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Infectious Etiology of Childhood Acute Lymphoblastic Leukemia, Hypotheses and Evidence

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1. Introduction

Research on the role of infectious agents in the etiology of cancer has grown remarkably in recent decades. A causal association between infection events and the development of different types of cancer has been strongly suggested in epidemiologic studies, while the direct oncogenic capacity of a set of pathogens has been demonstrated in the laboratory.

It is now recognized that between 15 and 20% of all tumors are associated with infection by direct tumorigenic agents [1]. However, the transforming mechanisms of carcinogenic infectious agents are not restricted to the expression of oncogenes and their ability to modulate the expression and function of oncogenes and anti-oncogenes in target cells. Other routes of transformation have been described, in which, an agent participates through more indirect mechanisms, such as promoting immune suppression or chronic inflammation. Although, in indirect mechanisms of transformation the infectious agent usually does not reside in the cell that will form the tumor mass, it contributes to cancer development making favorable conditions for tumor initiation or growth.

One of the malignancies proposed to be etiologically related to infection is childhood acute lymphoblastic leukemia (ALL). ALL is a heterogeneous group of hematologic malignancies in which the process of differentiation and limited proliferation that characterizes normal lymphopoiesis is altered and replaced by a malignant clonal expansion of immature lymphocytes. ALL is the most common type of childhood malignancy worldwide, unfortunately, little is known about the origin of ALL, some cases are associated with genetic predisposition conferred by Down syndrome, Bloom syndrome, ataxia-telangiectasia, Nijmegen breakage syndrome or exposure to environmental agents such as ionizing radiation

or mutagenic chemicals, however these events account for less than 5% of ALL cases [2], therefore, discernible causal factors involved in cancer initiation or promotion are unknown for the bulk of primary leukemia.

Several etiologic factors have been proposed to cause ALL. One of the most reported in the literature and the subject of this chapter is related to infections. Independently, Greaves, Kinlen and Smith have suggested different mechanisms by which certain events related to infection may explain at least some cases of childhood leukemia [3-5]. Interestingly, the suggested role of infectious agents in leukemogenesis varies from one hypothesis to another, favoring either direct or indirect mechanisms of transformation. It is our main goal to describe these hypotheses highlighting the type of evidence in favor and against them and providing a biological frame in which to discuss possible mechanisms of leukemogenesis by the infectious agents. Due to the large number of publications in the field, this is not intended as an in-deep and complete review of all published literature but a summary in which to set the basis for discussion.

2. 'Delayed infection' hypothesis and 'two-hits' minimal model by Greaves

One of the most cited proposals on the infectious etiology of ALL is the *delayed infection* hypothesis, in which Greaves argues that some cases of the common B-ALL (CD10⁺ CD19⁺ preB cALL) observed in the peak age of 2 to 5 years could be associated with an aberrant immune response displayed by an immature immune system [3]. This hypothesis is based in the theory that early exposures to common infectious agents are required for the proper maturation of the immune system, lack of these exposures results in aberrant responses when children are finally in contact with the agent(s). In Greaves view, ALL develops in the biological context of an aberrant immune response due to delayed infections, and thus, the infectious agents are only an indirect trigger of the leukemogenic process.

More recently, Greaves has added to his proposal the most frequent chromosomal aberrations in pre-B cALL, hyperdiploidy and the translocation TEL-AML1 (also known as ETV6-RUNX1), as susceptibility factors. Molecular analysis has shown shared clonotypic TEL and AML1 breakpoints in leukemic blasts from monochorionic monozygotic identical twins [6]. The same result has been observed when comparing the patients' blood at diagnosis and their blood archived at birth (Guthrie cards) [7]. These results have supported that these genetic insults are often generated *in utero*, based on such findings, Greaves has proposed a minimal 'two-hits' model to explain the development of pre-B cALL [8]. According to this model, hyperdiploidy or the TEL-AML1 translocation originate *in utero* and provide the first oncogenic hit, which is not sufficient for the occurrence of the disease but generates a pre-leukemic clone. In the presence of additional postnatal oncogenic hits, this susceptible clone then evolves into a malignant leukemic clone. Such additional hits could be promoted indirectly by the aberrant immune response to infection of children growing in microbiological isolated environments.

Greaves' hypothesis is based on the observation of the steady increase of childhood leukemia parallel to the increase of upscale living conditions in developed countries. Since its publication, a series of epidemiological studies have been designed to test the *delayed infection* hypothesis. Evaluation of parity, breastfeeding, improved hygiene conditions, neonatal or infant infections, vaccination against some viruses, day care attendance [9-13] among others, have been used as markers of exposure to infectious agents during the first years of life. As we will see next, these studies have found heterogeneous and even contradictory results.

The United Kingdom Childhood Cancer Study (UKCCS), a nationwide, population based case-control study, was designed to investigate different hypotheses about risk factors in childhood cancer, one of them referred to the association between day care attendance during the first year of life and the risk of developing leukemia [11]. Day care attendance was used as a surrogate marker for exposure to infectious agents, assuming that as more contacts a child has, there is a larger chance for exposure to infections. Data were obtained through interviews with parents of 1286 children with ALL between 2 and 14 years of age and 3605 controls from 10 different regions of the UK. The results showed an inverse relationship between 'social activity' and the risk of leukemia, OR=0.73 (95% confidence interval (CI): 0.62-0.87), showing also a dose-response trend. The interpretation of these findings was that early exposure to infections, indicated by day care attendance, is a protective factor against childhood leukemia, thus supporting Greaves' *delayed infection* proposal [11].

Another study from the same UKCCS data set was published two years later. In this report, it was analyzed the relationship between neonatal infections and risk of leukemia; the data were extracted from primary-care records compiled before diagnosis and interviews with parents. According to this study, children with ALL (ages 2-5 years) had more clinically diagnosed neonatal infections than their counterpart control: episodes number=3.6 (95% CI: 3.3-3.9) *vs* 3.1 (95% CI:2.9-3.2) [14]. These results contrast with the ones from the previous UKCCS study and argue that early infections are a risk factor for ALL, and therefore, give no support to the *delayed infection* hypothesis.

The study by Cardwell and colleagues using hospital records of clinically diagnosed infections in the first year of life from the UK General Practice Research Database (GPRD), compared 162 ALL cases with 2215 matched controls, no differences were found between cases and controls OR=1.05 (95%CI:0.64-1.74), then this study provided no support to Greaves' hypothesis [15]. Another large group, the Northern California study group analyzed day care attendance and parental recall of children ear infections between 294 ALL cases (ages 1-14) and 376 matched controls. Both markers were found protective, OR=0.42 (95% CI:0.18-0.99) and OR=0.32 (95% CI:0.14-0.74), respectively, but only for non-Hispanic white children, supporting Greaves' hypothesis but suggesting ethnic differences in the etiology of ALL [16].

The number of children born in a family has also been used as a marker for microbiological exposure. Dockerty and colleagues investigated the association between parity and risk of ALL in children aged 0-14 from England and Wales. They found a statistically significant protective effect for ALL in children of houses with increasing parity, OR=0.5 (95% CI: 0.3-0.8) [9]. Infante-Rivard et al also evaluated parity and day care attendance in a population based study (491 leukemia cases of children under 10 years old and 491 matched con-

trols) in Québec Canada. This group found a protective association with day care attendance, OR=0.49 (95% IC:0.31-0.77) and breast-feeding OR=0.68 (95% IC:0.49-0.95), while having older siblings was associated with increased risk of leukemia, OR=2.12 (95% IC: 1.57-2.85) [12].

The study by Flores-Lujano evaluated the frequency of severe infections that required hospitalization in the first year of life in children with Down's syndrome (DS) with or without ALL (57 cases and 218 controls aged 19 years or younger). In this study, DS children were chosen because it is known that they have an around 10 to 30 fold higher incidence of B cell ALL. They also found an association between infection an increased risk of leukemia, OR=3.45 (95% CI:1.37–8.66), which is against the Greaves' hypothesis [17].

In summary, many studies have explored the *delayed infection* hypotheses with heterogeneous results, with some studies actually showing an increased risk given by infections in the first years of life. The lack of consistency among investigations deserves further analysis and it is beyond the aim of this chapter. Some of the variables among studies are concerned with the methodological approach, study design, statistical tests and the representativeness of the studied population, among many others that could explain the heterogeneity of the results. Many other considerations are more related to the biological aspects of the hypothesis as it is discussed in the integrated discussion with the other hypotheses concerning an infectious origin of childhood leukemia.

3. 'Population mixing' hypothesis by Kinlen

In early 1980, an unusual increase in the incidence of childhood leukemia was observed in young people living in the vicinity of nuclear reprocessing plants in Cumbria, England and Dounreay, Scotland. It was thought that such increase in leukemias was the result of radioactive contamination, which might have caused somatic or germinal line mutations in the population [18-20]. However, in deep tests showed no evidence of radioactive leaks (Committee on Medical Aspects of Radiation in the Environment) or many other types of population occupational exposures [20].

In 1988 Kinlen proposed that the observed leukemia clusters could result from the unusual population mixing occurring in regions receiving the influx of workers and their families who were attracted by new jobs in nuclear plants. Disease outbreaks associated with population growth and migration had been previously documented, and Kinlen hypothesized that this was also the case for the leukemia clusters. During populations mixing, resident people would be naive to infection by different agents carried by the newcomers and vice versa, exposure to such agents would cause an abnormal response leading to the outbreak [4].

Kinlen first proved his *population mixing* hypothesis in Thurso, Scotland, an isolated rural area that received large influxes of people who had migrated to work at a nuclear plant. The results showed that during the period when the population doubled (1951-1967) there was

an increased incidence of childhood leukemia, returning to normal numbers in subsequent years [4]. Other relevant studies of Kinlen's group are concerned with new military settlements; for instance, in post-war Britain between 1949 and 1950, when national military service was mandatory for all men reaching 18 years of age and the period of service was increased from 1 to 2 years. During the following years there was a significant increase of leukemia in areas with the highest proportion of military servicemen. A similar phenomenon was observed in Fallon, Nevada US when there was a considerable increase in the number of trainee recruits in the nearby naval base [21].

Virtually every study that has been led by Kinlen's working group has shown similar results, *i.e.* they have observed a significant increase in childhood leukemia matching large-scale mixing between rural and urban populations. [22-27]. In favor of Kinlen proposal, childhood leukemia clusters were more evident when people from urban regions were mixed with people from isolated areas with low population density, and those who develop leukemia were mostly children from the most immunologically isolated. Also, the leukemia peaks were transitory coinciding with the largest flow of people, arguing against a common source of a persistent chemical/radiation contaminant.

Other researchers have addressed the same question. For example, Koushik and colleagues conducted an ecologic study of childhood leukemia and population mixing in Ontario, Canada. The percent of population change was employed as indicator of mixing population. In this study, 1394 leukemia cases recorded between 1978 and 1992 were included. The results showed that population growth was also associated with a high incidence of leukemia, but only in rural and not in urban areas [28]. Other studies have shown no support for the Kinlen's hypothesis, among them is Laplanche & de Vathaire's [29]. This study included all French communities and covered the period between 1968 and 1990 during which occurred a rapid population increase. According to the results during the mentioned period, deaths from leukemia in children or young adults under 25 years of age were slightly lower than the expected estimate and no differences in risk according to the size of population increase or region were found. Another French study carried around the nuclear reprocessing plant of La Hague found no evidence of increase in childhood leukemia cases [30].

Although, not all the studies carried out around areas of population mixing have correlated with clusters of childhood leukemia, it is relevant that most do. It is also important that, although the original observation was done around nuclear plants, there is evidence of a similar phenomenon occurring in many other regions around non-nuclear sites, including military settlements. From his observations, Kinlen proposed that a common infectious agent could be responsible and adults are the main transmitters, thus population mixing could be responsible for the leukemia cases seen even in the first year of life.

If Kinlen proposal is true, it is possible that the data against his hypothesis had different explanations: 1) the effect may be dose dependent, so, high levels of contact might be necessary; 2) the hypothesis has been proposed for large-scale rural-urban population mixing and many studies might not reach the required population threshold, and 3) other genetic and/or environmental differences might be affecting the outcome [4, 22].

Similar to the Greaves' hypothesis, the identity of the infectious agent(s) involved in Kinlen model is still not known. In fact, most of the population mixing studies had failed to find an increase in a symptomatic infection in adults or children, paralleling the increase in leukemia incidence. Considering that there are viruses of recognized leukemia causality in animals and one human's leukemia caused by a virus, Kinlen has proposed that the agent involved could be a prevalent virus causing an uncommon infection [31]. Kinlen also considers that the putative causative virus is not transmitted as a typical acute infection virus, a characteristic common of tumorigenic viruses. However, the viral family known to be involved in animal leukemia is the retroviridae, and specifically for adult humans the causative agent is the human T cell leukemia/lymphoma virus type 1 (HTLV-1), which is endemic of areas with no recognized peaks of childhood leukemia. Because both Kinlen and Greaves models fail to identify the causative agent, both hypotheses seem similar pointing out to a common mechanism of response rather than a possible direct mechanism of infection.

4. Direct viral leukemogenesis hypothesis by Smith

A third hypothesis regarding the infectious etiology of childhood leukemia was proposed by Smith and colleagues. According to the *delayed infection* hypothesis, children exposed to infectious agents during the first months of life (e.g. in developing countries) should have almost no leukemogenic potential, whereas children that become infected later (e.g. in affluent societies), exposure to the same agent would be potentially leukemogenic. Smith disagrees with this scenario, especially for children aged 2 and 3, which represent the larger proportion of children within the peak incidence of 2 to 5 years old, and suggested that there should be an alternative mechanism by which the infection leads to leukemia and that could explain all age-related peaks of disease, including infant leukemias [5].

In his publication *Considerations on a possible viral etiology for B-precursor acute lymphoblastic leukemia of childhood* Smith proposed that the infectious process leading to leukemia occurs during intrauterine life by mother to fetus transmission [5]. *De novo* infected seronegative women or those in which the agent reactivation occurred during pregnancy were especially vulnerable to infect their fetus. This hypothesis also considers possible infections during the first year of life of children from seronegative mothers unable to passively immunize their offspring. According to Smith's hypothesis, the pathogen acts through a direct mechanism of B cell infection, initiating or complementing the process of cellular transformation together with additional oncogenic hits either intrauterine or postnatal.

Considering that more than 60% of cases of ALL-B are associated with chromosomal abnormalities, Smith hypothesized that the agent involved should be a virus, since many viral agents present a variety of mechanisms that promote genetic instability. According to Smith's hypothesis the putative virus should have the ability to cross the placenta, to infect B lymphocytes and to have oncogenic potential. However, such agent should not have the ability to induce severe abnormalities, since ALL is not associated with other cancers or birth defects. Thus, an important difference of Smith's hypothesis is that the infection *per se*

carries the power to trigger the chromosomal abnormalities often present in childhood leukemia, while for Greaves, the genetic insult is already present and the infection indirectly promotes the acquisition of additional hits.

Several viral families fulfill Smith's criteria for a causative agent. Members of the adenovirus, herpesvirus and polyomavirus are transmitted very early pre- or post-natally, have tropism for bone marrow cells and have oncogenic potential; we know that most of the population carries all these viruses asymptotically, with only a few of them developing a related-neoplasia. On the other hand, the retroviruses are also good candidates, as they already have been implicated in leukemias. Several transforming mechanisms have been described for all of these viruses, including expression of constitutively active viral signaling proteins, transcriptional activation of cellular oncogenes and/or disruption of tumor suppressor genes, and importantly, induction of genetic instability; for instance Epstein Barr Virus (EBV or human herpesvirus-4) is associated with Burkitt's lymphoma, in which it also correlates with translocation of the cellular oncogene c-Myc [32].

Studies showing that maternal infections are associated with an increased risk of ALL supported Smith's model. Lehtinen et al analyzed sera of the first trimester from 342 Finnish and Icelandic mothers of children with ALL, searching for antibodies against herpesvirus EBV, cytomegalovirus and HHV-6 (human herpesvirus-6). Only an increase of anti-EBV antibodies was found correlating with leukemia cases, OR=2.9 (95% CI:1.5-5.8) [33]. Because of the nature of the antibodies found, this data suggested EBV reactivation as a potential event leading development of ALL. This same group confirmed the above observation with an additional 304 mothers: anti-EBV reactivation antibodies, OR=1.9 (95% CI:1.2-3.0) [34]. The possible role of EBV reactivation during pregnancy is still awaiting confirmation from other groups. Naumberg's group also found a similar positive association when the mother had lower genital tract infections, OR=1.78 (95% CI:1.2-2.7), especially in children older than 4 years of age at diagnosis, OR=2.01 (95% CI:1.1-3.8) [35].

Many other studies have shown conflicting results between viral infection during pregnancy and subsequent childhood leukemia in offspring, either by influenza virus or by other unspecified common infections [10, 12, 36]. On the other hand, several small studies have found an association between maternal varicella-zoster virus (causing chicken-pox) reactivation and childhood leukemia [37, 38]. Note, however, that none of these approaches have addressed viruses with recognized oncogenic potential and that they are epidemiological studies based on the mother recalled history of infection during pregnancy.

A distinct approach to explore direct transformation occurring *in utero* has been conducted through retrospective analyses of children who developed leukemia; in these studies, viral genomes have been searched in archived blood spots collected at birth with very heterogeneous results. For instance, an early study found blood spots positive to adenovirus-C in two children that developed leukemia, but other groups have not reproduced such result [39]. Bogdanovic et al searched for viral genomes from herpesvirus EBV and HHV-6, polyomavirus JCV and BKV (from the patients' initials from whom the viruses were isolated) and parvovirus 19 in Guthrie cards from 54 Swedish patients, finding no association [40-42]. Parvovirus B19 was another good candidate for causality since it has been associated with sev-

eral childhood hematological diseases. One should consider that, although the search for viral genomes in Guthrie cards is more stringent, the negative result does not mean that there is not increased viral infection/reactivation during pregnancy and the titer and type of antibodies are probably more reliable markers for this.

Based on Smith's original proposal, the notion of a direct oncogenic mechanism in the etiology of childhood leukemia was widened to include infections with a transforming agent occurring postnatally but prior to the onset of the disease. In this possible leukemogenic mechanism, infection is not necessarily the first oncogenic hit. To test this proposal derived from Smith's hypothesis, different viral agents have been screened directly in the leukemia blast (Table 1). One study evaluated the presence of the viral genome of polyomavirus JCV and BKV in 15 samples at diagnosis of pre-B ALL and a second study included 25 samples in which the viral genome of JCV, BKV and SV40 (simian virus 40) were searched. In both studies, the screening was performed by PCR without finding any of these viruses present in the leukemia samples [43, 44].

Virus	Leukemia subtype	Age (years)	Sample	Screening method	N	Ref.
Polyomaviruses JVC and BKV	B-precursor ALL	1-12	BM or PB	Endpoint PCR	15	[43]
Polyomaviruses JVC, BKV and SV40	B-precursor ALL	2-5	BM	Real-time PCR	25	[44]
Polyomaviruses JVC and BKV	B-precursor ALL T-ALL	0.75-17	Archived neonatal blood spots	Nested PCR	50 4	[40]
Herpesviruses EBV y HHV-6	B-precursor ALL T-ALL	0.75-17	Archived neonatal blood spots	Nested PCR	50 4	[41]
Herpesviruses EBV, HHV-6, -7 and -8	B-precursor ALL	1.5-13	BM or PB	Southern blot (only for EBV) and Real-time PCR	47	[45]
Parvovirus B19	B-precursor ALL T-ALL	0.75-17	Archived neonatal blood spots	Nested PCR	50 4	[42]
Retrovirus BLV	ALL	≤16	BM and PB	Southern blot	131	[46]
Annelovirus TT	ALL	us	BM, PB and CFS	Nested PCR, dot blot and Southern blot	28	[47]

* In this study, the samples were obtained at diagnosis or during treatment. BM: bone marrow, PB: peripheral blood, CSF: cerebrospinal fluid, us: unspecified.

Table 1. Screening for viral sequences in ALL.

Mackenzie et al searched for human herpesvirus-4 (EBV), -6, -7 and -8 (KSHV); 20 peripheral blood or bone marrow samples were tested by Southern blot (EBV) or conventional PCR (HHV -6, -7 and -8). The authors found that seven samples were positive for some of these viruses; however, the low viral load found indicated that the viral genome was not present in every leukemia blast and therefore the result did not support that infection was part of the initial insult that preceded the malignant clonal expansion [45].

Bender et al screened for Bovine leukemia virus (BLV) years before the publication of Smith's proposal. BLV is an exogenous retrovirus whose direct role in the genesis of bovine leukemia has been well documented. 131 samples of ALL (the article did not address a specific subtype of leukemia) and 136 controls were screened by Southern blot for the BLV genome. Cases and controls were negative to the virus arguing against a positive role of BLV in childhood leukemia [46]. Screening for transfusion-transmitted virus (TTV) have also been negative [47].

In summary, different studies have failed to identify viral agents within the leukemia cells indicative of a viral direct leukemogenic mechanism. However, it is important to consider that these studies included only a small number of samples, 50 or less. These studies at the most suggest that if an infectious agent is involved in leukemogenesis, this would occur in a limited number of cases. A larger number of samples from more geographical regions and different social strata should be included for a more definitive conclusion.

The list of candidate viruses is not exhausted yet and the pathogen involved in the genesis of leukemia (if any) could still be unknown, Kaposi sarcoma associated herpesvirus (KSHV) and Merkel cell polyomavirus (MCPV) were discovered a few years ago and have already been associated with several neoplasias including the ones from which the virus were isolated, Kaposi's sarcoma and Merkel cell carcinoma, respectively [48]. Under this idea, the study of MacKenzie et al was designed to identify undescribed members of the Herpesviridae family by a degenerate PCR, but no new herpesviruses were found in any of the 18 samples analyzed [45]. As the individual virus "hunt" is a limited method, next generation sequencing technologies are an attractive approach to ask for the presence of known and unknown infectious agents in leukemic cells.

5. Space-time clustering of childhood leukemia by Alexander

As we learn in the previous section, childhood leukemia has been shown to be a disease often presented in space and time clusters correlating with communities with large influx of people. Population based morbidity/mortality maps are used in public health to inform us of points of an excess of cases (the cluster) relative to the expected incidence, which are then unlikely to have happened by chance and points out to possible etiological factors and the population at risk. Leukemia aggregates have been studied for decades and to date, a number of studies have reported an unusual increase in the number of cases associated with space-time patterns, some of them have been anecdotal reports but others have been discovered through employment of formal statistical analysis. We describe next some cluster stud-

ies that have been specifically designed to test the hypothesis of the involvement of infectious agents in the development of childhood leukemia.

Alexander's work is one of the pioneering reports using rigorous statistical methods to determine the existence of spatial temporal clusters as indirect evidence of an infectious etiology for childhood leukemia. The analysis was performed using data obtained from the censuses of 1971 and 1981 in England, Wales and Scotland and was restricted to wards whose contribution to spatial clustering test exceeded an expected, arbitrarily established threshold, from a Poisson distribution on uniform risk of the disease. The report included 487 cases of ALL and other unspecified leukemias. The location at birth was extrapolated from the location data at diagnosis (assuming no changes in residence). The association infection-leukemia was tested from 3 hypothesis envisioned from three different scenarios based on the period of exposure and age of disease presentation:

Period of exposure	Age at presentation
I In utero or around the time of birth	5 years or older
II Post-natal	Under 5 years
III Recent first exposure previous to the onset	'Childhood peak' (ages 2-4 years)

Table 2.

To test these hypotheses, the cases were divided into series A and B, the 'susceptibles' (not exposed) and the 'infectives'. To evaluate spatial and temporal associations, the data were analyzed as pairs of cases; spatial linkage was defined based in location within the same electoral ward. Temporal linkage was an overlap of at least 3 months between the time of presumed susceptibility of the child in series A and infectivity of the child in series B.

The results of this study showed support for the hypothesis I: exposure around the time of birth leads to an increased risk of leukemia whose onset takes place at 5 years or older. At the biological level, the authors interpreted the silent and persistent infection of an agent acquired *in utero* as potentially contributing to the development of the malignancy at any time prior to its presentation. The authors exemplified the process similar to an infection by pestivirus, which however, has not been associated with carcinogenic processes in animals and they are known to induce death even *in utero*. According to this paper, infections did not explain the cases in the 2-5 years old peak, which is the most common in developed countries such as those included in this study [49].

The report of Birch et al, included 798 cases of acute leukemia diagnosed between 1954 and 1985 taken from the Manchester Children's Tumour Registry (MCTR) and aimed to evaluate various scenarios for the infectious etiology of leukemia (cluster criteria were established *a priori* as less than 5 km and less than 1 year apart). To support Greaves', Kinlen's and Smith's proposals, two working hypotheses were established: H1 is true (Greaves and Kinlen hypotheses) and H2 is false (Smith hypothesis). This study also considered 4 possible space-time interactions in which the potentially leukemogenic infection would occur. The

different hypothetical scenarios and their associated proposals depending on the type of interaction were as follows:

Interactions	Hypothetical Scenery	Support to
I Between times and places of birth	The infection occurred <i>in utero</i> or in early infancy	Smith's hypothesis
II Between times and places of diagnosis	The infection occurred before diagnosis	Greaves' and Kinlen's hypothesis
III Between time of diagnosis and place of birth	The infection occurred before diagnosis	Greaves' and Kinlen's hypothesis
IV Between time of birth and place of diagnosis	No plausible according to previous results	---

Table 3.

To analyze the data, different statistical tests were used and the authors considered mobilization of children from the records of changes of residence. The results showed evidence of space-time clustering based on place of birth and time of diagnosis for the sub-groups aged 0-4 years, but no evidence based on place and time of birth, thus the results lent support to Greaves and Kinlen hypotheses but they did not support Smith's [50].

Methodologies used to search time clusters have also been used to address seasonal variation for childhood leukemia. According to this idea, if an infection is associated with disease, then a seasonal pattern would be expected, either at birth or diagnostic. Perhaps the largest study of this type is the one conducted by Higgins et al, using the population based data from the UK National Registry of Childhood Tumors that included 15,835 leukemia cases from children born and diagnosed between 1953-1995. No seasonality was found in this study after leukemia classification by age, gender or immunophenotype [51]. Similar studies have been conducted in the USA, Singapore and Sweden, founding the same negative result. In all of these studies only some temporal peaks (but no evidence of seasonality) have been observed [52].

Many other studies have provided evidence for space-time clustering of childhood leukemia [53-56]. Some have not addressed a possible infectious explanation but correlated with population mixing. An extreme example was Greece, which experienced one of the largest influx of people from rural to urban settings and presented one of the highest incidences of childhood leukemia around that time [57]. Although, these studies based on the observation of space-time clusters are considered an indirect evidence of the involvement of infectious agents in the etiology of leukemia, the identity of such agent(s) is unknown and therefore the participation of other environmental factors cannot be presently ruled out.

6. Integrative discussion

Indirect evidence supports an association between infections in childhood leukemia, and three hypotheses have been proposed to explain and/or address this question with variable and even opposite results. From these hypotheses, the *delayed infection* by Greaves argues for an indirect role for infection, Smith's hypothesis for a direct causative role and Kinlen's seems to sit in the middle, favoring a direct infection of the cell that will become the leukemic blast but also an indirect mechanism of response still unexplained. In other words, for Greaves, infections in early life are protective and for Kinlen and Smith are a risk factor; for Greaves and Kinlen almost any type of infectious agents (for Kinlen mostly viral) able to trigger aberrant immune or cellular responses could be the causative agent, for Smith it would be viruses with direct oncogenic capacities.

Based mainly in adult cancers, we now know that pathogens contribute to neoplasia through different mechanisms. The classical ones are those in which the agents infect cells and promote oncogenic transformation 'from within', through altering signaling pathways and gene expression programs (supports Smith). Indirect roles (supports Kinlen) include promotion of an inflammatory microenvironment, loss of cancer immune surveillance and a cofactor role helping the tumor through secretion of growth and angiogenic factors. The latter one is the mechanism proposed to explain cytomegalovirus oncomodulatory role in high-grade gliomas and it is thought to be a tumor maintenance rather than an initiating mechanism [58]. From these mechanisms, a direct role would be very possible but so far multiple studies have failed to find evidence of infection by oncogenic agents in the leukemic blast. On the other hand, an inflammatory role is very unlikely because it is generally associated with chronic diseases lasting decades (e.g. *Helicobacter pylori* and hepatitis B and C virus infections). A cofactor or immune suppressive roles are possible, especially for pre-leukemic clones (e.g. the ones with an early chromosomal abnormality).

Considering all these mechanisms, it is important to acknowledge that the term childhood leukemia harbors many different biological entities, and it is very likely that they involve different mechanisms of origin. Examples of important known differences are the lineage origin of the leukemic blast, myeloid *vs* lymphoid or T cell *vs* B cell. Also, there are at least three recognized B cell immature developmental stages where the leukemia is originated: early proB, preB-I and large preB-II, which are recognized for the differential expression of lineage- and stage- specific antigens and are dependent on the activity of different signaling pathways and transcriptional programs [59].

As mentioned before, childhood leukemia is also associated to chromosomal abnormalities: hyperdiploidy, hypodiploidy and translocations t(12;21)(p13;q22) (TEL-AML1), t(1;19)(q23;p13) (E2A-PBX1), t(9;22)(q34;q11) (BCR-ABL) and t(4;11)(q21;q23) (MLL-AF4) are among the most common in B-ALL. These genetic abnormalities affect specific signaling pathways and favor transcriptional expression profiles related to the developmental stage of the B cell leukemic blast. Therefore, the risk and protection factors driven these known and still many unknown different childhood leukemia entities are probably different and models of the origin of the disease should be restrained to specific subtypes. Because most reports

group together several subtypes of leukemia, ethnic, stage, age and genetic insult, it is difficult to interpret whether they support or reject the different hypothesis of the infectious origin of the disease.

It should be noted that Greaves' hypothesis concerns the common form of B-cell ALL (CD19⁺, CD10⁺). This form comprises most of the ALL cases that peak at 2–5 years of age observed in developed countries or in affluent communities that have improved their living standards and have become 'more hygienic' [60]. Through comparison of international reports, variations in the peaks of childhood ALL have been identified. The aforementioned peak at 2–5 years of age is reduced, or even absent, for Black Africans and for other developing communities [61–63]. In Mexico, for example, two incidence peaks have been reported; the first occurring at 2–3 years of age and the second at 6–9 [64]. There is also the infant leukemia (of children under one year old) that is of very bad prognostic and is at least 80% positive to MLL translocations, supporting different etiologies between age groups.

Although, the *delayed infection* and *mixing population* hypotheses exhibit several points in common, they exhibit important differences too, for instance, Greaves' hypothesis is concerned to common childhood leukemia seen in age group of 2 to 5 years, while, Kinlen has not associated the leukemia clusters with a particular subtype of disease and has interpreted his results as all types of childhood leukemia might have a common cause. This argument could weak his hypothesis since a common etiologic mechanism for the different subtypes of the disease is difficult to envision. Also, the largest increase in leukemia cases has been reported for developed countries and Kinlen has not provided an explanation of how his model of large mixing of urban and rural populations can be extrapolated to or represent an affluent or aseptic setting.

Given the multifactorial nature of cancer, the role of other environmental and genetic factor in Kinlen's proposal is also missing. Kinlen's studies often seem to be based in the sole action of an infectious agent, but there are not known examples of infectious agents or oncogenic insults with full penetrance.

Some studies supporting the population mixing proposal have observed a specific increase of infant leukemia, which is mostly associated with MLL translocations. It has been reported that topoisomerase II inhibitors, consumed in some foods during pregnancy or present in drugs commonly used to treat cancer, are a risk factor for this type of translocation [65, 66]. There are not infectious agents known to promote MLL translocation or to inhibit topoisomerase II enzyme. Therefore, how other environmental insults take part in events of population mixing should also be considered. In this scenario, Greaves model seems more complete, since it includes the genetic lesions that characterize childhood leukemia. Greaves uses these genetic lesions to frame a biologically plausible mechanism in which children are more vulnerable to a leukemogenic process after an untimely infection episode. Still, in Greaves model, the first and perhaps more important oncogenic hit happens by chance and therefore his model does not provide an easy target for controlled intervention.

The rise of several types of diseases in recent years, mainly in developed countries, has been proposed to be associated with increasing hygienic conditions. This hygiene hypothesis

states that lack of early childhood exposure to microorganisms triggers the appearance of disease [67]. Although Greaves has modeled his *delayed infection* proposal in the *hygiene hypothesis*, there might be subtle differences between both hypotheses. While the *hygiene hypothesis* is highly concerned with acquisition of the human normal flora, Greaves is also concerned with pathogens that are not life threatening when acquired early. There are many examples of the latter and several diseases have been associated to delayed infection, examples of them are EBV or cytomegalovirus-related infectious mononucleosis, measles and chickenpox. In these cases, infection in the first years of life leads to mild to no symptoms, but when acquired late leads to serious and even life threatening diseases and in the case of EBV, it has been proposed that it predisposes to lymphoma. However, the window in which these infections become dangerous are usually beyond the years of the higher incidence peak of childhood leukemia and could only explain leukemia of the teenager or young adult.

Studies in mice are strongly indicative that animals grown in germ free conditions are often immunologically unsuited to fight infections and that perhaps one of the most important components of the immune instruction program is the normal flora [68]. Inoculation of probiotics in germ free mice is associated with development of a regulatory immune response in mucosa (based mainly in frequencies of regulatory T cells and levels of cytokines IL-10 and TGF- β) and equilibrated Th1/Th2/Th17 environments. Same results have been obtained after inoculation with several members of *Bifidobacterium*, which are normal residents of infant feces [69]. Animals without a normal microbiota often develop fatal responses when they are challenged with low doses of otherwise controllable pathogens. These results have supported a model in which humans have co-evolved with their flora and this flora is more than a passive passenger providing multiple benefits to the host. Several lines of evidence support that a normal microbiota is necessary for a healthy host metabolism, and also for what is now known as the microbial immunotraining.

The example of germ free animals, although extreme, points out that looking for infection markers of childhood common pathogens might not be indicative of the normal development and equilibrium of the human microbiota and the immune system, and if it is true that leukemia is the result of an aberrant immune response, then markers of infections are not representative of homeostatic acquisition of the normal human flora. In a similar scenario, infections that may confer risk or protection for leukemia are not necessarily symptomatic, thus, data collection of infections with clinical symptoms would exclude relevant infections to normal immune system development. Also, studies that finding that early symptomatic infections are a risk factor for leukemia might only be reflecting on the antibiotics used to treat those infections and the effects that they had on the establishment of the children normal microbiota.

There are many diseases with increased incidence in affluent societies. Among the most studied are asthma, allergies and type 1 diabetes; several studies have tried to link some of these diseases to childhood leukemia. Linabery et al published a meta-analysis of the different studies searching for association between childhood leukemia and allergy, asthma, eczema and hay fever. Although, this meta-analysis shows a protective effect of these diseases, OR=0.69 (95% CI:0.54-0.89), OR=0.79 (95% CI:0.61-1.02), OR=0.74 (95% CI:0.58-0.96), OR=0.55 (95% CI: 0.46-0.66), respectively, the authors observed high heterogeneity of the data with

several studies failing to find an effect [70]. Moreover, the overall protection observed argues that the same hygienic conditions driving allergies are protecting from childhood leukemia. An alternative explanation is that the molecular pathway leading to allergy and the leukemogenesis pathway are mutually exclusive.

A similar approach has been proposed for parasitic infections, since the fall of this type of infection has been parallel to the increase in allergies and childhood leukemia in most developed countries. Furthermore, many parasites drive Th1 immune responses while allergies are associated with Th2 responses providing a feasible biological frame for protection to allergies and perhaps childhood leukemia. A few studies have found a correlation between lack of infection of intestinal parasites and childhood leukemia [71]. However, many autoimmune diseases also explained by the hygiene hypothesis are triggered by Th1 responses, such as type 1 diabetes, confusing a mechanistic explanation for this phenomenon and arguing against a common origin for all these diseases.

7. Conclusion

Different hypotheses have tried to relate the origin of childhood acute lymphoblastic leukemia to infections and epidemiological, clinical and molecular evidence have been searched to support them with highly variable results. ALL is a common term that harbors several diseases varying in their age of presentation, associated genetic lesions, cellular origin and prognosis, probably reflecting different biological origins and thus suggesting different causative factors. Hence, although some of the accumulated evidence favors one or other of the hypotheses there is not a consensus whether infections participate and this participation is through direct or indirect mechanisms of transformation. Although, the postulated mechanisms differ from each other, they are not mutually exclusive. The causal factors of leukemia most probably are influenced by complex environmental and genetic interaction with some of them having greater or lesser roles in different individuals or subtypes of the disease. New approaches and methodologies should be used to provide further data supporting the role of infections. In that scenario, more direct markers of aberrant immune responses should be analyzed to support Greaves proposal. Th1/Th2/Th17 and/or regulatory immune environments should be tested as early as during pregnancy, lactation or in stored newborn blood. Next generation technologies should be used to identify novel infectious agents in ALL samples and to study the microbiota of patients. All these efforts together will result in a better understanding of the role of infectious agents in childhood ALL, their mechanisms of leukemogenesis and will provide better points for disease control.

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References

- [1] Parkin DM. The global health burden of infection-associated cancers in the year 2002. *International journal of cancer Journal international du cancer* 2006;118(12) 3030-3044.
- [2] Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet* 2008;371(9617) 1030-1043.
- [3] Greaves MF. Speculations on the cause of childhood acute lymphoblastic leukemia. *Leukemia* 1988;2(2) 120-125.
- [4] Kinlen L. Evidence for an infective cause of childhood leukaemia: comparison of a Scottish new town with nuclear reprocessing sites in Britain. *Lancet* 1988;2(8624) 1323-1327.
- [5] Smith M. Considerations on a possible viral etiology for B-precursor acute lymphoblastic leukemia of childhood. *Journal of immunotherapy* 1997;20(2) 89-100.
- [6] Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, et al. Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet* 1999;354(9189) 1499-1503.
- [7] Gale KB, Ford AM, Repp R, Borkhardt A, Keller C, Eden OB, et al. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *Proceedings of the National Academy of Sciences of the United States of America* 1997;94(25) 13950-13954.
- [8] Greaves M. Childhood leukaemia. *British Medical Journal*. 2002;324(7332) 283-287.

- [9] Dockerty JD, Draper G, Vincent T, Rowan SD, Bunch KJ. Case-control study of parental age, parity and socioeconomic level in relation to childhood cancers. *International journal of epidemiology* 2001;30(6) 1428-1437.
- [10] McKinney PA, Juszczak E, Findlay E, Smith K, Thomson CS. Pre- and perinatal risk factors for childhood leukaemia and other malignancies: a Scottish case control study. *British journal of cancer* 1999;80(11) 1844-1851.
- [11] Gilham C, Peto J, Simpson J, Roman E, Eden TO, Greaves MF, et al. Day care in infancy and risk of childhood acute lymphoblastic leukaemia: findings from UK case-control study. *British Medical Journal* 2005;330(7503) 1-6.
- [12] Infante-Rivard C, Fortier I, Olson E. Markers of infection, breast-feeding and childhood acute lymphoblastic leukaemia. *British journal of cancer* 2000;83(11) 1559-1564.
- [13] Groves FD, Gridley G, Wacholder S, Shu XO, Robison LL, Neglia JP, et al. Infant vaccinations and risk of childhood acute lymphoblastic leukaemia in the USA. *British journal of cancer* 1999;81(1) 175-178.
- [14] Roman E, Simpson J, Ansell P, Kinsey S, Mitchell CD, McKinney PA, et al. Childhood acute lymphoblastic leukemia and infections in the first year of life: a report from the United Kingdom Childhood Cancer Study. *American journal of epidemiology* 2007;165(5) 496-504.
- [15] Cardwell CR, McKinney PA, Patterson CC, Murray LJ. Infections in early life and childhood leukaemia risk: a UK case-control study of general practitioner records. *British journal of cancer* 2008;99(9) 1529-1533.
- [16] Ma X, Buffler PA, Wiemels JL, Selvin S, Metayer C, Loh M, et al. Ethnic difference in daycare attendance, early infections, and risk of childhood acute lymphoblastic leukemia. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2005;14(8) 1928-1934.
- [17] Flores-Lujano J, Perez-Saldivar ML, Fuentes-Panana EM, Gorodezky C, Bernaldez-Rios R, Del Campo-Martinez MA, et al. Breastfeeding and early infection in the aetiology of childhood leukaemia in Down syndrome. *British journal of cancer* 2009;101(5) 860-864.
- [18] Black D, editor. Investigation of the Possible Increased Incidence of Cancer in West Cumbria. *Proceedings of Report of the Independent Advisory Group HMSO*. 1984; London.
- [19] Heasman MA, Kemp IW, Urquhart JD, Black R. Childhood leukaemia in northern Scotland. *Lancet* 1986;1(8475) 266.
- [20] Gardner MJ, Snee MP, Hall AJ, Powell CA, Downes S, Terrell JD. Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. *British Medical Journal* 1990;300(6722) 423-429.

- [21] Francis SS, Selvin S, Yang W, Buffler PA, Wiemels JL. Unusual space-time patterning of the Fallon, Nevada leukemia cluster: Evidence of an infectious etiology. *Chemico-Biological Interaction* 2012;196(3) 102-109.
- [22] Kinlen LJ. Epidemiological evidence for an infective basis in childhood leukaemia. *British journal of cancer* 1995;71(1) 1-5.
- [23] Kinlen LJ, Balkwill A. Infective cause of childhood leukaemia and wartime population mixing in Orkney and Shetland, UK. *Lancet* 2001;357(9259) 858.
- [24] Kinlen LJ, Clarke K, Hudson C. Evidence from population mixing in British New Towns 1946-85 of an infective basis for childhood leukaemia. *Lancet* 1990;336(8715) 577-582.
- [25] Kinlen LJ, Hudson C. Childhood leukaemia and poliomyelitis in relation to military encampments in England and Wales in the period of national military service, 1950-63. *British Medical Journal* 1991;303(6814) 1357-1362.
- [26] Kinlen LJ, John SM. Wartime evacuation and mortality from childhood leukaemia in England and Wales in 1945-9. *British Medical Journal* 1994;309(6963) 1197-1202.
- [27] Kinlen LJ, Dickson M, Stiller CA. Childhood leukaemia and non-Hodgkin's lymphoma near large rural construction sites, with a comparison with Sellafield nuclear site. *British Medical Journal* 1995;310(6982) 763-768.
- [28] Koushik A, King WD, McLaughlin JR. An ecologic study of childhood leukemia and population mixing in Ontario, Canada. *Cancer causes & control : CCC* 2001;12(6) 483-490.
- [29] Laplanche A, de Vathaire F. Leukaemia mortality in French communes (administrative units) with a large and rapid population increase. *British journal of cancer* 1994;69(1) 110-113.
- [30] Guizard AV, Boutou O, Pottier D, Troussard X, Pheby D, Launoy G, et al. The incidence of childhood leukaemia around the La Hague nuclear waste reprocessing plant (France): a survey for the years 1978-1998. *Journal of Epidemiology and Community Health* 2001;55(7) 469-474.
- [31] Kinlen L. Childhood leukaemia, nuclear sites, and population mixing. *British journal of cancer* 2011;104(1) 12-18.
- [32] Sompallae R, Callegari S, Kamranvar SA, Masucci MG. Transcription profiling of Epstein-Barr virus nuclear antigen (EBNA)-1 expressing cells suggests targeting of chromatin remodeling complexes. *PLoS One* 2010;5(8) e12052.
- [33] Lehtinen M, Koskela P, Ogmundsdottir HM, Bloigu A, Dillner J, Gudnadottir M, et al. Maternal herpesvirus infections and risk of acute lymphoblastic leukemia in the offspring. *American journal of epidemiology* 2003;158(3) 207-213.

- [34] Tedeschi R, Bloigu A, Ogmundsdottir HM, Marus A, Dillner J, dePaoli P, et al. Activation of maternal Epstein-Barr virus infection and risk of acute leukemia in the offspring. *American Journal of Epidemiology* 2007;165(2) 134-137.
- [35] Naumburg E, Bellocco R, Cnattingius S, Jonzon A, Ekbom A. Perinatal exposure to infection and risk of childhood leukemia. *Medical and pediatric oncology* 2002;38(6) 391-397.
- [36] Little J. *Epidemiology of Childhood Cancer*. 1st edition. 1999.
- [37] Blot WJ, Draper G, Kinlen L, Wilson MK. Childhood cancer in relation to prenatal exposure to chickenpox. *British Journal of Cancer* 1980;42(2) 342-344.
- [38] Adelstein AM, Donovan JW. Malignant disease in children whose mothers had chickenpox, mumps, or rubella in pregnancy. *British Medical Journal* 1972;164(5841) 629-631.
- [39] Vasconcelos GM, Kang M, Pombo-de-Oliveira MS, Schiffman JD, Lorey F, Buffler P, et al. Adenovirus detection in Guthrie cards from paediatric leukaemia cases and controls. *British Journal of Cancer* 2008;99(10) 1668-1672.
- [40] Priftakis P, Dalianis T, Carstensen J, Samuelsson U, Lewensohn-Fuchs I, Bogdanovic G, et al. Human polyomavirus DNA is not detected in Guthrie cards (dried blood spots) from children who developed acute lymphoblastic leukemia. *Medical and Pediatric Oncology* 2003;40(4) 219-223.
- [41] Bogdanovic G, Jernberg AG, Priftakis P, Grillner L, Gustafsson B. Human herpes virus 6 or Epstein-Barr virus were not detected in Guthrie cards from children who later developed leukaemia. *British Journal of Cancer* 2004;91(5) 913-915.
- [42] Isa A, Priftakis P, Broliden K, Gustafsson B. Human parvovirus B19 DNA is not detected in Guthrie cards from children who have developed acute lymphoblastic leukemia. *Pediatric Blood Cancer* 2004;42(4) 357-360.
- [43] MacKenzie J, Perry J, Ford AM, Jarrett RF, Greaves M. JC and BK virus sequences are not detectable in leukaemic samples from children with common acute lymphoblastic leukaemia. *British journal of cancer* 1999;81(5) 898-899.
- [44] Smith MA, Strickler HD, Granovsky M, Reaman G, Linet M, Daniel R, et al. Investigation of leukemia cells from children with common acute lymphoblastic leukemia for genomic sequences of the primate polyomaviruses JC virus, BK virus, and simian virus 40. *Medical and pediatric oncology* 1999;33(5) 441-443.
- [45] MacKenzie J, Gallagher A, Clayton RA, Perry J, Eden OB, Ford AM, et al. Screening for herpesvirus genomes in common acute lymphoblastic leukemia. *Leukemia* 2001;15(3) 415-421.
- [46] Bender AP, Robison LL, Kashmiri SV, McClain KL, Woods WG, Smithson WA, et al. No involvement of bovine leukemia virus in childhood acute lymphoblastic leukemia and non-Hodgkin's lymphoma. *Cancer Research* 1988;48(10) 2919-2922.

- [47] Shiramizu B, Yu Q, Hu N, Yanagihara R, Nerurkar VR. Investigation of TT virus in the etiology of pediatric acute lymphoblastic leukemia. *Pediatric Hematology and Oncology* 2002;19(8) 543-551.
- [48] Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008;319(5866) 1096-1100.
- [49] Alexander FE. Space-time clustering of childhood acute lymphoblastic leukaemia: indirect evidence for a transmissible agent. *British Journal of Cancer* 1992;65(4) 589-592.
- [50] Birch JM, Alexander FE, Blair V, Eden OB, Taylor GM, McNally RJ. Space-time clustering patterns in childhood leukaemia support a role for infection. *British Journal of Cancer* 2000;82(9) 1571-1576.
- [51] Higgins CD, dos-Santos-Silva I, Stiller CA, Swerdlow AJ. Season of birth and diagnosis of children with leukaemia: an analysis of over 15 000 UK cases occurring from 1953-95. *British Journal of Cancer* 2001;84(3) 406-412.
- [52] Gao F, Nordin P, Krantz I, Chia KS, Machin D. Variation in the seasonal diagnosis of acute lymphoblastic leukemia: evidence from Singapore, the United States, and Sweden. *American Journal of Epidemiology* 2005;162(8) 753-763.
- [53] Alexander FE, Boyle P, Carli PM, Coebergh JW, Draper GJ, Ekbom A, et al. Spatial temporal patterns in childhood leukaemia: further evidence for an infectious origin. EUROCLUS project. *British Journal of Cancer* 1998;77(5) 812-817.
- [54] Alexander FE, Boyle P, Carli PM, Coebergh JW, Draper GJ, Ekbom A, et al. Spatial clustering of childhood leukaemia: summary results from the EUROCLUS project. *British Journal of Cancer* 1998;77(5) 818-824.
- [55] Alexander FE, Chan LC, Lam TH, Yuen P, Leung NK, Ha SY, et al. Clustering of childhood leukaemia in Hong Kong: association with the childhood peak and common acute lymphoblastic leukaemia and with population mixing. *British Journal of Cancer* 1997;75(3) 457-463.
- [56] McNally RJ, Alexander FE, Birch JM. Space-time clustering analyses of childhood acute lymphoblastic leukaemia by immunophenotype. *British Journal of Cancer* 2002;87(5) 513-515.
- [57] Kinlen LJ, Petridou E. Childhood leukemia and rural population movements: Greece, Italy, and other countries. *Cancer Causes Control* 1995;6(5) 445-450.
- [58] Michaelis M, Doerr HW, Cinatl J. The story of human cytomegalovirus and cancer: increasing evidence and open questions. *Neoplasia* 2009;11(1) 1-9.
- [59] Perez-Vera P, Reyes-Leon A, Fuentes-Panana EM. Signaling proteins and transcription factors in normal and malignant early B cell development. *Bone Marrow Research* 2011;502751.
- [60] Greaves M. Childhood leukaemia. *British Medical Journal* 2002;324(7332) 283-287.

- [61] Brown WM, Doll R. Leukaemia in Childhood and Young Adult Life. *British Medical Journal* 1961;1(5231) 981-988.
- [62] Hewitt D. Some features of leukaemia mortality. *British Journal of Preventive & Social Medicine* 1955;9(2) 81-88.
- [63] Ramot B, Magrath I. Hypothesis: the environment is a major determinant of the immunological sub-type of lymphoma and acute lymphoblastic leukaemia in children. *British Journal Haematology* 1982;50(2) 183-189.
- [64] Bernaldez-Rios R, Ortega-Alvarez MC, Perez-Saldivar ML, Alatorre-Medina NE, Del Campo-Martinez Mde L, Rodriguez-Zepeda Mdel C, et al. The age incidence of childhood B-cell precursor acute lymphoblastic leukemia in Mexico City. *Journal of Pediatric Hematology/Oncology* 2008;30(3) 199-203.
- [65] Felix CA. Secondary leukemias induced by topoisomerase-targeted drugs. *Biochimica et Biophysica Acta* 1998;1400 233-255.
- [66] Spector LG, Xie Y, Robison LL, Heerema NA, Hilden JM, Lange B, Felix CA, Davies SM, Slavin J, Potter JD, Blair CK, Reaman GH, Ross JA. Maternal diet and infant leukemia: the DNA topoisomerase II inhibitor hypothesis: a report from the children's oncology group. *Cancer Epidemiology, Biomarkers and Prevention* 2005;14(3) 651-655.
- [67] Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites and the hygiene hypothesis. *Science* 2002;296(5567) 490-494.
- [68] Tlaskalova-Hogenova H, Stepankova R, Kozakova H, Hudcovic T, Vannucci L, Tuckova L, et al. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. *Cellular and Molecular Immunology* 2011;8(2) 110-120.
- [69] Qiurong L. Reciprocal Interaction between commensal microbiota and mucosal immune system. *Journal of Immunodeficiency and Disorders* 2012;1(1) 1-2.
- [70] Linabery AM, Jurek AM, Duval S, Ross JA. The association between atopy and childhood/adolescent leukemia: a meta-analysis. *American Journal of Epidemiology* 2010;171(7) 749-764.
- [71] Rivera-Luna R, Cardenas-Cardos R, Martinez-Guerra G, Ayon A, Leal C, Rivera-Ortega F. Childhood acute leukemia and intestinal parasitosis. *Leukemia* 1989;3(11) 825-826.

