ASSOCIATION BETWEEN POLYCHLORINATED BIPHENYLS AND CIRCULATORY IMMUNE MARKERS: RESULTS FROM NHANES 1999–2004

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SUMMARY

Objectives: Polychlorinated biphenyls (PCBs), a family of persistent toxic and organic environmental pollutants, were associated with multiple organ damages in humans once accumulating. However, association between PCBs exposure and circulatory immune markers were not clear.

Methods: Data was collected from participants enrolled in the National Health and Nutrition Examination Survey in 1999–2004. PCBs were categorized by latent class analysis (LCA). Weighted quantile sum (WQS) regression was used to investigate effects of PCBs exposure on circulatory immune markers including leukocyte counts, monocyte-lymphocyte ratio (MLR), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII).

Results: There were 3,109 participants included in the final analysis with blood PCBs levels presented as 3 classes. The high PCBs group had a higher rate of comorbidities. Leukocyte, lymphocyte and neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and system immune-inflammation index (SII) were significantly lower in the high PCBs group than in the low PCBs group (all p-values < 0.05). After adjusting for covariant variables, the low PCBs group was positively associated with SII ($p = 0.021$) and NLR ($p = 0.006$) in multivariate regression. Significantly negative correlations between PCBs classification and SII ($\beta = -14.513$, $p = 0.047$), and NLR ($\beta = -0.035$, $p = 0.017$) were found in WQS models. LBX028LA showed the most significant contribution in the associations between PCBs and SII, and LBX128LA contributed most significantly to associations with NLR.

Conclusion: Our study adds novel evidence that exposures to PCBs may be adversely associated with the circulatory immune markers, indicating the potential toxic effect of PCBs on the human immune system.

Key words: polychlorinated biphenyls, circulatory immune markers, latent class analysis, weighted quantile sum regression, systemic immune-inflammation index, platelet-lymphocyte ratio

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INTRODUCTION

Disorders of the immune system were found in a variety of conditions including cardiovascular disease, neural disease, oncology, endocrine disease, etc. (1–3) Early detection of disorders of the immune system is certainly important for the diagnosis and treatment of the disease. The circulatory immune markers may have prognostic significance in detecting long-term events (4). Presently, easily accessible circulatory immune markers are widely used in evaluation of disease prognosis (5–7), including the platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), monocyte-to-lymphocyte ratio (MLR), and system immune-inflammation index (SII), based on neutrophil, lymphocyte, monocyte and/or platelet counts, also known as systemic inflammatory biomarkers.

Polychlorinated biphenyls (PCBs), a collection of synthetic persistent organic environmental pollutants with chemical stability, are difficult to decompose and commonly found in water, soil and air (8). Although the production of PCBs has been ceased in many countries, people may still be exposed to them through contact with media contaminated by PCBs. The high lipophilicity of PCBs leads to their accumulation in organisms and further enrichment through the food chain, causing a range of adverse effects in animals and humans, such as carcinogenesis, cognitive and behavioural abnormalities, and teratogenicity and endocrine metabolism disorders (9, 10).

The human immune cells are highly toxic-sensitive to PCBs. Nagayama et al. (11) found that perinatal exposures to HCE, chlordane and dioxins were significantly associated with the increase in the percentages of CD8+ and CD3+ T lymphocytes, and CD4+/CD8+ T cell ratios, respectively. On the other hand, in the research with small number of participants, monocyte percentages were found to be significantly decreased in subjects exposed to PCBs compared to the controls (12). In addition to

exerting an effect on the macroscopic indicator of cell number, PCB exposure played a role in telomere, an important biomarker and target of cellular life cycle. It was reported that telomeres of leukocytes were a target for the toxicity of PCBs in rats and PCB exposures were associated with increased leukocyte relative telomeres length (13). The previous report showed that high exposure to PCBs was associated with lower total white blood cell (WBC) count, red blood cells (RBC), haemoglobin, and haematocrit, which was the first report on an association between PCBs and WBC and RBC in the general population (14). On the other hand, some studies have shown that lymphocyte phenotypes or subpopulation numbers are not associated with serum PCBs, or that PCBs are weakly correlated with biochemical indices (15, 16). Further research is needed to uncover these potential relationships. Despite these mixed findings, data related to the effect of PCBs on circulatory immune biomarkers from general population were still scarce. To our knowledge, no researches have focused on associations between PCBs exposure and circulatory immune markers in general population.

Latent class analysis (LCA) is a method that used one or more categorical latent variables to investigate the complex correlations among multiple exogenous categorical variables. It has been widely used in medical research for its ability to conduct factor analysis and cluster analysis, and its classification criteria and results are more reasonable than those of traditional clustering (17, 18). In this study, we utilized data from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2004 to categorize PCBs using the LCA method. To explore the sociodemographic characteristics and the impact of different PCBs classes on circulating immune markers, we employed multivariate regression models with PCB concentrations as the independent variables and immune markers as the dependent variables. Additionally, we constructed weighted quartiles sum (WQS) models to further analyse these relationships, thereby providing a basis for targeted interventions in diverse population groups.

MATERIALS AND METHODS

Participants

The population selection for NHANES is carried out through a computer program that first selects areas of the country, then neighbourhoods, households, and finally, individuals for random sampling. Participants aged 12 years and older from the NHANES survey cycles conducted in 1999–2004 were obtained online. Each survey is a nationally representative sample of the US civilian, noninstitutionalized population based on a complex probability sampling design. The survey was conducted periodically before 1999 and continuously thereafter. Details of the study design and data collection have been described previously (19). The Ethics Review Board have approved the study (NCHS IRB/ERB Protocol Number: Protocol #98-12).

PCBs and Circulatory Immune Markers

Details of the NHANES laboratory measurements are available online for 1999–2004. For the PCB content measurement relevant to our study, samples were collected during participants' clinical examinations, which are part of NHANES overall health assessment. Serum samples were collected and exposure assessment for environmental chemicals was conducted by the Laboratory Sciences Division, the National Centre for Environmental Health, and the Environmental Health Laboratory of CDC. Briefly, non-dioxin-like polychlorinated biphenyls and mono-ortho-substituted polychlorinated biphenyls were measured in one-third subsample of participants aged 12 years and older. Special sample weights are required to analyse these data properly. PCBs were quantified by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry. The serum samples were measured on a whole-weight or per gram of total lipid basis to reflect the amount of PCBs stored in body fat, with lipid-adjusted serum concentrations (ng/g unless stated otherwise) for individual PCB congeners were used in this study. More detailed descriptions of analytical methods have been reported previously: dioxins, furans, and coplanar PCBs (L28DFP_C)[1]; and non-dioxin-like polychlorinated biphenyls (L28NPB_C): 2003–2004 data documentation, codebook, and frequencies[2]. The complete blood count (CBC) measurements with 5-part differential in whole blood were obtained based on the Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for haemoglobinometry (NHANES laboratory procedures manual)[3].

SII, NLR, PLR and MLR was calculated as followed:

$$SII = \frac{\text{platelet count} \times \text{neutrophil count}}{\text{lymphocyte count}}$$

$$NLR = \frac{\text{neutrophil count}}{\text{lymphocyte count}}$$

$$PLR = \frac{\text{platelet count}}{\text{lymphocyte count}}$$

$$MLR = \frac{\text{monocyte count}}{\text{lymphocyte count}}$$

Statistics

The statistical analysis was performed using SPSS Statistics version 21.0 software and R 4.03 software. Characteristics of the participating population were shown as mean (standard deviation, SD) or median (inter-quantile range, IQR) for continuous variables, and proportions for categorical variables. Crude data were compared using the chi-square test for the categorical variables, and the ANOVA (Kruskal-Wallis test was used in non-normally distributed data) for the numerical variables.

The normally distributed measures were expressed as mean (standard deviation), and analysed using the ANOVA or Kolmogorov-Smirnov test; the skewed measures were expressed as median (P25, P75), and the Kruskal-Wallis rank sum test was used

[1]http://wwwn.cdc.gov/nchs/nhanes/2003-2004/L28DFP_C.htm
[2]http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/L28NPB_C.htm
[3]http://www.cdc. gov/nchs/data/nhanes/nhanes_03_04/lab.pdf

for comparison between groups. The count data were expressed as relative numbers, and the χ^2 test was used for comparison between groups. All p-values < 0.05 were considered statistically significant. The LCA model was constructed using the poLCA package in R software, and the Bayesian information criterion (BIC) was used to evaluate the model fit test, with a smaller BIC value indicating a better model, and a log-maximum likelihood ratio test was performed. With entropy point above 0.7, the classification was considered accurate. To investigate the associations of PCB concentrations with immune markers, multivariate regression models and weighted quantile sum (WQS) regression (20) were used including unadjusted and two adjusted models, with medium PCB blood level considered as reference class. The WQS regression model is a method that combines an exposure percentile weighted score with a linear (continuous outcome) or logistic (binary outcome) regression, which allows a weighted index to be constructed in a supervised manner to assess the overall effect of environmental exposure and the contribution of each component of the mixture to the overall effect. Other covariates included in the models were age, sex, race, insurance, education level, family income to poverty ratio, marital status, physical activity, cigarettes smoking, alcohol consumption, BMI, etc. The covariates were selected because they were potential confounders as determined by subject-matter knowledge. The mixture effect of PCB exposure was inferred from the WQS index in the regression model. The WQS regression was performed using the gWQS package (version 3.0.3) in R software.

RESULTS

Characteristics of the Study Participants in Assembled NHANES Cohort

After excluding samples with missing data of immune markers, or missing data on relevant covariates, among 3,361 participants in NHANES spanning 1999 to 2004, there were 3,109 participants

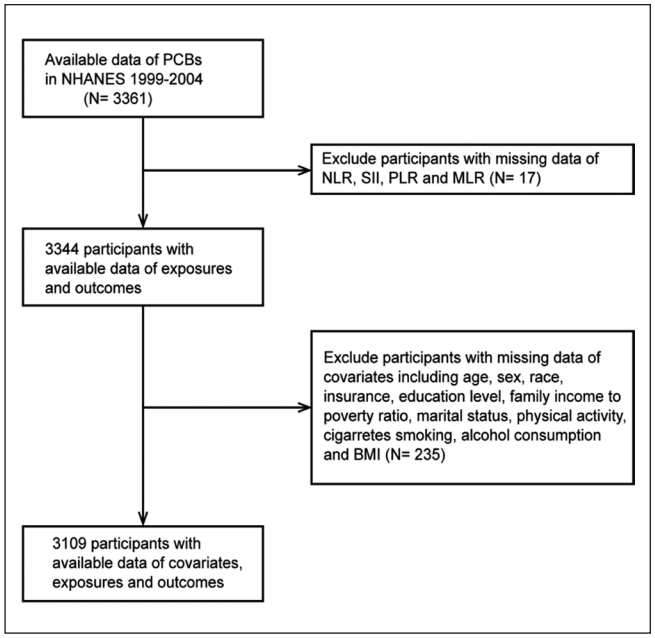

Fig. 1. *Flow chart of this study.*

Table 1. *Baseline characteristics*

Variables	n (%)
Demographic parameters	
Age (years), mean (SD)	41.3 (21.2)
Sex	
Male	1,491 (48.0)
Female	1,618 (52.0)
Race/ethnicity	
Mexican American	832 (26.8)
Other Hispanic	135 (4.3)
Non-Hispanic White	1,396 (44.9)
Non-Hispanic Black	623 (20.0)
Other race, including multi-racial	123 (4.0)
Socioeconomic status	
Education level	
Less than 9th grade	379 (12.2)
9–12th grade or equivalent	1574 (50.6)
Some college or above	1156 (37.2)
Family income-to-poverty ratio, mean (SD)	2.4 (1.6)
Insurance	
Insured	2,441 (78.5)
Uninsured	668 (21.5)
Marital status	
Married/cohabited	1,537 (49.4)
Widowed	222 (7.1)
Divorced/separated	260 (8.4)
Unmarried	1,090 (35.1)
Anthropometry	
BMI, mean (SD)	27.5 (6.4)
Lifestyle indicators	
Smoking status	
Ever-smoker	813 (26.1)
Non-smoker	1,588 (51.1)
Current smoker	708 (22.8)
Average drinks per day*	0.0 (0.0–0.3)
Physical activity	
Non-active	1,315 (42.3)
Active	1,794 (57.7)
Comorbidities	
Cardiovascular disease	95 (4.0)
Stroke	67 (2.8)
Cancer	198 (8.3)
Emphysema	38 (1.6)
Chronic bronchitis	146 (6.2)
Hypertension	773 (26.1)
Diabetes	237 (7.6)

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Circulating immune markers	Mean (SD)
Total leukocyte count (1,000 cell/uL)	7.3 (2.2)
Absolute lymphocyte count (1,000 cell/uL)	2.1 (0.7)
Absolute neutrophil count (1,000 cell/uL)	4.4 (1.8)
Absolute monocyte count (1,000 cell/uL)	0.6 (0.2)
Platelet count (1,000 cells/uL)	267.8 (68.6)
Systemic immune-inflammation index	611.2 (412.2)
Neutrophil-lymphocyte ratio	2.3 (1.2)
Monocyte-lymphocyte ratio	0.3 (0.1)
Platelet-lymphocyte ratio	138.2 (54.8)

(92.5%) included in the analyses (Fig. 1). The mean age of the study participants was 41.3 years (±21.2 SD), and the mean body mass index (BMI) was 27.5 (±6.4 SD). The baseline characteristics of the study population are shown in Table1.

Classes of PCBs Based on LCA

When PCBs was classified by LCA, 3 classes were selected as the best models based on the best performing indicators including likelihood ratio tests and information indices. The C1 class accounted for 23.4%, the C2 class for 29.5%, and the C3 class for 47.1%; they were named medium PCB group, low PCBs group and high PCBs group, respectively. Among the PCBs measured in blood, significant differences were observed between the different groups (all $p<0.001$, as determined by ANOVA), with the highest PCB153 levels (lipid-adjusted) observed in high PCBs group (49.1±41.5), followed by medium PCBs group (13.5±7.5) and low PCBs group (10.2±7.1) (Fig. 2). These data are presented in Table 2.

Socio-demographic and Immune Marker-related Characteristics of Different PCBs Classes

There were no statistically significant differences in the composition ratios of the 3 PCBs group for gender, smoking, average daily alcohol consumption, physical activity, and chronic bronchitis (all $p>0.05$). In contrast, the differences in age, percentage of race, household income and poverty rate, insurance, education level, marital status, and BMI were statistically significant (all $p<0.05$). The ratio of comorbidities including cardiovascular disease, stroke, cancer, emphysema, hypertension, and diabetes were significantly higher in the high PCBs group than in the low PCBs group ($p<0.01$) and medium PCBs groups ($p<0.01$). Among circulatory immune markers, total leukocyte count, absolute lymphocyte count, absolute neutrophil count, NLR, MLR, and SII are significantly lower in high and medium PCBs group than in low PCBs group ($p<0.05$) (Table 3).

Association of PCBs Classes with Immune Markers in Multivariate Regression

To explore the relationship between PCBs and immune markers, we perform multivariate regression using PCBs classes as the dependent variable and immune markers as independent variables.

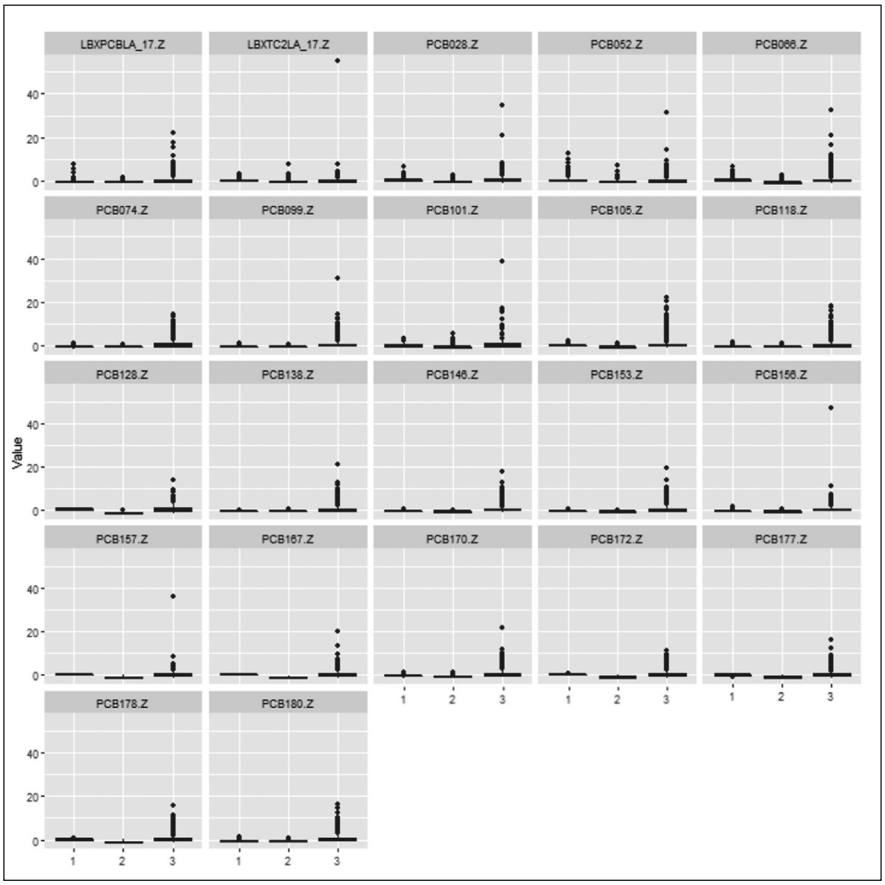

Fig. 2. *Lipid adjusted PCBs levels of 3 classes.*

Table 2. *Characteristics of each class of PCBs*

PCBs (ng/g)	Class 1 (n = 729) Mean (SD)	Class 2 (n = 917) Mean (SD)	Class 3 (n = 1,463) Mean (SD)	p-value
PCB28 lipid adj	10.7 (2.9)	5.4 (2.9)	10.2 (5.5)	< 0.001
PCB52 lipid adj	6.6 (4.3)	3.7 (2.3)	5.6 (4.1)	< 0.001
PCB66 lipid adj	5.4 (1.7)	1.9 (1.4)	5.0 (3.8)	< 0.001
PCB74 lipid adj	5.4 (2.8)	3.5 (2.2)	16.5 (15.7)	< 0.001
PCB99 lipid adj	4.7 (1.8)	3.1 (1.7)	12.3 (13.6)	< 0.001
PCB101 lipid adj	5.7 (2.2)	2.3 (1.6)	5.9 (5.4)	< 0.001
PCB105 lipid adj	4.0 (0.5)	1.3 (1.0)	5.6 (5.3)	< 0.001
PCB118 lipid adj	6.0 (3.8)	4.3 (2.8)	21.6 (25.0)	< 0.001
PCB128 lipid adj	4.0 (0.4)	0.7 (1.1)	3.3 (2.2)	< 0.001
PCB138 and 158 lipid adj	13.5 (7.5)	10.2 (7.1)	49.1 (41.5)	< 0.001
PCB146 lipid adj	4.0 (0.6)	1.8 (1.2)	9.2 (7.9)	< 0.001
PCB153 lipid adj	19.0 (11.6)	13.5 (9.8)	69.4 (55.3)	< 0.001
PCB156 lipid adj	4.3 (1.3)	2.2 (1.9)	10.4 (7.3)	< 0.001
PCB157 lipid adj	4.0 (0.4)	1.0 (1.1)	4.1 (1.8)	< 0.001
PCB167 lipid adj	4.0 (0.4)	0.9 (1.1)	4.3 (2.3)	< 0.001
PCB170 lipid adj	6.5 (3.7)	4.2 (3.6)	20.0 (14.8)	< 0.001
PCB172 lipid adj	4.0 (0.4)	1.0 (1.1)	4.6 (2.6)	< 0.001
PCB177 lipid adj	4.0 (0.5)	1.3 (1.1)	5.3 (3.6)	< 0.001
PCB178 lipid adj	4.0 (0.4)	1.2 (1.1)	5.1 (3.0)	< 0.001
PCB180 lipid adj	13.4 (11.1)	10.8 (10.3)	51.7 (42.3)	< 0.001
PCB183 lipid adj	4.0 (0.4)	1.5 (1.1)	6.2 (4.2)	< 0.001
PCB187 lipid adj	5.0 (2.2)	3.2 (2.6)	16.7 (15.8)	< 0.001

Table 3. *Comparison of variables and circulating immune markers based on the three latent classes of PCBs*

Variables	Medium (n = 729) n (%)	Low (n = 917) n (%)	High (n = 1,463) n (%)	p-value
Demographic parameters				
Age (years), mean (SD)	31.3 (15.6)	31.9 (15.4)	52.0 (21.6)	< 0.001
Sex				
Male	347 (47.6)	437 (47.7)	707 (48.3)	0.928
Female	382 (52.4)	480 (52.3)	756 (51.7)	
Race/ethnicity				
Mexican American	237 (32.5)	278 (30.3)	317 (21.7)	< 0.001
Other Hispanic	39 (5.3)	41 (4.5)	55 (3.8)	
Non-Hispanic White	285 (39.1)	388 (42.3)	723 (49.4)	
Non-Hispanic Black	140 (19.2)	168 (18.3)	315 (21.5)	
Other race, including multi-racial	28 (3.8)	42 (4.6)	53 (3.6)	
Socioeconomic status				
Education level				
Less than 9th grade	74 (10.2%)	91 (9.9%)	214 (14.6%)	< 0.001
9–12th grade or equivalent	396 (54.3%)	477 (52.0%)	701 (47.9%)	
Some college or above	259 (35.5%)	349 (38.1%)	548 (37.5%)	
Family income-to-poverty ratio, mean (SD)	2.4 (1.6)	2.3 (1.6)	2.5 (1.6)	0.001

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Insurance				
Insured	532 (73.0)	678 (73.9)	1,231 (84.1)	<0.001
Uninsured	197 (27.0)	239 (26.1)	232 (15.9)	
Marital status				
Married/cohabited	317 (43.5)	431 (47.0)	789 (53.9)	<0.001
Widowed	18 (2.5)	20 (2.2)	184 (12.6)	
Divorced/separated	52 (7.1)	61 (6.7)	147 (10.0)	
Unmarried	342 (46.9)	405 (44.2)	343 (23.4)	
Anthropometry				
BMI, mean (SD)	26.7 (6.3)	27.8 (6.6)	27.7 (6.3)	0.001
Lifestyle indicators				
Smoking status				
Ever-smoker	183 (25.1)	238 (26.0)	392 (26.8)	0.192
Non-smoker	394 (54.0)	449 (49.0)	745 (50.9)	
Current smoker	152 (20.9)	230 (25.1)	326 (22.3)	
Average drinks per day*	0.0 (0.0–0.3)	0.0 (0.0–0.3)	0.0 (0.0–0.3)	0.708
Physical activity				
Non-active	312 (42.8)	382 (41.7)	621 (42.4)	0.886
Active	417 (57.2)	535 (58.3)	842 (57.6)	
Comorbidities				
Cardiovascular disease	7 (1.4)	9 (1.4)	79 (6.4)	<0.001
Stroke	11 (2.2)	9 (1.4)	47 (3.8)	0.009
Cancer	22 (4.5)	18 (2.8)	158 (12.7)	<0.001
Emphysema	3 (0.6)	8 (1.2)	27 (2.2)	0.046
Chronic bronchitis	26 (5.3)	40 (6.3)	80 (6.4)	0.674
Hypertension	86 (12.7)	153 (17.6)	534 (37.6)	<0.001
Diabetes	21 (2.9)	34 (3.7)	182 (12.4)	<0.001
Circulating immune markers, mean (SD)				
Total leukocyte count (1,000 cell/uL)	7.3 (2.0)	7.7 (2.4)	7.1 (2.1)	<0.001
Absolute lymphocyte count (1,000 cell/uL)	2.1 (0.7)	2.2 (0.7)	2.1 (0.7)	<0.001
Absolute neutrophil count (1,000 cell/uL)	4.3 (1.7)	4.7 (2.0)	4.2 (1.7)	<0.001
Absolute monocyte count (1,000 cell/uL)	0.6 (0.2)	0.6 (0.2)	0.6 (0.2)	0.641
Platelet count (1,000 cells/uL)	270.3 (63.1)	272.9 (67.0)	263.4 (71.9)	0.003
Systemic immune-inflammation index	588.9 (342.4)	642.0 (382.8)	602.9 (458.4)	0.020
Neutrophil-lymphocyte ratio	2.2 (1.1)	2.4 (1.3)	2.3 (1.2)	0.013
Monocyte-lymphocyte ratio	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	<0.001
Platelet-lymphocyte ratio	135.8 (49.8)	135.3 (47.9)	141.3 (60.8)	0.013

In the unadjusted model, low PCBs and high PCBs class were positively correlated with both SII and NLR, and high PCBs was positively correlated with both PLR and MLR. After adjusting for age, sex, race, social security, economics, marital status, activity, smoking status, and BMI in model 1, low PCBs class remained positively associated with SII and NLR. In model 2, all positive correlations disappeared after adjusting for the same factors as model 1 plus comorbidities including hypertension, diabetes, cancer, stroke, cardiovascular disease, heart failure, hyperlipidaemia, emphysema, and chronic bronchitis (Table 4).

Associations of PCB Classification with Immune Markers in WQS Models

Given the significant differences we observed among circulating immune markers across different PCB classes groups, we used the WQS regression to minimize the incorrectly grouping and estimate weights of each variable for the WQS regression index for immune markers. It was shown that a significant negative correlation of PCBs classification was with both SII ($\beta = -14.513$, $p = 0.047$) and NLR ($\beta = -0.035$, $p = 0.017$) (Table 5). Of all PCBs, LBX028LA contributed most to associations with

Table 4. *Associations of PCB classification with immune markers in unadjusted and multivariate regression*

Variables	Unadjusted		Model 1		Model 2	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
SII						
Medium	Reference		Reference		Reference	
Low	43.33 (18.03, 68.64)	0.001	46.64 (7.00, 86.28)	0.021	51.43 (−23.57, 126.43)	0.179
High	23.38 (0.64, 46.11)	0.044	13.23 (−26.58, 53.05)	0.515	−1.55 (−73.24, 70.14)	0.966
NLR						
Medium	Reference		Reference		Reference	
Low	0.18 (0.10, 0.25)	< 0.001	0.16 (0.05, 0.28)	0.006	0.16 (−0.05, 0.37)	0.137
High	0.19 (0.11, 0.26)	< 0.001	0.03 (−0.09, 0.15)	0.590	−0.04 (−0.24, 0.16)	0.721
PLR						
Medium	Reference		Reference		Reference	
Low	−0.89 (−4.38, 2.59)	0.615	0.00 (−5.29, 5.30)	0.999	2.44 (−6.82, 11.70)	0.606
High	3.54 (0.41, 6.67)	0.027	1.64 (−3.68, 6.96)	0.545	0.92 (−7.93, 9.77)	0.838
MLR						
Medium	Reference		Reference		Reference	
Low	−0.01 (−0.01, 0.00)	0.146	−0.01 (−0.02–0.01)	0.260	−0.00 (−0.02, 0.02)	0.904
High	0.02 (0.02, 0.03)	< 0.001	0.00 (−0.01–0.01)	0.762	−0.00 (−0.03, 0.01)	0.473

Model 1 was adjusted for age, sex, race, insurance, education level, family income to poverty ratio, marital status, physical activity, cigarettes smoking, alcohol consumption, and BMI; Model 2 was adjusted for age, sex, race, insurance, education level, family income to poverty ratio, marital status, physical activity, smoking status, alcohol consumption, BMI, hypertension, diabetes, cancer, stroke, cardiovascular disease, heart failure, hyperlipidaemia, emphysema, and chronic bronchitis using appropriate sampling weights

Table 5. *Associations of PCB classification with immune markers in WQS models*

Variables	Model 1		Model 2	
	β (95% CI)	p-value	β (95% CI)	p-value
SII	−14.513 (−28.832, −0.193)	0.047	−9.301 (−22.791, 4.190)	0.177
NLR	−0.035 (−0.065, −0.006)	0.017	−0.022 (−0.072, 0.028)	0.393
PLR	−0.384 (−1.891, 1.122)	0.617	0.559 (−1.420, 2.538)	0.560
MLR	0.002 (−0.001, 0.004)	0.272	0.002 (−0.003, 0.007)	0.433

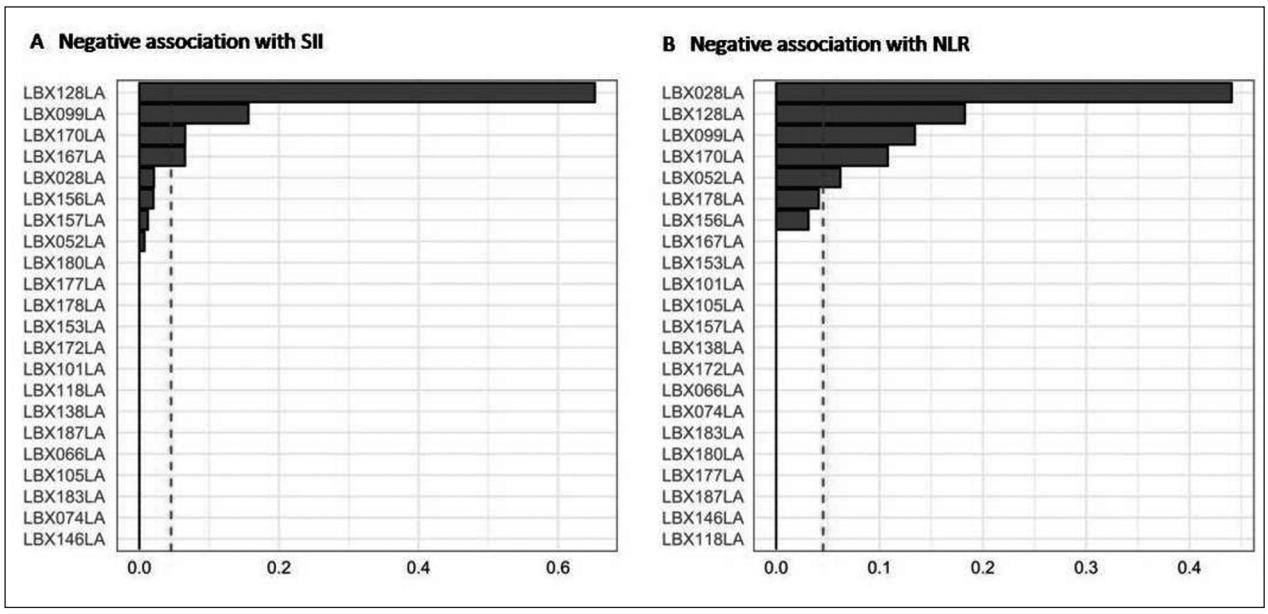

Fig. 3. *Estimated weights of PCBs for weighted quantile sum (WQS).*

SII, followed by LBX0128LA, LBX099LA and LBX170LA. And for NLR, LBX128LA contributed most to associations, followed by LBX099LA, LBX170LA and LBX167LA (Fig. 3). No correlation of WQS index with PLR or MLR was observed. However, except for socioeconomic and demographic variables, after adjusting for comorbidities including hypertension, diabetes, cancer, stroke, cardiovascular disease, heart failure, hyperlipidaemia, emphysema, and chronic bronchitis in multivariate regressions, no correlation of PCB classification with any immune markers was observed.

DISCUSSION

PCBs, as commonly used materials in industry and chemistry, were found to be toxic to human body. In our cross-sectional study, using NHANES data from 1999 to 2004, concentration of PCBs from participants were identified as in low, medium and high groups by LCA. The high PCBs exposure group in the general population had a higher rate of co-morbidities, while total leukocyte count, absolute lymphocyte count, absolute neutrophil count, NLR, NLR, and SII were significantly lower than in the low PCB exposure group. Besides, in multivariate regression model, after adjusting with socioeconomic and demographic variables, low PCBs class was positively associated with both SII and NLR, which suggested that higher PCBs exposed people are more susceptible to immune cells inhibition in general population. The mix effect of PCB exposure to immune markers was inferred from WQS model and it was shown that PCBs was significantly negatively associated with both SII ($\beta = -14.513$, $p = 0.047$) and NLR, among which, LBX028LA contributed most to associations with SII and LBX128LA contributed most to associations with NLR.

The International Agency for Research on Cancer (IARC) lists PCBs as known human carcinogens (21). Lots of studies have shown that single PCBs exerted hazards on animal and human through multiple molecular level (13, 22, 23). However, in reality, exposure to these chemicals nearly always occurs as mixtures rather than individual PCB congeners. As a result, it appears to be more meaningful to explore the hazards of PCB mixture on human body. To achieve this, the latent class analysis of PCBs exposure and regression models were adopted to investigate the mixed effects of PCBs on circulating immune markers. Consistent with previously reported studies (14), we found that high PCBs exposure was predominantly found in older individuals. The age difference may reflect the bioaccumulation of these chemicals over the years in older individuals. PCB congeners have been shown to be associated with increased risk of adult NHL, diabetes, cardiovascular disease, and stroke in clinical studies (24–26). The present study also showed a higher prevalence of co-morbidities in the high PCBs group, which could be attributed to PCB exposure. Of particular interest was our report indicating that the prevalence of emphysema was significantly different in populations with different PCBs exposures, which was rarely reported recently. And all these co-morbidities were associated with inflammation.

The neutrophil-to-lymphocyte ratio, a promising inflammatory indicator that combined neutrophil and lymphocyte, is widely studied in diseases and comprehensively considered as an indicator of inflammatory status of human body (27). It was reported that NLR value in general population ranged from 0.78 to 3.53 (28). In this study, the normal range of NLR in general population with PCB exposure was 2.3 ± 1.2, which was not significantly different from the normal population. However, high PCBs class group has a lower NLR than low PCBs class group, suggesting that immune cells were likely to be inhibited in people with high PCBs exposure from general population. Presently, it was still not clear how lower or higher NLR impact on general population. The vast majority of current reports on NLR were studied in patients with diseases that suggested excessively high or low NLR is abnormal. Researches on different cancers reveal different roles of NLR in prognosis. High NLR values in lung and brain tumours was a typical feature and may represent an immunosuppressive phenotype attributable to neutrophils rather than lymphocytes (29, 30). Yet in advanced gastric cancer, patients with low NLR had better disease-free survival and overall survival (31). Considering that high PCBs group has a higher prevalence of cancer for unknown reasons, we suggest that further research is still needed on the physio-pathological mechanisms and diagnostic value of NLR in the general population exposed to PCBs. Besides NLR, SII is another parameter that integrates lymphocytes, neutrophils and platelets for evaluating system inflammatory status. Retrospective studies on inflammatory diseases had shown that SII was associated with severity and activity of diseases, and in acute pancreatitis SII was more sensitive to predict the severity than NLR and PLR (32). Long-term elevation of SII is also an independent risk factor for developing a solid cancer (33). At present, rare studies have reported the immune inflammation in the general population after exposure to PCBs. With the understanding that no model is considered inherently superior (34), we would rather explore the association of PCBs with circulating immune markers using multivariate regression and WQS. In our study, low PCBs class was found to be positively associated with SII in multivariate regression model after adjusting for socio-demographic variables, which could be inferred that exposure to low doses of PCBs may activate the moderate inflammatory response. In WQS model, we found that LBX128LA contributed most significantly for the negative correlation between PCBs and SII, which had the highest weight among all PCB chemicals. However, unfortunately, after adjusting for socio-demographic and comorbidities variables both in multivariate regression and WQS model, correlations disappeared. It was not clear whether PCBs are indeed not correlated with circulating immune markers or whether this was due to confounding of concurrent diseases as tumours, diabetes, hypertension, etc., which are themselves presented with elevated circulating immune markers (33, 35, 36).

This study has some limitations. Firstly, this is a cross-sectional study which limits the inference of a causal relationship between PCBs and circulating immune markers. Secondly, the conclusions we obtained were from statistical analysis and need to be verified by laboratory and other evidence. Although this study focused on the association of PCBs with circulating immune markers using regression analysis after LCA and obtained meaningful results, it is not necessarily the most optimal approach.

CONCLUSION

In summary, our present study showed that exposure to PCBs in the general population could be categorized into three class:

low, medium and high, each with significantly different demographic socioeconomic characteristics, and comorbidities ratio. In a multivariate regression analysis, PCBs in the lower levels were positively associated with SII and NLR after adjusting for socioeconomic and demographic variables. And specific PCBs that contributed most to the association with SII and NLR were identified as LBX128LA and LBX028LA, respectively. These associations were noteworthy as it implies activation or inhibition of circulatory immune cells may be associated with different PCBs mixture level.

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Conflicts of Interest

None declared

Authors' Contributions

All authors contributed to the study conception and design; MY, LW, LY – material preparation, data collection and analysis; MY, LW, LY contributed equally to this work and share the first authorship; CY, HK – the first draft of the manuscript. All authors read and approved the final manuscript.

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