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# Dose–response relationships between polycyclic aromatic hydrocarbon exposure and blood cell counts among coke oven workers: a sex-stratified analysis

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## ABSTRACT

**Objectives** To explore sex differences and dose–response relationships between nine urinary polycyclic aromatic hydrocarbon (PAH) metabolites and neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR) and complete blood counts among coke oven workers.

**Design and setting** A cross-sectional study with stratified sex was conducted in Shanxi, China.

**Participants** A total of 458 male workers and 226 female workers were selected.

**Primary and secondary outcome measures** General linear models, p values for trend tests and natural cubic spline models were used to explore the dose–response relationships between nine urinary PAH metabolites and NLR, PLR and complete blood counts.

**Result** Compared with male workers, female workers had lower exposure level of PAH (0.95 ng/mL vs 1.38 ng/mL). Only among female workers did we observe that a 1-unit increase in lg(1-OHPyr) was related to a 0.149 (95% CI: 0.055 to 0.242; p for trend=0.041) and 0.103 (95% CI: 0.025 to 0.181; p for trend=0.007) increase in lg(NLR) and lg(PLR), and a 0.116 (95% CI: −0.179 to −0.054; p for trend=0.007) decrease in lg(lymphocyte counts (LYMs)). A 1-unit increase in lg(2-OHNap) was related to a 0.045 (95% CI: 0.003 to 0.086; p for trend=0.037) increase in lg(PLR) and a 0.029 (95% CI: −0.056 to −0.002; p for trend=0.030) and 0.016 (95% CI: −0.029 to −0.003; p for trend=0.010) decrease in lg(white blood cell counts (WBCs)) and lg(haemoglobin (HGB)).

**Conclusion** Female workers' NLR, PLR, WBCs, HGB and LYMs may be more susceptible than those of male workers when affected by PAH.

## Strengths and limitations of this study

► We conducted a sex stratification analysis of coke oven workers and found sex differences in the exposure to polycyclic aromatic hydrocarbon (PAH).

► Eleven urinary monohydroxylated PAH (OH-PAH) in worker urine samples were measured using high-performance liquid chromatography with tandem mass spectrometry.

► The neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) were a balance of inflammation and immunity, so it is more stable than single blood cells when affected by PAH.

► Natural cubic spline model and p values for trend test were used to assess shapes of dose–response relationships between urinary OH-PAH levels and NLR, PLR and complete blood counts.

► This was a cross-sectional study and cannot prove causal relationship.

## INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH) are organic micropollutants, produced by incomplete combustion of fossil fuels and carbon-containing organic matter.[1] PAH commonly exist in the environment and can enter the human body through inhalation, ingestion and dermal contact.[2 3] Coke oven workers are a typical population with long-term exposure to PAH.[4] Long-term exposure to mixtures of

PAH can exert multiple health effects, such as increased risk of oxidative stress, cardiovascular disease and cancers.[5–9] In particular, PAH are highly lipid soluble and thus readily absorbed by the body, and all kinds of blood cells in human peripheral blood are vulnerable to PAH exposure. Therefore, changes in blood cells may reflect the toxic effects of PAH. Although there have been studies on PAH and blood cells, in most studies only male workers were recruited, or they did not perform a sex stratification analysis. As we know, sex plays a significant role in blood cells. There were demonstrations of sex differences in air pollution (including PAH) exposure-related adverse influences, indicating that females were more susceptible.[10–17] Thus, exploring the sex difference in PAH exposure and blood cell changes will be possible to explain sex difference mechanisms for cancer, and cardiovascular and cerebrovascular diseases triggered by air pollution.

The blood cells obtained from routine blood tests can be used to calculate the neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR). The NLR and PLR emerged as new indicators that reflect the degree of inflammation and oxidative stress.[18–23] Neutrophil counts (NEUTs) represent a subclinical inflammation stage.[24] Conversely, reduced lymphocyte counts (LYMs) reflect physiological stress that is related to the immune system.[25] Platelet counts (PLTs) have been suggested to be markers of inflammatory and immune responses.[26 27] Thus, the NLR and PLR are balances of inflammation and stress responses, and are more stable than single blood cells.[19] Studies have found that PAH exposure is associated with PLR among children and the general population, but they did not perform a sex stratification analysis.[27 28] Although we are unaware of studies exploring the effect of PAH exposure on NLR, a study found that an increased NLR was related to smoking status.[29] They did not perform a sex stratification analysis either. Therefore, the effects of PAH on the NLR and PLR in both males and females need to be further explored.

In addition, the association and dose–response relationships between PAH exposure and blood cells alone were inconsistent in previous studies. Epidemiological studies have found that PAH exposure was related to a decrease in white blood cell counts (WBCs) among coke oven workers or male workers.[30 31] Other studies found that PAH exposure was related to an increase in WBCs among the general population and was more evident among males.[32 33] Female rats exposed to PAH had markedly increased WBC counts.[34] In addition, studies have shown no associations between urinary PAH metabolites or benzo[a]pyrene and WBCs among the general population and in rats.[27 35 36] In addition, when PAH levels were higher, red blood cell counts (RBCs) and haemoglobin (HGB) levels were lower in male workers and female rats, while no relationship was found between them only in males.[31 32 34]

In this context, we further determined sex differences and relationships between PAH exposure and NLR, PLR and complete blood counts. Both in all participants and stratified by sex, we examined the association of nine urinary monohydroxylated PAH (OH-PAH) with NLR, PLR and complete blood counts and further explored their dose–response relationships.

## SUBJECTS AND METHODS
### Study population
All subjects in this study were recruited from a coking plant located in Shanxi, China. This was a cross-sectional survey, and the duration of the survey was 7 days. A total of 776 coke oven workers were investigated in this study, but 76 participants were excluded due to a lack of urine samples. Seventeen participants were excluded due to a lack of blood indicators. Then 684 participants were included in the final analysis. The present study protocol was approved by the Ethical Committee of Shanxi Medical University, and informed consent was obtained from all study participants prior to the study.

### Personal interview questionnaire
A questionnaire designed by our research group and practised before investigation was used to obtain the subjects' sociodemographic characteristics during their working period. For example, we recorded age, sex, nationality, occupational history, length of service, smoking and drinking habits, frequency of eating fried and coal-baked foods, histories of personal and family diseases, symptoms and medication used, and others. Smoking was defined as currently smoking no less than one cigarette per day over the last 6 months, and drinking was defined as currently drinking wine, beer or spirits no less than three times a week for the last 6 months. A total of 684 exposed workers completed the questionnaire and were included in the study.

### Biological sample collection and analysis
All participants were asked to deliver their urine samples using special urine containers. The urinary samples were transported to the laboratory immediately, and urinary gravity was measured at first. After that, the urinary samples were repackaged into 5 mL cryogenic vials and then stored at −80°C until chemical analyses. Urine samples were measured for a suite of PAH metabolites: 2-hydroxynaphthalene (2-OHNap), 1-hydroxynaphthalene (1-OHNap), 3-hydroxyfluorene (3-OHFlu), 2-hydroxyfluorene (2-OHFlu), 9-hydroxyphenanthrene (9-OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 1-hydroxyphenanthrene (1-OHPhe), 1-hydroxypyrene (1-OHPyr), 3-hydroxychrysene (3-OHChr), 6-hydroxychrysene (6-OHChr) and 9-hydroxybenzo(a)pyrene (9-OHBap), by high-performance liquid chromatography (HPLC) with tandem mass spectrometry (Shimadzu, Kyoto, Japan).[37] The linearity (expressed as $R^2$), limit of detection (LOD), precision (expressed as relative standard deviation) and mean recovery rate were 0.9930–0.9998, 0.02–0.094 ng/mL, 2.7%–11.6% and 71.4%–109.4%, respectively. Reagent blanks and quality control samples along with urine samples were analysed for the accuracy and comparability of the measure results. HPLC separation conditions and mass spectrometry detection programmes were described.[38 39]

### Blood indicators
Blood indicators (including WBCs, RBCs, HGB, PLTs, NEUTs, LYMs) were derived from participants' routine blood examination using a fully automated haematology analyser. The NLR and PLR were calculated simultaneously.

### Statistical analysis
Continuous variables were expressed as mean±SD or median and IQR. T-test and Wilcoxon rank-sum test were used to compare distribution differences of categorical data (age, WBCs, RBCs, HGB, PLTs, NEUTs, LYMs, NLR, PLR, 2-OHNap, 1-OHNap, 3-OHFlu, 2-OHFlu, 2-OHPhe, 9-OHPhe, 1-OHPhe, 1-OHPyr, 9-OHBap). The categorical data were expressed by actual frequencies and percentages.

**Table 1** Baseline characteristics of participants

| Variables | All (n=684) | Male (n=458) | Female (n=226) | P value |
|---|---|---|---|---|
| Basic information | | | | |
| Age, year | 39.52±9.71 | 40.41±10.36 | 37.70±7.96 | **0.003** |
| Education, n | | | | |
| Middle school and below | 155 (27.9) | 112 (30.2) | 43 (22.7) | 0.180 |
| High school | 161 (25.3) | 110 (25.3) | 51 (25.1) | |
| College and above | 368 (46.9) | 236 (44.4) | 132 (52.2) | |
| BMI, Kg/m$^2$ | | | | |
| 0–24 | 290 (42.0) | 198 (42.5) | 92 (41.0) | 0.803 |
| 24–28 | 279 (41.0) | 185 (40.9) | 94 (41.2) | |
| ≥28 | 115 (17.0) | 75 (16.6) | 40 (17.8) | |
| Exercise, n | | | | |
| Never | 219 (31.8) | 136 (29.9) | 83 (35.8) | 0.117 |
| Occasionally | 276 (38) | 187 (38.2) | 89 (37.6) | |
| Often | 187 (30) | 134 (31.7) | 53 (26.2) | |
| Smoking, n | | | | |
| Never | 371 (52.5) | 146 (30.9) | 225 (99.5) | <0.001 |
| Current | 282 (42.4) | 281 (61.7) | 1 (0.5) | |
| Former | 31 (5.0) | 31 (7.4) | _ | |
| Drinking, n | | | | |
| Never | 483 (68.2) | 265 (55.3) | 218 (96.1) | <0.001 |
| Current | 187 (29.3) | 179 (41) | 8 (3.9) | |
| Former | 13 (2.3) | 13 (3.4) | _ | |
| Blood parameters | | | | |
| WBC, $10^9$ L | 5.69 (4.84–6.69) | 5.93 (5.01–6.93) | 5.26 (4.48–6.24) | <0.001 |
| RBC, $10^{12}$ L | 4.90 (4.50–5.10) | 5.00 (4.80–5.20) | 4.40 (4.20–4.60) | <0.001 |
| HGB, g/L | 151.00 (138.00–159.00) | 156.00 (151.00–162.00) | 133 (125.00–139.00) | <0.001 |
| PLT, $10^9$ L | 199.00 (171.00–231.50) | 191.00 (166.00–220.00) | 220.50 (186.00–254.00) | <0.001 |
| NEUT, $10^9$ L | 3.23 (2.60–4.03) | 3.35 (2.63–4.12) | 3.15 (2.49–3.83) | **0.006** |
| LYM, $10^9$ L | 1.83 (1.55–2.22) | 1.90 (1.60–2.33) | 1.66 (1.44–2.02) | <0.001 |
| NLR, % | 1.77 (1.37–2.23) | 1.74 (1.36–2.22) | 1.86 (1.38–2.28) | 0.202 |
| PLR, % | 107.22 (86.39–133.98) | 97.43 (80.93–123.33) | 123.85 (102.72–156.76) | <0.001 |

P values <0.05 are marked in bold.
Data are presented as mean±SD, N (%) or median (IQR).
BMI, body mass index; HGB, haemoglobin; LYM, lymphocyte count; NEUT, neutrophil count; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; PLT, platelet count; RBC, red blood cell; WBC, white blood cell.

The $\chi^2$ test was used to compare distribution differences of categorical variables (including body mass index (BMI), education level, exercise status, smoking status and drinking status). Covariates in the general linear model were selected based on previous literature and univariate analysis results. Covariates including age, BMI, education level, smoking status, drinking status and exercise status were selected. To improve normality, urinary OH-PAH, NLR, PLR and complete blood counts were log-transformed based on 10 before further analysis due to skewed distributions. The rate of 3-OHChr and 6-OHChr in urine was less than 50%; therefore, the two urinary PAH metabolites were not included in our final analysis. For other PAH metabolites,

the concentrations of PAH metabolites below the LOD were assigned a value corresponding to one-half the LOD. General linear models were used to estimate the associations between X (urinary OH-PAH) and Y (NLR, PLR, complete blood counts) among coke oven workers. The obtained β coefficient (95% CI) indicated that a unit increase in X was associated with an average unit increase in Y. The multicollinearity diagnosis was made using the variance expansion factor (VIF) method (online supplemental table S1), and sensitivity analysis was conducted (online supplemental table S2). The p value for trend test and the natural cubic spline model were used to assess dose–response relationships between urinary OH-PAH levels and NLR, PLR and complete blood counts.

**Table 2** Urinary-specific gravity-corrected concentrations of the 11 PAH metabolites (ng/mL)

| PAH metabolites | Percent detection | Median (IQR) | Range |
|---|---|---|---|
| 2-OHNap | 99.57 | 0.29 (0.13–0.69) | 0.00–29.28 |
| 1-OHNap | 77.29 | 0.03 (0.01–0.06) | 0.00–5.88 |
| 3-OHFlu | 85.57 | 0.03 (0.01–0.06) | 0.00–0.55 |
| 2-OHFlu | 96.00 | 0.15 (0.07–0.29) | 0.00–2.82 |
| 2-OHPhe | 100.00 | 0.20 (0.12–0.32) | 0.02–3.67 |
| 9-OHPhe | 99.43 | 0.11 (0.07–0.18) | 0.00–6.95 |
| 1-OHPhe | 89.71 | 0.06 (0.03–0.11) | 0.00–1.44 |
| 1-OHPyr | 99.86 | 0.09 (0.06–0.17) | 0.01–6.68 |
| 3-OHChr | 41.57 | 0.01 (0.00–0.03) | 0.00–0.15 |
| 6-OHChr | 26.00 | 0.00 (0.00–0.01) | 0.00–0.11 |
| 9-OHBap | 50.71 | 0.01 (0.00–0.03) | 0.00–0.49 |

PAH, polycyclic aromatic hydrocarbon.

**Table 3** Sex comparisons of PAH metabolites' median and IQR (ng/mL)

| PAH metabolites | Male (n=458) | Female (n=226) | P value |
|---|---|---|---|
| ΣOHPAH | 1.38 (0.85–2.01) | 0.95 (0.63–1.63) | <0.001 |
| 2-OHNap | 0.27 (0.13–0.64) | 0.32 (0.13–0.72) | 0.495 |
| 1-OHNap | 0.03 (0.01–0.06) | 0.02 (0.01–0.05) | **0.022** |
| 3-OHFlu | 0.04 (0.02–0.07) | 0.02 (0.01–0.04) | <0.001 |
| 2-OHFlu | 0.18 (0.10–0.35) | 0.09 (0.04–0.17) | <0.001 |
| 2-OHPhe | 0.21 (0.13–0.37) | 0.17 (0.10–0.27) | <0.001 |
| 9-OHPhe | 0.13 (0.08–0.21) | 0.09 (0.06–0.14) | <0.001 |
| 1-OHPhe | 0.06 (0.04–0.12) | 0.05 (0.02–0.09) | **0.002** |
| 1-OHPyr | 0.10 (0.07–0.19) | 0.07 (0.04–0.12) | <0.001 |
| 9-OHBap | 0.01 (0.00–0.03) | 0.02 (0.00–0.04) | **0.001** |

P values <0.05 are marked in bold.
Data are presented as median (IQR).
PAH, polycyclic aromatic hydrocarbon.

The natural cubic spline with 3 df for PAH exposure term was used in the fully adjusted general linear model.[40 41] Linearity was tested by comparing the model fit of the linear and spline models using a $\chi^2$ test.[42] The data analysis software used was SAS V.9.4 and R V.4.0.1.

### Patient and public involvement statement

In this study, we collected data on coking plant workers. Coke plant workers who participated in the 2018 baseline survey and agreed to participate in the follow-up study will be selected as participants in this follow-up study. Workers who completely voluntarily fill in the informed consent form and provide blood and urine samples were involved in this study. Patients were not involved in the recruitment to and conduct of the study. The information provided by the workers will be kept confidential under scientific numbering and strict management systems. The urine and blood samples provided by the workers will be destroyed after the study. We will disseminate the results to study participants by phone. This study was a cross-sectional survey and not a randomised controlled trial, so there was no burden of the intervention.

### RESULTS
### Demographic characteristics of the participants

Table 1 presents the distribution characteristics of selected variables among eligible coke oven workers (n=684) in this study. There were 458 male workers and 226 female workers grouped by sex. For basic information, compared with the female workers, male workers were older and more likely to smoke and drink (p<0.05), but there were no significant differences in the distribution of education level, BMI status and exercise status between male and female workers(p>0.05). For blood parameters, compared with the female workers, male workers' WBC, RBC, HGB, NEUT and LYM were higher (p<0.05), and their PLT and PLR were lower, but there were no significant differences

in the distributions of NLR between male and female workers (p>0.05).

Table 2 presents the urinary gravity-corrected concentration distribution of 11 PAH metabolites as well as their LOD and detection rate. The median and IQR of PAH metabolites, including 2-OHNap, 1-OHNap, 3-OHFlu, 2-OHFlu, 2-OHPhe, 9-OHPhe, 1-OHPhe, 1-OHPyr, 3-OHChr, 6-OHChr and 9-OH Bap, were 0.29 (0.00–29.28) ng/mL, 0.03 (0.00–5.88) ng/mL, 0.03 (0.00–0.55) ng/mL, 0.15 (0.00–2.82) ng/mL, 0.20 (0.02–3.67) ng/mL, 0.11 (0.00–6.95) ng/mL, 0.06 (0.00–1.44) ng/mL, 0.09 (0.01–6.68) ng/mL, 0.01 (0.00–0.15) ng/mL and 0.00 (0.00–0.11) ng/mL, respectively. The median value of 2-OHNap was the highest (0.29 ng/mL) in this study. When stratified by sex, it was found that except for 2-OHNap, there were significant differences in the concentration distribution of PAH metabolites between male and female workers (table 3). Compared with the female workers, levels of male workers' 1-OHNap, 3-OHFlu, 2-OHFlu, 2-OHPhe,9-OHPhe, 1-OHPhe and 1-OHPyr were higher, and their 9-OHBap levels were lower.

### Dose–response relationships between PAH exposure and NLR and PLR before and after sex stratification

Figure 1 shows the results regarding general linear models between urinary OH-PAH and NLR and PLR both before and after sex stratification. They were considered stable results because both the generalised linear model and p value for trend test (figure 2) were statistically significant. No statistically significant result was found among all participants and male workers. Among female workers, a 1-unit increase in lg(2-OHNap) was related to a 0.045 (95% CI: 0.003 to 0.086; p for trend=0.037) increase in lg(PLR), and a 1-unit increase in lg(1-OHPyr) was related to a 0.149 (95% CI: 0.055 to 0.242; p for trend=0.041) and 0.103 (95% CI: 0.025 to 0.181; p for trend=0.007)
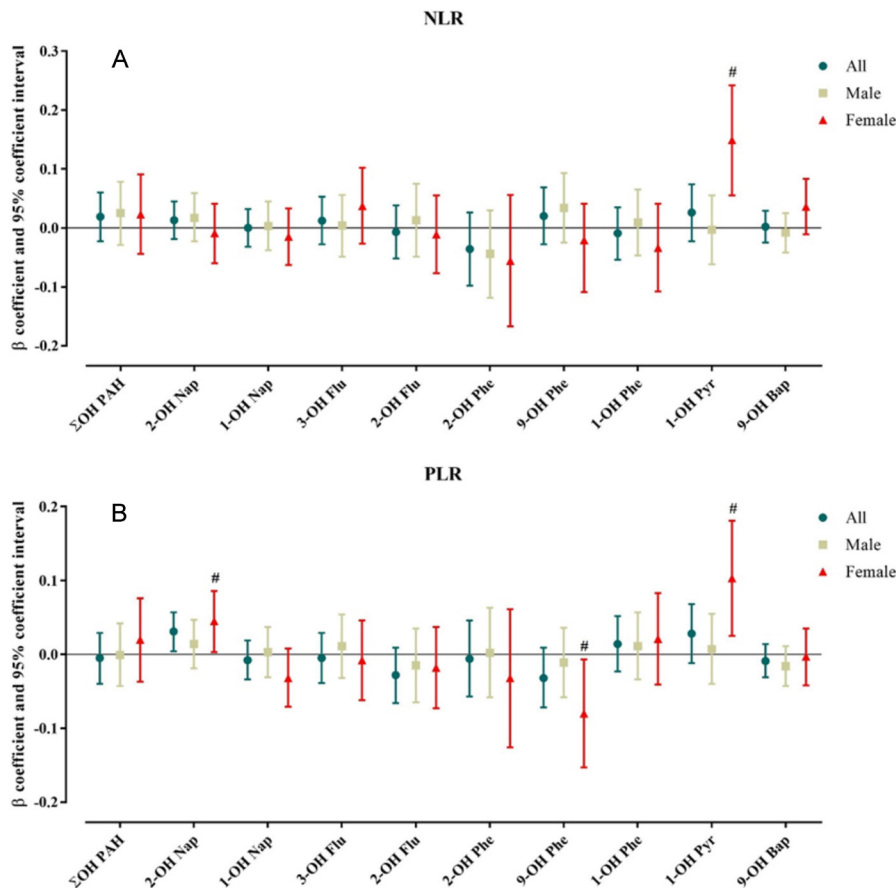
**Figure 1** Results regarding general linear models between changes in NLR, PLR and increase in PAH metabolites both among all participants, male workers and female workers after being adjusted for other eight PAH metabolites, age, education, body mass index, smoking status, drinking status and exercise status (sex was also adjusted among all participants). # represents both the results of generalised linear models and p value for trend test p<0.05. NLR, neutrophil–lymphocyte ratio; PAH, polycyclic aromatic hydrocarbon; PLR, platelet–lymphocyte ratio.

increase in lg(NLR) and lg(PLR), respectively. However, a 1-unit increase in lg(9-OHPhe) was related to a 0.080 (95% CI: −0.153 to −0.007; p for trend=0.007) decrease in lg(PLR). The natural cubic spline model also suggested that non-linear relationships existed between 1-OHPyr and PLR (adjusted model) (p=0.037), and the association between 1-OHPyr and NLR, 2-OHNap and PLR, and 9-OHPhe and PLR was linear (adjusted model) (both p>0.05) (figure 3).

**Dose–response relationships between PAH exposure and complete blood counts before and after sex stratification**

Figure 4 shows the results regarding general linear models between urinary OH-PAH and complete blood counts both before and after sex stratification. All participants' results of p value for trend test are shown in online supplemental figure 1. After being stratified by sex, the p value for trend test results are shown in online supplemental figures 2 and 3. They were considered stable results when both the generalised linear model and p value for trend test were statistically significant. Among all participants, a 1-unit increase in lg(9-OHPhe) was related to a 0.044 (95% CI: 0.018 to 0.071; p for trend=0.002) and 0.052 (95% CI: 0.015 to 0.090; p for trend=0.008) increase in

lg(WBCs) and lg(NEUTs). Among male workers, a 1-unit increase in lg(9-OHPhe) was related to a 0.046 (95% CI: 0.013 to 0.079; p for trend=0.001) and 0.059 (95% CI: 0.013 to 0.105; p for trend=0.001) increase in lg(WBCs) or lg(NEUT). Among female workers, a 1-unit increase in lg(2-OHNap) was related to a 0.029 (95% CI: −0.056 to −0.002; p for trend=0.030) and 0.016 (95% CI: −0.029 to −0.003; p for trend=0.010) decrease in lg(WBCs) or lg(HGB). A 1-unit increase in lg(1-OHPyr) was related to a 0.116 (95% CI: −0.179 to −0.054; p for trend=0.007) decrease in lg(LYMs).

The non-linear relationship between urinary OH-PAH and complete blood counts was checked using the natural cubic spline model. Among all participants, it was found that the results of the natural cubic spline model suggested that the association between 9-OHPhe and WBCs, and 9-OHPhe and NEUTs was linear (adjusted model) (both p>0.05) (online supplemental figure 4). Among male workers, it was found that the results of the natural cubic spline model suggested that non-linear relationships essentially existed between 9-OHPhe and NEUTs (adjusted model) (p=0.038), and the association between 9-OHPhe and WBCs was linear (both p>0.05)
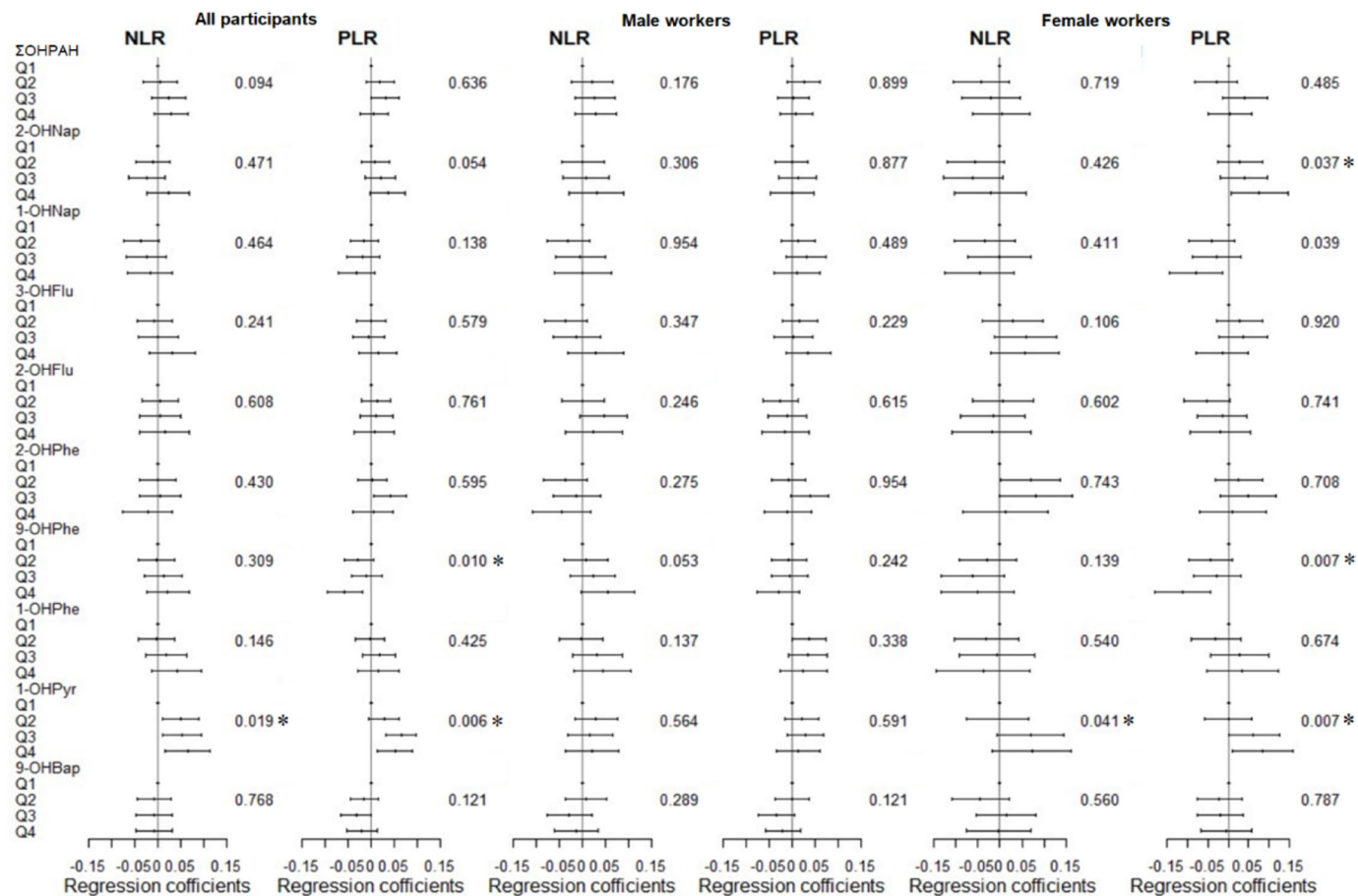
**Figure 2** The p value for trend test of NLR, PLR and increase in PAH metabolites both among all participants, male workers and female workers after being adjusted for other eight PAH metabolites, age, body mas index, smoking status, drinking status and exercise status (sex was also adjusted among all participants). * represents the results of p value for trend test p<0.05. NLR, neutrophil–lymphocyte ratio; PAH, polycyclic aromatic hydrocarbon; PLR, platelet–lymphocyte ratio.

(online supplemental figure 5). Among female workers, the results of the natural cubic spline model suggested that the association between 2-OHNap and WBCs, 2-OHNap and HGB, and 1-OHPyr and LYMs was linear (both p>0.05) (online supplemental figure 6).

## DISCUSSION

To our knowledge, this was the first study to explore sex differences in NLR, PLR and complete blood counts caused by PAH exposure among coke oven workers. It was found that NLR, PLR and LYMs were affected by 1-OHPyr; PLR, WBCs and HGB were affected by 2-OHNap; and PLR was affected by 9-OHPhe for female workers, who were more susceptible than male workers. Dose–response relationships mainly existed for 9-OHPhe and inflammation-related indicators WBCs and NEUTs among all participants and male workers.

Benzene exposure has potential haematotoxicity and leads to a decrease in blood cells.[43–45] However, PAH are hydrocarbons containing more than two benzene rings, and their haematotoxicity remains controversial. In addition to the effect of PAH on blood cells, sex has been known to have a significant role in blood cells. Before

the sex stratification analysis, there were no correlations between PAH and NLR, PLR, HGB or LYMs in this study. Thus, if the sex stratification analysis was not conducted or only male workers were recruited, the influence of PAH on the NLR, PLR and complete blood counts might be undervalued. This result implies that the sex difference was significant for PAH exposure.

It was found that most PAH metabolites had positive correlations in our study, so we controlled for other PAH metabolites when assessing the associations between a PAH metabolite and NLR, PLR, complete blood counts and multicollinearity diagnosis for both VIFs <3 (online supplemental table S1). In this study, the 2-OHNap and 1-OHPyr levels in female workers were not significantly higher than those of male workers (median value: 0.32 ng/mL vs 0.27 ng/mL; median value: 0.10 ng/mL vs 0.07 ng/mL), but were lower than those reported in other studies.[46] Thus, we believed that NLR, PLR, WBCs, HGB and LYMs for female workers were more susceptible than male workers to the effects of 2-OHNap and 1-OHPyr in this study. In addition, male workers had higher levels of urinary OH-PAH and 9-OHPhe than did female workers (median value: 1.38 ng/mL vs 0.95 ng/mL; 0.13 ng/
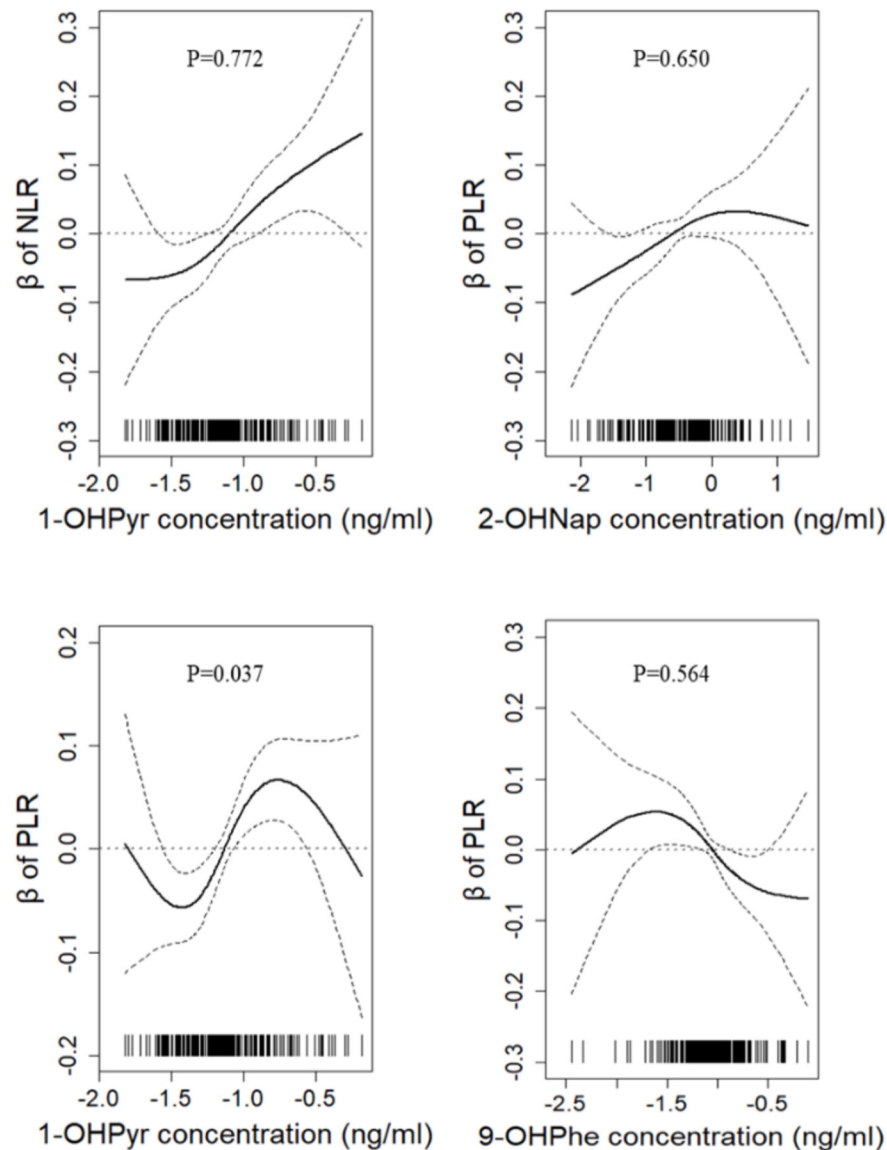
**Figure 3** Results regarding the natural cubic spline models between urinary OH-PAH levels and NLR and PLR in female workers after being adjusted for other eight PAH metabolites, age, education, body mass index, smoking status, drinking status and exercise status (p value was by comparing the fit of the spline model with a linear model). NLR, neutrophil–lymphocyte ratio; OH-PAH, monohydroxylated PAH; PAH, polycyclic aromatic hydrocarbon; PLR, platelet–lymphocyte ratio.

mL vs 0.09 ng/mL), and these values were lower than in other studies.[46] Thus, in this study the effect of 9-OHPhe on male workers may be more pronounced.

NLR and PLR are inexpensive and reproducible biomarkers of inflammation, oxidative stress and autoimmune connective tissue disorders.[17–22 47] Studies found PAH exposure was associated with increased PLR among general population.[26 27] In this study, among female workers, the effects of 1-OHPyr exposure were consistent with those of previous studies; however, 9-OHPhe was related to a decrease in the PLR. The root cause was the difference in the molecular interactions of 9-OHPhe and 1-OHPyr. A study found that a low concentration of 9-OHPhe could promote the activity of catalase, but

1-OHPyr inhibited the activity of catalase, which plays an important role in protecting cells from oxidative damage.[48] That study provides support for our findings. However, we are unaware of studies exploring the effect of PAH exposure on the NLR; nevertheless, a study suggested that smoking status was related to an increased NLR among the general population.[28] Tobacco smoke is also a source of PAH. In this study, the relationship between 1-OHPyr exposure and NLR in female workers was consistent with tobacco smoke exposure. It was found that 1-OHPyr was related to a decrease in LYMs, which explained the increase in NLR and PLR caused by 1-OHPyr in this study and indicated that PAH exposure mainly affected the physiological stress related to
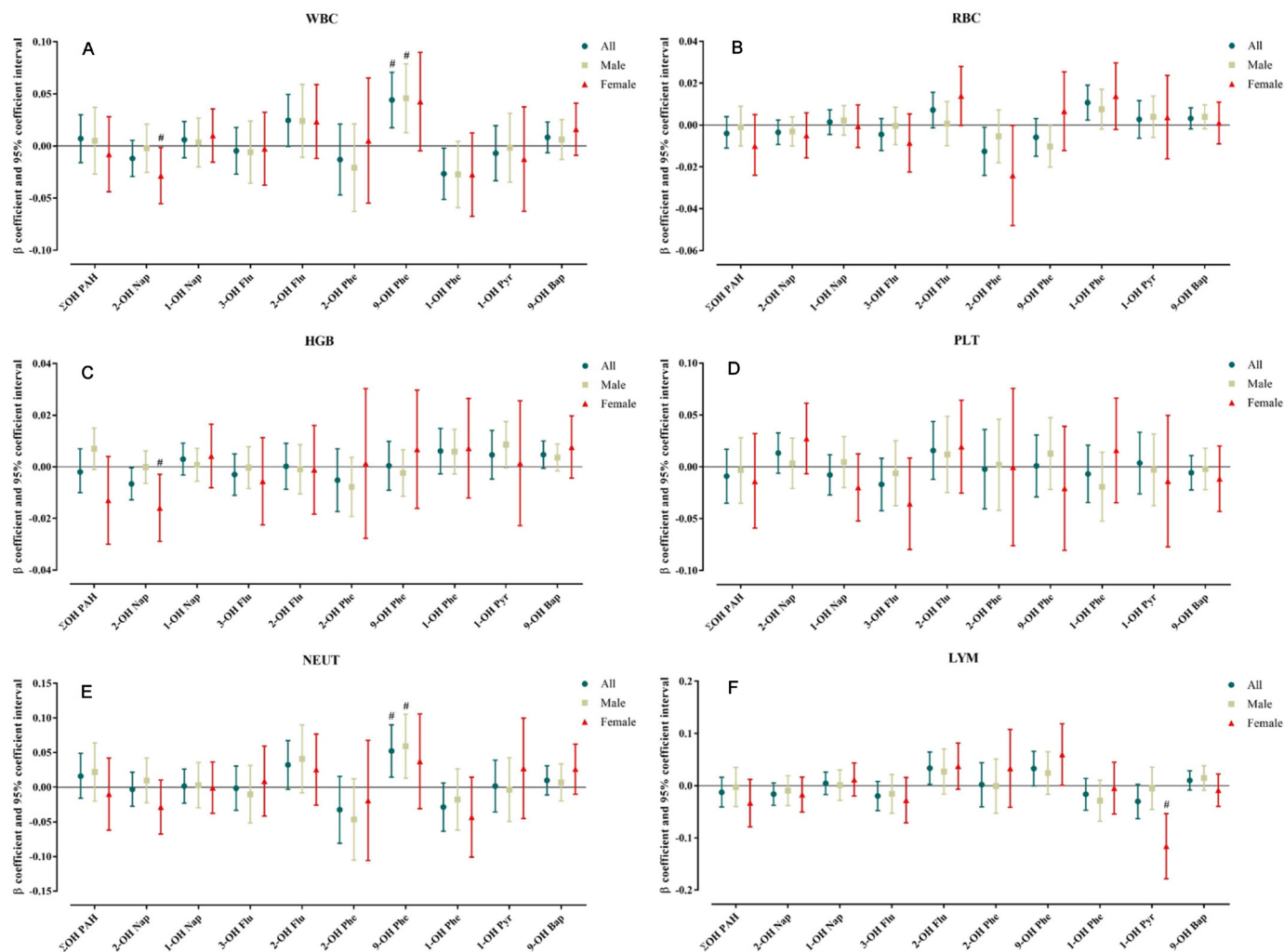
**Figure 4** The results of general linear models between changes in complete blood counts and increase in PAH metabolites among all participants, male workers and female workers after being adjusted for other eight PAH metabolites, age, education, body mass index, smoking status, drinking status and exercise status (sex was also adjusted among all participants). # represents both the results of generalised linear models and p value for trend test p<0.05. HGB, haemoglobin; LYM, lymphocyte count; NEUT, neutrophil count; PAH, polycyclic aromatic hydrocarbon; PLT, platelet count.

immune function. From what has been discussed above, we believe that there are sex differences in the NLR and PLR induced by PAH. The reasons for the sex difference may be that females were more susceptible than males to oxidative stress induced by PAH.[15 49 50] Oxidative stress could also induce HGB oxidation and nitration, and the nitration of HGB was an indicator of RBC damage.[51] Thus, HGB, inflammation and oxidative stress may be linked indirectly.[52] It was found that 2-OHNap was related to a decrease in HGB among female workers, but not among male workers. There was no association between PAH and HGB in a study that only males were recruited either.[30] And in in vivo studies, HGB was reduced in both male and female rats when they were subchronically exposed to PAH or benzo[a]pyrene.[35 53] Therefore, exposure to PAH leads to a reduction in HGB, which may be more pronounced in females.

Among blood cells, WBCs are universally measured, and they increase significantly in response to infection, trauma, inflammation and certain diseases.[52 53] It was

found that 9-OHPhe exposure could induce an increase in WBCs and NEUTs among male workers and all participants, consistent with other studies among male workers and in vivo studies.[30 49] This may be due to higher exposure levels of 9-OHPhe in male workers. We also found that 2-OHNap was associated with decreased WBCs among female workers, consistent with studies that found that increased 2-OHNap was associated with decreased WBCs among coke oven workers.[29] The reason for the sex difference may be the different influences of PAH on oxidative stress in males and females, and oxidative stress levels were related to PAH-induced haematotoxicity.[15 30] In female coke oven workers who are more sensitive to oxidative stress, the effect of haematotoxicity may have been produced.

However, this study still needs to be further improved. First, a single-spot urine sample was collected in this study. Multiple-point urine sample measurements in future studies should be used to evaluate the individual long-term exposure to environmental PAH. Second, we

did not measure oxidative stress, which would be informative to validate sex difference. Third, other environmental compounds, such as particulates, benzene and heavy metals in coke oven emissions, may have affected the analysis.

## CONCLUSION

This study, mainly from the perspective of epidemiology, examined the association of nine urinary OH-PAH with complete blood counts, NLR and PLR and further explored their dose–response relationships in all coke oven workers, male coke oven workers and female coke oven workers. Our study indicated that urinary OH-PAH levels were related to changes in NLR, PLR, WBCs, NEUTs, LYMs, HGB and existing dose–response relationships. Moreover, there were marked differences between male and female workers, in that NLR, PLR, WBCs and HGB for female workers were more susceptible than male workers to PAH.

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