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EXPOSURE TO OXYGEN AND PHOTOTHERAPY DURING THE PERINATAL PERIOD AND THEIR POTENTIAL EFFECTS ON ACUTE LYMPHOCYTIC LEUKEMIA

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EXPOSURE TO OXYGEN AND PHOTOTHERAPY DURING THE PERINATAL PERIOD AND THEIR POTENTIAL EFFECTS ON AUCTE LYMPHOCYTIC LEUKEMIA

By

Ali Artaman

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ABSTRACT

EXPOSURE TO OXYGEN AND PHOTOTHERAPY DURING THE PERINATAL PERIOD AND THEIR POTENTIAL EFFECTS ON AUCTE LYMPHOCYTIC LEUKEMIA

By

Ali Artaman

A growing body of evidence suggests that perinatal factors may increase the risk of acute lymphocytic leukemia (ALL), the most common hematological malignancy in children. I propose to conduct a matched case-control study to investigate the relationship of ALL to 3 perinatal interventions: maternal oxygen therapy, neonatal oxygen therapy, and neonatal phototherapy for jaundice. Children with histologically-confirmed ALL diagnosed before age 10 and born in one of the 13 largest hospitals in Michigan from 1985-2002 (n = 200) will be ascertained in the Michigan Cancer Registry. In Phase I, records of ALL cases will be linked by the Michigan Department of Community Health to their birth certificates and to hospital discharge abstracts from the Michigan Inpatient Database. Phase II of this study expands the search for perinatal exposure information by going to hospitals of birth and care of cases and controls, and reviewing maternal and neonatal hospital and NICU medical records. The proposed study would be the first case-control study of this topic in the US, and will provide a more robust estimate of the association of perinatal oxygen and phototherapy use to ALL risk than is available now.

DEDICATION

I dedicate this thesis to my relatives and friends around the world.

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge the support provided by the members of my thesis guidance committee at Michigan State University (MSU): Dr. M. H. Rahbar for his intellectual and academic support for the development of this thesis and sharing his thoughts on relevant methodological and analytical issues, Dr. N. S. Paneth for his critical and constructive feedback particularly on perinatal issues, and Dr. S. A. Omar for his thoughtful advices on clinical implications and issues related to the topic of my thesis.

Secondly, I would like to acknowledge the role of all my instructors throughout my training in the Department of Epidemiology at MSU. The completion of this thesis could have really not been possible without the background knowledge gained through every one of the classes I took in this department.

Finally, I would like to acknowledge the support of the State Registrar, Mr. Glenn Copeland, and his colleagues at Michigan Department of Community Health (MDCH) in providing me with preliminary data and technical information.

PREFACE

The Department of Epidemiology at Michigan State University has different options for completing the requirements of thesis for Master of Science in Epidemiology. For my thesis, I have chosen the option of developing a research proposal.

The topic and the content of my thesis has generated significant enthusiasm among several faculty members at MSU which has led to a productive collaboration among MSU, MDCH and several hospitals in the State of Michigan. These efforts successfully resulted in the preparation and submission of an R03 research grant proposal, in response to Program Announcement PAR-06-294, to the National Institutes of Health (NIH) in November 2006.

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LIST OF ABBREVIATIONS

ALL	Acute lymphocytic (or lymphoblastic) leukemia
AML	Acute myeloid leukemia
BW	Birth weight
CI	Confidence interval
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CNS	Central nervous system
CRIRB	Community Research Institutional Review Board
DB	Data base
DNA	Deoxyribonucleic acid
ELBW	Extremely low birth weight
HR	Hazard ratio
IRB	Institutional Review Board
LBW	Low birth weight
MLBDB	Michigan Live Birth Data Base
MCR	Michigan Cancer Registry
MIDB	Michigan Inpatient Data Base
MDCH	Michigan Department of Community Health
MSU	Michigan State University
NAACCR	North American Association of Central Cancer Registries
NCI	National Cancer Institute

NICU	Neonatal intensive care unit
O_2	Oxygen
OR	Odds ratio
ROS	Reactive oxygen species
SEER	Surveillance, Epidemiology and End Results
SIR	Standardized incidence ratio
VLBW	Very low birth weight

CHAPTER 1: BACKGROUND

SECTION 1.A: INTRODUCTION

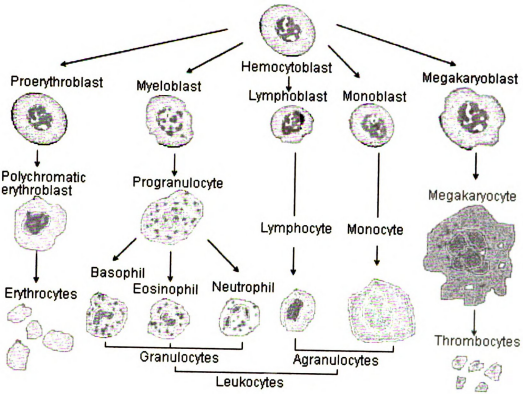
Leukemia (or leukaemia) is a cancer of the blood or bone marrow characterized by an abnormal proliferation of blood cells, usually white blood cells (leukocytes). It is part of the broad group of diseases called hematological neoplasms.

Acute leukemia is characterized by the rapid growth of immature blood cells. Chronic leukemia is distinguished by the excessive build up of relatively mature, but still abnormal, blood cells. When leukemia affects lymphoid cells (lymphocytes and plasma cells), it is called *lymphocytic leukemia*. When myeloid cells (eosinophils, neutrophils, and basophils) are affected, the disease is called *myeloid* or *myelogenous leukemia*.

The signs and symptoms of ALL are variable but follow from bone marrow replacement and/or organ infiltration. They include: generalized weakness and fatigue; anemia; frequent or unexplained fever and infections; weight loss and/or loss of appetite; excessive bruising or bleeding from wounds, nosebleeds, petechiae; bone pain, joint pains; breathlessness; enlarged lymph nodes, liver and/or spleen.

Figure 1 shows a schematic view of blood cell lineage.

Figure 1: Blood cell lineage



Source: SEER's Training Website, NCI

SECTION 1.B: INCIDENCE OF LEUKEMIA

Leukemias comprise about 3% of incident cancers worldwide,[1] but their etiologies remain largely unknown. A relatively high incidence is found in the USA, Canada, Western Europe, Australia and New Zealand.[2]

Table 1 shows age-standardized incidence rates of leukemia from selected cancer registries for the age group 0-14 years.

Table 1: Age (world) standardized incidence rate of leukemia per 100,000, male, age 0-14 years (1997)

Registry	ASR (W)	Cases
Canada	4.96	143
USA, Michigan, Detroit: Black	4.01	6
USA, Michigan, Detroit: White	3.40	10
USA, SEER: Black	3.27	14
USA, SEER: White	4.56	92
USA, Hawaii	6.40	8
China, Hong Kong	3.18	19
India, Bombay	2.87	51
Kuwait: Kuwaitis	1.91	3
Czech Republic	4.82	41
Sweden	5.83	48
UK, England, Oxford	3.99	10
UK, Scotland	7.17	33
Australia, Victoria	4.89	24
New Zealand	7.08	31

Source: Cancer Incidence in Five Continents, International Agency for Research on Cancer[3]

Among children, *leukemia* is the most common cancer, accounting for approximately a third of all childhood malignancies.[4] Of the approximately 2,500 new cases of childhood leukemia diagnosed annually in the US, 80% are ALL, 15% are acute monocytic leukemia (AML), and 5% are chronic leukemias.[5]

Based on cases diagnosed in 2000-2003 from 17 SEER geographic areas, the median age at diagnosis for ALL was 13 years of age. The age-adjusted incidence rate of ALL was 1.5 per 100,000 men and women per year. The median age at death for ALL was 46 years of age. The age-adjusted death rate was 0.5 per 100,000 men and women per year.[6]

Table 2 shows childhood cancer incidence rates in children 0-14 years by site for the period 1998-2002.

Table 2: Childhood cancer incidence rates in children 0-14 years, by site, 1998-2002

Site	Male	Female	Total
All sites	15.6	14.3	15.0
Leukemia	4.9	4.2	4.6
ALL	3.9	3.4	3.6
Brain/ONS	3.6	3.3	3.5
Soft tissue	1.1	0.9	1.0
Non-Hodgkin lymphoma	1.2	0.6	1.0
Kidney and renal pelvis	0.8	1.0	0.9
Bone and Joint	0.6	0.6	0.6
Hodgkin lymphoma	0.6	0.5	0.5

*Per 100,000, age-adjusted to the 2000 US standard population.

ONS: Other nervous system

Source: SEER Program, 1975-2002, Division of Cancer Control and Population Sciences, National Cancer Institute, 2005.

Table 3 shows childhood cancer death rates in children 0-14 years by site for the period 1998-2002.

Table 3: Childhood cancer death rates in children 0-14 years, by site, 1998-2002

Site	Male	Female	Total
All sites	2.7	2.3	2.5
Leukemia	0.8	0.7	0.8
ALL	0.4	0.3	0.4
Brain/ONS	0.8	0.7	0.7
Non-Hodgkin lymphoma	0.1	0.1	0.1
Soft tissue	0.1	0.1	0.1
Bone and Joint	0.1	0.1	0.1
Kidney and Renal pelvis	0.1	0.1	0.1

*Per 100,000, age-adjusted to the 2000 US standard population.

ONS: Other nervous system

Source: SEER Program, 1975-2002, Division of Cancer Control and Population Sciences, National Cancer Institute, 2005.

The overall 5-year relative survival rate for 1996-2002 from 17 SEER geographic areas was 63.7%. Five-year relative survival rates by race and sex were: 63.2% for white men; 65.9% for white women; 52.7% for black men; 56.1% for black women. Based on rates from 2001-2003, 0.12% of men and women born today will be diagnosed with ALL at some time during their lifetime. This number can

also be expressed as 1 in 857 men and women will be diagnosed with ALL during their lifetime.[6]

Some children who are cured experience diminished quality of life because of the long-term effects of their cancer diagnosis and treatment. Simple enumeration of hospital days does not include important elements of *burden* of therapy, such as days in the intensive care unit, operative procedures, radiographic studies, laboratory tests, and consultations. Hospital stay may not reflect outpatient expenses, families' indirect expenses, or the intangible benefits and burdens of treatment, nor does it reflect the possible late adverse effects of therapy.[7]

SECTION 1.C: LITERATURE REVIEW REGARDING THE ETIOLOGY OF ALL

EPIDEMIOLOGIC FEATURES OF ALL SUGGESTING PERINATAL ORIGINS

Childhood leukemia is more prevalent in boys than girls,[4] but unlike most other cancers, ALL has a distinctive age distribution characterized by a rapid increase in incidence after birth, a sharp rise between 2 and 5 years of age, and a decline to a steady low incidence for the rest of childhood.[8] The distinct peak of childhood ALL at ages three to four seen in virtually all surveyed populations,[4] has suggested that a *leukemogenic* event may occur during the fetal or perinatal

period.[9,10] Powerful evidence for this proposition comes from the recent work of Taub et al, who have detected leukemic clones on newborn genetic screening cards (Guthrie cards) of Michigan children later diagnosed with leukemia.[11]

Pregnancy exposures can contribute to human cancer risk, as illustrated most clearly by the finding, in women exposed prenatally to diethylstilbestrol (DES), of a very large excess of clear-cell adenocarcinoma of the vagina.[12] Ionizing radiation during pregnancy raises ALL risk,[13] and another suggestion of prenatal influences derives from the consistent finding that a high rate of fetal growth is linked to ALL.[14] A meta-analysis of 18 studies of the association between birth weight and ALL showed a 30% excess of ALL (OR 1.3, 95% CI 1.2, 1.4) in children who had weighed 4,000 grams or more at birth as well as a dose-response relationship for birth weight (OR 1.14 for each 1,000-g increase, 95% CI 1.08, 1.20).[15]

For reasons to be explained below, two additional perinatal factors, O_2 exposure and phototherapy, might also be risk factors for ALL. In addition, unlike high birth weight, both of these commonly used perinatal interventions are under medical control, and may well be overused in current perinatal practice, opening a door to prevention should causality be established.

THE BIOLOGICAL RATIONALE FOR LINKING O_2 TO ALL

O_2 saturation is <60% in the fetus, a level that would be considered pathological if experienced later in life.[16] While the healthy newborn normally experiences a rise in blood and tissue levels of O_2 upon exposure to room air, in one study median O_2 saturation was just 63% (interquartile range of 53%-68%) in healthy infants at 1 minute of age, and rose gradually to 90% (79%-91%).[17] The process of transitioning to a normal postnatal O_2 saturation requires at least 5 minutes in healthy newborns breathing room air.[18]

The widespread practice of using pure oxygen immediately after birth to resuscitate depressed newborns may thus easily overshoot physiological norms, and has become an increasingly controversial therapy in newborn medicine. A randomized trial comparing room air to 100% O_2 has shown that asphyxiated term infants resuscitated with pure O_2 take longer to produce their first cry than do controls treated with room air (1.7 ± 0.5 vs.1.2 ± 0.6 minutes), and need longer to achieve a sustained respiratory pattern when ventilated (7.5 ± 1.8 vs. 4.6 ± 0.7 minutes).[19]

Higher and more persistent levels of biochemical markers of cardiac and renal damage in asphyxiated infants have also been found in asphyxiated infants treated with pure O_2 compared to room air recipients.[20] Indeed, a systematic

review of five trials of this topic found evidence of higher mortality in infants resuscitated with 100% O_2.[21] It should be noted, however that in the judgment of a Cochrane review, only two of the trials were randomized and blinded, while the other three were *quasi-randomized* and not blinded.[22] Moreover, a more recent published report of the International Liaison Committee on Resuscitation (ILCOR) recommends use of supplemental O_2 if resuscitation in room air has not produced a satisfactory clinical response in 90 seconds.[23]

Of particular relevance to the hypothesis of the proposed study is the finding that O_2 supplementation in newborns produces persistence of reactive O_2 species (ROS), detectable for as long as 30 days after even brief exposures.[24] In one trial comparing 100% O_2 to room air for immediate newborn resuscitation only, the reduced-to-oxidized glutathione ratio was significantly lower in the pure O_2 group, revealing protracted oxidative stress, and the activities of superoxide dismutase and catalase in erythrocytes were 69% and 78% higher in the pure O_2 group at 28 days of age.[17]

Oxidative stress may contribute to newborn tissue injury through mechanisms similar to those produced by inflammation,[25] and may also participate in the carcinogenic process. The complex series of cellular and molecular changes in cancer development may include endogenous damage arising from oxygen-free radicals (OFRs), intermediates of oxygen reduction that attack both DNA bases

and the deoxyribosyl backbone of DNA. Improvements in analytical techniques in recent decades have permitted the identification and quantification of adducts of OFRs with DNA. The most extensively studied lesion is 8-hydroxydeoxyguanine (8-OH-dG), which causes mutations by producing GC→TA transversions. But OFRs can attack other cellular components such as lipids, leaving behind reactive species that can also couple to DNA bases. ROS can also act as secondary messengers in intracellular signaling cascades, which induce and maintain the oncogenic phenotype of cancer cells.[26] These observations have led to the widely held view that OFRs are an important class of carcinogens,[27] and that intervention with antioxidants may be an important preventive modality in cancer.[28]

The widespread use of O_2 supplementation in newborns, the persistence of ROS in newborn blood for at least a month, the known physiological susceptibility of newborns to oxygen toxicity, and the strong suggestion that ROS are carcinogenic, provide a solid biological substrate for the epidemiologic observations linking oxygen exposure in newborns to childhood cancer, and especially leukemia, to be reviewed below.

EPIDEMIOLOGIC EVIDENCE FOR THE LINK OF O_2 TO ALL

Five studies have examined childhood leukemia in relation to exposure to O_2 in the newborn period (Table 4). Four Swedish case-control studies, some of which did not clearly distinguish leukemia subtypes, have found significant odds ratio (OR)'s of 2-3 for the association of childhood leukemia with exposure to O_2. Cnattingius et al showed that risk of ALL, across all childhood age groups, increased with use of supplementary O_2 (OR 2.3; 95% CI 1.5-3.6).[29] Notably, the risk was stronger than that found for postpartum asphyxia (OR 1.8; 95% CI 1.2-2.6), which is a major risk factor for O_2 administration.

A more recent Swedish case-control study showed that resuscitation with 100% O_2 with a facemask and bag immediately postpartum was associated with a significantly increased risk of ALL (OR 2.6, 95% CI 1.2-6.8).[30] The risk increased further if ventilation lasted for 3 minutes or more (OR 3.5, 95% CI 1.2-10.8). Low Apgar scores at 1 and 5 minutes were associated with an increased risk of ALL in this study, but not significantly so.

Table 4: Childhood leukemia or cancer and neonatal O_2 exposure (*significant OR's bolded*)

Principal Author, Publication Year	Population	Cancer Type	Study Design	# Cases	# Controls	Main Exposure	OR
Zack, 1991	Sweden	ALL, AML	Matched case-control	411	2,055	Supplemental O_2 ICD-8 codes 93.32 and 93.70)	**2.6**
Cnattingius, 1995	Sweden	AML CML, type unspecified	Matched case-control	98	490	Supplemental O_2	2.3 *
Cnattingius, 1995	Sweden	ALL, CLL, type unspecified	Matched case-control	613	3,065	Supplemental O_2	**2.3**
Naumburg, 2002	Sweden	ALL, CLL, type unspecified	Matched case-control	578 Excluding Down syndrome	578	100% O_2 by face mask postpartum (Ref: No O_2 by mask)	**2.6**
						100% O_2 > 3 minutes (Ref: No O_2 by mask)	**3.5**
Spector, 2005	USA	All childhood cancers	Historical Cohort (CPP Cohort)	48 16 ALL or AML	54,795 live births	Open/positive -pressure O_2 <2 minutes (Ref: No O_2 exposure)	0.7 **
						Open/positive -pressure O_2 > 3 minutes, all children	**2.9** **

Table 4
(continued)

Open/positive -pressure O_2 > 3 minutes, children > 1 year	2.0 **

* 1.7 if Down syndrome cases excluded (84 cases and 420 controls)

** Hazard Ratio (HR)

In a recent large cohort study in the USA, the hazard ratio (HR) for all childhood cancers was 2.9 (95% CI 1.5 to 5.7) with exposure to 3 or more minutes of O_2.[31] Exposure for 2 minutes or less did not raise the risk. In this study too, markers of birth asphyxia were found more commonly in children who later developed childhood cancer, but as in the other two studies, the effect was not as strong as O_2 exposure.

Paneth underlined in his editorial comment on this US study, "on the grounds of both biologic plausibility and strength of association, O_2 seems a more likely determinant of cancer than does birth depression".[32] This editorial comment also noted that if this relationship was truly causal, 1 in 7 childhood cancers would be prevented by avoidance of use of supplemental O_2 in neonates.

The US study, the only cohort study among those summarized in the table, did not have sufficient power to assess the effect of neonatal O_2 therapy on ALL alone.

Laboring women are also commonly treated with 100% O_2, but the subject of maternal oxygen supplementation has not so far been investigated for its relationship to offspring childhood cancer. Maternal O_2 supplementation improves the transport of O_2 to fetus but does not appear to have dramatic effects on fetal oxygen saturation levels.[33] Since maternal O_2 supplementation in

the perinatal period is a very common exposure, I propose to assess it in the proposed study for its potential link to ALL.

THE BIOLOGICAL RATIONALE FOR LINKING PHOTOTHERAPY TO ALL

Approximately two thirds of the more than 4 million neonates born annually in the US become clinically jaundiced.[34] Phototherapy is a simple, easy-to-use tool that reduces serum indirect hyperbilirubinemia by breaking down bilirubin into other molecules thereby preventing the kernicteric brain damage that can occur in neonates with severe hyperbilirubinemia. Approximately 9% of newborns in the US receive phototherapy,[35,36] even though the vast majority do not have jaundice severe enough to lead to significant harm if left untreated.

Physiologic jaundice in premature neonates is more severe than in full-term neonates, with mean peak total serum bilirubin (TSB) concentrations reaching 10 to 12 mg/dl (171 to 205 μmol/L) by the fifth day of life.[37] Thus premature babies, including babies born mildly prematurely and not treated in newborn intensive care units, are especially likely to receive phototherapy.

Despite its widespread use, phototherapy is not well standardized in practice in the US, and a variety of devices delivering phototherapy with varying efficacies are produced and used. Bilirubin absorbs light maximally in the blue range (340 to 540 nm), and daylight and cool white lamps which have a spectral emission of

370 to 430 nm may be less effective than blue lamps which have a narrower spectral range which peaks between 420 and 480 nm.[38] Many light sources used in phototherapy produce significant quantities of UV light.[39]

Ben-Sasson and Davis offer the hypothesis that exposure to photosensitizing lighting immediately after birth may be a contributing cause of ALL because fluorescent lamps and other light sources with strong illumination – around 400 nm – are protoporphyrin-activating. Activation of protoporphyrin produces superoxides and free radicals that can induce breaks in DNA.[40] Untoward effects of phototherapy on DNA have been demonstrated in vitro.[41] However, the specific chemical nature of DNA damage induced by fluorescent light has not been well determined. A team of investigators have detected induction of oxidative DNA lesions in cultured cells irradiated with fluorescent light.[42]

As early as 1937, Najib-Farah demonstrated a protective effect of bilirubin in bacterial infections.[43] Enzymatic conversion of biliverdin to bilirubin by the enzyme biliverdin reductase results in protection from oxidative stress.[44] The bilirubin-biliverdin interconversion cycle amplifies the *antioxidant* actions of bilrubin.[45] While VLBW infants are more susceptible than term infants to CNS toxicity from indirect hyperbilirubinemia, bilirubin may play a particularly beneficial role as an antioxidant and enzyme inducer in these vulnerable infants[46] The carcinogenicity of phototherapy may thus arise from either or both of two

pathways. In the process of breaking down bilirubin, phototherapy may increase ROS, or phototherapy may release carcinogenic substances as a result of bilirubin breakdown.

EPIDEMIOLOGIC EVIDENCE FOR THE LINK OF PHOTOTHERAPY TO ALL

Three studies have examined childhood leukemia following phototherapy in the newborn period (Table 5), two of which addressed ALL. A Dutch register-based matched case-control study reported a higher frequency of phototherapy among 519 children (1.3 percent) with ALL than among 507 control children (0.2 percent).[47] However, the total number of exposed children was small and the difference in relative frequency was not statistically significant.

Table 5: Childhood leukemia or cancer and phototherapy

Principal Author, Publication Year	Population	Cancer Type	Study Design	# Cases	# Controls	Main Exposure	OR
Van Steensel-Moll, 1992	The Netherlands	ALL	Matched case-control	238	196	Phototherapy (blue light 420-470 nm)	3.6
Cnattingius, 1995	Sweden	AML CML, type un-specified	Matched case-control	98	490	Phototherapy	7.5 *
Olsen, 1996	Denmark	All childhood cancers	Cohort	87 (28 ALL)	55,120 Mature neonates with hyper-bilirubinemia	Phototherapy (prolonged irradiation with light at wavelengths of 420-470 nm), all childhood cancers	1.0 **
						Phototherapy ALL	1.1 **

*4.3 if Down syndrome cases excluded.

** Standardized incidence ratio

In a Danish study, among 55,120 newborns, 9% received phototherapy, which consisted of irradiation with light at wavelengths of 420-470 nm.[48] Linkage of the birth cohort with the national cancer registry through 1991 revealed 34 children with leukemia (28 with ALL), and no excess risk among those receiving neonatal phototherapy (SIR 1.2, 95% CI 0.8-1.7). Subgroup analyses revealed no remarkable patterns for any category of leukemia subtype, gender, or age at diagnosis (follow-up age of up to 14 years).

In contrast, Cnattingius et al found that both physiological jaundice (OR 2.5; 95% CI 1.2-5.0) and phototherapy (OR 7.5; 95% CI 1.8-31.9) were associated with leukemia.[49] Thus the evidence on the role of phototherapy in the process of leukemogenesis is more limited than is the case for O_2, but is nonetheless suggestive, with a strong and highly significant OR in the largest study. No studies have yet assessed the possibility that phototherapy and oxygen treatment might interact or be synergistic in their contribution to risk of ALL.

SECTION 1.D: CRITICAL APPRAISAL OF THE EXISTING LITERATURE

OXYGEN THERAPY

During the past two decades, a number of studies have been published that focus separately on risk factors for lymphocytic and non-lymphocytic leukemias.

Compared with *lymphocytic* leukemia among children, less is known about specific risk factors for childhood *myeloid* leukemia, a malignancy with a much lower 5-year survival rate. Because less than 20% of all childhood leukemias are myeloid, conclusions from analytical studies on total childhood leukemia may not apply to myeloid leukemia.

The study published by Zack et al in 1991,[50] as stated by Cnattingius et al in1995,[29] was the first to link use of supplementary oxygen to increased risk of childhood *lymphatic* leukemia. Zack's study describes an *exploratory population-based* study of maternal and perinatal risk factors for childhood leukemia in Sweden. The Swedish National Cancer Registry ascertained 411 cases in successive birth cohorts from 1973 through 1984 recorded in the Swedish Medical Birth Registry. Using the latter, the investigators matched 5 controls without cancer to each case by *sex* and *month* and *year* of birth.

Mothers of children with leukemia were more likely to have been exposed to nitrous oxide anesthesia during delivery than mothers of controls [OR 1.3; 95% CI 1.0, 1.6]. Children with leukemia were more likely than controls to have Down syndrome (OR 32.5; 95% CI 7.3, 144.0) or cleft lip or cleft palate (OR 5.0; 95% CI 1.0, 24.8); to have had a diagnosis associated with difficult labor but unspecified complications (OR 4.5; 95% CI 1.1, 18.2) or with other conditions of the fetus or newborn (OR 1.5; 95% CI 1.1, 2.1), specifically, uncomplicated

physiological jaundice (OR 1.9; 95% CI 1.2, 2.9); or to have received supplemental oxygen (OR 2.6; 95% CI 1.3, 4.9).

Because multiple potential risk factors were analyzed in this study, future studies were needed to check these findings. Zack et al did not confirm the previously reported higher risks for childhood leukemia associated with being male, having a high birth weight, or being born to a woman of advanced maternal age. Bilirubin measurements were unavailable from the data sources used for this study; the presence of kernicterus or the use of phototherapy or exchange transfusions was not mentioned in the records of the study subjects.

The *new* findings of this study included an increased risk of childhood leukemia associated with the mother's exposure to nitrous oxide anesthesia during delivery, the child's physiological jaundice without demonstrable contributory conditions, the child's use of supplemental oxygen, and diagnosis of cleft lip or cleft palate in the child.

The use of supplemental oxygen, in Zack's study, was rare; only 1.5% of controls and 4% of cases received supplemental oxygen. The use of supplemental oxygen was not always recorded when it may have or should have been used (e.g., when asphyxia occurred soon after birth). The association of ALL with jaundice could be confounded by phototherapy, which Zack et al did not assess.

In the study conducted by Cnattingius et al and published in 1995,[49] which extends the number of births, cohorts, and follow-up through 1989, the analyses of maternal and perinatal risk factors were based on all childhood *myeloid* leukemias, as well as restricted to myeloid leukemias without Down syndrome. Cases were children born in Sweden from 1973 through 1989 who had been registered in the Birth Register and who had subsequently been diagnosed with myeloid leukemia and ascertained in the National Cancer Register through 1989.

A total of 98 cases of myeloid leukemia were identified. For each case, the investigators selected 5 controls (490 in all) matched by *sex*, birth *year*, and birth *month* from those in the source population who, according to the Register of Causes of Death, survived at least to the age that their matched case was diagnosed, without themselves having been diagnosed with myeloid leukemia before that date.

Children with myeloid leukemia were overall more than twice as likely to have had neonatal physiological jaundice (ICD-8 codes 775 and 778.93-778.98). Exposure to phototherapy, a treatment for neonatal jaundice, increased risk for myeloid leukemia more than 7-fold, and incubator use increased this risk by more than 3-fold. When cases with Down syndrome were excluded, odds ratios for these exposures decreased, and their lower 95% confidence limit overlapped 1.0.

Cnattingius et al state, "Our previous study of childhood leukemia[50] is one of the few that separately evaluated possible associations of pregnancy-related and perinatal risk factors for myeloid leukemia of childhood." Cnattingius' study extends the number of birth cohorts and the duration of follow-up so that the number of cases more than doubled. A notable increased risk for myeloid leukemia was above all associated with Down syndrome in both studies. It should be pointed out that the 9 cases of Down syndrome reported in the previous study were also included among the 14 cases of Down syndrome in the present study.

In both studies, birth defects in general, heart defects, and physiological jaundice also increased risk for *myeloid* leukemia. In Cnattingius' study, this risk was also increased among children whose mothers were <20 years old at delivery, had a history of at least one spontaneous abortion, had smoked cigarettes daily during early pregnancy, had hypertension during pregnancy, or had undergone Cesarean section. Children who had been a member of a multiple birth, who had undergone phototherapy, or who had used an incubator also had an increased risk for myeloid leukemia.

The population-based, nationwide selection of cases and comparable controls suggests that *selection bias* is unlikely to account for these associations. Furthermore, exposure data are obtained prospectively in a standardized fashion for virtually all births. As the collection of exposure data precedes the diagnosis

of myeloid leukemia, this precludes *recall bias*. Although some exposures may have been underreported, this underreporting should not differ between cases and controls but would reduce statistical power to detect any effects.

When children with Down syndrome diagnosed at or soon after birth were excluded, risks of myeloid leukemia, although reduced, continued to be elevated (not-significantly) among neonates with physiological jaundice and among those treated with phototherapy or in an incubator. Physiological jaundice has been associated previously with *lymphatic* rather than myeloid leukemia, and the suggested pathway is that exposure to phototherapy generates free radicals that carcinogenically transform lymphoblasts in the newborn.

The first paper primarily *focused* on assessing the association between supplementary oxygen and leukemia was published by Naumburg et al in 2002.[30] He, indeed, published the most recent *case-control* study on this topic. Cnattingius, the first author of the two previous studies on birth-related risk factors of childhood leukemia was a co-investigator of this study. Naumburg et al state, "In a previous register-based case–control study,[29] exposure to supplementary oxygen was for the first time reported as a risk factor for childhood *lymphatic* leukemia."

With more detailed exposure data and a large number of cases, Cnattingius' study on childhood *lymphatic* leukemia confirmed this association, particularly

among older children, even after adjustment was made for postpartum asphyxia. Because the investigators were unable to quantify exposure to oxygen, they could not investigate possible *dose-response* relationships between oxygen exposure and the risk of *lymphatic* leukemia.

In Cnattingius' study, the exposure data were crude, and information on dose and duration, and to some extent the underlying reasons for oxygen treatment, were lacking. The objective of Naumburg's study was to achieve a better understanding of the association between supplementary oxygen treatment, other perinatal factors and the subsequent risk of childhood lymphatic leukemia, through conducting a population-based case–control study in Sweden.

In Naumburg's study, cases were children with lymphatic leukemia (ICD-7 and ICD-8 codes 204.0, 204.1, 204.3, 204.4, 204.7, 204.9, 206.0, 206.9, 207.0, 207.9) up to the age of 16 years born and diagnosed with lymphatic leukemia between 1973 and 1989. In total, 658 cases were identified through the Swedish Cancer Register, which includes 97% of all patients with cancer. Controls were randomly selected from the Swedish Birth Register, which includes data on 99% of all births in Sweden. One control was individually matched to each case subject with regard to *gender*, birth *year* and *month*. The control had to be alive and without cancer on the date when the case was diagnosed with lymphatic leukemia. By linkage to the Swedish Cancer Register, the Register of Causes of Death and the Swedish Medical Birth Register, using the *unique* 10 digit national

registration number, the living and non-cancer status of the control was confirmed.

In fact, a majority of subjects in this study had been identified through Cnattingius' study, and chance could not be ruled out. However, the data collection of Naumburg's study was blinded with regard to case or control status and the information was based on medical records and not register data as in Cnattingius' study.

The strengths of Naumburg's study are the population-based design, the large number of cases, the small amount of missing information and the small number of excluded cases. Only 12% of the cases and controls had to be excluded from the original study base because the medical records of the case or control were not found. The loss of subjects was non-differential with regard to exposure to oxygen. The medical records used at antenatal and delivery centers in Sweden were standardized and the records were retrieved in a uniform way. Adjustments were made for potential confounders (such as mother's age, parity and maternal smoking habits), and children with Down syndrome, an accepted risk factor for lymphatic leukemia, were excluded.

> *Naumburg et al underline,"owing to the high number of neonates exposed to oxygen in medical practice, further studies are warranted."*

The US study published by Spector et al in 2005 [31] has several obvious shortcomings some of whom commented on by Paneth.[32] The analysis of Spector et al of the venerable data of the National Collaborative Perinatal Project (NCPP) describes a slightly higher risk of cancer in children exposed to >3 minutes of oxygen in the delivery room than in children without oxygen exposure. A range of variables were examined, and none seemed capable of explaining the association by confounding. Low Apgar score at 1 minute (but oddly, not at 5 minutes) was also associated with cancer risk, but less convincingly than oxygen.

Paneth states, "It is not unreasonable to suppose that if oxygen is a cancer risk, a low Apgar score would appear to be also, because the two phenomena necessarily cluster together, but the article does not mention whether controlling for oxygen eliminates the low Apgar association."

The results of Spector's study are consistent with those reported by Naumburg et al,[30] who had found a significant odds ratio of 2.6 for resuscitation with 100% oxygen with a face mask and bag after birth, and increased to 3.5 if manual ventilation lasted for 3 minutes or more. The largest fraction of cancer cases in Spector's study were leukemias. The study concluded that Resuscitation with 100% oxygen ventilation immediately postpartum is associated with an increased risk of childhood *lymphatic* leukemia, and the risk was even more pronounced when the resuscitation lasted for 3 min or more. The association between resuscitation with 100% oxygen and childhood lymphatic leukemia was even

stronger when the duration of exposure was 3 min or more. This can be interpreted as a dose-response relationship. However, the results are based on small numbers (oxygen by mask 3-10 min, 14 cases, 4 controls, OR 3.54, 95%CI 1.16-10.80, p-value 0.026) and chance cannot be ruled out.

The investigation conducted by Spector et al is the *only* study in the US assessing association of *oxygen* therapy and *childhood cancer.* However, the data used for this study is not population-based (tendency towards *selection bias*). The Collaborative Perinatal Project (CPP) enrolled approximately 48,000 women, who had about 60,000 pregnancies, at 12 university-affiliated medical centers between 1959 and 1966.[51] Since phototherapy began to become a standard intervention for the treatment of neonatal jaundice in 1970s it was not included in this study.

Briefly, trained observers in delivery rooms recorded the details of 54,795 live births using a standardized methodology. Children received multiple physical examinations in the first year of life and again at age 7 years. Parents were interviewed several times in the first 2 years and annually thereafter until their children were age 7 or 8 years (depending on the study site).

Low power of Spector's study is a major concern. Fifty-one cancers were identified in the cohort and subsequently confirmed by 2 pediatricians. Three infants who were diagnosed with cancer within the first week of life were

excluded from the analysis. Supplemental O_2 was recorded with respect to the mode of delivery (*open* oxygen or *positive-pressure* oxygen), with the duration of each recorded in whole minutes. Newborns were considered to be exposed to O_2 if there was an affirmative response to either variable. Length of O_2 was the sum of the 2 durations.

Spector et al state, "this study's major strength is its prospective design; its major weakness is its small number of cases. Several other caveats also apply in the interpretation of these results."

An interesting point with respect to the measurement of the main exposure is that O_2 was not significantly associated with childhood cancer when analyzed as a *continuous* variable. There was a long right tail to the distribution of O_2 exposure duration such that 14.5% of exposed children (n = 1115) had durations greater than 10 minutes. Spector et al underline, "The fact that no cancers occurred among the children with the very longest durations of exposure diminishes the argument for an association of O_2 with cancer in childhood."

The percentage of O_2 that the newborn children in the CPP cohort received was not documented. Also, the investigators counted infants as exposed if O_2 was delivered by *hose* or *loose mask*, either of which can administer a lower fraction of inspired oxygen than is intended. Thus the investigators could not verify the

concentration of O_2 received by each infant. In contrast, Naumburg et al found a significant association of leukemia specifically with administration of 100% oxygen by *bag* and *mask*.

According to Spector et al, an alternate explanation for these results is that O_2 may be a proxy for a poor transition to extrauterine life. The investigators adjusted for markers of neonatal distress to examine this possibility. The association of prolonged O_2 with childhood cancer remained significant when adjusted for low 5-minute Apgar score and the presence of meconium at birth. Adjustment for low 1-minute Apgar score attenuated the association, but this may have been a function of missing data. Their findings appeared to be independent of at least 2 markers of neonatal distress.

Finally, the magnitude of the association that Spector et al observed was low and thus could be due to confounding by a factor that they did not examine. They also cannot rule out the possibility that their findings were due to chance. However, their findings do corroborate those of a few previous case-control studies, and there is some plausibility to the idea that such a brief exposure as O_2 could have long-term sequelae.

> *Spector et al recommend, "Special consideration may be given to the effect of O_2 on preterm infants...Many studies of childhood cancer have obtained delivery records as part of data collection. We encourage any*

investigators who have such data to examine the history of oxygen supplementation."

PHOTOTHERAPY

In the study conducted by van Steensel-Moll et al in the Netherlands,[47] based on stratified analysis of the ALL cases born in hospital, an OR of 3.6 (95% CI 0.9-57.4) was reported for hospitalization due to neonatal hyperbilirubinemia. As this investigation was not designed to study the association between fluorescent light and childhood leukemia, no detailed information concerning *intensity* and *duration* of potential exposures was available. The investigators, therefore, underline, "to confirm the hypothesis of Ben-Sasson and Davis,[40] epidemiologic studies with specific questions concerning fluorescent light exposure in the neonatal period will be necessary."

To test the hypothesis that exposure to high intensity lightning (around 400 nanometers) in neonatal nurseries increases the incidence of childhood leukemia, over 55,120 newborn children treated with phototherapy for hyperbilirubinemia were identified from the Danish Hospital Discharge Register for 1977-89.[48] The large series of over 55,000 newborn children of whom 85 to 90 percent were exposed to intense light during treatment for hyperbilirubinemia revealed no increase in childhood leukemia or other childhood cancers.

According to Olsen et al, "children with hyperbilirubinemia were identified from the Hospital Discharge Register prior to the registration of the cancer outcome, so that any bias caused by selection of study subjects is unlikely. The study was population-based, and the cases were identified from a high-quality national cancer registry, with little evidence for underreporting."

SECTION 1.E: THE PROPOSED STUDY

The proposed study would be the first case-control study of neonatal exposure to either supplementary O_2 or to phototherapy in relation to childhood leukemia conducted in the US. It would also be the first ever epidemiologic study on the association between maternal exposure to supplementary O_2 and childhood leukemia. No study of neonatal O_2 exposure and leukemia in the US has been conducted since the CPP, which studied births from 1959-1966.[31]

I plan to model the association between outcome and exposures, controlling for several variables and taking account of effect modification. Except for the CPP, whose data base is now more than 40 years old, no epidemiologic study of this topic has used a data source other than an administrative data base. Thus the proposed study, which will include review of medical records of mothers and babies, is innovative in this regard as well.

SECTION 1.F: AIMS OF THE PROPOSED STUDY

OVERALL OBJECTIVE

The objective of the proposed study is to provide a better understanding of perinatal factors associated with ALL, the most common hematological malignancy in children, with particular attention to maternal and neonatal oxygen (O_2) therapy and neonatal phototherapy.

SPECIFIC AIMS

1. To examine the association between supplemental neonatal O_2 therapy and childhood ALL. *I hypothesize that supplemental neonatal O_2 therapy for 3 minutes or longer increases the risk of childhood ALL, controlling for potential confounders.*

2. To examine the association between supplemental maternal O_2 therapy during delivery and childhood ALL. *I hypothesize that maternal O_2 therapy for 30 minutes or longer during delivery increases the risk of childhood ALL, controlling for potential confounders.*

3. To examine the association between neonatal phototherapy and childhood ALL. *I hypothesize that neonatal phototherapy for 1 day or longer increases the risk of childhood ALL, controlling for potential confounders.*

4. To use multivariate methods to carefully assess potential confounding variables, particularly factors such as birth depression and jaundice, that lead to use of O_2 and phototherapy, to determine the independent contribution of these treatments to ALL risk, and to examine interactions of interest. *I hypothesize that after adjustment for risk factors for treatment and other potential confounders, the associations of maternal and neonatal O_2 and phototherapy with ALL will remain.*

CHAPTER 2: METHODS

SECTION 2.A: OVERVIEW OF STUDY DESIGN

I propose to undertake a matched case-control study in the State of Michigan, a state with a large and heterogeneous population and well-established cancer and birth registries. I will link childhood ALL cases in the state to 3 other databases, the Michigan Live Birth Data Base (MLBDB), the Michigan Inpatient Data Base (MIDB) of discharge abstracts for mothers and infants and to hospital records of mothers and infants.

The first phase of this study is the linkage of cancer cases to the state live birth and inpatient data bases, all three of which are computerized files housed in the MDCH, and the latter two are already linked. This phase will provide information on neonatal exposures to ventilatory therapy, the only measure of neonatal O_2 exposure on birth certificates (personal communication from MDCH indicates that from 1991-2004, among 1,902,365 Michigan births, 35,944 (1.9%) received *assisted ventilation*).

It will also provide access to duration of hospital stay, which, like assisted ventilation, identifies a subset likely to have received O_2, as well as major procedures, including phototherapy use. Although the birth certificate provides limited exposure information, the file provides the pool from which to identify

matched control infants. Once ALL cases are selected, the MLBDB will be used to identify controls as described below.

In the second phase of the study, the study team will conduct hospital and NICU medical record abstraction in the 13 largest hospitals of the state to obtain data on the exposures of interest in cases and controls. Medical records are the only source that allows assessment of neonatal exposure to O_2, maternal O_2 therapy, neonatal phototherapy, and exposure to potential confounding and interacting variables.

Neither phase of this study involves contact with study subjects and/or their legal guardians. No biological samples will be collected nor analyzed as part of this study. Only the recorded data archived at the state-wide cancer, live birth, and inpatient registries and designated hospitals will be used as research material for this study.

SECTION 2.B: DATA SOURCES

Michigan Cancer Registry (MCR): This population-based register receives documentation of cancer cases from pathology departments, medical records departments, and death certificates. Although the NCI-funded Detroit SEER Registry has conducted comprehensive cancer surveillance since 1969 for Wayne (including Metropolitan Detroit), Oakland and Macomb counties since

1973, the rarity of childhood cancers means that three counties will not provide enough statistical power to conduct the proposed study.

The MCR follows the registration guidelines provided by the NAACCR, a professional organization that develops and promotes uniform data standards for cancer registration, provides education and training and certifies population-based registries, and has received that organization's highest rating (personal communication with MDCH). In 1994, Paneth et al conducted a study that assessed the completeness of the then new Michigan Cancer Registry. In 12 randomly selected hospitals in Michigan, better than 92% of cancers identified in discharge summaries were found in the cancer registry. For all cancers < age 21 years, the figure was 100%.[52]

Michigan Live Birth Data Base (MLBDB): In 2004, 128,572 live births were entered in the MLBDB, which maintains computerized records of all live births in the state since 1970. The filing of birth certificates occurs in local registrars' offices located throughout the state. These local offices receive certificates from hospitals, and review, file and forward the documents to the state office where each is incorporated into the MLBDB.

Michigan Inpatient Data Base (MIDB): The MIDB is a comprehensive source of all-payer, patient-level data, which includes virtually all inpatient activity at Michigan acute care hospitals. MIDB contains more than 100 clinical and

nonclinical variables included in a hospital discharge abstract, such as: principal and secondary diagnoses, principal and secondary procedures, admission and discharge status, patient demographics (e.g., gender, age, and, for some States, race), expected payment source (e.g., Medicare, Medicaid, private insurance, self-pay; for some States, additional discrete payer categories, such as managed care), total charges and length of stay.

Hospital Records of mothers and newborns: Appropriate documentation of neonatal health status in delivery room and/or neonatal intensive care unit and maternal health status in delivery room, particularly in the largest hospitals in the State of Michigan, provides an opportunity for detailed measurement of hospital-related exposures. The hospitals designated for the proposed study have security guidelines for clinical research to ensure that all confidential health information is protected.

SECTION 2.C: SOURCE POPULATION

Live births in Michigan from 1985 through 2002 are the source population for study subjects. From 1985 to 2002, 2,160 children under 21 years of age resident in the state of Michigan at the time of diagnosis with leukemia are recorded in the Michigan Cancer Registry (personal communication, MDCH). It can be assumed that the vast majority of these cases were born in Michigan.

Table 6 shows age-specific incidence rates of ALL, AML, CLL, and CML in the State of Michigan in the period 1985-2002.

Table 6: Numbers of leukemia cases and age-specific incidence rates per 100,000 population in Michigan residents under age 10 years, 1985 - 2002

Age at Diagnosis (y)	ALL		AML		CLL		CML	
	Number	Rate	Number	Rate	Number	Rate	Number	Rate
< 5 years	678	5.4	119	1.0	2	*	22	0.2
5-9 years	333	2.6	56	0.4	3	*	10	0.1

** Rate cannot be reliably estimated due to small sample size.*

SECTION 2.D: STUDY SUBJECTS

Case subjects are children up to 10 years of age (the age group with the highest risk of ALL) who were born in one of 13 designated hospitals in Michigan from 1985 through 2002, who are listed as live births in the Michigan Live Birth Data Base (MLBDB), and who were diagnosed with ALL (histologically confirmed), and are recorded in the Michigan Cancer Registry (MCR). Medical diagnoses have been coded according to ICD-9 (code 204.0 for ALL). Cases of Down syndrome with leukemia will be excluded from our study.

Table 7 shows that for ALL under the age of 10, the target population, more than 1,000 cases are available. The designated hospitals are the largest delivery services in the state and account for about 40% of all births (2004 data). Thus more than 400 cases should be available for sample selection. Restriction to the

largest hospitals is done for efficiency, and to avoid having to obtain IRB approval and to arrange record abstraction from small hospitals with very few cases. While the proposed study is thus not state-wide, it does include a large cross-section of hospitals in major cities in Michigan.

SECTION 2.E: CONTROL SUBJECTS

Controls will be selected from the MLBDB. I will match each case to two control children surviving infancy, born of the same *gender*, *ethnicity* (Hispanic, Black, Non-Hispanic White, Asian, Other), *birth weight* (± 250 grams) and *gestational age* (± two weeks) and born in the same *month* as the case in the same *hospital*. Matching on these variables should minimize potential confounding by birth characteristics that may be correlated with O_2 use or cancer.

Cases and the controls will also be linked to the hospital discharge abstracts which are recorded in the MIDB, which is already linked to the MLBDB. MDCH will assign a random number to all live births, exclude any cases from the file and then select controls matching the selection criteria in a random sequence.

SECTION 2.F: INCLUSION OF WOMEN AND MINORITIES

The proposed study includes mothers and their infants. ALL is about equally divided between males and females and there is no strong evidence of racial or ethnic differences in prevalence. No racial/ethnic group will be excluded or

included preferentially. The study team will attempt to recruit a diverse groups of study subjects whose racial and ethnic backgrounds reflects the proportion of these minorities in their area (2000 census data for Michigan: 80% white, 14% black, 0% Native Hawaiian, 2% Asian, < 1% American Indian, 3% other races, and 3% Hispanic) as well as an equal number of males and females. Table 7 shows the number of ALL cases by year of diagnosis, race and gender in the State of Michigan for the period 1985-2003.

Table 7: Number of ALL cases by year of diagnosis, race and gender (Michigan residents, 1985-2003)

Numbers of Acute Lymphoblastic Leukemia (ALL) Cases

by Year of Diagnosis, Race and Gender

Michigan Residents, 1985 - 2003

Year of Diagnosis	All Races			White			Black		
	Total	Male	Female	Total	Male	Female	Total	Male	Female
1985	148	76	72	138	71	67	9	4	5
1986	150	100	50	128	88	40	18	11	7
1987	150	80	70	137	75	62	11	5	6
1988	155	90	65	135	81	54	16	6	10
1989	176	89	87	159	78	81	14	9	5
1990	156	100	56	137	85	52	15	12	3
1991	181	104	77	154	91	63	23	10	13
1992	139	79	60	125	72	53	9	4	5
1993	166	105	61	141	91	50	17	10	7
1994	152	89	63	135	81	54	11	5	6
1995	130	70	60	113	62	51	11	5	6
1996	140	74	66	124	70	54	9	4	5
1997	144	88	56	132	81	51	9	6	3
1998	133	82	51	111	68	43	18	12	6
1999	124	71	53	112	64	48	10	5	5
2000	165	96	69	151	89	62	13	7	6
2001	110	56	54	97	52	45	6	2	4
2002	99	61	38	83	54	29	10	5	5
2003	86	52	33	71	43	27	7	6	1

Source : MDCH, October 2006

SECTION 2.G: INDEPENDENT VARIABLES

From the linked dataset the study team will examine the frequency of the following groups of variables in cases and controls:

- maternal level of education;
- mother's place of residence;
- mother's age at the time of delivery;
- biological father's age (if available);
- mother's reproductive history (to the extent available in the linked dataset);
- pregnancy complications such as pre-eclampsia (for the index child); and
- delivery complications such as cesarean section or breech birth (for the index child).

Table 8 provides a partial list of variables of interest for the proposed study, including both main exposure and potentially confounding variables or effect modifiers.

Table 8: A partial list of variables considered for the proposed study

Variables	Categories	Data Source
Maternal education		MLBDB
	< high school	
	high school	
	> high school	
Maternal age		MLBDB
	≤ 35 years	
	> 35 years	
Race of child		MLBDB
	Black	
	White	
	Other	
Apgar score at 1 minute		MLBDB, hospital records
	7 to 10	
	0 to 6	
Apgar score at 5 minutes		MLBDB, hospital records
	7 to 10	
	0 to 6	
Presence of meconium		MLBDB, hospital records
	No	
	Yes	
Birth weight (g)	≤ 1500	MLBDB, hospital records
	1500-1999	
	2000-2499	
	2500-2999	
	3000-3499	
	3500-3999	
	4000-4499	
	≥ 4500	
Gestational age (week)		MLBDB, hospital records
	≤ 36	
	37-42	
	≥ 43	
Birth weight for gestational age (GA)		Hospital records
	Appropriate	
	Small for GA	
	Large for GA	
Mode of delivery		MLBDB, hospital records
	Vaginal	
	Instrumental	
	Cesarean	

Table 8 (continued)

Pulmonary disorder		MLBDB
	No	
	Yes	
Congenital heart defects		MLBDB
	No	
	Yes	
Twins		MLBDB
	No	
	Yes	
Neonatal and maternal supplemental oxygen exposure		
Neonatal oxygen exposure in the delivery room		Hospital records
	None	
	≤ 2 minutes	
	≥ 3 minutes	
Cumulative neonatal oxygen exposure during hospital stay		Hospital records
Duration (hour)		
	None	
	≤ 24	
	≥ 25	
Type of oxygen (% pure)		Hospital records
	None	
	≤ 50	
	50 to 70	
	≥ 70	
Maternal oxygen exposure during delivery		Hospital records
	Yes	
	No	
Maternal exposure to nitrous oxide anesthesia during delivery		Hospital records
	Yes	
	No	
Phototherapy exposure following birth		
Duration (hour)		Hospital records
	None	
	≤ 24	
	≥ 25	

From the live birth file variable group, *Abnormal Conditions of the Newborn*, I will record use of assisted ventilation for < 30 mins (code 5), and for > 30 mins (code 6) to identify the most severe subset of study subjects exposed to O_2, since it can be assumed that mechanical ventilation shortly after birth nearly always includes O_2 therapy.

By linking to MIDB, the study team will obtain additional information on length of hospital stay, major diagnoses (e.g. respiratory distress syndrome, necrotizing enterocolitis) and major interventions (e.g. parenteral nutrition, mechanical ventilation), all of which will be contrasted in cases and controls. The hospital records of mothers and infants will allow the study team to further expand exposure information on cases, controls and their mothers.

In Phase II of the study, exposure data will be gathered from the 4^{th} data source, obstetric and neonatal hospital records, which the study team will abstract blind to case or control status in a uniform manner across designated hospitals. Maternal records will be abstracted for factors prompting O_2 therapy such as maternal chronic diseases, maternal asthma, prolonged labor, abnormal fetal heart rate patterns, fetal acidosis or presumed fetal asphyxia. Neonatal records will be abstracted for variables related to indication and duration of O_2 use as well as other factors such as need for resuscitation, respiratory distress and sepsis.

In infants, the type and time of resuscitation with 100% O_2 will be recorded, including duration of *bag* and *mask* ventilation immediately after birth (divided into three groups to replicate the exposure studied by Spector et al: 0–1 min, 1-2 min and 3 min or more), and flushing of O_2 without mask ventilation. Resuscitation with room air or lower O_2 concentrations will also be recorded. Information will also be collected about O_2 treatment during the first 2 weeks after birth (grouped according to percentage of supplementary O_2 (21–40%, 50–60%, >70%), and duration of treatment (≤ 24 h and ≥ 25 h).

The duration of neonatal phototherapy will be recorded, as will the type of phototherapy unit, so that the light frequency in use can be determined. All measured bilirubin levels, both direct and indirect, recorded in records will be abstracted, with their date and time of measurement recorded as well, so that cumulative exposure to bilirubin levels can be estimated.

Birth depression is critical to record, as it often conditions O_2 use. The Apgar score will be used as an indicator of birth depression and will be grouped in two different ways:

(i) according to the World Health Organization (WHO) International Classification of Diseases (10th revision), which classifies severe birth asphyxia as an Apgar score at 1 min of 3 or less; and

(ii) an Apgar score at 5 min of 6 or less.

An important potential confounding factor to consider may be exposure to x-ray irradiation. Infants receiving more than brief O_2 therapy are likely to have a chest X-ray ordered. I will also effectively control, in the process of data analysis, for important risk factors for phototherapy. Similarly, I will also control for factors that may cause a rapid rise in bilirubin and earlier or longer use of phototherapy, such as degree of jaundice, blood group (Rh and ABO) incompatibility, sepsis, and dehydration.

SECTION 2.H: DATA COLLECTION PROCEDURES

Data collection procedures of the proposed study are as follows

- Phase I
  - Record linkage of study subject cancer files to MLBDB and MIDB by MDCH to identify cases by MDCH
  - Selection of matched controls from selected hospitals from MLBDB by MDCH
- Phase II
  - Identification of medical records of cases and controls at study hospitals by collaborating neonatologists
  - Neonatal medical record abstraction on-site

RECORD LINKAGE

Records will be linked by MDCH using a multistage probabilistic linkage procedure which requires linkage of several variables that define the unique identity of an individual.[53] A *linked birth* is defined as a linkage between a case in the cancer registry file and any birth in the birth registry file. Variables contained within the *cancer* registry file that are available for linkage to the birth certificate records include: name (first, middle, and last), birth date, and place of birth. While Michigan is among 5 states that record maternal social security number on birth certificates, that information is not recorded in the cancer registry.

The linkage procedure and preliminary file handling will be performed by the MDCH staff responsible for maintaining both the state cancer registry and the Michigan vital records data. In this procedure, a string of relevant variables will be created for each step. In successive phases of the multi-step process, selected variables will be substituted or removed from the linkage process, with decreasingly stringent requirements. The content of the strings in each step will be prioritized in the order of the completeness of the linkage information. All records (and not just those remaining unlinked after the previous step) will be utilized in each linkage step, allowing measurement of the number of potentially linked births based on different variable combinations. Using all available identifying variables permits the identification of potentially linked births, while allowing for some disparities between identifiers in cancer registry files and the live birth records.

After all automated steps have been performed, MDCH staff will conduct a manual review of potentially linked records using an organized listing of the cancer registry and birth file identifier fields, to check for accuracy of the linkage. A link will be confirmed when all data in the cancer registry file and the birth record file will be identically linked. Minor errors in the cancer registry or birth record may include simple misspellings of names. Other information available to confirm links during this manual review step includes the parent's address at cancer diagnosis (as indicated in the cancer registry), and mother's address at birth (per the birth records). MIDB information is already linked to birth certificates, and will therefore become available to the investigators in the same linkage step.

HOSPITAL RECORDS

For the purpose of this study, the study team will focus on the 13 hospitals in Michigan in which 40% of total deliveries of the state in 2004 were performed. In each hospital, the study team will have a neonatologist collaborator. The study team will also locate *maternal* medical records at the same hospital where the neonate was born or hospitalized and abstract variables pertinent to the study's research aims.

SECTION 2.I: SAMPLE SIZE CALCULATION AND STATISTICAL ANALYSIS

SAMPLE SIZE CALCULATION

Using PS software,[54] I have calculated the value of n (required number of study cases) for various OR's ranging from 1.8-3.0 as indicated in Table 9. I set significance levels at α=.05 and β=.20 (80% power). For the purpose of sample size calculation, I consider the prevalence of exposure to oxygen and to phototherapy in controls to be 0.09.

Table 9: Study sample size

Exposure	Case: Control Ratio	Odds Ratio (OR)	Sample Size (# case)
0.09	1:2	1.8	319
0.09	1:2	2.0	219
0.09	1:2	2.2	**162**
0.09	1:2	2.5	114
0.09	1:2	3.0	74

I have assumed that there is a relatively high correlation between O_2 and phototherapy. Therefore, an inflation of approximately 20% (adjustment for multivariate analysis) has been taken into account in the calculation of the required sample size. I thus plan on matching 200 cases with 400 controls, in order to detect an OR of at least 2.2.

DATA ANALYSIS

For analysis of the data collected through this case-control study, I will consider comparisons of O_2 and photo-therapy, as the primary exposure variables, among cases (of ALL) and controls. *Descriptive* information about these variables as well as other variables of interest, which might be potential confounders or effect modifiers, will be arrayed using descriptive statistical techniques. Descriptive statistics will be used to characterize the cases and controls and will include distributions of the duration of O_2- and photo-therapy, gestational age, Apgar scores and several other birth and maternal variables.

Several perinatal factors might be potential confounders or effect modifiers on the pathway between O_2 supplementation, phototherapy, and ALL. It may be, for example, that the high birth weight–ALL association reflects more O_2 therapy in large infants because of birth depressing conditions such as shoulder dystocia. Certainly low birth weight infants, not heretofore recognized as at high risk for ALL, do receive more O_2. For these reasons, I plan to match on birth weight, to control for birth weight itself and factors associated with birth weight, including treatments.

Univariate and multivariate statistic analyses will be performed using conditional logistic regression to study association between ALL and exposure to supplementary O_2 treatment, phototherapy and other perinatal factors of interest. Maximum likelihood estimates of the OR, p-values and 95% CI will be obtained,

taking into account potential confounding factors or effect modifications. The statistical procedure (PROC PHREG) in SAS (version 9.0; SAS Institute Inc., Cary, NC) will be used to develop a final conditional logistic model for this 1:2 matched case–control study. The final model will include adjustments for potential confounders, such as mother's age, parity, maternal smoking habits, neonatal bilirubin level and Apgar score. All tests of hypotheses will be two-sided at significance level $\alpha = .05$.

SECTION 2.J: HUMAN SUBJECTS

RECRUITMENT AND INFORMED CONSENT

The research team at MSU will not have any interaction with the study subjects. Informed consent will not be obtained. Rather, the study team will have third parties (at MDCH and study hospitals) abstract the relevant material and create an anonymized data file for me to analyze. Subjects will only be identified by coded study ID. Thus the study will store no material with identifiers. In the following list, research sites that are part of CRIRB are bolded. All others sites will be contacted for IRB approval after obtaining CRIRB approval.

1. **Department of Epidemiology at MSU**
2. **Michigan Department of Community Health**
3. **Sparrow Hospital, Lansing**
4. Hurley Medical Center, Flint

5. Gensys Regional Medical Center, Flint

6. Bronson Methodist Hospital, Kalamazoo

7. Spectrum Health, Grand Rapids

8. William Beaumont Hospital, Royal Oak and Troy

9. Covenant Health Care, Saginaw

10. St. Joseph Mercy Hospital , Ann Arbor,

11. University of Michigan Hospitals, Ann Arbor

12. St John Hospital and Medical Center, Detroit

13. Hutzel Hospital, Detroit

PROTECTION AGAINST RISK

It is not feasible to contact individuals in this study or their parents and obtain consent for record review. Therefore, I propose to have the initial linkage of records be performed by the MDCH. After the abstraction of medical records (which requires the study team to use identifiers), all identifying information will be removed from the data file the study team will use. The study team will maintain no individual identifiers of any sort. After its matching protocol is complete, MDCH will provide a list of cases and controls with identifiers to the study hospitals.

At each designated hospital, a neonatologist collaborator will coordinate abstraction of neonatal and maternal medical records on-site without providing

any identifiable information to the study investigators. This list will be provided to the hospital abstractors who will identify the required information. Upon entry of all data into computer storage, all identifiers will be removed. In both phases of the proposed study, the identity of the individuals will not be known to the investigators. Thus there is no risk to subjects, not even a risk of identity disclosure. The research team at MSU will not be able to identify any subjects in this study once the medical record abstraction is complete. The de-identification process will take place at the hospitals that obtain medical records. Once a unique number is assigned to each case and control, including indication of case/control status, all other identifying information such as name and address of child or parent, or any other piece of information that could identify the child or parent will be removed from the data file.

POTENTIAL BENEFIT TO INDIVIDUAL SUBJECTS AND SOCIETY

No benefits are anticipated for subjects. The potential benefit to society through better understanding of perinatal etiologies of ALL is substantial. The benefit-risk ratio is very favorable given the potentially large societal benefits and absence of any risk to the participants.

CHAPTER 3: DISCUSSION AND CONCLUDING REMARKS

SECTION 3.A: STRENGTHS AND LIMITATIONS OF THE PROPOSED STUDY

Despite the rarity of childhood cancers, it appears that our study team can obtain a very large sample of cases in the State of Michigan. However, general limitations of case-control studies will apply to the proposed study, but the most important limitation, *recall bias*, will not operate, as exposure information was collected years prior to the diagnosis. In addition, I believe relatively *diverse* and *representative* study population has several merits. The study population comes from all over the state (minimal s*election bias*), resulting in high external validity. I will conduct hospital record review in identical fashion for cases and controls (minimal *Information bias*).

The proposed study's major strength is its design which includes a two-phase approach, allowing the study team to obtain a matched control sample in the first phase, and to collect detailed information on exposures and interventions (minimal *measurement bias*) in the second phase, resulting in high *internal validity*.

SECTION 3.B: PUBLIC HEALTH IMPLICATIONS

Exposures acting before birth and early in life have long been thought to be important determinants of leukemia.[55] Approximately, 10% of newborns require

some assistance to begin breathing at birth, 1% require extensive resuscitative measures,[56] and close to 10% receive phototherapy. Inasmuch as both of the interventions I propose to study are very commonly used in infants with relatively mild perinatal conditions, it is critical to know whether they have any carcinogenic potential. Moreover, both 100% O_2 and phototherapy could be replaced – if not in all, certainly in many cases – by other forms of intervention. For reasons not necessarily related to carcinogenesis, some pediatricians have called for minimizing O_2 exposure, and for using room air rather than 100% O_2 whenever possible for newborn resuscitation.[32,57,58] Likewise, careful observation may be able to replace phototherapy in milder degrees of neonatal jaundice.

At times cancer epidemiology must investigate exposures whose removal or amelioration is difficult to achieve, either because individuals are powerfully addicted to the exposure (as with smoking) or because major industrial reorganization would be required (as in the discharge of certain chemicals into the environment). In this case, however, the putative carcinogenic exposures might easily be replaced, as a matter of clinical policy, by available alternatives. Neonatal medicine has repeatedly altered its practices after the discovery of hazards to susceptible newborns of, for example, unmonitored oxygen administration, sulfonilamides, chloramphenicol, feeding restriction and many other practices.

REFERENCES

1. Stewart BW, Kleihues P, International Agency for Research on Cancer. World cancer report. Lyon: IARC Press, 2003.

2. Little J, International Agency for Research on Cancer. Epidemiology of childhood cancer. IARC scientific publications, no.149. Lyon; Oxford: International Agency for Research on Cancer ; Distributed by Oxford University Press, 1999.

3. Ferlay J, International Agency for Research on Cancer., International Association of Cancer Registries. CI5VII electronic database of Cancer incidence in five continents, vol. VII. Lyon, France: International Agency for Research on Cancer, 1997.

4. Parkin DM, Stiller CA, Draper GJ, Bieber CA. The international incidence of childhood cancer. Int J Cancer 1988;42(4):511-20.

5. Chan KW. Acute lymphoblastic leukemia. Curr Probl Pediatr Adolesc Health Care 2002;32(2):40-9.

6. Ries LAG, Harkins D, Krapcho M, Mariotto A, Miller BA, Feuer EJ, Clegg L, Eisner MP, Horner MJ, Howlader N, Hayat M, Hankey BF, Edwards BK. SEER Cancer Statistics Review, 1975-2003. Bethesda, MD, http://seer.cancer.gov/csr/1975_2003/, based on November 2005 SEER data submission, posted to the SEER web site: National Cancer Institute, 2006.

7. Gaynon PS, Bostrom BC, Hutchinson RJ, Lange BJ, Nachman JB, Steinherz PG, Sensel MG, Lee MK, Stram DO, Sather HN. Duration of hospitalization as a measure of cost on Children's Cancer Group acute lymphoblastic leukemia studies. J Clin Oncol 2001;19(7):1916-25.

8. Linet MS, Devesa SS. Descriptive epidemiology of childhood leukaemia. Br J Cancer 1991;63(3):424-9.

9. Taub JW, Ge Y. The prenatal origin of childhood acute lymphoblastic leukemia. Leuk Lymphoma 2004;45(1):19-25.

10. Burjanivova T, Madzo J, Muzikova K, Meyer C, Schneider B, Votava F, Marschalek R, Stary J, Trka J, Zuna J. Prenatal origin of childhood AML occurs less frequently than in childhood ALL. BMC Cancer 2006;6:100.

11. Taub JW, Konrad MA, Ge Y, Naber JM, Scott JS, Matherly LH, Ravindranath Y. High frequency of leukemic clones in newborn screening blood samples of children with B-precursor acute lymphoblastic leukemia. Blood 2002;99(8):2992-6.

12. Hatch EE, Herbst AL, Hoover RN, Noller KL, Adam E, Kaufman RH, Palmer JR, Titus-Ernstoff L, Hyer M, Hartge P, Robboy SJ. Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States). Cancer Causes Control 2001;12(9):837-45.

13. Anderson LM, Diwan BA, Fear NT, Roman E. Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. Environ Health Perspect 2000;108 Suppl 3:573-94.

14. Hjalgrim LL, Rostgaard K, Hjalgrim H, Westergaard T, Thomassen H, Forestier E, Gustafsson G, Kristinsson J, Melbye M, Schmiegelow K. Birth weight and risk for childhood leukemia in Denmark, Sweden, Norway, and Iceland. J Natl Cancer Inst 2004;96(20):1549-56.

15. Hjalgrim LL, Westergaard T, Rostgaard K, Schmiegelow K, Melbye M, Hjalgrim H, Engels EA. Birth weight as a risk factor for childhood leukemia: a meta-analysis of 18 epidemiologic studies. Am J Epidemiol 2003;158(8):724-35.

16. Saugstad OD. Oxygen saturations immediately after birth. J Pediatr 2006;148(5):569-70.

17. Kamlin CO, O'Donnell CP, Davis PG, Morley CJ. Oxygen saturation in healthy infants immediately after birth. J Pediatr 2006;148(5):585-9.

18. Rabi Y, Yee W, Chen SY, Singhal N. Oxygen saturation trends immediately after birth. J Pediatr 2006;148(5):590-4.

19. Vento M, Asensi M, Sastre J, Garcia-Sala F, Pallardo FV, Vina J. Resuscitation with room air instead of 100% oxygen prevents oxidative stress in moderately asphyxiated term neonates. Pediatrics 2001;107(4):642-7.

20. Vento M, Sastre J, Asensi MA, Vina J. Room-air resuscitation causes less damage to heart and kidney than 100% oxygen. Am J Respir Crit Care Med 2005;172(11):1393-8.

21. Saugstad OD, Ramji S, Vento M. Resuscitation of depressed newborn infants with ambient air or pure oxygen: a meta-analysis. Biol Neonate 2005;87(1):27-34.

22. Tan A, Schulze A, O'Donnell CP, Davis PG. Air versus oxygen for resuscitation of infants at birth. Cochrane Database Syst Rev 2004(3):CD002273.

23. The International Liaison Committee on Resuscitation (ILCOR) consensus on science with treatment recommendations for pediatric and neonatal patients: neonatal resuscitation. Pediatrics 2006;117(5):e978-88.

24. Vento M, Asensi M, Sastre J, Lloret A, Garcia-Sala F, Vina J. Oxidative stress in asphyxiated term infants resuscitated with 100% oxygen. J Pediatr 2003;142(3):240-6.

25. Saugstad OD. Oxidative stress in the newborn--a 30-year perspective. Biol Neonate 2005;88(3):228-36.

26. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160(1):1-40.

27. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004;266(1-2):37-56.

28. Granot E, Kohen R. Oxidative stress in childhood--in health and disease states. Clin Nutr 2004;23(1):3-11.

29. Cnattingius S, Zack MM, Ekbom A, Gunnarskog J, Kreuger A, Linet M, Adami HO. Prenatal and neonatal risk factors for childhood lymphatic leukemia. J Natl Cancer Inst 1995;87(12):908-14.

30. Naumburg E, Bellocco R, Cnattingius S, Jonzon A, Ekbom A. Supplementary oxygen and risk of childhood lymphatic leukaemia. Acta Paediatr 2002;91(12):1328-33.

31. Spector LG, Klebanoff MA, Feusner JH, Georgieff MK, Ross JA. Childhood cancer following neonatal oxygen supplementation. J Pediatr 2005;147(1):27-31.

32. Paneth N. The evidence mounts against use of pure oxygen in newborn resuscitation. J Pediatr 2005;147(1):4-6.

33. Meschia G. Placental respiratory gas exchange and fetal oxygenation. In: Creasy RK, Resnik R, Iams JD, eds. Maternal-fetal medicine. 5th ed. Philadelphia, Pa.: W.B. Saunders Co., 2004;xviii, 1362 p.

34. Maisels MJ, Gifford K, Antle CE, Leib GR. Jaundice in the healthy newborn infant: a new approach to an old problem. Pediatrics 1988;81(4):505-11.

35. Vreman HJ, Wong RJ, Stevenson DK. Phototherapy: current methods and future directions. Semin Perinatol 2004;28(5):326-33.

36. Stoll BJ, Kliegman RM. The high-risk infant. In: Behrman RE, Kliegman R, Jenson HB, eds. Nelson textbook of pediatrics. 17th ed. Philadelphia, PA: Saunders, 2004.;p547.

37. Cashore WJ, Oh W. Unbound bilirubin and kernicterus in low-birth-weight infants. Pediatrics 1982;69(4):481-5.

38. Wong RJ, DeSandre GH, Sibley E, Stevenson DK. Neonatal jaundice and liver disease. In: Fanaroff AA, Martin RJ, Walsh MC, eds. Fanaroff and Martin's neonatal-perinatal medicine : diseases of the fetus and infant. 8th ed. Philadelphia, Pa.: Mosby Elsevier, 2006.

39. Gies HP, Roy CR. Bilirubin phototherapy and potential UVR hazards. Health Phys 1990;58(3):313-20.

40. Ben-Sasson SA, Davis DL. Neonatal exposure to protoporphyrin-activating lighting as a contributing cause of childhood acute lymphocytic leukemia. Cancer Causes Control 1992;3(4):383-7.

41. Stoll BJ, Kliegman RM. Digestive system disorders. In: Behrman RE, Kliegman R, Jenson HB, eds. Nelson textbook of pediatrics. 17th ed. Philadelphia, PA: Saunders, 2004;p598.

42. Lipinski LJ, Hoehr N, Mazur SJ, Dianov GL, Senturker S, Dizdaroglu M, Bohr VA. Repair of oxidative DNA base lesions induced by fluorescent light is defective in xeroderma pigmentosum group A cells. Nucleic Acids Res 1999;27(15):3153-8.

43. Najib F. Defensive role of bilirubinaemia in pneumococcal infection. The Lancet 1937;229(5922):505-6.

44. Sedlak TW, Snyder SH. Bilirubin the beneficent: In reply. Pediatrics 2004;114(6):1741-2; author reply 1742-3.

45. Sedlak TW, Snyder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. Pediatrics 2004;113(6):1776-82.

46. Bratlid D. Criteria for treatment of neonatal jaundice. J Perinatol 2001;21 Suppl 1:S88-92; discussion S104-7.

47. van Steensel-Moll HA, van Duijn CM, Valkenburg HA, van Zanen GE. Predominance of hospital deliveries among children with acute lymphocytic leukemia: speculations about neonatal exposure to fluorescent light. Cancer Causes Control 1992;3(4):389-90.

48. Olsen JH, Hertz H, Kjaer SK, Bautz A, Mellemkjaer L, Boice JD, Jr. Childhood leukemia following phototherapy for neonatal hyperbilirubinemia (Denmark). Cancer Causes Control 1996;7(4):411-4.

49. Cnattingius S, Zack M, Ekbom A, Gunnarskog J, Linet M, Adami HO. Prenatal and neonatal risk factors for childhood myeloid leukemia. Cancer Epidemiol Biomarkers Prev 1995;4(5):441-5.

50. Zack M, Adami HO, Ericson A. Maternal and perinatal risk factors for childhood leukemia. Cancer Res 1991;51(14):3696-701.

51. Hardy JB. The Collaborative Perinatal Project: lessons and legacy. Ann Epidemiol 2003;13(5):303-11.

52. Paneth N, et al. DOW Chemical Company Report. 1994.

53. Herman AA, McCarthy BJ, Bakewell JM, Ward RH, Mueller BA, Maconochie NE, Read AW, Zadka P, Skjaerven R. Data linkage methods used in maternally-linked birth and infant death surveillance data sets from the United States (Georgia, Missouri, Utah and Washington State), Israel, Norway, Scotland and Western Australia. Paediatr Perinat Epidemiol 1997;11 Suppl 1:5-22.

54. Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. Control Clin Trials 1990;11(2):116-28.

55. Lightfoot T. Aetiology of childhood leukemia. Bioelectromagnetics 2005;Suppl 7:S5-S11.

56. American Heart Association (AHA, 2005) guidelines for cardiopulmonary resuscitation (CPR) and emergency cardiovascular care (ECC) of pediatric and neonatal patients: neonatal resuscitation guidelines. Pediatrics 2006;117(5):e1029-38.

57. Tan A, Schulze A, O'Donnell CP, Davis PG. Air versus oxygen for resuscitation of infants at birth. Cochrane Database Syst Rev 2005(2):CD002273.

58. Saugstad OD, Ramji S, Vento M. Oxygen for newborn resuscitation: how much is enough? Pediatrics 2006;118(2):789-92.