaemia per degree of urbanization could be found for all age groups together (Linos et al., 1978). In contrast in Brisbane, Australia, the incidence of ALL among 0 – 15-year-old children was higher than in the less highly urbanized areas surrounding the city. This study was based on morbidity data taken from the Queensland Childhood Malignancy Registry (McWhirter & Bacon, 1980).

In The Netherlands too the incidence of all leukaemias is slightly higher in the cities than in rural areas. With increasing urbanization the incidence of all types of leukaemia was found to be 3.11, 3.42 and 3.88 per 100,000 child-years, respectively. However, in view of these small differences, we hesitate to draw any conclusions. In addition the difference in the incidence of ALL, the most common type of leukaemia, between cities and rural areas, was even less pronounced and a significant trend in the incidence of ALL per degree of urbanization could not be demonstrated at all. Our analysis of the incidence of leukaemia in relation to the degree of urbanization was restricted to the municipalities in which the children lived at the time of diagnosis and not where they were born. In a rural population the municipality where the child is born is often not the same municipality where the mother lived during pregnancy, because about 50% of these children are born in hospital (data for 1970 from Kloosterman et al., 1981). An analysis of the municipality where these children were born is therefore of little value. This is not true for larger geographic units, such as COROP regions and provinces.

In conclusion it can be stated that in The Netherlands in the period studied (1973 – 1980) there were some differences in the incidence of childhood leukaemia per geographical unit. On the basis of this

investigation however, no conclusions can be drawn concerning the causes of these differences in geographical distribution. In order to gain some insight into possible aetiological factors of childhood leukaemia, a case-control study is now in progress.

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Addendum 3

Time space distribution of childhood leukaemia in The Netherlands

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Abstract

In the western part of The Netherlands, during 1973 – 1980 leukaemia was diagnosed in 293 patients aged under 15 years. An overall incidence rate of 2.91 per 100,000 person years was calculated. No seasonal influence on month of birth, or month of diagnosis of these patients could be traced by the method of Edwards. Time space clustering was looked for by both methods of Mantel and Knox. No significant time space clustering of date and place of diagnosis of childhood leukaemia was found in all types of leukaemia, acute lymphocytic leukaemia (ALL), ALL in boys and girls, ALL in children under 6 years at diagnosis and in acute non-lymphocytic leukaemia.

Introduction

The results of studies of temporal and spatial distribution of childhood leukaemia are not consistent and are even contradictory (Caldwell & Heath, 1976). Clustering of cases might be caused by an environmental agent acting in a limited geographical area for a limited period. This could be most easily detected in childhood leukaemia because of the limited mobility of children compared with adults, which may facilitate the observation of clusters.

The existence of a complete seven year nation-wide registry of childhood leukaemia offered us the opportunity to look for time space clustering in the densely populated Western part of the country.

Material and methods

The morbidity registry of the Dutch Childhood Leukaemia Study Group (DCLSG) was established in 1972. It covers the whole country which in 1976 had a childhood population (< 15 years) of nearly 3.4 million (total population 13.9 million).

Nearly 160 paediatricians in The Netherlands collaborate in the DCLSG in an effort to optimize the treatment of children with leukaemia. They routinely send blood and bone marrow slides of each child with leukaemia, or under suspicion of this disease to the DCLSG laboratory. These slides are reviewed according to previously determined criteria by two independent experts. Before 1975 the DCLSG laboratory used its own diagnostic criteria based on morphology and cytochemistry (Sudan black and periodic acid-Schiff (PAS)). Since 1975 the French-American-British (FAB) classification has been used (Van Wering & Vissers-Praalder, 1979; Bennet, et al., 1976). The treatment of these children is centrally coordinated and clinical information is uniformly collected.

In 1980 the completeness of the morbidity registration was checked by sending a questionnaire to all 462 paediatricians in The Netherlands. They were asked to give the names, dates of birth, dates of diagnosis and sex of all patients with leukaemia whom they had treated or consulted between 1973 and 1980. The overall response rate to the questionnaire was 92.6% and 17 cases, hitherto unknown, were reported. Bone marrow slides of 12 of these patients were still available and the diagnosis of leukaemia could be confirmed at the DCLSG laboratory. We estimate that over 95% ascertainment of patients had been achieved in the period 1973 – 1980.

The patients with leukaemia who were accepted for this study were all under 15 at the time of diagnosis (1 January 1973 until 1 January 1980). The diagnosis was confirmed by bone marrow slides at the DCLSG laboratory. The cluster analysis was limited to the Western part of The Netherlands comprising the provinces of North Holland, South Holland and Utrecht, because the home address at diagnosis of each patient could be located on a geographical map with a scale of 1:50,000. Within this map the addresses in larger towns were identified with the use of large scale street maps. This part of The Netherlands covers approximately 7677 square kilometers. In 1976 the childhood population (0 – 15 years) was 1.44 million. It has the highest

population density in The Netherlands and is nearly uniformly urbanized. This has the advantage that the same distance between patients does have an equal meaning.

For the cluster analysis, the date of the diagnostic bone marrow sample was chosen, and this represents the date of diagnosis. A seasonal trend in month of birth or month of diagnosis of leukaemic patients was examined by using the analysis of monthly frequencies of Edwards (1961).

The methods of Knox (1964) and Mantel (1967) were used to investigate time space interaction. Both analyses are based on examination of time and distance intervals between all possible pairs of cases. The hypothesis that pairs of patients who are close in time are also close in space is tested. Mantel's method uses a time versus space regression. For each possible pair of patients, say patient i and patient j, a spatial measure of Xij and a temporal measure Yij is obtained. A clustering measure is given by test statistic:

$$Z = Xij.Yij$$

Evidence of time space clustering is given if the observed Z differs significantly from the permutational expectation of Z

$$X = (Z - E(Z)) \times (SE(Z))^{-1}$$

In concept, the set of time and space locations for the patients are split apart and then put back together at random. Each possible way of putting them back together results in a new value for Z. Mantel describes how to determine approximately the expected Z and the standard error of Z under normal assumptions, so that the departure of the observed Z from its expected value can be tested for significance.

In this study the reciprocal value of time and distance intervals between pairs of patients was used in Mantel's cluster analysis. In this way the short distances will be spread out while collapsing the range of great distances. Temporal and space distances equal to zero were replaced by the value three.

In the method of Knox, each pair is classified as being close or far apart in time and close or far apart in space to form a 2x2 contingency table. The expected number of pairs may be calculated from the marginal totals. The observed number of close pairs is tested as a

Poisson deviate from the expected number. In this model critical time and distance intervals are arbitrary chosen.

In our analysis two kilometers distance intervals (2, 4, 6, 8 and 10 kilometer) and time intervals of two months (2, 4, 6, 8, 10 and 12 months) were used.

Analyses according to Edwards, Mantel and Knox were performed for the total group of patients and for different subgroups classified according to type of leukaemia, tumour load (initial leucocyte counts over 50,000/mm^3), age (younger than 6 year at diagnosis) and sex (Knox, 1964; Glass et al., 1971).

Results

During 1973 − 1980 in the Western part of The Netherlands leukaemia was diagnosed in 293 patients aged under 15 year. This amounts to an incidence rate of 2.91 per 100,000 person years (standard error (SE) = 0.17). Acute lymphocytic leukaemia (ALL) was the most common type compared with acute non-lymphocytic leukaemia (ANLL) and chronic myeloid leukaemia (CML, including the juvenile and adult type) that is, 79.5%, 16.7% and 2.4%, respectively. In 1.4% the type could not be classified.

The patients' months of diagnosis or months of birth were examined for evidence of a seasonal trend. The analyses were performed for all patients, patients with ALL, the subgroups of boys and girls with ALL, patients with ALL younger than 6 years at diagnosis, patients with initial counts $\geqslant 50{,}000$ leucocytes/mm^3 and patients with ANLL. No significant seasonal variation was found. Table 1 gives the results for ALL.

Table 2 gives the results of time space clustering using Mantel's method. No significant clustering of date and place of diagnosis was detected neither for all leukaemic patients nor for the different subgroups of patients (all p-values for analysis > 0.10).

Table 3 shows the results of Knox's analysis of children with leukaemia at a critical space distance of two kilometer and three critical time distances of two, four and six months. The null expectation did not show a departure from the observed number of close pairs classified according to type, age and sex (p-values > 0.10).

Only clustering of patients with ALL with initial count $\geqslant 50{,}000$ leucocytes/mm^3 was found. This consists of a higher number of pairs with a distance separation of under two kilometers and a time

Table 1. Seasonal incidence of acute lymphocytic leukaemia

Months	1	2	3	4	5	6	7	8	9	10	11	12
With regard to diagnosis	17	23	22	16	19	18	20	20	15	17	29	17
With regard to birth	26	17	12	16	24	28	9	25	14	27	20	15

separation of under six months. Four pairs were found while the expected number was 1.4. This difference was statistically significant ($\chi = 2.20$, one-sided $p < 0.025$). In addendum 8B detailed tables, with all performed time space cluster analyses according to Knox, are described.

Discussion

Knox (1964) did not find a seasonal influence on the months of birth for children with leukaemia. This agrees with our results. Most previous investigations (Till et al., 1967; Ederer et al., 1964), except one study in England (Knox, 1964), did not establish any significant seasonal variaton for the months of diagnosis. Neither in this study was any seasonal influence on the month of diagnosis found.

Knox (1964) in Northumberland and Durham, Till et al. (1967) in London and Gunz and Spears (1968) in New Zealand, detected weak evidence of clustering of date and place of diagnosis in patients with leukaemia, aged under six years at diagnosis, with Knox's statistical method. In London even clustering of date and place of

Table 2. Time space cluster analysis by Mantel's method

	All types	ALL	ALL boys	ALL girls
Number	293	233	133	100
Z	20.91	12.17	3.51	2.50
E(Z)	21.90	13.64	3.94	2.93
SE	1.75	1.29	0.57	0.89
X	-0.56	-1.14	-0.75	-0.48

	ALL < 6 years	ALL $\geqslant$ 50,000/mm^3	ANLL
Number	159	43	49
Z	5.37	0.71	0.50
E(Z)	6.17	0.52	0.55
SE	0.72	0.39	0.15
X	1.11	0.49	0.33

ALL = Acute lymphocytic leukaemia

ANLL = Acute non-lymphocytic leukaemia

Table 3. Time space cluster analysis by Knox's method

Time separations between pairs of cases in months		⩽2 kilometer space separation between pairs of cases			
		All types	ALL	ALL boys	ALL girls
⩽2	obs.	12	6	0	1
	exp.	13.5	9.0	2.3	2.2
	X	-0.41	-1.00	-1.52	-0.81
⩽4	obs.	27	15	0	4
	exp.	26.3	17.4	4.2	4.3
	X	0.14	-0.58	-2.05	-0.14
⩽6	obs.	40	26	3	5
	exp.	39.2	25.8	6.3	6.2
	X	0.13	0.04	-1.31	-0.48

Time separations between pairs of cases in months		⩽2 kilometer space separation between pairs of cases		
		ALL <6 years	ALL ⩾50,000/mm^3	ANLL
⩽2	obs.	2	1	0
	exp.	3.6	0,4	0.2
	X	-0.84	0.95	-0.45
⩽4	obs.	4	1	0
	exp.	6.8	0.9	0.5
	X	-1.07	0.10	-0.71
⩽6	obs.	6	4	0
	exp.	9.9	1.4	0.7
	X	-1.24	2.20*	-0.84

* Significant $p < 0.025$

birth was shown. Mantel's cluster analysis was also used on the data in New Zealand (Glass et al., 1971).

The results confirm time and space clustering of childhood leukaemia. The authors, however, suggest that this finding is perhaps due to shifting population patterns and unequal population growth.

In Connecticut and Michigan, cases of childhood leukaemia were analysed for time space interaction (Ederer et al., 1964; Stark & Mantel, 1967). In these studies discrete units of space function as "cells" in which the distribution of the patients over time is tested for random distribution. The time and distance boundaries were arbitrary chosen. The results show no significant clustering.

In San Francisco and Lewisham no time space interaction of childhood leukaemia was detected with Knox's method (Klauber & Mustacchi, 1970; Lock & Merrington, 1967).

A reanalysis of the death certificates of leukaemic children in London with a generalised approach of Knox's analysis, in which time and distance intervals and arbitrary periods of latency and susceptibility of leukaemia were taken into account, established no evidence of time space clustering (Smith et al., 1976).

The studies about time space clustering in childhood leukaemia have either produced negative findings or weak positive findings. In this study we found no evidence for time space clustering in the total group of patients, nor in the subgroups classified according to type, age, and sex.

The method of analysis can influence the results of time space clustering. In the Western part of The Netherlands, time space analysis of leukaemic patients do only show clustering with Knox's method for patients with ALL with high initial leucocyte counts ($\geqslant 50{,}000/mm^3$).

Mantel's method, however, could not confirm this finding. Considering the exploratory nature of Knox's analysis, which uses varying critical distance intervals, the clustering could be a change event.

In The Netherlands we found no seasonal variation in months of birth or months of diagnosis of children with leukaemia. Also no significant time space clustering of childhood leukaemia was traced. The restrictions of the statistical methods of cluster analysis have to be taken into account. Long latency periods of aetiological factors could mask clustering. Furthermore, the patients were classified according to cytomorphological criteria and one wonders if these leukaemia subgroups are homogeneous with regard to the aetiology.

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Addendum 4

Childhood leukaemia and parental occupation: a register-based case-control study

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Abstract

To explore possible aetiological factors, a case-control study on childhood leukaemie was performed in The Netherlands. The cases were selected from a complete nation-wide register of cases of childhood leukaemia between 1973 and 1980. Controls were matched with cases for year of birth, sex, and place of residence at the time of diagnosis. The information about possible exposures was collected by a postal questionnaire addressed to the parents. This report concerns the results of the analysis of parental occupations and occupational exposures for 519 patients with acute lymphocytic leukaemia and 507 controls. During pregnancy, more mothers of patients were working in "hydrocarbon-related" occupations; the relative risk was 2.5 (95% confidence interval 0.7 − 9.4). Likewise, greater occupational exposure to chemicals (paint, petroleum products and unspecified chemicals) during pregnancy was found for mothers of patients (relative risk 2.4, 95% confidence interval 1.2 − 4.8). The kind of work being performed by the mothers one year before diagnosis did not differ between cases and controls. No relation was found between a "hydrocarbon-related" job or occupational chemical exposure of the father and leukaemia in the offspring.

Introduction

Several investigators have studied the relation between parental occupation and the risk that an offspring will develop a childhood

malignancy. In one study the risk of dying of a malignant disease was twice as high for children whose fathers were working in "hydrocarbon-related" occupations (Fabia & Thuy, 1974). Other studies,however, could not confirm this finding (Hakulinen et al., 1976; Zack et al., 1980; Kwa et al., 1980; Hemminki et al., 1981; Sanders et al., 1981). Few studies have included an evaluation of the mother's occupation during the pregnancy under study. In Finland, a register-based case-control study showed an increased odds ratio for childhood leukaemia for the offspring of mothers working as pharmacists (Hemminki et al., 1981). The Texas Childhood Cancer Study, which included all types of childhood malignancies, did not find a correlation between mothers with a "hydrocarbon-related" occupation and cancer in their children (Zack et al., 1980)

To explore possible aetiological factors of childhood leukaemia, a case-control study was undertaken. Since a complete nation-wide childhood leukaemia register is maintained in The Netherlands, a sufficient number of patients could be collected for a study of one specific type of childhood malignancy. Part of this exploratory investigation included evaluation of both the maternal and the paternal occupations during the pregnancy as well as in the year before the disease was diagnosed in the child. In addition, the mailed questionnaire included a list of specific occupational risk factors to determine exposure during the relevant pregnancy.

Material and methods

Patients

In The Netherlands a complete nation-wide morbidity register of childhood leukaemia has been maintained since 1972. The completeness of this register was checked and is estimated to be 95.6 − 99.9% (van Steensel-Moll et al., 1983). The diagnosis was confirmed by histological examination of bone marrow smears in the laboratory of the Dutch Childhood Leukaemia Study Group. These slides were reviewed, according to previously determined criteria, by two independent experts (Bennet et al., 1976).

Excluding the 44 foreign children, 713 Dutch children with leukaemia were eligible for this study. They were all < 15 years at the time of disease onset and were diagnosed between 1 January 1973 and 1 January 1980. At the time of this case-control study 50.5% of the leukaemic patients, irrespective of type, had died.

Controls

A population control group was obtained from the Dutch municipal registries. The controls were randomly drawn from the available group of children with the same date of birth (within 2 months) and sex who lived in the same municipality as the patient at the time of diagnosis. Thus, they were matched with the cases according to age, sex and geography. Two controls were selected for each leukaemic patient; the second served as replacement in case of non-response of the first control.

Data collection

The data were collected between 1 October 1981 and 1 December 1982 by means of questionnaires mailed to the children's parents.

The consent to approach the parents was obtained from the attending paediatricians. One month before the questionnaires were to be sent to the parents, the paediatricians had the opportunity to introduce the study to the parents, if this was considered necessary.

The mailed questionnaires were accompanied by an introductory letter to the parents of the patients and the controls. In the letter to the parents of the cases the objective of the study was explained, i.e. to investigate possible causes of childhood leukaemia. The letter to the parents of the controls described a study to determine possible causes of leukaemia, cancer and other serious chronic illnesses in childhood. In these letters a telephone number was given for eventual further information.

Three standard questionnaires were sent with the introductory letter: one for the natural father, one for the natural mother and one concerning the relevant child. The complete list of items of the questionnaires is given in addendum 8G.

A section of the questionnaires concerned the occupations and a list of possible occupational exposures of both parents during pregnancy. The occupations one year before diagnosis were also evaluated.

The occupations during pregnancy and one year before diagnosis were coded according to the Occupation Index of the Institute of Sociology of the University of Nijmegen (1973). This classification was used to construct the main occupational categories (9) and more detailed occupations (110) (Rothman & Boice, 1979). The definition of "hydrocarbon-related" occupations, according to Zack et al. (1980),

includes: machinist, automobile mechanic, painter, gas station attendant, laundry operator, printer, pharmacist, chemical analyst, chemical and petroleum industries, cleaner and dyer.

Parents who did not respond within 5 weeks received a reminder; if necessary a second reminder followed another 4 weeks later. For controls, the replacement control was approached if no answer was obtained from the first control after the second reminder.

Adopted children were excluded from both the control and the case group. Patients were also excluded whenever the paediatrician judged it undesirable to mail questionnaires to those particular parents.

Response

The response rates for the parents of leukaemic patients and controls were 88% and 70%, respectively (Table 1).

At least 50% of the paediatricians took the opportunity to approach the parents before the questionnaire was sent. There was no difference in response rate, however, between informed and non-informed parents.

The control group was supplemented with 121 replacement controls, who were contacted in the same way as the first controls. A total of 625 patients with leukaemia and 615 controls was available for analysis.

Analysis

The present analysis is restricted to the 519 patients with acute lymphocytic leukaemia (ALL) and 507 controls. It was assumed that ALL is more homogeneous with regard to aetiology than the group of all leukaemias. Since ALL accounts for 82% of all leukaemias, a sufficient number of patients was available for analysis. To measure the strength of association the relative risk (RR) was used. Since the cases and controls were matched for age and sex, the relative risk was computed over sex and three age strata (0 – 4, 5 – 9, 10 – 14 years) by means of the Mantel-Haenszel odds ratio. The 95% confidence interval (CI) of the RR was computed using the test-based interval estimation procedure proposed by Miettinen (Rothman & Boice, 1979).

Table 1. Response of parents of leukaemia patients and controls to the mailed questionnaire

	Cases	%	Controls	%
Response	625	88.0	494	69.9
Non-response	56	7.9	184	26.0
Refusal	13	1.8	17	2.4
Adopted child	2	0.3	5	0.7
Moved	14	2.0	7	1.0
	710*		707**	

* Paediatrician refused consent to contact the parents (3)

** Municipal authorities refused to cooperate (6)

Results

Maternal occupation during pregnancy

More mothers of patients worked outside the home during pregnancy than control mothers (RR = 1.3, 95% CI 1.0 – 1.7). The relative risks for various main job categories are presented in Table 2. These risks were computed relative to the category without a job outside the home during pregnancy. More mothers of leukaemic patients worked in

Table 2. Relative risk of leukaemia based on mother's job during pregnancy and one year before diagnosis (adjusted for age and sex)

Maternal	At the time of pregnancy			One year before diagnosis		
Occupational groups	Ca	Co	RR(95% CI)	Ca	Co	RR(95% CI)
Manual & mechanical skills	15	8	2.0 (0.8 – 4.7)	4	7	0.5 (0.2 – 1.9)
Trade & transport	27	22	1.3 (0.7 – 2.3)	15	15	0.9 (0.4 – 1.9)
Administration & office work	42	40	1.1 (0.7 – 1.8)	12	9	1.3 (0.5 – 3.0)
Domestics, hotel & catering	26	10	2.8 (1.3 – 5.7)	15	7	1.9 (0.8 – 4.6)
Medical & social services	21	23	1.0 (0.5 – 1.8)	8	16	0.5 0.2 – 1.1)
Education	16	13	1.3 (0.6 – 2.8)	8	12	0.6 (0.2 – 1.5)
Agriculture, forestry & horticulture	3	7	0.4 (0.1 – 1.7)	3	8	0.4 (0.1 – 1.3)
Manager, police, fire brigade & military	1	0	–	2	0	–
Scientific & artistic	2	2	1.0 (0.2 – 7.5)	2	3	0.6 (0.1 – 3.7)
All occupations	184	153	1.3 (1.0 – 1.7)	69	77	0.8 (0.6 – 1.2)
No occ. outside the home	333	351		450	425	

Ca = case, Co = control, RR = relative risk, CI = confidence interval

factories or as manual labourers. Of these, eight case mothers and two control mothers worked in the textile industry (RR = 4.2, 95% CI 1.0 – 17.7) and four case mothers and two control mothers were factory workers (unspecified) during pregnancy. In addition, more mothers of children with leukaemia worked as domestics or in the hotel and catering industry (service jobs); this usually involved a part-time job as charwoman.

Seven mothers of patients and three mothers of controls had had "hydrocarbon-related" occupations, including printer, dyer, gas station attendant, pharmacist and chemical analyst; this resulted in an increased relative risk of 2.5 (95% CI 0.7 – 9.4).

The answers to the standardized close-ended questions concerning exposure to various occupational risk factors during pregnancy are described in Table 3. Exposure to chemicals (paint, petroleum products and other unspecified chemicals) was reported significantly more often by mothers of cases than of controls. Exposure to cleaning products was also more common among the mothers of patients. This is in agreement with the finding that more case mothers worked as part-time charwomen. During pregnancy exposure to insecticides, pesticides, herbicides and animals was greater for the control group. Similarly occupations in agriculture and horticulture were reported more often by mothers of controls than by mothers of patients (Table 2).

Maternal occupation one year before diagnosis

We also analysed the occupation of the mothers one year before the diagnosis leukaemia in the child was established (Table 2). "Hydrocarbon-related" occupations (petroleum and chemical industry, pharmacist and gas station attendant) were reported by three mothers of patients and three mothers of controls (RR = 1.0, 95% CI 0.2 – 4.7). For this period, however, no data concerning specific occupational exposures were collected.

Paternal occupation at the time of pregnancy

The distribution of the job categories at the time of pregnancy among the fathers of patients with leukaemia and the control fathers was approximately the same, with the exception of the medical and social services (Table 4). The difference found for the medical and social services can be explained almost entirely by the higher number of social workers among the fathers of children with leukaemia. There is

Table 3. Occupational exposure of the mother and father during pregnancy (adjusted for age and sex)

	Mothers at the time of pregnancy			Fathers at the time of pregnancy		
Exposure	Ca	Co	RR(95% CI)	Ca	Co	RR(95% CI)
Pigment (dyes)	22	13	1.8 (0.9 – 3.6)	25	16	1.6 (0.8 – 3.3)
Chemicals*	25	11	2.4 (1.2 – 4.8)	140	113	1.2 (0.8 – 1.7)
Tar or asphalt	1	0	–	26	23	1.1 (0.6 – 2.0)
Plastic or rubber	1	0	–	24	12	2.0 (0.9 – 4.0)
Cleaning products	9	5	1.9 (0.6 – 5.8)	21	16	1.4 (0.7 – 2.8)
Radioactivity	4	1	4.2 (0.6 – 31.8)	13	9	1.4 (0.6 – 3.5)
Exhaust gases	4	0	–	89	70	1.3 (0.8 – 1.9)
Animal products	9	12	0.8 (0.3 – 1.9)	25	26	0.9 (0.5 – 1.7)
Animals	10	12	0.9 (0.4 – 2.1)	36	38	1.0 (0.6 – 1.6)
Sun	8	15	0.6 (0.2 – 1.3)	82	68	1.2 (0.8 – 1.9)
Pesticides, herbicides & insecticides	4	6	0.7 (0.2 – 2.5)	36	35	1.0 (0.6 – 1.7)

*Paint, petroleum products & other chemicals

Ca = case, Co = control, RR = relative risk, CI = confidence interval

Table 4. Relative risk of leukaemia based on father's job during pregnancy and one year before diagnosis (adjusted for age and sex)

Paternal	At the time of pregnancy			One year before diagnosis		
Occupational groups	Ca	Co	RR(95% CI)	Ca	Co	RR(95% CI)
Manual & mechanical skills	188	194	1.0 (0.7 – 1.3)	183	170	1.1 (0.8 – 1.5)
Trade & transport	78	79	1.0 (0.7 – 1.5)	67	81	0.8 (0.6 – 1.3)
Domestics, hotel & catering	7	5	1.5 (0.4 – 5.5)	9	4	2.5 (0.6 – 7.9)
Medical & social services	18	7	2.8 (1.1 – 7.2)	19	8	2.3 (1.0 – 5.2)
Agriculture, forestry & horticulture	35	39	0.9 (0.5 – 1.5)	32	38	0.9 (0.5 – 1.5)
Manager, police, fire brigade & military	10	13	0.8 (0.3 – 1.8)	11	13	0.8 (0.3 – 1.8)
Scientific & artistic	13	16	0.8 (0.4 – 1.8)	13	15	0.9 (0.4 – 1.9)
Administration & education (reference category)	115	114		126	127	

Ca = case, Co = control, RR = relative risk, CI = confidence interval

no clear explanation for this finding, but it does not seem to be related to a specific exposure. Medical and paramedical occupations were reported by ten case fathers and seven control fathers.

Because only 44 fathers did not work during the pregnancy, the category administration and education was used as reference category for computing the relative risks.

During this period fathers of leukaemic patients did not occupy more "hydrocarbon-related" jobs (Table 5), nor did they report more occupational exposure to chemicals (Table 3).

Paternal occupation one year before diagnosis

Table 4 lists the jobs of the fathers one year before diagnosis of the child's disease. Again medical and social occupations were reported more often by fathers of patients. The "hydrocarbon-related" occupations in this period were distributed equally among fathers of cases and controls (Table 5).

Discussion

The present study covered all children with acute lymphocytic leukemia in The Netherlands diagnosed between 1973 and 1980 (van Steensel-Moll et al., 1983). In all cases the diagnosis was confirmed by a histological examination of a bone marrow preparation. Therefore selection bias seems unlikely. In view of the high response rate for the parents of children with leukaemia, response bias seems unlikely. Since the patients belonged to the total Dutch population, the control group was also selected from that population. Since the families of controls would have less interest in the study than the families of the patients, the response of the former can be expected to be lower (Cole, 1979). In this study the response rate for the control parents was 70%; in order to ensure reliable analyses we decided to include replacement controls. Because the replacement controls were drawn from the same municipality and were selected in the same way and at the same time as the first controls and since the response was comparable to that of the first controls (67%), bias introduced by the inclusion of substitute controls seems unlikely.

Table 5. Paternal "hydrocarbon-related" occupations during pregnancy and one year before diagnosis

	During pregnancy			One year before diagnosis		
"Hydrocarbon-related occupations"	Ca	Co	RR(95% CI)	Ca	Co	RR(95% CI)
Auto mechanic, machinist, gas station attendant & miner	18	23	0.8 (0.4 − 1.5)	16	19	0.8 (0.4 − 1.7)
Painter, cleaner & dyer	8	5	1.6 (0.5 − 5.0)	8	6	1.3 (0.4 − 4.0)
Petroleum & chemical industry	5	4	1.2 (0.3 − 4.8)	6	3	2.0 (0.5 − 8.0)
Pharmacist, printer & chemical analyst	6	4	1.5 (0.4 − 5.4)	6	3	2.0 (0.5 − 8.0)
All "hydrocarbon-related" occupations	37	36	1.0 (0.6 − 1.7)	36	31	1.2 (0.7 − 2.0)
Administration & education	115	114		126	127	

Ca = case, Co = control, RR = relative risk, CI = confidence interval

Maternal occupation

More mothers of patients worked in factories or as manual labourers as well as domestics or in hotel and catering industries during the relevant pregnancy, than control mothers. These main job categories are rather heterogeneous. The textile industry was listed significantly more often by mothers of patients than mothers of controls. However, no exposure to chemicals was reported by this group of (mainly) dressmakers.

During pregnancy "hydrocarbon-related" occupations, defined by Zack et al. (1980), gave an increased relative risk (RR = 2.5, 95% CI 0.7 − 9.4).

However, the number of mothers with such an occupation was small. The computed relative risk was not significant at the 5% level. In agreement with this finding more mothers reported occupational exposure to chemicals during pregnancy. Since more mothers of patients worked outside the home, the RR's for "hydrocarbon-related" occupations and exposure to chemicals were also calculated relative to the category of working women with other jobs or without such exposure, respectively: the RR's decreased slightly and were 2.0 (95% CI 0.5 − 7.6) and 2.0 (95% CI 1.0 − 4,2), respectively. The total number of mothers who experienced exposure to chemicals is higher than the number with "hydrocarbon-related" jobs, as defined by the list of occupations. Since for the purpose of analysis paint, petroleum products and other unspecified chemicals were grouped together, "exposure to chemicals" represents a diversity of exposures. This could explain the lower number of "hydrocarbon-related" jobs relative to the higher occupational exposure to chemicals. This finding applies for both the cases and the controls. All mothers with a "hydrocarbon-related" occupation during pregnancy reported exposure to chemicals.

The Texas Childhood Cancer Study, however, did not find an association with "hydrocarbon-related" occupations for the mothers of children with cancer relative to the controls (Zack et al., 1980). In a register-based case-control study in Finland, pharmacists were more frequently reported among mothers of patients with leukaemia.

With regard to occupational exposure of the parents to chemicals, both prenatally and postnatally but before diagnosis, Gold et al.(1979), could not demonstrate a difference between children with brain tumours and either the cancer control group or the population control group. Another case-control study of children with brain

tumours, however, showed that parents of cases were more likely than those of controls to report maternal occupations involving chemicals (Peters et al., 1981). In our study more mothers of controls worked in the fields of agriculture, horticulture and forestry; this also applied for the fathers of controls. However, in the Finnish Study of all childhood malignancies a slightly increased relative risk was found for women living on a farm (RR = 1.7; Hemminki et al., 1981).

Paternal occupation

The paternal occupations during pregnancy and one year before diagnosis were evaluated. As far as the main job categories are concerned more case fathers had a job in the social services, which does not seem to be related to a specific exposure. The other job categories were about equally distributed among cases and controls.

Fathers of leukaemic patients did not work more frequently in "hydrocarbon-related" occupations, neither during pregnancy nor one year before diagnosis. This finding is in accordance with the results of several studies (Hakulinen et al., 1976; Zack et al., 1980; Kwa et al., 1980; Hemminki et al., 1981; Sanders et al., 1981) carried out subsequent to a positive report from Quebec, Canada (Fabia & Thuy, 1974). In the Canadian study, children born to fathers with "hydrocarbon-related" occupations had approximately twice the risk of dying of leukaemia before their 5th birthday when compared with the children of fathers with other occupations. In the Finnish study leukaemia appeared to be particularly more common among the children of motor vehicle drivers (Hemminki et al., 1981). In our study the relative risk for this category was calculated to be 1.4(95% CI 0.7 − 2.5) during pregnancy and 1.0(95% CI 0.6 − 2.0) one year before diagnosis. Thus we could not confirm this finding. Nor did the fathers of patients with leukaemia report more frequent exposure to exhaust gases (Table 3).

Possible bias

Occupational exposure to chemicals may have been exaggerated by the mothers of children with leukaemia as questions about specific exposures may have been asked several times of the cases but only once of the controls (recall bias - Sackett, 1979). Furthermore, knowledge of the child's disease status may have influenced both the intensity and the outcome of the search for exposure to a putative risk factor. (exposure suspicion bias - Sackett, 1979). However, if these biases play

an important role then one would expect an association between exposure to chemicals not only for the maternal but also for the paternal occupation. Another argument against bias is that those maternal jobs during pregnancy defined as "hydrocarbon-related" exhibit a higher relative risk. All of these mothers reported occupational exposure to chemicals during pregnancy. Since a generally accepted occupation code book was used, it seems unlikely that the code "hydrocarbon-related" occupation was chosen more often for cases. If these biases play a role, the same result should be seen for paternal "hydrocarbon-related" occupations in both periods and for maternal occupations one year before diagnosis. This, however, was not the case in our study.

If overreporting influenced the rate of exposure to chemicals for the case mothers then the answers for the other exposures, especially insecticides, herbicides and pesticides, should be influenced in the same way. However this was not apparent, for either maternal or paternal occupational exposures.

To obtain an insight into the role of possible bias, we again examined the job-related RR's (110 occupations) for all mothers within the category with exposure to chemicals and the category without exposure to chemicals during pregnancy (Tockman, 1982). Of the mothers who were exposed to chemicals, there were more nurses in the case group; in contrast, of the mothers who did not report exposure to chemicals, there were more nurses in the control group. For the other jobs this phenomenon was not found. If the nurses are excluded from the analysis, then a two-fold risk for leukaemia still exists for the offspring of mothers exposed to chemicals during pregnancy (RR = 2.2, 95% CI 1.0 – 4.7).

In general, bias with respect to occupational exposures of the mother during pregnancy cannot be excluded. The lack of detailed occupational histories prevents speculation about specific carcinogens. In studies on leukaemia in adults an association with benzene has been established (Aksoy, 1980).

In summary, no association between "hydrocarbon-related" occupations of the father and childhood leukaemia was found. On the other hand more mothers of leukaemic patients reported occupational exposure to chemicals during pregnancy compared with population controls and more of these so-called case mothers had a "hydrocarbon-related" occupation. However, these findings are based on low exposure frequencies and may only explain to a very small degree the

incidence of leukaemia. The influence of possible recall or exposure suspicion bias cannot be excluded completely. This study does not yield any data about the type, duration or intensity of exposure to chemicals.

Further studies on the relation between maternal jobs during pregnancy and childhood malignancies are necessary. Nowadays more women work during pregnancy. Furthermore, the kind of job undertaken by women has been extended and is no longer restricted to the "typically female" administrative jobs.

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Addendum 5

Risk factors in childhood leukaemia: a case-control study of prenatal factors.

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Abstract

To explore possible aetiological factors of childhood leukaemia, a case-control study was conducted in The Netherlands. The cases were obtained from a complete nation-wide register of childhood leukaemia and consisted of all patients recorded between 1973 and 1980. Controls were matched with the cases for year of birth, sex and place of residence at the time of diagnosis.

Information about the exposure of both parents to potential risk factors in the year before pregnancy as well as the exposure of the mother during pregnancy was collected via a mailed questionnaire addressed to the parents. The present paper concerns 519 patients with acute lymphocytic leukaemia and 507 controls. Subfertility (relative risk (RR) = 6.0; 95% confidence interval (CI) 0.9 − 38.2), "hormonal" treatment (RR = 2.8; 95% CI 0.7 − 10.5) and a history of two or more miscarriages (RR = 1.6; 95% CI 1.0 − 2.7) were more frequently reported among mothers of children with leukaemia. The use of oral contraceptives was also slightly higher (RR = 1.2; 95% CI 0.9 − 1.6). Paternal exposures in the year before pregnancy could not be associated with childhood leukaemia. For the period of pregnancy we confirmed increased RR's for diagnostic radiation (RR = 2.2; 95% CI 1.2 − 3.8) and threatened abortion (RR = 1.6; 95% CI 1.0 − 2.6). There was also an increased use of "drugs to maintain pregnancy" in this period (RR = 1.9; 95% CI 1.0 − 3.5). No association between childhood leukaemia and prenatal viral infections, smoking or alcohol

was found. Interaction between a history of two or more miscarriages and prenatal exposure to radiation was confirmed.

Introduction

In animal studies an increased cancer risk has been found for offspring exposed to carcinogens during pregnancy (Tomatis & Mohr, 1973). The suggestion that intra-uterine exposure to carcinogens can also cause malignancy in humans, is supported by the occurrence of vaginal clear-cell adenocarcinoma in women whose mothers received diethylstilboestrol during pregnancy (Herbst et al., 1971). In addition, the development of a neuroblastoma in three children with the foetal hydantoin syndrome suggests an increased risk of tumours after in-utero exposure to hydantoins (Seeler et al., 1979).

Furthermore, retrospective as well as prospective studies have revealed an association between exposure to radiation during pregnancy and childhood leukaemia (Stewart et al., 1958; MacMahon, 1962). Acute lymphocytic leukaemia constitutes 82% of all leukaemias in childhood and is relatively uncommon in older age groups. The distribution of this malignancy with respect to age peaks at 3 − 4 years. This peak incidence could indicate that leukaemia originates during intra-uterine development of the child or in early infancy.

To explore possible aetiological factors of childhood leukaemia, a case-control study was undertaken in The Netherlands. The existence of a complete nation-wide morbidity register of childhood leukaemia offered the opportunity to conduct a study with a sufficient number of patients with one specific childhood malignancy.

As part of this study the relation between childhood leukaemia and exposure to potential risk factors of both the father and the mother in the year before pregnancy, thus including maturation of germ cells and conception, as well as exposure of the mother during pregnancy was evaluated.

Subjects and methods

For the selection of patients and controls as well as data collection and the response of the parents, see "material and methods" of addendum

4 (childhood leukaemia and parental occupation: a register-based case-control study).

Three standard questionnaires were sent with the introductory letter: one for the natural father, one for the natural mother and one concerning the relevant child.

One section of the questionnaires for both parents concerned their medical history, alcohol consumption and smoking habits in the year before the relevant pregnancy. Furthermore, the mother was asked about the use of oral contraceptives, miscarriages, still births, medication, illnesses, X-ray exposure, vaccination, alcohol consumption and smoking habits during pregnancy.
To achieve optimal standardization of the questionnaires (mark sensing forms), most questions were closed-ended (yes or no answers).

The present analysis is restricted to the 519 patients with acute lymphocytic leukaemia (ALL) and 507 controls. To measure the strength of association the relative risk (RR) was used. Since the cases and controls were matched for age and sex, the relative risk was computed over sex and three age strata (0 – 4, 5 – 9, 10 – 14 years) by means of the Mantel-Haenszel odds ratio. The 95% confidence interval (CI) of the RR was computed using the test-based interval estimation procedure proposed by Miettinen (Rothman & Boice, 1979).

In order to adjust for the possible confounding effect of several factors simultaneously, multiple logistic regression analysis by means of the method of maximum likelihood was applied to some of the risk factors (Breslow & Day, 1980).

Furthermore, interactions and relationships between potential risk factors before and during pregnancy were tested (Lilienfeld, 1982).

Results

General and obstetric variables

The maternal and paternal age at the time of the child's birth did not differ significantly between cases and controls. The classification into eight educational categories was used as a measure of social class. A relative risk of 1.2 for the five upper educational classes relative to the three lower classes (vocational, primary or lower schooling) was found (Table 1).

An equal number of mothers of patients and controls reported ever having had a miscarriage. A history of two or more miscarriages,

Table 1. The relative risk (RR) for some general and obstetric variables (adjusted for age and sex)

Variable	Ca	Co	RR(95% CI)
Maternal age >35	51	58	0.8(0.5 − 1.3)
Paternal age >35	76	94	0.8(0.6 − 1.1)
Social class	251	228	1.2(0.9 − 1.5)
Ever miscarriage	116	115	1.0(0.7 − 1.3)
$\geqslant 2$ Miscarriages	39	23	1.6(1.0 − 2.7)
Still births	21	14	1.4(0.7 − 2.8)
Oral contraceptives before pregnancy	171	146	1.2(0.9 − 1.6)
Total respondents	519	507	

Ca = case, Co = control, RR = relative risk, CI = confidence interval

however, was more frequently reported by mothers of patients. After adjustment for possible confounders, i.e. number of deliveries, maternal age and social class, the RR for repeated miscarriages ($\geqslant 2$) relative to no miscarriages was 1.7; 95% CI 1.0 − 2.9. The relative risks for the use of oral contraceptives (OC) and for the duration of use before the relevant pregnancy ($<$ 5 years, $\geqslant 5$ years) were adjusted

for mother's age, year of birth of the child, social class and birth order of the child; the RR for OC was then 1.2(95% CI 0.9 − 1.7) and for both < 5 years and ⩾5 years duration of OC use, 1.3.

Exposure of the mother to potential risk factors in the year before pregnancy (Table 2)

Among the mothers of cases and controls who had ever used oral contraceptives (OC) before pregnancy 74% and 70% respectively reported use of OC in the year preceding the pregnancy. More mothers of patients used oral contraceptives in this year. Adjustment for maternal age, year of birth of the child, social class and birth order of the child did not change the RR for OC use.

The overall use of medicines is slightly higher for mothers of patients (Table 2). The use of hormones for non-contraceptive purposes was nearly three times greater for the case mothers: for the case group, clomiphene (2), "drugs to induce pregnancy" - probably clomiphene (3), regulation of menstruation (1) and threatened abortion (2), and for the control group, regulation of menstruation (1) and threatened abortion (2). An RR for hormonal treatment of 2.5 (95% CI 0.7 − 9.7) was computed after adjustment for maternal age, number of miscarriages preceding the relevant pregnancy and oral contraceptive use in the year preceding the pregnancy. Mothers of cases and controls who did not take any medication were used as the reference category. "Drugs to induce pregnancy" (usually clomiphene) were reported by 5 case mothers and none of the control mothers. In accordance with this finding more mothers of children with leukaemia were hospitalized or had visited a gynaecologist for problems of fertility. Miscarriages and curettages showed a relative risk of nearly two.

In total, 58 mothers of patients and 46 mothers of controls were hospitalized or had visited a specialist. Specialist care was required for infections (10 cases and 7 controls), fractures (1 case and 1 control), epilepsy (2 cases and 2 controls), gynaecological operations (2 cases and no controls), skin diseases (2 cases and 9 controls) and labour (3 cases and 4 controls). All other disorders were reported in very low frequencies and showed no obvious differences between the mothers of patients and controls.

We also included questions about the occurrence of chickenpox, measles, mumps, rubella, herpes zoster and other viral infections. No differences, however, between the mothers of cases and controls were

Table 2. The relative risk for variables in the year before pregnancy for the mother and the father (adjusted for age and sex)

Variable	Mother			Father		
	Ca	Co	RR (95% CI)	Ca	Co	RR (95% CI)
Oral contraceptives	127	103	1.3 (1.0 – 1.8)			
Drugs:	73	54	1.5 (1.0 – 2.1)	61	48	1.3 (0.9 – 1.9)
female "hormones"	8	3	2.8 (0.7 – 10.5)			
"hormones" to induce pregnancy	5	0	–			
Hospitalization/ consultation:						
subfertility	6	1	6.0 (0.9 – 38.2)	1	0	–
miscarriages & curettages	11	6	1.8 (0.7 – 5.0)			
Viral infections	20	15	1.3 (0.6 – 2.5)	15	12	1.0 (0.4 – 2.3)
Alcohol consumption	323	298	1.2 (0.9 – 1.5)	430	433	0.8 (0.6 – 1.2)
Smoking	232	225	1.0 (0.8 – 1.3)	363	364	0.9 (0.7 – 1.3)

Ca = case, Co = control, RR = relative risk, CI = confidence interval

found. Furthermore no relation could be found between alcohol, smoking habits, the frequency of alcohol consumption (daily, weekly or less than once per week), the quantity of cigarettes smoked or any combination of these and leukaemia in the offspring.

Exposure of the father to potential risk factors in the year before pregnancy (Table 2)

Table 2 shows the use of drugs, alcohol and cigarettes for the fathers and reveals no significant differences between case and control fathers. Forty fathers of leukaemic patients and 34 fathers of controls were hospitalized or had visited a specialist for a diversity of reasons. No differences, however, between cases and controls were found. Infections were reported by the fathers of 9 cases and 7 controls, trauma by the fathers of 4 cases and 5 controls. Other illnesses were reported in very low frequencies. The occurrence of viral infections, the same as those listed in the questionnaire for the mother, was the similar for the case and control fathers.

Exposure of the mother to potential risk factors during pregnancy (Table 3)

The RR's for different drugs taken during pregnancy are given in Table 3. The overall drug intake is somewhat higher for the mothers of cases compared with the mothers of controls, i.e. 158 and 119, respectively. When those drugs which are available without a prescription are excluded, the mothers of 135 cases and 111 controls reported the use of medicines during pregnancy (RR = 1.3, 95% CI 1.0 − 1.7). Twice as many mothers of patients reported use of "drugs to maintain the pregnancy" (hormones, medication for threatened abortion, drugs to inhibit early contractions of the uterus or early labour). The RR for these drugs together was adjusted for maternal age (RR = 2.0, 95% CI 1.1 − 3.9) and number of preceding miscarriages (RR = 1.9, 95% CI 1.0 − 3.8) and hormonal medication the year before the relevant pregnancy (RR = 2.6, 95% CI 1.2 − 5.5) simultaneously.

Complications of the pregnancy, such as maternal bleeding, threatened abortion, solutio placentae and early labour, which required hospitalization or a gynaecologist consultation, were slightly more frequent among the mothers of leukaemic patients (Table 3). In accordance with this finding more mothers of cases underwent echographic examination. Diabetes and hypertension were almost

Table 3. The relative risk for potential risk factors during pregnancy (adjusted for age and sex)

Variable	Ca	Co	RR(95% CI)
Medication:			
"drugs to maintain pregnancy"	27	17	1.9(1.0 – 3.5)
antibiotics	13	14	1.1(0.5 – 2.3)
analgesics	8	3	3.1(0.9 – 11.0)
anti-epileptic drugs	3	2	1.7(0.3 – 10.2)
diuretics	3	2	1.7(0.3 – 10.2)
iron preparations	56	49	1.3(0.9 – 2.0)
sedatives or sleeping pills	15	6	2.9(1.2 – 7.2)
others	33	26	1.4(0.8 – 2.5)
Hospitalization/consultations			
diabetes	6	4*	1.5(0.4 – 5.3)
hypertension	8	9	0.9(0.3 – 2.3)
other complications of pregnancy**	14	8	1.7(0.7 – 4.1)
infections	10	7	1.4(0.5 – 3.9)
Vaccination:	15	16	0.8(0.4 – 1.6)
rubella vacc.	9	14	0.6(0.3 – 1.4)
influenza vacc.	4	2	2.0(0.4 – 10.3)
Viral infections	16	11	1.4(0.7 – 3.1)
Radiation	41	19	2.2(1.2 – 3.8)
Threatened abortion	46	29	1.6(1.0 – 2.6)
Alcohol	188	186	1.0(0.8 – 1.2)
Cigarettes	165	164	1.0(0.7 – 1.3)

* 1 juvenile diabetes

** ICD 640, 641, 644; bleeding, threatened abortion, early labour

Ca = case, Co = control, RR = relative risk, CI = confidence interval

equally reported, as was the concurrent occurrence of the two diseases (2 cases, 3 controls).

A diversity of other illnesses was reported by the mothers but the frequency of each disease was very low and no obvious relation with case-control status was found.

Vaccinations or viral infections in the prenatal period did not yield increased relative risks for childhood leukaemia. The question about viral diseases was presented in the same way as that pertaining to the one-year period before pregnancy.

Radiodiagnostic examinations were experienced significantly more frequently by the mothers of patients. The RR was not changed by simultaneously including the possible confounders maternal age, birth year of the child, hospitalization during pregnancy and birth order of the child in the logistic model.

The question whether there was a period of threatened abortion during pregnancy of the child concerned was answered in the affirmative by more mothers of children with leukaemia (Table 3). Adjustment for maternal age, number of miscarriages and social class did not change the RR noticeably. Alcohol and smoking habits were not related to childhood leukaemia.

Interaction and relationship between different variables (Tables 4 & 5)
The interaction between some of the variables is shown in Table 4. When the potential risk factors "two or more miscarriages" and "prenatal exposure to radiation" were present together, the RR non-significantly doubled (RR = 4.0). However, in only a few mothers these two events occurred simultaneously.

We also tested the interactions between "problems of fertility and menstruation in the year before the relevant pregnancy" and "exposure to radiation", "hormonal therapy" or "threatened abortion" during the relevant pregnancy. Those categories with two positive risk factors, however, consisted of too few subjects to be able to calculate the RR.

Interaction between hormonal treatment during pregnancy and prenatal exposure to radiation, smoking, alcohol consumption, viral infections or occupational exposure to chemicals during pregnancy was not found (Table 5). The latter risk factor is described in another paper (van Steensel-Moll et al., submitted for publication).

Table 4. Interaction between the variables miscarriage and maternal age or prenatal exposure to radiation

	No miscarriages	⩾2 Miscarriages
<35 Year	1.0	1.6(0.9 − 2.9)
⩾35 Year	0.8(0.5 − 1.3)	1.7(0.6 − 5.1)
No radiation	1.0	1.6(0.9 − 2.8)
Radiation	2.4(1.3 − 4.6)	4.0(0.5 − 30.7)

Discussion

The present study covered all children with acute lymphocytic leukaemia in The Netherlands diagnosed between 1973 and 1980 (van Steensel-Moll et al., 1983). In all cases the diagnosis was confirmed by histological examination of a bone marrow preparation. In view of the high response rate for the parents of patients with leukaemia, selection bias seems unlikely.

The control group was selected from the total Dutch childhood population since the patients were derived from that population. Since the population controls would have less interest in the study than the patient's family, the reponse of the former can be expected to be lower

(Cole, 1979). In this study the response rate for control parents was 70%; for analytical purposes it was then decided to add replacement controls. Since the replacement controls were drawn from the same municipality and were selected in the same way and at the same time as the first controls and since the response was similar to that of the first controls (67%), bias introduced by the inclusion of substitute controls seems unlikely.

In this report the emphasis is placed upon a selected number of items. The mothers of leukaemic children had a history of two or more miscarriages more often than the mothers of the controls (adjusted for sex, age of the child at diagnosis, maternal age, number of deliveries and social class). This is in accordance with other studies (Stewart et al., 1958; Miller, 1963). Over 50% of all clinically recognizable spontaneous abortions are due to chromosomal abnormalities (Jacobs, 1977); however, hormonal disorders or infectious diseases could also play a role.

Exposure to potential risk factors in the year before the pregnancy
A period of one year before pregnancy was chosen because it covers the maturation of the germ cells and conception. No relationship was seen between the case-control status of the fathers and the following variables: drugs, diseases which required hospitalization or a specialist consultation, viral infections, smoking habits or alcohol consumption.

Use of oral contraceptives (OC) in the year before pregnancy was reported by 127 mothers of patients and 103 mothers of controls. We did not find a relation between case-control status and duration of OC use.

In a study of aetiological factors of brain tumours in children, it was found that the mothers of these children did not differ from the mothers of the cancer control group or the population control group as far as the use of oral contraceptives prior to the birth of the relevant child is concerned (Gold et al., 1979). There is also no evidence that children born to women who used oral contraceptives prior to pregnancy exhibit a higher incidence of birth defects (Rothman & Louik, 1978; Royal College of General Practitioners, 1976). In contrast, chromosomal abnormalities in foetuses have been reported to be more common following the use of oral contraceptives (Alberman, 1976), although these findings have not been confirmed (Klinger et al., 1976). The period between discontinuation of oral contraceptives and pregnancy was longer for mothers of patients (RR

Table 5. Interaction between hormone therapy during pregnancy and prenatal diagnostic radiation, smoking, alcohol,viral infections or occupational exposure to chemicals

	No "hormones"	"Hormones"
No radiation	1.0	2.3(1.4 − 3.8)
Radiation	2.8(1.4 − 5.6)	0.6(0.1 − 6.6)
No smoking	1.0	1.6(0.6 − 4.0)
< 10 Cigarettes	1.1(0.8 − 1.7)	1.5(0.3 − 6.9)
⩾ 10 Cigarettes	0.8(0.6 − 1.3)	1.9(0.5 − 7.9)
No alcohol	1.0	2.9(1.1 − 7.4)
Alcohol	1.2(0.9 − 1.7)	0.9(0.3 − 2.7)
No viral infection	1.0	1.7(0.8 − 3.3)
Viral infection	1.2(0.4 − 3.3)	0/0
No occupational exposure to chemicals	1.0	1.1(0.5 − 2.4)
Occupational exposure to chemicals	3.1(1.2 − 7.8)	0/1

⩽ 2 months = 1.0, RR 2 − 5 months = 1.3, RR ⩾ 5 months = 1.5), which could suggest a relative decrease in fertility among the mothers of patients. However, we did not inquire into the use of other contraceptives during this period.

Five mothers of patients had used drugs "to induce pregnancy" (2 x clomiphene, 3 x probably clomiphene). In accordance with this finding the mothers of the cases reported more fertility problems (RR = 6.0; 95% CI 0.9 − 38.2). In addition more mothers of leukaemic children reported miscarriage or currettage in the year before pregnancy (RR = 1.8; 95% CI 0.7 − 5.0).

In the literature conflicting reports have appeared on chromosomal abnormalities in embryos of mice and rabbits after superovulation (Tagaki & Sasaki, 1976; Fechheimer & Beatty, 1974). Congenital defects have been reported in 11 children of 10 women who received clomiphene to induce ovulation (IARC, 1979). The results of a follow-up study suggested that subfertility in women might be associated with an increased prevalence of congenital defects in the offspring (Ahlgren et al., 1976). Other studies, however, do not corroborate this finding (Hack et al., 1972). No relationship was found between the case or control status of the mother and smoking habits or alcohol consumption.

The results of case-control studies based on the parents' or patients' memory could be influenced by recall and exposure suspicion bias (Sackett, 1979). The overall use of drugs was slightly increased among mothers of children with leukaemia, which might suggest bias. Among these drugs, the use of antibiotics was slightly increased (18 cases/12 controls). Other drugs, however, were reported with nearly equal frequency (iron preparations 7/6, analgesics 9/7, anti-epileptic drugs 3/2, others 18/19). These findings make it rather unlikely that the higher consumption of drugs "to induce pregnancy" can be explained by bias.

Events such as hospitalization or a visit to a gynaecologist for problems of fertility are not likely to be easily influenced by memory bias. Skin diseases (dermatitis, psoriasis and acne) were reported more frequently by controls (2 cases/9 controls). If overreporting of diseases by the mothers of patients plays an important role, one would not expect a higher number of such minor diseases as skin diseases among the controls.

Exposure to potential risk factors during pregnancy

In our study more mothers of children with leukaemia reported use of "drugs to maintain pregnancy" and "pregnancy complications".

A case-control study in Finland revealed no association between leukaemia and female hormonal treatment during pregnancy

(Salonen, 1976). From their data we calculated the RR to be 0.7 (95% CI 0.3 – 1.6). A case-control study of children with brain tumours, a cancer control group and a population control group also yielded no association in this respect (Gold et al., 1979). A recent report of the Oxford Childhood Cancer Survey (OCCS) gave a RR for hormones during pregnancy, stratified for X-ray exposure during pregnancy, of 1.3 (recalculated 95% CI 1.0 – 1.5). The group that did not take this specific medication was used as reference category (Kinnier Wilson et al., 1981). In our study the RR for "drugs to maintain pregnancy" relative to those who did not take these drugs was 1.7 (95% CI 0.9 – 3.1).

During pregnancy the overall use of drugs among the mothers of children with leukaemia was slightly increased. However, only a higher intake of hormones, analgesics and sedatives or sleeping pills was observed; for other drugs no essential differences were found. This indicates that systematic overreporting by the mothers of patients alone seems an unlikely explanation.

The results with respect to hormones suggest three possibilities: an effect of the disorder which required hormonal treatment; an effect of the drug, analogous to the effect of diethylstilboestrol on the occurrence of vaginal clear-cell adenocarcinoma or an association between hormones and another unidentified risk-determining factor (Kinnier Wilson et al., 1981). In accordance with the OCCS threatened abortion and threatened early labour were associated with case-control status (RR = 1.6, 95% CI 1.0 – 2.6).

Several retrospective as well as prospective studies have evaluated the possibility that maternal-foetal infections might be associated with the subsequent development of childhood malignancies (Munoz, 1976). An 11-year follow-up study of 16,750 British women with a history of influenza during pregnancy shows a ninefold increase in childhood leukaemia and other neoplasms of lymphatic or haematopoietic tissues (Fedrick & Alberman, 1972). The OCCS found a RR of 1.5 for all malignancies if influenza was reported during pregnancy. In this ongoing study an increased RR for chickenpox was not observed (Blot et al., 1980). Other studies did not find an association between prenatal influenza and cancer in the offspring (Munoz, 1976).

In our study no clear relation between prenatal viral infections and childhood leukaemia was found (RR = 1.4). Two mothers of patients and one mother of a control reported varicella infection (RR = 2.0;

95% CI 0.2 − 20.8). Other viral infections were reported by 14 cases and 10 controls.

In a preliminary communication in 1956 the association between malignant diseases in childhood and diagnostic radiation in utero was reported (Stewart et al., 1956). For leukaemia the RR for prenatal exposure to radiation was 1.5; 95% CI 1.3 − 1.6 (Bithell & Stewart, 1975).

A prospective study reported that prenatal exposure to diagnostic X-rays increased the frequency of childhood leukaemia, cancer of the nervous system and other cancers (MacMahon, 1962). After prolongation of this study the original risk ratio for leukaemia (RR = 1.5) remained virtually unchanged , but that for solid tumours was no longer elevated (Miller, 1982). Thus prenatal exposure to radiation seems to be associated specifically with childhood leukaemia. In our study the RR for prenatal exposure to radiation was 2.2 (95% CI 1.2 − 3.8). The RR for exposure to radiation in the first month of pregnancy was 7.2 (95% CI 1.2 − 43.7). All except one of these mothers reported routine thorax X-rays during the first month of pregnancy. According to Bithell and Stewart (1975) the early months appear to carry a much higher risk.

Since the mothers provided the information about the roentgenological examination, no data on the number of X-rays were available. Others have already demonstrated a dose-response relation between the number of films and cancer risk (Bithell & Stewart, 1975).

In a follow-up study of 89,302 mothers a relative risk of 1.3 (95% CI 0.8 − 2.2) was found for cancer among the offspring of women who smoked during pregnancy (Neutel & Buck, 1971). The few reported case-control studies of maternal smoking were not able to demonstrate a correlation (Everson, 1980). We also could not find an association between smoking of the mother during pregnancy and leukaemia in the offspring.

Since ALL peaks at 3 − 4 years of age, we compared the prenatal exposure to potential risk factors of the youngest age group with that for the two older age groups: 5 − 9 and 10 − 14 years. However, the distribution of the different risk factors over cases and controls did not differ markedly within the three strata.

Interaction and relationship between different variables
Biologically, cancer is most probably the end result of a complex multistage process and may therefore be due to a sequence of exposures to different agents (Farber & Cameron, 1980). For this reason we studied the interactions between different potential risk factors. A doubling of the RR was found when habitual abortion ($\geqslant 2$ miscarriages) and prenatal exposure to radiation were both present. However, the presence of both risk factors was encountered in only four patients and one control. This interaction was also found in another case-control study (Gibson et al., 1968).

It has been suggested that hormones may stimulate carcinogenesis by providing a background - either normal (permissive) or abnormal (teratogenic) - for subsequent tumourigenesis by chemical, physical or viral agents (IARC, 1979). We tested the interaction between hormonal therapy during pregnancy and the following variables: prenatal exposure to radiation, smoking, alcohol, viral infections and occupational exposure to chemicals. However, the RR for hormonal treatment was not increased by adding one of these variables. The numbers in each exposure category, however, were very small.

In summary, subfertility, hormonal treatment in the year preceding pregnancy and habitual abortion were more frequently reported among the mothers of children with leukaemia. Furthermore, an increased use of hormones and sedatives or sleeping pills during pregnancy and more threatened abortions were observed in this group. Interaction of the risk factors "two or more miscarriages" and "prenatal exposure to radiation" was found. We confirmed an increased RR for diagnostic X-rays during pregnancy. In agreement with some studies no association between reported viral infections during pregnancy and childhood leukaemia was found.

Our study indicates the necessity of further inquiries into the relation between childhood leukaemia and subfertility of the mother as well as the probably associated hormonal treatment during pregnancy.

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Addendum 6

Childhood leukaemia and infectious diseases in the first year of life: a register-based case-control study

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Abstract

In The Netherlands a nation-wide register of children with leukaemia formed the basis for a case-control study. All cases of childhood leukaemia diagnosed between 1973 and 1980 were eligible for the study. Population controls were matched with the cases for year of birth, sex and place of residence at the time of diagnosis. The information was collected by mailed questionnaires addressed to the parents. The analyses are based on 492 children with leukaemia and 480 controls.

Common colds, periods of fever and primary childhood infections showed after adjustment for the potential confounders birth order, family size, social class and residential space (number of rooms) slightly decreased RR's of 0.8, 0.9 and 0.8, respectively. Furthermore, significantly fewer cases reported infectious diseases which required hospitalization (RR = 0.7; 95% confidence interval (CI) 0.4 − 1.0).

The general infection risk profile is compatible with the lower infection risk for patients: RR for first-born children 1.8 (95% CI 1.1 − 2.7), RR one-child family size 1.4 (0.8 − 2.3), RR higher educational class 1.2 (0.9 − 1.5) and ⩾7 rooms in the house 1.4 (0.6 − 2.6). On the basis of the literature three considerations for this finding are suggested. Firstly, the smaller number of leukaemic children with serious infections is due to selective death. Secondly, decreased stimulation of the immune system or postponement of infections until older age might play a role in the development of

leukaemia. Thirdly, infections might play a modulating role in the development of either leukaemia or the localized malignancy, lymphoma.

Introduction

The findings of some epidemiological studies are compatible with the hypothesis that the immune system plays a defensive role in the development of neoplasms of the lymphatic and haematopoietic system (Stewart, 1980). In studies of adults, both renal transplantation and treatment with immunosuppressive drugs are associated with an increased incidence of non-Hodgkin lymphoma (Kinlen et al., 1979). An increased incidence of these lymphomas was also found among patients who were treated by dialysis alone. These findings suggest that immunosuppression of any type enhances the risk of the development of non-Hodgkin lymphoma (Kinlen et al., 1980). Furthermore, the risk of a malignancy is also increased for patients with primary immunodeficiency diseases. The majority of these patients have either a lymphoma or leukaemia (Kersey et al., 1973). There is slight evidence that autoimmune diseases occur more frequently in families of children with acute lymphocytic leukaemia (Till et al., 1979).

A role of the immune system or infectious diseases in the development of leukaemia may be suggested by the fact that leukaemia occurs more frequently in young children than in older children,i.e. adolescents, and that the incidence of leukaemia is higher for populations with a low mortality rate for infections compared with countries with a high mortality rate for infections (Olisa et al., 1975; Ramoth & Magrath, 1982).

A case-control study of possible aetiological factors based on a nation-wide childhood leukaemia morbidity register was conducted in The Netherlands. As a part of this exploratory study the occurrence of infectious diseases in the first year of life was evaluated together with a general profile for infections (birth order, family size, social class and housing).

Material and methods

For the selection of patients and controls as well as data collection and response of the parents see "material and methods" of addendum 4

(childhood leukaemia and parental occupation: a register-based case-control study).

Three standard questionnaires went with the introductory letter: one for the natural father, one for the natural mother and one concerning the relevant child.

One section of the questionnaire covered the medical history of the child in the first year of life and the following general variables: birth order, family size at the time of diagnosis, social class and housing. The answers were coded by trained personnel according to a standard code book.

The present analysis is restricted to the patients with acute lymphocytic leukaemia (ALL) and their controls. Since ALL represents 82% of all leukaemias, a sufficient number of patients was available for analysis.

The patients who developed leukaemia in the first year of life and their controls were excluded from the analysis of infections (cases, $n = 27$; controls, $n = 27$). To measure the strength of association, the relative risk (RR) was used. Since the cases and controls were matched according to age and sex, the relative risk was computed after stratification for sex and age ($0 - 4$, $5 - 9$, $10 - 14$ years) by means of the Mantel-Haenszel odds ratio. The 95% confidence interval (CI) of the RR was computed using the test-based interval estimation procedure proposed by Miettinen (Rothman & Boice, 1979).

Birth order, family size, social class and number of rooms in the home could influence the risk of infection for the child. Therefore, different infectious diseases were adjusted for these possible confounders simultaneously by multiple logistic regression analysis by means of the method of maximum likelihood (Breslow & Day, 1980).

Results

A number of variables which could be considered to influence the risk profile for infantile infections are presented in Table 1.

As measure for social class the educational level was used. The relative risk (RR) for ever breast-fed was 1.1. The number of children receiving more than one month of breast-feeding was also equal for patients and controls (relative risk (RR) $= 1.0$; 95% confidence interval (CI) $0.7 - 1.3$). Children with leukaemia were more often first-born children from small families living in larger houses (more rooms). Their fathers had a higher educational level compared to the controls.

Table 1. Exposures of the child (adjusted for age and sex)

Exposure	Ca	Co	RR(95% CI)
Breast-feeding	321	302	1.1(0.8 − 1.4)
No breast-feeding	195	198	
Birth order:			
1	230	203	1.8(1.1 − 2.7)
2 or 3	239	230	1.5(1.0 − 2.3)
> 4	46	68	1.0
Family size:			
1 child	108	99	1.4(0.8 − 2.3)
2 − 3 children	338	325	1.2(0.8 − 1.8)
> 4 children	69	89	1.0
Social class	251	228	1.2(0.9 − 1.5)
Number of rooms:			
0 − 3	14	18	1.0
4 − 6	394	385	1.3(0.6 − 2.7)
> 7	97	92	1.4(0.6 − 2.9)

Ca = case, Co = control, RR = relative risk, CI = confidence interval

The type of housing, i.e. multiple dwellings, one-family houses and single houses, however, did not differ between the two groups (all RR's 1.0).

Adjustment of the RR of birth order for family size caused a slight change. For first-born children the RR became 2.0 (95% CI 0.9 − 4.3) and for second or third born children 1.4 (0.8 − 2.7).

Table 2 gives the relative risks for leukaemia when the child had had infectious or other diseases during the first year of life. Bronchitis and primary childhood infections, i.e. measles, rubella, chickenpox or mumps, were more common in the first year of life of the patients. However, adjustment for the confounders birth order, family size, social class and number of rooms in the house (residential space) reduced the RR's to 1.1 (95% CI 0.6 − 1.9) and 0.8 (0.4 − 2.0), respectively.

Furthermore, common colds and periods of fever (defined as a temperature of more than 38^{0}C for two days or longer) were reported slightly more often by the controls (Table 2).

When the RR's were adjusted for the effects of confounding by birth order, family size, social class and residential space, the RR for common colds was 0.8 (95% CI 0.6 − 1.0) and remained 0.9 for periods of fever. The RR computed for two common colds or less was 0.8 (95% CI 0.6 − 1.0) and for more than two colds 1.1 (0.8 − 11.5). After adjustment for confounding these RR's decreased to 0.6 (0.4 − 0.8) and 0.9 (0.6 − 1.4), respectively. For one period of fever, 2 − 5 periods and more than five periods of fever the adjusted RR's relative to no fever were 0.8 (95% CI 0.6 − 1.1), 1.0 (0.7 − 1.5) and 1.2 (0.5 − 3.1),respectively.

In the questionnaire we specifically asked about hospitalization or specialist consultation during the first year of life. More controls had a serious infection which required hospitalization or specialist consultation during this period. After adjustment for the possible confounders the RR remained decreased (RR = 0.7; 95% CI 0.4 − 1.2).

The most frequently reported infections were; pneumonia, bronchitis, meningitis, otitis, tonsillectomy, skin infections, urinary tract infections and unspecified fever or viral infections. Other disorders which required hospitalization or specialist consultation such as Rhesus incompatibility, congenital defects and seizures were more frequently reported for children with leukaemia. Of the four children with Rhesus incompatibility, three required an exchange transfusion. As far as the congenital abnormalities are concerned: heart diseases (4 cases/2 controls), cleft lip or palate (1/1), pyloric hypertrophy (2/3), hernias (12/7), hemangioma (1/1), Hirschsprung's disease (1/0) and others (4/5) were listed. Among these patients three children had Down's syndrome, two a congenital heart disease and one Hirschsprung's disease. In this respect it should be

Table 2. Exposures of the child in the first year of life (adjusted for age and sex)

Exposure	Ca	Co	RR(95% CI)
Bronchitis	33	28	1.2(0.7 – 2.0)
Primary infections*	16	12	1.3(0.6 – 2.7)
Otitis	36	47	0.7(0.5 – 1.1)
Common colds	285	293	0.9(0.7 – 1.1)
Periods of fever	147	153	0.9(0.7 – 1.2)
Hospitalization/ consultation:	99	95	1.0(0.7 – 1.4)
infections	35	51	0.7(0.4 – 1.0)
congenital defects	26	19	1.4(0.7 – 2.5)
rhesus incompatibility	4	0	–
diarrhoea	3	10	0.4(0.2 – 1.1)
Prematuritas/ Dysmaturitas	9	8	1.1(0.4 – 2.9)
Birth trauma	1	1	1.0(0.1 – 15.7)

* Primary infections: measles, chickenpox, mumps or rubella.

Ca = case, Co = control, RR = relative risk, CI = confidence interval

noted that out of the total series of ALL patients eight children had Down's syndrome, while none of the controls suffered this condition.

Diarrhoea was reported more often for the control children. For five controls the cause of the diarrhoea was listed as infection; for the cases and the other controls it was unknown (Table 2).

We also asked about the use of drugs during the first year of life, but these answers were invalidated since the drugs used during hospitalization are often unknown to the parents.

Discussion

We decided to include questions about the first year of life in the investigation since in general the autologous immune response and further development of the immune system start in this period together with the first contacts with many antigens. If a dysfunctioning immune system or the frequent occurrence of infections is associated with leukaemia, then this might be expressed in this period.

In this study a slightly lower frequency of infections in the first year of life was reported for children with leukaemia. This finding was most pronounced for infectious diseases which required hospitalization or specialist consultation (RR = 0.7).

Since birth order, family size, social class and residential space could influence the risk for infections, we adjusted for these variables. Adjustment reduced the RR for bronchitis and common or primary infections but changed the RR for infections which required hospitalization or specialist consultation only slightly.

It should be realized that primary childhood infections are relatively rare in the first year of life, yielding small groups for both cases and controls, since in the first months of life specific immunoglobulins from the mother protect the child. In this respect it is noteworthy that measles, chickenpox, rubella and mumps were equally reported by mothers of patients and controls in the period preceding the relevant pregnancy.

General evidence for a possible lower infection risk for children with leukaemia is provided by the finding of more first-born children of small families and slightly higher social class living in large houses among leukaemic patients.

Biased reporting of birth order, family size, education and residential space (number of rooms) seems very unlikely. In the mailed questionnaire it was explicitly stated on a separate page that "the

following questions concern the first year of life". Secondly in each of these questions the phrase "the first year of life" was repeated. Therefore, it seems unlikely that a systematic misclassification was introduced in one of the two groups.

Minor illnesses such as common colds were frequently reported for cases as well as controls, i.e. 59% and 62%, respectively. From another source, the incidence of urinary tract infections and sepsis in the neonatal period was calculated to be 10 – 13 and 1 – 5 per 1000 live births, respectively (Glasgow & Overall, 1979). For the nearly 500 controls in this study the numbers were 5 and 1, respectively, which is an argument against overreporting due to misclassification.

Infections in the first year of life might be well remembered by the mother since such spells are a reason for delaying infant vaccinations until a later visit to the child health centre. In this study the overall number that was hospitalized or visited a consultant was equal for the children with leukaemia and their controls. Infections were reported more often for the controls and other disorders such as congenital defects and Rhesus incompatibility were reported more often for the cases.

Hence the lower infection rate could not be explained by a general underreporting of all illnesses by the parents of patients, nor by an overreporting of diseases among the controls due to misclassification.

Furthermore, if we consider the three age categories separately (1 – 4, 5 – 9, 10 – 14 years) we find this lower infection risk for each age group. The RR's were 0.6, 0.8 and 0.7, respectively, although the calculation for the older age category was based on rather small numbers.

Some studies did not find a relation between birth order and leukaemia (Fasal et al., 1971; Graham et al., 1966) whereas others observed decreasing RR's for leukaemia with increasing birth order (MacMahon, 1962 ;Stewart et al., 1958).

The probability that a neoplasm of the lymphatic or haematopoietic system will develop in childhood seems to be slightly increased for the higher social classes (Kneale & Stewart, 1976 ; McWirther & Bacon, 1982) although others have not corroborated this finding (Teppo et al., 1976 ;Birch et al., 1981).

In the Oxford Childhood Cancer Survey (OCCS) the occurrence of infections in children with leukaemia was compared with that found for children with solid tumours and a population control group of live children. This was broken down into different periods preceding the

death of the diseased children and comparable periods for the controls (Kneale & Stewart, 1978). A heightened sensitivity to infections in children with malignancies, especially leukaemia, was found for the period before death occurred. Most marked was the difference in infectious diseases occurring within the year of death. They concluded that neoplasms with direct involvement of the immune system begin to undermine the resistance to infections before the malignant disease is clinically recognizable (Kneale & Stewart, 1978 ;Stewart, 1980). These data, however, give no insight into the risk of infection before disease onset but concern the period preceding death, which includes the period of disease when the susceptibility to infections is high.

Studies in adults suggest that immunosuppression can enhance the development of non-Hodgkin lymphoma (Kinlen et al., 1980). For patients with primary immunodeficiency diseases the risk of lymphoma and leukaemia is increased. Furthermore, an association between auto-immune diseases and these neoplasms is suggested (Fudenberg, 1975; Till et al., 1979;Isomaki et al., 1978)

Reviewing the data in the literature together with those of the present study, three lines of causal thinking emerge:

1. Children who are prone to develop leukaemia die in their first year of life as a result of infections. The leukaemia could be either the underlying cause of these lethal infections or an impairment of the immune system as such (pre-leukaemia) could be the basic abnormality (Kneale, 1971).

   This phenomenon of early death could explain the lack of leukaemic patients with serious infections in our data. However, the advanced treatment of infectious diseases today renders this conclusion very unlikely. Before the antibiotic era an increasing childhood leukaemia mortality rate correlated with a decreasing mortality rate for pneumonia (Stewart & Kneale, 1969).
2. A lower infection rate in the first year of life for children who develop leukaemia in later life could lead to the hypothesis, that a decreased stimulation of the immune system in early life or possibly the postponement of infections to older age groups, might play a role. The latter is suggested for Hodgkin's disease: "this disease may be a rare consequence of a common infection, with the probability of oncogenesis increasing with age at the time of infection" (Gutensohn et al., 1979).

   Further evidence, although poor, for this hypothesis is the low incidence of infection and the high incidence of leukaemia in

developed countries in contrast to a high mortality rate for (parasitic) infections and a low mortality rate for leukaemia in developing countries. The distribution of the variables: birth order, family size, social class and residential space are compatible with this hypothesis. However, the low incidence of leukaemia in young adulthood as compared with childhood does not corroborate this latter suggestion.

3. Infections or the immune system as such may have a modulating effect on the development of malignancies. This hypothesis, which assumes that peculiar features of lymphatic and haematopoietic neoplasms are the result of the reaction of the immune system, is based on the following observations (Stewart, 1980).
   The OCCS data revealed a higher incidence of infections four or more years before death among patients with a lymphoma in comparison with children with leukaemia. A relation between Epstein-Barr virus and the development of Burkitt's lymphoma is suggested (The et al., 1978), but the viral hypothesis does not explain why the tumour is so common in areas with holoendemic malaria and is practically unknown elsewhere (Stewart, submitted for publication).
   We could speculate that the lower frequency of infections in the first year of life, and therefore the reduced stimulation of the immune system, as found in our study might enhance the conditions under which a neoplasm disseminates, i.e. to develop leukaemia. However, the occurrence of infections in children with lymphoma was not evaluated in this study.

It will be necessary to investigate the role of infectious diseases and the immune system in the development of childhood leukaemia and lymphoma in future studies. For this purpose it is advisable that in future case-control studies serological confirmation of past viral infections will be obtained, in addition to the anamnestic data, for newly registered patients.

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Addendum 7

Response to the mailed questionnaire

In case-control studies high response rates are crucial to avoid response bias, which is important for interpretation of the results. Mailed questionnaires are said to have relatively low response rates (Hoppener, 1981). In the published case-control studies on aetiological factors in childhood cancer, the interview was always the method-of-choice for gathering information (Stewart et al., 1958; Zack et al., 1980; Gold et al., 1979). The use of only mailed questionnaires in such studies has to my knowledge never been described. In preliminary discussions on the design of this case-control study it was decided that for economical and methodological reasons standardized mailed questionnaires were preferable. As both the cases and the controls were distributed over the entire country, it would have been very costly to have to visit all potential participants at home. Moreover this approach eliminates interviewer bias, which is particularly important when several interviewers are needed.

However, some of the treating paediatricians expressed their reservations about a questionnaire sent by a medical investigator, who is unknown to the parents of the patients. The fear was that the parents might become upset and resent further interference with and probing into their personal tragedy. This concerned especially the parents who had lost their child due to the disease. At the time of this study nearly 50% of the children had died of their leukaemia.

In order to estimate the magnitude of this emotional problem it was decided to conduct a pilot study to see what the response rate and the reaction of the parents would be. The pilot study yielded response rates of 82% for the cases and 70% for the controls.

The parents answered the extra questions about the emotional acceptability of the investigation in the affirmative in all cases. Moreover the remarks accompanying the answers expressed a positive attitude towards the investigation. Four parents (8%) refused to participate for emotional reasons. However, a few months later one of these parents sent back the completed questionnaires.

Table 1. The response rates listed according to prior contact of the treating paediatrician with the parents

	Contact		No contact		Contact unknown		Total
	Nr.	Rel.%	Nr.	Rel.%	Nr.	Rel.%	
Response	332	89.9	100	86.2	193	84.6	625
Non-response	19	5.2	12	10.3	25	11.0	56
Refusal	8	2.2	1	0.9	4	1.8	13
Excluded from the study*	10	2.7	3	2.6	6	2.6	19
Total	369		116		228		713
Rel.%	(52%)		(16%)		(32%)		(100%)

* Adopted children, paediatrician advised not to send the questionnaires, moved and new address unknown

The main study was carried out as follows: one month before the questionnaires were dispatched to the parents, the attending paediatricians received a list of the patients to be contacted. During that month they were able to approach the parents, whenever they felt this to be necessary. One oncological centre telephoned all of the parents and asked their consent for the study. Others mailed an

introductory letter to the parents. In total 52% of the paediatricians contacted the parents and 16% did not; the approach of the remaining 32% is unknown. The reponse rates for the parents were not clearly influenced by prior contact with the paediatrician (Table 1); treatment results however did have some influence on the response rates (Table 2). The treatment results were those reported to the register of the DCLSG on or before 1 May 1982.

The parents who did not respond within five weeks received a first reminder and eventually a second one four weeks thereafter. The primary response rate (within five weeks) for the parents of deceased children was relatively lower than that for parents of children who were off treatment (Table 3). The former group of parents probably needed more time to start answering all of the questions concerning their child.

The response rates varied between 84% and 94% for the different years of diagnosis of the leukaemia in the child. However, no trend with the year of diagnosis was found.

In this study population controls were used since the leukaemia cases were population-based. However, a disadvantage is that the parents of such controls are often less cooperative because they are less interested in the study than the parents of the patients. Therefore the response tends to be lower (Cole, 1979). In this study the cases and controls reached response rates of 88% and 70%, respectively (Table 4 & 5).

The response rates for the reminders are listed in Table 5. The primary response as well as the response after the first reminder is somewhat lower for the parents of control children compared with the high rates for the parents of leukaemia patients. The Oxford Childhood Cancer Survey reported response rates on interviews for cases and population controls, selected from birth registers, of 83% and 60%, respectively (Stewart, 1958). A recent interview study of young Hodgkin patients and population controls achieved rates of 86% and 69%, respectively (Gutensohn et al., 1981). Although our questionnaire was long (a minimum of 384 and a maximum of 718 questions) and was sent by mail, the response rates for this study are clearly comparable to those of other studies. A major factor in obtaining a high response rate for a mailed questionnaire may be the quality and content of the accompanying letter (Vandenbroucke, 1983; see appendices 8D & 8F for covering letters). The response rates are a reflection of the willingness of parents to participate in the study.

Table 2. Response listed according to treatment results

	Relapse		Under treatment		Without treatment		Deceased	
	Nr.	Rel.%	Nr.	Rel.%	Nr.	Rel.%	Nr.	Rel.%
Response	87	90.6	6	85.7	227	91.3	304	84.4
Non response	6	6.2	1	14.3	12	4.8	37	10.3
Refusal	1	1.0	–	–	1	0.4	11	3.1
Excluded from the study	2	2.1	–	–	9	3.6	8	2.2
Total	96		7		249		360	
Rel.%	(13.5%)		(1.0%)		(35.0%)		(50.5%)	

For one child the treatment result was unknown

$X^2 = 11.0$ (2 degrees of freedom, $p < 0.005$) for response and non-response + refusal in the categories relapse, without treatment and deceased

Table 3. Response rates listed according to the first and second reminder for each of the 4 categories of treatment results for leukaemia patients

Time	Treatment results						
	Relapse		Under treatment	Without treatment		Deceased	
	Nr.	Rel.%	Nr.	Nr.	Rel.%	Nr.	Rel.%
Before first reminder	53	61.0	1	145	63.9	169	55.6
Between first and second reminder	27	31.0	3	72	31.7	116	38.2
After second reminder	7	8.0	2	10	4.4	19	6.2
Total response	87		6	227		304	

$X^2 = 6.0$ (4 degrees of freedom, $p = 0.10$)

On the other hand an impression of the parental reactions could also provide information about their attitude to the investigation.

Table 4. Response of the control parents

	Nr.	Rel.%
Response	494	69.9
Non response	184	26.0
Refusal	17	2.4
Excluded from the study	12	1.7
Total number	707*	

* 6 Controls were not selected because the municipal authorities refused to cooperate

In accordance with other studies (Bellman, 1982; Rothman, 1981; Vermeer, 1981), the reaction of the parents of the patients to our study was overwhelmingly positive. Some parents of deceased children responded: "Although the study is of no use to my own child, I hope it will prevent this terrible disease in other children. We know what it means to have had a child with leukaemia". It was this group of patients in particular that some attending paediatricians wanted to

protect from the grief which would arise upon receiving questionnaires on the disease of their child. It should be realized, however, that parents will never forget an event like the death of one of their children (Bellman, 1981).

The parents of the patients were very cooperative in that some went to their general practitioner to gather extra information and others wrote extensive letters suggesting possible causes of the leukaemia of their child. Still others described cancer or leukaemia clusters in their neighbourhood, town or school. Sometimes details of events preceding the diagnosis, the period of illness and/or the death of their child were reported.

Of course some parents (12) refused to participate for emotional reasons. One father refused because the mother of the child had died 9 years ago. Three general practitioners informed us that due to emotional difficulties a specific family would not be answering the questions. Seven parents telephoned to tell us this. Some regretted that they could not summon up the courage to help in this study.

In reaction to the announcement of the study in the newspapers, one mother offered to participate as a control mother, another mother wrote to tell us about the death of her son due to leukaemia in 1959. Parents of deceased leukaemia patients who had not yet received the questionnaires, called to express their willingness to participate in the study.

Similarly, the parents of control children were cooperative and interested in the study, even though the mean time required to fill out the questionnaire was 1 – 1.5 hour (data of the pilot study). Several telephoned to ask why they were selected for the study. Two control parents were worried about the fact that the questionnaire had been sent to them, because cancer or leukaemia had been diagnosed in their family recently.

We received 17 refusals for control parents (Table 4). In three cases the death of the mother of the child concerned rendered answering the questionnaires impossible. The others would not cooperate for reasons of divorce, family problems and, in one case illnesses in the family. In half of the cases the reason for refusal was not mentioned.

In conclusion, the high response rate for the study, the additional remarks on the questionnaires, the telephone calls and letters from the parents of several leukaemia patients, express a highly positive attitude toward this investigation.

In fact we believe that these impressions of parental reactions

Table 5. Response rates for cases and controls in relation to the first and second reminder

	Cases			Controls		
	Nr.	Abs.%	Rel.%	Nr.	Abs.%	Rel.%
Before first reminder	368	51.8	58.9	288	40.7	58.3
Between first and second reminder	219	30.8	35.0	148	20.9	30.0
After the second reminder	38	5.3	6.1	58	8.2	11.7
Total response		87.9			69.8	

contradict the opinion of some treating physicians (Hermann et al., 1981). For future studies on sensitive subjects, a pilot study involving a restricted number of cases and controls could provide useful information about the attitude of the patients and their relatives.

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Addendum 8A

ALL	acute lymphocytic leukaemia
ANLL	acute non-lymphocytic leukaemia
CI	confidence interval
CML	chronic myeloid leukaemia
DCLSG	Dutch Childhood Leukaemia Study Group - Stichting Nederlandse Werkgroep Leukemie bij Kinderen (SNWLK)
OCCS	Oxford Childhood Cancer Study
RD	rate difference
SE	standard error

Addendum 8B

Knox's cluster analysis of all patients (Table 1)

Knox's cluster analysis of all patients with ALL (Table 2)

Knox's cluster analysis, boys with ALL (Table 3)

Knox's cluster analysis, girls with ALL (Table 4)

Knox's cluster analysis of all patients with ALL $<$ 6 years at diagnosis (Table 5)

Knox's cluster analysis of patients with ALL $< 50 \times 10^9$ leuc/l (Table 6)

Knox's cluster analysis of patients with ALL $\geqslant 50 \times 10^9$ leuc/l (Table 7)

Knox's cluster analysis of patients with ANLL (Table 8)

Table 1. Knox's cluster analysis of all patients, all types (n = 293)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	5	11	20	37	61	1009
	exp.	7.0	15.9	25.5	40.8	59.5	
	X	0.77	1.22	1.10	0.59	0.19	
⩽2	obs.	12	20	36	60	105	1940
	exp.	13.5	30.5	49.1	78.4	114.4	
	X	0.41	1.90	1.87	2.08	0.88	
⩽3	obs.	19	36	58	95	156	2839
	exp.	19.8	44.7	71.9	114.7	167.4	
	X	0.17	1.30	1.64	1.84	0.88	
⩽4	obs.	27	50	81	134	212	3770
	exp.	26.3	59.3	95.4	152.4	222.4	
	X	0.14	1.21	1.48	1.49	0.69	
⩽5	obs.	33	66	105	176	272	2952
	exp.	32.8	74.1	119.2	190.3	277.7	
	X	0.03	0.94	1.30	1.04	0.34	
⩽6	obs.	40	82	130	222	332	4622
	exp.	39.2	88.4	142.3	227.1	331.5	
	X	0.13	0.68	1.03	0.34	0.03	
⩽8	obs.	50	108	166	282	429	4622
	exp.	51.4	116.1	186.8	298.2	435.2	
	X	0.20	0.75	1.52	0.94	0.30	
⩽10	obs.	62	134	211	355	533	5683
	exp.	63.4	143.2	230.5	367.9	536.9	
	X	0.18	0.77	1.28	0.67	0.17	
⩽12	obs.	72	161	255	413	617	6669
	exp.	74.3	167.8	270.0	431.1	629.1	
	X	0.27	0.53	0.91	0.87	0.48	
All time separations		298	673	1083	1729	2523	42778

Table 2. Knox's cluster analysis of all patients with ALL (n = 233)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	2	6	10	18	33	623
	exp.	4.6	10.5	16.6	25.3	37.7	
	X	1.20	1.38	1.61	1.46	0.77	
⩽2	obs.	6	12	21	35	64	1225
	exp.	9.0	20.6	32.6	49.8	74.1	
	X	1.00	1.89	2.03	2.10	1.18	
⩽3	obs.	11	23	35	54	88	1788
	exp.	13.1	30.0	47.6	72.7	108.2	
	X	0.58	1.28	1.82	2.19	1.94	
⩽4	obs.	15	31	47	75	120	2373
	exp.	17.4	39.9	63.1	96.5	143.6	
	X	0.57	1.40	2.03	2.19	1.97	
⩽5	obs.	19	41	62	104	161	2952
	exp.	21.6	49.6	78.5	120.0	178.7	
	X	0.56	1.22	1.87	1.46	1.32	
⩽6	obs.	26	54	82	136	199	3523
	exp.	25.8	59.2	93.7	143.3	213.2	
	X	0.04	0.67	1.21	0.61	0.98	
⩽8	obs.	34	73	111	177	268	4622
	exp.	33.9	77.6	123.0	187.9	279.8	
	X	0.02	0.53	1.08	0.80	0.70	
⩽10	obs.	44	92	142	227	339	5683
	exp.	41.6	95.5	151.2	231.1	344.0	
	X	0.37	0.35	0.75	0.27	0.27	
⩽12	obs.	52	110	172	268	399	6669
	exp.	48.9	112.0	177.4	271.2	403.7	
	X	0.45	0.19	0.41	0.19	0.23	
All time separations		198	454	719	1099	1636	27028

Table 3. Knox's cluster analysis, boys with ALL (n = 133)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	0	2	2	7	12	200
	exp.	1.2	3.0	5.0	7.7	11.9	
	X	1.08	0.57	1.35	0.27	0.03	
⩽2	obs.	0	2	4	10	17	389
	exp.	2.3	5.8	9.7	15.1	23.1	
	X	1.52	1.58	1.84	1.31	1.28	
⩽3	obs.	0	4	7	14	24	547
	exp.	3.2	8.2	13.7	21.2	32.5	
	X	1.78	1.46	1.81	1.56	1.50	
⩽4	obs.	0	6	10	18	32	716
	exp.	4.2	10.7	17.9	27.7	42.6	
	X	2.05	1.43	1.88	1.85	1.62	
⩽5	obs.	1	10	17	31	49	903
	exp.	5.2	13.5	22.6	35.0	53.7	
	X	1.85	0.95	1.18	0.67	0.64	
⩽6	obs.	3	13	20	40	58	1086
	exp.	6.3	16.2	27.2	42.1	64.6	
	X	1.31	0.80	1.38	0.32	0.82	
⩽8	obs.	4	16	26	50	77	1437
	exp.	8.3	21.4	36.0	55.7	85.5	
	X	1.51	1.18	1.67	0.76	0.91	
⩽10	obs.	8	23	37	67	103	1795
	exp.	10.4	26.8	45.0	69.5	106.7	
	X	0.75	0.73	1.19	0.30	0.36	
⩽12	obs.	10	27	44	78	121	2098
	exp.	12.2	31.3	52.6	81.3	124.8	
	X	0.63	0.77	1.18	0.36	0.34	
All time separations		51	131	220	340	522	8778

Table 4. Knox's cluster analysis, girls with ALL (n = 100)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	0	0	1	3	5	117
	exp.	1.1	2.1	3.2	4.8	6.9	
	X	1.03	1.46	1.22	0.83	0.72	
⩽2	obs.	1	2	3	7	11	240
	exp.	2.2	4.4	6.5	9.9	14.1	
	X	0.81	1.13	1.37	0.92	0.83	
⩽3	obs.	2	4	6	11	16	344
	exp.	3.1	6.3	9.3	14.2	20.2	
	X	0.64	0.90	1.09	0.84	0.94	
⩽4	obs.	4	6	9	17	23	470
	exp.	4.3	8.5	12.7	19.4	27.6	
	X	0.14	0.87	1.04	0.54	0.88	
⩽5	obs.	4	7	11	21	30	567
	exp.	5.2	10.3	15.3	23.4	33.3	
	X	0.51	1.03	1.11	0.49	0.58	
⩽6	obs.	5	11	16	28	39	687
	exp.	6.2	12.5	18.6	28.3	40.4	
	X	0.48	0.42	0.60	0.06	0.22	
⩽8	obs.	7	16	23	36	50	895
	exp.	8.1	16.3	24.2	36.9	52.6	
	X	0.40	0.07	0.25	0.15	0.36	
⩽10	obs.	10	21	31	48	66	1076
	exp.	9.8	19.6	29.1	44.3	63.3	
	X	0.07	0.32	0.35	0.55	0.35	
⩽12	obs.	11	25	36	54	74	1245
	exp.	11.3	22.6	33.7	51.3	73.2	
	X	0.09	0.50	0.40	0.38	0.09	
All time separations		45	90	134	204	291	4950

Table 5. Knox's cluster analysis of all patients with ALL < 6 years at diagnosis (n = 159)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	0	0	3	6	13	310
	exp.	1.9	4.0	6.7	10.7	16.8	
	X	1.37	2.01	1.43	1.45	0.92	
⩽2	obs.	2	3	9	15	28	589
	exp.	3.6	7.6	12.7	20.4	31.9	
	X	0.84	1.68	1.04	1.20	0.69	
⩽3	obs.	2	5	13	21	36	854
	exp.	5.2	11.1	18.4	29.6	46.2	
	X	1.39	1.83	1.26	1.58	1.50	
⩽4	obs.	4	8	18	28	50	1130
	exp.	6.8	14.7	24.4	39.1	61.2	
	X	1.07	1.74	1.29	1.78	1.43	
⩽5	obs.	5	11	23	37	63	1390
	exp.	8.4	18.0	30.0	48.1	75.2	
	X	1.18	1.66	1.28	1.61	1.41	
⩽6	obs.	6	13	29	49	78	1635
	exp.	9.9	21.2	35.3	56.6	88.5	
	X	1.24	1.78	1.06	1.01	1.12	
⩽8	obs.	11	22	42	66	108	2149
	exp.	13.0	27.9	46.4	74.4	116.3	
	X	0.56	1.11	0.64	0.98	0.77	
⩽10	obs.	16	32	56	89	140	2667
	exp.	16.1	34.6	57.5	92.4	144.4	
	X	0.03	0.44	0.20	0.35	0.36	
⩽12	obs.	20	39	70	104	164	3126
	exp.	18.9	40.6	67.4	108.3	169.2	
	X	0.25	0.25	0.31	0.41	0.40	
All time separations		76	163	271	435	680	12561

Table 6. Knox's cluster analysis of patients with ALL $< 50 \times 10^9$ leuc/l (n = 187)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	0	1	3	9	19	417
	exp.	2.5	6.2	10.4	15.4	23.4	
	X	1.59	2.09	2.29	1.62	0.90	
⩽2	obs.	3	6	13	23	44	813
	exp.	5.0	12.1	20.2	30.0	45.5	
	X	0.88	1.75	1.61	1.27	0.23	
⩽3	obs.	7	15	24	37	62	1187
	exp.	7.2	17.6	29.6	43.8	66.5	
	X	0.09	0.62	1.02	1.02	0.55	
⩽4	obs.	9	19	31	50	82	1564
	exp.	9.5	23.2	38.9	57.6	87.6	
	X	0.17	0.87	1.27	1.01	0.60	
⩽5	obs.	10	24	41	67	104	1930
	exp.	11.8	28.6	48.1	71.1	108.1	
	X	0.51	0.87	1.02	0.49	0.39	
⩽6	obs.	16	34	54	86	125	2304
	exp.	14.0	34.2	57.4	84.9	129.0	
	X	0.52	0.03	0.44	0.12	0.36	
⩽8	obs.	19	45	72	113	169	3036
	exp.	18.5	45.0	75.6	111.9	170.0	
	X	0.12	0.01	0.41	0.10	0.08	
⩽10	obs.	27	57	93	145	218	3722
	exp.	22.7	55.2	92.7	137.2	208.5	
	X	0.91	0.24	0.03	0.67	0.66	
⩽12	obs.	33	68	114	172	254	4331
	exp.	26.4	64.3	107.8	159.6	242.6	
	X	1.28	0.47	0.59	0.98	0.73	
All time separations		106	258	433	641	974	17391

Table 7. Knox's cluster analysis of patients with ALL $\geqslant 50 \times 10^9$ leuc/l (n = 43)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	1	1	1	2	3	12
	exp.	0.1	0.3	0.4	0.6	0.9	
	X	2.23	1.36	0.92	1.71	2.24	
⩽2	obs.	1	1	1	2	3	30
	exp.	0.4	0.7	1.0	1.6	2.2	
	X	0.95	0.36	0.03	0.32	0.52	
⩽3	obs.	1	1	1	2	3	51
	exp.	0.6	1.2	1.8	2.7	3.8	
	X	0.48	0.17	0.57	0.43	0.40	
⩽4	obs.	1	1	1	2	3	70
	exp.	0.9	1.6	2.4	3.7	5.2	
	X	0.10	0.49	0.91	0.89	0.96	
⩽5	obs.	3	3	3	5	7	94
	exp.	1.1	2.2	3.2	5.0	7.0	
	X	1.73	0.55	0.13	—	0.01	
⩽6	obs.	4	4	4	7	9	113
	exp.	1.4	2.6	3.9	6.0	8.4	
	X	2.20	0.85	0.06	0.41	0.21	
⩽8	obs.	7	7	8	11	13	153
	exp.	1.9	3.6	5.3	8.1	11.4	
	X	3.76	1.82	1.20	1.01	0.49	
⩽10	obs.	7	8	9	13	15	183
	exp.	2.2	4.3	6.3	9.7	13.6	
	X	3.20	1.81	1.08	1.05	0.39	
⩽12	obs.	7	8	10	14	18	225
	exp.	2.7	5.2	7.7	12.0	16.7	
	X	2.57	1.21	0.82	0.59	0.32	
All time separations		11	21	31	48	67	903

Table 8. Knox's cluster analysis of patients with ANLL (n = 49)

Time separations between pairs of cases in months		Distance separations between pairs of cases (km)					
		⩽2	⩽4	⩽6	⩽8	⩽10	All distances
⩽1	obs.	0	0	0	1	1	21
	exp.	0.1	0.2	0.4	0.8	1.1	
	X	0.33	0.46	0.65	0.22	0.12	
⩽2	obs.	0	0	0	1	1	45
	exp.	0.2	0.5	0.9	1.7	2.4	
	X	0.48	0.68	0.96	0.55	0.91	
⩽3	obs.	0	0	0	1	1	70
	exp.	0.4	0.7	1.4	2.7	3.7	
	X	0.60	0.85	1.20	1.03	1.42	
⩽4	obs.	0	0	0	2	4	97
	exp.	0.5	1.0	2.0	3.7	5.2	
	X	0.70	0.99	1.41	0.89	0.52	
⩽5	obs.	0	0	1	3	5	122
	exp.	0.6	1.2	2.5	4.7	6.5	
	X	0.79	1.12	0.94	0.77	0.60	
⩽6	obs.	0	0	1	4	7	147
	exp.	0.7	1.5	3.0	5.6	7.9	
	X	0.87	1.22	1.15	0.69	0.31	
⩽8	obs.	0	0	1	4	9	187
	exp.	1.0	1.9	3.8	7.2	10.0	
	X	0.98	1.38	1.44	1.18	0.32	
⩽10	obs.	1	1	3	6	12	244
	exp.	1.2	2.5	5.0	9.3	13.1	
	X	0.22	0.94	0.89	1.09	0.30	
⩽12	obs.	1	2	4	7	15	291
	exp.	1.5	3.0	5.9	11.1	15.6	
	X	0.40	0.56	0.80	1.24	0.15	
All time separations		6	12	24	45	63	1176

Addendum 8C

STICHTING NEDERLANDSE WERKGROEP LEUKEMIE BIJ KINDEREN
Centraal Bureau
p/a Juliana Kinderziekenhuis,
Postbus 60604
2506 LP 's-Gravenhage

's-Gravenhage, datum

Zeer geachte Heer en Mevrouw,

Via de kranten, de tijdschriften en de televisie wordt de indruk gewekt dat het ontstaan van leukemie samenhangt met factoren uit onze omgeving. Bij ouders die een kind met leukemie hebben, maar ook bij andere ouders ontstaat hierover ongerustheid. Ongetwijfeld zult U zelf hebben nagedacht over gebeurtenissen die mogelijk van belang zijn geweest bij het ontstaan van de ziekte bij Uw kind.

Om te weten te komen welke factoren wel en welke factoren juist geen rol spelen bij het ontstaan van leukemie wordt een landelijk onderzoek verricht.

Gaarne vragen wij uw medewerking hiervoor en verzoeken U een vragenlijst in te vullen. Het onderzoek vindt plaats na overleg en met instemming van Uw kinderarts. De gegevens worden anoniem verwerkt en blijven geheim.

Het onderzoek wordt verricht door de Stichting Nederlandse Werkgroep Leukemie bij Kinderen (SNWLK) in samenwerking met het Instituut Epidemiologie van de Erasmus Universiteit Rotterdam. Het Ministerie van Volksgezondheid en Milieuhygiene heeft voor dit onderzoek geld ter beschikking gesteld. De SNWLK is een landelijk samenwerkingsverband van kinderartsen die kinderen met leukemie behandelen. Het Instituut Epidemiologie verricht al vele jaren onderzoek naar oorzaken van chronische ziekten. Van daaruit vindt ook de uitvoering plaats.

Voor het onderzoek zullen vele vragen gesteld worden. Misschien zullen niet alle vragen even gemakkelijk te beantwoorden zijn. Misschien wel moet U daarvoor jaren teruggaan in Uw herinnering. Het is daarom dat wij U verzoeken de vragen zo goed mogelijk te beantwoorden. Wilt U daarom de instructie bij de vragenlijst goed doorlezen. Om vast te stellen welke factoren wel en welke factoren niet van belang zijn, zullen de vragen ook gesteld worden aan gezinnen waar de ziekte niet is voorgekomen. Een heleboel gebeurtenissen spelen zich immers in ieders leven af en het is dan ook noodzakelijk om dezelfde vragen te stellen aan ouders van gezinnen, waarin deze ziekte niet is voorgekomen.

Mocht U nog vragen hebben, dan kunt U mij telefonisch bereiken op het Instituut Epidemiologie van de Erasmus Universiteit Rotterdam onder nummer 010 – 634455 bgg 010 – 634457.

Wij doen nogmaals een dringend beroep op Uw medewerking en danken U bij voorbaat voor de moeite die U wilt nemen voor het invullen van de vragenlijst.

Met de meeste hoogachting,
namens het bestuur van de SNWLK

H.A. van Steensel-Moll, arts.

Addendum 8D

DUTCH CHILDHOOD LEUKAEMIA STUDY GROUP
Central Bureau
c/o Juliana Children's Hospital
Post Office Box 60604
2506 LP The Hague

The Hague, date

Dear Madame, Dear Sir,

As a result of reports in newspapers and magazines as well as on television, many have gained the impression that the development of leukaemia can be related to environmental factors. Parents who have a child with leukaemia, as well as other parents, have become deeply concerned about this. Without a doubt, you yourself have wondered whether certain events influenced the development of the disease in your child.

In order to determine which factors play a role in the development of leukaemia and which factors do not, it was decided that a national study should be carried out.

We would like to ask you to cooperate in this study by filling in a questionnaire. The study will be conducted after consultation with and with the approval of your paediatrician. The data is to be processed anonymously and will remain confidential.

The study will be carried out by the Dutch Childhood Leukaemia Study Group (DCLSG) in cooperation with the Institute of Epidemiology of the Erasmus University Rotterdam. The Ministry of Public Health and Environmental Hygiene has provided the funds for this study. The DCLSG is a national cooperation of paediatricians who treat children with leukaemia. The Institute of Epidemiology has carried out many studies of chronic diseases in the past. This study too is to be conducted under their direction.

For this study many questions must be asked. Some questions may not be easy to answer; you may have to delve deep into your memory. For this reason we ask you to answer the questions as accurately as possible. Therefore please read the instructions of the questionnaire carefully.

In order to determine which factors are important and which factors are not, the questionnaire will also be sent to families in which the disease has not occurred. After all many events occur in the course of one's life and that is why it is necessary that the same questions be answered by the parents of children who do not have this disease.

If you should have any questions, you can call me at the Institute of Epidemiology of the Erasmus University in Rotterdam (tel: 010 – 634455 or 010 – 634457).

Once again we would like to urge you to cooperate and, on behalf of the DCLSG, I would like to take this opportunity to thank you for taking the trouble to fill in the questionnaire.

Sincerely yours,

H.A. van Steensel-Moll, M.D.

Addendum 8E

ERASMUS UNIVERSITEIT ROTTERDAM
Instituut Epidemiologie
Postbus 1738
3000 DR Rotterdam
Telefoon: 010 – 634455

Rotterdam, datum

Zeer geachte Heer en Mevrouw,

Via de kranten, de tijdschriften en de televisie wordt vaak de indruk gewekt dat ernstige en chronische ziekten op de kinderleeftijd, zoals kanker, leukemie en andere ziekten samenhangen met factoren uit onze omgeving.

Bij ouders die een kind met deze ziekte hebben, maar ook bij andere ouders ontstaat hierover begrijpelijkerwijs ongerustheid. Om meer inzicht te krijgen in mogelijke oorzakelijke factoren wordt een landelijk onderzoek verricht door de Stichting Nederlandse Werkgroep Leukemie bij Kinderen (SNWLK) in samenwerking met het Instituut Epidemiologie van de Erasmus Universiteit Rotterdam. Het Ministerie van Volksgezondheid en Miliehygiene heeft voor dit onderzoek geld ter beschikking gesteld.

Om vast te stellen welke invloeden van belang zijn, zullen de vragen niet alleen gesteld worden aan ouders van zieke kinderen maar ook aan ouders van gezinnen, waarin de ziekte niet is voorgekomen. Een heleboel gebeurtenissen spelen zich immers in ieders leven af en het is bij zo'n onderzoek dan ook noodzakelijk om de antwoorden van ouders van zieke kinderen te vergelijken met die van ouders van kinderen, die deze ziekte niet hebben.

Voor dit onderzoek zullen vele vragen gesteld worden. De vragen zullen misschien niet altijd even gemakkelijk te beantwoorden zijn. U zult daarvoor vaak jaren terug moeten gaan in Uw herinnering. Daarom verzoeken wij U de vragen zo goed mogelijk te beantwoorden. Pas als wij de zekerheid hebben dat deze vragen zowel door de ouders van zieke als door de ouders van gezonde kinderen zo volledig mogelijk zijn ingevuld, kunnen wij iets meer zeggen over factoren die misschien verband houden met het ontstaan van ernstige ziekten op de kinderleeftijd. Wilt U daarom de instructie bij de vragenlijst goed doorlezen? Gaarne vragen wij Uw medewerking voor dit onderzoek. Uiteraard worden de gegevens anoniem verwerkt en blijven geheim.

Mocht U nog vragen hebben dan kunt U mij telefonisch bereiken onder nummer 010 – 634455 bgg 010 – 634457.

Wij doen nogmaals een dringend beroep op Uw medewerking en danken U bijvoorbaat voor de moeite die U wilt nemen voor het invullen van de vragenlijst.

Met de meeste hoogachting,

H.A. van Steensel-Moll,arts.

Addendum 8F

ERASMUS UNIVERSITY
Institute of Epidemiology
Post Office Box 1738
3000 DR Rotterdam
Tel: 010 – 634455

Rotterdam, date

Dear Madame, Dear Sir,

As a result to reports in the newspapers and magazines as well as on television, many have gained the impression that the development of serious and chronic childhood diseases, such as cancer, leukaemia and other illnesses, might be related to environmental factors. Understandably parents who have a child with one of these diseases, as well as other parents, have become deeply concerned about this.

In order to acquire a better insight into possible causative factors, a national study is to be carried out by the Dutch Childhood Leukaemia Study Group in cooperation with the Institute of Epidemiology of the Erasmus University Rotterdam. The Ministry of Public Health and Environmental Hygiene has provided funds for this study.

To identify the factors that might be important, not only the parents of sick children but also the parents of children who do not have the disease will receive a questionnaire. After all, many events occur in the course of one's life, which is why it is important for such a study that the same questions be answered by the parents of children who do not have the disease.

For this study many questions must be asked. Some of the questions may not be easy to answer; you may have to delve deep into your memory. For this reason we ask you to answer the questions as accurately as possible. Only when we can be certain that these questions have been answered as completely as possible by the parents of sick as well as the parents of healthy children can we gather more information about those factors that might be related to the development of serious illnesses in childhood. Will you therefore please read the instructions that accompany the questionnaire carefully.

We would like to request your cooperation in this study. It goes without saying that the data will be processed anonymously and will remain confidential.

Should you have any questions, please call me (010 – 634455 or 010 – 634457).

Once again we urgently request you to cooperate and we would like to take this opportunity to thank you for taking the trouble to fill in the questionnaire.

Sincerely yours,

H.A. van Steensel-Moll, M.D.

Addendum 8G

Questionnaire for the mother and father

The questionnaire for the father is, except the specific obstetric questions, the same as for the mother.

general variables
- year of birth
- education
- occupations
- number and sex of liveborn and stillborn children
- miscarriages
- deceased children and cause of death
- consanguinity

ever one of the following exposures
- infectious diseases (checklist)
- therapeutic radiation
- congenital defect

exposures before pregnancy
- use of oral contraceptives
- miscarriages
- infectious diseases (checklist)
- cancer, hospitalization or specialist consultation

exposures one year before pregnancy
- oral contraceptives, drugs
- viral infections, vaccinations
- hospitalization or specialist consultation
- alcohol consumption (daily, weekly, less than once a week)
- smoking habits (maximum daily intake)
- pets
- list of occupational exposures

exposures during pregnancy
- smoking habits ,alcohol consumption
- drugs

viral infections, vaccinations
hospitalization or specialist consultation
diagnostic irradiation
ultrasound examination
threatened abortion
pets, hobbies
list of occupational exposures

first degree relatives of the mother/father
number of brothers and sisters
number and causes of deaths
cancer, leukaemia, checklist of other diseases
congenital anomalies and Down's syndrome

Questionnaire for the child

general variables
born at home, medical indication for birth at the hospital
breast feeding
congenital anomalies
tonsillectomy
influenza

exposures concerning brothers or sisters of the child
congenital anomalies, Down's syndrome
infectious diseases
epilepsy, diabetes, asthma
operations, other diseases

exposures concerning brothers or sisters before the diagnosis in the child
hepatitis
infectious mononucleosis
influenza, disorders of the lymph gland
hospitalisation or specialist consultation

exposures concerning the index child before diagnosis

infectious diseases
epilepsy, diabetes, asthma
X-rays
drugs (repeated short courses or regularly, one month or longer)
visiting school

first year of life of the child

infectious diseases or periods of fever
hospitalization or visiting a consultant
drugs
X-rays

the child's environment

place of residence at diagnosis and place of birth
kind of house, number of rooms, type of heating
location in relation to traffic, factories, airport, railways and high tension wires

end questions

who answered the child's questionnaire?
contact with general practioners, paediatricians or others?

Curriculum vitae

Henriëtte Albertine van Steensel-Moll was born on July 9th, 1955 in Vlaardingen, The Netherlands. She passed her secondary school exam in 1972 at the "Groen van Prinsteren Lyceum" in Vlaardingen. She started her medical training in the same year at the Erasmus University Rotterdam. In 1979 she obtained her medical degree. From September 1979 to January 1980 she was a resident in paediatrics at the "Zuiderziekenhuis" in Rotterdam (Head of the paediatrical department: Prof. Dr. C.J. de Groot).

In February 1980 she started the investigation described in this thesis under the auspices of the Dutch Childhood Leukaemia Study Group in The Hague (Chairman: Dr. G.E. van Zanen) and the Institute of Epidemiology, Erasmus University Rotterdam (Head: Prof. Dr. H.A. Valkenburg).

In April 1983 she started her training in paediatrics at the Sophia Children's Hospital in Rotterdam (Head: Prof. Dr. H.K.A. Visser).