

# Infant Acute Leukemia and Maternal Exposures during Pregnancy

Maria S. Pombo-de-Oliveira,<sup>1</sup> Sergio Koifman,<sup>2</sup> and Brazilian Collaborative Study Group of Infant Acute Leukemia

<sup>1</sup>Division of Experimental Medicine, Research Center, Instituto Nacional de Câncer and <sup>2</sup>Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

## Abstract

Infant acute leukemia (IAL) has a unique profile characterized by the high incidence of translocations involving the *MLL* gene located at the 11q23 region. To test the potential role of intrauterine and perinatal factors linked to the risk of IAL development, a hospital-based case-control study was conducted in different cities of Brazil. A total of 202 children (ages 0-21 months) with newly diagnosed IAL was enrolled (1999-2005), and 440 age-matched controls were selected from the same hospitals wherein IAL cases were treated. A statistically significant association between maternal use of hormones during pregnancy and IAL was observed [odds ratio (OR), 8.76; 95% confidence interval (95% CI), 2.85-26.93] in a multivariable analysis. The association of certain exposures during pregnancy (hormones, dipyrone, metroni-

dazole, and misoprostol) and *MLL* gene rearrangements was tested using a case-case approach. Despite the lack of statistical significance, the magnitude of the OR for maternal exposure to dipyrone (OR, 1.45; 95% CI, 0.75-2.86), metronidazole (OR, 1.72; 95% CI, 0.64-4.58), quinolones (OR, 2.25; 95% CI, 0.70-25.70), and hormones (OR, 1.88; 95% CI, 0.50-7.01) may suggest the occurrence of interactions between such maternal exposures during pregnancy and *MLL* rearrangements, yielding into IAL development. The strong and statistically significant association between IAL and estrogen exposure during pregnancy observed in this study deserves further investigation to investigate its role in intrauterine leukemogenesis. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2336-41)

## Introduction

The understanding of leukemogenic pathways in childhood has been improved markedly by identification of a series of critical and consistent mutations occurring during pregnancy (1, 2). The causes of these genetic alterations are not known, but the different pediatric leukemia subtypes are thought to have distinct etiologies (3). Most cases of infant acute leukemia (IAL) have rearrangements involving the *MLL* gene at region 11q23 and arise *in utero* as confirmed by retrospective analyses of neonatal blood spots of affected infants (1, 4). This biologically and clinically unique leukemia (5-7) has an extremely short latency period. Moreover, *MLL* fusions resemble those found in secondary acute myelogenous leukemia resulting from exposure to topoisomerase II (topo-II) inhibitors. This has led to the proposal that inadvertent exposure to biochemically similar topo-II inhibitors during pregnancy may be involved in the causation of IAL (8, 9). Given the rarity of this leukemia, there have been very few epidemiologic studies focused especially on IAL with *MLL* fusions (10).

Nevertheless, in an international collaborative study, significant associations between exposures to DNA-damaging drugs and mosquitocidals during pregnancy and *MLL*<sup>+ve</sup>, but not *MLL*<sup>-ve</sup>, leukemia were noted (11). However, because there were few molecularly classified cases in this study, further independent analysis was needed to confirm or refute these apparent associations (11).

We conducted a case-control study in Brazil to explore the hypothesis that certain environmental exposures could increase the risk of IAL with *MLL* gene rearrangements. In this report, we present the preliminary data exploring the association of maternal exposure to environmental risk factors and IAL.

## Materials and Methods

**Study Population.** The Brazilian Collaborative Study Group of Infant Acute Leukemia is a national cooperative group supported by Instituto Nacional de Câncer-Ministério da Saúde (Rio de Janeiro, Brazil) and by a network of academic medical centers and hospitals located in 10 different states in Brazil as listed in Appendix A. Fifteen institutions and >45 physicians from different Brazilian cities enrolled patients (cases and controls) to this study. Participants were recruited from hospitals situated in the following cities: Rio de Janeiro, Campinas, Belo Horizonte, Salvador, Recife, João Pessoa, Brasília, Goiania, and Florianópolis, Santa Maria, and São Paulo. We estimate that ~91% of the ascertainment of IAL cases for each Brazilian participating region were notified to the Brazilian Collaborative Study Group of Infant Acute Leukemia during the period of the study. This estimate was ascertained taking into account data provided by the population-based cancer registries established in all Brazilian regions.

**Epidemiologic Design.** A hospital-based case-control study was conducted to evaluate the magnitude of association between IAL exposure to selected environmental risk factors during pregnancy. The association between *MLL* rearrangements and selected environmental exposures was also explored using either a case-case study (12) or a case-only study approach (13).

**Case Definition.** Cases were eligible if (a) they were diagnosed with acute lymphoblastic leukemia or acute

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**Requests for reprints:** Maria S. Pombo-de-Oliveira, Instituto Nacional de Câncer, Centro de Pesquisa, Rua André Cavalcanti 37, CEP 20231-050, Rio de Janeiro, Brazil. Phone: 55-21-3233-1324; Fax: 55-21-3233-1470. E-mail: mpombo@inca.gov.br

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**Table 1. Sociodemographic variables distribution, IAL cases and controls, Brazil, 1999-2005**

	Cases (n = 202), n (%)	Controls (n = 440), n (%)	P
Age at hospitalization (mo)			
0-15	103 (51.0)	262 (59.5)	0.04
16-21	99 (49.0)	178 (40.5)	
Sex			
Male	100 (49.6)	233 (53.0)	0.488
Female	102 (50.4)	207 (47.0)	
Race (skin color)*			
White	106 (52.5)	156 (35.6)	0.004
Intermediate	58 (28.7)	142 (32.2)	
Black	38 (18.8)	142 (32.2)	
Maternal age (y)			
<18	4 (2.0)	39 (8.9)	0.002
18-24	66 (32.7)	185 (42.0)	
25-34	99 (49.0)	162 (36.8)	
>35	33 (16.3)	54 (12.3)	
Population density (residence area)			
<10,000	11 (5.4)	18 (4.0)	0.001
10,000-39,999	28 (13.9)	36 (8.2)	
40,000-99,999	27 (13.4)	52 (11.9)	
100,000-499,000	50 (24.8)	83 (18.9)	
500,000-1,000,000	18 (8.9)	39 (8.9)	
>1,000,000	68 (33.7)	212 (48.1)	
Geographic origin in Brazil			
Southeast	117 (57.9)	251 (57.1)	0.001
Northeast	51 (25.2)	104 (23.6)	
South	24 (11.9)	51 (11.6)	
Central plateau	10 (5.0)	34 (7.7)	
Mother education (y)			
<4	104 (51.5)	208 (47.2)	0.001
5-9	74 (36.6)	198 (45.0)	
≥10	24 (11.9)	34 (7.7)	
Monthly family income †			
<400	99 (49.0)	276 (62.7)	0.001
400-999	50 (24.8)	111 (25.2)	
1,000-1,999	35 (17.3)	42 (9.5)	
≥2,000	18 (8.9)	11 (2.5)	

\*According to Parra et al. (14).

†In Real (\$R, the Brazilian currency).

myelogenous leukemia according to standard classifications (14) at age  $\leq 21$  months and (b) bone marrow aspirates were available for immunophenotyping and molecular analysis. The cut point of 21 months for eligibility was determined with consideration to the frequent delay in identification of acute leukemia in some areas of Brazil. Had cases been limited to those diagnosed at  $<1$  year of age, there would have been a significant loss for those with *MLL* gene rearrangements. The analyses to characterize *MLL* status were done by conventional karyotype, by reverse transcription-PCR assay, and/or by fluorescence *in situ* hybridization. Details of the immunophenotyping and molecular data of this study are described elsewhere (15).

**Control Selection.** All controls were age frequency matched with IAL cases selected among hospitalized children in the same regional hospitals. The mothers of the controls were approached for participation when the case was still hospitalized. Controls that presented with severe life-threatening conditions to minimize recall bias were selected. The pathologies included were the following: trauma ( $n = 28$ , 6.3%), cardiopathy ( $n = 40$ , 9.1%), infectious diseases ( $n = 87$ , 19.7%), metabolic disorders ( $n = 18$ , 4.1%), neurologic diseases ( $n = 16$ , 3.6%), sickle cell anemia ( $n = 56$ , 12.7%), nonsyndrome defects ( $n = 18$ , 4.1%), allergy/asthma ( $n = 64$ , 14.5%), pneumonia ( $n = 72$ , 16.3%), nutritional disturbances ( $n = 30$ , 6.8%), and seizures ( $n = 12$ , 2.7%).

**Exclusion Criteria.** Cases and controls presenting clinical syndromes resulting from chromosomal abnormalities, such

as Down syndrome, and other selected conditions, such as myelodysplastic syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia, and neurofibromatosis, were excluded. The absence of a well-established diagnosis and the inaccessibility to the biological mother and/or children  $>21$  months at diagnosis were also exclusion criteria from the study enrollment.

**Race Criteria.** The Brazilian population has a historical background of intensive intermarriage among different ethnic groups, and consequently, ethnic/race stratification is very difficult to characterize by applying the same criteria usually used elsewhere. Skin color denotes the Brazilian equivalent of the English term "race" and is based on a complex phenotypic evaluation that takes into account besides skin complexion, hair type, and nose and lip shape. In this study, we categorized the children by skin color as whites, intermediates, and blacks according to criteria described by Parra et al. (16).

**Data Collection.** Mothers were interviewed in person in the hospital with the aid of a well-structured questionnaire divided in two major sections. The first part of the questionnaire was devoted to childbirth and nursing and the second part of the questionnaire to exposures during pregnancy. Questions about demographics included family income, maternal age, and education level. Maternal history of diseases and reasons for use of medications were obtained from questions about the use of different types of drugs taken due to infectious illnesses, previous fetal loss threat, anemia, backache, etc. [e.g., antibiotics, herbal medicines, vitamins, pain killers, and hormones (oral contraceptives, hormones for pregnancy retention, and thyroid hormones)]. This analysis included information about maternal exposures during the 3 months before the index pregnancy, during the index pregnancy, and during nursing of the index child.

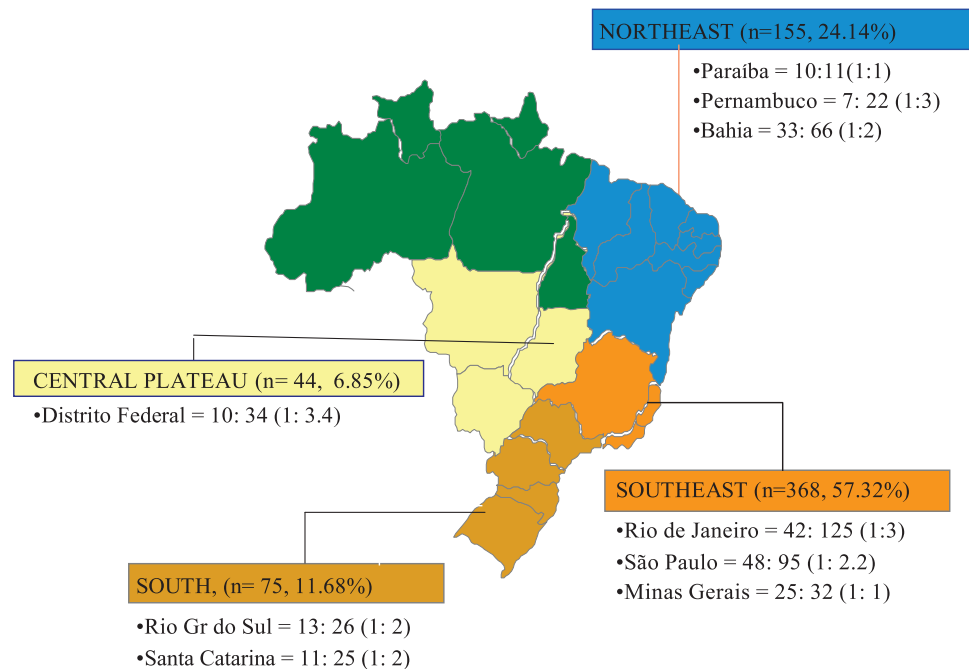
The mothers of 96% of IAL cases and 95% of potential controls consented to interview. The median (range) of the interval between the dates of IAL diagnoses and the date of mothers' interviews was 9 (0.1-36) months.

**Ethical Aspects.** All collaborating institutions approved the study protocol, and a written consent was obtained for diagnostic procedures and interview.

**Statistical Analysis.** Statistical analyses on the association between exposure to selected environmental variables and IAL during the period of index pregnancy were carried out toward the use of unconditional logistic regression with regular methods implemented by the Statistical Package for the Social Sciences version 13 (SPSS, Chicago, IL). All analyses were adjusted for region of residence, sex, income, maternal age, and birth weight. Results are expressed as odds ratios (OR) with 95% confidence intervals (95% CI), and all *P* values are two sided. Case-control analyses were conducted for combined IAL and IAL stratified by *MLL* gene status as a case-case analysis (12). The association between *MLL* rearrangements and selected variables was ascertained using ORs and 95% CI adjusted for the aforementioned variables.

## Results

The study accrued 642 subjects (202 cases and 440 controls), with enrollment starting in January 1999 and ending in July 2005. The main demographic characteristics of IAL cases and controls distribution are summarized in Table 1, and the geographic origins of the cases and controls are outlined in Fig. 1. According to age at enrollment, 51.0% of cases and 59.5% of controls were ages  $\leq 15$  months ( $P = 0.04$ ). Mean age



**Figure 1.** Distribution of cases and controls (ratio) by geographic regions, Brazil, 1999-2005.

at diagnosis in the study was 12.9 months for IAL cases, and mean age at entry into the study was 14.1 months for controls ( $P = 0.580$ ). A higher proportion of white children were observed among IAL cases (52.5%) than controls (35.6%;  $P < 0.0001$ ).

The majority of the children in this series, either cases or controls, came from urban or semiurban environments surrounding the largest participating cities, with population sizes usually  $>1$  million people. The majority of cases (57.9%) and controls (57.1%) were enrolled in the southeastern cities, with the northeast cities running second (25.2% and 23.6%, respectively). Mothers of cases showed an age distribution older than mothers of controls ( $P = 0.002$ ).

There were 140 cases of acute lymphoblastic leukemia, mainly B-cell precursors, and 62 cases of acute myelogenous leukemia, whose immunophenotypes and *MLL* status are described elsewhere (15). The highest frequency of *MLL*

rearrangements was found in pro-B acute lymphoblastic leukemia ( $P < 0.001$ ). Other chromosomal abnormalities, such as *TEL/AML1*, *AML1/ETO*, and *PML/RARA*, were also detected in this series of IAL cases, but the frequency distribution was too small for individual associations with risk factors (15).

The results of the main variables related to exposures during pregnancy of IAL cases and controls are shown in Table 2. Maternal exposure to smoking tobacco or marijuana and alcohol intake during pregnancy (OR, 0.87; 95% CI, 0.63-1.21) were not associated with IAL in this study. Medication consumption ascertained according to the reason for use during the pregnancies was explored. These medications included vitamins, pain relievers, antibiotics for urinary or respiratory tract infections, antiemetics, antidiarrheals, antifungal, fertility medications or abortive drugs, and herbal medicines.

**Table 2. Maternal exposures during pregnancy, IAL cases and controls, Brazil, 1999-2005**

	IAL, n (%)	Controls, n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*
Tobacco	37 (18.3)	101 (23.0)	0.75 (0.49-1.15)	0.89 (0.631-1.25)
Marijuana	7 (3.5)	17 (3.9)	0.90 (0.37-2.20)	0.87 (0.63-1.20)
Pain relievers				
Dipyroné	124 (61.4)	228 (51.9)	1.48 (1.05-2.08)	1.45 (1.02-2.06)
Others <sup>†</sup>	50 (24.8)	111 (25.2)		0.97 (0.66-1.43)
Antibiotics				
Amoxicillin	25 (12.4)	62 (14.1)	0.86 (0.52-1.42)	0.88 (0.63-1.25)
Ciprofloxacin (quinolone)	5 (2.5)	11 (2.5)	0.99 (0.34-2.89)	0.94 (0.32-2.77)
Vitamins/iron supplement	73 (36.1)	169 (38.4)	0.77 (0.52-1.14)	0.90 (0.63-1.28)
Folic acid	28 (13.9)	47 (10.7)	1.35 (0.82-2.22)	1.22 (0.73-2.05)
Antiemetic	18 (8.9)	25 (5.7)	1.62 (0.86-3.04)	1.69 (0.87-3.28)
Antifungic (metronidazole)	38 (13.9)	44 (10.1)	1.45 (0.87-2.40)	1.39 (0.82-2.34)
Abortive drugs				
All <sup>‡</sup>	40 (19.8)	90 (20.5)	0.96 (0.63-1.45)	0.81 (0.53-1.25)
Misoprostol	6 (3.0)	7 (1.8)	1.28 (0.40-4.06)	1.23 (0.38-4.02)
Hormones <sup>§</sup>	18 (8.9)	4 (0.9)	10.66 (3.56-31.94)	8.76 (2.85-26.93)
Herbal infusions	4 (2.0)	5 (1.1)	1.76 (0.47-6.62)	1.93 (0.49-7.58)
Pesticides	91 (45.3)	119 (27.0)	2.23 (1.58-3.16)	2.18 (1.53-2.13)

\*Adjusted for sex, income, maternal age, and birth weight.

<sup>†</sup>Paracetamol, aspirin, hyoscine, and codeine.

<sup>‡</sup>Misoprostol, herbal infusions, and other compounds used as abortive.

<sup>§</sup>Oral contraceptives, antiabortive progesterone treatment, and thyroid hormones.

**Table 3. Hormonal intake during preconception and pregnancy and IAL according to *MLL* status, Brazil, 1999-2005**

Hormones intake	IAL, n (%)	<i>MLL</i> <sup>+ve</sup> , n (%)	<i>MLL</i> <sup>-ve</sup> , n (%)	Controls, n (%)	IAL vs controls, OR (95% CI)*	<i>MLL</i> <sup>+ve</sup> vs controls, OR (95% CI)*	<i>MLL</i> <sup>-ve</sup> vs controls, OR (95% CI)*
Preconception †							
Present	24 (12.2)	14 (21.5)	6 (7.7)	23 (5.3)	2.26 (1.21-4.21)	3.34 (1.51-7.36)	1.13 (0.40-3.14)
Absent	173 (87.8)	51 (78.5)	72 (92.3)	409 (94.7)			
1st trimester							
Present	17 (8.4)	5 (7.4)	4 (5.0)	3 (0.7)	11.35 (3.20-40.20)	10.57 (2.33-47.91)	7.55 (1.50-37.94)
Absent	185 (91.6)	63 (92.6)	76 (95.0)	438 (99.3)			
2nd trimester							
Present	7 (3.5)	1 (1.5)	2 (2.5)	3 (0.7)	4.49 (1.07-18.87)	2.62 (0.15-17.56)	3.52 (0.51-24.02)
Absent	195 (96.5)	67 (98.5)	78 (97.5)	438 (99.3)			
3rd trimester							
Present	6 (3.0)	1 (1.5)	3 (3.8)	3 (0.7)	2.32 (0.60-8.98)	1.02 (0.10-9.93)	3.94 (0.80-19.28)
Absent	196 (97.0)	67 (98.5)	77 (96.2)	438 (99.3)			

\*Reported hormonal intake 1 year before pregnancy.

†*MLL* status and hormonal exposure OR (case-case approach) adjusted for sex, income, maternal age, and birth weight.

As shown in Table 2, a multivariate analysis revealed significant increased risks for IAL cases associated with maternal use of hormones during pregnancy after adjustments for sex, income, birth weight, maternal age, and history of cancer in first-degree relatives. The review of IAL mother's records who reported having used hormonal substances during pregnancy revealed that four of them did so to prevent fetal loss considering previous personal antecedents. The remaining 14 women reported use of contraceptives for abortion purposes or because they were not aware of the current pregnancy. Although an elevated OR was found for herbal medicine exclusively for the total series of IAL compared with controls, no significant association was found.

Results for maternal exposure during pregnancy to domestic pesticides and dipyrone reveal a moderate statistically significant association with IAL (Table 2). Joint exposure to dipyrone and metronidazole during pregnancy, reported by 54 mothers, was tested and revealed no association (OR, 1.05; 95% CI, 0.32-3.41).

In the multivariable analysis using logistic modeling, the environmental exposures that showed statistically significant association with IAL were the following: hormones (OR, 8.76; 95% CI, 2.85-26.93), pesticides at home (OR, 2.18; 95% CI, 1.53-2.13), and dipyrone (OR, 1.4; 95% CI, 1.02-2.06).

Hormonal exposure before and during pregnancy was associated to IAL compared with controls, both with and without *MLL* gene rearrangements. The mothers of 18 cases reported the consumption of hormones during pregnancy: 12 (66.7%) of them as oral contraceptives, 4 (3.2%) as thyroid hormones, and 2 (1%) as therapeutic drugs for pregnancy retention. We also explored whether the timing of the exposure (pregnancy trimester) would be associated with IAL risk magnitude, and for most medications, the direction of the OR remained similar to the overall OR, although a small decrease in risk estimates was observed in the second and third trimester in *MLL*<sup>+ve</sup> cases (Table 3). The highest magnitudes of association were observed for consumption of hormones during the first trimester of pregnancy [OR, 11.35

(95% CI, 3.20-40.20) for all IAL cases; OR, 10.57 (95% CI, 2.33-47.91) for *MLL*<sup>+ve</sup> cases; and OR, 7.55 (95% CI, 1.50-37.94) for *MLL*<sup>-ve</sup> cases]. An association between *MLL*<sup>-ve</sup> and hormonal exposure during the first trimester of pregnancy (OR, 7.55; 95% CI, 1.50-37.94) was also observed. In the case-case approach, the associations of dipyrone, metronidazole, quinolones, and hormones with *MLL* rearrangements showed ORs higher than the unity, without statistical significance (Table 4).

## Discussion

Previous studies support the hypothesis that IAL with *MLL* rearrangements could be caused by exposures to compounds *in utero* that could inhibit topo-II activity (11, 17). We conducted a case-control study of IAL aiming to evaluate a selected maternal exposures during pregnancy and to assess previously reported associations with DNA-damaging substances. Despite the fact that this study was not designed as a population-based study, we estimate that it includes ~91% of the acute leukemia cases in children ages <12 months diagnosed in the participating centers in this particular time period, taking into account the expected number of IAL cases according to the population-based cancer registries data in Brazil and the number of cases of IAL ascertained in this study.

There were no major differences in this study about the distributions of demographic features among Brazilian regions. However, the overrepresentation of cases in the southeastern cities and the vast difference in the numbers of controls and cases in the highest population density areas are explained by the easier access to health care and by health care practices. Brazilian health care is public and available to all members of the population by law. Because private hematology-oncology care is very expensive, even affluent people make use of the public health care. However, for less expensive treatments, the middle and upper classes turn to private institutions. This socioeconomic phenomenon may

**Table 4. Association of selected environmental exposures during pregnancy and *MLL* status, IAL cases, in a case-case analysis, Brazil, 1999-2005**

Exposure	Exposed and <i>MLL</i> <sup>+ve</sup> (n)	Unexposed and <i>MLL</i> <sup>+ve</sup> (n)	Exposed and <i>MLL</i> <sup>-ve</sup> (n)	Unexposed and <i>MLL</i> <sup>-ve</sup> (n)	Crude OR (95% CI)*	Adjusted OR † (95% CI)
Dipyrone	47	23	44	34	1.58 (0.80-3.08)	1.45 (0.75-2.86)
Metronidazole	12	54	7	72	2.29 (0.84-6.19)	1.72 (0.64-4.58)
Quinolones	2	65	1	79	2.43 (0.21-27.41)	2.25 (0.70-25.70)
Hormones	6	57	4	76	2.00 (0.54-7.42)	1.88 (0.50-7.01)
Misoprostol	3	17	2	10	0.88 (0.12-6.21)	0.44 (0.50-7.01)

\*Interaction OR between *MLL* gene status and selected exposures (case-only approach).

†ORs for *MLL* gene status and selected exposures adjusted for sex, income, maternal age, and birth weight.

explain why we have a more "deprived" control population. The concern about introducing bias in the maternal hormone result, as the more affluent group (cases) might have better access to health care/contraception, should be ruled out because birth control pills are distributed freely in the public health care system. Although a higher proportion of white children were observed among cases than controls ( $P < 0.0001$ ), we consider that such statistical difference should be analyzed cautiously considering the intense genetic mixing of the Brazilian population (16). Several interviewers in the different participation centers carried out the characterization of skin complexion, presenting subjective variability in such classification procedures. Therefore, the results observed in this study must be analyzed prudently for the aforementioned reasons. We believe that maternal education and income distribution in cases and controls reflect the social environment experienced by all participants more so than the ethnic profile.

Lower socioeconomic status, as measured by income and maternal education, was associated with an increased risk of IAL, which is similar to previous reports in a more developed country (18). These results contrast with children ages >24 months from middle and upper classes who tend to have an increased risk of developing common acute lymphoblastic leukemia associated with overprotection and high living standards (4, 19). This difference is plausible and can be explained by the inadvertent exposure to environmentally harmful compounds among mothers with lower education/income status and generally less knowledge about health risk factors in general.

As a whole, this study suggested that some environmental exposures during pregnancy might yield an increased risk of IAL in offspring. A markedly higher statistically significant risk was observed for hormonal exposure during pregnancy (OR, 8.76; 95% CI, 2.85-26.93), which deserves further scrutiny. A positive association with maternal exposure to dipyrene and household pesticides and IAL offers support for previous studies (11, 20). Dipyrene consumption during pregnancy was previously found to be associated with Wilms' tumor (21) and was found in this study to be associated with IAL with *MLL* rearrangements, suggesting that dipyrene should be considered hazardous during pregnancy, especially in Brazil where it is a cheap compound and may show a high attributable risk for childhood malignancies (11, 21).

The mechanisms of leukemogenesis in IAL are related to the fact that the growing fetus is more sensitive to the effects of potential DNA damage insults during the early stage of pregnancy. The gene fusion resulting from chromosomal translocation is assumed to be the initiating event in leukemogenesis (2, 3). Because reciprocal rearrangements of the *MLL* gene are the most common genetic feature in IAL, it is important to understand how these fusion genes might possibly have originated as an effect of transplacental exposures. It is well known that chemotherapeutic drugs that target topo-II, which inhibit the resealing of broken DNA strand ends, trigger the formation of *MLL* translocations (22). Among the topo-II inhibitors are benzene metabolites, such as benzoquinone, isoflavones, anthraquinone, and quinolone antibiotics (8). The potential role of exogenous estrogens in breast cancer studies was shown in descriptive studies and in experimental models, pointing out that the results of the DNA damage were induced by their metabolites causing mainly single-strand breaks (23-25). As the metabolite products in the estrogen biosynthesis are semiquinone and quinone, a pathway mimicking the same topo-II inhibitors could explain the high association found in this series of IAL.

Although it is speculated that the gene fusion resulting from *MLL* rearrangements in infant acute lymphoblastic leukemia might occur in a restricted period of the beginning of B

lymphopoiesis, clonotypic DJ rearrangements of the immunoglobulin heavy chain genes indicate that the second event leading to overt leukemia could happen at a later time during fetal development (26). The association between hormonal exposure either before or during pregnancy and *MLL* status, ascertained using a case-case approach (12), revealed an association in all studied periods, although higher during the first trimester of pregnancy. However, due to the diversity of *MLL* gene rearrangements with several gene partners, false-negative results could occur and this misclassification would artificially decrease the magnitude of association (27).

The present study provides evidence that hormone exposure during pregnancy should be studied in more depth as cause-effect *in utero* leukemogenesis.

## Appendix A. Brazilian Collaborative Study Group of Infant Acute Leukemia

Members: Paulo Ivo C. Araújo,<sup>3</sup> Dora Márcia Alencar,<sup>4</sup> Silvia R. Brandalise,<sup>5</sup> Eni Guimarães Carvalho,<sup>6</sup> Virginia M. Coser,<sup>7</sup> Imaruí Costa,<sup>7</sup> José Carlos Córdoba,<sup>8</sup> Mariana Emerenciano,<sup>1</sup> Jane Dobbin J,<sup>1</sup> Maria Célia Moraes Guerra,<sup>3</sup> Venâncio Gumes Lopes,<sup>4</sup> Isis Q. Magalhães,<sup>8</sup> Núbia Mendonça,<sup>4</sup> Andrea Gadelha,<sup>9</sup> Gilson Guedes,<sup>9</sup> Flávia Pimenta,<sup>9</sup> Vitória P. Pinheiro,<sup>5</sup> Waldir Pereira,<sup>7</sup> Gilberto Ramos,<sup>10</sup> Terezinha J.M. Salles,<sup>11</sup> Denise Bousfield da Silva,<sup>12</sup> Marcelo P. Land,<sup>3</sup> Elaine Sobral,<sup>3</sup> Fernando Werneck,<sup>13</sup> Carlos Scridelli,<sup>14</sup> Luis Gonzaga Tone,<sup>14</sup> Lincoln Vermondi,<sup>12</sup> Luis Fernando Lopes,<sup>15</sup> Wellington Mendes.<sup>15</sup>

Affiliations:

1. Centro de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, Brazil
2. Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil
3. Instituto de Pediatria e Puericultura Martagão Gesteira, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
4. Sociedade de Oncologia da Bahia, Salvador, Bahia, Brazil
5. Centro Infantil de Investigações Hematológicas D. Boldrini, Campinas, São Paulo, Brazil
6. Hospital Martagão Gesteira, Salvador, Bahia, Brazil
7. Departamento de Hematologia, Universidade de Santa Maria, Rio Grande do Sul, Brazil
8. Hospital de Apoio Brasília, Unidade de Onco-Hematologia Pediátrica, Brasília, Brazil
9. Hospital Napoleão Laureano, João Pessoa, Paraíba, Brazil
10. Departamento de Pediatria da Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
11. Hospital Oswaldo Cruz, Centro de Oncologia Pediátrica, Recife, Pernambuco, Brazil
12. Serviço de Oncologia do Hospital Joana de Gusmão Florianópolis, Santa Catarina, Brazil
13. Departamento de Pediatria, Hospital dos Servidores do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
14. Departamento de Pediatria, Hospital das Clínicas, Ribeirão Preto, São Paulo, Brazil
15. Hospital do Câncer AC Camargo, São Paulo, Brazil

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Maria S. Pombo-de-Oliveira and Sergio Koifman

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