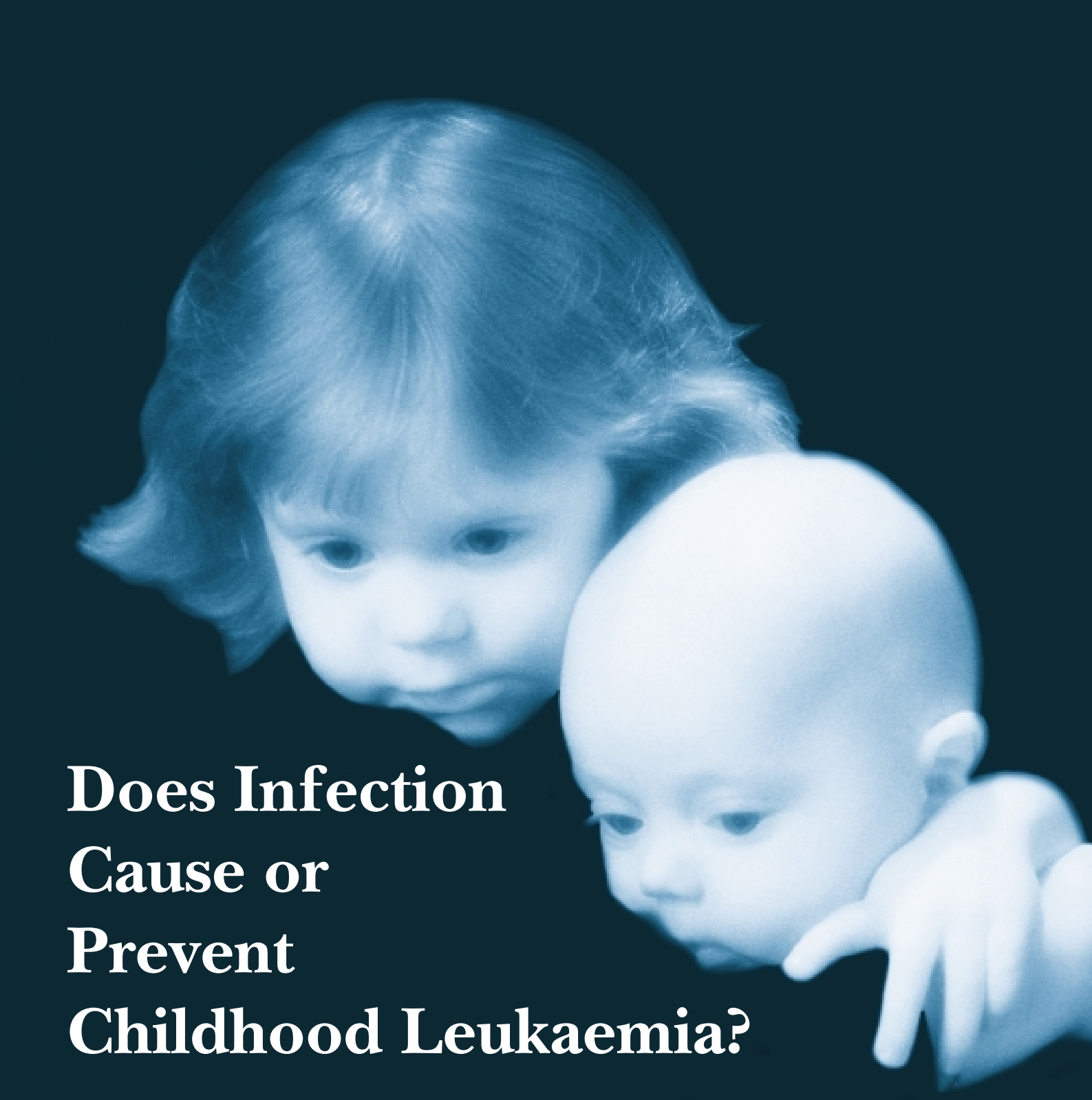

Does Infection Cause or Prevent Childhood Leukaemia?

A review of the scientific evidence

Prepared for CHILDREN with LEUKAEMIA
by Dr Kirsten Edgar and Dr Adrienne Morgan

Fighting Britain's biggest childhood cancer

CHILDREN with LEUKAEMIA
Registered Charity No. 298405. Inaugurated in 1988 by Diana, Princess of Wales in memory of Jean and Paul O'Gorman

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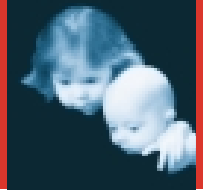

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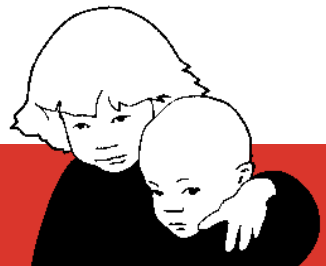


Executive Summary

What causes childhood leukaemia? This review is the second in a series produced by the charity CHILDREN with LEUKAEMIA into the causes of childhood leukaemia. The first looked at exposure to electric and magnetic fields and this report examines the effect of infectious exposure.

Childhood leukaemia

Leukaemia is the most common childhood cancer. Developments in treatment and care have led to a dramatic increase in survival rates in recent years, but it still kills about one in five affected children and those who survive must go through years of gruelling treatments that put them at risk of long-term side effects. Finding out what causes childhood leukaemia is the first step towards preventing this devastating childhood cancer.

Types of childhood leukaemia

Leukaemia can be classified as either *lymphoid* or *myeloid,* denoting the type of white blood cell affected, and as either *acute* or *chronic,* reflecting the speed of progression. Almost all childhood leukaemias are of the acute form. **Acute lymphoblastic (lymphoid) leukaemia (ALL)** accounts for more than 80 per cent of all cases of childhood leukaemia and **Acute myeloid leukaemia (AML)** for most of the remaining cases. **Chronic myeloid leukaemia (CML)** accounts for less than 3 per cent of childhood leukaemias and **chronic lymphocytic leukaemia (CLL)** is unheard of in children. Children in the two to five year age group are at greatest risk of developing childhood leukaemia, and specifically of developing ALL. The risk rises sharply after birth, peaks around the third or fourth year of life and then declines. This is known as the *childhood peak* which is almost entirely common or cALL.

The genetics of childhood leukaemia

Different genetic mutations underlie the multiple types of the leukaemias. Considerable advances have been made in understanding the biological mechanisms underpinning these different types of leukaemia. These have been of immense importance in developing novel treatments for childhood leukaemia, but do not reveal the underlying causes of this disease. Childhood leukaemia is generally initiated during pregnancy but additional post-natal events are required for the child to develop full-blown leukaemia. This is known as the 'two hit hypothesis'.

Causes of childhood leukaemia

There is no single external factor to which a child must be exposed if they are to develop leukaemia. Nor is there a single factor, exposure to which is guaranteed to result in the development of leukaemia. The only generally accepted cause of childhood leukaemia is exposure to ionising radiation (X-rays and gamma rays) but this can not account for all cases. Suggested causes of leukaemia include non-ionising radiation, chemical exposure and infectious exposure.

Increasing incidence of childhood leukaemia

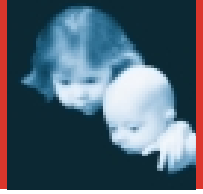
The incidence of childhood leukaemia has increased steadily throughout the 20th century. Since our genetic make-up does not change significantly over such a short time scale, the increasing incidence of childhood leukaemia must be a reflection of some aspect of our changing lifestyle or environment.

Three infection hypotheses

One of the possible causes of childhood leukaemia is infectious exposure (and quite possibly the subsequent inflammatory process). There are three main hypotheses:
1) infectious exposure during pregnancy or around the time of birth increases the risk of childhood leukaemia;
2) delayed infectious exposure beyond the first two years of life prevents normal immune system development and increases the risk of childhood leukaemia following subsequent infection; and
3) unusual population mixing introduces a new infection to previously unexposed populations and childhood leukaemia is an unusual result of such an infection.

Maternal infection

Epidemiological evidence suggests that maternal infection, particularly influenza and chicken pox, during pregnancy can **increase** the risk of childhood leukaemia. However,



the studies are reliant on small numbers of cases and for the most part rely on retrospective questionnaires which are inherently problematic. Reactivation of maternal Epstein-Barr virus and adenovirus-C in the newborn also appear to be risk factors but further work needs to be done in other research groups to confirm these findings.

Infections in childhood

About half of the epidemiological studies into a direct association between childhood infections and leukaemia demonstrate a **protective** effect of infection in early life. The questionnaire-based studies tended to show a protective effect for infection. Two studies looking for direct evidence of previous infections indicate that, for the infections measured, infection **strongly protects** against leukaemia. However, recent studies using patient's records found that any infectious episode was associated with an **increased risk** of childhood leukaemia. Vaccination, which stimulates the immune system in a similar way to an infection, is generally **protective.** It is also reasonably clear that childhood leukaemia is not directly caused by a virus.

Surrogate (proxy) measures of infection

A number of surrogate or indirect measures (proxies) of exposure to infection have been studied to determine what, if any, association they might have with childhood leukaemia. Some of these proxies have shown a strong protective association with leukaemia, such as day care, breast-feeding, socio-economic status and allergy. Studies involving these proxies generally support the hypothesis that exposure to infection within the first two years of life decreases the risk of developing childhood leukaemia and of the childhood peak of ALL in particular. The results of studies concerning other proxies are suggestive of a similar association but require further work to confirm whether this is real or an artefact of the study designs. The problem common to all of these proxies is that, while they may be individually associated with both infection and childhood leukaemia, it is not necessarily the case that infection is thereby associated with leukaemia.

Genetics of immune response

The study of the genetic components of our immune system also offers evidence that infection is involved in the causation of childhood leukaemia. The discovery of molecules in the immune system that afford either increased risk for, or protection against, ALL could have important implications for the design of prophylactic vaccines.

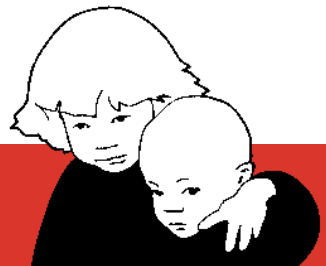
Clustering

Clear time and space clusters of childhood leukaemia have been observed and population mixing (a new population entering into an otherwise isolated community) appears to be a significant factor in the cause of childhood leukaemia. There is an increased incidence of childhood leukaemia after population mixing in areas that were originally very isolated, this is particularly noticeable for the childhood leukaemia 1-4 years peak in rural wards with a high diversity of origin of in-migrants. In contrast, population mixing in urban areas, where there is already a high level of mixing, results in a decreased rate of leukaemia. However, the association of these clusters and population mixes with infection is unclear and thus they do not provide particularly strong evidence for the role of infection in the causation of childhood leukaemia.

In summary

There is a substantial body of **epidemiological evidence** associating a **protective** effect of early childhood exposure to infection and childhood leukaemia. Whereas, delayed exposure to common infections, when the child is more than approximately two years of age, is associated with an **increase** in the risk of childhood leukaemia.

Further work is needed to extend these epidemiological associations to determine the biological mechanism by which infection, or the inflammation that it causes, might influence the development of childhood leukaemia.



1. Introduction

Leukaemia is the most common childhood cancer. Developments in treatment and care have led to a dramatic increase in survival rates in recent years but it still kills about one in five affected children. Those who survive must go through years of gruelling treatments which put them at risk of long-term side effects.

The causes of leukaemia remain generally unknown. There are some factors which are known to cause the disease (such as high dose radiation), but these cannot account for all cases. Incidence of the disease increased throughout the 20th century, suggesting that aspects of our changing lifestyle may be partly to blame.

The idea of a causal association between infection and childhood leukaemia is not new. Clinicians were speculating as to the involvement of infections in the causation, or aetiology, of childhood leukaemia as early as 1922 [1]. Many hundreds of papers have followed.

This review is the second in a series produced by the charity CHILDREN with LEUKAEMIA into the causes of childhood leukaemia. The first looked at exposure to electric and magnetic fields. The purpose of this review is to summarise the evidence supporting the role of infection in the aetiology of childhood leukaemia. This is not an exhaustive review of the literature surrounding this question but aims to cover the main theories that have been proposed and the main body of support for each of these theories.

1.1 Childhood leukaemia

The term 'leukaemia' describes a group of cancers involving an abnormal production (proliferation) of blood cells, usually white blood cells. Leukaemias are the most common childhood cancers, accounting for up to one third of all cases in the UK (aged 0 to 14 years).

Leukaemic cells develop from cells in the bone marrow ("haemopoietic stem cells") that go on to become blood cells. Blood cells include white blood cells (which fight infections), red blood cells (which transport oxygen around the body) and platelets (for blood clotting).

Leukaemia arises from a mutation in the DNA of a single cell. This single cell generates an expanding clone of abnormal cells via repeated cell divisions. The result is a proliferation of abnormal white blood cells and a disruption of the production of normal blood cells.

Haemopoietic cells divide frequently – an adult will make more than 6 million new blood cells per second. Even more cells are made *in utero* when the embryo is growing rapidly. The cells that go on to become one type of white blood cell (lymphocytes) undergo DNA rearrangement to create the large number of different cells which make up the immune system. This process is intrinsically prone to DNA replication errors (mutations) – which may occur either spontaneously or as a result of exposure to external carcinogens.

This frequent division and rearrangement of the haemopoietic cells to make the components of the immune system carries with it an inherent vulnerability to cancer development. This vulnerability can be seen as a potential evolutionary trade-off for the otherwise highly advantageous properties of the human immune system[2].

1.1.1 Types of childhood leukaemia

Leukaemia can be classified as either *lymphoid* or *myeloid*, denoting the type of white blood cell affected, and as either *acute* or *chronic*, reflecting the speed of progression.

Almost all childhood leukaemias are of the acute form. **Acute lymphoblastic (lymphoid) leukaemia (ALL)** accounts for more than 80 per cent of all cases of childhood leukaemia and now more than 90% survive. **Acute myeloid leukaemia (AML)** accounts for most of the remaining cases. Chronic leukaemias are very rare in childhood. Chronic myeloid leukaemia (CML) accounts for less than 3 per cent of childhood leukaemias (less than 15 children per year in the UK) and chronic lymphocytic leukaemia (CLL) is unheard of in children.

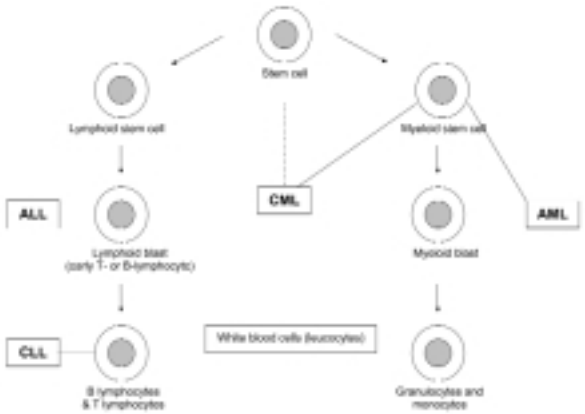


Figure 1. White blood cell lineages and the major leukaemias present in adults and children

ALL can be further divided as being from either T- or B-lymphocyte precursor cells. Approximately 80% of childhood ALL diagnoses involve B-lymphocyte precursors which are further subdivided into a large group with a common antigen (common ALL or cALL) and a small group that lack the antigen (pro-B ALL).

1.1.2 Incidence of childhood leukaemia

Childhood leukaemia incidence (per 1,000,000 population) has increased steadily throughout the 20th century.

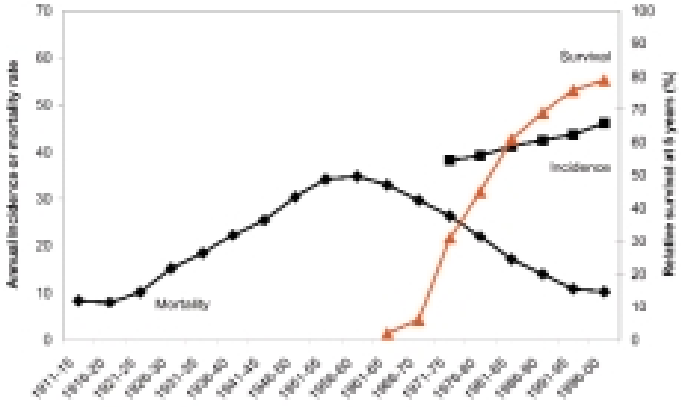
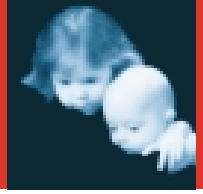


Figure 2. Trends in childhood leukaemia incidence, survival and mortality, 0-14 years, England and Wales 1911-2000

Incidence and mortality rates per million for England and Wales [3,4] five-year relative survival rates (%) for children diagnosed in South-East England 1960-88[5] and in Great Britain 1993-97[6]



Incidence data were not collected prior to the 1970s but until the introduction of the first combination therapies in the 1960s, childhood leukaemia was almost inevitably fatal. Consequently, the mortality figures provide a fairly accurate representation of incidence figures. In the 1960s the two curves dramatically diverge as survival rates begin to grow.

Since our genetic make-up does not change significantly over such a short time scale, the increasing incidence of childhood leukaemia must be a reflection of some aspect of our changing lifestyle or environment.

1.1.3 The childhood peak of ALL

Children in the two to five year age group are at greatest risk of developing childhood leukaemia, and specifically of developing ALL. The risk rises sharply after birth, peaks around the third or fourth year of life and then declines. This is known as the *childhood peak*. This peak is almost entirely made up of the B-lymphocyte precursor ALL and its subset cALL. The childhood peak appeared to develop around 1930 in England and Wales and continued to increase into the 1990s. This peak developed at different times in different countries and is absent in developing countries [7], strongly suggesting that varying rates of lifestyle change have an impact on the incidence of cALL internationally.

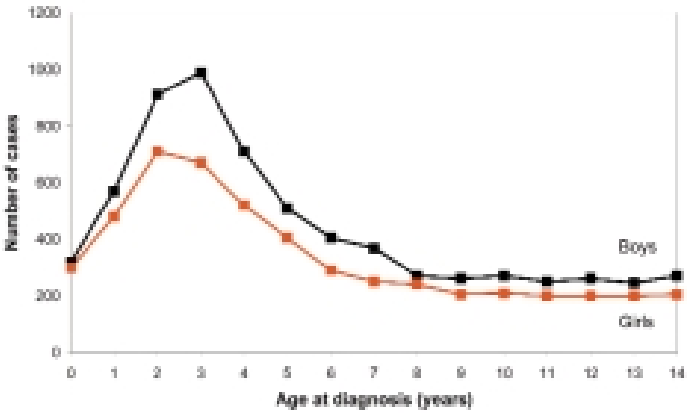


Figure 3. Childhood leukaemia – new cases by age and sex, Great Britain, 1971-1997 [8]

1.1.4 The genetic basis of leukaemia

Leukaemic cells can carry a variety of abnormalities including chromosome gain or loss or structural changes like chromosome deletions, duplications, inversions and translocations.
In childhood leukaemia, these abnormalities arise mainly before birth. However, they arise more frequently than childhood leukaemia [9]. For example, a common chromosomal abnormality, the 'TEL-AML1' fusion, is present in nearly one per cent of new born babies but fewer than one per cent of these will go on to develop leukaemia.

The development of childhood leukaemia is generally initiated *in utero* but additional post-natal events are required for the child to develop full-blown leukaemia. This is known as the 'two hit hypothesis' and the very first evidence for this was derived from studies of identical twins where the same leukaemic cells were found in each set of twins demonstrating that the mutation must have occurred *in utero*. [10, 11]. Further confirmation of this hypothesis came from very recent work with a twin pair, one pre-leukaemic and one with leukaemia. Hong *et al* established a lineal relationship between the cancer cells in the leukaemic twin and pre-leukemic cells in the pre-leukaemic child, both of which carried the same TEL-AML1 fusion. This demonstrated that TEL-AML1 functions as a first-hit mutation in leukaemia development [12].

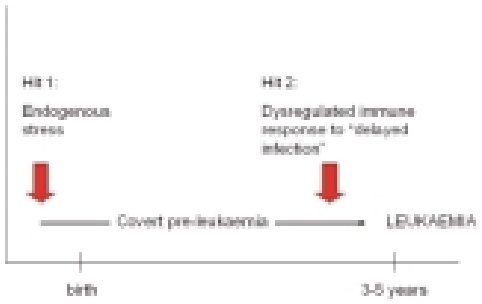


Figure 4. A schematic representation of Greaves' two-hit hypothesis for ALL

The exception to the two-hit model is infant leukaemia, i.e. diagnosis at less than one year of age, in which it is thought that all necessary changes take place in utero *and the concordance rate in identical twins is greater than 50%*

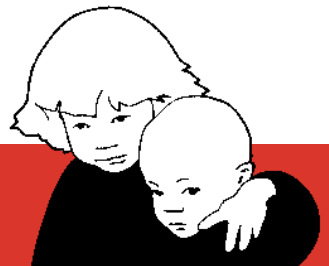
1.1.5 The causes of childhood leukaemia

There are different types of leukaemia and a number of different genetic mutations underlying them. Considerable advances have been made in understanding the biological mechanisms underpinning these different disease types and scientists have mapped out many of the chromosomal changes involved, such as the TEL-AML1 fusion described above.

Understanding these biological mechanisms is necessary for developing novel treatments for childhood leukaemia, but do not reveal the underlying causes (aetiology) of this disease. **There is no single external factor to which a child must be exposed if they are to develop leukaemia. Nor is there a single factor, exposure to which is guaranteed to result in the development of leukaemia.**

It is thought, instead, that childhood leukaemia is multi-causal and multi-factorial, meaning that there are many different factors which can cause leukaemia and exposure to more than one of these is probably necessary. The multiple exposures to these causative factors may not coincide with each other in terms of timing, and indeed it is considered probable that the exposures may occur at different stages in a child's life.

One particular theory regarding the timing of exposure to factors which may cause leukaemia, the "two-hit hypothesis" has already been discussed. However, we do not yet know what factors (other than spontaneous DNA replication error) cause the initial chromosome translocations and what factors are the 'second hits' which cause children with these translocations to go on to actually develop leukaemia.



Some of the main factors for which associations have been identified are set out in the paragraphs which follow. Space does not permit a detailed discussion of these factors, and we do not claim to have provided an exhaustive list of the evidence for and against each of the suggested factors. Instead, we have aimed to call the reader's attention towards some of the relevant research.

Factors which may cause leukaemia include ionising and non-ionising radiation, chemical exposure and infectious exposure. Within these categories, the only generally accepted cause of leukaemia is ionising radiation. Epidemiological evidence for this comes from studies of *in utero* irradiation of the foetus through obstetric x-rays and studies of Japanese atomic bomb survivors. It is estimated that background ionising radiation is implicated in around 25% to 34% of cases of childhood leukaemia, with exposure both *in utero* and early in life being important. See Wakeford [13] for a review.

Our increasing exposure to light at night has been suggested to increase the risk of leukaemia by disrupting our circadian rhythms and suppressing production of the hormone melatonin[14]. It has also been suggested that extremely low frequency electric and magnetic fields (EMF) have a role to play in the development of childhood leukaemia. This potential factor and the research, and surrounding controversy is discussed in more detail in the review: *Do electric and magnetic fields cause childhood leukaemia? A review of the scientific evidence* [15]

Several types of chemical exposures have been suggested as causative factors with varying degrees of evidence. These include exposure to air pollution [16, 17], pesticides [18], and compounds found in food such as N-nitroso compounds (found in cured meats and hotdogs) [19] and DNA topoisomerase inhibitors. These latter compounds are found in certain fruits, tea, coffee, wine, soy, cocoa and other substances and are also used in some chemotherapy drugs [20, 21]. They inhibit DNA repair and are strongly associated with one of the chromosome rearrangements common in infant leukaemia [22].

Parental exposure to chemicals has also been implicated in the aetiology of childhood leukaemia. Evidence regarding the link between parental smoking and childhood leukaemia is inconsistent. At least one study has shown a trans-placental effect[23]. Similarly there are some studies that show a link between maternal drug use and increased risk of childhood leukaemia, including prescription drugs (antihistamines and allergy remedies [24], anti-emetic medication [25]) and recreational drugs (alcohol [26] and marijuana [24, 25]).

Possible protective factors include diet with evidence from one study suggesting that there is a strong protective effect of consumption of oranges and bananas in early life [27]. Curcumin and turmeric have been shown to inhibit cancer [28] (including childhood leukaemia) at initiation, promotion and progression stages of development [29-31] (see also www.leukaemiaconference.org). There is some evidence that maternal folate supplementation during pregnancy may protect against childhood leukaemia [32], and differences detected in the way that individuals metabolise folate may be important[33].

Other factors which are associated with childhood leukaemia, but are not necessarily causative or protective, are birth weight[34], gender [35, 36], ethnicity [37] and maternal history of miscarriages [38]. Many of these factors may well be correlated with each other.

1.2 Infection and childhood leukaemia

Speculations concerning a link between infections and childhood leukaemia were first published as long ago as the 1920s, with observations that the age distribution of the disease was similar to that of common childhood infectious diseases and that many patients had a record of infections around the time of diagnosis.

1.2.1 Background – what is the rationale for suspecting a link?

Doctors at Great Ormond Street Hospital postulated that 'the solution to the problem of leukaemia lies rather in some peculiar reaction to infection rather than in the existence of some specific infective agent' [1] whilst others believed that a specific infection was involved [39].

The connection between leukaemia and infection fell largely out of favour until the 1980s when links between other human blood cell cancers and infections were made [including the link between Epstein-Barr virus (EBV) and Burkitt lymphoma and the link between human T-cell lymphotropic virus 1 (HTLV1) retrovirus and adult T-cell leukaemia/lymphoma]. The discovery that leukaemias in domestic cattle, cats and chickens were also viral in origin further contributed to the re-emergence of infection hypotheses in childhood leukaemia [40].

There are now three main hypotheses concerning the possible role played by infection in childhood leukaemia:

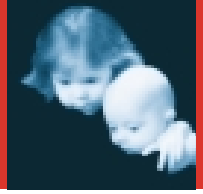
- infectious exposure *in utero* or around the time of birth increases the risk of childhood leukaemia;
- delayed exposure beyond the first year of life to common infections increases the risk of childhood leukaemia; and
- unusual population mixing introduces new infections to previously unexposed populations and childhood leukaemia may be an unusual result of such an infection.

1.2.1.1 Infectious exposure *in utero*

Smith [41] proposed that the childhood peak of ALL (typically cALL), which classically occurs between 2 to 5 years of age in populations of children in developed countries, is due to *in utero* exposure to infection.

His model suggests that the infectious agent causes a primary infection in the mother which is subsequently passed on to the foetus and, as a result of this infection, the child is more likely to develop leukaemia in the next few years, perhaps because of stress and genetic instability caused by the infection.

Smith identified a number of characteristics that the causative infectious agent should possess: i) ability to produce genomic instability; ii) specific effects on B-lymphocytes and not on T-lymphocytes; iii) higher rates of infection in regions with lower



socioeconomic status; iv) limited general cancer causing potential; v) minimal symptoms associated with the primary infection; and vi) ability to cross the placenta and infect the foetus but not cause severe foetal abnormalities. He identified the JC virus (a virus very common in the general population that is normally without symptoms, but that can, in cases of immunodeficiency such as AIDS, cause progressive damage of the white matter of the brain) as a possible candidate virus.

1.2.1.2 Delayed exposure

In 1988, Greaves put forward a 'delayed infection' hypothesis for childhood leukaemia which also sought to explain the childhood peak of cALL. This is, in part, based on the hygiene hypothesis first proposed by Strachan in 1989 [42] which suggests that infections and unhygienic contact with older siblings or through other exposures may confer protection from the development of allergic illnesses. This protection may come from either overt or unapparent infections with viruses and bacteria, non-invasive microbial exposures in the environment, or some combination of the two [43].

The 'delayed infection' hypothesis is based on the premise that the immune system is programmed to anticipate infectious exposure in early childhood and that the absence of such exposure prevents the immune system from developing normally. Greaves proposed that delayed exposure to infection causes an aberrant immune response which acts as the second or post-natal 'hit' in his two-hit model described above [44].

1.2.1.3 Population mixing

Observed clusters of childhood leukaemia and non-Hodgkin lymphomas around the nuclear processing plants of Sellafield and Dounreay in 1983 attracted a great deal of public attention, with speculation that the clusters must be related to radiation exposure.

Kinlen was unconvinced by the evidence implicating radiation. He proposed that herd immunity to a postulated widespread viral infection (to which leukaemia is a rare response) would tend to be lower than average in such isolated communities and that the large influxes of workers might have caused epidemics of such infections.

He tested his hypothesis using the town of Glenrothes in Scotland – a town identified as the only other rural area that received a large influx at the same time – and found a threefold increase in the incidence rate of childhood leukaemia at the time of the influx of newcomers [45].

This provided a basis for suggesting that some childhood leukaemia clusters might be an unusual outcome of a common but relatively non-pathological infection arising in non-immune individuals following 'population-mixing'.

1.2.2 Infection and inflammation

We generally respond to an infection with a protective inflammatory response, this is what makes us feel so ill. It is possible that, in susceptible individuals carrying pre-leukaemic cells, one of the unintentional consequences of this inflammatory response is childhood leukaemia. Many insults to the body generate an inflammatory response including exposure to ionising radiation [46]. Cells that have not been directly irradiated can receive damaging inflammatory signals produced by irradiated cells. This suggests that environmental agents, including infections, may be promoting secondary genetic changes rather than actually causing them [46] and could be Greaves' "second hit" to a pre-leukaemic cell in childhood leukaemia.

Inflammation is already implicated in the cause of a number of cancers. Individuals with chronic inflammatory disease of the colon have a 10-fold higher likelihood of developing colorectal carcinoma, and gastric cancer can develop in individuals chronically infected with Helicobacter pylori. Similarly, inflammatory conditions of the liver, such as chronic hepatitis and cirrhosis, are well-established risk factors for the development of hepatocellular carcinoma. Non-infectious inflammation is further associated with lung cancer, which can be caused by asbestos exposure and silica exposure [47-49].

Recent research has highlighted an important role for inflammation in cancer – in particular two molecules produced by the inflammatory process, tumour necrosis factor (TNF- α) and interleukin (IL)-6 [48].

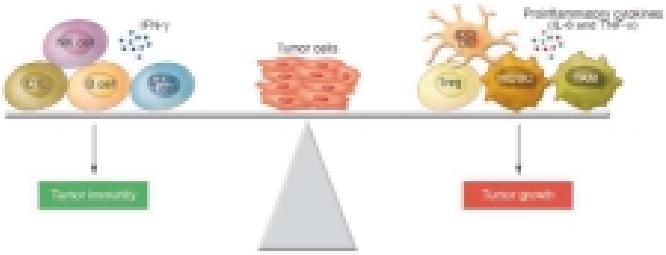
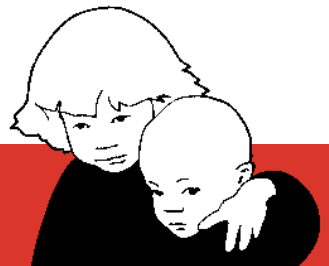


Figure 5. Some of the immunological factors that affect tumour development [48]

Prolonged production of TNF- α causes a specific signalling pathway (the NF- κB kinase/ NF- κB pathway) inside the cell to turn on anti-cell death genes which then encourage the growth of cancerous cells. TNF- α also causes new blood vessels to grow to provide cancer cells with nutrients and protects cancer cells from death signals [48, 49].

It is, therefore, possible that inflammation may promote pre-leukaemic cells to become leukaemic in childhood leukaemia by creating an environment where cells of the immune system are stimulated to grow. There has, in fact, been a recent report of work linking TNF- α with the development of acute myeloid leukaemia [50]. When haematopoietic stem cells (those cells that leukaemic cells develop from) were exposed to TNF- α for a long period (30 days), abnormal clones of cells were produced. These cells, grown in mice, led to the development of acute myeloid leukaemia (AML). Although this study relates to AML it is possible that something very similar could happen with ALL.



1.3 How do we investigate the link between childhood leukaemia and infections?

Direct laboratory measurements and epidemiological studies have attempted to identify a link between infections and the risk of childhood leukaemia.

1.3.1 Laboratory studies

Some attempts have been made to identify specific infections. This is not a straightforward undertaking, however. It is unclear which infections may be important and it is difficult to know where to start. Even with candidate infections, in the UK neither maternal serum nor cord blood is routinely banked; thus, the likely importance of the timing of exposures renders the use of specimens obtained at or after diagnosis less useful.

Some studies have used Guthrie spots (spots of blood routinely taken from babies at the time of birth, also known as Guthrie cards) to look for evidence of infectious exposure *in utero,* with some success, even though the amount of tissue is small and may not be in good condition.

Other laboratory studies have looked for evidence of infection within the children using blood samples taken at leukaemia diagnosis. One approach has been to look for evidence of viral DNA within leukaemic cells of newly diagnosed children, prior to treatment. Again these have been problematic due to uncertainties over which infections to look for. One avenue that is showing some success is that of studying the genomic make-up of the immune systems of both healthy and leukaemic children. The rationale is that if infection plays a role, then potential genetic differences in the immune systems of the patients versus the healthy population may promote or inhibit the development of leukaemia.

1.3.2 Epidemiological studies

Epidemiology is the study of the distribution of disease in populations and of the factors that influence this distribution. It is concerned with the patterns of disease among groups rather than with treating the disease at the individual level. Epidemiology is very valuable in assessing risks to human health from certain factors, but it does have limitations. These are discussed below.

To investigate an association in a disease such as childhood leukaemia, epidemiologists generally use a **case-control study**, which is a way of comparing people who already have a particular disease (cases) with people who do not (controls). In the case of infections, epidemiologists will recruit a group of people who have (or have had) leukaemia and a group of people who do not and compare the exposure of the two groups to measures of infection to see if there are any differences. The controls are chosen to be as similar as possible to the cases in all characteristics except their leukaemia.

However, it is well known that there is a difference in the socio-economic status of cases and controls as noted by McNally *et al*: "In case-control studies, controls are very consistently from higher socio-economic groups than cases. Therefore the findings [of these studies] may be due to confounding factors and not represent a true causal relationship" [51]. This difference in socio-economic status is limited to studies that involve active participation of the cases and controls: for example, studies that gather data by questionnaire. This will have confounding effects in studies where there is an intrinsic difference in behaviour according to socio-economic status, for instance different diets, or breast-feeding, where mothers from a higher socio-economic bracket are more likely to breast-feed their children.

The other main type of epidemiological study is a **cohort, or longitudinal, study,** a systematic study of a group of people which can be conducted either prospectively or retrospectively. In a prospective study, a group of people are systematically followed up for a defined period of time or until the onset of a specific event in order to observe their pattern of disease. A retrospective cohort study involves a group where data on the group's exposure and disease experience are already known. This type of study is very difficult to do for childhood leukaemia. Because of the small number of children diagnosed each year, the cohort would have to be very large for the case group to be statistically significant. This can be seen in, for example, the cohort study reported by Groves *et al*, which followed all children born in Finland, 1987-89 [52]. In this study, of 125,129 live births, 80 children went on the develop leukaemia, giving a very small sample size of the population of interest.

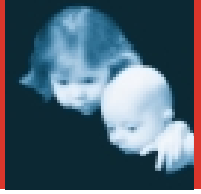
The main problems with using an epidemiological approach to investigate the association between infection and childhood leukaemia are as follows:

- **Recall difficulties.** Many studies rely on parental recall to assess the child's exposure to infection. There is evidence to suggest that this may be unreliable. A large study looking into the association between allergy and childhood leukaemia compared parental recall with medical records. The reliability of recall differed between different allergic conditions, with eczema history being substantially under-reported. They found no difference, however, in reliability of recall between cases and controls [53]. Some, but not all, infections will be recorded in a child's medical records.

- One way in which this is addressed is by the use of **proxy measures.** This can be more reliable than recall. For example, some studies have reasoned that children who attend day care from an early age will be exposed to more infections in early childhood and therefore use attendance at day care as a proxy for infectious exposure. Other proxy measures which have been used include breast-feeding history and sibship (the number of individuals born to a particular pair of parents).

- **Which infections** are we looking for? The other major problem is that we don't know what infections might be important. By looking at infections in general, we may be masking any associations that are present. Some studies have sought associations with candidate infections, with mixed success. In general, however, positive findings have not been replicated.

- Different **sub-types of leukaemia.** Infection may only be involved in the aetiology of a sub-type of childhood leukaemia, perhaps the childhood peak (mostly cALL) that



has increased significantly over the last 50 years. It is therefore quite possible that studies into leukaemia in general may be non-significant due to the dilution effect of studying all of the sub-types.

- **Timing of infection.** Given the Greaves "Two-Hit" and "delayed infection" hypotheses, it is important to study the timing of any infectious exposure but to do this can be very difficult. For example, maternal exposure to infection during pregnancy may well increase the risk of the child developing leukaemia through the first hit, infection within the first two years of life may have a protective effect and later on may result in the development of childhood leukaemia.

- **Sample size.** Childhood leukaemia is a mercifully rare disease and although some studies are large most are not and often do not have sufficient resolving power to demonstrate any statistically significant association between infection (or its proxies) and the sub-types of childhood leukaemia. The Childhood Leukaemia International Consortium (CLIC) has recently been formed and aims to build up a very large set of leukaemic cases and controls internationally (currently over 10,000 cases and over 5,000 with associated biological material).

1.3.3 Relative risks and odds ratios

Epidemiologists often express their results in terms of relative risks (RR) and odds ratios (OR). These are slightly different statistical ways of expressing the proportion of people affected by specific factors. Risk is the probability that an event will happen, calculated by dividing the number of events by the number of people. Risk ratios or relative risks are calculated by dividing the risk in the treated or exposed group by the risk in the control or unexposed group. In comparison, odds are calculated by dividing the number of times an event happens by the number of times it does not happen. Odds ratios are calculated by dividing the odds of having been exposed to a risk factor by the odds in the control group.

In both relative risk and odds ratios, if a value of 1 is returned this indicates that there is no difference in risk between the exposed and unexposed groups. Ratios of an event greater than 1 indicate that the rate of that event is increased in cases compared to controls. Inversely, ratios of less than 1 indicate that the rate of event is decreased in cases compared to controls.

Both of these statistical expressions of risk or odds are frequently given with their 95% confidence intervals (95% CI). We can be fairly sure that the "true value" lies in the range given by this interval. If the 95% confidence interval quoted by an author does not include 1, the result is considered statistically significant. As an example, if the RR or OR is above 1 and the lowest value of 95% CI is still greater than 1, this result is considered a statistically significant increase in risk. Some other studies quote 'p' values to show statistical significance. If the p value is less than 0.05, the result is considered statistically significant. Even results that are not statistically significant due to small sample numbers may be considered suggestive, in that they point out where the field would benefit from further studies. Possible variations are shown graphically in Figure 6.

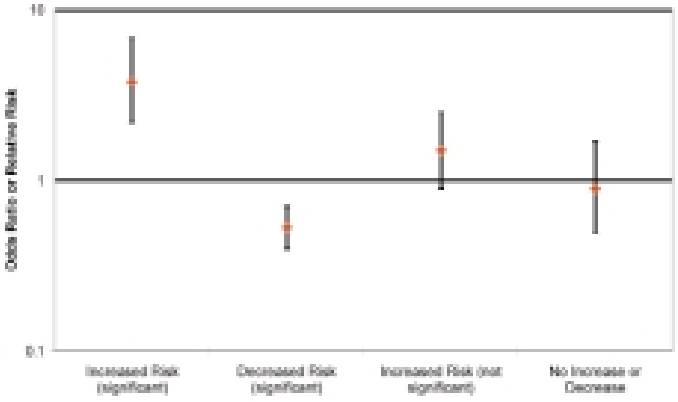


Figure 6. Examples of odds ratios
Showing: ex 1 statistically significant increased risk; ex 2 statically significant decreased risk; ex 3 not statistically significant but suggestive increased risk; ex 4, not statistically significant

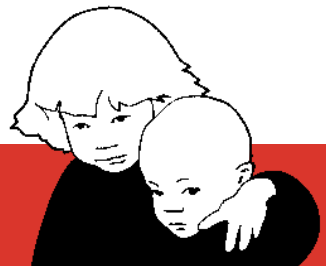
Relative risk tends to come closer to what most people consider when comparing the relative likelihood of events and is often used in cohort studies. However, in some research designs, it is impossible to calculate this value. Thus in case-control studies, odds ratios have to be used instead. Odds and risk tend to give similar values when comparing rare events, as is the case for studies concerning the incidence of childhood leukaemia, but may be substantially different for more common events.

1.3.4 Assessing the evidence

This report is not intended to provide a systematic critical analysis of the data on the association between childhood leukaemia and infection; rather, it is intended to provide an overview of the subject, pointing out areas of significance in the debate. As such, there is only a limited critical assessment of the evidence presented.

The papers discussed in the text of this report, and earlier papers which are not discussed but which provide further evidence regarding particular associations, are summarised in tables throughout the text. These tables provide information such as the number of subjects, the results of the study, any numerical results, and any 95% confidence intervals or p-values associated with those results. Studies with larger sample sizes, i.e. those that are based on more cases, tend to have greater statistical significance, and this size is frequently noted in the text.

Where possible throughout this report, we have also endeavoured to present graphically the results of both the papers explicitly discussed in the text and the earlier papers. This provides an immediate visual means to see trends in the data reported.



2. Infection and childhood leukaemia – the evidence

The idea of a causal association between infection and childhood leukaemia is not new. Speculations concerning a link between infections and childhood leukaemia were first published as long ago as the 1920s. An infectious cause has been long suspected by clinicians and many hundreds of studies have been done. This section reviews the evidence for infection causing or preventing childhood leukaemia.

2.1 Childhood leukaemia and maternal infections during pregnancy

Is there an infectious agent which causes a primary infection in the mother and which may be passed on to the foetus? As a result of this maternal infection is the child is more likely to develop leukaemia in the next few years?

2.1.1 Is there an association between childhood leukaemia risk and maternal infections during pregnancy?

Both epidemiological and laboratory studies have been performed to attempt to answer this question.

2.1.1.1 Epidemiological evidence

Epidemiological studies looking for a connection between childhood leukaemia risk and infections during pregnancy have produced contradictory evidence. There are several good studies showing a high risk with viral infections (OR of 6 or more), but there are also good studies which show no such association.

There have been a number of case control studies which have looked for a link between infectious exposure (in general) and childhood leukaemia risk. Some of the most recent are as follows:

Kwan *et al* used data (1995-2002) from the Northern California Childhood Leukemia Study with 365 cases and equal number of controls. Data on maternal illnesses were **obtained by interview.** Maternal history of influenza/pneumonia was associated with a statistically significant increased risk of ALL in the offspring (OR = 1.89, 95% CI 1.24-2.89), although the risk was non-significant for common ALL (OR = 1.41, 95% CI: 0.75-2.63). A similar pattern of increased risk was found for history of sexually transmitted disease (OR = 6.33, 95% CI 1.65-24.27) [54].

Naumburg *et al* looked at children born and diagnosed with leukaemia between 1973 and 1989 in Sweden. Their cases comprised 578 children with ALL and 74 with AML. Information on infection was **retrieved from hospital medical records.** A history of maternal infection in general was not significantly associated with childhood leukaemia (OR = 1.25, 95% CI 0.95-1.65). However, a specific maternal infection, lower genital tract infection, was significantly associated with an **increased** risk of childhood leukaemia (OR = 1.78; 95% CI 1.17-2.72), with the strongest association found in children over 4 years of age at diagnosis (OR = 2.01, 95% CI 1.12-3.80) [55].

Infante-Rivard *et al* carried out a study in Quebec, Canada, in 1989-95 including 491 cases diagnosed between 1980 and 1993 and aged between 0 and 9 years and an equal number of matched controls. **Using a questionnaire** to collect data from the mothers, they found that recurrent infections during pregnancy was **not a risk factor** (OR=1.09, 95% CI 0.65-1.84) although this study relied on maternal recall [56].

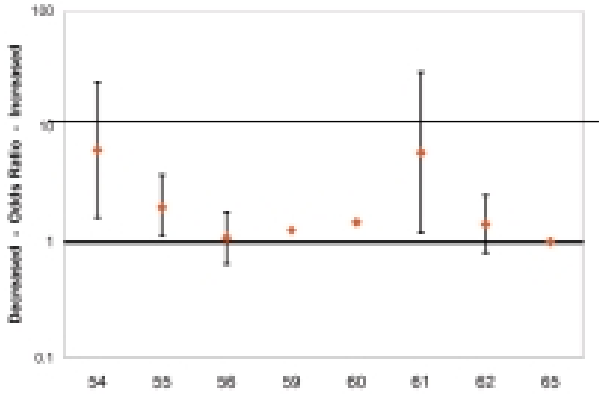


Figure 7. Maternal exposure to infection and risk of childhood leukaemia

The numbers in this and all subsequent figures relate to the reference listed in the tables and in the text

Table 1 Epidemiological evidence for an association between childhood leukaemia risk and maternal infections during pregnancy

Study and reference	Study design	Time period	No of cases	Results	Figures given†
Oxford Survey of Childhood Cancers (Stewart *et al.*, 1958) 57	Mortality cohort (leukaemia or cancer)	1953-1955	677 CL cases, 739 other cancer cases	Malignant disease might follow maternal infection during pregnancy	13 cases/1 control
Miller (Miller *et al.*, 1969) 58	Single case	N/A	1	Neonatal leukaemia death reported with a history of mother having an influenza-like illness early in the 2nd trimester	
Oxford Survey of Childhood Cancers (Gilman *et al.*, 1989) 59	Case-control	1964-1979	8059 childhood cancer cases	Increased risk of CL with febrile illnesses in mother	RR = 1.27 ($p < 0.05$)
Children's Cancer Group (Buckley *et al.*, 1994) 60	Case-control		547 ALL cases	The risk for B-ALL was significantly elevated for any maternal infection	OR = 1.5
Southern England (Roman *et al.*, 1997) 61	Case-control	1962-1992	113 ALL cases, 15 AML	Increased risk of CL with documented viral infections in pregnancy	OR = 6.0 (1.2-29.7)
Scotland (McKinney *et al.*, 1999) 62	Case-control	1991-1994	144 CL cases, 146 other cancer cases	Increased risk of CL with any infection in pregnancy, especially those diagnosed aged 0-4 years	OR = 1.44 (0.81-2.55)
Sweden (Naumburg *et al.*, 2002) 55	Case-control	1973-1989	578 ALL cases, 74 AML cases	Increased risk of CL diagnosed at age 4y+ with lower genital tract infections during pregnancy	OR = 2.01 (1.12-3.80)
Northern California Childhood Leukemia Study (Kwan *et al.*, 2007) 54	Case-control	1995-2002	365 CL cases	Increased risk of CL with maternal history of sexually transmitted diseases (Chlamydia, genital herpes and HPV)	OR = 6.33 (1.65-24.27)

† Unless otherwise noted, all figures in brackets are 95% confidence intervals in this and all subsequent tables

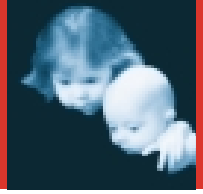


Table 2 Epidemiological evidence against an association between childhood leukaemia risk and maternal infections during pregnancy

Study and reference	Study design	Time period	No of cases	Results	Figures given
Oxford Survey of Childhood Cancers (Knox *et al.*, 1980) 63	Case-control	1956-1960	1652 CL cases, 1202 other cancer cases	No difference between cases and controls in terms of illnesses during pregnancy	
Oxford Survey of Childhood Cancers (Knox *et al.*, 1983) 64	Case-control	1953-1979	1321 CL cases	No evidence from family composition that infection of mother contributes to aetiology of CL	
Inter Regional Epidemiological Study of Childhood Cancer (McKinney *et al.*, 1987) 65	Case-control	1980-1983	171 CL cases and 63 lymphoma cases	Infection during pregnancy (influenza, urinary tract infection) failed to show any major positive association with CL	(RR < 2 and not significant at the 5% level)
Canada (Infante-Rivard *et al.*, 2000) 56	Case-control	1980-1995	491 ALL cases	No effect of recurrent maternal infections	OR = 1.09 (0.65-1.84)

Table 3. Epidemiological evidence for an association between childhood leukaemia risk and maternal influenza during pregnancy

Study and reference	Study design	Time period	No of cases	Results	Figures given
National Child Development Study (Fedrick & Alberman, 1972) 67	Birth cohort, first week of March 1958	1958-1969	20 cancer cases out of 16750 children	Increased risk of childhood leukaemia following maternal influenza infection	OR = 4.0
Finland (Hakulinen *et al.*, 1973) 68	Birth cohort, 1953-1959	1953-1969	179 leukaemia cases out of 610,733 births	Increase of childhood leukaemia in Finland following maternal exposure to 1957 'Asian' flu during pregnancy	Exposed incidence rate = 68.1/million, unexposed incidence rate = 44.2/million
Oxford Survey of Childhood Cancers (Bithell *et al.*, 1973) 69	Case-control	1953-1972	> 9,000 childhood cancer cases	Increased risk of childhood cancer after influenza during pregnancy	OR = 1.52 (1.11-2.14)
California (Austin *et al.*, 1975) 70	Birth cohort of leukaemic children, 1950-1972	1950-1972	1317 CL cases	Increased risk of childhood leukaemia after flu in first trimester of pregnancy in 0-4y age group	RR = 3.4
Northern California Childhood Leukemia Study (Kwan *et al.*, 2007) 54	Case-control	1995-2002	365 CL cases	Increased risk of (1.) childhood leukaemia and especially (2.) ALL with maternal influenza/pneumonia during pregnancy and 3 months prior	1. OR = 1.77 (1.17-2.68) 2. OR = 2.02 (1.28-3.18)

Table 4. Epidemiological evidence against an association between childhood leukaemia risk and maternal influenza during pregnancy

Study and reference	Study design	Time period	No of cases	Results	Figures given
Manchester (Leck & Steward, 1972) 71	Birth cohort, 1951-1968	1951-1968	575 CL cases	No increase in childhood leukaemia after maternal infection during influenza epidemics in Manchester	Incidence ratio exposed/unexposed = 0.97
Southern U.S.A. (Randolph & Heath, 1974) 72	Birth cohort of children with leukaemia or lymphoma, 1957-1971	1957-1971	342 CL and lymphoma cases	No consistent increase in incidence of childhood leukaemia in Atlanta or Texas after influenza during pregnancy	
Northern England (Nyari *et al.*, 2003) 66	Birth cohort, 1975-1986	1975-2001	508 CL and NHL cases out of 404,106 births	No association with influenza during pregnancy was found	

Several studies have looked for associations between childhood leukaemia and specific infections during pregnancy, in particular, influenza and chicken pox.

Going back over many years, there have been suggestions of a link between maternal influenza and an increased risk of childhood leukaemia although these studies had low case and control numbers. Two recent large studies give conflicting results. The Northern California Childhood Leukemia Study described above, with 365 cases, showed a **doubling of risk** [54]. In contrast, Nyari *et al*, who used data from a retrospective cohort study in the northern region of England from 1975-1986 and had a similar number of cases to the Kwan study found **no increase in risk** [66].

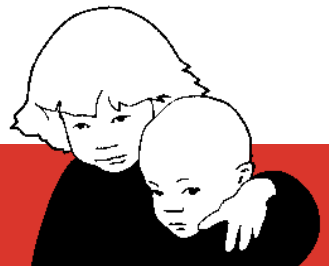


Table 5. Epidemiological evidence for an association between childhood leukaemia risk and maternal chicken pox

Study and reference	Study design	Time period	No of cases	Results	Figures given
Oxford Survey of Childhood Cancers (Stewart *et al.*, 1958) 57	Mortality cohort (leukaemia or cancer)	1953-1955	677 CL cases, 739 other cancer cases	Excess of childhood leukaemia after mother had zoster (reactivated chicken pox known as shingles)	3 cases/1 control
England (Adelstein & Donovan, 1972) 73	Birth cohort, 1951-1952 (mothers suffered viral infection)	1950-1971	1006 children with maternal viral infection	Increased risk of childhood leukaemia in children of mothers who had chicken pox but not mumps or rubella during pregnancy	Expected number of deaths 0.15, observed 2
Oxford Survey of Childhood Cancers (Bithell *et al.*, 1973) 69	Case-control	1953-1972	> 9,000 childhood cancer cases	Excess of leukaemia after mother had chicken pox	Tentative relative risk estimated at 3.7
New York State (Vianna & Polan, 1976) 74	Incidence of leukaemia matched to seasonal infections	1950-1970	777 CL cases	Maternal chicken pox during pregnancy increase risk of childhood leukaemia	63 mothers, expected incidence << 1, observed incidence = 3
England (Till *et al.*, 1979) 75	Cases diagnosed in a single London hospital	1973-1975	54 ALL cases	Maternal chicken pox and herpes zoster during pregnancy	2 of 54 cases – higher than expected
Oxford Survey of Childhood Cancers (Blot *et al.*, 1980) 76	Case-control	1971-76	2800 childhood cancer cases	Maternal chicken pox during pregnancy	No excess leukaemia with maternal chickenpox

The effect of maternal chicken-pox infection on the risk of childhood leukaemia has also been studied for many years and suggests an association with an increased risk of childhood leukaemia. Indeed, no study failed to find an association. Nevertheless, these were studies with small numbers of cases and controls which would need repeating with larger numbers to be confident of an association.

Summarising these studies, over half offer some epidemiological evidence to suggest that maternal infection during pregnancy increases the risk of developing childhood leukaemia. This provides some support for Smith's hypothesis regarding the role of infectious exposure *in utero* and fits as the possible first hit in Greaves' two-hit hypothesis.

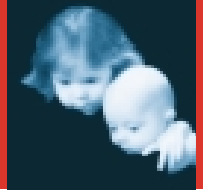
However, the largest objective study to date, by Naumberg *et al*, did not produce a significant association between generic maternal infections and childhood leukaemia [55]. This may reflect the difference between studies based on interview data as opposed to medical records as interviews are more likely to detect minor infections that would not necessarily require medical attention. There are enough other studies that show an inverse or null effect of maternal infection on the risk of developing childhood leukaemia to suggest that further work is necessary in this field to confirm any association.

2.1.1.2 Can any infections be found during pregnancy or in the newborn?

Very few studies have attempted to look for specific infections during pregnancy and the risk of childhood leukaemia. This is largely because of the Herculean nature of the task. Where specific infections have been sought, only maternal Epstein-Barr virus (EBV) reactivation [77-79] and neonatal adenovirus-C infection [80] were found to be associated with an increased risk of leukaemia. This work, performed in Finland, Iceland and Sweden, has yet to be repeated elsewhere and a Swedish group looking for pre-natal EBV infection failed to find significant elevation of EBV in Guthrie spots [81].

Lehtinen *et al* used data from a joint study cohort comprising the offspring of 550,000 mothers in Finland and Iceland. The cohort was followed up for cancer in the offspring before they reached 15 years of age during 1975-1997 through national cancer registries. For each index mother-case pair, three or four matched mother-control pairs were identified from national population registers. Blood samples taken during the first three months of pregnancy were retrieved from mothers of 342 ALL and 61 other leukaemia cases and from the mothers of 1,216 controls and were tested for antibodies (a measure of infection) to cytomegalovirus, Epstein-Barr virus (EBV), and human herpes virus 6 (HHV-6). The presence of maternal antibodies to EBV was associated with a highly significant increased risk of ALL in the offspring (adjusted OR = 2.9, 95% CI 1.5-5.8) [77]. If the mother had the virus at the time of birth, the child had an OR of 2.0 (95% CI 0.3-12) of developing ALL. The group further analysed maternal blood for antibodies to other parts (antigens) of EBV that are found when the virus is reactivated; these were associated with an increased risk of non-ALL in the offspring (odds ratio = 5.6, 95% confidence interval: 1.1, 29, respectively). All of this suggests that EBV reactivation may be associated with a proportion of childhood leukaemia [77, 79] but as the sample sizes are small the work needs to be repeated by other groups.

In contrast, Bogdanovic *et al* analysed Guthrie cards from 54 patients in Sweden with ALL (along with 47 healthy controls) and found that all tested negative for both HHV-6 and EBV DNA. These negative findings suggest that childhood ALL is unlikely to be associated with an *in utero* infection in the foetus with EBV or HHV-6 [81]. The same group also failed to find Human polyomaviruses JCV and BK 82 and Human Parovirus B19 [83]. However, EBV rarely passes through the placenta [84] and a maternal infection would not have been detected by this group so this does not necessarily mean that the mother did not have reactivated EBV that could potentially have an impact on the child *in utero* through and inflammatory pathway.



Lehtinen *et al* also found antibodies to *Helicobacter pylori* (a relatively common gastric infection that produces no symptoms in most infected people) were associated with increased risk of childhood leukaemia in offspring in Iceland (OR = 2.8, 95% CI 1.1-6.9) [78].

Recently this same group have found an association between adenoviruses and the risk of developing childhood leukaemia [80]. The common, endemic species C adenoviruses are capable of interfering with DNA repair mechanisms and as such were considered good initiators of the hallmark genetic mutations of childhood ALL. Analysing the Guthrie cards of the above described cases and controls, Gustafsson *et al* found a significant association between species C adenovirus DNA and ALL. The calculated odds ratio is 5.2 (1.3-31). This association is particularly interesting in light of proposed interactions between adenoviruses and some of the molecular components (HLA) of the human immune system [85]. Section 2.2.2.7 develops this idea further.

In summary, the laboratory evidence for maternal infection in the aetiology of childhood leukaemia appears to be evenly split between increased risk and no effect. The EBV studies are persuasive but need to be repeated by other groups. The evidence for adenovirus infection in the newborn child is also very interesting and would merit further work.

2.1.1.3 Conclusion

Is there an association between childhood leukaemia risk and maternal infections during pregnancy?

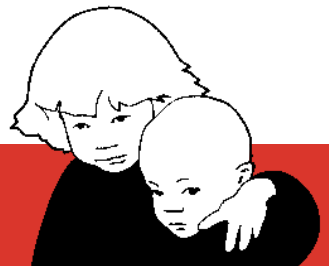
On balance, the evidence suggests that maternal infection increases the risk of childhood leukaemia. Epidemiological studies have found an association between maternal infection, particularly influenza and chicken pox, and the incidence of childhood leukaemia. But, the case numbers are generally small and most rely on retrospective questionnaires with all their associated problems. The small number of laboratory-based studies of maternal and newborn infection appears to be contradictory. Although there is some evidence for reactivation of the Epstein-Barr virus, the lack of viral DNA in the newborn does not necessarily mean that a maternal infection during pregnancy did not have an effect on the foetus. Most recently, adenovirus-C DNA was found in the newborn. Further work needs to be done in other research groups to confirm these findings.

Table 6. Laboratory evidence for an association between childhood leukaemia risk and maternal infection during pregnancy

Study and reference	Study design	Time period	No of cases	Results	Figures given
Finland and Iceland (Lehtinen *et al.*, 2003) 77	Case-control	1975-1997	342 ALL cases, 61 other leukaemia cases	Increased risk of ALL (diagnosed at 2-6 years) in children of mothers with antibodies to EBV	OR = 2.9 (1.2-7.4)
Finland and Iceland (Lehtinen *et al.*, 2005) 78	Case-control	1975-1997	341 ALL cases, 61 other leukaemia cases	Increased risk of childhood leukaemia in children of mothers with antibodies to H. pylori	OR = 2.8 (1.1-6.9)
Finland and Iceland (Tedeschi *et al.*, 2007) 79	Case-control	1975-1997	304 ALL cases, 39 other leukaemia cases	Presence of maternal EBV primary infection antibodies increases risk of (1.) ALL and (2.) non-ALL; presence of maternal EBV reactivation antibodies associated with increased risk of (3.) non-ALL	1. OR = 1.9 (1.2-3.0) 2. OR = 5.6 (1.1-29) 3. OR = 4.5 (1.3-16)
Sweden (Gustafsson *et al.*, 2007) 80	Case-control	1980-2001	49 ALL cases	Increased risk of ALL with adenovirus C infection during pregnancy	OR = 5.2 (1.3-31)

Table 7. Laboratory evidence against an association between childhood leukaemia risk and maternal infection during pregnancy

Study and reference	Study design	Time period	No of cases	Results	Figures given
Sweden (Priftakis *et al.*, 2003) 82	Case-control	1980-2001	54 ALL cases	Human polyoma viruses JCV and BK were not detected in Guthrie cards from children who later developed ALL	
Sweden (Isa *et al.*, 2004) 83	Case-control	1980-2001	54 ALL cases	Human parovirus B19 was not detected in Guthrie cards from children who later developed ALL	
Sweden (Bogdanovic *et al.*, 2004) 81	Case-control	1980-2001	54 ALL cases	Herpes and EBV viruses were not detected in Guthrie cards from children who later developed ALL	
Finland and Iceland (Lehtinen *et al.*, 2005) 78	Case-control	1975-1997	341 ALL cases, 61 other leukaemia cases	No evidence of antibodies for Chlamydia or M pneumonia in mothers of children with childhood leukaemia	



2.2 Childhood leukaemia and infections in early life

There is a long history of research into an association between childhood infections and leukaemia. In the 1950s, Stewart *et al* observed that children who developed leukaemia had had fewer infections than children who did not [57].

2.2.1 Is there an association between infections in early life and risk of childhood leukaemia?

Since the 1950s, there have been many studies looking for an association between childhood infections and leukaemia with about half demonstrating a **protective** effect of infection in early life. Two major studies published by in 2007 found an **increased** risk of childhood leukaemia with early infection.

Most studies have used questionnaires to obtain information about prior infections. As discussed in the introduction, this approach has inherent problems, in particular, the reliability of parental recall. Two studies used blood samples taken at diagnosis which gives an objective measure of prior infection – but is restricted to the infections measured. It also gives no information about the window of exposure, which is likely to be important. Two further studies use patient records to provide information about prior infections.

2.2.1.1 Questionnaire-based studies

The most recent study, by Ma *et al* (2005), part of the extensive Northern California Childhood Leukemia Study (NCCLS), included a total of 294 ALL cases (ages 1-14 years) and 376 individually matched controls. The authors found that self-reported ear infection during infancy was **protective** and was associated with a **significantly reduced** risk of common ALL (cALL) (OR = 0.32; 95% CI, 0.14-0.74) in non-Hispanic White children. In Hispanic children however, no association was observed between early infections and risk of childhood ALL or cALL [86].

Jourdan-Da Silva *et al* (2004) conducted a major case-control study in France, including 473 cases of acute leukaemia diagnosed between 1995 and 1998, age, sex and region-matched with 567 controls. A slight **protective** effect of early infections was observed (OR=0.8; 95% CI 0.6-1.0). The association was strongest for early gastrointestinal infections [87].

Canfield *et al* (2004) investigated a possible association between early life infections and leukaemia in children with Down Syndrome (DS). Children with DS are highly susceptible to leukaemia, with an estimated 10- to 30-fold increased risk compared to children without DS. It is generally assumed that the aetiology of leukaemia in children with DS is different and accordingly children with DS are excluded from most aetiological studies. The authors enrolled 158 children with DS diagnosed with leukaemia between 1997 and 2002 and 173 DS controls, selected from the cases' primary care clinics and frequency matched on age at leukaemia diagnosis. The authors found evidence of a **protective effect** of early-life infections, with a **significant negative association** between acute leukaemia and any infection in the first two years of life (adjusted OR = 0.55, 95% CI 0.33-0.92; OR=0.53, 95% CI 0.29-0.97; and OR = 0.59, 95% CI 0.28-1.25 for acute leukaemia combined, ALL, and AML respectively) [88].

Chan *et al* (2002) also reported a **protective effect** of early life infections in their case-control study of childhood leukaemia diagnosed at ages 2-14 years in Hong Kong [89]. 98 children with leukaemia and 228 controls took part in the study in which interviews with mothers were used to ascertain infectious exposure. Reported roseola and/or fever and rash in the first year of life significantly **reduced** the risk of leukaemia (OR = 0.33, 95% CI 0.16 - 0.68).

A French study [90], which included 280 leukaemia cases and 288 hospital controls, reported a statistically-significant **protective** effect for repeated early common infections (≥ 4 per year before age 2 years, OR = 0.6, CI 0.4-1.0) as well as for surgical procedures for ear-nose-throat infections before age 2 years (OR = 0.5, CI 0.2-1.0). The joint effect of day care attendance and early common infections was to further reduce the risk of childhood leukaemia (OR = 0.3, CI 0.1-0.8).

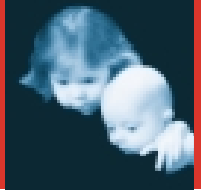
A case-control study in the US by Neglia *et al* (2000), consisting of 1,842 newly diagnosed cases of ALL under age 15 years, and 1,986 matched controls [91], also reported a **protective effect** of infection during early childhood: ear infections during infancy were **less common** among cases with a dose-response relationship – i.e. more episodes of infection conferred increasing protection.

Schuz *et al* (1999) used 1,010 leukaemia cases in their German case-control study looking at the association of childhood leukaemia with factors relating to the immune system. Overall, there did not appear to be an association between infections during childhood and leukaemia risk. When the different infectious diseases were analysed separately, a **protective effect of exposure to chicken pox** (OR = 0.8, 0.7–1.0) but not of **pneumonia** (OR = 1.3, 1.0–1.9) emerged. The chicken pox association did not remain significant when restricted to cALL whereas the pneumonia association strengthened when cALL was considered separately (OR = 1.7, 1.2-2.3) [92].

A national case-control study in New Zealand by Dockerty *et al* (1999), using 121 children aged 0-14 years with leukaemia and controls selected from birth records reported **an increased risk of leukaemia in relation to reported influenza infection** during the first year of life (adjusted odds ratio 6.8, 1.8-25.7). However, the number of exposed cases and controls were low (9 and 4 respectively) and the authors themselves expressed concerns that this could be a chance finding due to multiple comparisons. The authors found **no association** between number of infections and B-cell precursor ALL although a small effect could not be ruled out with a study of this small size [93].

In the 1980s, the Inter-Regional Epidemiological Study of Childhood Cancer (IRESCC) in the UK [65, 94] collected interview and medical information relating to the child's past medical experiences from parents of 555 children diagnosed with cancer, and parents of 1,110 unaffected matched controls. Increasing numbers of illnesses under 6 months of age appeared to be associated with an **increased** risk of ALL.

In the Netherlands, a national register of children with leukaemia formed the basis for a case-control study of the association between infectious diseases in the first year of life and ALL [95]. Analysis included 492 cases (diagnosed with ALL between 1-15 years) and 480 controls matched on age, sex and place of



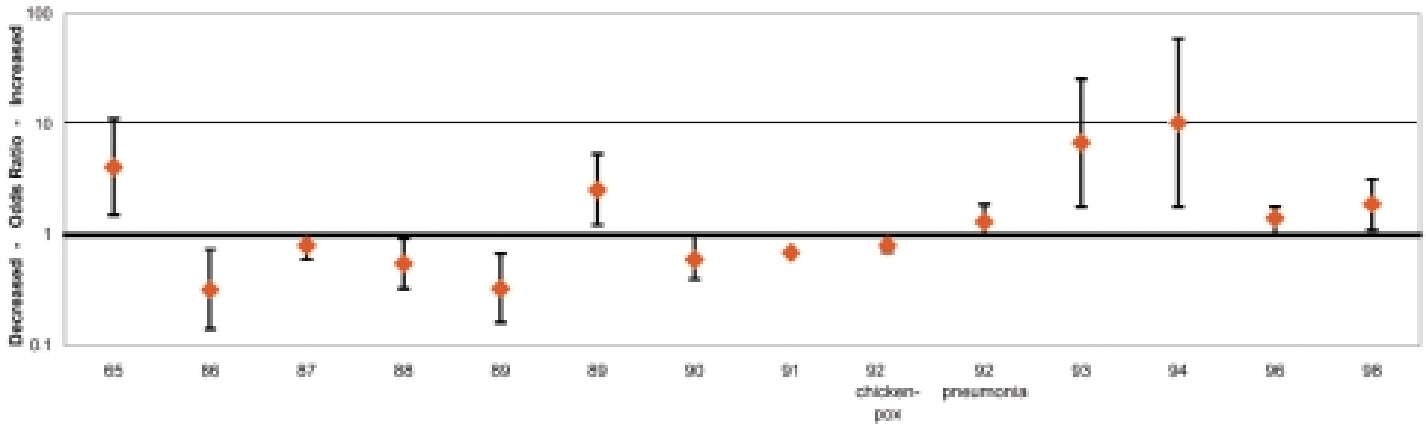


Figure 8. Early childhood exposure to infection and risk of childhood leukaemia (questionnaire based)

residence at diagnosis, with data collected by mailed questionnaires. The study found weak evidence of a **protective effect** of serious infections (those requiring hospitalisation/specialist consultations) in early childhood: there was a lower frequency of serious infections in the first year of life for cases (relative risk = 0.6; 95% CI 0.4 – 1.0). However, this finding was not significant when adjusted for birth order, family size, social class, and number of rooms. No significant associations were found between ALL and any of the other infections included in the analysis (bronchitis, measles, chickenpox, mumps, rubella, otitis, common colds or fever).

2.2.1.2 Direct medical evidence from records and blood samples
One of the most recent studies to use objective measures, part of the United Kingdom Childhood Cancer Study (UKCCS) [96], examined the relationship between childhood cancer and preceding exposure to infectious diseases using patient records of 425 children diagnosed with ALL at ages 2-5 years (1991-1996) and matched controls. The authors reported that **children who developed ALL at the childhood peak, between the ages of 2 and 5 years, had more clinically diagnosed infections in the first year of life** than unaffected children had. For documented viral infection in pregnancy, the odds ratio (OR) was 6.0 [95% confidence interval (CI) 1.2-29.7]. The average number of infectious episodes was 3.6 (95% CI: 3.3 - 3.9) versus 3.1 for controls (95% CI: 2.9 - 3.2). This excess was apparent from the first month of life, with children with more than one neonatal episode of infectious illness developing their leukaemia at a younger age than children with only one episode or none.

The excess of infectious episodes in cases was further confirmed in a larger UKCCS study which used both patient records and maternal interviews for 811 children diagnosed with leukaemia (0-14 years of age, 1991-1996) and 1288 controls.[97] During the course of the comparison of maternal recall and medical records it was determined that there was a high degree of under-reporting of infection in both case and control mothers. More than one in four mothers who took their child to a GP with an infectious illness did not report doing so at interview. The authors also determined that the case mothers had a greater degree of under-reporting than the control mothers did. Odds ratios for infectious illness estimated from **interview** data all tended to be below one indicating a **protective** effect whereas those from **medical records** tended to be above one indicating an **increased** risk.

The UKCCS study required active participation of both cases and controls which inevitably introduced participation bias [51]. Using medical records includes only infections which resulted in a medical consultation. This approach gives less weight to a mother's memory of her child's level of infections and also neglects the impact of lesser infections. These studies are also at odds with the findings of other (objective) studies which have found a protective effect of early childhood infections.

Petridou *et al* (2001) reported mixed results from a study which used blood samples from case and control children in Greece to measure past exposure to 10 common infections. They found little evidence for an association between ALL and any of the studied infectious agents among the very young children. In children aged 5 years or older, **exposure to EBV or HHV-6 had a significantly protective effect,** whereas **exposure to parainfluenza increased the risk of ALL** [98]. However, it is not possible to determine the window of exposure using this method and this is likely to be important.

In a case-control study of Scottish children with leukaemia (aged 0-14 years) who were age and sex matched to two population-based controls, details of neonatal infections were taken from the mother's hospital obstetric notes and swab tests performed on the children [62]. 144 leukaemias (including 124 ALLs) were analysed. The presence of a neonatal infection had a protective effect, **significantly reducing** the risk of ALL (OR = 0.49, 95% CI 0.26-0.95), particularly in the under-fives.

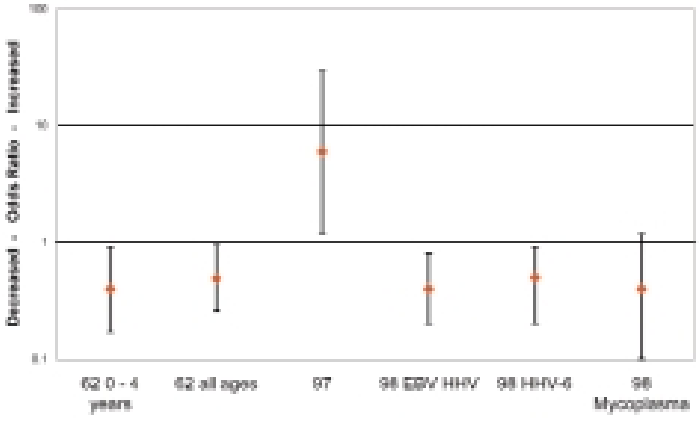


Figure 9. Early childhood exposure to infection and risk of childhood leukaemia (objective measures)

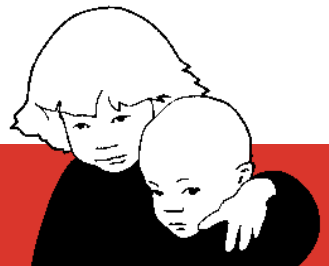


Table 8. Epidemiological evidence for infections in early life reducing the risk of childhood leukaemia

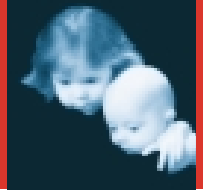
Study and reference	Study design	Time period	No of cases	Results	Figures given
Scotland (McKinney *et al.*, 1999) 62	Case-control	1991-1994	144 CL cases	Reduced risk of ALL with neonatal infection (as confirmed by swab tests) in (1.) 0-14 year olds, strengthened in (2.) 0-4 year olds	1. OR = 0.49 (0.26-0.95) 2. years OR = 0.40 (0.17-0.90)
Children's Cancer Group (Neglia *et al.*, 2000) 91	Case-control	1989-1993	1842 ALL cases	Ear infections during infancy were less common among cases (1. 1 episode; 2. 2-4 episodes; 3. 5+ episodes; 4. continuous)	1. OR = 0.86 2. OR = 0.83 3. OR = 0.71 4. OR = 0.69 (p for trend = 0.026)
Greece (Petridou *et al.*, 2001) 98	Case-control	1993-1997	94 ALL cases	Decreased risk of ALL with some early childhood infections (1. EBV; 2. HHV-6; 3. mycoplasma)	1. OR = 0.4 (0.2-0.8) 2. OR = 0.5 (0.3-0.9) 3. OR = 0.4 (0.1-1.2)
France (Perrillat *et al.*, 2002a) 90	Case-control	1995-1999	240 ALL cases, 40 AML cases	Repeated common infections (4 or more) in early life protective	OR = 0.6 (0.4-1.0)
Hong Kong (Chan *et al.*, 2002) 89	Case-control	1994-1997	98 AL cases	Roseola and/or fever and rash in first year of life reduced risk of acute leukaemia	OR = 0.33 (0.16-0.68)
France (Jourdan-Da Silva *et al.*, 2004) 87	Case-control	1995-1998	473 CL cases	(1.) More than 4 early infections reduces the risk of acute leukaemia, (2.) particularly gastrointestinal infections	1. OR = 0.8 (0.6-1.0) 2. OR = 0.1 (0.03-0.6)
Children's Oncology Group (Canfield *et al.*, 2004) 88	Case-control	1997-2002	97 ALL cases, 61 AML cases	Any infection in first two years of life reduced risk of acute leukaemia in children with Down syndrome	OR = 0.55 (0.33-0.92)
Northern California Leukemia Study (Ma *et al.*, 2005) 86	Case-control	1995-2002	294 ALL cases, 376 controls	Self-reported ear infection during infancy associated with reduced risk of c-ALL in non-Hispanic White children	OR = 0.32 (0.14-0.74)
UK Childhood Cancer Study (Simpson *et al.*, 2007) 97	Case-control	1991-199	811 CL cases	From interview data infection is associated with reduced risk of childhood leukaemia	OR < 1 for most infections

Table 9. Epidemiological evidence for infections in early life increasing the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Oxford Survey of Childhood Cancers (Stewart *et al.*, 1958) 57	Mortality cohort (leukaemia or cancer)	1953-1955	677 CL cases, 739 other cancer cases	Infection prior to 2 years before diagnosis more common in cases than controls	613 cases, 566 controls
Inter Regional Epidemiological Study of Childhood Cancer (McKinney *et al.*, 1987) 65	Case-control	1980-1983	171 CL cases, 63 lymphoma cases	Viral disease under age of 6 months increased risk of ALL and lymphoma	RR = 4.1 (1.5-11.3)
Inter Regional Epidemiological Study of Childhood Cancer (Hartley *et al.*, 1988) 94	Case-control	1980-1983	148 ALL cases, 23 other leukaemia cases	Perinatal infections increases risk of ALL	RR = 10.31 (1.8-59.26)
Germany (Schuz *et al.*, 1999) 92	Case-control	1992-1997	1184 CL cases	(1.) Increased risk of cALL with pneumonia, (2.) stronger if in year prior to diagnosis and also present with (3.) bronchitis	1. OR = 1.7 (1.2-2.3) 2. OR = 2.6 (1.4-4.8) 3. OR = 1.9 (1.3-2.7)
New Zealand (Dockerty *et al.*, 1999) 93	Case-control	1990-1993	97 ALL cases, 24 other leukaemia cases	Little effect of infections in first year of life except influenza (but small study)	OR = 6.8 (1.8-25.7)
Greece (Petridou *et al.*, 2001) 98	Case-control	1993-1997	94 ALL cases	Increased risk of ALL with early parainfluenza 1,2 or 3 infection	OR = 1.9 (1.1-3.2)
Hong Kong (Chan *et al.*, 2002) 89	Case-control	1994-1997	98 AL cases	Tonsillitis 3-12 months prior to diagnosis increases risk	OR = 2.56 (1.22-5.38)
England (Kroll *et al.*, 2006) 99	Time trend in incidence of leukaemia matched to influenza epidemics	1974-2000	11,790 CL cases	Influenza epidemic preceded peak of cALL but not other childhood leukaemias	
UK Childhood Cancer Study (Roman *et al.*, 2007) 96	Case-control	1991-1996	455 ALL cases	(1.) At least one infection ≤ 12 months of age increases risk of ALL (diagnosed 2-5 years), (2.) strengthened for ≤ 1 month of age	1. OR = 1.2 (0.9-1.7) 2. OR = 1.4 (1.0-1.8)
UK Childhood Cancer Study (Simpson *et al.*, 2007) 97	Case-control	1991-1996	811 CL cases	From medical records case children averaged significantly more infections in first year of life than controls	Cases: 3.2 episodes (3.1-3.3). Controls: 2.9 episodes (2.8-3.0)

Table 10. Epidemiological evidence for infections in early life having no effect on the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Northern England (Nyari *et al.*, 2003) 66	Birth cohort, 1975-1986	1975-2001	508 CL and NHL cases out of 404,106 births	Infection within first 6 months of life had no significant effect on risk of leukaemia and non-Hodgkin's lymphoma	



In conclusion, about half of the **questionnaire**-based studies showed a **protective** effect for infection in early life and two studies looking for **direct evidence** of previous infections indicate that, for the infections measured, infection **strongly protects** against leukaemia. The recent large UKCCS studies using patient's records for any infectious episode found that previous documented infection **increased** the risk of childhood leukaemia.

Li *et al* studied a "natural" experiment - the impact of SARS, and the strict hygiene measures imposed as a result of the epidemic on the development of ALL. Compared with ALL in Hong Kong between 1994 and 2005, i.e. from 10 years before the SARS epidemic to 2 years afterwards there was a **decrease** of ALL in 2003, concurrent with a **decrease** in the number of notifiable communicable diseases (chickenpox, measles and scarlet fever). In the two years after 2003 the number of cases of ALL then increased back to the usual range [100].

The authors argue that there is a direct relationship between decreased infectious exposure, reflected in the reported cases of notifiable communicable diseases, and rate of ALL and support an infectious aetiology for ALL. Although, this appears contrary to the evidence of infection in early life being protective, children of all ages had lower levels of infection. As such, this evidence does not necessarily negate Greaves' delayed infection hypothesis and can be seen as supportive of infection causing the "second hit" in the "two hit hypothesis".

The situation of Hong Kong will hopefully provide an interesting ongoing experiment. Those children born just before and during the SARS epidemic will have been strongly affected by the strict hygiene measures introduced, in that they will have had very little or no exposure to the infectious agents common in infancy. Their immune systems would be expected to be highly dysregulated and, if Greaves' hypothesis of delayed exposure is correct, this cohort of children may be more prone to childhood leukaemia than children who had more normal infant immune system challenges.

2.2.1.3 Are any viruses found in leukaemic cells?
Epstein-Barr virus (EBV) is known to cause Burkitt's lymphoma and human T-cell lymphotropic virus 1 (HTLV1) retrovirus causes adult T-cell leukaemia/lymphoma. Leukaemias in domestic cattle, cats and chickens are also caused by viruses [40].

Is childhood leukaemia caused directly by a virus is a similar way? Ruth Jarret's group have been looking for viral DNA in leukaemic cells and so far have not found any. DNA from the following viruses was not found: Polyomas JC & BK [101, 102] and herpes [103, 104]. They went on to compare normal cell DNA and tumour DNA (representational difference analysis) and found no foreign DNA in the tumour cells – i.e. no viruses [104].

This suggests that it is unlikely that a single, direct carcinogenic virus causes childhood leukaemia. However, it is always difficult to prove a negative. There is a 5% chance that they would not detect a retrovirus, and viruses with small genomes [104]. But, the group believe that they are very unlikely to have missed viruses as large as HHV-6 and are unlikely to have missed a complete adenovirus.

Despite the lack of evidence of a direct viral mechanism from Jarret's group we know that some viruses cause cancer indirectly (helicobacter pylori infection and adult gastric lymphoma [105], HIV and Kaposi's sarcoma [106]). Whilst a direct mechanism appears not the case for childhood leukaemia, it does not argue against any inflammatory or immunostimulatiory effects of a previous infection on the body.

2.2.1.4 Is vaccination protective?
Vaccination stimulates the immune system in a similar way to an infection, providing protective immunity without having to have the actual infection, and so can be regarded as a direct measure of infective exposure.

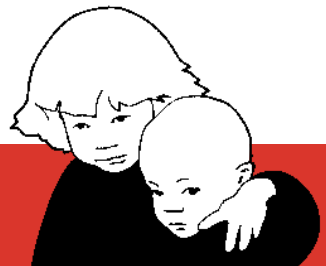
The effect of vaccination on the risk of childhood leukaemia is difficult to assess because almost every child in developed countries is immunised. However, the Hib vaccine was introduced relatively recently (1992), and so there are vaccinated and non-vaccinated children to compare. The evidence is reasonably strong that the Hib vaccination is **protective**.

The majority of studies so far have used **questionnaires** to obtain information about a variety of vaccinations. Three found vaccination to be **protective**. In the UK IRESCC study McKinney *et al* found that fewer children diagnosed with leukaemia had been immunised compared with the control groups (OR=0.2) [65, 94]. In Japan, BCG vaccination, measles infection, and measles vaccination were found to be strongly protective (RR=0.1-0.2) [107] as were at least four routine immunizations in Germany (OR=0.3) [92].

Other questionnaire-based studies found **no significant protection.** One group in the USA and Canada found the MMR vaccine to be a risk factor (OR=1.7) [60]. Others found no effect in New Zealand with any vaccination, measles alone or MMR [93]; in Greece with anti-viral vaccinations [108]; and in France with any vaccination [109].

The Hib vaccine was shown to be **protective** in the USA using data taken from medical records (OR=0.57) [110]. The protective association was also shown in The Northern California Childhood Leukemia Study with controls randomly selected from the state-wide birth files and vaccination data obtained from medical records [111]. This protective effect was confirmed in further studies [52, 111-114].

In conclusion, the impact of vaccination on the risk of developing childhood leukaemia is intrinsically difficult to study due to the widespread nature of vaccination in the western world, and indeed studies looking at well-established vaccines such as MMR produce contradictory reports. However, the relatively recent introduction of the Hib vaccine offered an excellent opportunity to observe the impact of early immune stimulation on the incidence of leukaemia. All studies show a protective effect of this vaccination, supported by a study on Hib infection, which shows that early life infection is protective whereas infection after the age of five increases the risk of leukaemia. There is also some evidence of a dose-response relationship.



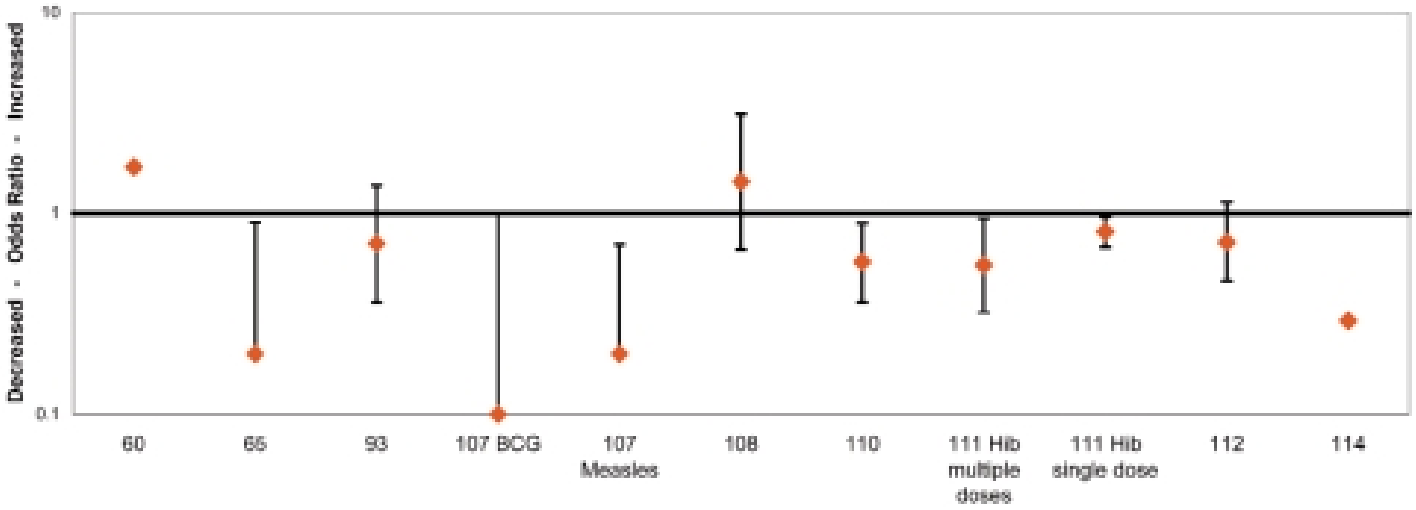


Figure 10. Effect of vaccination on childhood leukaemia risk

Table 11. Epidemiological evidence for vaccination reducing the risk of childhood leukaemia

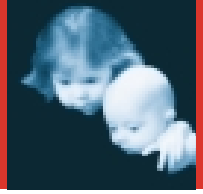
Study and reference	Study design	Time period	No of cases	Results	Figures given
Inter Regional Epidemiological Study of Childhood Cancer (McKinney *et al.*, 1987) 65	Case-control	1980-1983	171 CL cases, 63 lymphoma cases	Vaccinations decrease risk of childhood leukaemia	RR = 0.2 (0.1-0.9)
Inter Regional Epidemiological Study of Childhood Cancer (Hartley *et al.*, 1988) 94	Case-control	1980-1983	148 ALL cases, 23 other leukaemia cases out of 555 childhood cancer cases	Never being immunised increases the risk of childhood cancer	RR = 3.58 (1.57-8.15)
Japan (Nishi & Miyake, 1989) 107	Case-control	1981-1987	63 ALL cases	(1.) BCG and (2.) measles vaccinations in early childhood protective against ALL	1. RR = 0.1 (0.0-1.0) 2. RR = 0.2 (0.1-0.7)
U.S.A. (Groves *et al.*, 1999) 110	Case-control	1989-1993	439 ALL cases	Protective effect of conjugated Hib vaccination against ALL	RR = 0.57 (0.36-0.89)
Germany (Schuz *et al.*, 1999) 92	Case-control	1992-1997	1184 CL cases	Increasing numbers of vaccinations are protective against childhood leukaemia (0-3 vaccinations cf > 6 vaccinations)	OR = 3.2 (2.3-4.6)
Northern California Childhood Leukemia Study (Ma *et al.*, 2005b) 111	Case-control	1995-2002	294 ALL cases	Each dose of Hib vaccine reduces the risk of ALL (1. single dose; 2. 3 or more doses)	1. OR = 0.81 (0.68-0.96) 2. OR = 0.55 (0.32-0.94)
Finland (Auvinen *et al.*, 2000; Groves *et al.*, 2000) 112, 113	Birth cohort, 1985-1987	1985-1996	77 CL cases out of 114,000 births	Hib vaccination protective against childhood leukaemia (but results not statistically significant)	Multiple vs single doses OR = 0.72 (0.46-1.13)
Canada (Groves *et al.*, 2001) 114	Case-control	1966-1970	42 ALL cases	Effect of Hib infection depends on age of ALL diagnosis: protective in children diagnosed at or before age of 5	OR = 0.29 (p = 0.12)
Finland (Groves *et al.*, 2002) 52	Birth cohort, 1987-1989	1987-1999	80 CL cases out of 125,129 births	Protective effect of Hib vaccine but no difference between various conjugated forms	

Table 12. Epidemiological evidence for vaccination increasing the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Children's Cancer Group (Buckley *et al.*, 1994) 60	Case-control	1964-1989	547 ALL cases	Increased risk of B-ALL with MMR vaccination	OR = 1.7

Table 13. Epidemiological evidence for vaccination having no effect on the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Greece (Petridou *et al.*, 1997) 108	Case-control	1993-1994	153 CL cases	No effect of viral vaccination	OR = 1.44 (0.66-3.13)
New Zealand (Dockerty *et al.*, 1999) 93	Case-control	1990-1993	97 ALL cases, 24 other leukaemia cases	No significant effect of any vaccination alone, measles only, or MMR	OR = 0.71 (0.36-1.38)
ESCALE (Mallol-Mesnard *et al.*, 2007) 109	Case-control	2003-2004	726 AL cases	No association between any vaccination and the risk of childhood leukaemia	



2.2.1.5 Conclusion - Is there an association between childhood leukaemia risk and infections in early life?

In this section we have looked at the evidence for a direct association between infection and the aetiology of childhood leukaemia including questionnaire based epidemiological studies, medical records and laboratory studies. There is a suggestion of a **protective** effect of infections in early life - particularly for vaccination, a good simulation of infection in the effect it has on the infant immune system.

Despite being suggestive of a protective effect of early infection, overall the data is inconsistent, with about half the epidemiological studies showing an increased risk of childhood leukaemia with infection in early life. One possible reason for the inconsistency of the data is the window of exposure studied. Some reports specifically exclude any data from 3 months prior to diagnosis as the developing leukaemia reduces resistance to infections. The inclusion of these data in other studies may give rise to conflicting results.

Not all of the studies addressed the impact of infection on the same type of leukaemia. Some looked at leukaemia as a whole, whereas others studied ALL, or cALL. It is possible that infection plays a role in the aetiology of only one particular type of leukaemia, e.g. cALL, which makes up the majority of the childhood peak and has increased significantly over the last century.

2.2.2 Is there an association between proxy measures of infection and risk of childhood leukaemia

Measuring the level, or even existence, of infections within an individual or population poses major challenges. Most studies are forced to rely on retrospective recall of infections and direct measures of infections are not always possible. Many investigators have therefore used indirect or proxy measures of infection in an attempt to overcome these problems. To be a good proxy, it needs to be reasonably well linked to infectious exposure.

Two proxy measures which have been widely used are: the level of social and physical contacts (usually assessed by the child's attendance at nursery or day care) and birth order. These are discussed below. Breast feeding, seasonal variation, socio-economic status, and allergy – four other factors which have been associated with both childhood leukaemia and infection – are also discussed.

2.2.2.1 Social and physical contacts

Children who have more social and physical contact with other children have more infections [115-117] which makes day care/nursery a good proxy for infection. It is also reasonably easy to obtain accurate data even in retrospective questionnaires.

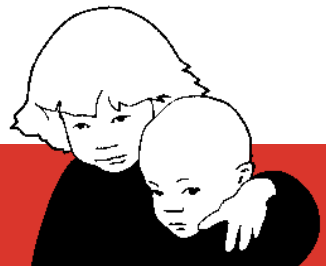
Three major recent studies [86, 118, 119] as well as a number of smaller studies [56, 87, 90, 120] have reported a **protective** effect of day care attendance. No studies have reported an increased risk of childhood leukaemia with day care attendance although several have reported no protective effect [89, 91, 108, 121, 122].

The first of the major studies, undertaken by Kamper-Jorgensen *et al*, is a nationwide case-control study based on Danish register data. All cases of ALL diagnosed in children aged 0-15 years between 1989 and 2004 were included (559 cases) and matched with 5590 controls on date of birth and sex (10 controls per case)[118]. The authors found that ever attending day care resulted in a **significantly protective** effect, giving a rate ratio of 0.68 (0.48-0.95), an association that was observed across all sub-types of ALL.

The second study, the United Kingdom Childhood Cancer Study (UKCCS), is a population based case-control study into the causes of childhood cancer which includes 3,140 children with cancer (aged 2-14 years) and 6,305 similar aged controls. The cases were diagnosed between 1991 and 1996 and included 1,286 children with ALL[119]. Any social activity outside the family in the first year of life **significantly reduced** the risk of ALL. There was a consistent and statistically significant reduction in risk for each level of activity in the first year of life and a dose-response trend across increasing levels of activity. They obtained similar results for cALL and other ALL sub-groups.

The third major study, the Northern California Childhood Leukemia Study (NCCLS), included 294 children with ALL (ages 1-14 years) and 376 individually matched, healthy controls in their analysis of day care attendance[86]. Day care attendance, measured by child hours, was associated with a **significantly reduced** risk of cALL in non-Hispanic white children, with a larger effect for day care attendance during infancy than for day care attendance before diagnosis. This effect was not apparent in Hispanic children. The association with cALL in the non-Hispanic white children persisted when day care attendance was cut-off one year before the diagnosis date, thus eliminating the possibility that pre-clinical symptoms could have affected day care attendance and biased the results. The magnitude of effect was stronger for day care attendance during infancy than later in childhood. They highlight an important ethnic difference, but it is not clear whether this may be due to cultural/environmental factors or biological characteristics.

The largest study to find **no effect** of day care was carried out by the US Children's Cancer Group and the National Cancer Institute[91]. This case-control study used 1,842 newly diagnosed cases of ALL under age 15, and 1,986 matched controls. Neither attendance at day care nor time at day care was associated with risk of ALL overall or 'common' ALL. These data do not support the hypothesis that a decrease in the occurrence of common childhood infection increases risk of ALL. But it is argued that the study is unsound because it used random digit dialling to select controls, an approach which is now regarded as flawed by most epidemiologists [40].



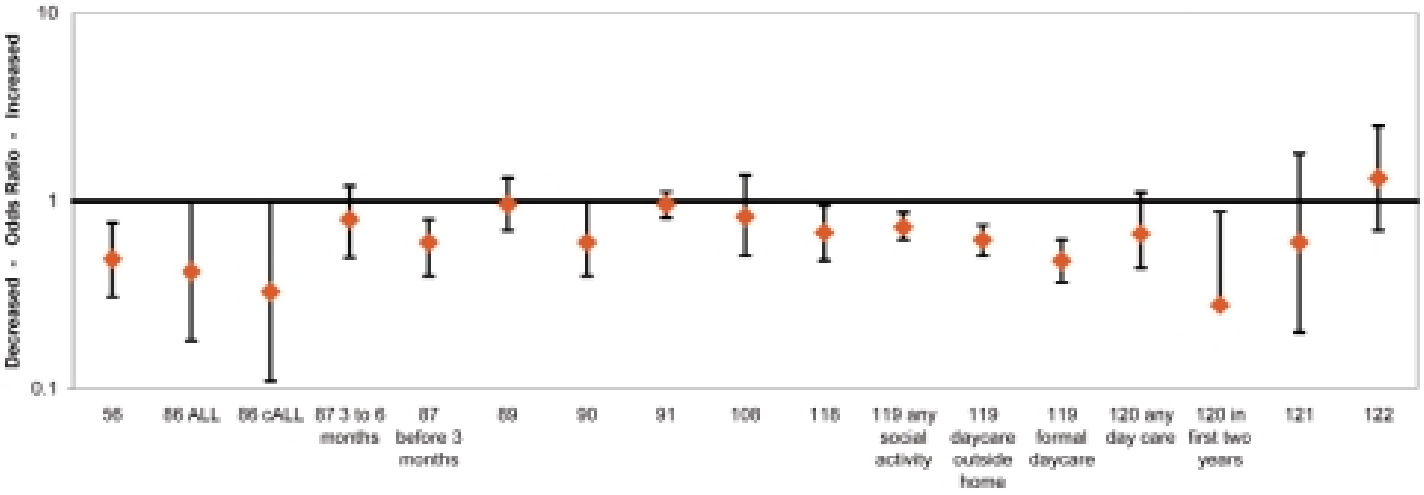


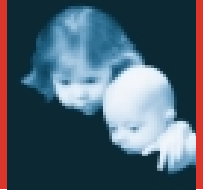
Figure 11 Effect of social and physical contacts on childhood leukaemia risk

Table 14. Epidemiological evidence for social and physical contact reducing the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Greece (Petridou *et al.*, 1993) 120	Case-control	1987-1991	136 CL cases	Children who (1.) ever attended day care had a lower risk of developing leukaemia, especially (2.) those who attended for at least three months during the first two years of life	1. OR = 0.67 (0.44-1.11) 2. OR = 0.28 (0.09-0.88)
Canada (Infante-Rivard *et al.*, 2000) 56	Case-control	1980-1995	491 ALL cases	Early day care attendance is significantly protective against ALL	OR = 0.49 (0.31-0.77)
France (Perrillat *et al.*, 2002a) 90	Case-control	1995-1999	240 ALL cases, 40 AML cases	Early attendance at day care is protective against childhood leukaemia	OR = 0.6 (0.4-1.0)
France (Jourdan-Da Silva *et al.*, 2004) 87	Case-control	1995-1998	473 CL cases	Early day care decreases risk of acute leukaemia, with a stronger effect for children who started earlier (starting at 1. < 3 months; 2. 3-6 months)	1. OR = 0.6 (0.4-0.8) 2. OR = 0.8 (0.5-1.2)
Northern California Childhood Leukemia Study (Ma *et al.*, 2005a) 86	Case-control	1995-2002	294 ALL cases	Protective effect against (1.) ALL and (2.) cALL of > 5,000 child-hours day care in white children, especially during infancy	1. OR = 0.42 (0.18-0.99) 2. OR = 0.33 (0.11-1.01)
UK Childhood Cancer Study (Gilham *et al.*, 2005) 119	Case-control	1991-1996	1286 ALL cases	Increasing levels of social activity (1. any social activity; 2. regular day care outside the home; 3. formal day care at a facility) associated with decreasing risk of ALL	1. OR = 0.73 (0.62-0.87) 2. OR = 0.62 (0.51-0.75) 3. OR = 0.48 (0.37-0.62)
Denmark (Kamper-Jorgensen *et al.*, 2007) 118	Case-control	1989-2004	559 ALL cases	Ever attending day care has significantly protective effect against ALL	RR = 0.68 (0.48-0.95)

Table 15. Epidemiological evidence for social and physical contact having no effect on the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Southern England (Roman *et al.*, 1994) 121	Case-control	1972-1989	51 CL and NHL cases	Attendance at day care for three months or more in year before diagnosis had no significant effect on risk of ALL	RR = 0.6 (0.2-1.8)
Greece (Petridou *et al.*, 1997) 108	Case-control	1993-1994	153 CL cases	No effect of day care on risk of childhood leukaemia	OR = 0.83 (0.51-1.37)
Children's Cancer Group (Neglia *et al.*, 2000) 91	Case-control	1989-1993	1842 ALL cases	No association of day care with ALL observed	OR = 0.96 (0.82-1.12)
New York State (Rosenbaum *et al.*, 2000) 122	Case-control	1980-1991	255 ALL cases	Non-significant effect of day care on risk of ALL studying never cf > 36 months attendance	OR = 1.32 (0.70-2.52)
Hong Kong (Chan *et al.*, 2002) 89	Case-control	1994-1997	98 AL cases	Attendance at daycare during first year of life has no effect on risk of acute leukaemia	OR = 0.96 (0.70-1.32)



In conclusion, children who have more social and physical contact with other children have more infections and there is a correlation between increased risk of infection and day care/nursery attendance. There is a reasonable consensus that attendance at day care is protective against childhood leukaemia from three major recent studies and a number of smaller studies. No studies have reported an increased risk of leukaemia with day care attendance although several older studies reported no effect. The protective effect is most marked against ALL, in particular the subtype cALL. There is also some evidence that there is a dose-response trend in this effect; that increasing numbers of hours' exposure to increasing numbers of children has a stronger impact on the risk of developing ALL. This is suggestive of an infectious aetiology – but not proof. How reliable a proxy is day care attendance for infectious exposure? What else might it be a proxy for? For example, different levels of nutrition, exposure to environmental carcinogens, socio-economic status or birth order all of which are also associated with leukaemia risk.

2.2.2.2 Birth order

Birth order is used as a proxy for infection as it is assumed that children in larger families – especially those lower down the birth order – are exposed to more infections in early childhood due to close proximity with older siblings. No clear consensus has emerged from the epidemiological studies. Of 13 recent case-control studies, five showed an increased risk of childhood leukaemia associated with larger families, seven found no effect and two showed a protective effect.

The three most recent studies illustrate some of the issues:

In a case-control study in Quebec, Canada, Infante-Rivard *et al* (2000) analysed 491 cases diagnosed between 1980 and 1993, between 0 and 9 years of age, and an identical number of matched controls. They showed the importance of both age of exposure and age of diagnosis. Having a school age sibling **during the first year of life was significantly protective for those older than 4 years at the time of diagnosis. In contrast,** having a school age sibling **at the time of diagnosis significantly increased the risk in all children, but most markedly in those diagnosed before 4 years of age**[56].

Using 3,878 leukaemia cases (of which 3,153 were ALL) cases taken from the National Registry of Childhood Tumours in England and Wales (diagnosed age 0-14 years), Dockerty *et al* (2001) reported a **strong and significant** protective effect of increasing parity on risk of childhood ALL, with the most marked effect in children aged between 1 and 5 years at diagnosis (i.e. those children most likely to be diagnosed with cALL) [123].

Jourdan-Da Silva *et al* (2004) conducted a case-control study in France, including 473 leukaemia cases, 408 of which were ALL. They reported an association between a birth order of four or more and a **significantly increased risk** of ALL[87].

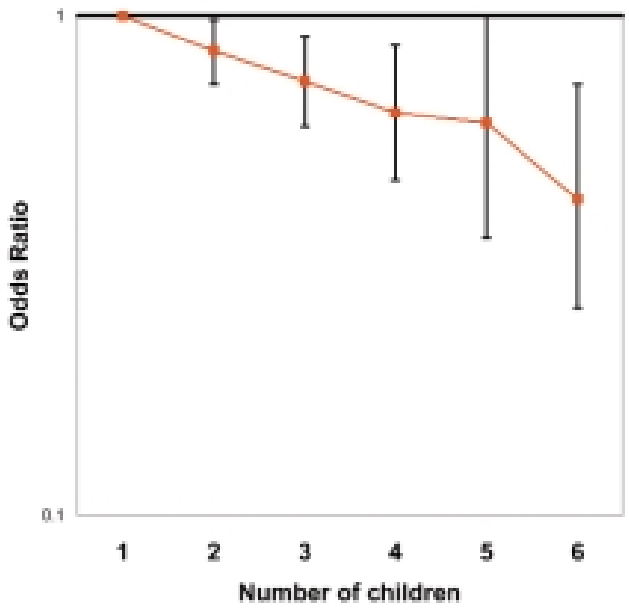


Figure 12. Birth order and the risk of developing childhood leukaemia between the ages of 1 and 5 years. CCRG data Dockerty *et al.*, 2001[123]

Table 16. Epidemiological evidence for decreasing risk of childhood leukaemia with increasing birth order

Study and reference	Study design	Time period	No of cases	Results	Figures given
Greece (Petridou *et al.*, 1997) 108	Case-control	1993-1994	153 CL cases	Inverse association of birth order with childhood leukaemia risk	OR = 0.74
Canada (Infante-Rivard *et al.*, 2000) 56	Case-control	1980-1995	491 ALL cases	Having a school age sibling during first year of life if diagnosed ≥ 4years has a protective effect against ALL	OR = 0.46 (0.22-0.97)
England (Dockerty *et al.*, 2001) 123	Case-control	1968-1986	10,162 childhood cancer cases	Increasing parity is increasingly protective against ALL diagnosed at 1-5 years (1. 1st child; 2. 2nd child; 3. 3rd child; 4. 4th child; 5. 5th child; 6. 6th or more child)	1. OR = 1 2. OR = 0.85 (0.73-0.98) 3. OR = 0.74 (0.60-0.91) 4. OR = 0.64 (0.47-0.87) 5. OR = 0.61 (0.36-1.03) 6. OR = 0.43 (0.26-0.73)

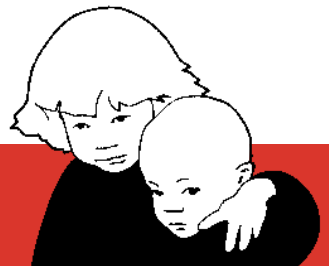


Table 17. Epidemiological evidence for increasing risk of childhood leukaemia with increasing birth order

Study and reference	Study design	Time period	No of cases	Results	Figures given
Canada (Infante-Rivard *et al.*, 2000) 56	Case-control	1980-1995	491 ALL cases	Having a school age sibling at the time of diagnosis is diagnosed < 4 years of age	OR = 4.54 (2.27-9.07)
United Arab Emirates (Bener *et al.*, 2001) 124	Case-control	1983-1997	117 ALL, HL and NHL cases	Increasing risk of ALL with increasing birth order	Mean birth order cases = 4.1, controls = 2.9; OR = 1.80 (1.13-2.86)
Children's Cancer Group (Ou *et al.*, 2002) 125	Case-control	1989-1993	1842 ALL cases	Increased risk of ALL for high birth order (4th cf 1st born)	OR = 2.0 (1.3-3.0)
California (Reynolds *et al.*, 2002) 37	Case-control	1988-1997	1407 ALL cases, 240 ANLL cases	No effect of parity on ALL but ≥ 2 previous live births increases risk of (1.) acute non-lymphoblastic leukaemia and (2.) acute myeloid leukaemia	1. OR = 1.59 (1.00-2.53) 2. OR = 1.85 (1.04-3.31)
France (Jourdan-Da Silva *et al.*, 2004) 87	Case-control	1995-1998	473 CL cases	Birth order of 4 or more increases risk of ALL	OR = 2.0 (1.1-3.7)

In conclusion, when using birth order as a proxy for infection there is little consensus between studies. Dockerty's very large study in England and Wales shows a strong protective association with increasing parity but other studies show the reverse. Earlier studies tend to find that increasing parity is associated with protection whereas more recent studies find an association with an increased risk of developing childhood leukaemia. This may reflect some impact of changing social conditions in the countries studied and over time.

There may also be an explanation other than infection for these results. The strong association of ALL with a history of abortion(s) may be a confounding factor[126]. It is not only being the first child but also being the only child which is associated with a high risk. Thus the firstborn association may be a reflection of reproductive failure within the same families, resulting in families with only one or two children. Previous studies generally lack a distinction between the firstborn and the only child, making it difficult to distinguish between this possibility or the link with infection.

2.2.2.3 Socio-economic status

The use of socio-economic status (SES) as a proxy measure for infection is based on the assumption that people with a higher socio-economic status encounter fewer infectious diseases as a result of smaller family size, less crowding and later interaction with other children. However, SES could also be a proxy for other potential carcinogens for example, chemical exposure in diet or environment or exposure to non-ionising radiation. Early studies have shown a clear **protective** association with social deprivation, but later studies do not always confirm this.

A number of studies has shown that incidence of ALL is higher in areas of high social class (e.g. Alexander 1991[127], Stiller & Parkin, 1996[128], Borugian, 2005[129]). Evidence from developing countries suggests that incidence of ALL in children aged 1-4 years is rising with improved socio-economic conditions (Hrusak, 2002[130]).

However, SES is intrinsically difficult to study in epidemiological studies. Poole *et al*[131] extensively reviewed case–control studies in North America since 1980. They found that studies that **required participation did not show a positive** association of childhood leukaemia with SES, whereas studies that with no participation showed a **positive** association of childhood leukaemia with SES. They concluded that in case-control studies that required active participation, higher SES controls are more likely to be involved than lower SES controls. This results in a study in which the controls are of a higher socio-economic status than the cases. This disparity means that, if higher SES is associated with an increased risk of ALL and the controls are at a higher risk than the cases, the size of any effect of SES would be reduced. Studies which do not require participation can avoid such a disparity.

Studies undertaken since Poole's analysis include the recent Committee on Medical Aspects of Radiation in the Environment 11th Report[132]. This is the largest study to date, using a dataset comprising 10,737 cases of leukaemia registered with the National Registry of Childhood Tumours between 1969 and 1993. The study found that SES (as measured using the Carstairs Index[133]) is strongly associated with leukaemia incidence with the highest incidence rates in the second highest SES group very closely followed by the incidence in the highest SES group. Data from the same source was also published in 2005[134] and gives a very similar result for registrations from 1962 to 1995 (see graph). These studies did not involve any participation.

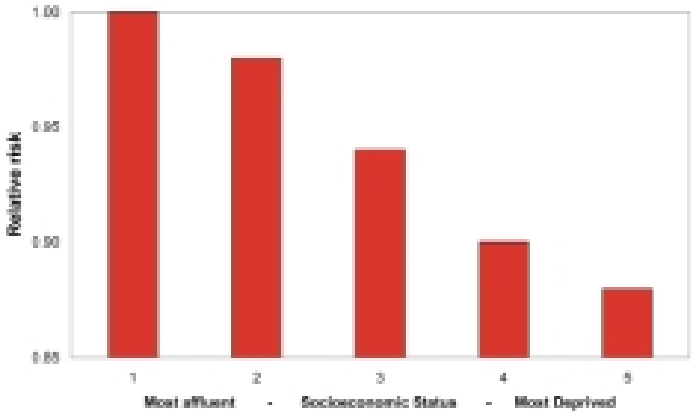
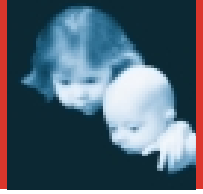


Figure 13. Socioeconomic status and the risk of developing childhood leukaemia, CCRG data 1962-1995 from Draper *et al*[134]



Similarly, Borugian *et al*'s large Canadian-based study showed a positive association between childhood leukaemia and SES. Involving 5,240 children (0-19 years) diagnosed with leukaemia between 1985 and 2001, it showed that the rate ratio of children who lived in the lowest economic quintile neighbourhoods at the time of diagnosis developing ALL compared with the richest neighbourhoods was 0.86 (95% CI = 0.78-0.95) [129]. This study did not involve any participation, nor did it need to use controls.

However, the recent UKCCS study comes to a different conclusion [135]. 4,430 children (0-14 years) newly diagnosed with cancer (of which 1,578 were ALL) in the UK between 1991 and 1996 took part in the study. The authors state that no differences in area-based measures of deprivation or father's occupation were observed between cases and controls at time of birth or diagnosis for ALL. They conclude that "the comprehensive nature of the data, coupled with complete case-ascertainment and representative population-based controls suggests that SES in the UK is not a determinant of ALL in children", and argue that previously reported effects may be artefactual.

The recently published discussions between Borugian and Smith [136, 137] explore the differences in results reported by these two groups. Borugian points out that their results are actually rather similar in that the Canadian study, using 4,024 children with ALL and comparing the poorest of the five categories with the richest, found a rate ratio of 0.86 as mentioned above, while the UKCCS study, using 1578 ALL cases, found an odds ratio of 0.90 (95% CI = 0.75-1.07). Some of the differences in interpretation may therefore be related to resolving power and opinions regarding levels of statistical significance.

In conclusion, older epidemiological studies have shown a clear protective association with social deprivation but later studies do not always confirm this. Studies that do not require participation consistently find a **positive** association between childhood leukaemia and SES and that case-control studies that involve participation tend not find this association. However, this association does not necessarily support an infectious aetiology for childhood leukaemia. SES could also reflect different exposure to other potential carcinogens for example, chemical exposure in diet or environment or exposure to non-ionising radiation.

2.2.2.4 Breast-feeding

Breast-feeding has a protective effect against infection in infants by providing passive immunity from the mother. Breast milk is also anti-inflammatory and immunomodulatory – a major positive influence over the infant's developing immune system (reviewed [138]). There is evidence that breast-feeding is associated with protection against childhood leukaemia and, although not all studies agree, only two studies have shown an increased risk. A point of caution regarding studies about the impact of breast-feeding is that there is a well-documented difference between breast feeding rates according to the mother's socio-economic status, and as discussed previously, case-control studies consistently have controls of a higher socio-economic status thus causing potential for confounding in the results [51]. The data is reviewed in the following studies: [139-142]. Only one of these is discussed here as the studies on the effect of breast-feeding are relatively consistent.

Kwan *et al* (2004) reviewed the published evidence from 14 studies for an association between breast-feeding and childhood leukaemia risk. They observed a significant, **protective** effect of long-term breast-feeding on risk of both ALL (OR = 0.76, 0.68 - 0.84) and AML (OR = 0.85, 0.73 - 0.98). Short-term breast-feeding was similarly protective for ALL and AML. Results for studies that adjusted and did not adjust for socio-economic status were not significantly different from the results for the 14 studies combined. This meta-analysis of epidemiological studies showed that both short-term and long-term breast-feeding is associated with a reduced risk of childhood ALL and AML [139].

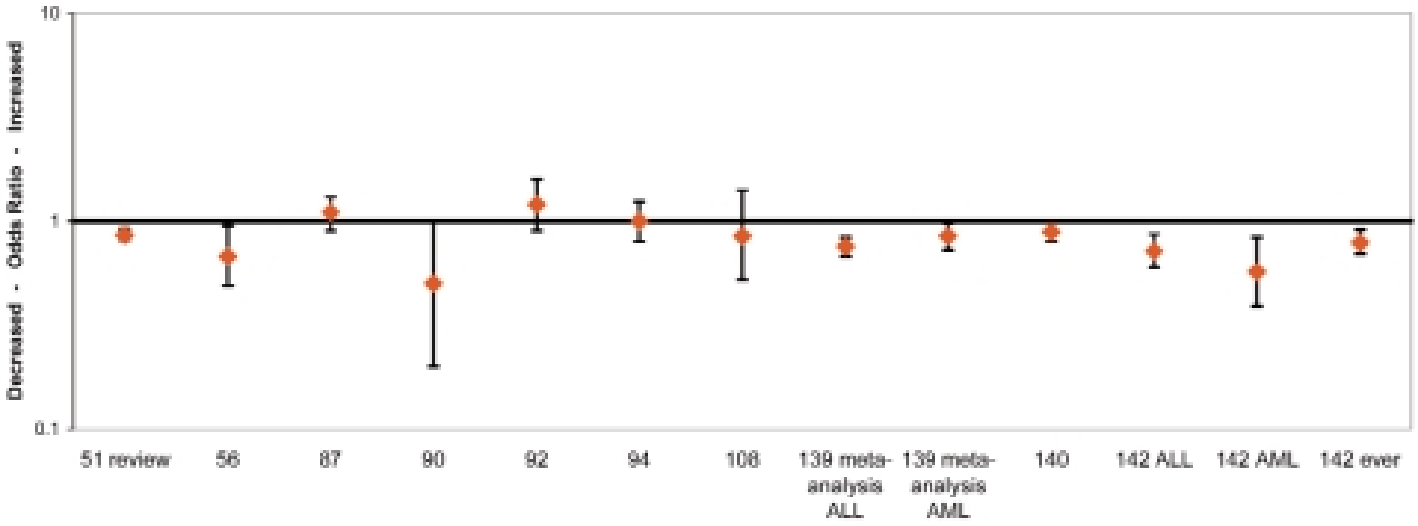


Figure 14. Effect of breast-feeding on childhood leukaemia risk

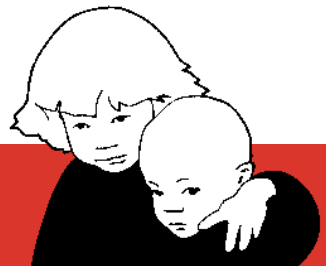


Table 18. Epidemiological evidence for breast-feeding reducing the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Greece (Petridou *et al.*, 1997) 108	Case-control	1993-1994	153 CL cases	Weak non-significant inverse association with breast-feeding	OR = 0.85 (0.52-1.41)
Children's Cancer Group (Shu *et al.*, 1999) 142	Case-control	1989-1993	1744 ALL cases, 456 AML cases	(1.) Ever having been breast-fed is protective against childhood leukaemia, breast-feeding for 6 months strengthens the effect for both (2.) ALL and (3.) AML	1. OR = 0.79 (0.70-0.91) 2. OR = 0.72 (0.60-0.87) 3. OR = 0.57 (0.39-0.84)
Canada (Infante-Rivard *et al.*, 2000) 56	Case-control	1980-1995	491 ALL cases	Protective effect of any breast-feeding for ALL	OR = 0.68 (0.49-0.95)
UK Childhood Cancer Study (UKCCS, 2001) 140	Case-control	1991-1996	1401 ALL cases, 214 AML cases, 22 other leukaemia cases	Breast-feeding decreases risk of childhood leukaemia	OR = 0.89 (0.80-1.00)
United Arab Emirates (Bener *et al.*, 2001) 124	Case-control	1983-1997	117 ALL, HL and NHL cases	**Shorter** duration of breast-feeding has increased risk of ALL (< 6 months cf > 6 months)	OR = 2.47 (1.17-5.25)
France (Perrillat *et al.*, 2002a) 90	Case-control	1995-1999	240 ALL cases, 40 AML cases	Breast-feeding for 6 months or more is protective for (1.) ALL, but not statistically significant for (2.) AML	1. OR = 0.5 (0.2-1.1) 2. OR = 0.6 (0.1-2.9)
Meta-analysis (Kwan *et al.*, 2004b) 139	Meta-analysis of 14 reports	N/A	N/A	Protective effect of breast-feeding shown for (1.) ALL and (2.) AML	1. OR = 0.76 (0.68-0.84) 2. OR = 0.85 (0.73-0.98)
Review (McNally & Eden, 2004) 51	Review of literature reports	N/A	N/A	Breast-feeding is protective against childhood leukaemia	OR = 0.86 (0.81-0.92)

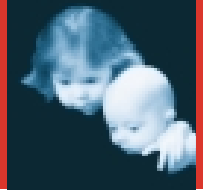
Table 19. Epidemiological evidence for breast-feeding having no effect on the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Inter Regional Epidemiological Study of Childhood Cancer (Hartley *et al.*, 1988) 94	Case-control	1980-1983	148 ALL cases, 23 other leukaemia cases	No significant association of childhood leukaemia with breast-feeding	RR = 1.00 (0.80-1.24)
Germany (Schuz *et al.*, 1999) 92	Case-control	1992-1997	1184 CL cases	Short duration of breast-feeding has no impact on risk of acute leukaemia (1 month cf > 6 months)	OR = 1.2 (0.9-1.6)
France (Jourdan-Da Silva *et al.*, 2004) 87	Case-control	1995-1998	473 CL cases	No association of childhood leukaemia with breast-feeding, irrespective of duration (ever cf never)	OR = 1.1 (0.9-1.5)

Table 20. Epidemiological evidence for breast-feeding increasing the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Germany (Kaatsch *et al.*, 1996) 143	Case-control	1988-1993		Children with leukaemia were more frequently breast-fed	

It appears that breast-feeding **protects** infants against infection and is also associated with protection against ALL. Although not all studies agree, only two studies have shown an increased risk. For example, prior to the introduction of the Hib vaccine in Sweden Silfverdal *et al* [144] found that breast-feeding was significantly protective against Hib, with a dose-response relationship for the number of weeks of exclusive breast-feeding. Whilst this appears contrary to the hypothesis that infection has a protective effect, breast-feeding stimulates the infant's immune system in a similar way to early infection and vaccination and potentially provides a protective effect against childhood leukaemia.



2.2.2.5 Seasonal variation

Seasonal variation in the timing of birth or diagnosis may be a proxy for infectious exposure since many infectious diseases have characteristic seasonal onsets. If infections are involved with childhood leukaemia, then it reasonable to expect that there may be a seasonal pattern at birth and/or diagnosis. Despite a considerable amount of work in this area there is no consistent evidence for seasonal variation with childhood leukaemia.

The authors of the most recent paper[145] estimated the dates of ALL peak diagnosis over a range of geographic locations including Singapore (1968-1999), Hawaii and mainland United States (1973-1999), and western Sweden (1977-1994). Despite the wide range of geographic localities studied, the authors only found evidence for seasonality in western Sweden where a peak in early January was observed, most strongly for males and those less than 20 years of age. They concluded that there was little evidence for seasonality in most of the populations studied.

The largest study to date by Higgins *et al* (2001) used population-based data from the National Registry of Childhood Tumours of 15,835 cases of childhood leukaemia born and diagnosed in the UK between1953-95. They found no evidence of seasonality in either month of birth or month of diagnosis overall or in any subgroups by age, sex, histology or immunophenotype. They did, however, find a significant February peak in month of birth for cases born before 1960 and a significant August peak in month of diagnosis for those diagnosed before 1962[146].

Six studies found seasonal variation in month of diagnosis or first reported symptom;[147-152] one did not[153]. Three studies found seasonal variation in month of birth;[74, 152, 154] two did not[153, 155].

Table 21. Epidemiological evidence for seasonality in month of diagnosis or first symptom of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
U.S.A. (Harris *et al.*, 1987) 147	Time trend in incidence of leukaemia matched to seasonality	1973-1980		Trimodal increases in ALL risk observed (April, August, December for latitudes above 40°N, February, July, October for other locations)	
East Anglia (Badrinath *et al.*, 1997)148	(As above)	1971-1994	271 ALL cases, 67 AML cases	Observed 40% summer excess of ALL	May-October 158 vs November-April 113 1.40 (1.16-1.64)
Manchester (Westerbeek *et al.*, 1998) 149	(As above)	1954-1996	1070 ALL cases, 244 AML cases	Significant seasonal variation in date of first symptom of cALL, peak in November; significant summer excess in date of diagnosis of total ALL cases	
Children's Cancer Group (Ross *et al.*, 1999) 151	(As above)	1989-1991	5532 ALL cases, 1153 AML cases	Seasonal variation for ALL with a peak in summer, strengthened if the analysis is confined to northern USA (>40°N). Variation in AML not statistically significant but suggestive of a summer peak	
United Kingdom (Higgins *et al.*, 2001) 146	(As above)	1953-1995	15,835 CL cases	August peak in month of diagnosis for cases diagnosed prior to 1962	Amplitude = 10.8%
Denmark (Sorensen *et al.*, 2001) 152	(As above)	1950-1994	458 ALL cases	Peak in October for ALL diagnosis month in under-4's	Peak to trough ratio = 1.6 (1.2-2.0)
Iran (Karimi & Yarmohammadi, 2003) 150	(As above)	1996-2000	211 ALL cases, 63 AML cases, 5 CML cases	Peak in month of first symptom for ALL in October, peak in month of diagnosis for ALL in November	ALL winter:summer ratio = 1.157 ($p < 0.001$)
Gao (Gao *et al.*, 2005) 145	(As above)	1968-1999	10,146 ALL cases	Peak in month of diagnosis in western Sweden in January for cases < 20 years of age, and in December for males of all ages	

Table 22. Epidemiological evidence for seasonality in month of birth of children with leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
New York State (Vianna & Polan, 1976) 74	Time trend in incidence of leukaemia matched to seasonality	1950-1970	777 CL cases	Seasonal variation in month of birth for lymphatic leukaemia cases was observed: under 2 age group (Jan-Apr), 2-3 age group (May-Oct) and 4-9 age group (Aug-Dec)	
Northern England (Feltbower *et al.*, 2001) 154	(As above)	1950-1995	4427 childhood cancer cases	Peak in month of birth for ALL diagnosed between 1-6 years: Cumbria peak in Feb-Mar, Yorkshire peak in September	
United Kingdom (Higgins *et al.*, 2001) 146	(As above)	1953-1995	15,835 CL cases	February peak in month of birth for cases diagnosed prior to 1960	Amplitude = 8.1%
Denmark (Sorensen *et al.*, 2001) 152	(As above)	1950-1994	458 ALL cases	Peak in birth month in April for ALL in under-4's	Peak to trough ratio = 1.4 (1.0-2.0)

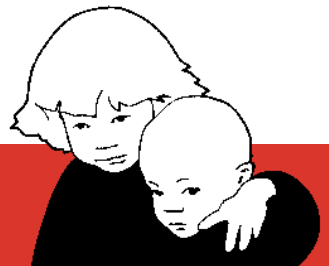


Table 23. Epidemiological evidence for No seasonality with childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Dutch Childhood Leukaemia Study Group (van Steensel-Moll *et al.*, 1983) 153	Time-space clustering of leukaemia cases	1973-1980	293 CL cases	No seasonal influence on moths of birth of diagnosis found	
Hungary (Kajtar *et al.*, 2003) 155	Time trend in incidence of leukaemia matched to seasonality	1988-2000	814 ALL cases	No association between month of birth and ALL	

Although the data provide modest support for seasonal variation in ALL, there is very little agreement in terms of the season of peak risk. The months of first reported symptom and diagnosis range from across the entire year, with approximately half reports quoting a summer peak and half a winter peak. Risk by birth month seems to be concentrated around spring and summer, but there are only three studies reported, making it difficult to draw a definite conclusion. In addition, some of the studies divide the year into very large units, e.g. summer versus winter and so any association of childhood leukaemia with a specific time would be masked, as would any small effects.

2.2.2.6 Allergy

If an abnormal immune response to an infection is involved in the development of childhood leukaemia, then there may be associations to be found with other abnormal immune responses, for example allergy or atopy. Allergic responses happen when the immune system mistakenly responds to something innocuous, like grass pollen, as if it were a foreign invading pathogen. Atopic individuals have a tendency to develop a wide range of disorders like eczema, allergies and asthma which are caused by a dysfunctional immune system.

The prevalence of allergies is steadily increasing in the developed world and appears to be associated with modern lifestyle. It is hypothesised that modern living standards and hygienic conditions are correlated with an increased risk for the development of an allergic disease. This so-called "hygiene hypothesis" (originally described by Strachan, 1989 [42] and recently reviewed by von Mutius, 2007 [43]) states that due to reduced exposure to microbial components, the proposed allergy-preventing potential of these factors is no longer present in sufficient qualities and/or quantities, which leads to an imbalance of the immune system with a predisposition to the development of allergic disorders. This is very similar to Greaves' delayed exposure hypothesis so it is reasonable to look for a link.

Most epidemiological studies into allergy and childhood leukaemia have shown an inverse association, in that children who have allergic conditions are less likely to develop leukaemia. These studies are summarised below.

The most recently published UKCCS study obtained information about the clinically diagnosed allergies of 839 cases and 1,337 controls from primary care records [53]. More than a third of subjects had at least one allergy diagnosed prior to leukaemia diagnosis or pseudo-diagnosis, and the study found that histories of eczema or hayfever were associated with significant **reduction** in risk for total ALL and cALL. For eczema this was OR=0.70 (95% CI 0.51-0.97) and OR=0.68 (95% CI 0.48-0.98) respectively, and for hayfever the risk was OR=0.47 (95% CI 0.26-0.85) and 0.62 (0.33-1.16) respectively. No such patterns of inverse association were seen either for the combination of asthma and ALL, or for the combination of any allergy and AML.

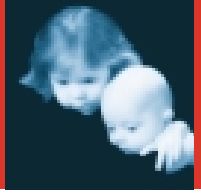
Rosenbaum *et al* assessed allergy histories in ALL using cases (n = 255) diagnosed between 1980 and 1991 at one of four referral centres in a 31-county area of New York State. Data were collected by mailed questionnaire, completed by case and control parents in 1995. For allergy histories before the age at leukaemia diagnosis for cases and an equivalent age for controls they found that a positive history of any allergy had an inverse association with ALL (OR=0.58, 95% CI 0.38-0.88) [156].

A French case-control study, conducted from 1995 to 1998 and including 408 cases of ALL and 65 cases of AML, used self-administered questionnaires to collect data on the medical history of the child and his/her environment. A history of asthma was associated with a decreased risk of ALL (OR=0.5; 95% CI 0.3-0.90) [87].

Schuz and his co-workers undertook a German case-control study comprising more than 1000 children with acute leukaemia. Information was gathered by both questionnaire and interview. A decreased risk of acute leukaemia with a history of any allergy was seen, giving an odds ratio of 0.6 (0.5–0.8) [92].

This work was confirmed and extended by the group in a later population-based case-control study with self-reported information on allergies of the children and their first-degree relatives. The study included a total of 1,130 cases of ALL, 164 cases of AML and 2,957 controls. The authors found that hay fever, neurodermatitis and contact eczema are underrepresented within the group of children with ALL, with odds ratios of 0.45 (95% CI 0.31-0.66) for hay fever, of 0.49 (95% CI 0.34-0.71) for neurodermatitis, and of 0.62 (95% CI 0.39-0.99) for eczema, respectively. The effect of these atopic diseases was stronger than that of other allergies (OR 0.89, 95% CI 0.66-1.21) [157].

Wen *et al* compared the histories of selected allergic disorders (asthma, hay fever, food or drug allergies, eczema, and hives) obtained by interview of 1842 cases of ALL with those of 1986 individually matched controls. The combined history of any one or more of the five allergic disorders evaluated was associated with a significant reduced risk of ALL (adjusted OR = 0.7, 95% CI 0.6-0.8), as were histories of four specific allergic disorders (asthma, hay fever, food or drug allergies, and eczema). The combined history of any one or more of the five allergic disorders among any of the siblings of the study subjects also revealed a significant inverse association (adjusted OR = 0.9, 95% CI 0.8-1.0) [158].



The risk profile of childhood leukaemia in Greece was studied through a case-control investigation that included all 153 cases diagnosed throughout the country during 1993 and 1994, and two hospital controls for every case. Inverse associations were observed with previous hospitalisation for allergic diseases [108].

A study was performed in Hokkaido, Japan, with 63 cases diagnosed between 1981 and 1987 in the Hokkaido Prefecture. The study found that atopy was significantly less likely to be associated with non-T cell acute lymphoblastic leukaemia, with an odds ratio of 0.26 (0.11-0.59) [107].

There are two reports which contradict the above-described findings with a history of allergy associated with an increased risk of childhood leukaemia. In the most recent of these Spector *et al* abstracted data on five allergic conditions from the medical records of 180 cases of childhood acute lymphoblastic leukaemia (ALL) and 718 matched controls in the Western United States. Allergies were divided into late and early diagnoses (those made within the year before the matched case's ALL diagnosis and those made earlier, respectively). Atopy or hives was significantly associated with ALL for both the early and late diagnoses (early and late respectively: OR=2.20; 95% CI: 1.16-4.16, OR=3.78; 95% CI: 1.00-14.29) and late diagnosis of asthma was also associated with a significant increase in risk (OR=3.10; 95% CI: 1.39-6.95)[159].

The US Children's Cancer Group reported the results of an analysis of data obtained from parents of children with ALL (and a control group of children without cancer), linked to a clinical database. They found that children with allergies had an elevated risk of developing a rare sub-type of ALL (pre-B ALL). However, the analysis had very few cases of pre-B ALL (38) so the result was not statistically significant [60].

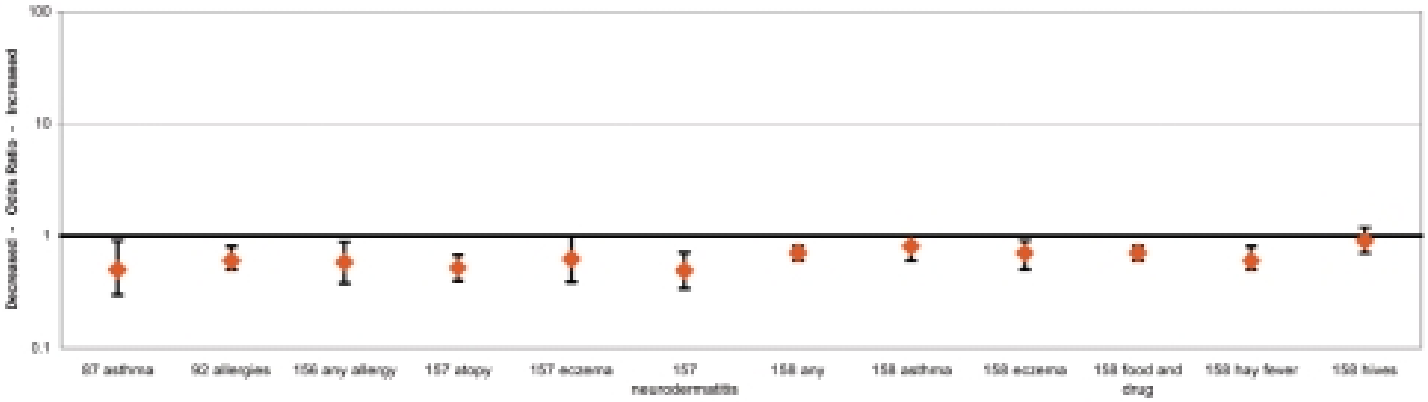


Figure 15. Allergy and the risk of developing childhood leukaemia (data from interviews)

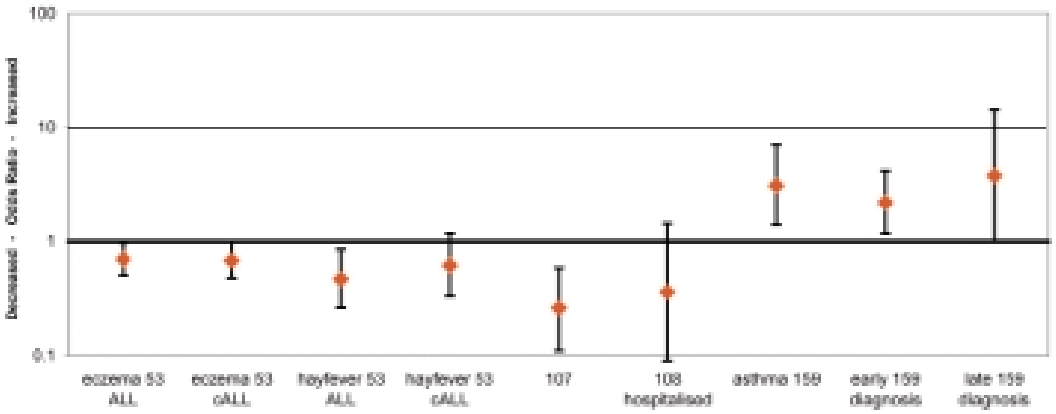


Figure 16. Allergy and the risk of developing childhood leukaemia (data from medical notes)

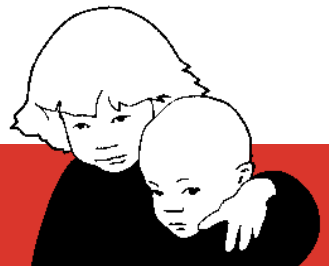


Table 24. Epidemiological evidence for history of allergy reducing the risk of childhood leukaemia

Study and reference	Study design	Time period	No of cases	Results	Figures given
Japan (Nishi & Miyake, 1989) 107	Case-control	1981-1987	63 ALL cases	History of atopy inversely associated with ALL	OR = 0.26 (0.11-0.59)
Greece (Petridou *et al.*, 1997) 108	Case-control	1993-1994	153 CL cases	Indicative inverse association of childhood leukaemia with previous hospitalisation with allergic diseases	OR = 0.36 (0.09-1.43)
Germany (Schuz *et al.*, 1999) 92	Case-control	1992-1997	1184 CL cases	Inverse association of allergy with acute leukaemia	OR = 0.6 (0.5-0.8)
Children's Cancer Group (Wen *et al.*, 2000) 158	Case-control	1989-1993	1842 ALL cases	Inverse association of allergy (1. asthma; 2. hay fever; 3. food and drug; 4. eczema; 5. hives; 6. any of the above) with ALL	1. OR = 0.8 (0.6-1.0) 2. OR = 0.6 (0.5-0.8) 3. OR = 0.7 (0.6-0.8) 4. OR = 0.7 (0.5-0.9) 5. OR = 0.9 (0.7-1.2) 6. OR = 0.7 (0.6-0.8)
Germany (Schuz *et al.*, 2003) 157	Case-control	1980-1994	1130 ALL cases, 164 AML cases	Atopic diseases (1. hay fever; 2. neurodermatitis; 3. eczema) more strongly inversely associated with ALL than (4.) other allergies	1. OR = 0.45 (0.31-0.66) 2. OR = 0.49 (0.34-0.71) 3. OR = 0.62 (0.39-0.99) 4. OR = 0.89 (0.66-1.21)
France (Jourdan-Da Silva *et al.*, 2004) 87	Case-control	1995-1998	473 CL cases	History of asthma associated with a decreased risk of ALL	OR = 0.5 (0.3-0.9)
New York State (Rosenbaum *et al.*, 2005) 156	Case-control	1980-1991	255 ALL cases	History of any allergy is protective against ALL	OR = 0.58 (0.38-0.88)
UK Childhood Cancer Study (Hughes *et al.*, 2007) 53	Case-control	1991-1996	720 ALL cases, 101 AML cases, 18 other CL cases	Eczema associated with decrease in risk of (1.) ALL and (2.) cALL. Hay fever also associated with decrease in risk of (3.) ALL and (4.) cALL	1. OR = 0.70 (0.51-0.97) 2. OR = 0.68 (0.48-0.98) 3. OR = 0.47 (0.26-0.85) 4. OR = 0.62 (0.33-1.16)

Table 25. Epidemiological evidence for history of allergy increasing the risk of childhood leukaemia

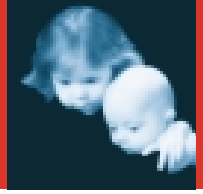
Study and reference	Study design	Time period	No of cases	Results	Figures given
Children's Cancer Group (Buckley *et al.*, 1994) 60	Case-control		547 ALL cases (including 38 pre-B ALL cases)	Children with allergies have an elevated risk of developing pre-B ALL	
Western U.S.A. (Spector *et al.*, 2004) 159	Case-control	1985-1999	180 ALL cases	Late diagnosis (within year before ALL diagnosis) of (1.) atopy or hives and (2.) asthma; and early diagnosis (prior to that year) of (3.) atopy or hives increased risk of ALL	1. OR = 2.20 (1.16-4.16) 2. OR = 3.10 (1.39-6.95) 3. OR = 3.78 (1.00-14.29)

Despite the final two reports discussed, the great majority of the studies indicate that there is a reciprocal association between the risk of developing childhood ALL and having a history of allergy and atopy. Atopic individuals have a tendency to develop a wide range of disorders like eczema, allergies and asthma which are caused by a dysfunctional immune system but are less likely to develop leukaemia. It is suggested that lack of exposure to common pathogens in early infancy can lead a dysfunctional immune system. It is possible that allergy/atopy and childhood ALL are divergent pathological responses to "delayed" common infections and that the response is dependent upon genetic background. Some possible variations in the genetic background for immune responses are explored in the following section.

2.2.2.7 The genetics of immune response to infection

Our ability to fight infections is determined, in part, by our genes. There is a group of genes that code for molecules on our lymphocytes (known as HLA molecules) and determine how our lymphocytes respond to invading pathogens. Different people have different HLA molecules. Therefore, they will respond differently to infections. In a population, there are many hundreds of different types of HLA molecules. This has had a survival advantage with no one pathogen being able to kill the entire human population. If the connection between infection and childhood leukaemia represents an underlying feature of the immune system, there should be some sort of association with particular HLA molecules.

Dorak *et al* considered the association of one HLA molecule (HLA-DR) in childhood ALL in 114 patients from a single centre and 325 local newborn controls. He found that one particular type of HLA molecule (HLA-DRB4*01) was **more prevalent** in patients (21.1% v 8.3%; OR = 2.9, P =.0005) and was **even more prevalent** in male ALL patients (32.8% v 4. 0%; OR = 11.7, 95% CI = 4.9 - 28.0). This difference strongly suggests that this particular HLA molecule is one of the genetic risk factors for childhood ALL [160].



The same group went on to investigate 114 high-risk or relapsed patients with childhood ALL and 118 healthy controls. A different HLA molecule (HLA-DRB3 (DR52)) was **underrepresented** in patients most significantly for HLA-DRB1*12 (P=0.0007) and HLA-DRB1*13 (P=0.0001). They confirmed their previous results that HLA-DRB4 was **more prevalent** in males with childhood ALL (67.4%) compared to controls (42.1%, P=0.003) and female patients (35.7%, P=0.004). Besides being a general marker for increased susceptibility to childhood ALL in males, HLA-DRB4 was over-represented in high-risk patients [161].

Using the UKCCS tissue bank, Taylor *et al* compared the frequency of children with leukaemia (n = 982) with another HLA molecule (HLA-DPB1) and two groups of non-leukaemic children, children with solid tumours excluding lymphomas (n = 409) and normal infants (n = 864). **Significantly** more children with cALL and T-ALL had a particular type of HLA molecule (DPB1*0201) as compared with children with solid tumours [odds ratio (OR), 95% confidence interval (CI) for cALL: 1.76, 1.20-2.56; T-ALL: 1.93, 1.01-3.80] and normal infants (OR, 95% CI for cALL: 1.83, 1.34-2.48; T-ALL: 2.00, 1.10-3.82) [162].

The same group looked at ALL cases (n=776) with the chromosomal changes of either the TEL-AML1 rearrangement (approximately 25%), or too many chromosomes (high hyperdiploid karyotype 30%) in comparison with newborn controls (n = 864). One particular group of HLA molecules (HLA-DPB1) conferred **significant protection** against TEL-AML1+ ALL (odds ratio (OR), 95% confidence interval (95% CI): 0.42, 0.22-0.81; $p < 0.005$) and high hyperdyploid karyotype ALL (OR; 95% CI: 0.44, 0.30-0.65; $p < 0.0001$). These negative associations were almost entirely due to a single HLA molecule (DPB1*0101)[163]. These results suggest that this particular HLA molecule may protect against the development of the major sub-types of childhood leukaemia. This could be as the result of an improved immune response to leukaemia-associated proteins. Identifying this protein could have important implications for the design of prophylactic vaccines.

Later work reported by the same group attempted to explain why this particular HLA molecule, (DPB1*0101) might have this significant protective effect [163]. A leukaemic cell with the TEL-AML1 chromosomal rearrangement produces the TEL-AML1 fusion protein. This protein is not normally made in the body and may stimulate an immune response which would be useful because it could kill the leukaemia. This could be as the result of an improved immune response to leukaemia-associated proteins. Identifying this protein could have important implications for the design of prophylactic vaccines.

In conclusion, there appears to be a reasonable body of evidence that specific HLA molecules are associated with childhood leukaemia, resulting in either increased risk or a protective effect, depending on which particular HLA molecule is investigated. The identification of a significantly protective HLA molecule (HLA-DBP1*0101), if confirmed by independent research, offers a potential starting point in the design of a vaccine for childhood leukaemia. In particular, the recent proposal by Taylor *et al*, described above, of a biological mechanism for this HLA molecule interacting with a genetic mutation common to ALL cases (the TEL-AML1 fusion) may lead to a vaccine preventing the emergence of pre-leukaemic cells.

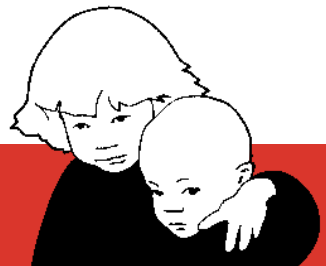
As discussed earlier in this review, there is some evidence that adenovirus C and EBV infections during pregnancy are positively associated with an increased risk of developing ALL. Adenovirus is known to infect cells that can become leukaemic (B lymphocytes), is very good at evading an immune response [164-167] and parts of it look very similar to an HLA molecule (HLA-DR53)[168]. Dorak proposes that an interaction between this HLA molecule and specific adenovirus types could stimulate pre-leukaemic cells to overt leukaemia [85]. This could also be happening with EBV. B lymphocytes are a major target for EBV infection [164, 167, 169] and parts of EBV also look very similar to the same HLA molecule (HLA-DR53) [168]. This proposal provides a mechanism linking infection, the immune system, and childhood ALL.

2.2.2.8 Conclusion - What is the weight of evidence for an association between childhood leukaemia risk and proxy measures of infections in early life?

Several possible proxies for exposure to infection have been studied to determine what, if any, association they might have with childhood leukaemia. Some of these proxies have shown a fairly strong association with leukaemia, such as day care, breast-feeding socio-economic status, and allergy. The results of others are suggestive of an association but require further work to confirm whether or not it is an artefact of the study designs. There are also two proxies commonly studied for which there is very little consensus in their association with childhood leukaemia: birth order and seasonal variation in the date of birth, first symptom, or diagnosis.

The problem common to all of these proxies is that while they may be individually associated with both infection and childhood leukaemia, it is not necessarily true that infection is thereby associated with leukaemia. There may be alternative explanations for these associations with leukaemia, for instance variations in nutrition or exposure to environmental carcinogens.

The study of the genetic components of our immune system suggests that infection is involved in the aetiology of childhood leukaemia. The discovery that different types of HLA molecules are associated with either an increased risk, or protection against, ALL suggests a biological mechanism for the association between infection and leukaemia.



2.3 Childhood leukaemia clusters and population mixing

One of the features of childhood leukaemia is a tendency to cluster – as defined as an excess number of cases found within a defined area compared with general population risk. This is important because a cluster of any disease is often the starting point for finding a cause - the earliest example being John Snow demonstrating that the water pump in Broad Street in London was associated with the Cholera Outbreak of 1854. Removing the pump handle resulted in no more cases of Cholera in that area.

A cluster of childhood leukaemia was first recorded in Niles, Chicago, Illinois in 1957 to 1960. Eight cases of childhood leukaemia occurred during this period in a single small area. All the cases and/or their older siblings attended the same school. A 'rheumatic-like' illness was recorded at diagnosis. The authors drew the conclusion that the leukaemias were most likely a consequence of infection, possibly tied in with the coincident rapid population expansion within the parish [170].

Many other clusters have since been reported. The most recent is in the small isolated rural town of Fallon, Nevada, where 14 cases of childhood leukaemia were diagnosed between 1999 and 2003. The probability of a cluster of this size occurring in the United States by chance has been calculated by Steinmaus *et al* as being once every 22,000 years [171].

There now seems little doubt that clusters of childhood leukaemia do exist (most notably the childhood aged 1-4 peak of cALL) and that they are not simply due to chance. The most recent Committee on Medical Aspects of Radiation in the Environment (COMARE) 11th report used a dataset comprising 10,737 cases of leukaemia registered with the National Registry of Childhood Tumours at the Childhood Cancer Research Group in Oxford between 1969 and 1993 [132]. They conclude that, after looking for geographical (**space** only) clusters over the 25-year period, "there is some good evidence for weak case aggregation of acute lymphoblastic leukaemia. The term weak is used because the average numbers of cases in each ward is low, but the results reinforce the concepts that case occurrence is not entirely random." Cases occurring within a few years and a few kilometres of each other (**space-time**) show clear clustering for acute lymphoblastic leukaemia. When the results of the two methods are compared, both methods highlight the fact that acute lymphoblastic leukaemia exhibits clustering.

The question, therefore, is what is causing this clustering?

When clusters of childhood leukaemia are reported, initial attention turns to environmental exposures, such as radiation exposures in the Sellafield and Dounreay clusters and leakage of jet fuel in the Fallon cluster cluster (as well as the very powerful LORAN navigation transmitter, aircraft radar, etc.). Proponents of the infectious aetiologies of childhood leukaemia propose that such clusters are actually the result of unusual patterns of population mixing and also that areas of high population density will have a higher level of infections and thus stronger and more varied herd immunity. We will examine the evidence supporting these hypotheses.

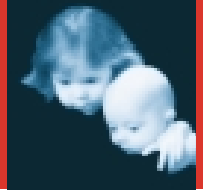
2.3.1 Population mixing

In 1983 a Yorkshire Television programme (Windscale: the Nuclear Laundry) exposed an apparent increase of leukaemia cases near the nuclear reprocessing plant in Sellafield. The presence of the cluster was confirmed in the Black report [172], but it also concluded that the estimated radiation dose from the Sellafield discharges and other sources received by the local population could not account for the observed increased leukaemia incidence. The first report of the Committee on the Medical Aspects of Radiation in the Environment (COMARE [173]) confirmed the cluster round Windscale (Sellafield) [174] and found another around Dounreay [175].

In 1987, Doll [176] looked at published levels of radioactive discharge and concluded that the levels were insufficient to cause leukaemia, which was supported by the second COMARE report [177]. Doll suggested that there may be other explanations. This argument does, however, seem somewhat counter-intuitive, radioactivity being the only established cause of childhood leukaemia from studies of the atom bomb survivors [178] and diagnostic X-rays [179], as reviewed by Wakeford [180]. The very recent publications showing that children who developed leukaemia between 1980 and 2003 were 2.19 times more likely than control children, to live near German nuclear power stations, has now re-opened this debate [238 239]. The assumption remained that the clustering resulted from either environmental radioactive contamination from the plant or mutations inherited from exposed parents working at the plant [181].

Unconvinced by the evidence implicating radiation, Kinlen [45] proposed that the increased incidence of childhood leukaemia and non-Hodgkin lymphoma might have infectious origins that were due to the unusual population mixing that occurred when the isolated communities surrounding the power plants were enlarged to accommodate the influx of workers. He argued that the plants were built in unusually isolated places, "where herd immunity to a postulated widespread virus infection (to which leukaemia is a rare response) would tend to be lower than average", a situation conducive to epidemics. He argued further that in this situation, it will be a specific infection that leads to the development of childhood leukaemia as opposed to a response to a non-specific infection.

To test this hypothesis, Kinlen analysed a parallel 'rural new town', Glenrothes in Scotland, which he argued was the only other rural area that received a similar large influx of people at the same time as Sellafield. He found a transient increase (around threefold) in the incidence rate of childhood leukaemia at the same time as a relatively abrupt influx of substantial numbers of newcomers [45]. A significant increase of leukaemia below age 25 was found (10 observed, expected 3.6), with a greater excess below age 5 (7 observed, expected 1.5). This and subsequent studies throughout the UK provided a basis for suggesting that some childhood leukaemia clusters might be an unusual outcome of a specific common but relatively non-pathological infection arising in individuals who were non-immune and following contact or 'population-mixing' with carriers. Some of these studies are described below.



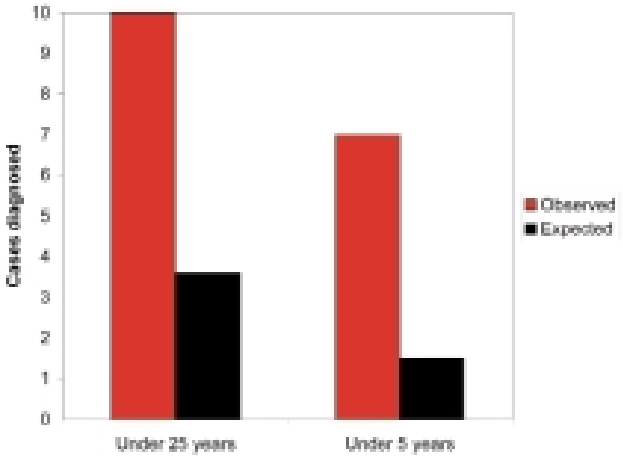


Figure 17. Excess numbers of childhood leukaemia cases in Glenrothes

Looking at evidence from population mixing in British New Towns from 1946-85, using reported death rates from leukaemia and census information, Kinlen found an excess of childhood leukaemia in the 0-4 age group in rural New Towns but not in the overspill towns of London and Glasgow [182]. He argued that this was due to the differing conditions present in the rural versus the overspill New Towns. Rural New Towns had a higher density of children than the areas from which their residents would have originated, whereas overspill towns had a lower density of children than their origins. Residents of the rural towns also had a greater diversity of origin than the overspill towns. Kinlen's argument is that these two factors would encourage a higher level of an underlying infection in the rural towns, leading to the excess cases of childhood leukaemia.

Population mixing is further linked to the cause of childhood leukaemia by Kinlen in several studies. He attributes the clusters around the west Berkshire nuclear installations of Aldermaston and Burghfield to increased contact between adults due to increasing commuting, in that a significant increase in the incidence of childhood leukaemia was observed in the towns which had the highest levels of commuting [183, 184]. After ranking districts according to extent of commuting increase, a significant trend in leukaemia incidence was found at ages 0-14 (P less than 0.05) and a suggestive one at ages 0-4 (P = 0.055). Among ten similar sized groups of county districts ranked by commuting increase, the only significant increases (P less than 0.001) of leukaemia in 1972-85 at ages 0-4 and 0-14 were in the highest tenth for commuting increase. These excesses persisted after excluding Reading, a major part of an area where an excess of leukaemia has been linked to the nearby nuclear establishments at Aldermaston and Burghfield.

Similarly, Kinlen attributes an increase in both childhood leukaemia and polio around Military Encampments during National Service 1950-63, particularly in rural areas to an influx of large numbers of servicemen [185]. In 1950-3 but not subsequently a significant excess of leukaemia in children under 15 was found in the fifth of county groupings with the highest proportions of servicemen. This was due mainly to a significant excess in children under 2 years (and especially in those under 1 year) in rural districts. It was confirmed among the tenth of local authority districts with the highest proportion of servicemen. These rural areas also showed significantly more notifications of, and deaths from, poliomyelitis among children than the rural average.

According to Kinlen, the cluster around the Dounreay nuclear site was due to an influx of North Sea oil workers [186]. A significant excess of leukaemia and non-Hodgkin's lymphoma was found in 1979-83 in the group of rural home areas with the largest proportion of oil workers, following closely on large increases in the workforce. The area near the Dounreay nuclear installation, where an excess of leukaemia is already well known, was within the rural high oil category.

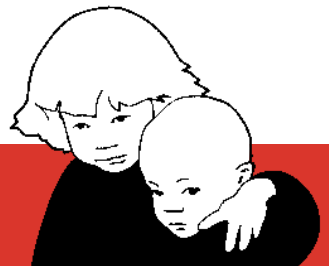
Kinlen further suggests that the wartime evacuation increased the incidence of childhood leukaemia in rural areas [187]. The 476 rural districts of England and Wales were ranked according to the ratio of government evacuees (two thirds of them children) to local children in September 1941. The districts were divided into three categories, each with similar numbers of children in 1947 but with different ratios of evacuees to local children ("low," "intermediate," "high"). There was a 47% excess of leukaemia at ages 0-14 years that occurred in 1945-9 in the rural "high" category for evacuees relative to the "low" category, with a significant trend across the three categories.

The impact of the wartime evacuation is, however, debated by Wolff [188]. He argues that, despite the evacuation of almost half the nation's schoolchildren from cities over the course of four days in September 1939, there was no "sudden increase" in childhood leukaemia mortality in the periods 1940-44 or 1945-49.

However, Wolff looked only at children age 0-4 years, whereas Kinlen studied deaths in children age 0-14 years. When this larger age group is broken down, Kinlen found that the excess of leukaemia was more pronounced in the 5-14 year olds (RR=1.91, 95% CI 1.17-3.45 high evacuee category relative to low). In comparison the result for the younger children was not statistically significant (RR=1.24, 95% CI 0.82-1.91). Thus these two studies may not be incompatible. Also, in the 0-4 year age group in Britain, leukaemia mortality did almost double over that 20 year period [189].

Further studies suggest that high childhood leukaemia mortality in Greece and Italy in the 1950s and 1960s was due to high levels of population movements [190]. Greece and Italy, two countries with unusually high levels of rural migration in the 1950s and 1960s, also had unusually high mortality rates from childhood leukaemia. Greece was most affected proportionally by these population movements and from 1958 to 1972 had the highest recorded childhood leukaemia mortality in the world.

Kinlen found an excess of childhood leukaemia among children living near large construction projects in Britain since 1945, situated more than 20 km from a population centre, involving a



workforce of more than 1000, and built over three or more calendar years [191]. A 37% excess of leukaemia and non-Hodgkin's lymphoma at 0-14 years of age was recorded during construction and the following calendar year. The excesses were greater at times when construction workers and operating staff overlapped (72%), particularly in areas of relatively high social class. For several sites the excesses were similar to or greater than that near the nuclear site of Sellafield (67%).

Kinlen attributes a 3.6 fold increase of childhood leukaemia in Orkney and Shetland during World War II, where local people were outnumbered by servicemen stationed there, to wartime population mixing [192].

During the Second World War, explosives production factories were built and operated at Drigg and Sellafield, and a shell filling factory at Bootle, in west Cumbria, requiring substantial numbers of construction workers to be brought into this remote and isolated area. An excess of leukaemia deaths at ages 1-14 was found during the construction period (observed 3; observed/expected (O/E) 2.2, 95% confidence interval (CI): 0.6, 6.0), which was more marked and statistically significant during the overlap with operations (O 3; O/E 4.5, 95% CI: 1.1, 12.2), especially at ages 1-4 (O 2; O/E 7.1, CI: 1.2, 23.6) [193].

In the Fallon cluster, Kinlen points out that there had recently been a huge increase in military personnel assigned to the Naval Air Station reaching the level of 55,000 in 2000 (local Fallon population 7,536). He argues that this supports his population mixing hypothesis [194] and that this is a unique opportunity to study the role of infection in childhood leukaemia [195].

Other authors have also linked population mixing to the causation of childhood leukaemia. Langford [196] found a significantly increased risk of childhood leukaemia mortality for 0-14 year olds in areas that experienced more than a 50% increase in population over the period 1969 to 1973 (RR 1.408, 95% CI 1.126-1.761). Wartenberg, using US National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) Program, found that increases in rural county population sizes from 1980 to 1989 were associated with an increase in childhood leukaemia - especially in the 0-4 age group [197]. His results are presented below.

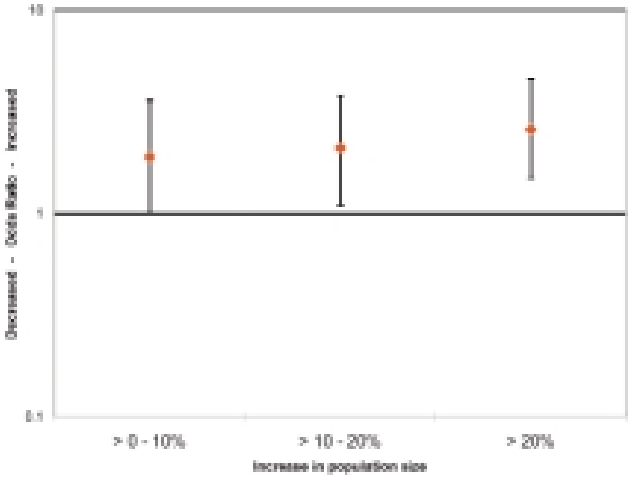


Figure 18. Population increases in rural counties and risk of childhood leukaemia mortality [197]

Dickinson & Parker [198] developed a new statistical model to evaluate the Seascale/Sellafield cluster and concluded that population mixing was a significant risk factor for ALL - especially in young children (i.e. most likely to be cALL), accounting for over 50% cases in Cumbria and most cases in Seascale. In 1999, Sir Richard Doll said, "With Dickson & Parker's paper, the time may now have come when Kinlen's hypothesis of population mixing as a cause of childhood leukaemia may be regarded as established" [199].

But the population mixing hypothesis is far from being generally accepted.

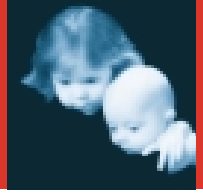
Roman *et al* found an increased number of cases of childhood leukaemia close to Aldermaston and Burghfield [200, 201] and associated these with paternal radioactivity exposure – similar to the ideas originally proposed by Gardner (described later in 2.3.3) around the Sellafield cluster [181, 202]. But Kinlen [184] argued that the data could be reinterpreted and increased the case for population mixing as described above.

In Hong Kong, no increase in risk was seen with increased population mixing [203]. In Yorkshire, Parslow *et al* found that the incidence of all childhood leukaemias was significantly lower in areas of high population mixing (incidence rate ratio (IRR) 0.72, 95% CI 0.54-0.97) and higher in areas of low population mixing (IRR 1.56, 95% CI 0.73-3.34) [204]. The UKCCS investigators [205] analysed their 1991-6 database of 3,838 cases and 7,669 matched controls and found elevated levels of ALL in areas of low diversity of origin i.e. low levels of migration - estimated with census data - the implication being that higher population densities and migrations are protective. They conclude, "This study, and a survey of 17 published reports on population mixing, suggests that a low diversity of migrant backgrounds may be associated with acute lymphoblastic leukemia. These findings do not support the population mixing hypothesis." The findings do, however, support the Greaves delayed infection hypothesis.

However, in the studies described above, they did not look for the effect of population mixing on a previously isolated community as Kinlen has done but instead studied largely urban population mixing. Wartenberg states that "an alternative explanation for the protective effect of high levels of urban population mixing reported by Parslow *et al* is decreased susceptibility of the children in such areas as a result of the greater herd immunity that tends to typify urban areas" [206].

Recently, Louise Parker's group found a significant increase in risk (RR 2.1) in childhood leukaemia in children born in areas and times of higher population mixing in South Hungary [207]. The effect was more pronounced for boys (RR 3.1). They reviewed 13 other studies and concluded that 10 studies agree with their analysis. Taylor [208] responded to this study by reiterating that the UKCCS study [205] showed the opposite but suggested that a consensus needs to be reached on a definitive measure of population mixing, a suggestion with which Parker agreed [209]. The use of such a definitive measure may help to resolve the differences between studies.

There is still, however, some degree of controversy surrounding the use of population mixing as a proxy for infection. The evidence being largely circumstantial and not involving any



formal dosimetry. Authors also disagree on what they mean by population mixing and many of the studies reported here have used different definitions, so the results are not necessarily directly comparable.

A comparison of different measure of population mixing has been undertaken using hospital admission data for infectious cases in Eastern England, the West Midlands and Scotland as a reliable indicator of infection rates [237]. The authors of this study determined that commuting distance had the most consistent association with infectious admissions in children age 0-14 years. In comparison, other common population mixing measures such as migration (including volume, diversity of origins and distance migrated), deprivation, and population density did not show a strong association with hospital admissions. Furthermore, the authors found that commuting distance (and, more weakly, a number of the other measure studied) showed a negative association with infectious admissions. This means that in areas of high population mixing there were lower rates of infection.

In conclusion, there appear to be inconsistent findings in the population mixing evidence. There is an increased incidence of childhood leukaemia after population mixing, in areas that were originally very isolated, especially the cALL childhood peak. In comparison, population mixing in urban areas, where there is already a high level of mixing, results in a decreased rate of leukaemia. These two findings are not necessarily incompatible, and may in fact be seen as complimentary in terms of herd immunity. Isolated areas could have a low level of herd immunity to common infections compared with urban areas and thus it might be expected that responses to infections would differ between these types of areas.

This is supported by a recent population-based study of acute lymphoblastic leukaemia diagnosed among children aged under 15 years in England and Wales during 1986-1995. Stiller *et al* 2008 [240] analysed incidence at ward level from the 1991 census. Incidence at ages 1-4 years was higher in rural wards and increased with the diversity of origin wards from which in-migrants had moved during the year before the census. They found no evidence of association with population mixing for ALL diagnosed at ages 0 or 5-14 years. This apparent specificity to the 1-4 age group suggests that these associations are particularly marked for cALL, with the disease more likely to occur when delayed exposure to infection leads to increased immunological stress, as predicted by Greaves. The association with diversity of incomers, especially in rural areas, is also consistent with the higher incidence of leukaemia predicted by Kinlen, where population mixing results in below average herd immunity to an infectious agent.

However, all these population mixing studies could also reflect changing environmental factors associated with increases in population, such as increasing light at night or air pollution.

The diversity of measures of population mixing and questions regarding its use as a proxy for infection within communities means that much more work is needed in this field before an association between population mixing, infection and childhood leukaemia can be considered firmly established.

2.3.2 Population density

Another approach that has developed out of the population mixing hypothesis is to look at population density, the rationale being that areas of high population density will have greater numbers of infections passed around than more rural areas and that this will affect the rate of leukaemia incidence.

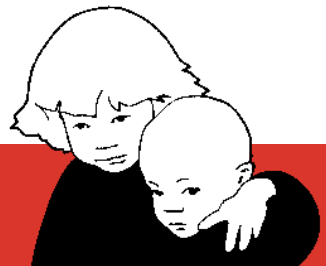
A recent study by McNally used the Manchester Children's Tumour Registry to examine the geographical distribution of the incidence of acute leukaemia and lymphoma [210]. A total of 910 children were included, all of whom had leukaemia or lymphoma, diagnosed 1976-2000. The authors observed an association between acute lymphoblastic leukaemia (ALL) incidence and population density (P = 0.05) such that **higher rates** were seen in more **densely populated** areas.

Several other studies have also found a similar association between high childhood leukaemia incidence and high population density. One example is Muirhead's report, in which, in three metropolitan areas of the USA, a significant **increase** in risk was found with **increasing population density.** Comparing the highest to the lowest population density areas, the authors found a relative risk of 1.4 (95% CI 1.1-2.0) for white children [211]. Similarly, in the UK **high rates** of childhood leukaemia mortality were found in areas of high **population density** [212], and urban areas of Taiwan showed a higher incidence of childhood leukaemia (RR 1.3), especially in the cALL group (RR 1.5)[213]. In Sweden, a **higher risk** of ALL was found in **population centres**[214], and in North West England, a **higher incidence** of ALL was found in areas of g**reater population density** [210].

In contrast, a study of 13,551 cases of childhood leukaemia diagnosed between 1980 and 1989 in 17 countries of Europe and Australia (EUROCLUS), found that childhood leukaemia incidence was **higher in moderately densely populated areas as compared with higher or lower density** [215-217].

The 11th COMARE Report, with 10,737 cases of leukaemia registered with the National Registry of Childhood Tumours at the Childhood Cancer Research Group in Oxford between 1969 and 1993, also studied the impact of population density on the risk of developing childhood leukaemia. They found that incidence **increases** as the population density **decreases,** i.e. areas of low population density have higher rates of childhood leukaemia [132]. There was a significant trend across the quintiles of population density in Scotland, but this trend was not significant in England and Wales. However, narrowing the analysis to ALL specifically strengthens this trend and the effect of population density is strongest when analysing the data for children in 0-4 year age group only. This suggests that the association observed is most likely to be related to cALL and the childhood peak.

Alexander and McKinney's group has also found, however, evidence implicating an association between low population density and childhood leukaemia. Using the Data Collection



Survey, a specialist registry of leukaemias and lymphomas covering about half of England & Wales, and also census information, they found that there was a **higher incidence** of leukaemia in **isolated rural towns** compared to built-up areas [127, 218-221]. They revisited this work in 1996 after the suggestion that the higher incidence could be attributed to higher proportions of households owning cars but found no evidence that the excesses of ALL observed in isolated areas was related to benzene exposure in these children[222]. They concluded, "the previous explanation for the small area variation of childhood ALL in terms of geographical isolation and interpretation in terms of exposure to common infections continues to be justified."

In conclusion, many studies have looked for an association between population density on the risk of developing childhood leukaemia but have not yet reached a consensus. Some report that high population density is associated with increased rates of leukaemia, other studies low and mid-population densities. This may be related to different methodologies used to define and describe population density. It is also not clear that population density is a good proxy for infection as comparisons of hospital admission rates with a variety of population measures showed that population density did not have a strong association with infectious episodes.

2.3.3 Other explanations for clusters

There have been many other suggested explanations for the observed clusters of childhood leukaemia cases. Some of these are discussed below.

In what is referred to as the Gardner hypothesis, parental preconceptional irradiation has been suggested as a potential cause of some of the clusters of childhood leukaemia. Gardner's work on the Sellafield cluster showed a 6.42 relative risk for children of fathers receiving a preconceptional radiation dose of 100 mSv or more [181]. The following year Urquhart [223] did a similar study in Caithness near the Dounreay nuclear power plant and found no association with paternal exposure to ionising radiation before conception. Gardner points out, however, that occupational radiation doses have fallen over the years, and the workers at Dounreay had a lower exposure [202].

The association between parental employment in the nuclear industry and the development of childhood leukaemia was also studied by Roman *et al* in west Berkshire, Basingstoke and North Hampshire [201]. Between 1972 and 1985 they found an excess of childhood leukaemia in children under 5 years living within 10km of the atomic weapons establishments at Aldermaston and Burghfield [200]. In the follow up study the authors found that, of the parents whose exposure to external penetrating ionising radiation was measured, three fathers of cases and two fathers of controls had been monitored prior to the conception of their child (relative risk 9.0, 95% confidence interval 1.0-107.8), but in no case was the exposure greater than 5 mSv.

In 2002, Parker's group [224] re-visited the whole issue of parental exposure, undertaking a birth cohort study that extended the boundaries of Gardner's 1990 work by including all children born to mothers who lived in Cumbria between 1950 and 1991. They found evidence to support the original 1990 observations by Gardner in that the children of radiation workers had an increased risk of developing leukaemia and non-Hodgkin's lymphoma (RR = 1.9, 95% CI 1.0-3.1). Adjusting for population mixing decreased the risk in the village of Seascale but not elsewhere. The authors also observed a dose-response such that the risk increased with the increasing level of the father's preconceptional external irradiation dose.

The hypothesis that paternal pre-conceptional exposures may be a factor in aetiology of childhood leukaemia was recently supported by Pearce *et al* [225]. In their study of paternal occupational exposure in the North of England, they found that exposure to electromagnetic fields resulted in a significant increased risk of ALL for the male children of those fathers (ALL diagnosis under the age of 6 OR=1.81, 95% CI 1.19-2.75). This provides further evidence that some DNA mutations may cross generations.

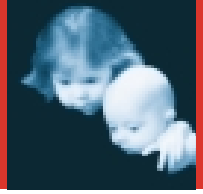
There is also evidence from animal model systems of radiation effects in parents being passed on to offspring. This includes the induction of a significant increase in heritable tumours following the treatment of parent mice with x-rays [226] and the development of a second-generation excess of leukaemia in mice whose sires had been injected with radioactive plutonium [227].

Studies on the radiation effects on a specific DNA sequence (the minisatellite locus CEB1) in children with cancer and their parents compared with controls [228] showed a 50% increase in the inheritable (germ line) mutation rate and that the mutations in children with cancer were paternal in origin. However, a more recent, leukaemia-specific study by Davies *et al* of the same germ line mutation found no significant difference in the mutation rates between leukaemic cases and controls [229]. This suggests that more work needs to be done in this area to determine if inheritable mutations are a plausible cause of childhood leukaemia.

Environmental factors have also been suggested as causes for the clustering of childhood leukaemia cases. An excess of cases around estuaries has been found by both Alexander and Badrinath [218, 230]. Alexander *et al* attribute the estuarine excess to the presence of heavy metals and radio actinides in the water. In comparison, Badrinath *et al* argued that both recreational and occupational activities in the estuaries would increase exposures to resins, solvents, paints and petroleum products. A recent study by Stark *et al*, however, did not find an excess of coastal cases of childhood leukaemia [231] but this was not limited to estuarine areas and covered the Solway Firth coast of Dumfries and the Galloway Health Board area, and so may not be directly comparable.

Knox looked at leukaemia clusters in Britain and concluded that there were indeed clusters, and they were associated with the use of fossil fuels - especially petroleum [232]. This conclusion was challenged by Draper's group [233] who argued that Knox's findings could be "largely or entirely artefactual".

The recent publication of maps of emissions of many different substances by the National Atmospheric Emissions Inventory and "hotspots" for 2001 allowed Knox to revisit the issue of air pollution, combustion process gases and volatile organic



compounds more recently[16, 17]. Air pollution is an intrinsically difficult subject to study using the traditional epidemiological methods as exposure is ubiquitous. As such, Knox used a "case centred" method to evaluate the effect of air pollution on rates of leukaemia incidence. In this method, the case acts as their own control, and their address at birth is compared to their address at death, with the distance from an emission hazard being the particular point of interest. Knox argues that the excesses of outward over inward migrations show an increased prenatal or early infancy risk.

Using this method, Knox determined that there were significant birth proximity relative risks within 1.0 km of hotspots for a variety of airborne pollutants such as carbon monoxide, volatile organic compounds, benzene, and dioxins[16]. He also found excess relative risks within 1.0 km of bus stations, hospitals, heavy transport centres, railways and oil installations[17]. He argues that this increase in risk with birth proximity to pollutants indicates that maternal inhalation of these materials leads to transmission across the placenta to the foetus, raising the risk of developing childhood cancers. If this can be confirmed, it could potentially indicate a role for air pollution as the first hit in the development of childhood leukaemia.

A proposed environmental factor in the aetiology of the Fallon cluster is airborne tungsten and cobalt. Sheppard *et al* used a number of techniques to study the concentration of these metals around Fallon [234-236]. Specifically, by using dendrochemistry (the study of the concentration of elements through time in tree rings), the authors showed that the concentration of tungsten and cobalt had been either high or increasing relative to nearby comparison towns since the early to mid 1990's. This result suggests a temporal correspondence between the increase in the airborne concentration of these metals and the onset of the observed cluster of leukaemia cases. The authors note that although there is no direct evidence linking the two, previous research has shown that combined exposure to these two elements can be carcinogenic to humans.

The recent publications from Germany have re-opened the entire issue of clusters around nuclear power plants[238 239] and the contribution of man-made radiation to the aetiology of childhood leukaemia. A case control study included 593 childhood leukaemia cases under 5 years, (1980 to 2003) and registered at the German childhood cancer registry, and 1,766 matched controls. Residential proximity to the nearest nuclear power plant was determined for each subject individually (with a precision of about 25 m). They found a statistically significant trend for 1/distance, in that cases lived closer to one of Germany's 16 nuclear power plants than the randomly selected controls. They determined a statistically significant odds ratio of 2.19 (lower 95%-CL: 1.51) for residential proximity within 5 km compared to residence outside this area.

2.3.4 Clustering conclusion

Different camps have been emerging within the field of leukaemia clusters:

Kinlen argues that specific infections brought into areas of isolated and low population density (and therefore low herd immunity) cause clusters of childhood leukaemia. This idea is supported by McNally and Parker. Some scientists associated with the UKCCS argue that high density living and high migration are protective, bringing in and maintaining herd immunity and stimulating the immune system. This argument supports Greaves' hypothesis. Gardner, Knox and other authors argue instead that childhood leukaemia is unrelated to infection but is instead caused by other environmental factors, e.g. radiation and hydrocarbons, which may potentially provide the first hit of the two-hit hypothesis.

Population mixing is a challenging subject as many of the authors who report an effect have used different definitions, depending on what data are available for a particular region and time. This makes comparison of results between groups difficult. It is also possible that population mixing is not, in fact, a good proxy for infection.

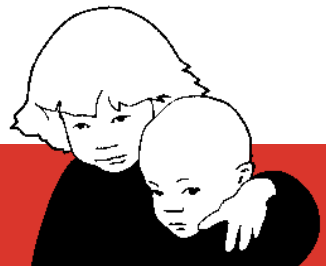
The associated population density studies are highly contradictory, and do not appear to provide proof for either a positive or a negative association of childhood leukaemia with high density living, or even a suggestive association. Some studies report that high population density results in increased rates of leukaemia, other studies low and mid-population densities.

There is a reasonable body of evidence associating environmental factors with the observed clusters of leukaemia, but these vary from cluster to cluster. Some involve an effect on the parents of children with leukaemia, and others identify localised sources of environmental carcinogens. This will only be resolved with further work.

In summary, childhood leukaemia clusters, in both space and time, and population mixing do appear to be associated with the aetiology of childhood leukaemia. There is an increased incidence of childhood leukaemia after population mixing, in areas that were originally very isolated and had low levels of herd immunity. It is particularly noticeable for the childhood leukaemia 1-4 years peak in rural wards with a high diversity of origin of in-migrants. In comparison, population mixing in urban areas, where there is already a high level of mixing, results in a decreased rate of leukaemia.

However, it is very difficult to look at the timing of any infectious exposure in these studies. Given the Greaves "Two-Hit" and "delayed infection" hypotheses, it is very likely that the timing of any infectious exposure is critical to the risk of developing childhood leukaemia. Maternal exposure to infection during pregnancy probably increases the risk of the child developing leukaemia through the first hit. Exposure of the child to infection within the first two years of life probably has a protective effect but delayed exposures may result in the development of childhood leukaemia.

But, it is far from proven that childhood leukaemia clusters have an infectious aetiology and thus do not provide strong evidence for the role of infection in the causation of childhood leukaemia.



3. Conclusion

In this review we have looked at the evidence for and against infection causing or preventing some cases of childhood leukaemia. We have looked at the epidemiological association between maternal infections during pregnancy and subsequent risk of childhood leukaemia. We also reviewed direct epidemiological associations with infectious episodes during childhood and also with vaccination. We have looked at the evidence for viruses causing leukaemia directly. A number of proxy measures of infection have been reviewed, including social and physical contacts, birth order, socio-economic status, allergy, breast feeding and seasonality. Finally we considered the evidence for the different hypotheses for the cause of leukaemia clusters.

How does this evidence in this review relate to the three infection hypotheses discussed in introduction to this review?

Most of the evidence in this review supports Greaves' delayed infection hypotheses. This does not necessarily exclude either Smith's maternal infection theory, or Kinlen's population mixing theory, but these might be seen as complimentary hypotheses. (Nor does it suggest that infection is the only cause of childhood leukaemia). Maternal infections may contribute to the first hit in the two-hit hypothesis and population mixing the delayed exposure of a group of children with low herd immunity to previously unknown infections.

Smith infectious exposure *in utero*

Smith[41] proposed that the childhood peak of ALL (typically cALL), which classically occurs between 2 to 5 years of age in populations of children in developed countries, is due to *in utero* exposure to infection. There is no direct clinical evidence to support an infectious agent causing a primary infection in the mother, subsequently passed on to the foetus, increasing the risk of childhood leukaemia. From epidemiological studies, any infection during pregnancy, and particularly influenza and chicken pox, appears to be positively associated with an increased risk of childhood leukaemia. Also, the evidence that our immune response genes are related to childhood leukaemia, resulting in either increased risk or a protective effect and the positive association with adenovirus C and EBV infections during pregnancy, suggests that there may be some *in utero* effect of infections on childhood leukaemia risk. It is possible that the genetic damage of the "first-hit" in ALL is caused by stress to the foetus *in utero* perhaps caused by the mother's inflammatory response to infection.

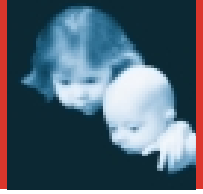
Greaves 'delayed infection' hypothesis

Greaves[44] sought to explain the childhood peak of cALL based on the hygiene hypothesis [42] which suggests that infections and unhygienic contact with older siblings or through other exposures may confer protection from the development of allergic illnesses. The 'delayed infection' hypothesis for childhood leukaemia is based on the premise that the immune system is programmed to anticipate infectious exposure in early childhood and that the absence of such exposure prevents the immune system from developing normally, Greaves proposed that delayed exposure to infection causes an aberrant immune response which acts as the second or post-natal 'hit' in his two-hit model described in the introduction.

Epidemiological studies looking at actual exposure of the child to infection suggest that exposure within the first two years may have a protective effect by stimulating the immune system. With a child not exposed to infection in early life, later exposures result in a hyperactive response from the immune system that provokes the development of childhood leukaemia. In this, an accurate measure of any infectious exposure and the timing it is critical. Unfortunately, this sort of epidemiological data is difficult to obtain reliably, so these direct measures of infection are merely suggestive. Because of this, many studies have looked for links with proxy measures of infection. In general, the evidence from proxy measures of infection strongly support Greaves' hypothesis. The evidence is strongest for social and physical contacts, breast feeding, socio-economic status, and allergy. The birth order evidence is contradictory with only the earlier studies being supportive and the seasonal incidence inconclusive. But, how good a proxy for infections are these measures and what else they could be associated with, for example environmental carcinogens?

Kinlen population mixing

It is quite clear that childhood leukaemia clusters in both space and time. Kinlen[45] proposed that these clusters are an unusual outcome of a specific, common but relatively non-pathological infection arising in non-immune individuals following 'population-mixing'. He proposed that herd immunity to a specific widespread viral infection (to which leukaemia is a rare response) would tend to be lower than average in such isolated communities and that the large influxes of workers might have caused epidemics of such infections.



None of the studies described in this review have identified one specific infection and several different types of infections or infection in general appear to have an association with leukaemia. This does not support Kinlen's hypothesis of population mixing, where he suggests that an unusual reaction to a **specific infection** leads to a child developing leukaemia. However, it is not necessarily in conflict with Kinlen's hypothesis for the situations for which it was formulated, i.e. sudden population influxes into previously very isolated areas, where herd immunity was low to infections that might be common elsewhere. The studies reviewed tend to be national studies that do not concentrate on such small areas and thus any association of leukaemia with a specific infection in a specific area might be masked. Increased levels of all the proxy measures of infection could be caused by population mixing but there is no direct evidence to support this.

Kinlen's hypothesis is supported by the evidence that there is an increased incidence of childhood leukaemia after population mixing, in areas that were originally very isolated and is particularly noticeable for the childhood leukaemia 1-4 years peak in rural wards with a high diversity of origin of in-migrants. In comparison, population mixing in urban areas, where there is already a high level of mixing, results in a decreased rate of leukaemia.

But, it is not at all clear that childhood leukaemia clusters are caused by infection. Some would argue that cancer clustering in space and time occurs routinely by chance. Recent publications from Germany have re-opened the argument for radioactivity as a cause of childhood leukaemia clusters and there is good evidence implicating environmental carcinogens. Also, Kinlen's hypothesis that there will be a specific infection will be very hard to prove, and while it may be appropriate for a single isolated community, it can not account for the apparent widespread general impact of infection.

Inflammation and infection

Many insults to the body generate an inflammatory response - including exposure to ionising radiation, other environmental carcinogens and infection. One of the unintentional consequences of this primarily protective inflammatory response could be childhood leukaemia. Inflammation is already implicated in the cause of a number of cancers. Inflammatory mediators create an environment where cells of the immune system are stimulated to grow. In childhood leukaemia this could provide selective growth pressure to a pre-existing pre-leukaemic cell. Environmental agents and infections may be promoting secondary genetic changes rather than actually causing them. Inflammation could be Greaves' "second hit". Thus, the inflammatory response, induced by infection and many other environmental agents, may provide a unifying hypothesis for the epidemiological evidence for a diverse range of environmental and infectious agents in the aetiology of childhood leukaemia.

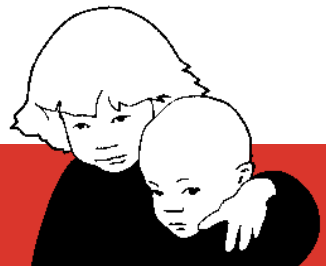
So, does infection cause or prevent childhood leukaemia?

Yes and no. Infection is associated with the causation of some of the childhood peak of ALL (cALL). Maternal exposure to infection during pregnancy is associated with an increase in the risk of a child developing leukaemia possibly through the mutagenic "first hit" to the foetus. Exposure of the child to infection within the first two years of life is associated with a reduced risk of childhood leukaemia and delayed exposures may result in the development of childhood leukaemia. However, the statistically significant evidence for this comes mainly from indirect (proxy) epidemiological measures of infection. There are clusters of childhood leukaemia cases but the case for these being caused by infections is unproven. Sudden population influxes, of diverse origin, into previously very isolated areas, where herd immunity was low is associated with an increased risk of cALL. But, there is also good evidence for associations between childhood leukaemia clusters and known environmental carcinogens.

And finally

On behalf of CHILDREN with LEUKAEMIA, thank you for reading this report. We hope you have found it interesting and informative. If you would like to communicate with the authors please email adrienne@leukaemia.org.

Dr Kirsten Edgar and Dr Adrienne Morgan March 2008



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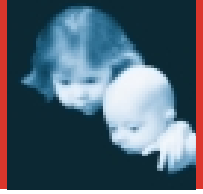

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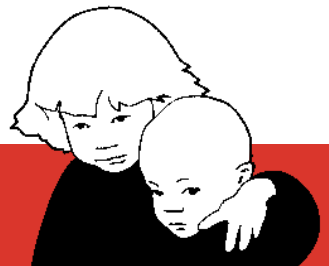

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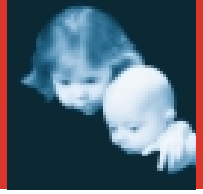

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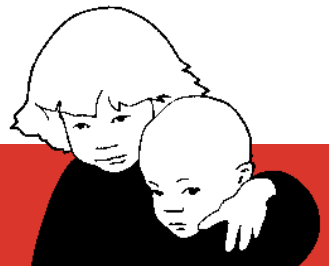

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CHILDREN with LEUKAEMIA is the UK's leading charity dedicated to fighting Britain's biggest childhood cancer through funding research into new and improved treatments, funding research into causes and prevention and funding welfare projects to minimise the stress of treatments.

We want all children diagnosed with leukaemia to be cured and for the cure to be effected with minimum disruption to their lives. Ultimately our aim is to understand what causes children to develop leukaemia, so that the rising incidence of the disease can be halted and reversed.

To date, the charity has raised over £90million pounds for vital research into treatment and prevention, support for leukaemic children and their families and to campaign on behalf of children with leukaemia and at risk of leukaemia.

Established in 1988 in memory of 14 year old Paul O'Gorman who was killed by leukaemia, we have contributed to the development of a network of childhood leukaemia research centres around the UK. Teams of scientists in these centres are at the very forefront of advances in research into the causes and treatment of childhood leukaemia.

Fighting Britain's biggest childhood cancer

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