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Resting Heart Rate Mediates the Association Between Circulating Neutrophil Count and Arterial Stiffness Progression: The Kailuan Study

Zhaogui Wu[1,*], Dan Wu[2,3,*], Shuohua Chen[4], Sichi Xu[5,6], Shunming Zhang[7], Shouling Wu[4]

[1]Department of Cardiology, Xiangyang No. 1 People's Hospital, Hubei University of Medicine, Xiangyang, Hubei, 441000, People's Republic of China; [2]Department of Pediatrics, Second Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, People's Republic of China; [3]Centre for Precision Health, Edith Cowan University School of Medical and Health Sciences, Joondalup, WA, Australia; [4]Department of Cardiology, Kailuan General Hospital, Tangshan, Hebei, 063001, People's Republic of China; [5]Department of Cardiology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430014, People's Republic of China; [6]Key Laboratory for Molecular Diagnosis of Hubei Province, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430014, People's Republic of China; [7]School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, People's Republic of China

*These authors contributed equally to this work

Correspondence: Shouling Wu, Department of Cardiology, Kailuan General Hospital, No. 57, Xinhuadong Street, Lubei District, Tangshan, Hebei, 063001, People's Republic of China, Tel +86 13363283666, Email drwusl@163.com; Shunming Zhang, School of Public Health, Xi'an Jiaotong University Health Science Center, No. 76, Yanta West Road, Xi'an, Shaanxi, 710061, People's Republic of China, Tel +86 19502920539, Email zhangshunming@xjtu.edu.cn

Objective: This study aimed to longitudinally investigate the association between circulating neutrophil count and the progression of arterial stiffness and to ascertain whether resting heart rate (RHR) mediates this association.

Methods: The current study included 56,760 participants with brachial-ankle pulse wave velocity (baPWV) measurements from a real-life, prospective cohort in China. The associations of circulating neutrophil (exposure) with baseline baPWV, baPWV progression, and arterial stiffness (outcomes), as well as RHR (mediator) were assessed using multivariable linear and Cox regression models and mediation analysis.

Results: After adjusting for cardiometabolic risk factors, for each 1-SD increase in neutrophil count, the corresponding increase was 13.5 cm/s (95% CI, 11.1 to 15.9 cm/s, $P<0.001$) for the baseline baPWV and 3.10 cm/s (95% CI, 1.51 to 4.69, $P<0.001$) for the annual change in baPWV. Over a median follow-up period of 4.08 (IQR: 2.37 to 6.21) years, there were 3,376 incident cases of arterial stiffness among the 23,263 participants. Each 1-SD increase in neutrophil count was associated with a 7% increase in the risk of developing arterial stiffness (HR: 1.07; 95% CI: 1.04 to 1.10, $P<0.001$) in the multivariable-adjusted model. In the mediation analyses, 20.0% (95% CI: 16.8% to 24.2%), 12.6% (95% CI: 8.16% to 26.4%), and 16.7% (95% CI: 9.94% to 51.0%) of the observed associations of neutrophil counts with baPWV at baseline, baPWV progression and developing arterial stiffness, respectively, were mediated by RHR.

Conclusion: The present study underlines that circulating neutrophil count is significantly associated with arterial stiffness progression and that the RHR is, in part, a mediator of this association.

Keywords: arterial stiffness, inflammation, neutrophil count, resting heart rate, mediator, cohort study

Introduction

According to the Global Burden of Disease Study, cardiovascular disease (CVD) is a significant global health issue that continues to be the primary cause of mortality globally.[1] Maintaining cardiovascular health and reducing CVD-related mortality rates are therefore promising strategies for extending life expectancy.[2] Arterial stiffness, a prominent feature of vascular aging, exerts a direct impact on the cardiovascular system through the deterioration of arterial elasticity and the elevation of pulse pressure.[3,4] There has been consistent evidence that arterial stiffness is associated with future ischemic cardiovascular events as well as all-cause mortality.[5,6] Due to the

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chronic and progressive nature of arterial stiffness pathology, timely identification of individuals at high risk for arterial stiffness is crucial for implementing effective preventive strategies to halt disease progression.

Inflammation is widely acknowledged as a risk factor and pivotal pathogenic mediator in the development of arterial stiffness.[3,7] Epidemiological studies have demonstrated a positive association of elevated inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP), interleukin, and neutrophil-to-lymphocyte ratio, with arterial stiffness.[8–10] However, despite the merits of simplicity of measurement, high reproducibility, cost-effectiveness, and widespread accessibility with utilizing neutrophil count for assessing inflammatory status, longitudinal data on the correlation between neutrophil counts and arterial stiffness are scarce.

Resting heart rate (RHR) serves as a useful, simple, and noninvasive metric of autonomic nervous system balance and basal metabolic rate. Compelling evidence from epidemiological studies has established a positive association between RHR and arterial stiffness.[11,12] According to the findings of the Kailuan study, the long-term RHR trajectory pattern is a powerful predictor of arterial stiffness.[13] Additionally, inflammation was reported to be strongly linked to RHR.[14,15] Intriguingly, it was suggested that the effects of inflammation on cardiovascular health tended to be strongly amplified by a faster RHR.[16] In this context, a scientific question was raised regarding whether RHR mediates the association of neutrophil counts with arterial stiffness.

To address this knowledge gap, based on the Kailuan study, a real-life, community-based, prospective cohort in China, a series of analyses were conducted to thoroughly investigate the association between neutrophil counts and arterial stiffness assessed by brachial-ankle pulse wave velocity (baPWV). Thereafter, a mediation analysis was executed to further investigate the mediated effect of RHR in the above-established association.

Methods

Study Population and Design

The Kailuan study is a large prospective, ongoing cohort study established at the Kailuan General Hospital and its 11 affiliated hospitals in Tangshan, China, as previously described.[17] Briefly, from 2006 to 2007, participants from the Kailuan Group were recruited to participate in the initial survey, which included questionnaires, physical examinations, and laboratory assessments, with subsequent follow-up biennially. Since 2010, the Kailuan Study Arterial Stiffness Subcohort has been established, with baPWV tests used to evaluate the artery wall's health status.[18] Following the Declaration of Helsinki, the Kailuan study was approved by the Kailuan General Hospital's ethics committee (No. 200605). Written informed consent was obtained from all participants.

To comprehensively evaluate the association of neutrophil counts with arterial stiffness, this present study was designed to examine the associations of neutrophil counts with baseline baPWV, baPWV progression (main analysis), and the risk of arterial stiffness. Initially, 61,035 participants with at least one baPWV test from January 2010 to June 2022 were included. Then, 2,135 participants with incomplete neutrophil count data and 2,140 participants who had a history of CVD (including myocardial infarction and stroke) were excluded, leaving 56,760 participants for the baseline baPWV analysis. From 27,519 participants with repeated baPWV measurements, 239 participants with less than 60 days of follow-up were further excluded to minimize confounding, as arterial stiffness progression is unlikely to occur within such a short timeframe of neutrophil exposure,[19,20] leaving 27280 participants for the baPWV progression analysis. Finally, 4,009 participants with a baseline arterial stiffness as defined by a baPWV value $\geq$1800 cm/s[21] and 8 participants with a follow-up period shorter than 60 days were excluded, resulting in 23,263 individuals for the analysis of arterial stiffness risk. The participants' selection process is illustrated in Figure 1. The first baPWV examination date was defined as the index date. The follow-up period for the analysis of baPWV progression commenced at the index date and was extended until the last baPWV examination date. Similarly, the follow-up period for the analysis of arterial stiffness risk began at the index date and continued until the occurrence of arterial stiffness or the last baPWV examination date.

Data Collection and Definition

Demographics, medical history, and lifestyle data were collected via a structural questionnaire. Current smoking was ascertained as smoking more than one cigarette daily over the past year. Current drinkers were defined as those who

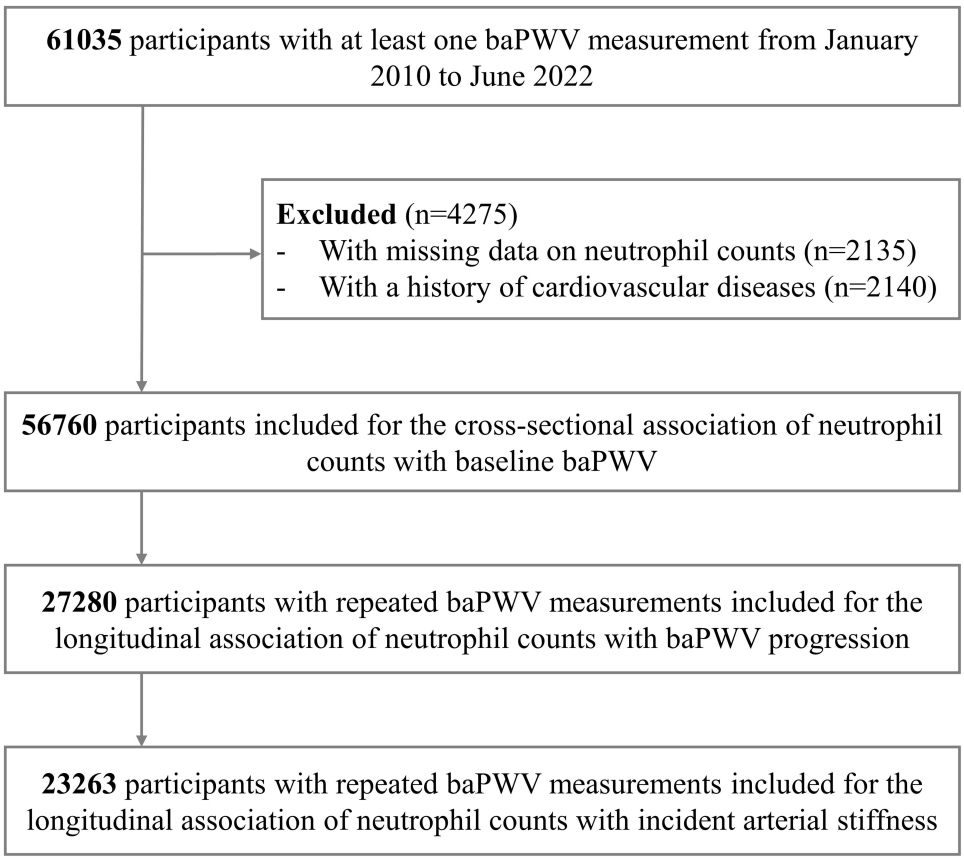

Figure 1 Flowchart of the study participants.
Abbreviation: baPWV, brachial-ankle pulse wave velocity.

drank at least once per month for the past six months.[22] Physical activity was classified as active or inactive, with active physical activity defined as having physical activity for ≥30 minutes per session and ≥3 times per week. Education level was categorized as less than high school, or high school or above. Anthropometric data, including weight and height, were assessed by well-trained staff. Body mass index (BMI) was determined as weight (kg) divided by height (m) squared.

RHR and blood pressure were assessed with participants sitting for at least 5 minutes. Before these measurements, participants refrained from smoking or consuming alcohol, tea, or coffee for at least 3 hours and had not engaged in physical exercise for the previous 30 minutes. A 10-second, 12-lead electrocardiogram was utilized to assess the RHR following 5 minutes of supine rest, with the results converted to beats per minute (bpm). Blood pressure was assessed on the left arm with a mercury sphygmomanometer, with the mean value of three readings recorded for both systolic blood pressure (SBP) and diastolic blood pressure (DBP). Hypertension was characterized by SBP or DBP measurement ≥140/90 mmHg, a medical history of hypertension, or the usage of antihypertensive agents.

Blood samples were obtained following an overnight fast of 8 to 12 hours. Neutrophil count measurements were obtained as part of routine blood tests using a Sysmex XT-1800i full blood count analyzer from Sysmex Corporation. Biochemical indices, such as fasting blood glucose, lipid profiles, and renal function, were assessed with a Hitachi 747 autoanalyzer (Hitachi, Tokyo, Japan). The Chronic Kidney Disease Epidemiology Collaboration creatinine equation was utilized to compute the estimated glomerular filtration rate (eGFR).[23] Diabetes was defined as a fasting blood glucose level of ≥7.0 mmol/L, a self-report of physician-diagnosed type 2 diabetes, or the usage of glucose-lowering agents.

BaPWV Measurement

In the Kailuan study, baPWV was assessed by a BP-203 RPE III networked arterial stiffness detection device manufactured by Omron Health Medical [China] Co., Ltd., as described previously.[18,24] Measurements were taken twice for all participants, with the second measurement as the definitive value. The higher baPWV value of the left and right sides was adopted for analysis.[21] The annual growth rate of baPWV was determined by the following equation: the change in baPWV (baPWV values at the final visit minus baseline baPWV values) was divided by the duration of follow-up in years.

Statistical Analysis

Normally distributed continuous data are described using the mean and standard deviation (SD), compared with the one-way ANOVA. Skewed continuous data are summarized by the median and interquartile range, compared with the Wilcoxon rank-sum test. Categorical variables are described as frequencies and percentages, compared with the $\chi 2$ test. To address the missing values in the covariates, as detailed in Supplementary Tables 1–3 where all covariates exhibited a missing rate of less than 6%, a multiple imputation technique utilizing chained equations was employed as previously described.[25]

Multivariable linear regression models were employed to examine the associations of neutrophil counts with baseline baPWV and the annual growth rate of baPWV. Additionally, multivariable Cox proportional hazards models were employed to determine the risk of incident arterial stiffness (hazard ratios (HRs) with 95% confidence intervals (CIs)) with exposure to neutrophil counts, assuming that the proportional hazards assumption holds. Two multifactorial adjustment models were constructed: Model 1, controlled for age and sex; Model 2, controlled for age, sex, BMI, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), eGFR, smoking, alcohol intake, education background, physical activity, diabetes, hypertension, and baseline baPWV (only for the analysis of baPWV progression). The variance inflation factor analysis indicated that multicollinearity among the covariates in Model 2 was found to be acceptable (Supplementary Figure 1). The assignment of covariables is shown in Supplementary Table 4, and the covariables were ascertained based on previously published high-quality studies.[21,26]

To examine the risk heterogeneity, the association of neutrophil counts with baPWV progression (main analysis) was further assessed with stratification by age, sex, BMI, diabetes status, and hypertension status. The potential interactions were evaluated by examining the statistical significance of multiplicative interaction terms between the stratified variables and neutrophil counts. To ascertain the reliability of the results, the following sensitivity analysis was conducted: (1) excluding participants with a follow-up period shorter than one year; (2) excluding participants with cancer at baseline; (3) excluding participants who took hypotensive, lipid-lowering, or hypoglycemic medications at baseline; and (4) excluding participants with incomplete covariate data and performing a full case analysis.

Mediation analyses were conducted to ascertain the indirect impact of neutrophil counts on arterial stiffness mediated through RHR. The linear model was utilized to examine the association between the predictor (neutrophil counts, continuous variable, per 1-SD increase) and the mediator (RHR, continuous variable). Linear models or logistic models were employed to evaluate associations of the predictor and mediator with outcomes, including baseline baPWV (continuous variable), baPWV progression (continuous variable), and arterial stiffness (binary variable), as deemed appropriate. The mediation analysis was conducted using the user-written command "medeff" in STATA, with the results calculated through bootstrapping with 1000 iterations.[27]

All analyses were conducted with Stata/MP 17.0 (Stata Corp LLC, College Station, TX). A bilateral $P<0.05$ was deemed statistically significant.

Results

Baseline Characteristics

Overall, 56,760 participants for the baseline baPWV analysis (mean age: 49.7±13.7 years), 27,280 participants for the baPWV progression analysis (mean age: 48.0±12.7 years), and 23,263 participants for the arterial stiffness risk analysis (mean age: 45.6 ±11.2 years) were enrolled in the present study. The baseline characteristics of participants involved in the baPWV progression analysis are compiled in Table 1. Compared with participants in the lowest quartile of neutrophil counts ($< 2.90*10^9$/L), participants in the highest quartile ($\geq 4.40*10^9$/L) were predominantly characterized by advanced age, male sex, current smokers, and current drinkers. In addition, they also exhibited higher levels of BMI, LDL-C, TG, RHR, and baPWV, along with lower

Table 1 Baseline Characteristics of Participants for the baPWV Progression Analysis

Characteristics	Neutrophil Counts, 10^9/L				*P*-value
	Quartile 1 (< 2.90)	Quartile 2 (≥ 2.90,<3.60)	Quartile 3 (≥ 3.60,<4.40)	Quartile 4 (≥ 4.40)	
Participants, n	7278	7120	6527	6355	
Age, years	47.7 (12.9)	47.9 (12.6)	48.1 (12.4)	48.2 (12.8)	<0.001
Male, n (%)	4347 (59.7)	4747 (66.7)	4639 (71.1)	4736 (74.5)	<0.001
BMI, kg/m^2	24.1 (3.33)	24.8 (3.38)	25.2 (3.49)	25.4 (3.60)	<0.001
HDL-C, mmol/L	1.47 (1.23, 1.77)	1.41 (1.19, 1.70)	1.38 (1.16, 1.66)	1.36 (1.15, 1.62)	<0.001
LDL-C, mmol/L	2.58 (2.07, 3.13)	2.63 (2.12, 3.18)	2.65 (2.15, 3.18)	2.67 (2.15, 3.21)	<0.001
TG, mmol/L	1.09 (0.75, 1.64)	1.25 (0.85, 1.92)	1.33 (0.90, 2.02)	1.42 (0.97, 2.20)	<0.001
eGFR, mL/min/1.73m^2	95.0 (26.6)	92.4 (26.3)	91.4 (26.6)	89.7 (27.6)	<0.001
Current smoker, n (%)	1628 (22.4)	1965 (27.6)	2068 (31.7)	2429 (38.2)	<0.001
Current drinker, n (%)	2806 (38.6)	2842 (39.9)	2788 (42.7)	2698 (42.5)	<0.001
Active physical activity, n (%)	1398 (19.2)	1332 (18.7)	1136 (17.4)	1064 (16.7)	<0.001
High school or above, n (%)	3430 (47.1)	3223 (45.3)	2881 (44.1)	2586 (40.7)	<0.001
Diabetes, n (%)	778 (10.7)	961 (13.5)	971 (14.9)	1047 (16.5)	<0.001
Hypertension, n (%)	1667 (22.9)	1952 (27.4)	1895 (29.0)	1994 (31.4)	<0.001
RHR, beats/min	75.8 (16.7)	76.5 (16.7)	77.7 (16.8)	80.2 (18.6)	<0.001
baPWV, cm/s	1439 (338)	1468 (332)	1491 (361)	1519 (359)	<0.001

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; RHR, resting heart rate; baPWV, brachial-ankle pulse wave velocity.

levels of HDL-C, eGFR, physical activity, and educational attainment. Furthermore, a higher prevalence of hypertension and diabetes was observed in this group. The baseline characteristics of participants in the other two analyses are detailed in Supplementary Tables 5 and 6.

Neutrophil Counts and Baseline baPWV

Overall, a notable positive association was observed between neutrophil counts and baseline baPWV, as indicated in Table 2. Linear regression analysis revealed that for each 1-SD increase in neutrophil count, there was a corresponding 13.5 cm/s increase in baPWV (95% CI: 11.1 to 15.9 cm/s, $P<0.001$) after adjusting for various confounders, including age, sex, BMI, lipid profile, renal function, lifestyle habits, education, and comorbidities including diabetes and hypertension. When neutrophil count was tested as a categorical pattern (quartiles), individuals in the second, third, and highest quartiles of neutrophil counts showed adjusted β values of 15.7 (95% CI: 9.03 to 22.4, $P<0.001$), 33.4 (95% CI: 26.4 to 40.4, $P<0.001$), and 55.4 (95% CI: 48.7 to 62.2, $P<0.001$) in the adjusted Model 2, compared to those in the first quartile.

Neutrophil Counts and baPWV Progression

We further examined the association between neutrophil counts and baPWV progression, indicating a notable rise in the annual growth rate of baPWV among participants in the fourth quartile of neutrophil counts, as compared to those in the first quartile (Table 2). Specifically, for each 1-SD increase in neutrophil count, there was a corresponding annual increase of 3.10 cm/s in baPWV after accounting for all covariates (95% CI: 1.51 to 4.69, $P<0.001$). When neutrophil counts were divided into quartiles, individuals in the second, third, and highest quartiles had β values of 3.90 (95% CI: −0.44 to 8.25, $P=0.078$), 5.18 (95% CI: 0.71 to 9.66, $P=0.023$), and 9.94 (95% CI: 5.39 to 14.5, $P<0.001$) in adjusted Model 2, respectively, compared to those in the first quartile.

Neutrophil Counts and Arterial Stiffness Risk

Over a median follow-up period of 4.08 (2.37, 6.21) years, there were 3,376 incidences of arterial stiffness. The incidence rate demonstrated an upward trend across the four groups categorized by quartiles of neutrophil count: 27.3, 30.2, 32.4, and 36.8 per 1,000 person-years, respectively. The associations between neutrophil counts and developing arterial stiffness are detailed in Table 2. After adjusting for confounders in Model 2, the risk of developing arterial stiffness increased by 7% (HR: 1.07; 95% CI:

Table 2 Association of Blood Neutrophil Counts with Baseline baPWV, baPWV Progression, and Incident Arterial Stiffness

Neutrophil Counts (10^9/L)	Baseline baPWV		Progression of baPWV		Arterial Stiffness	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value	HR (95% CI)	*P*-value
Model 1						
Q 1	Ref.		Ref.		Ref.	
Q 2	23.3 (16.4, 30.2)	< 0.001	5.08 (0.73, 9.42)	0.022	1.10 (0.99, 1.21)	0.063
Q 3	44.9 (37.7, 52.1)	< 0.001	6.86 (2.41, 11.3)	0.003	1.22 (1.10, 1.35)	< 0.001
Q 4	74.3 (67.4, 81.2)	< 0.001	12.5 (7.94, 17.0)	< 0.001	1.43 (1.30, 1.57)	< 0.001
P for trend	< 0.001		< 0.001		< 0.001	
Per SD increase	19.0 (16.6, 21.5)	< 0.001	3.85 (2.26, 5.43)	< 0.001	1.10 (1.07, 1.13)	< 0.001
Model 2						
Q 1	Ref.		Ref.		Ref.	
Q 2	15.7 (9.03, 22.4)	< 0.001	3.90 (−0.44, 8.25)	0.078	1.03 (0.93, 1.14)	0.865
Q 3	33.4 (26.4, 40.4)	< 0.001	5.18 (0.71, 9.66)	0.023	1.10 (0.99, 1.22)	0.073
Q 4	55.4 (48.7, 62.2)	< 0.001	9.94 (5.39, 14.5)	< 0.001	1.22 (1.11, 1.35)	0.004
P for trend	< 0.001		< 0.001		< 0.001	
Per SD increase	13.5 (11.1, 15.9)	< 0.001	3.10 (1.51, 4.69)	< 0.001	1.07 (1.04, 1.10)	0.001

Notes: Model 1 was adjusted for age (continuous) and sex (male or female); Model 2 was adjusted for age (continuous), sex (male or female), BMI (continuous), HDL-C (continuous), LDL-C (continuous), TG (continuous), eGFR (continuous), education level (less than high school, or high school or above), physical activity (active or inactive), current smoking (yes or no), current drinking (yes or no), diabetes status (yes or no), hypertension status (yes or no), and baseline baPWV (continuous; only for progression of baPWV). β indicates coefficients in regression analysis.
Abbreviations: HR, hazard ratio; CI, confidential intervals; other abbreviations are as in Table 1.

1.04 to 1.10, $P<0.001$) with each 1-SD increase in neutrophil count. Compared with participants in the lowest quartile, those in the highest quartile had an adjusted HR of 1.22 (95% CI: 1.11 to 1.35).

Subgroup Analysis and Sensitivity Analysis

In subgroup analyses, as presented in Supplementary Table 7, the observed positive association of neutrophil counts with baPWV progression remained consistent across various demographic and health factors, including age, sex, BMI, diabetes, and hypertension (all *P* values for interaction: ≥0.277). Sensitivity analyses further confirmed significant associations between neutrophil counts and baseline baPWV, baPWV progression, and the risk of arterial stiffness after excluding individuals with a follow-up period shorter than one year, with cancer at baseline, with the use of hypotensive, lipid-lowering, or hypoglycemic agents at baseline, or with missing covariate values. (Supplementary Table 8).

Mediation Analysis

Mediation analyses were utilized to investigate the mediated effect of RHR on the association between neutrophil counts and arterial stiffness. Positive correlations were observed between neutrophil counts and RHR, as well as between RHR and baseline baPWV, baPWV progression, and the risk of arterial stiffness. Ultimately, 20.0% (95% CI: 16.8% to 24.2%), 12.6% (95% CI: 8.16% to 26.4%), and 16.7% (95% CI: 9.94% to 51.0%) of the observed associations of neutrophil counts with baseline baPWV, baPWV progression, and arterial stiffness risk, respectively, were mediated by the RHR (Figure 2).

Discussion

This study comprehensively explored the association between neutrophil counts and arterial stiffness across the general population via diverse methodological approaches. Based on a cross-sectional analysis of 56,760 individuals and two longitudinal analyses involving over 20 thousand participants, two primary findings were obtained: (1) elevated circulating neutrophil counts were significantly associated with increased baselinebaPWV, a faster annual progression rate of baPWV, and a higher risk of arterial stiffness regardless of age, sex, BMI, HDL-C, LDL-C, TG, eGFR, smoking, alcohol use, education levels, physical activity, diabetes, and hypertension; and (2) RHR plays a mediating role in the association of neutrophil counts with arterial stiffness.

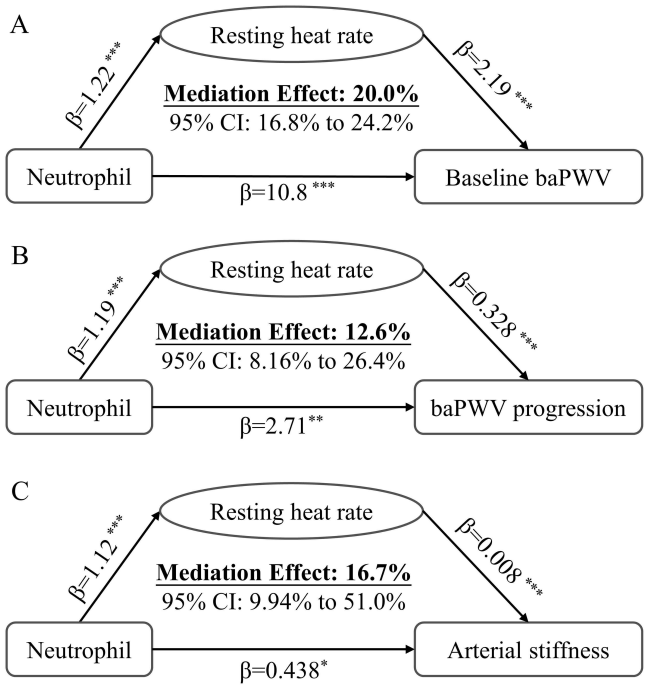

Figure 2 Mediation analyses.

Notes: The mediated effect of RHR on the association of neutrophil counts with baseline baPWV (**A**), the progression of baPWV (**B**), and the risk of developing arterial stiffness (**C**). All models were adjusted for age (continuous), sex (male or female), BMI (continuous), HDL-C (continuous), LDL-C (continuous), TG (continuous), eGFR (continuous), education levels (less than high school, or high school or above), physical activity (active or inactive), current smoking (yes or no), current drinking (yes or no), diabetes status (yes or no), hypertension status (yes or no), and baseline baPWV (continuous; only for progression of baPWV). "*" indicates "P < 0.05", "**" indicates "*P* < 0.01", and "***" indicates "*P* < 0.001". β indicates coefficients in regression analysis.

Abbreviation: abbreviations are as in Table 1.

As a result of reduced arterial wall elasticity, arterial stiffness is mainly attributed to changes in extracellular matrix proteins, including elastin fragmentation and collagen accumulation.[28] Converging studies have elucidated an association of increased arterial stiffness with an elevated risk of subsequent CVD and cardiovascular-related mortality.[5,29–31] As the pathology of arterial stiffening is a chronic progressive process, identifying susceptible individuals at an early stage via reliable biomarkers is crucial for the prevention of atherosclerosis and subsequent CVD.

Neutrophil counts are commonly evaluated in routine blood tests to provide valuable insights into systemic inflammatory status in clinical settings, and positive associations between neutrophil counts and the risk of CVD have been repeatedly confirmed.[26,32,33] In a recent large-scale epidemiological study,[26] Luo et al further solidified the association between neutrophil numbers and CVD risk with an observational analysis of 110,000 individuals and, for the first time, provided causal evidence supporting neutrophil counts as drivers of CVD using Mendelian randomization analyses.

To date, there has been no study reported on the association of neutrophil counts with arterial stiffness. In our study, the cross-sectional analysis provides the first evidence of positive associations between neutrophil counts and increased baPWV. Furthermore, longitudinal analyses revealed a significant independent association between neutrophil counts and baPWV progression and the onset of arterial stiffness. These results align with prior epidemiological studies that showed positive associations between other inflammatory markers (such as hs-CRP, interleukin, and monocyte-to-lymphocyte ratio) and arterial stiffness.[8–10] Our results indicate that neutrophil count is a potential risk candidate for arterial stiffness, a condition that is often silent and difficult to detect in its early stages. Therefore, this cost-effective method could aid in the early identification of arterial stiffness.

The association of neutrophil counts with arterial stiffness could be attributed to inflammation, as evidenced by epidemiological studies linking elevated levels of circulating inflammation to heightened arterial stiffness.[34–36] Indeed, inflammation is believed to promote endothelial dysfunction, smooth muscle cell proliferation, elastin fragmentation, collagen accumulation, and vascular fibrosis, ultimately resulting in increased arterial stiffness.[3,37] Remarkably, our results provide novel insight into the underlying mechanism of the above association beyond inflammation. Through mediation analysis, it was determined that

neutrophil counts contribute to the progression of arterial stiffness partially by elevating the RHR (Figure 2). The RHR serves as an indicator of autonomic nervous system function, with an increased RHR suggesting heightened sympathetic nervous system activity.[38] Previous studies have reported a positive association between indices of inflammation, such as CRP or interleukin levels, with the RHR, suggesting that inflammation might influence heart rate by affecting autonomic nervous activity.[14,15] Furthermore, consistent with our study, previous evidence from epidemiology studies as well as animal studies showed that a higher RHR was related to decreased arterial distensibility.[11,13,39,40] In a 6-year prospective study of patients who received treatment for hypertension, an elevated RHR was found to be significantly associated with an accelerated progression of arterial stiffness.[11] Another cohort study of 12,554 participants demonstrated that trajectory patterns with a higher level of RHR were associated with a greater arterial stiffness risk.[13] Mangoni and his colleagues[40] demonstrated that atrial pacing-induced elevated heart rates in rats result in significant decreases in carotid artery compliance. These studies indicate that increased RHR plays a key role in the development of arterial stiffness associated with systemic inflammation.

Strengths and Limitations

This study presents the first evidence regarding the association of neutrophil counts with arterial stiffness as evaluated by baPWV. By utilizing both cross-sectional and longitudinal designs, this study comprehensively investigated the association of neutrophil counts with arterial stiffness incidence and progression in a real-life, population-based cohort, which may contribute to the early identification of individuals vulnerable to arterial stiffness, thereby facilitating the implementation of targeted interventions to prevent CVD. Furthermore, this study revealed that neutrophil counts may contribute to the progression of arterial stiffness by increasing RHR, providing new insights into the underlying mechanisms of this association. Other merits of the study comprise the prospectively designed cohort, large sample size, high-quality longitudinal data, and relatively long-term follow-up.

It is essential to recognize the inherent constraints of the study. First, due to its observational nature, our analysis was unable to establish a causal association between neutrophil counts and arterial stiffness. Second, despite the inclusion of numerous demographic and clinical variables for adjustment, the possibility of residual or unmeasured confounders influencing the results cannot be ruled out. Third, the arterial stiffness onset date was ascertained by the baPWV measurement date, possibly leading to a discrepancy from the actual onset date, which may lead to an underestimation of the relationship between neutrophil counts and arterial stiffness risk. Therefore, additional study is essential to clarify the precise associations involved. Fourth, the lack of data on inflammatory conditions, such as rheumatoid arthritis and the use of steroids, constrained our capacity to evaluate their potential impact on the research findings. Finally, it should be noted that the study sample was drawn from the Kailuan Study conducted in China, implying that the results may not be generalizable to wider ethnic populations.

Conclusion

For the first time, the study demonstrated a positive association of neutrophil counts with arterial stiffness progression among the general population in northern China. Importantly, the RHR was identified as a potential mediator in the association between neutrophil counts and arterial stiffness. These findings imply that neutrophil counts may be a valuable and easily accessible marker for identifying individuals at increased risk for arterial stiffness and subsequent CVD.

Abbreviations

HR, hazard ratio; CI, confidential intervals; SD, standard deviation; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; RHR, resting heart rate; baPWV, brachial-ankle pulse wave velocity.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Ethics Approval and Consent to Participate

The Kailuan study was approved by the Kailuan General Hospital's ethics committee (No. 200605). Before participation, all individuals provided written informed consent.

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Disclosure

The authors declared no conflicts of interest in this work.

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