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Association of *Helicobacter pylori* infection and white blood cell count: a cross-sectional study

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ABSTRACT

Introduction *Helicobacter pylori* is a type of Gram-negative microaerobic bacteria that inhabits the gastric mucosal epithelium. It can cause various gastrointestinal diseases including gastritis, peptic ulcer and gastric cancer. White blood cells (WBC) are common immune cells, the increase in whose countoften indicates the presence of an infection. Currently, the relationship between *H. pylori* and WBC count remains full of controversy. This study aims to further elucidate the effects of *H. pylori* on WBC count in a population undergoing physical examination.

Methods and analysis A total of 864 participants who underwent physical examination and ^{14}C urea breath test (UBT) were retrospectively enrolled in this study from January to June 2021. The overall population was divided into *H. pylori*-negative (Hp−) and *H. pylori*-positive (Hp+) groups based on the disintegration per minute (DPM) value detected by UBT. Spearman's correlation analysis was used to assess the correlation between DPM and WBC count. General linear regression models were applied to assess the potential factors contributing to the increase in WBC count. Generalised additive model (GAM) was performed to identify the non-linear relationship between DPM and WBC count. Additionally, a piecewise linear regression was used to examine the threshold effect of the DPM on WBC count.

Results 403 subjects were diagnosed with *H. pylori* infection. The WBC and platelet (PLT) counts in the Hp+ group were significantly higher than those in the Hp− group. Additionally, the prevalence of *H. pylori* infection gradually increased with the WBC count quartiles (38.89% and 54.67% in quartile 1 and quartile 4, respectively). Spearman's correlation analysis showed that the DPM value significantly correlated with WBC count (r=0.089, p=0.009) and PLT count (r=0.082, p=0.017). The linear model revealed a positive independent association of *H. pylori* infection and DPM with WBC count (β_{Hp+}=0.398 (95% CI 0.170, 0.625), p<0.001; β_{DPM}=0.002 (95% CI 0.000, 0.0030), p=0.018). The results of the GAM and the piecewise linear regression suggested that the cut-off points of the association between DPM and WBC count were 40 and 155 of DPM, that is, the effect of DPM on WBC count varied with the difference of DPM <40, 40–155, and >155 (β_{DPM}=−0.005 (95% CI −0.017, 0.007), p=0.423; β_{DPM}=0.006 (95% CI 0.002, 0.013), p=0.047; and β_{DPM}=−0.007 (95% CI −0.012, −0.002), p=0.004, respectively).

Conclusions *H. pylori* infection was independently and positively correlated with WBC count; however, the effect of DPM on WBC count varied across different WBC count intervals, suggesting distinct immunological responses at different stages of infection.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ We qualitatively and quantitatively describe the association between disintegration per minute and white blood cells (WBC) count.
⇒ Our study may provide a clue about the potential effects of *Helicobacter pylori* infection on the immunological microenvironment.
⇒ Our study is limited due to its retrospective nature and the incomplete examinations.
⇒ Information on individual disease history, comorbidity, endoscopy and histopathological examinations is not available.
⇒ This research cannot directly prove a causal and temporal relationship between WBC count and *H. pylori* infection.

INTRODUCTION

Helicobacter pylori, a Gram-negative microaerophilic pathogenic bacterium, mainly inhabits the human gastric mucosal epithelium. It has infected 4.4 billion individuals around the world, and in China 50%–70% of the population suffer from this infection, which is closely linked to diet intake, lifestyle and the host's genetic predisposition.[1–4] With the aid of urease that it secretes and the specific interactions between adhesins and host cell receptors, *H. pylori* is able to survive and colonise in a highly acidic gastric environment. This persistent infection promotes damage to the gastric epithelium through release of effector proteins/toxins, ultimately leading to chronic and progressive gastritis, atrophy, intestinal metaplasia and even gastric cancer.[5–7] A large-scale prospective study revealed that gastric cancer develops only in *H. pylori*-infected individuals, confirming that this bacterium is a contributing factor

for gastric carcinogenesis.[8] Additionally, *H. pylori* infection is also associated with many extragastric diseases, such as cardiovascular diseases, cholecystitis, psoriasis and a range of autoimmune diseases.[9–11] Interestingly, several studies have also revealed the protective effects of *H. pylori* infection against asthma, coeliac diseases and inflammatory bowel diseases.[12] However, further studies are needed to provide deep insights into the local gastric and systemic effects of *H. pylori* infection and the mechanisms involved.

Despite its high infection rate, *H. pylori* only yields relatively few symptoms or pathologies.[12] This underscores the need to elucidate the potential chronic and subtle impacts of *H. pylori* infection on systemic inflammation and immunology among the general population. It has been reported that serum C reactive protein concentrations in patients infected with *H. pylori* are considerably higher than in patients negative for *H. pylori*.[13] Furthermore, the *H. pylori* protein JHP0290 and HP1286 can bind to multiple cell types, including gastric epithelial cell lines, monocyte-derived dendritic cells, and neutrophils, and trigger macrophage apoptosis.[14 15] These findings highlight the complexity and multifaceted nature of *H. pylori*'s interactions with the human immune system.

White blood cells (WBC) are recognised as the body's protective mechanism against invasion of foreign pathogenic micro-organisms. Various inflammatory reactions and infections could regulate the level of WBC.[16–18] Clinically, WBC count is usually used as an indicator of bacterial infection, offering advantages of easy access, mature detection methods, and rapid results.[18] Notably, the total WBC count tends to increase during *H. pylori* infection.[19] Similarly, Kondo *et al*[20] observed a reduction in the total counts of leucocytes, neutrophils and monocytes in peripheral blood following successful treatment of *H. pylori* infection. Our study aims to qualitatively and quantitatively explore the relationship between *H. pylori* infection and total WBC count, potentially providing clinical evidence on the pathogenesis and management of *H. pylori*.

MATERIALS AND METHODS

Participants

To avoid the influence of medications on the results of the ^{14}C urea breath test (UBT), those taking proton pump inhibitors in the preceding 15 days or any antibiotics within 30 days before the examination were excluded from the study. Volunteers presenting with malignant solid tumours, haematological malignancies, severe immunological diseases and active bacterial and viral infections were also excluded. When the volunteers went to the hospital's physical examination centre, the nurse verbally enquired about the history of the above-mentioned diseases and medications; no volunteers were found to have such histories. Finally, a total of 864 eligible volunteers were retrospectively included in this cross-sectional study from January 2021 to June 2021 (figure 1). These 864 eligible volunteers underwent health examinations and had UBT for *H. pylori* at the Department of Health Care Center, Northwest University Affiliated Shenmu Hospital (Shenmu, China). Overnight fasting blood samples for haematological parameter analysis and gas samples for UBT examination were collected once within the research period. Because clinical data was retrieved from the health-check project and no individual identifiable information was included, written consent from the included cases was not necessary and available.

^{14}C urea breath test

The detection of *H. pylori* was done by ^{14}C-UBT and was performed as follows. Briefly, after an overnight fast and routine oral cleaning, the participant took a ^{14}C urea capsule orally (27.8 kBq (0.75 µCi); CNNC Headway Biotechnology, Shenzhen, China) while seated and at rest. After 25 min expired air was collected anddetected

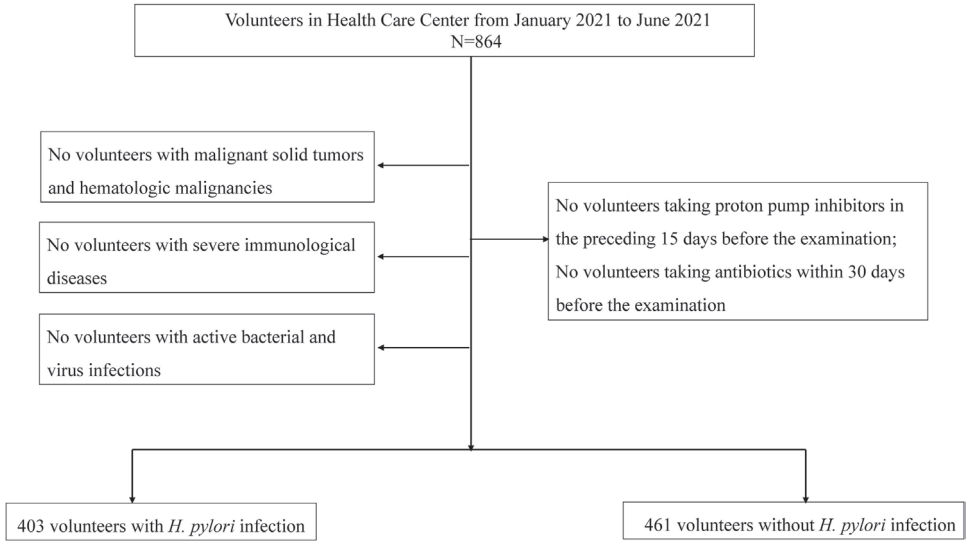

Figure 1 Flow chart of the inclusion and exclusion criteria. *H. pylori*, *Helicobacter pylori*.

using an *H. pylori* analyser (HUBT-20A2; CNNC Headway Biotechnology). The UBT result was presented as disintegration per minute (DPM), which indicates the amount of $^{14}CO_2$ produced from the metabolism following the administration of the labelled urea capsule, reflecting the metabolic activity of *H. pylori*. *H. pylori* infection was considered positive if the DPM was ≥50 and negative if it was ≤40. Meanwhile, a DPM within the 40–50 range indicated uncertainty about the presence of *H. pylori* and thus such cases were excluded. Subsequently, the overall population was divided into *H. pylori*-negative (Hp−) and *H. pylori*-positive (Hp+) groups according to whether the DPM was ≥50 or ≤40.

Anthropometry and biochemical measurements

A general medical examination provided information about age, gender, height and body weight, as well as blood pressure. Body mass index (BMI) was calculated by dividing the weight (kg) with squared height (m^2). Blood pressure was presented as the mean of two independent readings on sphygmomanometer at a 5 min interval in a quiet state. Overnight fasting venous blood samples were collected and processed within 30 min. Haematological parameters, including WBC, neutrophils, monocytes, lymphocytes, red blood cells (RBC), basophils, eosinophils and platelets (PLT), were evaluated on venepuncture samples using a haematology analyser (BC-6800; Mindray, Shenzhen, China). Lipid profile parameters, including serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), as well as kidney functional parameters (serum urea and creatinine) were also collected. Serum levels of gastrin-17 (G-17), pepsinogen I (PGI), pepsinogen II (PGII), and PGI/PGII were measured using ELISA on an automatic biochemical and fluorescence immunoanalyser (HIT-91A; Biouhan, Hefei, China). Plasma glucose concentration was evaluated using the glucose oxidase method, and glycated haemoglobin/haemoglobin A1c (HbA1c) was detected by Cobas C501 automatic biochemical analyser (Roche, Basel, Switzerland).

Statistical analysis

All data are presented as mean±SD, or median with range for continuous variables and percentage for categorical variables. For comparison of differences in continuous variables, an independent t-test or non-parametric Mann-Whitney U test was used to compare between two groups; one-way analysis of variance or Kruskal-Wallis test was used for comparison of variables among three groups or more. Spearman's correlation coefficient was used to analyse the correlation of data that did not conform to normal distribution. χ^2 test was used to analyse differences in categorical variables between different groups. General linear regression models were applied to assess the association between various baseline variables and WBC count in an unadjusted model, and in an age-adjusted, gender-adjusted and BMI-adjusted model (model 2), and then further adjusting for TC, TG, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and HbA1c (model 3). The interaction between DPM and other covariates, including age, gender, BMI and SBP, and their effect on WBC count were assessed using general linear regression model by introducing a DPM×covariate interaction term, which was analysed using the package 'interactions' in R software (http://www.R-project.org). Generalised additive model (GAM) was performed to identify the non-linear relationship of DPM and WBC count by smoothing plot using the package 'mgcv' of the R software. In the GAM, WBC was set as the dependent variable, and DPM and age as the independent variables (family=Gaussian, link function=identity). A further piecewise linear regression was used to examine the threshold effect of DPM on WBC count. The posteriori statistical power of this study was calculated using Stata SE V.12.0 software based on the difference of the mean and SD of the WBC count between the Hp+ and Hp− groups. The following parameters were input into the program: Hp−: WBC mean=6.74, SD=1.67, n1=461; Hp+: WBC mean=7.13, SD=1.73, n2=403; α=0.05 (two-tailed). The statistical power was 0.919. Other analyses were completed using SPSS V.26.0 software. A two-sided p value <0.05 was considered statistically significant.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

RESULTS

Baseline characteristics of the participants

A total of 864 eligible participates, consisting of 707 (81.8%) men and 157 (18.2%) women, with an average age of 36.80 years, were retrospectively enrolled in this cross-sectional study conducted at our centre from January 2021 to June 2021. Following ^{14}C-UBT screening, 403 (46.64%) subjects tested positive for *H. pylori* infection, exceeding the DPM value of 50. The overall population was then divided into Hp− and Hp+ groups based on their infection status. We then compared the demographics, metabolic, and anthropometric characteristics between the two groups. As shown in table 1, the two groups of participants had comparable age, gender, BMI, blood pressure, lipid profiles, and glucose metabolism and renal functional parameters (p>0.05). However, the Hp+ subjects exhibited substantially higher median serum G-17 concentration (1.65 pmol/L vs 5.66 pmol/L) and lower PGI/II ratio (16.33±8.80 vs 11.54±8.83) than the Hp− subjects, indicating a potential gastric pathological injury or functional impairment after the infection. When comparing the haematological parameters between the two groups, we found that WBC count significantly increased in the Hp+ subjects (6.74±1.67×10^9/L vs 7.13±1.73×10^9/L, p=0.001), in parallel with the increase in PLT count (221.17±46.00×10^9/L vs 228.02±47.76×10^9/L,

Table 1 Baseline characteristics of the enrolled participants infected and not infected with *H. pylori*

Characteristics	Overall (N=864)	Hp– (n=461)	Hp+ (n=403)	P value
Age (years)	36.80±9.91	37.22±10.31	36.32±9.41	0.184
Gender (male/female)	707/157	383/78	324/79	0.308
BMI (kg/m^2)	25.58±3.81	25.64±3.77	25.52±3.86	0.650
SBP (mm Hg)	127.64±15.40	127.53±16.17	127.48±15.57	0.963
DBP (mm Hg)	78.78±11.85	78.82±12.87	78.55±11.34	0.744
HR (beats per minute)	80.45±11.22	80.55±12.25	80.82±11.60	0.736
TC (mmol/L)	4.51±0.87	4.64±0.87	4.67±0.86	0.884
TG (mmol/L)	1.85±1.41	1.87±1.49	2.41±9.67	0.237
HDL-C (mmol/L)	1.27±0.30	1.28±0.29	1.27±0.32	0.903
LDL-C (mmol/L)	2.72±0.78	2.74±0.81	2.69±0.75	0.360
FBG (mmol/L)	4.68±1.10	4.60±0.79	4.77±1.37	0.088
HbA1c (mmol/L)	5.40±0.69	5.34±0.57	5.46±0.83	0.134
UA (µmol/L)	363.15±91.66	364.86±91.08	361.19±92.38	0.557
Urea (mmol/L)	5.42±2.83	5.35±2.48	5.50±3.18	0.455
Cre (µmol/L)	75.51±14.11	75.62±13.69	75.37±14.59	0.799
PGI/PGII	14.28±8.80	16.33±8.80	11.54±8.83	<0.001
G-17 (pmol/L)	2.83 (0.50, 60.7)	1.65 (0.50, 60.7)	5.66 (0.50, 60.4)	<0.001
WBC count (×10^9/L)	6.92±1.71	6.74±1.67	7.13±1.73	0.001
Neutrophils (%)	57.63±7.61	57.98±7.32	57.22±7.92	0.145
LYM (%)	34.02±6.83	33.83±6.84	34.24±6.82	0.380
Monocytes (%)	5.48±1.34	5.50±1.32	5.45±1.36	0.583
RBC (×10^{12}/L)	5.04±0.43	5.03±0.41	5.05±0.44	0.475
HB (g/L)	156.78±17.48	157.51±14.28	155.96±20.52	0.194
HCT (%)	46.98±7.18	46.75±4.69	47.26±9.24	0.305
MCV (fL)	91.89±7.32	92.24±6.77	91.50±7.89	0.139
RDW-CV (%)	13.20±4.84	12.88±0.63	13.57±7.03	0.069
PLT (×10^9/L)	224.37±46.93	221.17±46.00	228.02±47.76	0.011
NLR	1.83±0.64	1.83±0.64	1.80±0.71	0.398
PLR	6.82±2.12	6.82±2.12	6.96±2.16	0.327

Statistical analysis used the non-parametric Mann-Whitney U test for comparisons.
BMI, body mass index; Cre, creatinine; DBP, diastolic blood pressure; FBG, fasting blood glucose; G-17, gastrin-17; HB, haemoglobin; HbA1c, glycated haemoglobin/haemoglobin A1c; HCT, haematocrit; HDL-C, high-density lipoprotein cholesterol; Hp–, *Helicobacter pylori*-negative; Hp+, *Helicobacter pylori*-positive; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; LYM, lymphocyte; MCV, mean corpuscular volume; NLR, neutrophil to lymphocyte ratio; PGI, pepsinogen I; PGII, pepsinogen II; PLR, platelet to lymphocyte ratio; PLT, platelet; RBC, red blood cell; RDW-CV, red cell distribution width; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; UA, uric acid; WBC, white blood cell.

p=0.011). No substantial changes were observed in other haematological parameters in response to *H. pylori* infection.

Correlations between the UBT test DPM and haematological parameters

Based on the above results showing that the Hp+ group had higher counts of WBC and PLT, we hypothesised that individuals of the general population with Hp+ infection may be prone to having elevated WBC and PLT counts. To further demonstrate this, we conducted a Spearman's correlation analysis to assess the association between DPM detected by UBT and various haematological parameters across the entire study population. The analysis showed that DPM was positively correlated with WBC count (r=0.089, p=0.009) and PLT count (r=0.082, p=0.017). Meanwhile, DPM was adversely associated with haemoglobin concentrations (table 2). These findings indicate that *H. pylori* exposure and colonisation may directly or indirectly impact the haematological components and the immunological microenvironment.

Potential factors contributing to the increase in WBC count

We then focused on the association between *H. pylori* positive infection and elevation in WBC count. The overall population was divided into four groups according to WBC count quartiles, and the variables that affect WBC count were compared among these groups. The quartile cut-off values for WBC count were 5.75×10^9/L, 6.76×10^9/L, and 7.85×10^9/L, respectively. As shown in table 3, the

Table 2 Correlation between DPM values and haematological parameters

Variables	r	P value
WBC count (×10^9/L)	0.089*	0.009
Neutrophils (%)	−0.030	0.389
Lymphocytes (%)	0.030	0.378
Monocytes (%)	−0.050	0.142
Eosinophils (%)	0.066	0.054
Basophils (%)	−0.038	0.268
RBC (×10^{12}/L)	−0.012	0.726
HB (g/L)	−0.086*	0.012
HCT (%)	0.016	0.637
MCV (fL)	−0.022	0.525
PLT (×10^9/L)	0.082*	0.017
NLR	−0.033	0.330
PLR	0.047	0.168

*, p < 0.05.
DPM, disintegration per minute; HB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

prevalence of Hp+ infection gradually increased with increase in WBC count, with 38.89% infection rate in the quartile 1 subgroup and 54.67% in the higher quartile 4 subgroup (p=0.013). However, no significant difference in DPM was noted between WBC count quartiles. In addition, compared to individuals in the lower quartile of WBC count, those in the higher quartile exhibited higher levels of BMI, blood pressure, HR, TC, TG, and uric acid (UA) (p<0.05 for each comparison). Significant differences in HbA1c and G-17 were also found among the four groups (p<0.05 for each comparison).

Linear regression analysis of the association between *H. pylori* infection and WBC count

Multivariate general linear regression was performed to analyse the association between the contributing factors and WBC count by including variables from the univariate analysis with a cut-off p value of less than 0.2. As shown in table 4, in the unadjusted model 1, most of the included parameters increased the WBC count, while gender as well as high HDL-C reduced the WBC count. Hp+ infection and DPM were positively associated with WBC count (β_{Hp+}=0.398 (95% CI 0.170, 0.625), p<0.001; β_{DPM}=0.002 (95% CI 0.000, 0.003), p=0.018). After adjustment for age, gender, and BMI in model 2, HDL-C, LDL-C, and HbA1c showed no effect on WBC count. While the effect of Hp+ infection, DPM, blood pressure, HR, TC, TG, UA, and G-17 on WBC count remained statistically significant. After further adjustments for SBP, DBP, TC, TG, HR, and HbA1c in model 3, DPM (β_{DPM}=0.002 (95% CI 0.000, 0.003), p=0.001), Hp+ status (β_{Hp+}=0.427 (95% CI 0.216, 0.637), p<0.001), UA (β_{UA}=0.002 (95% CI 0.000, 0.003), p=0.038), and G-17 ($\beta_{G\text{-}17}$=0.017 (95% CI 0.006, 0.028), p=0.002) showed positive association with WBC level. Hp+ infection caused an increase in WBC by 0.427×10^9/L, and each unit increase in DPM value would lead to an elevation of WBC of 0.002×10^9/L. The linear regression plot is shown in figure 2.

Analysis of the interactive effects of DPM and covariates on WBC count

We further analysed the interaction between DPM and other covariates, including age, gender, BMI, and SBP on WBC count. The frequency of *H. pylori* active infection was compared among four quartiles of WBC and then further stratified by gender, age, BMI, and SBP. As shown in figure 3, the incidence of Hp+ infection increased with WBC count quartiles. The χ^2 analysis showed that there was statistical significance between the subgroups of male gender (p=0.045), age above 35 years old (p=0.016), and SBP less than 140 mm Hg (p=0.015). The interaction terms in the whole cohort population were then examined. Age, sex, BMI, and SBP were taken as covariables, respectively, and the DPM×covariate interaction term was introduced to the general linear regression. WBC count was taken as the dependent variable for analysis. The results showed that gender, BMI, and SBP had no significant interactions with DPM, although age seemed to interact with DPM in the interaction diagram but not significant (figure 4).

Non-linear model of the dose–response relationship between DPM and WBC count

Given that WBC count is a continuous variable, it is crucial to analyse its non-linear relationship with DPM. GAM was performed to identify the non-linear relationship of DPM and WBC count. As shown in table 5, the cut-off points of the association between DPM and WBC count were at 40 and 155 DPM. The threshold effects of DPM on WBC count were then analysed using piecewise linear regression (figure 5). It was shown that the DPM was not statistically correlated with WBC count as DPM was below the infection point of 40 (β_{DPM}=−0.005 (95% CI −0.017, 0.007), p=0.423), whereas when the DPM increased to 155 from 40 a significant positive correlation was indicated (β_{DPM}=0.006 (95% CI 0.002, 0.013), p=0.047). When the DPM was above 155, the WBC count decreased and there was a significant adverse association (β_{DPM}=−0.007 (95% CI −0.012, −0.002), p=0.004). These findings provide insights into the complex relationship between *H. pylori* infection, measured by DPM and WBC count, potentially indicating distinct immunological responses at different stages of infection.

DISCUSSION

H. pylori infection not only causes gastric symptoms, but also plays a role in a wide range of systemic diseases. Moreover, it is associated with alterations in haematological parameters, such as PLT indices, RBC, and

Table 3 Comparison of variables according to WBC count quartiles

Variables	Q1 (≤5.75) n=216	Q2 (5.76–6.76) n=219	Q3 (6.77–7.85) n=214	Q4 (≥7.86) n=214	F/χ^2	P value
Hp+ (%)	38.89	47.03	46.26	54.67	10.786	0.013
DPM	67.21±85.71	80.60±88.85	75.90±85.31	85.26±82.07	1.742	0.157
Gender (male/female)	158/58	172/47	187/27	189/25	23.032	<0.001
Age (years)	37.71±10.78	36.25±9.80	36.71±9.52	36.54±9.49	0.888	0.447
BMI (kg/m^2)	24.04±3.62	25.12±3.44	26.44±3.67	26.79±3.87	25.733	<0.001
SBP (mm Hg)	122.78±13.33	125.00±14.20	130.33±15.91	132.58±16.08	20.067	<0.001
DBP (mm Hg)	74.62±10.85	77.23±10.70	81.04±11.79	82.43±12.59	20.655	<0.001
HR (beats per minute)	78.37±11.34	78.50±10.89	81.49±10.49	83.56±11.42	11.052	<0.001
TC (mmol/L)	4.41±0.86	4.45±0.85	4.59±0.86	4.60±0.88	2.724	0.043
TG (mmol/L)	1.47±1.18	1.69±1.51	2.00±1.29	2.27±1.50	13.799	<0.001
HDL-C (mmol/L)	1.33±0.31	1.32±0.32	1.23±0.28	1.22±0.30	9.023	<0.001
LDL-C (mmol/L)	2.63±0.77	2.64±0.74	2.84±0.78	2.79±0.82	4.140	0.006
HbA1c (mmol/L)	5.31±0.53	5.26±0.54	5.58±1.04	5.40±0.46	3.223	0.023
FBG (mmol/L)	4.60±0.48	4.69±1.32	4.77±1.26	4.66±1.14	0.855	0.464
UA (µmol/L)	339.75±90.08	355.19±88.83	372.20±89.25	386.24±92.38	10.822	<0.001
Urea (mmol/L)	5.12±2.27	7.24±25.77	5.63±3.33	5.41±2.31	1.120	0.340
Cre (µmol/L)	73.91±14.48	75.40±13.89	76.29±12.49	76.44±15.42	1.446	0.228
PG (PGI/PGII)	15.46±6.59	15.46±11.27	13.22±7.76	13.24±10.40	1.234	0.298
G-17 (pmol/L)	5.37±9.49	7.43±11.11	5.69±9.17	8.68±12.19	3.905	0.009

Statistical analysis used the Kruskal-Wallis test for comparison.
BMI, body mass index; Cre, creatinine; DBP, diastolic blood pressure; DPM, disintegration per minute; FBG, fasting blood glucose; G-17, gastrin-17; HDL-C, high-density lipoprotein cholesterol; Hp+, *Helicobacter pylori*-positive; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; PGI, pepsinogen I; PGII, pepsinogen II; Q, quartile; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; UA, uric acid; WBC, white blood cell.

haemoglobin, in symptomatic patients, as demonstrated in several studies.[21–23] However, the potential chronic and subtle impacts of *H. pylori* on haematological parameters in the general population remain unexplored. Our current study sheds new light on this matter, revealing that individuals of the general population with active *H. pylori* infection are more likely to have higher WBC count. Furthermore, we confirmed the independent contribution of positive infection and activities of *H. pylori* to the changes in WBC count. Thus, our study qualitatively and quantitatively described the association between DPM and WBC count. The data suggested that *H. pylori* exposure and colonisation directly or indirectly alter the haematological components and the immunological microenvironment.

Prior researches have illuminated the relationship between *H. pylori* infection and changes in WBC count in the peripheral blood. Karttunen *et al*[19] observed an increase in WBC count during *H. pylori* infection, as well as in the number of lymphocytes and basophils, which may reflect the severity of the inflammation of the gastric mucosa. A large-scale, cross-sectional study revealed significant variations in WBC count quartiles associated with *H. pylori* infection among individuals undergoing general health screening, demonstrating an independent link with non-alcoholic fatty liver disease.[18] In a 19-year follow-up cohort study, the incidence of gastric cancer increased linearly with WBC level among the general Japanese population after adjustment for age and gender. Meanwhile, *H. pylori*-seropositive subjects in the highest WBC count quartile group showed a significantly greater risk of gastric cancer than those in the lower three quartile groups.[24] Kondo *et al*[20] also investigated the changes in peripheral WBC after eradication of *H. pylori* and found a decrease in total counts of peripheral WBC, neutrophils, and monocytes.[20] Our study delved into the effects of *H. pylori* infection on WBC count, noting that subjects with *H. pylori* infection tended to have higher WBC count. However, when UBT values surpassed 155, the WBC count declined. Thus, bacterial load may have a chronic effect on WBC count in a different mechanism. Human WBC can be affected by many factors. Specifically, we excluded patients receiving anti-inflammatory therapies within 30 days, those with active viral and bacterial infections, and those with other haematological and immunological diseases from this general population to minimise

Table 4 Multivariate generalised linear model of parameters in association with WBC count

	Model 1			Model 2			Model 3		
Variables	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
DPM	0.002	0.000, 0.003	0.018	0.002	0.000, 0.003	0.001	0.002	0.000, 0.003	0.001
Hp+	0.398	0.170, 0.625	<0.0001	0.407	0.188, 0.627	<0.0001	0.427	0.216, 0.637	<0.0001
Gender	−0.690	−0.983, −0.398	<0.0001						
BMI (kg/m^2)	0.109	0.080, 0.138	<0.0001						
SBP (mm Hg)	0.026	0.019, 0.034	<0.0001	0.017	0.009, 0.225	<0.0001			
DBP (mm Hg)	0.032	0.023, 0.041	<0.0001	0.030	0.021, 0.040	<0.0001			
HR (beats per minute)	0.030	0.020, 0.040	<0.0001	0.029	0.020, 0.039	<0.0001			
TC (mmol/L)	0.171	0.039, 0.303	0.011	0.097	0.067, 0.127	<0.0001			
TG (mmol/L)	0.268	0.188, 0.347	<0.0001	0.167	0.082, 0.252	<0.0001			
HDL-C (mmol/L)	−1.027	−1.399, −0.655	<0.0001	−0.367	−0.786, 0.052	0.086			
LDL-C (mmol/L)	0.172	0.026, 0.319	0.021	0.082	−0.063, 0.226	0.266			
HbA1c (mmol/L)	0.311	0.031, 0.592	0.029	0.242	−0.047, 0.531	0.101			
UA (μmol/L)	0.004	0.003, 0.005	<0.0001	0.002	0.000, 0.003	0.021	0.002	0.000, 0.003	0.038
G-17 (pmol/L)	0.014	0.003, 0.026	0.015	0.018	0.007, 0.029	0.002	0.017	0.006, 0.028	0.002

Model 1: unadjusted.
Model 2: adjusted for age, gender and BMI.
Model 3: adjusted for age, gender, BMI, TC, TG, SBP, DBP, HR and HbA1c.
BMI, body mass index; DBP, diastolic blood pressure; DPM, disintegration per minute; G-17, gastrin-17; HbA1c, glycated haemoglobin/haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; Hp+, *Helicobacter pylori*-positive; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; UA, uric acid; WBC, white blood cell.

the potential effect of other factors on WBC. Additionally, to clarify the contribution of *H. pylori* infection to WBC elevation, we constructed three different models that adjusted for confounding factors such as age, gender, BMI, and other covariates. These adjustments help mitigate the influence of these factors to some extent and enhance the reliability of our conclusion. However, there are still other potential confounding factors that have not been considered and adjusted for in these models, which is a limitation of our research. Furthermore, we found a significant association between DPM and WBC count among male rather than female participants. However, Hp+ infection (45.83% vs 50.32%) and the median values of UBT test in male and female participants showed no significant differences. Therefore, the positive ratio of *H. pylori* infection in the initial population of women might not be the main contributor to the gender difference in the association between DPM and WBC count. Gender-related difference in UBT results has also been noted in other studies. Moshkowitz *et al*[25] found that the mean UBT test values were significantly higher among females of all age groups, possibly representing an increased bacterial load among females and suggesting gender-associated differences in *H. pylori* host interactions.[25] Additionally, Petruzziello *et al*[26] showed that female adults exhibited a significantly higher delta over baseline compared with male adults in their geographical area. This effect may be due to hormonal differences, which can influence gastric emptying, bacterial load or even the production of urease by *H. pylori*; this, however, merits further investigation.[26]

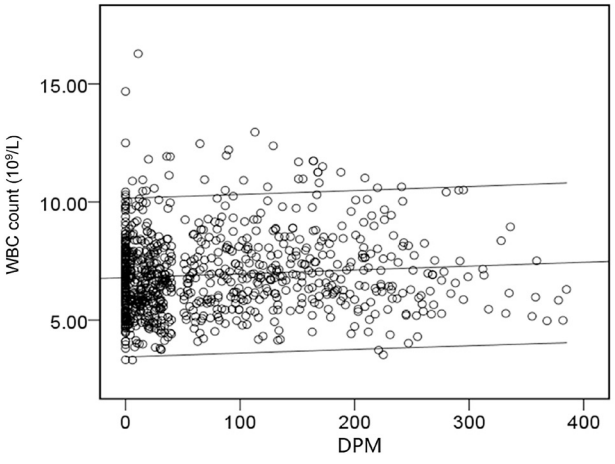

Figure 2 Fitted linear plot of DPM and WBC count. DPM, disintegration per minute; WBC, white blood cell.

In clinical practice, WBC count stands as a reliable, accessible, and cost-effective marker for assessing systemic inflammatory status.[18] This metric serves as a valuable indicator for diagnosing and predicting the prognosis of *H. pylori*-related diseases such as chronic gastritis, atrophic gastritis, and gastric cancer. It has been shown that gastritis with *H. pylori* is associated with an elevated serum leucocyte count in subjects who underwent health

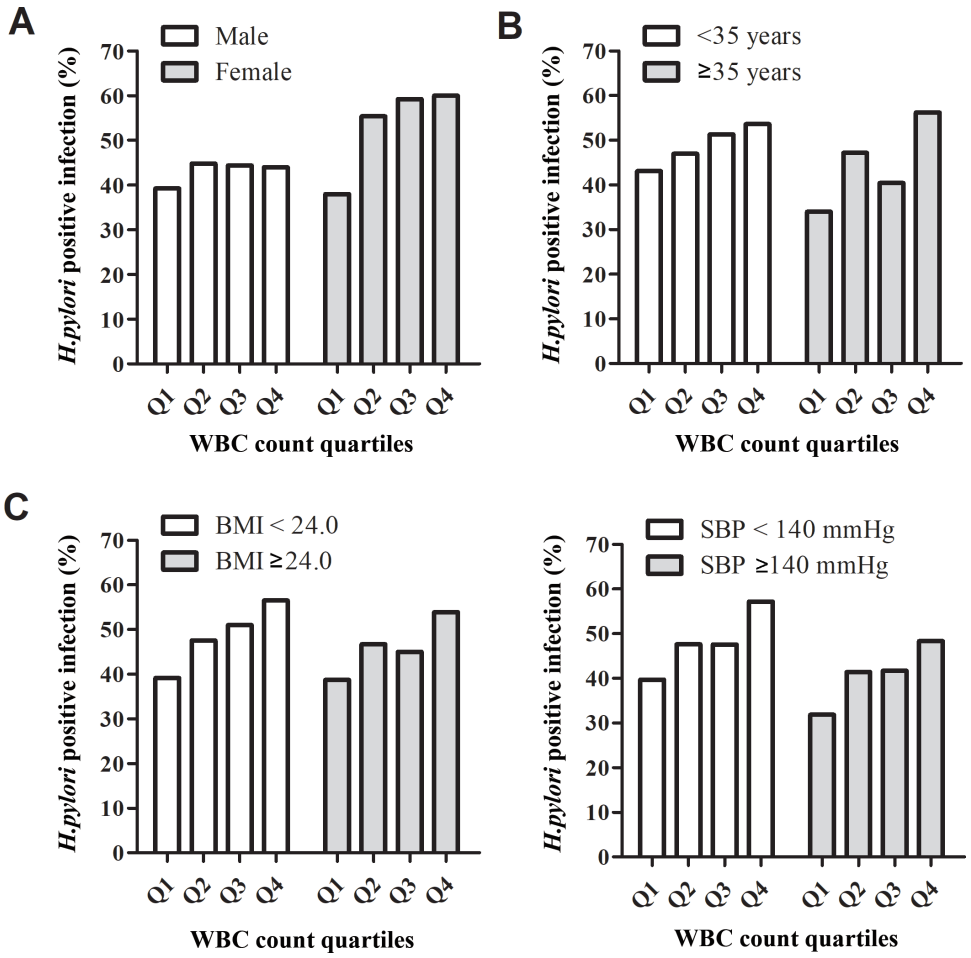

Figure 3 Subgroup analysis of the Hp+ infection ratio based on WBC count quartiles. The incidence of *Helicobacter pylori* infection was stratified by (A) gender, (B) age, (C) BMI and (D) SBP. BMI, body mass index; Hp+, *Helicobacter pylori*-positive; SBP, systolic blood pressure; WBC, white blood cell.

check-up and gastric biopsy, indicating an increased systemic inflammatory response.[27] A retrospective study showed that patients with gastric cancer exhibit higher level of WBC compared with patients with other benign gastric diseases (gastric polyp and benign gastric stromal tumour).[28] These observations raise the possibility that peripheral WBC count is likely to act as an intermediate factor between *H. pylori* infection and gastric cancer development. Based on our data and previous evidence that an *H. pylori* active infection was associated with elevation in WBC count, we speculated that, in the population undergoing health check-up, local *H. pylori* infection will affect inflammation and immune stress response at the system level. Our study only evaluated the association between DPM and WBC count in the general population, while evaluating their association in patients undergoing eradication therapy will strengthen the conclusion. This retrospective cross-sectional study failed to obtain more information about the treatment for the infection. A prior study revealed significant decrease in blood leucocytes, neutrophils, and monocytes after *H. pylori* eradication within 12 months.[20] Nevertheless, additional evidence on treatment conditions is warranted to provide a more comprehensive understanding.

After *H. pylori* invasion, the bacterium establishes direct contact with the host's epithelial cells and uses its type IV secretion system to introduce conserved structural components from its cell wall into the cytosol of gastric epithelial cells. This process triggers signalling cascades that converge on the transcription factor nuclear factor-κB, ultimately resulting in the production of inflammatory cytokines and the induction of inflammation in the host.[29] Additionally, *H. pylori* vacuolating toxin A stimulation inhibits interleukin 23 expression in dendritic cells and induces interleukin 10 and transforming growth factor-β in macrophages, activating the host immune response.[30] A meta-analysis indicated an association between *H. pylori* infection and psoriasis, with patients with psoriasis with *H. pylori* infection having more psoriasis areas and higher severity index scores.[31] Additionally, Zendehdel and Roham[10] summarised that *H. pylori* infection is associated with heart failure and bronchitis, attributed to inflammatory cytokine generation. However, a large population-based study found no independent association between serum immunoglobulin G for *H. pylori* infection and leucocyte count.[32] Interestingly, some studies have revealed that *H. pylori* protects against asthma, coeliac diseases, and inflammatory bowel

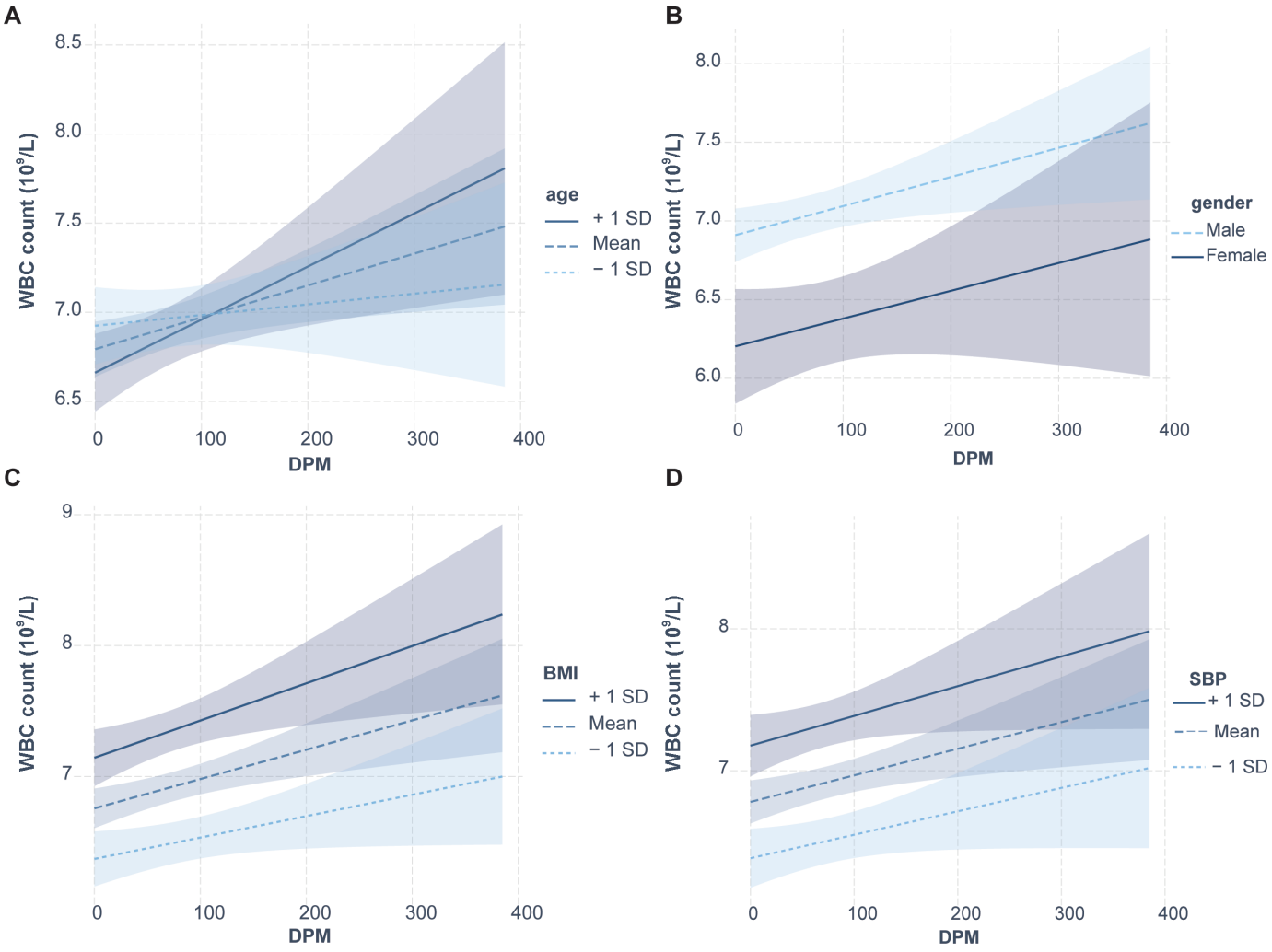

Figure 4 Linear regression model of the interaction terms in the whole cohort population. The interaction terms between DPM and (A) age, (B) gender, (C) BMI and (D) SBP. BMI, body mass index; DPM, disintegration per minute; SBP, systolic blood pressure; WBC, white blood cell.

diseases. For instance, Fouda *et al*[33] found that *H. pylori* seropositivity protects against childhood asthma and is inversely correlated to its clinical and functional severity. Lin *et al*[34] demonstrated that treatment for *H. pylori* infection is associated with a significant increase in the risk of inflammatory bowel diseases. These findings highlight the need for further evidence to clarify the specific mechanisms underlying *H. pylori*'s impact on systemic inflammation and immunology in the general population. In the current study, the threshold effect of DPM at around 155 is observed, indicating that lower *H. pylori* load will increase WBC count, while higher bacterial load will suppress WBC count to a certain extent. Many studies reported that UBT was more sensitive and accurate when used in the diagnosis of *H. pylori* infection, and DPM was significantly higher in peptic ulcer controls than in

Table 5 Results of two-piecewise linear regression model

Cut-off point	Effect size (β)	95% CI	P value
DPM≤40	−0.005	−0.017, 0.007	0.423
40<DPM≤155	0.006	0.002, 0.013	0.047
DPM>155	−0.007	−0.012, −0.002	0.004

DPM, disintegration per minute.

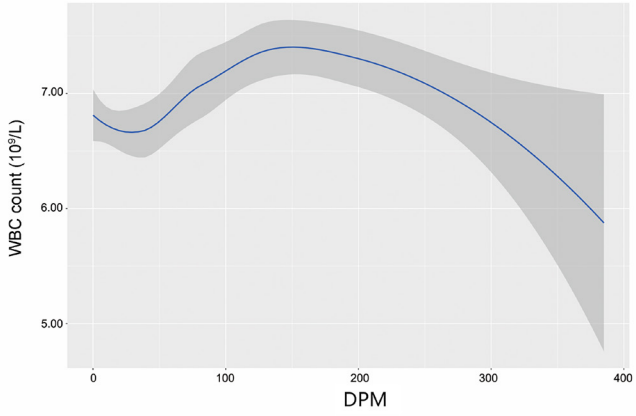

Figure 5 Fitted curve between DPM and WBC count. DPM, disintegration per minute; WBC, white blood cell.

patients with non-ulcer dyspepsia.[35] The DPM of the ulcer group was correlated significantly with the active inflammatory component of gastritis in the antrum, corpus, and fundus.[36] The association between the values of UBT as well as *H. pylori* severity and the impact of *H. pylori* activity on haematological parameters needs to be further validated.

Our study is limited due to its retrospective nature. Information on individual disease history, comorbidity, endoscopy, and histopathological examination is not available. Additionally, we only observed the effect of *H. pylori* on WBC count; profound details and variables on systemic inflammation and immunological response have not been recorded. Furthermore, male gender, overweight, middle-aged individuals, and young people account for a large proportion of the participants included in the study and thus the current results should be validated in more participants. Finally, we only evaluated the association between *H. pylori* infection and WBC count, and their relation after *H. pylori* eradication treatment should be further validated. Last but not least, this study cannot directly prove a causal and temporal relationship between WBC count and *H. pylori* infection, and the results can only serve as evidence of their association and therefore need to be verified by further randomised controlled trials. Nonetheless, this study proved the independent contribution of positive infection and activities of *H. pylori* to the changes in WBC count.

CONCLUSION

Our study found that in a population undergoing physical examination, those with *H. pylori* infection tend to have higher WBC count, and the prevalence of *H. pylori* infection gradually increases with increasing WBC count quartiles. These findings offer a clue on how *H. pylori* infection potentially affects the immunological environment resulting in the progression of many other chronic diseases. Future research should prospectively collect more samples and valuable variables to reveal the effect of *H. pylori* infection on systemic characteristics, which may provide theoretical basis for reducing the adverse effects and managing *H. pylori* infections in asymptotic individuals.

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Patient consent for publication Not required.

Ethics approval Ethical approval of this study was obtained from the Human Ethics Review Committee of Northwest University Affiliated Shenmu Hospital (SM020).

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