The Interplay of Cesarean-section Delivery and First-birth order as Risk Factors in Acute Lymphoblastic Leukemia

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Abstract

Background: Childhood B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) has been associated with early life exposures including birth by cesarean section (C-section), and a deficit of social exposure (first child). These exposures as proxies for microbiome acquisition in infancy are essential to prime the immune system and, restrain later dysregulated immune responses which can trigger ALL in susceptible individuals. We tested risk factors pertaining to immune stimulation that may impact BCP-ALL development.

Methods: Cases comprised 1126 children (0-12 years) with ALL (BCP-ALL: 78.5%) from the EMiLI study group in Brazil (2002-2020). Age and sex-matched controls (n=2252) were randomly selected from healthy children whose mothers participated in the National Placental and Umbilical Cord Blood Bank donation. Multiple logistic regression was run fitted and adjusted for selected covariates models.

Results: C-section delivery was associated with increased risk for ALL [Odds Ratio (OR)ALL:1.10, 95% Confidence Intervals (CIs):1.04-1.15; $OR_{BCP\text{-}ALL}$:1.09, 95% CIs:1.03-1.14], as well as being the first-born child. Interaction analysis showed a significant effect of first birth on the observed C-section associations ($p<0.0001$). Indeed, high-risk children, namely first-born children delivered via C-section were at increased risk for ALL (OR: 2.33, 95% CIs:2.40-4.84) compared to non-first, vaginally born children. An increased risk was found for first-born children delivered by C-section and non-breastfed with ALL (OR_{ALL}:2.32, 95%CIs: 1.27-4.24; $OR_{BCP\text{-}ALL}$:2.37, 95%CIs: 1.18-4.76).

Conclusions: Our observations are in accord with the prediction that exposures determining microbiome composition and adrenal pathway in infancy contribute to the risk of BCP-ALL.

Impact: These findings encourage the exploration of potential preventative interventions.

Introduction

Childhood acute lymphoblastic leukemia (ALL), the most common pediatric cancer account for about 35% of all childhood cancer cases with BPC-ALL being the most prevalent subtype (1). Implementation of risk-stratified, multi-agent treatment has led to impressive cure rates of approximately 90% (2). Relapse remains a clinical challenge, however, whereas the treatment is toxic with significant morbidity and long-term sequelae (3). This brings into focus the issue of ALL causation and the extent to which the disease might be preventable (4,5).

There is mounting support for a coherent and plausible causal explanation for BCP-ALL (5). According to this model risk of BCP-ALL is ascribed to a deficit of microbiome dependent immune priming in infancy with the consequence that later immune responses to common infections in childhood are dysregulated and can trigger ALL in susceptible children. The latter are children who have acquired clinically silent pre-malignant clones in utero (5).

This model is supported by robust epidemiological data derived from replicated case-control studies and meta-analyses which have identified Caesarean section birth (C–section) (6), lack of breastfeeding and a deficit of social contacts in infancy via older siblings or day care attendance as risk factors (7) 8). These social variables all reflect the different routes via which infants normally acquire a diverse gut microbiome (9,10). Previous studies have looked at these early-life social variables usually on an individual level. But given their likely essential and independent role in both acquisition and nourishment of a diverse and balanced infant gut microbiome ecosystem, we predicted they should interact or compound to amplify the risk of BCP-ALL.

Recently, a study using data from the Ministry of Health Live Birth Information System (SINASC in Portuguese) showed great improvements in maternal and childcare indicators, however, a high rate of C-section deliveries in both private and public sectors (11). Nonetheless, exclusive breastfeeding remains modest, namely less than 6 months in most settings (12). Obstetricians assist most deliveries

and, they are crucial in the decision about the type of delivery. When the Robson classification system was applied, the proportion of C-section deliveries was the same among women at low risk of maternal or fetal death suggesting that most C-sections were due to elective reasons (13). Herein, we tested this proposition in Brazil, a country experiencing one of the highest rates of C-section deliveries worldwide. Specifically, we considered that the highest risk group should be children born by C-section, first born and non-breastfed.

Material and Methods

Study Design and Subjects

The present hospital-based case-control study included children within incident diagnosis of ALL (less than 12 years) over the period 2002-2020 who participated in the project "*Epidemiology of Multi-institutional Study Group of Acute Leukemia* (EMiLI). The participants included in this study represent all geographic regions of Brazil in a network of studies linking patient ascertainment for diagnostic biomarkers' identification and epidemiological data. A flow diagram summarizing the study design with the selection process is detailed in Supplementary **Fig 1**. Briefly, cases were referred to the EMILi study with the purpose of immuno-molecular characterization of acute leukemia subtypes at the Pediatric Oncological-Hematological Research Program, National Cancer Institute-Rio de Janeiro, Brazil. There were 2178 incidents ALL cases. Cases with aberrant immunophenotype (n=44), children adopted (n,10), a genetic syndrome associated with leukemia (n,87), and children with more than 12 years at the diagnosis (n,453). The type of assistance healthcare received by 91.3% of children herein were within the Unified Health System [SUS; in which three-quarters of all Brazilian births take place (11)].

On-line forms with demography and maternal-childbirth characteristics, as well as clinical data was completed by the referring physicians according to their medical records. Once the case met the eligibility criteria, the mother was invited to answer an additional questionnaire (maternal interviews) about pregnancy characteristics, child delivery and healthcare during the first year of childhood. Pro-

B ALL, BCP-ALL or T-ALL subtypes were identified by flow cytometry including the most common cytogenetics and/or molecular aberrations mutual exclusively associated with ALL subtypes, such as numerical abnormalities (hyperdiploidy or hypodiploidy) and structural abnormalities, such as translocations (*ETV6-RUNX1, TCF3-PBX1, STIL-TAL1, KMT2A*-rearrangements and BCR-ABL1).

Controls ascertainment were children without leukemia, or any history of pediatric cancer selected from the National Placental and Umbilical Cord Blood Bank (NPUCBB, INCA-BR), whose mother voluntarily donated cord blood samples for hematopoietic progenitor cell transplantation as described in detail in the Supplementary Material. Controls were chosen from the total NPUCBB participants according to study-year and Brazilian macroregion, frequency-matched to cases on sex and age (by year of birth). Cases and controls were equally assisted within the Unified Health System (11,14).

In both cases and controls, children with Down syndrome and mothers who refused to participate in the enquiries and/or to sign the consent forms were excluded from the study. Quality control mechanisms were applied to the data recorded at baseline in NPUCBB along with comparisons to the national measurements of the variables included in this study, as well as to the inequalities in health indicators of the mode of delivery rate.

Maternal interviews were conducted within the first year after the ALL diagnosis by trained personnel either in person (period of 2002-2012) or in digital mode (after the year_2012). Only biological mothers of cases and controls were eligible. Information on pregnancy characteristics, birth delivery and the first year of child healthcare was verified by interviews throughout the Child Health Handbook (CHH) handled by the mothers. Controls 'information was also provided by the NPUCBB regarding the child's sex and ethnicity, mode of delivery, birth weight, gestational age, birth order and breastfeeding recorded at the maternal´ s chart.

The maternal questionnaires were approved by the Instituto Nacional de Cancer Ethics and Research Committee (CONEP # 1.394.043) and the parents signed the written consent form to participate in the study.

Statistical analysis

The distributions of the variables of interest were initially assessed through Pearson's chi-square test with Yates' continuity correction. To express the power calculation in odds ratio (OR), the sample size was calculated considering 2 controls per 1 case $\alpha=0.05$; $\beta=0.2$ for power=0.80, which provides us with the detection of a main effect of approximately OR,1.45, if the prevalence of exposure is 11.5% in cases and about 7% in controls. *(http://powerandsamplesize.com)*. First, we compared whether information collected in the control group reflects the Brazilian demographic and epidemiological profile on Maternal-Child health, such as the mean gestational age, birth size and mode of delivery. Then, controls were frequency matched to cases on age and sex in a 1:2 ratio.

To explore the impact of early life risk factors we have tested, (A) children aged 0-12 years with ALL, (B) children aged 1-12 years with BCP-ALL and, (C) infant-ALL (i-ALL, namely children less than 1 year of age at the diagnosis) controlling for potential confounders.

Multiple logistic regressions were fitted, and adjusted effect estimates (OR) and 95% confidence intervals (CIs) were calculated to assess the association of the mode of delivery with total ALL and BCP-ALL risk controlling for selected covariates. Specifically, the core model included the main variables of interest, namely, mode of delivery (C-section vs. vaginal); birth order (first born child as yes or no); child's ethnicity (White; non-White with mixed ethnicity), birth weight (≤2500, 2500-2999, 3000-3499, 3500-3999, ≥4000 grams) and gestational age (<37, 37-40, ≥41 weeks), maternal ages (<35; ≥35 year-old), maternal schooling (≤5, 6-12, >12 years).

Formal interaction analyses were thereafter conducted to assess the potential mediating effect of main variables of interest on the association of C-section delivery with total ALL, BCP-ALL and i-ALL (15). Simulation multiple logistic regression models were also run considering all controls with unknown birth order status as non-first born (best case scenario), and alternatively all controls with unknown birth order status as first born (worst case scenario).

We have also estimated the size of the effect among non-breastfed, first-born children who had been delivered by C-section (16). Similarly, we have tested the effect of breastfed and mode of delivery. Breast-feeding was defined using three categories, (1) none and/less than 29 days; (2) breast-feeding less than 3 months and, (3) more than 3 months.

Lastly, cytogenetics-molecular subtypes exclusively associated with BCP-ALL were also analyzed controlling for maternal age, child's age, sex, birth size, and ethnicity. All statistical analyses were performed using both R studio version R4.1.1 and the SAS software (SAS, Cary, NC).

Results

Out of eligible 1584 cases, 86 were excluded because their mothers refused to participate due to death of the index child, and there was loss of contact address for another 366, leaving 1132 cases (71.4%) for the analyses. The response rate for eligible cases were 71.4%% and for controls were 74%%.

The distributions of main variables of interest of 1126 children with ALL and their 2252 age and sex matched controls are shown in **Table 1**. Among cases, there were 130 (11.6%) pro-B ALL, 883 (78.1%) BCP-ALL and 112 (9.9%) T-ALL. As expected, most cases were 1-5 years old (mean age: 3.8 years), males and Whites. Among i-ALL subgroup a predominance of non-Whites was observed ($p<0.001$). Pregnancy duration of at least 37 weeks or more was predominant among cases (80.5%) and controls (87.9%), while being the first child and born by C-section was predominant among cases

(52.8% and 45.8% among controls). Maternal´ s age at childbirth was 26.1 (standard deviation 6.1, minimum 13 and maximum 47) and 86.3% were less than 35 years of age. The majority of mothers 'cases and controls have reported less than 12 years of schooling. For cases, mean 9.2 (standard deviation 4.5, minimum 0.1 and maximum 30), and mean 10.0 (standard deviation 3.1, minimum 0.1 and maximum 27) for controls.

Missing values (MV) were less than 5% for both cases and controls in the two main variables of interest, notably mode of delivery and birth order. The retrospectively collected data on breastfeeding had high proportion of MV among cases (23.4%) and controls (48.7%), and a high likelihood for recall bias among cases was observed.

In the multiple logistic regression (MLR) models **(Table 2**), low birth weight [(≤2500g), adjOR, 1.28 (95%CI, 1.12-1.47)], born less than 37 weeks (adjOR,1.18, 95%CI,1.02-1.28), C-section delivery (adjOR,1.10, 95%C,I 1.03-1.15) and being first born child (adjOR,1.06, 95%CI 1.02-1.10) were significantly associated with ALL, as well as BCP-ALL risk (adjOR, 95%1.14 (1.06-1.22). T-ALL were on the same direction [low birth weight: OR 2.9, 95%CI (0.69-2.72); C-section: OR 1.33, 95% CI, (0.81-2.19)], albeit not reaching statistical significance (Supplementary **Table 1**). We have also included gestational age as covariate, but no statistically significant changes were noted.

Simulation derived logistic regressions models for the association mode of delivery and first born showed no differences in the risk magnitude for ALL (OR, 1.23, 95% CI: 1.07-1.43) and BCP-ALL (OR, 1.21, 95% CI:1.04-1. 42) as shown in Supplementary **Table 2**. In the formal interaction analysis (**Table 3**), a positive relative excess risk due to interaction was noted regarding the effect of first birth on the association of C-section delivery with ALL (OR, 1.45 95% CI 1.78-8.39), BCP-ALL (OR, 1.41 95% CI 1.11-1.78), and i-ALL (OR, 1.81 95% CI 0.99-3.30).

To test the additive effect, in the both variables, a MLR was performed in 862 cases and 1150 controls according to the age break in ALL, BCP-ALL and i-ALL, test adjusted by maternal age and ethnicity (**Table 4**). The high-risk group, namely first-born children delivered by C-section were at

increased risk for ALL as well as BCP-ALL (OR_{ALL}: 2.33, 95% CIs: 1.20-4.54; $OR_{BCP\text{-}ALL}$: 2.41, 95% CIs: 1.20-4.84) compared to non-first, vaginally born children.

Because of the questionable quality of *posthoc* collected data about breastfeeding, sub-analyses were run only among the reported non-breastfed children to assess any increase in the risk on account of lack of breastfeeding. Indeed, in sub-analyses among non-breastfed children (Supplementary **Table 3**), despite small numbers of cases (n=128) and controls (n=93), the MLR showed that first born children were associated with ALL and BCP-ALL (OR: 2.32, 95% CIs:1.27-4.25). Of note, the odds for ALL and BCP-ALL among non-first-born child were of similar, albeit not statistically significant, among non-breastfed pointing to other factors that may also mediate the association.

Lastly, as shown in **Table 5**, we examined the most common cytogenetic-molecular subtypes of BCP-ALL (1-12 years) to test the hypothesis of *in utero* origin of ALL and risk factors that would be relevant to impart the additional hits necessary to lead to leukemic cells expansion. The MLR showed that either C-section and breastfed risk associations were null or not statistically significant, while the variable first born maintained the direction of the association in BCP-ALL with hyperdiploidy/*ETV6-RUNX1* (adjOR: 1.12, 95% CI:0.99-1.22) and other chromosomal abnormalities (adjOR: 1.10, 95% CI:1.01-1.21).

Discussion

Exploiting a large Brazilian case-control dataset, we have validated the prediction that early life risk factors for ALL, namely delivery by C-section (8,17,18) and, additionally being first born are consistently associated with increased risk as previously reported. Recently, Williams et al (2021) using populational-base information found similar results regarding C-section associated with ALL and hepatoblastoma (19), while Dwyer et al. (2021) have explored mode of delivery and birth characteristics in early-onset of non-Hodgkin lymphoma (20). The compound effect is essentially

additive as might be expected if the variables of birth process, breastfeeding, birth order and social contacts each had an independent contribution to establishment of a balanced gut microbiome.

Indeed, the established association with high birthweight was not found in the Brazilian data (21). Of note, however, about 47.1% of children in Brazil are born via elective C-section (13,14), which is scheduled earlier than normal delivery leading to a lower average birthweight compared to that expected through vaginal term deliveries. Increasing rates of prematurity and low birth weight in Brazil are associated to the increasing rates of Caesarean sections (22).

Regarding the low birth weight (LBW) association observed in our data, this is in line with the ALL association reported in the Junqueira et al study of São Paulo (18) in which, they found a positive association of LBW with ALL in a population different than the one in the current analysis. LBW was more likely observed in premature babies, children born by elective C-section and/or those of deprived population groups (23). Indeed, LBW maybe considered a proxy of increased gestational-prenatal stress due to deprivation conditions during such as shortage of nutrition and/or adverse environmental factors during pregnancy, leading to increased levels of glucocorticoids. The reported biological plausibility is speculative. A higher proportion of deliveries through elective C-section (although we did evaluate it herein) and LBW would decrease performance of all elements (epinephrine, cortisol levels and cytokines) necessary to establish the enteral feeding that can contribute to dysbiosis. Usually, low birthweight children receive formula and/or little mother's own milk, stay longer in the hospital environment, that might contribute to dysbiosis.

In any of the above conditions we speculate that the adrenal pathway and the microbiome composition may contribute to the ALL risk. Indeed, the adrenal hypothesis emerged from the ecological studies on age-related incidence of ALL, differences of incidence rate of ALL in developed and less developed societies and, the kinetics of disappearance of pre-leukemic clone throughout qualitative and quantitative changes in plasma cortisol levels (24). It was presented as a

plausible mechanism in the Greek study by Thomopoulos et al., that was confirmed later in CLIC data (6,17).

We did not evaluate other key variables of social exposure and established risk factor for BCP-ALL, namely lack of day care attendance in infancy, but Rudent et al (2015) have shown that day care attendance and breast feeding have a compound impact on ALL risk (8,25). Our findings suggest the possibility to identify a population of children at higher ALL/BCP-ALL risk who might benefit from preventive measures.

The biological plausibility underlying these associations need to be further explored, and several distinct pathways have been postulated, including the diminished cortical stress response in C-section compared to vaginal birth (26,27). In the study of Vogl et al.(2006), a significantly lower cortical stress umbilical cord concentration of cortisol was found, whereas children born via C-section had lower gestational age and birthweight compared to those born vaginally (28). However, the most likely biological mechanism at play with compounded early life exposures and risk of ALL is acquisition of the gut microbiome (9). We note that the same early life exposures are linked to risk of other more common immunopathology of children in developed societies including type 1 diabetes and allergies suggesting a shared underlying microbiome dependent immune deficit (29,30).

A diverse microbiome is essential to prime the infant immune system for well-regulated responses, avoiding chronic inflammation, in later life (31,32). This process depends upon microbial exposure during vaginal birth, breastfeeding, and social contacts especially in infancy and in the first few years of childhood and may be further compromised by antibiotic exposure (33). C-section birth, especially in pre-term babies is associated with a less diverse gut microbiome which is further compromised if mothers have a non-secretor (fucosyltransferase 2- *FUT2*) genotype with a lack of oligosaccharides in breast milk that nourish keystone microbiome species such as Bifidobacteria (34). Significantly, probiotic supplementation with probiotics including Bifidobacteria and Lactobacillus may restore the microbiome deficit in C- section born infants (35–37). We have no data on the gut microbiome status

of cases and controls in our study. However, there is published data showing that children with ALL have a less diverse gut microbiome than age matched controls (38,39).

Birth by C-section has increased in recent decades in many countries and Brazil has a very high incidence of around 70% in nulliparous women, in which a greater risk associated with unnecessary C-section was observed and regardless the socioeconomic status (13,14,22), albeit the highest C-section rates were found mainly in regions with high development indices such as the South, Southeast and Midwest macroregion of Brazil (13,22). Regarding breastfeeding, it is documented that C-section may prolong the mother surgery´s recovery, leading to delay of initiation of breastfeeding (40). Encouragement of normal vaginal birth when safe to do so along with more protracted breast feeding would be beneficial for several reasons and it should reduce risk of BCP-ALL along with a potential of more substantial risk reduction by probiotic supplementation (35). This latter intervention approach is already employed in clinical trials with encouraging early results for combating sepsis in infants (36), reducing risk of allergy, and restoring the microbiome of infants born by C-section. Given the prevalence of microbiome linked immune priming deficiencies, including ALL, in developed societies there may be a case for population wide probiotic supplementation (38,41). However, a more practical intervention to reduce the risk of childhood BCP-ALL, highlighted by our findings reported here, might be to follow recent clinical studies with specific bacterial probiotic supplementation in a large cohort of infants at higher risk by virtue of their C-section birth, paucity of breast feeding and lack of elder siblings.

The distribution of cytogenetic-molecular subtypes of B-cell ALL have important clinic-etiopathology characteristics (42). The evidence of *ETV6-RUNX1* or hyperdiploidy in utero origin as driver mutation and, age-associated ploidy/fusion gene abnormalities, it is not sufficient for overt leukemia unless complementary hits occur (5,43). We tested if they could have relevance in the interplay of C-section and first-born child´s models, being first born maintained the direction of the increased association in BCP-ALL. These risk factors impact ALL risk in the expected pattern,

supporting a risk scenario proposed in the combination of adrenal pathways hypothesis and microbiome composition where lack of immune priming increases ALL risk. The present study included the largest so far series of ALL cases in analytic epidemiology study in Brazil. The recall of mode of delivery, namely the main exposure variable, was nearly 100% accurate, validated in medically recorded information in both cases and controls. The data obtained from maternal questionnaires were cross-checked through the Child's Health Handbook handled by the mothers of cases during the interviews. Despite being geographically heterogeneous, the study is nationally representative, and the results reflect the country indicators, given that the Brazilian health system has national coverage largely funded and regulated by the federal government regarding specialized reference centers as well as maternal-child primary healthcare (11,14).

Regarding the statistical analyses- strengths, it is worth to mention that, over and beyond testing the primary hypothesis, namely whether C-section is also associated with BCP-ALL risk, we have exploited this dataset by testing the composite impact of being simultaneously first born and C-section delivered on the childhood ALL/BCP-ALL risk. We have also performed formal interaction analyses to assess whether there are additive effects among the main variables of interest namely mode of delivery and birth order which seem to be accurately reported in both cases and controls.

To bypass the inherent limitation of recall bias selectively among mothers of diseased children in this case control study regarding the breastfeeding duration, we performed sub-analyses exclusively among those children whose mothers reported not to have breastfeed the child, as well as, C-section and breast-feeding. Indeed, the high proportion of MV in the control series regarding breast feeding and its duration was an initial concern; the frequency of breast feeding, however, in the control series, after excluding the unknown breastfeeding values, was close to the nationwide figures, which gave some confidence to infer that the missing information was non differential and that we could proceed with the analyses among non-breastfed only. Regarding contribution to underlying biological

mechanisms, our dataset lacked information on the indication for C-section, which did not allow us to perform further sub-analyses and calculation of preventable fractions.

In conclusion, a sizeable increased risk was found for ALL subtypes for those children delivered by C-section, first born and non-breast fed. These findings endorse the causal hypothesis for childhood ALL associated with gut microbiome dysbiosis. The complex interplay of variables used as proxies of socialization and those shaping the microbiome of the newborn, such as birth order and breastfeeding, early exposure to infections and early day care attendance as well as specific reasons prelabour/scheduled C-section needs to be further explored in large cohort studies and concerted actions. Nevertheless, additive effects of first birth and non-breastfeeding on the association of C-section with BCP-ALL risk provide cues for clinical practices and the potential benefits of decreasing unnecessarily scheduled C-sections, especially among women giving birth to their first child, as well as for encouraging universal breastfeeding of the newborn. Our findings also identify a group of infants at significantly higher risk of ALL who might provide a target population for risk reduction via probiotic supplementation.

Authors´s Contributions.

M.S.Pombo-de-Oliveira, E.Th.Petridou, M.A.Karalexi, M.Greaves: Design study conception, data curation, statistics analysis, writings, and editings. **E. Ntzani, P.C. Neto, M.E.R. Junqueira, G.R.C.Mura, F. H. Paraguassu-Braga, L.F.Bouzas:** Constructing databases, formal statistics methodology. **L.F. Lopes and the EMiLi** study group participants have contributed with the recruitment and patients´ ascertainment, acquisition of data and management of enquires.

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References

1. Miranda-Filho A, Piñeros M, Ferlay J, Soerjomataram I, Monnereau A, Bray F. Epidemiological patterns of leukaemia in 184 countries: a population-based study. The Lancet Haematology. 2018;5:e14–24.

2. Pui C-H, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol. 2011;29:551–65.

3. Essig S, Li Q, Chen Y, Hitzler J, Leisenring W, Greenberg M, et al. Risk of late effects of treatment in children newly diagnosed with standard-risk acute lymphoblastic leukaemia: a report from the Childhood Cancer Survivor Study cohort. Lancet Oncol. 2014;15:841–51.

4. Greaves M, Cazzaniga V, Ford A. Can we prevent childhood Leukaemia? Leukemia. 2021;35:1258–64.

5. Greaves M. A causal mechanism for childhood acute lymphoblastic leukaemia. Nat Rev Cancer. 2018;18:471–84.

6. Marcotte EL, Thomopoulos TP, Infante-Rivard C, Clavel J, Petridou ET, Schüz J, et al. Caesarean delivery and risk of childhood leukaemia: a pooled analysis from the Childhood Leukemia International Consortium (CLIC). Lancet Haematol. 2016;3:e176-185.

7. Marcotte EL, Ritz B, Cockburn M, Yu F, Heck JE. Exposure to infections and risk of leukemia in young children. Cancer Epidemiol Biomarkers Prev. 2014;23:1195–203.

8. Urayama KY, Ma X, Selvin S, Metayer C, Chokkalingam AP, Wiemels JL, et al. Early life exposure to infections and risk of childhood acute lymphoblastic leukemia. Int J Cancer. 2011;128:1632–43.

9. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. Science. 2016;352:539–44.

10. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118:511–21.

11. Castro MC, Massuda A, Almeida G, Menezes-Filho NA, Andrade MV, de Souza Noronha KVM, et al. Brazil's unified health system: the first 30 years and prospects for the future. Lancet. 2019;394:345–56.

12. Boccolini CS, Boccolini P de MM, Monteiro FR, Venâncio SI, Giugliani ERJ. Breastfeeding indicators trends in Brazil for three decades. Rev Saude Publica. 2017;51:108.

13. Rudey EL, Leal M do C, Rego G. Cesarean section rates in Brazil: Trend analysis using the Robson classification system. Medicine (Baltimore). 2020;99:e19880.

14. Victora CG, Aquino EML, do Carmo Leal M, Monteiro CA, Barros FC, Szwarcwald CL. Maternal and child health in Brazil: progress and challenges. Lancet. 2011;377:1863–76.

15. Knol MJ, VanderWeele TJ, Groenwold RHH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. Eur J Epidemiol. 2011;26:433–8.

16. Kim RS, Kaplan RC. Analysis of secondary outcomes in nested case-control study designs. Stat Med. 2014;33:4215–26.

17. Thomopoulos TP, Skalkidou A, Dessypris N, Chrousos G, Karalexi MA, Karavasilis TG, et al. Prelabor cesarean delivery and early-onset acute childhood leukemia risk. Eur J Cancer Prev. 2016;25:155–61.

18. Junqueira MER, de Oliveira CT, Tone LG, Lee ML de M, de Andréa MLM, Bruniera P, et al. Caesarean sections, prenatal and postnatal conditions and childhood acute lymphoblastic leukaemia: A case-control study in the State of São Paulo, Brazil. Cancer Epidemiol. 2020;69:101851.

19. Williams LA, Richardson M, Spector LG, Marcotte EL. Cesarean Section Is Associated with an Increased Risk of Acute Lymphoblastic Leukemia and Hepatoblastoma in Children from Minnesota. Cancer Epidemiol Biomarkers Prev. 2021;30:736–42.

20. Dwyer KE, Wang R, Cozen W, Cartmel B, Wiemels JL, Morimoto LM, et al. Mode of Delivery, Birth Characteristics, and Early-Onset Non-Hodgkin Lymphoma in a Population-Based Case-Control Study. Cancer Epidemiol Biomarkers Prev. 2021;30:2286–93.

21. Milne E, Greenop KR, Metayer C, Schüz J, Petridou E, Pombo-de-Oliveira MS, et al. Fetal growth and childhood acute lymphoblastic leukemia: findings from the childhood leukemia international consortium. Int J Cancer. 2013;133:2968–79.

22. Barros FC, Rabello Neto D de L, Villar J, Kennedy SH, Silveira MF, Diaz-Rossello JL, et al. Caesarean sections and the prevalence of preterm and early-term births in Brazil: secondary analyses of national birth registration. BMJ Open. 2018;8:e021538.

23. Lee ACC, Katz J, Blencowe H, Cousens S, Kozuki N, Vogel JP, et al. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. Lancet Glob Health. 2013;1:e26-36.

24. Schmiegelow K, Vestergaard T, Nielsen SM, Hjalgrim H. Etiology of common childhood acute lymphoblastic leukemia: the adrenal hypothesis. Leukemia. 2008;22:2137–41.

25. Rudant J, Lightfoot T, Urayama KY, Petridou E, Dockerty JD, Magnani C, et al. Childhood acute lymphoblastic leukemia and indicators of early immune stimulation: a Childhood Leukemia International Consortium study. Am J Epidemiol. 2015;181:549–62.

26. Taylor A, Fisk NM, Glover V. Mode of delivery and subsequent stress response. Lancet. 2000;355:120.

27. Mears K, McAuliffe F, Grimes H, Morrison JJ. Fetal cortisol in relation to labour, intrapartum events and mode of delivery. J Obstet Gynaecol. 2004;24:129–32.

28. Vogl SE, Worda C, Egarter C, Bieglmayer C, Szekeres T, Huber J, et al. Mode of delivery is associated with maternal and fetal endocrine stress response. BJOG. 2006;113:441–5.

29. Thomas S, Izard J, Walsh E, Batich K, Chongsathidkiet P, Clarke G, et al. The Host Microbiome Regulates and Maintains Human Health: A Primer and Perspective for Non-Microbiologists. Cancer Res. 2017;77:1783–812.

30. Sevelsted A, Stokholm J, Bønnelykke K, Bisgaard H. Cesarean section and chronic immune disorders. Pediatrics. 2015;135:e92-98.

31. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host Microbe. 2015;17:690–703.

32. Tamburini FB, Andermann TM, Tkachenko E, Senchyna F, Banaei N, Bhatt AS. Precision identification of diverse bloodstream pathogens in the gut microbiome. Nat Med. 2018;24:1809–14.

33. Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG. 2016;123:983–93.

34. Korpela K, Blakstad EW, Moltu SJ, Strømmen K, Nakstad B, Rønnestad AE, et al. Intestinal microbiota development and gestational age in preterm neonates. Sci Rep. 2018;8:2453.

35. Korpela K, Salonen A, Virta LJ, Kekkonen RA, de Vos WM. Association of Early-Life Antibiotic Use and Protective Effects of Breastfeeding: Role of the Intestinal Microbiota. JAMA Pediatr. 2016;170:750–7.

36. Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, Chandel DS, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature. 2017;548:407–12.

37. Patole SK, Rao SC, Keil AD, Nathan EA, Doherty DA, Simmer KN. Benefits of Bifidobacterium breve M-16V Supplementation in Preterm Neonates - A Retrospective Cohort Study. PLoS One. 2016;11:e0150775.

38. Masetti R, Muratore E, Leardini D, Zama D, Turroni S, Brigidi P, et al. Gut microbiome in pediatric acute leukemia: from predisposition to cure. Blood Adv. 2021;5:4619–29.

39. Oldenburg M, Rüchel N, Janssen S, Borkhardt A, Gössling KL. The Microbiome in Childhood Acute Lymphoblastic Leukemia. Cancers (Basel). 2021;13:4947.

40. Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387:475–90.

41. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. J Allergy Clin Immunol. 2009;123:335–41.

42. Moorman AV. The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. Blood Rev. 2012;26:123–35.

43. Greaves M. In utero origins of childhood leukaemia. Early Hum Dev. 2005;81:123–9.

Table 1. Distribution of phenotypes and variables of A. 1126 children aged 0-12 years with acute lymphoblastic leukemia (ALL) and their 2252 age and sex matched controls; B. 883 children aged 1-12 years with B cell precursor acute lymphoblastic leukemia (Bcp-ALL) and their 1766 controls; and C. 131 infants with ALL (i-ALL <1 year) and their 262 controls by study variables: Brazil, 2002-2020.

Variable	A. ALL		A. Controls			B. Bcp-ALL		B. Controls			C. i-ALL		C. Controls		
	N	(%)	N	(%)	*p*-value	N,	(%)	N,	(%)	p-value	N,	(%)	N,	(%)	p-value
ALL Phenotypes															
Pro-B lymphoblastic	131	11.6	--			--		--			131		--		
Pre-B (CD10+)	883	78.5	--			883		--			0		--		
T cell lymphoblastic	112	9.9	--			--		--			0		--		
Child's sex				Matching Variable					Matching Variable					Matching Variable	
Female	516	45.8	1032	45.8		425	48.2	851	48.2		59	44.6	117	44.6	
Male	610	54.2	1220	54.2		458	51.8	915	51.8		72	55.4	145	55.4	
Child's age (years)				Matching Variable					Matching Variable					Matching Variable	
<1	136	12.0	272	12		--					--		--		
1-5	686	61.0	1374	61		636	72.0	1272	72.0		--		--		
6-12	304	27.0	608	27		247	28.0	494	28.0		--		--		
Child's ethnicity					0·496					0.786					0.001
White	592	52.6	1198	53.2		472	53.5	942	53.3		52	39.2	147	56.2	
Non-White	534	47.4	1028	45.6		411	46.5	802	45.4		79	60.8	114	43.5	
Missing	--	--	26	1.2		--		22	1.2		--		1	0.3	
Birth weight (grams)					0.001					0.001					0.159
≤2500	85	7.6	98	4.1		65	7.4	77	4.4		12	9.2	20	7.7	

2501-2999	188	16.7	383	17		162	18.3	295	16.7		16	12.3	55	21.2	
3000-3499	462	41.0	928	41.4		357	40.4	728	41.2		61	46.2	102	38.5	
3500-3999	288	25.6	636	28.2		219	24.8	509	28.7		34	26.2	63	24.2	
≥4000	68	6.0	187	8.3		52	5.9	141	8.0		6	4.6	20	7.7	
Missing	35	3.1	20	0.9		28	3.2	16	0.9		2	1.5	2	0.8	
Gestational age (weeks)					0.001					0.001					0.095
<37	90	8.0	95	4.2		78	8.8	71	4.0		4	3.1	13	4.6	
37-40	906	80.5	1975	87.9		705	79.8	1543	87.4		112	85.4	236	90.8	
≥41	99	8.8	175	7.8		76	8.6	146	8.3		13	10.0	13	4.6	
Missing	31	2.8	7	0.3		24	2.7	6	0.3		2	1.5	--		
First-Birth					0.001					0.003					0.086
Yes	594	52.8	1032	45.8		473	53.6	828	46.9		64	49.2	104	40.0	
No	505	44.8	1118	49.6		386	43.7	865	49.0		64	49.2	153	58.1	
Missing	27	2.4	102	4.5		24	2.7	73	4.1		3	1.5	5	1.9	
Mode of Delivery					0·088					0.336					0.389
Vaginal	552	49.0	1174	52.1		442	50.1	919	52.0		56	43.1	126	47.7	
Cesarean	574	51.0	1078	47.9		441	49.9	847	48.0		74	56.9	136	52.3	
Breastfeeding*					0.947					0.803					0.245
Yes	725	64.4	971	43.1		576	65.2	755	42.8		86	66.2	108	41.5	
No	137	12.1	185	8.2		102	11.6	129	7.3		27	20.8	47	18.1	
Missing	264	23.4	1096	48.7		205	23.2	882	49.9		18	13.1	107	40.4	
Maternal Age (years)δ					0.003					0.002					0.069
< 35	855	75.8	1944	86.3		675	76.3	1516	85.8		112	86.1	236	86.8	
≥ 35	95	8.4	294	13.1		69	7.8	233	13.2		18	13.8	36	13.2	
Missing	176	15.8	13	0.6		139	15.9	15	1.0		1	0.1	-	-	

Maternal Schooling (years) §														
≤5	207	18.4	219	9.7	p0.001	160	18.1	164	15.7		25	19.2	34	12.9
6-12	666	59	1475	65.5		527	59.5	1094	62		80	61.5	176	67.2
>12	181	16	337	15		139	15.7	306	17.3		25	19.2	37	14.1
Missing	72	6.6	221	9.8		57	6.7	202	11.4		1	0.1	25	9.5
Cytogenetics-Molecular tests§														
Successful			864	81.0	-	-	739	83.7	-	-	125	95.3		
Missing at least one			214	19.0	-	-	144	16.3	-	-	6	4.7		

* Further statistical evaluation was performed after missing values were excluded.§, The mother´s mean age at childbirth was 26.1 (standard deviation 6.0, minimum age 13 and maximum 47) years. The mean years of maternal schooling was 9.2 (standard deviation 6.0, minimum age 13 and maximum 47) years.

§ Cytogenetics-Molecular tests only in B-cell precursor acute Lymphoblastic leukemia and infant-Acute Lymphoblastic leukemia;

Table 2. Multiple logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CIs) for childhood. A.1068 children (0-12 years) acute lymphoblastic (ALL) and their 2106 controls, B.834 children B-cell precursor (Bcp-ALL; 1-12 years) and their 1660 controls, C.127 children infant (<1 years) acute lymphoblastic leukemia and their 253 controls, Brazil, 2002-2020

	A. ALL		B. Bcp-ALL		C. i-ALL	
Variables	**adjOR (95%CIs)**	***p*-value**	**adjOR (95%CIs)**	***p*-value**	**adjOR (95%CIs)**	***p*-value**
Child's ethnicity						
White	1.00 (reference)		1.00 (reference)			
Non-White	0.86 (0.81-0.91)	0.001	0.87 (0.82-0.94)	0.001	1.27 (0.51-3.20)	0.61
Birth weight (gram)						
≤2500	1.28 (1.12-1.47)	0.001	1.28 (1.12-1.47)	0.001	1.57 (0.41-5.94)	0.51
2501-2999	1.05 (0.98-1.13)	0.17	1.08 (0.99-1.17)	0.05	0.65 (0.27-1.67)	0.36
3000-3499	1.00 (reference)		1.00 (reference)		1.00 (reference)	
3500-3999	0.98 (0.97-1.04)	0.31	0.99 (0.93-1.04)	0.61	0.79 (0.39-1.59)	0.51
≥4000	0.93 (0.86-1.02)	0.13	0.92 (0.83-1.03)	0.13	0.61 (0.19-1.98)	0.41
Gestational age (weeks)						
<37	1.18 (1.04-1.31)	0.01	1.22 (1.09-1.37)	0.01	0.34 (0.05-2.14)	0.25
37-40	1.00 (reference)		1.00 (reference)		1.00 (reference)	
≥41	0.95 (0.87-1.03)	0.20	0.94 (0.84-1.04)	0.23	1.76 (0.67-4.65)	0.22
First birth						
No	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Yes	1.06 (1.02-1.10)	0.02	1.14 (1.06-1.22)	0.001	1.51 (0.66-3.42)	0.33
Mode of delivery						
Vaginal	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Cesarean section	1.10 (1.04-1.15)	0.001	1.08 (1.03-1.14)	0.003	1.57 (0.60-4.13)	0.36

All variables are in the model; Adj OR, adjusted maternal age.

Table 3. Formal interaction analysis tested for Cesarean section delivery and the first-born child risk factors and childhood acute lymphoblastic leukemia (ALL, 0-12 years), B-cell precursor-ALL (Bcp-ALL, 1-12 years) and, infant-ALL (≤1 1year), Brazil, 2002-2020.

Variables*	A. ALL				B. Bcp-ALL				C. i-ALL (<1 years)			
	Cases n, 1099	Controls n, 2147	OR (95%CIs)	*p*-value	Cases n, 860	Controls n, 1695	OR (95%CIs)	*p*-value	Cases n, 128	Controls n, 255	OR (95%CIs)	*p*-value
1.Vaginal/Non first-born	265 (24.1)	535 (24.9)	1.00 (reference)		207 (24.1)	425 (25.1)	1.00 (reference)		29 (22.7)	69 (27.1)	1.00 (reference)	
2.Vaginal/first-born	271 (24.7)	586 (27.3)	0.92 (0.74-1.13)	0.42	223 (25.9)	467 (27.6)	0.82 (0.64-1.04)	0.11	25 (19.5)	53 (20.8)	1.12 (0.59-2.13)	0.73
3.Cesarean/Non first-born	240 (21.8)	590 (27.5)	0.81 (0.66-1.01)	0.05	180 (20.9)	441 (26.0)	0.98 (0.77-1.23)	0.84	35 (27.3)	82 (32.2)	1.02 (0.56-1.82)	0.95
4.Cesarean/first-born	323 (29.4)	436 (20.3)	1.46 (1.78-8.39)	0.001	250 (29.1)	362 (21.4)	1.41 (1.11-1.78)	0.004	39 (30.5)	51 (20.0)	1.81 (0.99-3.30)	0.05
		AP*	SI*			AP*	SI*			AP*	SI*	
First birth		0.50	< 1			0.43	< 1			0.37	< 1	

AP, Attributable proportion to the interaction; SI, Synergy index; the proportion of first-born child born via C-section among cases was 54.3% and among controls was 42.6% (p value <0.0001) was calculated by Z-test.

Table 4. Multiple logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CIs) for confounders, A. 862 children (0-12 years) acute lymphoblastic (ALL) and their 1150 controls, B.678 children with B-cell precursor (Bcp-ALL; 1-12 years) and their 884 controls, and, C. 113 infant (<1 years) acute lymphoblastic leukemia and their 155 controls, Brazil, 2002-2020.

	ALL	Controls	OR*	*P*	Bcp-ALL	Controls	OR*	*P*	i-ALL	Controls	OR*	*P*
	n, (%)	n, (%)	95% CI		n, (%)	n, (%)	95% CI		n, (%)	n, (%)	95% CI	
Risk Variable:												
1. V/n-First Child	202 (23.4)	292 (25.4)	1.00*		158 (23.3)	228(25.8)	1.00*		26 (23.0)	48 (31.0)	1.00*	
2. V/First Child	205 (23.7)	389 (33.7)	1.08 (0.56-2.08)	0.83	169 (24.9	288 (34.1)	1.21 (0.61-2.41)	0.59	21 (18.6)	46 (28.0)	1.23 (0.12-6.59)	0.86
3. C/n-First Child	190 (22.1)	221 (19.2)	1.81 (0.92-3.56)	0.08	142 (20.9)	171 (18.4)	1.74 (0.86-3.53)	0.12	32 (28.3)	37 (23.6)	2.70 (0.25-12.53)	0.40
4. C/First Child	240 (27.8)	226 (19.7)	2.33 (1.20-4.54)	0.01	188 (27.6)	192 (21.7)	2.41 (1.20-4.84)	0.01	32 (28.3)	33 (21.3)	3.10 (0.31-15.10)	0.34
Missing	25 (2.9)	10 (0.9)			23 (3.2)	5 (0.6)			2 (1.8)	--	--	

The variables in model were, 1.V/n-First Child=vaginal birth and being non-first birth child, as reference; 2. V/First Child = vaginal birth and being first birth child; 3. C/n-First Child =Cesarean section and being non-first birth child; 4. C/First Child= Cesarean section and first birth child. Values corresponding to the number of cases and controls excluding the breastfeeding missing variable; Adj OR, adjusted by maternal age and ethnicity.

Table 5. Subgroup analyses by cytogenetics/molecular subtypes: unconditional multiple logistic regression-derived odds ratio (OR) and 95% Confidence Intervals (CI) for the association of mode of delivery with B-cell precursor acute lymphoblastic leukemia (Bcp-ALL;1-12 years; n,883), Brazil, 2002-2020

Subtype	Variable	Bcp-ALL	Controls (n=1766)	adjOR	95% CI	*p*-value
Hyperdiploid/ *ETV6-RUNX1*	Mode of delivery					
(n=332)	Vaginal	176	919	1.00	(reference)	
	Cesarean section	156	847	1.04	0.97-1.11	0.24
	Firstborn child					
	Yes	178	828	1.12	0.99-1.22	0.06
	No	146	865	1.00	(reference)	
	Missing	8	73			
Others[§]	Mode of delivery					
(n=154)	Vaginal	76	919	1.00	(reference)	
	Cesarean section	78	847	1.02	0.92-1.12	0.73
	Firstborn child					
	Yes	86	828	1.10	1.01-1.21	0.05
	No	63	865	1.00	(reference)	
	Missing	5	79			
All Negative [§§]	Mode of delivery					
(n=137)	Vaginal	66	919	1.00	(reference)	
	Cesarean section	71	847	1.02	0.85-1.21	0.85
	Firstborn child					
	Yes	71	828	1.09	0.98-1.21	0.11
	No	64	865	1.00	(reference)	
	Missing	2	73			

Missing	Mode of delivery					
(n=144)	Vaginal	73	919	1.00	(reference)	
	Cesarean section	71	847	1.11	0.94-1.32	0.22
	Firstborn child					
	Yes	75	828	1.06	0.76-1.23	0.78
	No	64	865	1.00	(reference)	
	Missing	5	73			

Abbreviation: n, number; p,p value; *adj.OR, Adjusted odd ratio for child's age, sex, maternal age and ethnicity;

[§] Others chromosomal-molecular aberrations, excluding infants. [§]Includes *TCF3-PBX1*, *BCR-ABL1*, near-hyperdiploid, hypodiploid. [§§]Normal karyotype/no fusion gene found; Missing are cases of unknown karyotype with negative fusion genes by fluorescence in situ hybridization and/or negative molecular results in the diagnostic evaluation of Bcp-ALL.