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Neonatal Inflammatory Markers Are Associated with Childhood B-cell Precursor Acute Lymphoblastic Leukemia

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Abstract

It has been proposed that children with acute lymphoblastic leukemia (ALL) are born with a dysregulated immune function that together with postnatal environmental exposures causes childhood ALL. Despite its importance for the understanding of ALL etiology, this hypothesis has been inadequately explored. In a population-based case–control study, we measured the concentrations of 10 cytokines and other inflammatory markers on neonatal dried blood spots from 178 children who at ages 1 to 9 years were diagnosed with B-cell precursor ALL and 178 matched controls. Through linkage with Danish nationwide registers, we also assessed whether neonatal inflammatory markers were associated with previously demonstrated risk factors for childhood ALL. Children who developed B-cell precursor ALL had significantly lower neonatal concentrations of IL8, soluble IL6 receptor (sIL6R) α, TGFβ1, monocyte chemotactic protein (MCP)-1, and C-reactive protein (CRP) and higher concentrations of IL6, IL17, and IL18

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.H. Søegaard, K. Rostgaard, J.L. Wiemels, K. Schmiegelow, H. Hjalgrim

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Hjalgrim

Study supervision: K. Rostgaard, K. Schmiegelow, H. Hjalgrim

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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compared with matched controls. Concentrations of IL10 were below the detection level for both patients and controls. Birth order (IL18 and CRP), gestational age (sIL6Rα, TGFβ1, and CRP), and sex (sIL6Rα, IL8, and CRP), but not maternal age, infections during pregnancy, birth weight nor mode of delivery were significantly associated with the neonatal concentrations of inflammatory markers. Our findings support the hypothesis that children who later develop B-cell precursor ALL are born with a dysregulated immune function.

Introduction

Infections early in life have long been suspected to play a role in the development of childhood acute lymphoblastic leukemia (ALL), in particular B-cell precursor ALL, the most common subtype. Many B-cell precursor ALL cases (e.g., high-hyperdiploidy and *ETV6-RUNX1*-translocated cases) are initiated in utero (1–3), and exposure to infections during pregnancy may increase offspring's risk of this intrauterine preleukemic development (4–7). Furthermore, it has been suggested that a lack of exposure to infections in early childhood can cause an abnormal immune response to infections later in childhood, leading to the transformation of preleukemic cells to leukemia. According to the delayed infection hypothesis, such abnormal immune response may be prevented by early exposure to infections (8). However, the hypothesis has been challenged by inconsistencies between epidemiologic studies of the role of early infections for the development of childhood ALL (9).

Inspired by a high incidence of clinically diagnosed infections in the first year of life among children who later developed ALL, it was proposed that children with ALL are born with a dysregulated immune function, causing them to react more vigorously to infections in early childhood (10, 11). This hypothesis was supported by Chang and colleagues (12) who compared inflammatory markers (interleukins) on neonatal dried blood spots between 116 children with ALL and 116 matched controls. In individual analyses, children who later developed ALL had statistically significantly lower neonatal concentrations of IL4, IL6, IL10, and IL13 than controls.

Although insight into the neonatal immune system is central for the understanding of the etiology of ALL, the study by Chang and colleagues (12) so far stands alone. To assess the association between neonatal concentrations of inflammatory markers and risk of childhood ALL, we therefore conducted a population-based matched case–control study with analysis of 10 cytokines and other inflammatory markers on neonatal dried blood spots from 178 children with B-cell precursor ALL and 178 matched controls. Through linkage with Danish nationwide registers, we assessed whether neonatal concentrations of inflammatory markers were associated with the risk of hospital contact due to infections and prescription for antimicrobials in the first years of life. Moreover, we assessed whether maternal exposures and child characteristics previously associated with childhood ALL risk were possible predictors of the child's neonatal concentrations of inflammatory markers.

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Patients and Methods

Study participants

Using the personal identification number recorded in the Civil Registration System (13) and assigned to all citizens in Denmark, we selected the study participants among singletons born in Denmark during 1995 to 2008. Information on vital status, birth characteristics, and birthplace of the child and its parents was obtained from the Civil Registration System and the Medical Birth Registry (14). The Nordic Society of Pediatric Hematology and Oncology database (15) holds information on all patients with ALL diagnosed in Denmark since 1981 with information on leukemia immunophenotype and karyotype, including retrospectively assessed information on *ETV6-RUNX1* karyotype for all patients diagnosed since 1992. Through linkage with this leukemia database, we identified 200 children who developed B-cell precursor ALL at ages 1 to 9 years. These patients were matched with 200 controls who were free of childhood leukemia and were alive at the diagnosis date of the index ALL patient. We matched patients and controls on potential confounders, including age (exact date of birth), sex, gestational age (±1 week), birth order (1, 2, or 3), mode of delivery (vaginal or by cesarean section), and selected only offspring of Danish-born parents (16–18). Furthermore, controls were matched as closely as possible to the index patient’s date of neonatal blood spot sampling. Additional matching on birth weight was not feasible. Moreover, we retrieved information on hospital contact due to infections and out-of-hospital prescription for systemic antimicrobials (henceforth referred to as “prescription for antimicrobials”) among mothers during pregnancy and among study participants before age 2 years through linkage with the Danish National Patient Register (NPR; ref. 19) and the Danish National Prescription Register (20). The former holds information on all in- and outpatient contacts since 1995 with diagnoses classified according to the International Classification of Diseases (ICD) 10th revision, whereas the latter includes information on all prescriptions redeemed at Danish pharmacies since 1995. ICD-10 codes for infections, also used in other studies (21), and Anatomical Therapeutic Chemical classification system codes for antimicrobials are listed in Supplementary Tables S1 and S2. Children with Down syndrome (ICD-10: Q90) were identified in the NPR and excluded from the cohort before matching.

Biological samples

Since 1982, neonatal dried blood spot samples collected for screening purposes from all children born in Denmark have been stored at −24°C in the Danish Neonatal Screening Biobank. According to national guidelines during the study period (1995–2008), neonatal blood spots were taken by a heel prick (capillary blood) at day 5 to 7 after birth (22). We retrieved neonatal blood spot samples from the 200 patients with B-cell precursor ALL and their 200 matched controls. However, the amount of residual dried blood spot material was insufficient for 22 patients. Thus, in total 178 children with B-cell precursor ALL and 178 matched controls were included for analyses.

Analysis of inflammatory markers

Samples were analyzed at Statens Serum Institut, Denmark, using a multiplex sandwich immunoassay (Meso-Scale). A total of 10 cytokines and other inflammatory markers

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possible to analyze with the applied immunoassay were selected *à priori* to provide a broad picture of the neonatal immune response (i.e., Th1, Th2, Th3, Th17, and acute inflammatory proteins). The investigated markers included IL6 and its soluble receptor sIL6Rα, IL8, IL10, IL12, IL17, IL18, TGFβ1, monocyte chemotactic protein (MCP)-1, and C-reactive protein (CRP). Two 3-mm disks were punched from each dried blood spot and extracted in 130 μL extraction buffer (23) shaking for 1 hour at room temperature. Further analysis was performed using Meso-Scale U-plex technology. The assay was made in-house according to the manufacturer's instructions using U-plex plates (Meso-Scale, K15235). Antibodies for all analytes were purchased from RnDsystems, except for MCP-1, which was purchased from BD Biosciences. Patients and their matched controls were analyzed on the same plates to avoid the influence of inter assay variation. Lower limits of detection were calculated as the zero calibrators + 2 standard deviations. Intra and inter assay variations are presented in Supplementary Table S3.

Statistical analysis

Concentrations of IL10 were below the detection level (9.42 pg/mL) for all patients and controls, and therefore not included in the statistical analyses. To minimize the influence of outliers on inferences with inflammatory markers as exposures, we used percentiles of the inflammatory marker distributions as predictors. Missing values for inflammatory markers, that is, concentrations too low to detect, were replaced by a value corresponding to the lowest percentile. We assessed the correlations between neonatal inflammatory markers by Pearson's and Spearman's correlation coefficients (Supplementary Table S4).

To assess the association between neonatal inflammatory markers and risk of childhood B-cell precursor ALL, we conducted conditional logistic regression with patient/control status as the outcome and each of the inflammatory markers as explanatory variables. In the logistic regression models, odds ratios (OR) were adjusted for birth weight and maternal age at delivery. In a second model, we also adjusted mutually for the concentrations of the other inflammatory markers. Variation in the inflammatory marker-ALL associations between the frequently prenatally initiated karyotypes high-hyperdiploidy and *ETV6-RUNX1* (1–3) compared with other ALL karyotypes was tested using conditional multinomial logistic regression (Supplementary Table S5).

We conducted a sensitivity analysis to assess the possible influence of differences in day after birth of neonatal blood spot sampling between patients and matched controls, including only patient–control pairs with identical date of sampling ($n = 87$ pairs). In this analysis, we found no marked changes in the association between the inflammatory markers and risk of childhood B-cell precursor ALL (Supplementary Table S6).

We also assessed whether reverse causality, that is, infections as an early symptom of childhood ALL, potentially affected the neonatal concentrations of inflammatory markers by excluding patients aged less than 2 years at diagnosis ($n = 21$) and their matched controls. ORs from these models did not differ from the primary analysis (Supplementary Table S7).

In subsequent analyses, we assessed whether maternal hospital contact due to infection or antimicrobial prescription during pregnancy (exposure, categorized as yes or no), maternal

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age at delivery, birth order, gestational age, birth weight (as continuous exposures), sex, and mode of delivery (as binary exposures) were associated with the neonatal concentrations of inflammatory markers (outcome, log-transformed) using linear regression models and multivariate analysis of variance to obtain joint test P values for each exposure. In these analyses, we adjusted for year of delivery, patient/control status, and day after birth of neonatal blood spot sampling and excluded $n = 34$ children with unknown sampling date. Model assumptions were assessed by diagnostic residual plots.

Among controls, we tested whether the neonatal concentrations of inflammatory markers were associated with risk of hospital contact due to infection or antimicrobial prescription before age 2 years. For the latter, we included only children born from July 1, 1996 when prescriptions redeemed to children in Denmark were recorded using the child's personal identification number ($n = 153$). We used the Andersen-Gill model for recurrent events (24), that is, whenever a child was at risk, the hazard rate was not dependent on the number of previously recorded outcomes. To model the hazard rate, we used Cox proportional hazards models with age as the underlying time scale. Children were followed from date of blood spot sampling until death, emigration or age 2 years, whichever occurred first. Children with unknown date of sampling were excluded ($n = 10$). We counted each hospital contact due to infection or redeemed antimicrobial prescription before age 2 years as an infectious disease episode. However, children were not considered at risk of a new infection the first 14 days following a previous hospital contact or antimicrobial treatment period defined as the date of antimicrobial redemption + standard treatment length for the antimicrobial in question (Supplementary Table S2). Hazard ratios (HR) were adjusted for maternal age at delivery, birth weight, and gestational age using linear terms. Furthermore, we stratified the baseline hazard on sex, birth order (1, 2, or 3), and mode of delivery. The proportional hazards assumption was assessed by graphical evaluation of the Martingale residuals.

Statistical analyses were carried out using SAS statistical software (9.4, SAS Institute, Inc.).

Approval

The study was carried out in keeping with Danish legislation and accordingly approved by the Danish Scientific Ethics Committee System granting exemption from rule of informed consent (protocol no. H-15017262), by the Danish Data Protection Agency (journal no. 2015–57-0102), and by the Steering Committee for the Danish Neonatal Screening Biobank.

Results

The 178 patients with B-cell precursor ALL in our study were diagnosed during 1998 to 2015. Median age at diagnosis was 3 years. The majority of patients had the frequently prenatally initiated karyotypes high-hyperdiploidy ($n = 78$) or *ETV6-RUNX1* ($n = 42$; in total 67.4%), whereas the remaining had other (29.8%) or missing karyotype (2.8%). Children with B-cell precursor ALL weighed on average 7 grams more at birth than controls and had slightly older mothers, while matching variables were evenly distributed among patients and controls (Table 1). In the vast majority of patient–control pairs ($n = 132$; 74%) age at blood spot sampling differed by 1 day between the patient and the control, the largest difference among the 178 pairs being 6 days.

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Table 2 presents the distribution of the neonatal concentrations of the 9 analyzed inflammatory markers among children who later developed B-cell precursor ALL and controls, respectively. A large proportion of patients and controls had IL6 and IL17 concentrations that were below the reliable detection limit.

Children who later developed B-cell precursor ALL had statistically significantly different neonatal concentrations of 8 of the 9 analyzed inflammatory markers compared with controls (Table 3). Neonatal concentrations of sIL6Rα, IL8, TGFβ1, MCP-1, and CRP were statistically significantly lower, whereas concentrations of IL6, IL17, and IL18 were statistically significantly higher among patients with B-cell precursor ALL compared with controls. Mutual adjustment for concentrations of the other inflammatory markers did not change the associations notably; however, the associations with sIL6Rα, IL17, IL18, and TGFβ1 were statistically non-significant in this model. The associations between inflammatory markers and B-cell precursor ALL risk did not differ statistically significantly between the prenatally initiated karyotypes as compared with other ALL karyotypes (Supplementary Table S5).

Birth order, gestational age, and sex were statistically significantly associated with the neonatal inflammatory marker concentrations (Table 4). Increasing birth order was associated with statistically significantly 9% higher IL18 and 14% lower CRP concentrations. Increasing gestational age (in weeks) was associated with statistically significantly 5% and 4% lower sIL6Rα and TGFβ1 concentrations, respectively, and 19% higher CRP concentrations. Boys had statistically significantly 9% and 8% lower sIL6Rα and IL8, and 22% higher CRP concentrations compared with girls. Neither maternal age at delivery, maternal hospital contact due to infection during pregnancy, maternal prescription for antimicrobials during pregnancy, birth weight nor mode of delivery were statistically significantly associated with the neonatal concentrations of inflammatory markers (Supplementary Table S8).

Among control children, 25 hospital contacts due to infections and 225 redemptions of antimicrobials were observed before age 2 years. Respiratory tract infections and antibacterials, respectively, accounted for the majority of these. Increasing neonatal concentrations of TGFβ1 and CRP were associated with statistically significantly decreased and increased risk of hospital contact due to infection, respectively, but no association between neonatal inflammatory markers and prescription for antimicrobials before age 2 years was observed (Supplementary Table S9).

Discussion

In this population-based case–control study, we measured concentrations of 10 inflammatory markers on neonatal dried blood spots of 178 children who developed B-cell precursor ALL and 178 controls. We found that patients with B-cell precursor ALL were born with abnormal concentrations of 8 of the 9 detectable inflammatory markers.

Our findings support those reported by Chang and colleagues (12) 2011, who in a case–control study observed statistically significant differences in 4 of 5 detectable inflammatory

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markers among children who developed ALL compared with controls, that is, of IL4, IL6, IL10, and IL13, and of IL10 in mutually adjusted analyses. Although the two studies are incongruent with regards to the difference in IL6 concentrations (i.e., we found higher concentrations, whereas Chang and colleagues found lower concentrations in patients with ALL compared with controls), the overall results of the two studies suggest that children with ALL are indeed born with an abnormal immune system.

This phenomenon could potentially explain the previously reported increased incidence of clinically diagnosed infections in infancy among children who later develop ALL observed in a United Kingdom childhood cancer study (10, 25, 26) as well as in Taiwan (11). In a Danish register-based study, however, the latter observation was not mirrored by an increased incidence of hospitalizations for infections during the first years of life among children who developed ALL (27).

As a consequence of an abnormal immune function at birth, it is possible that children who later develop ALL react more vigorously to infectious exposures in the first years of life, potentially causing preleukemia to progress to leukemia. In support of this, Simpson and colleagues (25) found that the risk of clinically diagnosed infections in infancy associated with exposure to infections through contact with other children was more pronounced among children who later developed ALL than among controls. Specifically, at birth order 2 or higher, the risk of infections in infancy was statistically significantly 1.6-fold higher among (future) patients with ALL than among controls. Conversely, our analyses of the link between neonatal concentrations of inflammatory markers and risk of infections before age 2 years (hospital contact or prescription for antimicrobials) showed no association for the majority of the 9 analyzed markers. However, our study was not designed to and may have been too small to detect such associations. Previous studies have reported associations between stimulated cytokine production in cord blood and the course and number of respiratory tract infections in early childhood (28, 29).

The presence of clone-specific mutations in patient neonatal blood spots has demonstrated a prenatal origin of most B-cell precursor ALL cases, namely those with high-hyperdiploidy or *ETV6-RUNX1*-translocation. Recently, it was suggested that up to 5% of all newborns harbor translocation *ETV6-RUNX1* (30). In combination with a constitutively aberrant immune system, it is possible that children who develop B-cell precursor ALL were unable to eliminate these preleukemic cells, thus allowing transformation to leukemia.

The aim of our investigation was to analyze the concentrations of 10 cytokines and other inflammatory markers chosen to provide a broad picture of the neonatal immune response and not to render a comprehensive mapping of the immune system in neonates destined to develop B-cell precursor ALL. Therefore, although some of the observed differences are compatible with a Th1/Th17 dominated immune system in the children who later developed B-cell precursor ALL, the small number of studied markers as well as the lack of examination of longitudinal sampling that may assess the subsequent balancing of immune function during infancy preclude further interpretations. This should be investigated in future studies.

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Some of the examined inflammatory markers also exhibited associations with potential and well-established ALL risk factors, specifically birth order, gestational age, and sex (16, 17). Importantly, however, if the ALL risk factor-inflammatory marker associations are interpreted in the context of the overall differences observed between patients with ALL and controls, the two sets of analyses are somewhat incongruent. For example, although the case–control comparison predicted that sIL6Rα, IL8, and CRP concentrations should be lower in boys than girls given the higher incidence of ALL in boys, this was not the case for CRP. Nevertheless, both analyses raise the interesting possibility that the effects of some ALL risk factors could be mediated by prenatal programming of immune function.

The major strengths of our study include its population-based design, including a random sample of future patients with B-cell precursor ALL born in Denmark during 1995 to 2008 and a randomly selected control group with individual matching on several factors, including exact birth date, sex, and a number of birth characteristics. The detailed information obtained from the Danish registers enabled us to assess whether the neonatal concentrations of inflammatory markers associate with certain maternal and child characteristics, and with the risk of infections in the child's first years of life. However, we cannot rule out that our study was too small to detect such associations. In particular, we had low statistical power to detect associations between neonatal inflammatory markers and maternal and child characteristics, which was evident from the wide confidence intervals presented in Table 4 and Supplementary Table S8 for some of the analyzed markers. Unfortunately, we were unable to reliably measure the concentrations of IL10, which have previously been reported to associate with ALL (12). Moreover, a large proportion of patients and controls had IL6 and IL17 concentrations that were below the reliable detection limit, thus results for these markers should be interpreted with caution.

In summary, we found that children with B-cell precursor ALL are born with abnormal concentrations of inflammatory markers. This observation supports the hypothesis that children with ALL have a dysregulated immune function, which is detectable already at birth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Significance:

Children who develop acute lymphoblastic leukemia are immunologically distinct at birth and could potentially react abnormally to infections in early childhood.

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Table 1.

Baseline characteristics of the 178 patients with B-cell precursor ALL and 178 matched controls born in Denmark during 1995–2008

	B-cell precursor ALL patients (*n* = 178) *n* (%)	**Controls (*n* = 178) *n* (%)**
Age at diagnosis (y)		
1–3	92 (51.7)	–
4–6	64 (36.0)	–
7–9	22 (12.3)	–
Year of birth		
1995–1998	54 (30.3)	54 (30.3)
1999–2002	51 (28.7)	51 (28.7)
2003–2005	42 (23.6)	42 (23.6)
2006–2008	31 (17.4)	31 (17.4)
Age at neonatal blood spot sampling (days)		
3–4	22 (12.4)	18 (10.1)
5–7	96 (53.9)	115 (64.6)
8	36 (20.2)	35 (19.7)
Missing	24 (13.5)	10 (5.6)
Mean	*6.3*	*6.1*
Sex		
Boys	95 (53.4)	95 (53.4)
Girls	83 (46.6)	83 (46.6)
Birth order		
1	72 (40.5)	72 (40.5)
2	78 (43.8)	78 (43.8)
3	28 (15.7)	28 (15.7)
Birth weight (gram)		
<3,000	8 (4.5)	8 (4.5)
3,000–3,499	58 (32.6)	55 (30.9)
3,500–3,999	72 (40.4)	78 (43.8)
4,000–4,499	30 (16.9)	31 (17.4)
4,500	10 (5.6)	6 (3.4)
Mean	*3658*	*3651*
Gestational age (weeks)		
37	5 (2.8)	5 (2.8)
38–39	60 (33.7)	61 (34.3)
40–41	101 (56.8)	106 (59.5)
42	12 (6.7)	6 (3.4)
Mean	*39.7*	*39.8*
Mode of delivery		

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	B-cell precursor	
	ALL patients (*n* = 178) *n* (%)	**Controls (*n* = 178) *n* (%)**
Vaginal	162 (91.0)	162 (91.0)
Cesarean section	16 (9.0)	16 (9.0)
Maternal age at delivery (y)		
<25	14 (7.9)	28 (15.7)
25–29	67 (37.6)	67 (37.7)
30–34	70 (39.3)	62 (34.8)
35	27 (15.2)	21 (11.8)
Mean	*30.1*	*29.2*
Maternal hospital contact due to infections during pregnancy		
Yes	15 (8.4)	8 (4.5)
No	163 (91.6)	170 (95.5)
Maternal antimicrobial prescription during pregnancy		
Yes	63 (35.4)	64 (35.9)
No	115 (64.6)	114 (64.1)

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Table 2.

Median and range of neonatal concentrations of inflammatory markers among patients with B-cell precursor ALL and controls

		B-cell precursor ALL patients (n = 178)				Controls (n = 178)			
	Detection Range pg/mL	Median[a] pg/mL	Range pg/mL	Below level of detection n (%)	Missing n (%)	Median[a] pg/mL	Range pg/mL	Below level of detection n (%)	Missing n (%)
IL6	0.97–5,000	0.99	0.06–7.79	81 (45.5)	12 (6.7)	0.78	0.001–7.51	108 (60.7)	12 (6.7)
sIL6Rα	29.01–200,000	2,307.09	725.49–7,091.02	0 (0)	0 (0)	2,617.23	999.78–7,115.50	0 (0)	0 (0)
IL8	0.46–5,000	1.66	0.68–506.68	0 (0)	0 (0)	1.92	0.51–6.29	0 (0)	0 (0)
IL12	4.08–5,000	10.27	4.34–24.48	0 (0)	0 (0)	10.47	1.57–75.07	4 (2.2)	0 (0)
IL17	3.79–5,000	4.99	0.31–14.85	55 (30.9)	16 (9.0)	4.55	0.19–12.83	64 (36.0)	19 (10.7)
IL18	0.28–5,000	14.61	4.21–45.65	0 (0)	0 (0)	13.41	2.42–49.57	0 (0)	0 (0)
TGFβ1	3.12–40,000	87.87	34.17–211.16	0 (0)	0 (0)	102.61	28.61–288.59	0 (0)	0 (0)
MCP-1	4.28–50,000	123.06	45.94–282.56	0 (0)	0 (0)	154.61	68.22–339.36	0 (0)	0 (0)
CRP (μg/mL)	<0.001–10	0.17	0.0034–1.27	0 (0)	0 (0)	0.24	0.012–1.89	0 (0)	0 (0)

[a] Before changing missing values to a value corresponding to the lowest percentile.

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Table 3.

Association between neonatal concentrations of inflammatory markers and odds ratios of childhood B-cell precursor ALL

	Adjusted OR (95% CI)[a]	Mutually adjusted OR (95% CI)[b]
IL6	1.19 (1.09–1.31)	1.45 (1.21–1.75)
sIL6Rα	0.82 (0.74–0.92)	0.88 (0.73–1.05)
IL8	0.84 (0.77–0.92)	0.83 (0.72–0.96)
IL12	0.94 (0.86–1.03)	1.02 (0.87–1.19)
IL17	1.12 (1.01–1.25)	1.16 (0.97–1.39)
IL18	1.08 (1.01–1.17)	1.12 (1.00–1.25)
TGFβ1	0.83 (0.76–0.92)	0.91 (0.79–1.05)
MCP-1	0.68 (0.59–0.77)	0.59 (0.49–0.73)
CRP	0.83 (0.75–0.91)	0.81 (0.70–0.94)

NOTE: The reported odds ratios correspond to one decile increase in the inflammatory marker. Conditional logistic regression with inflammatory markers ranked according to percentiles divided by 10 and modelled linearly. Abbreviations: CI, confidence interval.

[a]ORs were adjusted for birth weight (continuous in 500-g intervals) and maternal age at delivery (continuous).

[b]ORs were adjusted for birth weight (continuous in 500-g intervals), maternal age at delivery (continuous), and all other inflammatory markers in the table.

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Table 4.

Association between birth order, gestational age, and sex, respectively, and neonatal concentrations of inflammatory markers

	Birth order		Gestational age		Sex (boys vs. girls)	
	Percentage change (95% CI)	P^a	Percentage change (95% CI)	P^a	Percentage change (95% CI)	P^a
		0.02		<0.01		<0.01
IL6[b]	3.0 (−10.0–19.0)		0.6 (−7.5–9.5)		−2.4 (−20.7–20.1)	
sIL6Rα	−0.4 (−6.0–5.5)		−5.2 (−8.4 to −1.9)		−8.9 (−16.2 to −1.0)	
IL8	−2.5 (−8.0–3.2)		−2.8 (−6.1–0.6)		−8.4 (−15.7 to −0.5)	
IL12	3.9 (−1.1–9.3)		0.4 (−2.6–3.5)		2.8 (−4.4–10.6)	
IL17[c]	2.6 (−8.3–14.9)		−3.5 (−9.8–3.2)		−3.4 (−17.8–13.5)	
IL18	9.3 (1.0–18.4)		4.7 (−0.2–9.9)		2.2 (−9.1–14.8)	
TGFβ1	−1.4 (−6.7–4.1)		−4.2 (−7.3 to −1.0)		−7.6 (−14.6–0.0)	
MCP-1	3.7 (−0.6–8.3)		0.3 (−2.3–3.0)		−5.5 (−11.2–0.6)	
CRP	−13.9 (−24.3 to −2.0)		18.7 (9.9–28.1)		22.1 (1.1–47.5)	

NOTE: The reported estimates correspond to the percentage changes in the inflammatory markers with increasing birth order, increasing gestational age in weeks, and among boys vs. girls, respectively. Linear regression models with log-transformed concentrations of inflammatory markers as the outcomes, birth order and gestational age as continuous exposures, and sex as binary exposure, adjusted for birth year (continuous), patient/control status, and day after birth of neonatal blood spot sampling (continuous).

Abbreviations: CI, confidence interval.

[a] Multivariate analysis of variance P value for joint tests, excluding children with any missing concentrations ($n = 53$, leaving $n = 269$ for analysis).

[b] Excluding children with missing IL6 concentrations ($n = 24$, leaving $n = 298$ for analysis).

[c] Excluding children with missing IL17 concentrations ($n = 31$, leaving $n = 291$ for analysis).