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Cytokine profiles in cord blood in relation to prenatal traffic-related air pollution: The NELA cohort

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Abstract

Background: Outdoor air pollution may disturb immune system development. We investigated whether gestational exposure to traffic-related air pollutants (TRAP) is associated with unstimulated cytokine profiles in newborns.

Methods: Data come from 235 newborns of the NELA cohort. Innate response–related cytokines (IL-6, IFN-α, IL1-β, and TNF-α), Th1-related (IFN-γ and IL-2), Th2-related (IL-4, IL-5, and IL-13), Th17-related (IL-17 and IL-23), and immunomodulatory cytokine IL-10 were quantified in the supernatant of unstimulated whole umbilical cord blood cells after 7 days of culture using the Luminex technology. Dispersion/chemical transport modeling was used to estimate long-term (whole pregnancy and trimesters) and short-term (15 days before delivery) residential exposures to traffic-related nitrogen dioxide (NO_2), particulate matter ($PM_{2.5}$ and PM_{10}), and ozone (O_3). We fitted multivariable logistic regression, Bayesian kernel machine regression (BKMR), and weighted quantile sum (WQS) regression models.

Results: NO_2 during the whole pregnancy increased the odds of detection of IL-1β (OR per 10 μg/m^3 increase = 1.37; 95% CI, 1.02, 1.85) and IL-6 (OR per 10 μg/m^3 increase = 1.32; 95% CI 1.00, 1.75). Increased odds of detected concentrations of IL-10 was found in newborns exposed during whole pregnancy to higher levels of

Abbreviations: BKMR, Bayesian kernel machine regression; IFN, Interferon; IL, Interleukin; PM, Particulate matter; Th1, Helper T type 1 cells; Th2, Helper T type 2 cells; TNF, Tumor necrosis factor; TRAP, Traffic-related air pollution; WQS, Weighted quantile sum regression.

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NO_2 (OR per 10 μg/m^3 increase = 1.30; 95% CI 0.99, 1.69), PM_{10} (OR per 10 μg/m^3 increase = 1.49; 95% CI 0.95, 2.33), and $PM_{2.5}$ (OR per 5 μg/m^3 increase = 1.56; 95% CI 0.97, 2.51). Exposure to O_3 during the whole pregnancy increased the odds of detected IL-13 (OR per 10 μg/m^3 increase = 1.22; 95% CI 1.01, 1.49). WQS model revealed first and third trimesters of gestation as windows of higher susceptibility.

Conclusions: Gestational exposure to TRAP may increase detection of pro-inflammatory, Th2-related, and T regulatory cytokines in newborns. These changes might influence immune system responses later in life.

KEYWORDS

air pollution, birth cohort, cord blood, cytokines, traffic

1 | INTRODUCTION

Prenatal exposure to outdoor air pollutants has emerged as a threat for child respiratory health and risk of allergy. Epidemiological studies link in utero exposure to traffic-related air pollutants (TRAP) with increased risk of lower respiratory infections, impaired lung function, and asthma in childhood.[1–3] Although the mechanisms of these long-lasting effects are not fully understood, it is likely that a reshaped development of the immune system response may play a key role.[4]

Asthma and allergies are characterized by diverse clinical phenotypes that result from an exacerbated immune response to diverse stimuli in susceptible individuals. Developmental programming of the immune system by genetic, epigenetic, and environmental factors is critical for triggering this response. During fetal development, the immune system is physiologically skewed toward a predominant type 2 helper T cell (Th2) response characterized by producing IL-4, IL-5, and IL-13, which promotes a successful pregnancy maintenance.[5] At birth, the immunity begins a process of re-calibration toward a type 1 helper T cell (Th1) response.[6] The inception of asthma and allergic diseases in many cases is related to disturbances in the Th1/Th2 balance.[7] The importance of other immune system components including type 17 helper T (Th17)–related cells and T helper regulatory (Treg) cells is also growing in the last years.[8,9]

In vitro studies have shown that air pollution can impair antimicrobial and regulatory responses, including inflammatory cytokines release from epithelium and macrophages, and enhance Th2 and Th17 responses, as seen in allergy and asthma.[10,11] Growing evidence in humans also suggests that exposure to outdoor air pollution during critical developmental susceptibility windows disturbs cytokine responses. Gruzieva et al. showed that exposure to TRAP NO_2 during the first year of life was associated with increased IL-6 concentrations in serum at 8 years of age and correlated with IL-10, IL-13, and tumor necrosis factor (TNF) transcript levels at 16 years.[12] Knowledge on the influence of prenatal exposure to outdoor air pollution on cytokine patterns in the newborn is limited and focused on a few numbers of cytokines.[13–15] A first study focused on cytokine patterns related to short-term effects of exposure to moderate levels of outdoor air pollution in the last trimester of pregnancy showed higher PM_{10} concentrations to be associated with reduced secretion of IL-10, especially after exposure during the last 3 days of pregnancy, and increased secretion of IL-1β after exposure during the last 3 months of pregnancy.[13] Ashley-Martin et al.[14] reported increased concentrations at birth of both IL-33 and thymic stromal lymphopoietin (TSLP) among females but not males in relation to maternal exposure to outdoor NO_2, with stronger associations for exposures during the third trimester of gestation. More recently, Hahn et al.[15] found lower production of IL-6, TNF-α, and IL-10 in cord blood mononuclear cells from newborns prenatally exposed to higher levels of $PM_{2.5}$.

To the best of our knowledge, no study has characterized global cytokine profiles at birth nor examined different windows of prenatal susceptibility in response to outdoor air pollutants. In the present study, we aim to investigate whether exposure to TRAP during pregnancy is associated with unstimulated specific cytokine profiles in newborns and to explore gestational windows of higher susceptibility. For this purpose, we have designed a broad screening panel consisting of a biologically relevant collection of adaptive immunity and pro-inflammatory cytokines, estimated long- and short-term traffic-related levels of main air pollutants and applied different statistical approaches to evaluate the correlation between pollutants in a population-based birth cohort.

Key Message

Prenatal exposure to higher levels of traffic-related air pollutants may increase the concentrations of unstimulated pro-inflammatory (IL-1β and IL-6), Th2-related (IL-13), and immunomodulatory IL-10 cytokines in newborns. These changes might impact the immune system response of the offspring to respiratory microbes and allergens later in life.

2 | MATERIAL AND METHODS

2.1 | Study participants

The Nutrition in Early Life and Asthma (NELA) study (www.nela.imib.es) is a prospective population-based birth cohort set up between 2015 and 2018 in Murcia, a south-eastern Mediterranean region of Spain. The main objective of NELA is to unravel the developmental origins and mechanisms of asthma and allergy. The study protocol, recruiting methods, and data collection processes have been described elsewhere.[16] Among the 1350 women invited to participate, 738 (54%) were finally enrolled in the study. Umbilical cord blood samples were collected in 390 (53%) newborns and cytokine concentrations were determined in 235 (32%) who were included in the current study (Figure S1). The study protocol was reviewed and approved by the Ethics Committee of the Biomedical Research Institute of Murcia (IMIB-Arrixaca) in accordance with the guidelines of The Declaration of Helsinki (report 9/14; 29/09/2014). Written informed consents were obtained from parents at recruitment.

2.2 | Outdoor air pollutants exposure assessment

The Advanced Weather Research and Forecasting (WRF-ARW) model v3.9.1 + CHIMERE chemistry transport model (CTM) was coupled off-line for generating the outdoor concentrations for air pollutants during the entire duration of the NELA cohort study.[17] WRF has been coupled off-line on an hourly basis to CHIMERE.[18] This latter CTM includes gas-phase chemistry and aerosols and heterogeneous chemistry. The aerosol module was adapted to chemical species of health interest, both anthropogenic and natural. With respect to anthropogenic and natural emissions, a specific emission inventory was developed for the purpose of the NELA study in the Region of Murcia, including emissions from all possible sources. Biogenic emissions depend on WRF meteorological outputs. The final working resolution over the three target Health Areas of NELA recruitment is 0.5 km. Several twin sensitivity simulations were conducted with the aforementioned modeling chain to measure how pollutant concentrations at receptors respond to perturbations at sources using the zero-out method.[19] Particularly, we conducted simulations by using regional emission inventories, independently, for total emissions and isolating (setting to zero) the traffic emission. The difference between base-case simulations including total emissions in WRF+CHIMERE and simulations zeroing-out traffic emissions provided the levels of air pollution caused by traffic.

Residential address during pregnancy was geo-codified using Geographic Information System (GIS). Long-term (whole pregnancy and trimester-specific) and short-term (15 days before birth) mean concentrations of outdoor air pollutants (i.e., NO_2, $PM_{2.5}$, PM_{10}, and O_3) were estimated using dispersion/chemical transport modeling. We defined whole pregnancy as from date of conception to birth; first trimester as week 1–13; second trimester as week 14–26; and third trimester as the period from week 27 until birth. Date of gestation was calculated from the date of the last menstrual period reported at recruitment and confirmed using estimates based on ultrasound examination at 12 weeks of gestation.

2.3 | Cytokine profiles in umbilical cord blood

Blood was obtained from the umbilical cord vein, transferred from syringes to sterile heparinized tubes, and kept at room temperature until processing within 48 h.[20,21] Samples were diluted 1:7 with RPMI 1640 heparinized medium, and supernatants were collected after 7 days of culture,[22–25] aliquoted, and frozen at −80°C. Time point of cytokine measurement was based on previous publications and on our experiments comparing cytokine levels at 48 h and at 7 days of culture showing stable concentrations for most of the assessed cytokines (data not shown). Subsequently, supernatants were analyzed for cytokine with Luminex technology using the Human Cytokine Multiplex-Assay-Kit (Thermo Fisher) according to the manufacturer's instructions. Cytokines were selected based on innate and adaptive immune responses. The panel included innate response–related cytokines (IL-6, IFN-α, IL-1β, and TNF-α), Th1-related (IFN-γ and IL-2), Th2-related (IL-4, IL-5, and IL-13), Th17-related (IL-17 and IL-23), and immunomodulatory cytokine IL-10. Mixes of multiple standard cytokines were used to generate standard curves for each cytokine. We established a lineal range in the curve that we called detection range.

2.4 | Other variables

Questionnaires during the second and third trimesters of pregnancy obtained information about area of residency (urban, residential, and rural); maternal and paternal age; parental social class (defined as maternal or paternal occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the international ISCO88 coding system: I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled; and unemployed)[26]; maternal and paternal educational level (incomplete secondary or less, complete secondary, and university); maternal and paternal reported history of asthma (yes/no) and atopy (yes/no); maternal smoking during pregnancy and paternal smoking (yes/no); parity (0, nulliparous; vs. 1 or more, non-nulliparous); maternal pre-pregnancy body mass index (BMI) based on height and pre-pregnancy self-reported weight (kg/m^2) (categorized as normal BMI < 25, overweight 25 < BMI < 30, and obesity BMI ≥ 30); pets at home (yes/no); and maternal contact with farming animals during pregnancy (yes/no). Information related to child's sex, birthweight (g); gestational age (weeks); mode of delivery (vaginal non-instrumental, vaginal instrumental, and cesarean section); fever (yes/no) and use of antibiotics during labor (yes/no) was obtained from clinical records. Season of birth (spring, March-May; summer, June-August; fall, September-November; and winter, December-February) was also considered.

2.5 | Statistical analysis

Kruskal-Wallis's test for continuous variables and chi-square test for categorical variables were used to assess differences in maternal and neonate characteristics between included and excluded participants. The Spearman's rank correlation coefficient was computed to evaluate correlations between outdoor air pollutants.

First, we ran single-pollutant multivariate logistic and linear regression models to examine the associations between long-term (whole pregnancy and specific trimesters) and short-term (15 days before pregnancy) concentrations of residential TRAP with cytokine concentration in cord blood. For each cytokine, concentrations were categorized in "non-detectable," if values were below the lower limit of detection, vs. "detectable," if values were above the lower limit of detection. "Non-detectable" category was used as the reference. Associations are presented as odds ratios (OR) and their corresponding 95% confidence intervals (CI) that represent the odds of being above the lower limit of detection for each assessed cytokine per 10 μg/m^3 increase in NO_2, PM_{10}, and O_3 concentrations, and per 5 μg/m^3 increase in $PM_{2.5}$ concentration. Given that the amount of non-detectable values in umbilical cord blood of assessed cytokines was large and that non-detectable values were not missing at random, biases can be expected. To minimize this, we used multivariate multiple imputation methods to impute cytokine values below (LLOD) and above (ULOD) limits of detection (LOD) conditioning the imputation to the range (0, LOD) and (>ULOD).[27] Multiple imputation of non-detected values was performed using an interval regression imputation method and chained equations.[28,29] Twenty completed data sets were generated and analyzed separately, and the results were combined using Rubin's rules.[30] Generalized additive models (GAM) were used to graphically examine the shape of relationships between traffic-related levels and log-transformed cord blood cytokine concentrations. These did not show statistically significant evidence for a departure from (log)linear relationships (*p*-values for gain in linearity were between .10 and .40) (Figure 2). However, since the evidence was graphically not very strong, we performed multivariate linear regression models using log-transformed cytokine values and TRAP concentrations as categorical variables using quartiles as cutoffs.

Covariates included as cofounders were selected after running a series of models to assess the effect of additionally adjusting (forward selection) one by one each of the individual potential confounders on the coefficient of exposures of interest. Variables were retained in the multivariate models only if they showed at least marginally significant association ($p < .1$) or modified the coefficient of air pollutants by at least 5%. Final models were adjusted for newborn's sex, gestational age, birthweight, season of birth, maternal pre-pregnancy BMI (kg/m^2), parental social class, maternal atopy, and paternal smoking.

Bayesian kernel machine regression (BKMR) was applied to visualize the exposure-response function and verify assumptions using the R package *bkmr*.[31] The model was fit with 20,000 iterations using a Markov chain Monte Carlo method. The parameter r.jump2 was separately set to 0.5 to get suitable acceptance rates. We also analyzed the association between the quantile of the TRAP exposures and a healthy binary outcome (i.e., detectable vs. non-detectable cytokine levels) using a probit link function.[31,32] Each of the BKMR models was adjusted for the covariates described above.

Given that we did not identify non-linearity neither interaction within the mixture of air pollutants (Figures S2 and S3), we further applied weighted quantile sum (WQS) regression to estimate associations of prenatal TRAP mixtures with cytokine profiles at birth. We first run different WQS models for each window of susceptibility (whole pregnancy, trimesters, and 15 days before delivery), evaluating period-specific associations. Next, to account for the time-varying nature of TRAP concentrations over pregnancy, we evaluated all trimesters in the same model. WQS regression allows for the creation of a weighted linear index of correlated predictors that are weighted by their strength of association with the outcome of interest.[33] Before analysis, the data were randomly split into two data sets: a training data set (40%) and a validation data set (60%). 1000 bootstrap iterations were performed to calculate the CIs.

Data were analyzed in Stata Software (version 15.1, StataCorp), RStudio (version 1.1.463, RStudio), and GraphPad Prism Software (version 8.0.2, GraphPad Software Inc.).

3 | RESULTS

Characteristics of the 235 participants included in the present study are shown in Table 1. Compared to excluded newborns, those included had older parents, higher father education level, higher maternal pre-pregnancy BMI, higher maternal prevalence of reported asthma, higher gestational age, and lower proportion of births in summer.

Median of traffic-related outdoor air pollutant concentrations during whole pregnancy was 36.1 μg/m^3 for NO_2, 13.2 μg/m^3 for $PM_{2.5}$, 23.6 μg/m^3 for PM_{10}, and 20.3 μg/m^3 for O_3. The medians did not essentially change across assessed exposure periods (Table S1). Positive strong correlations (Spearman's $p > 0.7$) were detected between traffic-related NO_2, PM_{10}, and $PM_{2.5}$ (Table S2). Traffic-related O_3 showed moderate correlation with PM_{10} and $PM_{2.5}$ ($0.53 < p < 0.57$). There was not a correlation between traffic-related NO_2 and O_3 levels. Parents with high education level more probably lived in areas with high traffic-related concentrations. Births in spring were less common in areas with high traffic-related $PM_{2.5}$, PM_{10}, and O_3 levels (data not shown).

Unstimulated cytokine detection rates and concentrations in culture supernatants from umbilical cord blood of newborns are shown in Table 2. Cytokine detection rates ranged between 5% and 77%. IL-1β and IL-10 showed the highest detection rates (77% and 67%, respectively) and IL-17 the lowest (5%).

In adjusted single-pollutant models, exposure to higher prenatal levels of traffic-related NO_2 during whole pregnancy was associated with increased odds of detectable concentrations of

TABLE 1 Comparison of main characteristics between included and excluded participants, the NELA study, Spain (2015–2018)

	Included ($n = 235$)	Excluded ($n = 503$)	p value*
Maternal age (years)	33.3 ± 4.3	32.2 ± 4.8	.002
Nulliparous mothers	121 (51.5)	253 (50.3)	.763
Maternal education level			
Incomplete secondary or less	42 (17.9)	104 (20.7)	.146
Complete secondary	53 (22.6)	138 (27.4)	
University	140 (59.6)	261 (51.9)	
Maternal pre-pregnancy BMI, (kg/m^2)	24.4 ± 4.5	23.7 ± 4.4	.028
Normal (<25)	152 (64.7)	361 (71.8)	.135
Overweight (25–29.99)	59 (25.1)	97 (19.2)	
Obesity (≥30)	24 (10.2)	45 (9.0)	
Maternal asthma	33 (14.0)	48 (9.5)	.068
Maternal history of atopy	107 (45.5)	203 (40.4)	.185
Maternal smoking during pregnancy	39 (16.6)	89 (17.7)	.714
Paternal age (years)	35.6 ± 5.1	34.4 ± 5.4	.005
Paternal education level			
Incomplete secondary or less	57 (24.3)	161 (32.2)	.056
Complete secondary	84 (35.7)	145 (28.9)	
University	94 (40.0)	195 (38.9)	
Paternal asthma	22 (9.4)	44 (8.8)	.778
Paternal history of atopy	88 (37.6)	175 (34.9)	.469
Paternal smoking	83 (35.3)	175 (34.9)	.918
Area of study			
Urban	173 (73.6)	361 (71.8)	.790
Residential	34 (14.5)	73 (14.5)	
Rural	28 (11.9)	69 (13.7)	
Parental social class			
I–II, managers/ technicians	126 (53.6)	241 (47.9)	.252
III, skilled	52 (22.1)	110 (21.9)	
IV–V, semi-skilled and non-skilled	54 (23.0)	137 (27.2)	
Unemployed	3 (1.3)	15 (3.0)	
Pets at home	111 (47.2)	230 (45.7)	.702
Maternal contact with farming animals	44 (18.7)	88 (17.5)	.685
Newborn sex, female	119 (50.6)	244 (50.3)	.934
Gestational age (weeks)	39.8 ± 1.3	39.5 (1.6)	.033
Birthweight (g)	3275 ± 430.5	3226 ± 494.8	.335
Season of birth			
Autumn	74 (31.5)	128 (26.4)	.066
Spring	63 (26.8)	123 (25.4)	
Summer	53 (22.5)	155 (32.0)	
Winter	45 (19.2)	79 (16.2)	
Mode of delivery			
Vaginal non-instrumental	132 (56.2)	277 (58.0)	.822
Vaginal Instrumental	48 (20.4)	98 (20.6)	
Cesarean section	55 (23.4)	102 (21.4)	
Fever during labor	11 (4.7)	23 (5.0)	.882
Use of antibiotics during labor	68 (30.2)	110 (26.8)	.363

Note: Otherwise indicated data are expressed as *n* (%) or mean ± SD.
*p value derived from Kruskal-Wallis test for continuous variables and from Chi2 test for categorical variables.

pro-inflammatory cytokines IL-1β (OR per 10 μg/m^3 increase = 1.37; 95% CI 1.02, 1.85) and IL-6 (OR per 10 μg/m^3 increase = 1.32; 95% CI 1.00, 1.75) (Figure 1A). Moreover, higher levels of traffic-related NO_2 during the whole pregnancy were associated with elevated odds of detectable concentrations of immunomodulatory cytokine IL-10 (OR per 10 μg/m^3 increase = 1.30; 95% CI 0.99, 1.69) (Figure 1A). Odds of detectable concentrations of IL-1β tended to be increased in newborns exposed to higher prenatal exposure during the whole pregnancy to $PM_{2.5}$ (OR per 5 μg/m^3 increase = 1.62; 95% CI 0.97, 2.71) and PM_{10} (OR per 10 μg/m^3 increase = 1.77, 95% CI 1.02, 3.06). Moreover, odds of detectable immunomodulatory cytokine IL-10 tended to increase in relation to higher concentrations during the whole pregnancy of $PM_{2.5}$ (OR per 5 μg/m^3 increase = 1.49, 95% CI 0.95, 2.33) and PM_{10} (OR per 10 μg/m^3 increase = 1.56, 95% CI 0.97, 2.51). Exposure to higher levels of O_3 during the whole pregnancy was associated with increased odds of detectable concentrations of Th2-related cytokine IL-13 (OR per 10 μg/m^3 increase = 1.22; 95% CI 1.01, 1.49).

Traffic-related NO_2 levels during the whole pregnancy showed linear positive relationships with IL-1β (Figure 2A) and IL-6 (Figure 2B) concentrations in newborns. Moreover, the highest quartile of traffic-related NO_2 concentration (>39.6 μg/m^3) during pregnancy was positively associated with increased concentration of IL-1β (p for trend .027) and IL-6 (p for trend .060) (Table 3). Traffic-related O_3 levels showed a linear positive relationship with concentrations of IL-13 (Figure 2D), with the highest quartile (O_3 > 27.1 μg/m^3) during pregnancy positively associated with elevated concentration of IL-13 (p for trend .088) (Table 3). Stratified analyses by trimester of gestation showed similar associations between prenatal traffic-related NO_2, PM, and O_3 concentrations and cytokine detection in newborns across trimesters (Tables S3–S5).

Short-term exposure 15 days before birth to higher NO_2 concentrations was associated with increased odds of detectable pro-inflammatory IL-1β (OR per 10 μg/m^3 increase = 1.34; 95% CI 1.00,

TABLE 2 Distribution of cord blood cytokine concentrations (pg/ml) in newborns. The NELA study (n = 235), Spain (2015–2018)

	Detection rate (%)	Limits of detection and selected percentiles					
		LLOD	ULOD	p25	Mean ± SD	p50	p75
General inflammatory response							
IFN-α	52.8	0.5	550	<LLOD	2.2 ± 3.3	0.7	2.4
IL-1β	76.6	2.1	8800	2.2	31.2 ± 133.8	4.2	8.6
IL-6	31.5	29.7	7625	<LLOD	423.4 ± 1908.5	<LLOD	45.8
TNF-α	15.3	8.4	2163	<LLOD	<LLOD	<LLOD	<LLOD
Th1-related response							
IFN-γ	26.8	11.5	2950	<LLOD	13.3 ± 20.2	<LLOD	14.1
IL-2	43.8	20.1	5150	<LLOD	36.8 ± 60.2	<LLOD	41.5
Th2-related response							
IL-4	28.5	34.0	8725	<LLOD	37.3 ± 39.9	<LLOD	43.9
IL-5	52.3	12.1	3118	<LLOD	31.8 ± 43.1	13.3	43.0
IL-13	37.9	2.3	2400	<LLOD	3.4 ± 4.9	<LLOD	3.7
Immunomodulatory response							
IL-10	66.0	2.1	2175	<LLOD	10.4 ± 32.5	3.6	7.0
Th17-related response							
IL-17F	4.7	1.6	1712	<LLOD	<LLOD	<LLOD	<LLOD
IL-23	23.0	14.8	15,225	<LLOD	13.9 ± 16.5	<LLOD	<LLOD

Note: Cytokine concentrations were quantified in the supernatant of unstimulated whole umbilical cord blood cells after 7 days of culture with medium.

Abbreviations: LLOD, lower limit of detection; p25, 25th percentile; p50, 50th percentile; p75, 75th percentile; SD, standard deviation; Th1, Type 1 helper T cell; Th17, Type 17 helper T cell; Th2, Type 2 helper T cell; Treg, Regulatory helper T cell; ULOD, upper limit of detection.

1.80) and immunomodulatory cytokine IL-10 (OR per 10 μg/m^3 increase = 1.35; 95% CI 1.04, 1.74). Exposure to higher concentrations of O_3 15-days before birth was associated with increased odds of detectable Th2-related IL-13 (OR per 10 μg/m^3 increase = 1.20; 95% CI 1.00, 1.44) (Table S6).

Bayesian kernel machine regression models confirmed the linear positive dose-response relationships between exposure to TRAP mixture during pregnancy and cytokine concentrations in newborns (Figure S2). We observed no interaction among mixture TRAPs (Figure S3). Table 4 presents the WQS analyses for IL-1β, IL-6, IL-10, and IL-13 for each window of susceptibility. TRAP mixture, driven by NO_2, during the whole pregnancy, the first and the second trimesters was associated with increased odds of detectable IL-6 at birth. TRAP mixture, driven by O_3, during the whole pregnancy, the first, the second, and the third trimesters was associated with increased odds of detectable IL-13. Figure 3 shows the estimated WQS model regression index weights considering all trimesters in the same model. The highest weighted TRAPs for IL-1β were NO_2 in the first and the second trimesters and PM_{10} in the first trimester (Figure 3A). NO_2 in the first and the second trimesters was the highest weighted TRAP for IL-6 (Figure 3B). PM_{10} and O_3 in the first trimester were the highest weighted TRAPs for IL-10 (Figure 3C), and O_3 and PM_{10} in the third trimester were the highest weighted TRAPs for IL-13 (Figure 3D).

4 | DISCUSSION

To the best of our knowledge, this is most comprehensive prospective longitudinal cohort study characterizing cytokine profiles in newborns in relation to gestational exposure to TRAP using different statistical approaches to evaluate the correlation between pollutants. In single-pollutant models, we found long-term and short-term prenatal exposure to higher levels of TRAP to be associated with increased detection of unstimulated concentrations of pro-inflammatory (IL-1β and IL-6), Th2-related (IL-13), and immunomodulatory (IL-10) cytokines. Consistently, the BKMR model showed positive linear associations between exposure to mixed TRAP during pregnancy and detection levels of IL-1β, IL-6, IL-13, and IL-10 cytokines in newborns, and WQS model revealed the first and the third trimesters of gestation as the time windows of higher susceptibility.

A high proportion of samples was below detection limits of assessed cytokines, especially IL-17F and TNF-α that did not reach 20% of detection rate. These results are consistent with previous studies showing low detection rates of cytokines in cord blood of newborns.[13] Moreover, although intrauterine immune system activation is suggested,[34] our results support the hypothesis that uterus is supposed to be a sterile environment where the fetus interacts with a low number of antigens.

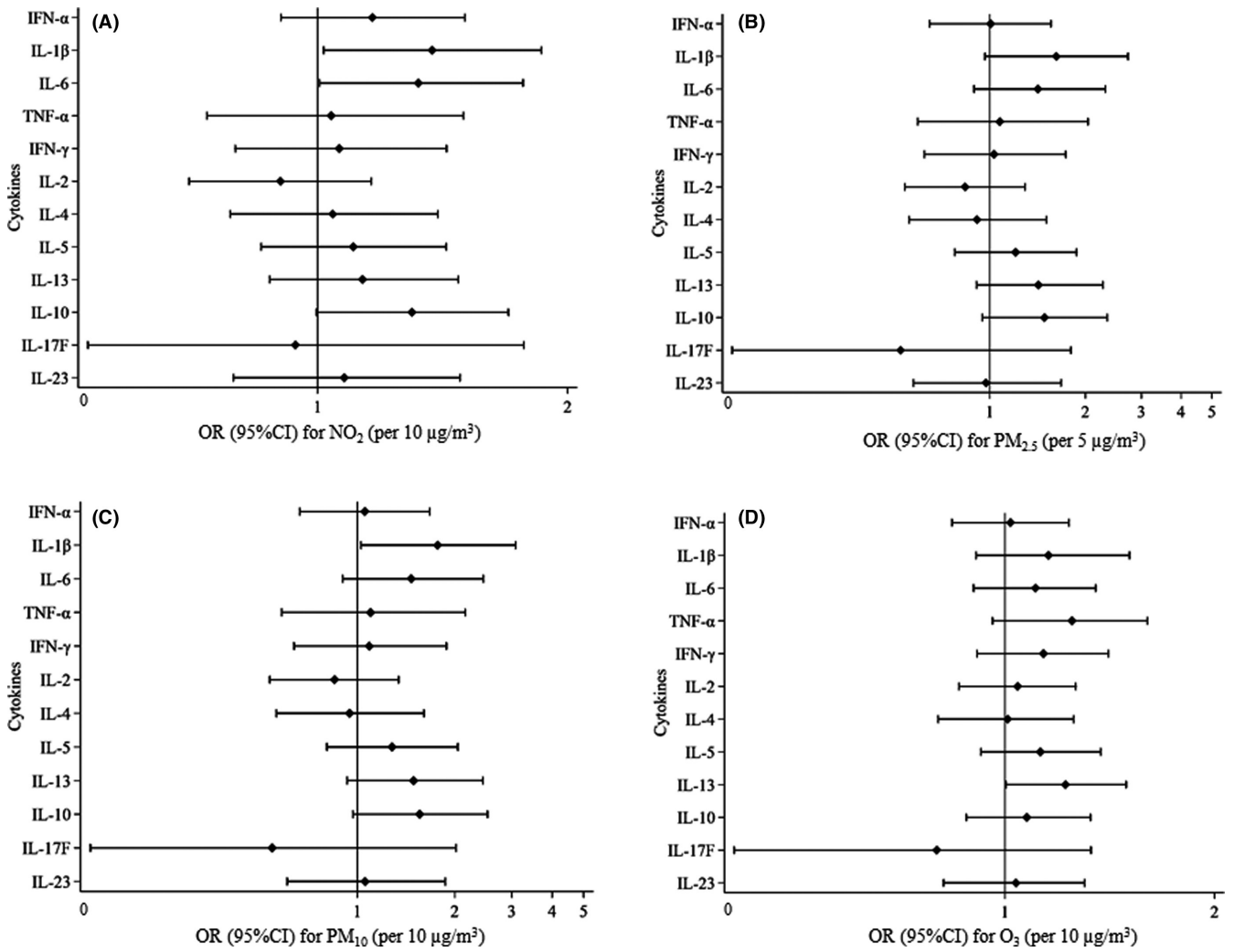

FIGURE 1 Adjusted odds ratios in log scale and the 95% confidence intervals (CI) for detectable (above the lower limit of detection) versus non-detectable (below the lower limit of detection) concentrations of cytokines in newborns in relation to traffic-related outdoor air pollutants during the whole pregnancy ($n = 235$). Cytokine concentrations were quantified in the supernatant of unstimulated whole umbilical cord blood cells after 7 days of culture with medium. All models adjusted for newborn's sex, gestational age, birthweight, season of birth, parental social class, maternal atopy, maternal pre-pregnancy body mass index, and paternal smoking. The NELA study (2015–2018) should be published in color

Our results suggest that a prenatal exposure to TRAP increased levels of pro-inflammatory cytokines (IL-1β and IL-6) in newborns. According to that, human exposure to air pollution has been associated with an inflammatory response.[10,35] Exposure to air pollution is suggested to induce reactive oxygen species (ROS) generation that leads to increase production of pro-inflammatory cytokines.[36] Although Hanh et al.[15] found prenatal exposure to moderate $PM_{2.5}$ concentrations during pregnancy to be associated with decreased IL-6 and TNF-α concentrations in mononuclear cell of newborns, other studies have shown exposure to moderate PM_{10} concentration during gestation to be associated with increased IL-1β in cord blood serum[13] and with a higher IL-6 production in serum from pregnant woman.[37] Regarding other stages of life, children 8 years of age exposed to TRAP NO_2 during the first year showed increased IL-6 serum levels[12]; and adults exposed to short-term and long-term particulate matter exhibited increased pro-inflammatory cytokine production (i.e., IL-1β, IL-6, and TNF-α).[38,39] Inflammation and oxidative stress have been associated with proliferation of Th2-related cells, a mechanism that might explain higher susceptibility to asthma and asthma exacerbation in response to air pollutants.[40–42]

Previous studies found decreased IL-10 production in cord blood from newborns whose mothers were exposed to moderate concentrations of $PM_{2.5}$ during pregnancy[13] and low PM_{10} levels in the last week of gestation.[15] However, a study conducted in three Bulgarian cities with differential particulate matter concentrations showed increased IL-10 concentrations in adolescents living in the higher polluted area.[43] Consistently with this, increased IL-10 expression was found in T and myeloid cells exposed to $PM_{2.5}$ in vitro.[44] Moreover, in vivo studies in mice have shown that prenatal exposure to ultrafine particles increased circulating IL-10.[45,46] Combined data from two European cities showed IL-10 changes only in the highest polluted area,[47] suggesting a dose-response

TABLE 3 Associations between residential traffic-related air pollution levels during pregnancy categorized in quartiles (Q) and cytokine concentrations in newborns ($n = 235$). The NELA study (2015–2018)

		IL-1β			IL-6			IL-10			IL-13		
	n	*β*	(95% CI)	*p* trend	*β*	(95% CI)	*p* trend	*β*	(95% CI)	*p* trend	*β*	(95% CI)	*p* trend
NO_2 (μg/m^3)													
Q1 (<23.1)	59	Ref.			Ref.			Ref.			Ref.		
Q2 (23.1–36.1)	59	8.7	(−19.3, 36.8)	.027	0.4	(−34.3, 35.1)	.060	2.2	(−23.5, 27.8)	.167	1.7	(−21.4, 24.7)	.266
Q3 (36.2–39.6)	59	10.9	(−17.1, 39.0)		6.5	(−26.0, 39.0)		20.0	(−4.4, 44.4)		2.7	(−21.3, 26.8)	
Q4 (>39.6)	58	33.3	(4.4, 62.1)		33.1	(−3.3, 69.4)		12.3	(−14.5, 39.0)		13.9	(−10.0, 37.9)	
$PM_{2.5}$ (μg/m^3)													
Q1 (<10.9)	59	Ref.			Ref.			Ref.			Ref.		
Q2 (10.9–13.2)	59	−14.4	(−42.4, 13.5)	.526	−3.9	(−37.2, 29.3)	.473	−4.1	(−30.9, 22.7)	.136	6.6	(−15.7, 28.8)	.176
Q3 (13.2–14.3)	59	−5.1	(−32.5, 22.4)		0.0	(−32.2, 32.2)		7.1	(−18.2, 32.3)		5.6	(−16.7, 27.9)	
Q4 (>14.3)	58	6.2	(−21.6, 34.0)		11.3	(−22.1, 44.7)		15.8	(−9.0, 40.6)		17.3	(−6.3, 40.8)	
PM_{10} (μg/m^3)													
Q1 (<18.9)	59	Ref.			Ref.			Ref,			Ref.		
Q2 (18.9–23.6)	59	−12.5	(−40.5, 15.5)	.304	−5.4	(−39.0, 28.1)	.366	1.2	(−25.3, 27.7)	.076	4.6	(−18.1, 27.3)	.155
Q3 (23.7–26.0)	59	−4.3	(−32.2, 23.6)		2.7	(−31.0, 36.4)		10.4	(−14.8, 35.5)		8.6	(−14.2, 31.4)	
Q4 (>26.0)	58	12.7	(−15.0, 40.4)		13.6	(−20.4, 47.7)		20.3	(−4.4, 45.0)		16.4	(−6.9, 39.7)	
O_3 (μg/m^3)													
Q1 (<14.5)	59	Ref.			Ref.			Ref.			Ref.		
Q2 (14.5–20.3)	59	22.2	(−5.0, 49.3)	.535	34.5	(2.3, 66.8)	.526	19.0	(−5.2, 43.3)	.392	12.5	(−10.6, 35.7)	.088
Q3 (20.4–27.1)	59	18.7	(−8.6, 46.0)		21.3	(−11.5, 54.0)		17.5	(−6.4, 41.5)		7.1	(−15.5, 29.7)	
Q4 (>27.1)	58	10.0	(−17.5, 37.6)		15.5	(−17.3, 48.2)		11.8	(−13.5, 37.2)		21.3	(0.0, 42.6)	

Note: Cytokine concentrations were quantified in the supernatant of unstimulated whole umbilical cord blood cells after 7 days of culture with medium. Values are regression coefficients (95% confidence interval) derived from linear regression models and reflect the difference (%) in each cytokine concentration. All models adjusted for newborn's sex, gestational age, birthweight, season of birth, parental social class, maternal atopy, maternal pre-pregnancy body mass index, and paternal smoking.

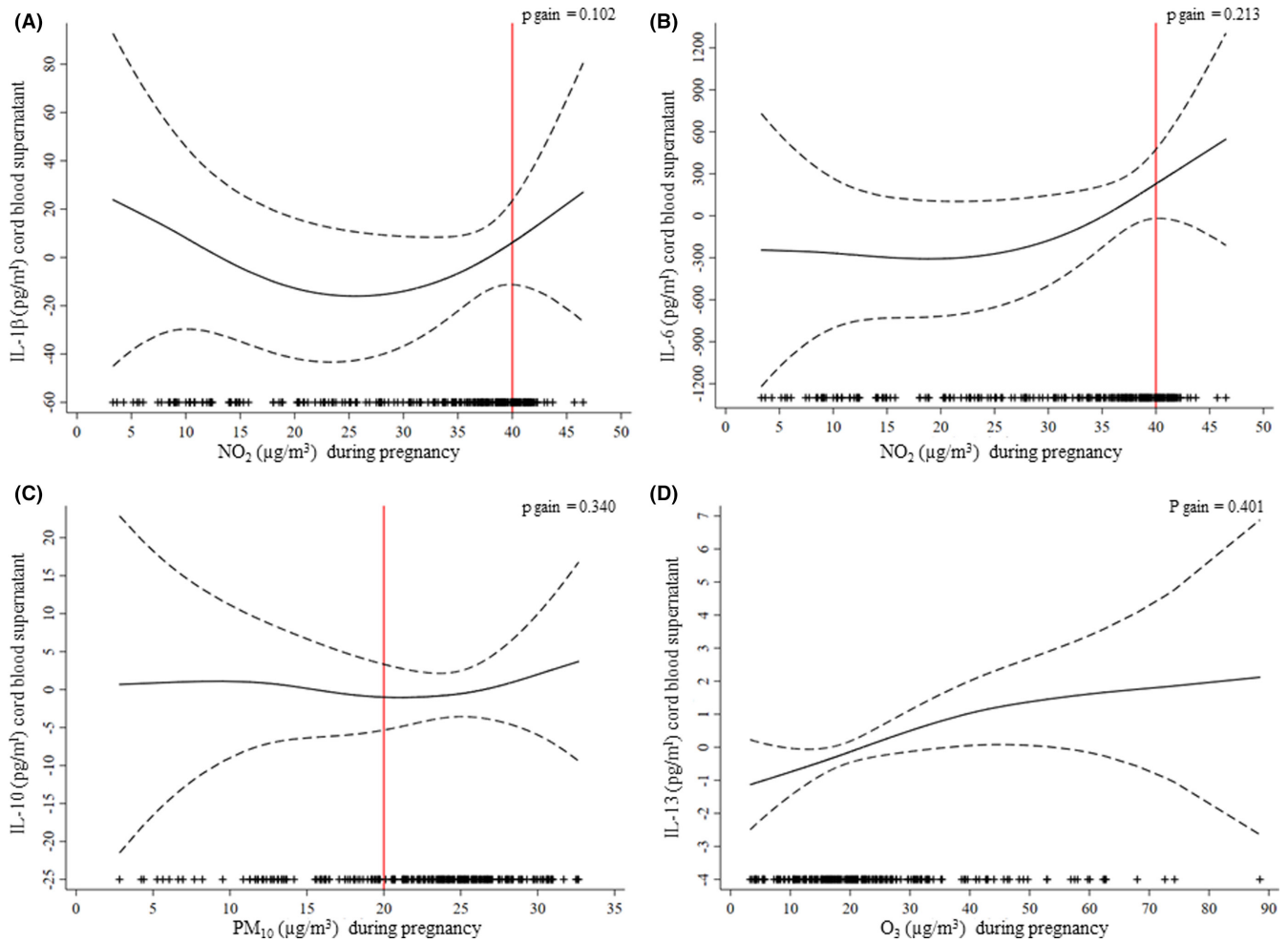

FIGURE 2 The relation (and 95% confidence levels) of residential traffic-related outdoor air pollutant levels during the whole pregnancy with cord blood cytokine concentrations: (A) NO_2 and IL-1β; (B) NO_2 and IL-6: (C) PM_{10} and IL-10; and (D) O_3 and IL-13. Cytokine concentrations were quantified in the supernatant of unstimulated whole umbilical cord blood cells after 7 days of culture with medium. General additive models adjusted for newborn's sex, gestational age, birthweight, season of birth, parental social class, maternal atopy, maternal pre-pregnancy body mass index, and paternal smoking. *p*-gain >.1 indicates that the relationship is not departure from linearity. The symbols (+) on the *X*-axis indicate air pollutant observations. Vertical lines indicate WHO recommended air quality standards should be published in color

effect that could explain differences in results from previous studies in cord blood and in our study, where participants were exposed to higher levels of PM. IL-10 is the most important anti-inflammatory cytokine that can suppress adaptive T cell response and impair response against pathogens, increasing susceptibility to infections.[48] Furthermore, increased IL-10 production might be a compensatory mechanism to suppressed Th2/Th17 response. In addition, asthmatic children showed a higher IL-10 concentration in response to NO_2 exposure during infancy.[12]

No previous studies have investigated the associations between prenatal exposure to O_3 and cord blood IL-13, a Th2-related cytokine involved in the development of Type-2 asthma phenotypes.[49] However, Ashley-Martin[14] found positive associations between prenatal exposure to air pollutants (i.e., NO_2) and cord blood concentrations of both IL-33 and TSLP, two cytokines also involved in Th2-related inflammation. Moreover, higher IL-13 production in cord blood has been related to skin prick test positivity and allergic manifestations in childhood.[50,51] In addition, the risk of chronic phlegm was associated with O_3 exposure.[52] Based on previous evidence, we hypothesize that prenatal exposure to O_3 may increase susceptibility to allergic diseases in early life through IL-13 pathways.

Our study has some limitations. The results are based on a subsample of the initial birth cohort. Although differences between included and excluded participants could impact to some extend the generalizability of results, because levels of TRAP did not differ, it should not affect their internal validity. We acknowledge that the small sample size may have affected the precision of estimates and the power of study. Cytokine concentrations were quantified after 7 days of culture of whole cord blood with medium, which could not depict the direct ex vivo cytokine concentrations in cord blood at birth. We had to dichotomize our outcomes (above versus below the detection limit) due to the relatively high proportion of cytokine measurements below the detection limit. This procedure could underestimate an association rather than to create a spurious

TABLE 4 Adjusted associations for detectable (above the lower limit of detection) versus non-detectable (below the lower limit of detection) concentrations of cytokines in newborns in relation to traffic-related outdoor air pollutants modeled as a mixture using weighted quantile sum (WQS) regressions (n = 235). The NELA study (2015–2018)

	IL-1β			IL-6			IL-10			IL-13		
Detectable/ Non-detectable	(180/55)			(77/158)			(155/80)			(89/146)		
	OR	(95% CI)	Average weight	OR	(95% CI)	Average weight	OR	(95% CI)	Average weight	OR	(95% CI)	Average weight
Whole pregnancy	1.17	(0.96, 1.44)		1.22	(1.02, 1.45)		1.11	(0.94, 1.31)		1.21	(1.02, 1.42)	
NO_2, μg/m^3			0.353			0.257			0.251			0.250
$PM_{2.5}$, μg/m^3			0.216			0.248			0.251			0.248
PM_{10}, μg/m^3			0.217			0.248			0.236			0.250
O_3, μg/m^3			0.214			0.248			0.261			0.252
1st Trimester	1.22	(0.98, 1.52)		1.27	(1.03, 1.55)		1.10	(0.92, 1.31)		1.24	(1.03, 1.49)	
NO_2, μg/m^3			0.278			0.254			0.228			0.249
$PM_{2.5}$, μg/m^3			0.231			0.245			0.221			0.250
PM_{10}, μg/m^3			0.254			0.250			0.292			0.250
O_3, μg/m^3			0.236			0.251			0.259			0.251
2nd Trimester	1.17	(0.94, 1.45)		1.22	(1.01, 1.47)		1.14	(0.96, 1.36)		1.20	(1.01, 1.43)	
NO_2, μg/m^3			0.370			0.261			0.262			0.249
$PM_{2.5}$, μg/m^3			0.209			0.248			0.237			0.247
PM_{10}, μg/m^3			0.210			0.246			0.243			0.247
O_3, μg/m^3			0.211			0.246			0.258			0.257
3rd Trimester	1.17	(0.94, 1.45)		1.13	(0.93, 1.37)		1.08	(0.90, 1.30)		1.24	(1.03, 1.50)	
NO_2, μg/m^3			0.329			0.260			0.279			0.241
$PM_{2.5}$, μg/m^3			0.226			0.247			0.248			0.241
PM_{10}, μg/m^3			0.220			0.245			0.225			0.242
O_3, μg/m^3			0.225			0.248			0.248			0.277
15-days before delivery	1.16	(0.92, 1.45)		1.17	(0.95, 1.44)		1.01	(0.83, 1.22)		1.00	(0.82, 1.21)	
NO_2, μg/m^3			0.289			0.254			0.254			0.252
$PM_{2.5}$, μg/m^3			0.258			0.253			0.258			0.243
PM_{10}, μg/m^3			0.223			0.246			0.246			0.249
O_3, μg/m^3			0.229			0.247			0.243			0.256

Note: Cytokine concentrations were quantified in the supernatant of unstimulated whole umbilical cord blood cells after 7 days of culture with medium. All models adjusted for newborn's sex, gestational age, birthweight, season of birth, parental social class, maternal atopy, maternal pre-pregnancy body mass index, and paternal smoking. The odds ratio (OR) and the 95% confidence intervals (CI) for detectable (above the lower limit of detection) versus non-detectable (below the lower limit of detection) concentrations of cytokines for one unit higher of the WQS index (representing a one-decile increase in chemical concentrations). Weights greater than 1/number (0.083, red dashed line) of chemicals and windows in the mixture are considered significant contributors to the overall mixture effect. Weights may not add to 1 due to rounding.

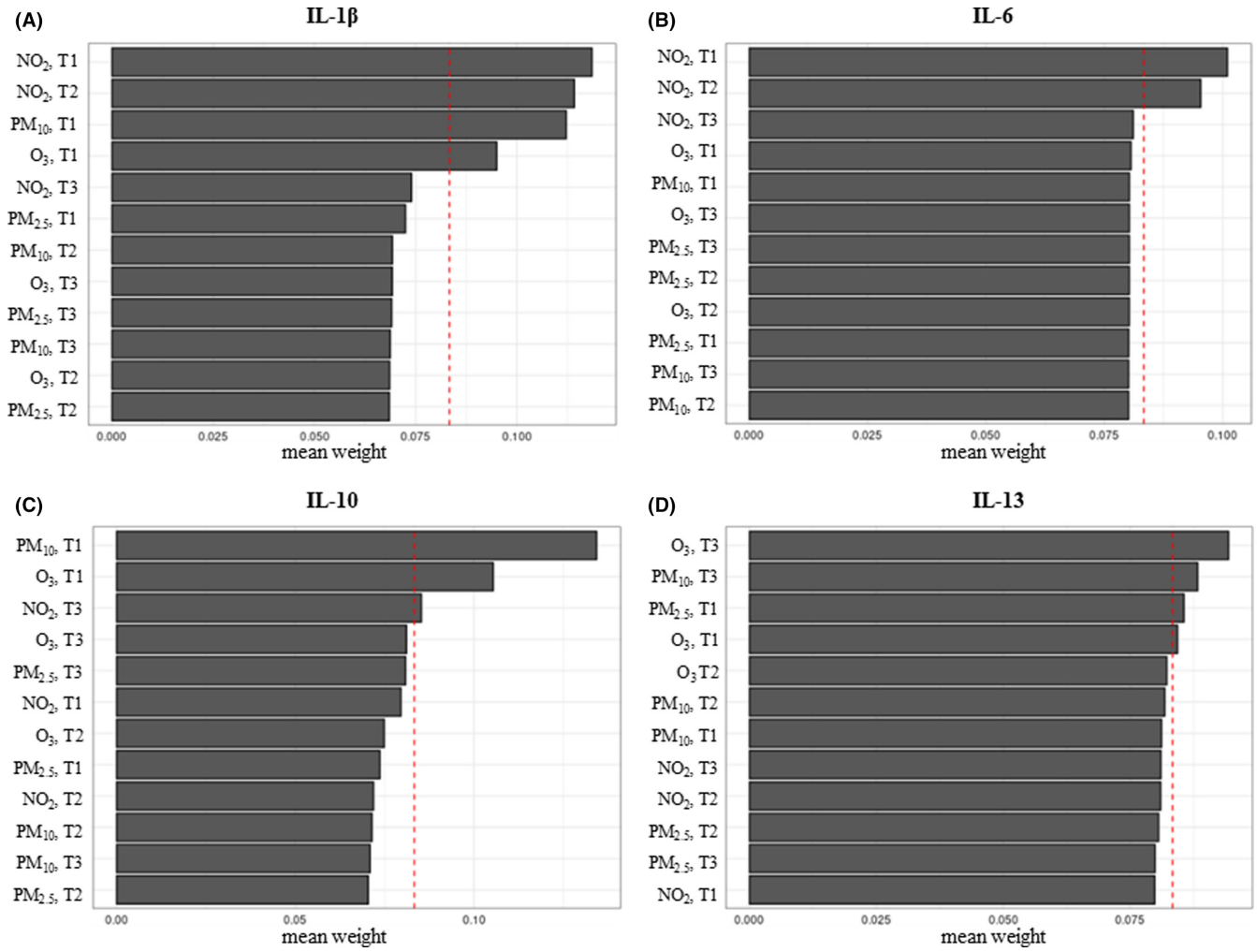

FIGURE 3 WQS model regression index weights for (A) IL-1β, (B) IL-6, (C) IL-10, and (D) IL-13. Cytokine concentrations were quantified in the supernatant of unstimulated whole umbilical cord blood cells after 7 days of culture with medium. Models were adjusted for newborn's sex, gestational age, birthweight, season of birth, parental social class, maternal atopy, maternal pre-pregnancy body mass index, and paternal smoking. T1: first trimester; T2: second trimester; T3: third trimester. The dashed red line represents the cutoff value of 0.083 (by default equal to the inverse of the number of elements in the mixture), discriminating which element has a significant weight should be published in color

association. However, after imputation of values out of the detection limits to minimize bias, GAM and linear regression models confirmed our main findings. In addition, we did not collect information on work location, time spent at home, or indoor pollutants, which may result in potential exposure misclassification. Finally, information on relevant clinical outcomes was not considered in the present study. Further follow-up of the NELA cohort is warranted to unravel the impacts of the observed changes in cytokine profiles at birth on respiratory health and the risk of asthma and allergy later in life.

Our study is strengthened by its population-based prospective design and the availability of extensive covariate data. Moreover, a broad panel of biologically relevant cytokines was used to characterize newborn's profile, which makes our study one of the most comprehensive to date. In addition, WRF models provide a high spatial and temporal resolution assessing air pollutants not routinely monitored and modeling both short- and long-exposure periods. In addition, applying mixtures statistical approaches allowed to better investigate and estimate mixture effects of prenatal TRAP.

In conclusion, our results suggest that exposure to residential TRAP during pregnancy may increase the levels of pro-inflammatory, Th2-related, and immunomodulatory cytokines in newborns. These changes might impact the immune system response of the offspring to respiratory viruses and allergens later in life.

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CONFLICT OF INTEREST

All authors have nothing to disclose.

AUTHOR CONTRIBUTION

Azahara M. García-Serna: Conceptualization (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Writing – original draft (lead); Writing – review & editing (lead). **Elena Martín-Orozco:** Conceptualization (supporting); Investigation (lead); Methodology (lead); Writing – review & editing (supporting). **Pedro Jiménez-Guerrero:** Methodology (lead); Writing – review & editing (supporting). **Trinidad Hernández-Caselles:** Methodology (supporting); Writing – review & editing (supporting). **Virginia Pérez-Fernández:** Formal analysis (supporting); Methodology (supporting); Writing – review & editing (supporting). **Esther Cantero-Cano:** Investigation (supporting); Resources (supporting); Writing – review & editing (supporting). **María Muñoz-García:** Methodology (supporting); Writing – review & editing (supporting). **María Dolores Molina-Ruano:** Resources (supporting); Writing – review & editing (supporting). **Encarna Rojo-Atenza:** Resources (supporting); Writing – review & editing (supporting). **Luis García-Marcos:** Conceptualization (supporting); Funding acquisition (supporting); Writing – review & editing (supporting). **Eva Morales:** Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Writing – original draft (lead); Writing – review & editing (lead).

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REFERENCES

1. Rice MB, Rifas-Shiman SL, Oken E, et al. Exposure to traffic and early life respiratory infection: a cohort study. *Pediatr Pulmonol*. 2015;50(3):252-259.
2. Cai Y, Hansell AL, Granell R, et al. Prenatal, early-life, and childhood exposure to air pollution and lung function: the ALSPAC cohort. *Am J Respir Crit Care Med*. 2020;202(1):112-123.
3. Bettiol A, Gelain E, Milanesio E, Asta F, Rusconi F. The first 1000 days of life: traffic-related air pollution and development of wheezing and asthma in childhood. a systematic review of birth cohort studies. *Environ Health*. 2021;20(1):46.
4. García-Serna AM, Martín-Orozco E, Hernández-Caselles T, Morales E. Prenatal and perinatal environmental influences shaping the neonatal immune system: a focus on asthma and allergy origins. *Int J Environ Res Public Health*. 2021;18(8):3962.
5. McFadden JP, Thyssen JP, Basketter DA, Puangpet P, Kimber I. T helper cell 2 immune skewing in pregnancy/early life: chemical exposure and the development of atopic disease and allergy. *Br J Dermatol*. 2015;172(3):584-591.
6. Diesner SC, Förster-Waldl E, Olivera A, Pollak A, Jensen-Jarolim E, Untersmayr E. Perspectives on immunomodulation early in life. *Pediatr Allergy Immunol*. 2012;23(3):210-223.
7. Zhang X, Zhivaki D, Lo-Man R. Unique aspects of the perinatal immune system. *Nat Rev Immunol*. 2017;17(8):495-507.
8. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol*. 2015;16(1):45-56.
9. Krusche J, Basse S, Schaub B. Role of early life immune regulation in asthma development. *Semin Immunopathol*. 2020;42(1):29-42.
10. Glencross DA, Ho TR, Camiña N, Hawrylowicz CM, Pfeffer PE. Air pollution and its effects on the immune system. *Free Radic Biol Med*. 2020;151:56-68.
11. De Grove KC, Provoost S, Brusselle GG, Joos GF, Maes T. Insights in particulate matter-induced allergic airway inflammation: Focus on the epithelium. *Clin Exp Allergy*. 2018;48(7):773-786.
12. Gruzieva O, Merid SK, Gref A, et al. Exposure to traffic-related air pollution and serum inflammatory cytokines in children. *Environ Health Perspect*. 2017;125(6):67007.
13. Latzin P, Frey U, Armann J, et al. Exposure to moderate air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. *PLoS One*. 2011;6(8):e23130.
14. Ashley-Martin J, Lavigne E, Arbuckle TE, et al. Air pollution during pregnancy and cord blood immune system biomarkers. *J Occup Environ Med*. 2016;58:979-986.
15. Hahn J, Gold DR, Coull BA, et al. Air pollution, neonatal immune responses, and potential joint effects of maternal depression. *Int J Environ Res Public Health*. 2021;18(10):5062.
16. Morales E, Alcantara-Lopez MV, Cabezas-Herrera J, et al. The Nutrition in Early Life and Asthma (NELA) birth cohort study: rationale, design, and methods. *Paediatr Perinat Epidemiol*. 2021. 10.1111/ppe.12826. online ahead of print.
17. Skamarock WC, Klemp JB, Dudhia J, Gill DO, Barker DM, Wang W, et al. A description of the Advanced Research WRF Version 2. NCAR Tech. Note NCAR/TN-468+STR, 2005; 88 pp.
18. Menut L, Bessagnet B, Khvorostyanov D, et al. CHIMERE 2013: a model for regional atmospheric composition modelling. *Geosci Model Dev*. 2013;6:981-1028.
19. Koo B, Wilson GM, Morris RE, Dunker AM, Yarwood G. Comparison of source apportionment and sensitivity analysis in a particulate matter air duality model. *Environ Sci Technol*. 2009;43:6669-6675.
20. Moldenhauer A, Wolf J, Habermann G, Genter G, Kiesewetter H, Salama A. Optimum storage conditions for cord blood-derived hematopoietic progenitor cells prior to isolation. *Bone Marrow Transplant*. 2007;40(9):837-842.
21. López MC, Palmer BE, Lawrence DA. Naïve T cells, unconventional NK and NKT cells, and highly responsive monocyte-derived macrophages characterize human cord blood. *Immunobiology*. 2014;219(10):756-765.
22. Bakker OB, Aguirre-Gamboa R, Sanna S, et al. Integration of multi-omics data and deep phenotyping enables prediction of cytokine responses. *Nat Immunol*. 2018;19(7):776-786.
23. Li Y, Oosting M, Smeekens SP, et al. A functional genomics approach to understand variation in cytokine production in humans. *Cell*. 2016;167(4):1099-1110.
24. Schirmer M, Smeekens SP, Vlamakis H, et al. Linking the human gut microbiome to inflammatory cytokine production capacity. *Cell*. 2016;167(4):1125-1136.e8.
25. Ter Horst R, Jaeger M, Smeekens SP, et al. Host and environmental factors influencing individual human cytokine responses. *Cell*. 2016;167(4):1111-1124.
26. Domingo-Salvany A, Regidor E, Alonso J, Alvarez-Dardet C. [Proposal for a social class measure. Working Group of the Spanish Society of Epidemiology and the Spanish Society of Family and Community Medicine]. *Aten Primaria*. 2000;25:350-363.

27. Uh HW, Hartgers FC, Yazdanbakhsh M, Houwing-Duistermaat JJ. Evaluation of regression methods when immunological measurements are constrained by detection limits. *BMC Immunol*. 2008;9:59.
28. van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. *Stat Med*. 1999;18:681-694.
29. Royston P. Multiple imputation of missing values: further update of ice, with an emphasis on interval censoring. *Stata J*. 2007;7(4):445-464.
30. Little RJA, Rubin DB. *Statistical Analysis with Missing Data*, 2nd ed. John Wiley & Sons; 2014.
31. Bobb JF, Valeri L, Claus Henn B, et al. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. *Biostatistics*. 2015;16(3):493-508.
32. Bobb JF, Claus Henn B, Valeri L, Coull BA. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. *Environ Health*. 2018;17(1):67.
33. Carrico C, Gennings C, Wheeler DC, Factor-Litvak P. Characterization of weighted quantile sum regression for highly correlated data in a risk analysis setting. *J Agric Biol Environ Stat*. 2015;20(1):100-120.
34. Zhivaki D, Lo-Man R. In utero development of memory T cells. *Semin Immunopathol*. 2017;39(6):585-592.
35. Liu C, Cai J, Qiao L, et al. The acute effects of fine particulate matter constituents on blood inflammation and coagulation. *Environ Sci Technol*. 2017;51(14):8128-8137.
36. Olvera Alvarez HA, Kubzansky LD, Campen MJ, Slavich GM. Early life stress, air pollution, inflammation, and disease: an integrative review and immunologic model of social-environmental adversity and lifespan health. *Neurosci Biobehav Rev*. 2018;92:226-242.
37. Buxton MA, Meraz-Cruz N, Sanchez BN, et al. Air pollution and inflammation: findings from concurrent repeated measures of systemic and reproductive tract cytokines during term pregnancy in Mexico City. *Sci Total Environ*. 2019;681:235-241.
38. Tsai DH, Riediker M, Berchet A, et al. Effects of short- and long-term exposures to particulate matter on inflammatory marker levels in the general population. *Environ Sci Pollut Res Int*. 2019;26(19):19697-19704.
39. Laratta CR, Kendzerska T, Carlsten C, et al. Air pollution and systemic inflammation in patients with suspected OSA living in an urban residential area. *Chest*. 2020;158(4):1713-1722.
40. Klümper C, Krämer U, Lehmann I, et al. Air pollution and cytokine responsiveness in asthmatic and non-asthmatic children. *Environ Res*. 2015;138:381-390.
41. Carthy P, Ó Domhnaill A, O'Mahony M, et al. Local NO2 concentrations and asthma among over-50s in Ireland: a microdata analysis. *Int J Epidemiol*. 2021;49(6):1899-1908.
42. Liu Y, Pan J, Zhang H, et al. Short-term exposure to ambient air pollution and asthma mortality. *Am J Respir Crit Care Med*. 2019;200(1):24-32.
43. Dobreva ZG, Kostadinova GS, Popov BN, Petkov GS, Stanilova SA. Proinflammatory and anti-inflammatory cytokines in adolescents from Southeast Bulgarian cities with different levels of air pollution. *Toxicol Ind Health*. 2015;31(12):1210-1217.
44. Ibironke O, Carranza C, Sarkar S, et al. Urban air pollution particulates suppress human T-cell responses to mycobacterium tuberculosis. *Int J Environ Res Public Health*. 2019;16(21):4112.
45. Rychlik KA, Secrest JR, Lau C, et al. In utero ultrafine particulate matter exposure causes offspring pulmonary immunosuppression. *Proc Natl Acad Sci USA*. 2019;116(9):3443-3448.
46. Jaligama S, Saravia J, You D, et al. Regulatory T cells and IL10 suppress pulmonary host defense during early-life exposure to radical containing combustion derived ultrafine particulate matter. *Respir Res*. 2017;18(1):15.
47. Mostafavi N, Vlaanderen J, Chadeau-Hyam M, et al. Inflammatory markers in relation to long-term air pollution. *Environ Int*. 2015;81:1-7.
48. Boonpiyathad T, Satitsuksanoa P, Akdis M, Akdis CA. Il-10 producing T and B cells in allergy. *Semin Immunol*. 2019;44:101326.
49. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity*. 2019;50(4):975-991.
50. Ohshima Y, Yasutomi M, Omata N, et al. Dysregulation of IL-13 production by cord blood CD4+ T cells is associated with the subsequent development of atopic disease in infants. *Pediatr Res*. 2002;51(2):195-200.
51. Uzuner N, Babayigit Hocaoglu A, Olmez Erge D, et al. Raised interleukin-13 levels in cord blood increases the risk of allergic sensitization at 5 years of age. *Iran J Allergy Asthma Immunol*. 2013;12(2):107-114.
52. Lee YL, Chen JH, Wang CM, Chen ML, Hwang BF. Association of air pollution exposure and interleukin-13 haplotype with the risk of aggregate bronchitic symptoms in children. *EBioMedicine*. 2018;29:70-77.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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