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Associations of Baseline and Changes in Leukocyte Counts with Incident Cardiovascular Events: The Dongfeng-Tongji Cohort Study

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Aim: The aim of the present study was to investigate the associations of baseline and longitudinal changes in leukocyte counts with incident cardiovascular disease (CVD).

Methods: We conducted a prospective study to investigate the associations of baseline and 5-year changes in leukocyte counts with incident CVD and its subtypes in middle-aged and elderly Chinese. We estimated the hazard ratios (HRs) and 95% confidence intervals (CIs) for CVD using the Cox proportional-hazards models.

Results: In the analyses of baseline total leukocyte count of 26,655 participants, compared with the lowest quartile ($<4.71\times10^9$/L), participants in the fourth quartile ($>6.70\times10^9$/L) had 11% higher risk for CVD. Consistent with total leukocyte count, neutrophil count also exhibited a significant positive association with the risk of CVD. In the analyses of 5-year changes in total leukocyte count of 11,594 participants, the changes in leukocyte count were categorized into three groups, i.e., the decreased group (<25%), stable group (25%–75%), and increased group (>75%). Compared with participants in the stable group (–1.18 to 0.44×10^9/L), participants in the increased group ($>0.44\times10^9$/L) had 14% higher risk for CVD. We also observed significant positive associations of the changes in neutrophil and monocyte counts with the risk of CVD. Furthermore, the total leukocyte count in the second or third tertile at the first follow-up with a 5-year increase was related to higher CVD risk.

Conclusion: High baseline total leukocyte count and a 5-year increase in total leukocyte count were related to higher CVD risk.

Key words: Leukocyte counts, Change, Prospective cohort, Cardiovascular disease

Introduction

Cardiovascular disease (CVD) is the leading cause of disease burden worldwide, and it is widely accepted as an inflammatory and immune disease[1, 2]. Leukocyte, one of the most low-cost and widely used markers of immune and inflammatory response, is recognized as a major contributor to a series of pathological processes of CVD, including oxidative stress, atherosclerotic plaque formation, plaque rupture, endothelial erosion, and thrombus formation[3-6]. Previous epidemiologic studies have demonstrated that several inflammatory markers, including high-sensitivity C-reactive protein and interleukin-6, are related to increased CVD risk[7-9]. In addition, randomized clinical trials have been conducted to prevent CVD through the inhibition of inflammation pathways[10, 11].

Several previous studies have demonstrated that total leukocyte count was epidemiologically associated with the risk of CVD events, but the results of these studies were inconsistent. The majority of previous

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studies found that high total leukocyte count was associated with greater risk of coronary heart disease (CHD) or stroke incidence[12-22], whereas others failed to find any significant association[23, 24]. Meanwhile, evidence on the relation of total leukocyte count with acute coronary syndrome (ACS) incidence is scarce, although ACS is one of the life-threatening subtypes of CHD. Results of the relation between leukocyte counts and stroke, such as ischemic stroke (IS) and hemorrhagic stroke (HS), were also inconsistent[20, 25, 26].

Conversely, limited prospective studies have reported conflicting relation of differential leukocyte counts with CVD incidence. Several studies demonstrated that higher neutrophil count increased the risk of CVD incidence[27-29], and monocyte count was also reported as a risk factor for CVD, CHD, and IS[15, 30, 31]. However, eosinophil and lymphocyte counts were reported to be inversely associated with the risk of CVD[32]. Moreover, the neutrophil-to-lymphocyte ratio (NLR) is a systemic inflammation biomarker that could balance the opposite effects of innate immunity (neutrophils) and adaptive immunity (lymphocytes) on arterial atherosclerosis[33]. Elevated NLR was reported to be linked to CHD incidence[34]. Together, associations of total and differential leukocyte counts with incident CVD and its subtypes remain to be elucidated.

Furthermore, different leukocyte subtypes exhibit lifespans varying from several hours to decades[35-38], which may change in response to age, sex, obesity, lifestyles such as smoking and drinking, and environmental factors[39-41], thus accelerating or decelerating CVD progression. To date, few studies have prospectively assessed the relationship of long-term changes in total leukocyte count with the risk of CVD events[42-44], and existing studies only reported significant associations of leukocyte count changes with incident CHD and mortality[42, 44]. Studies on the associations of longitudinal changes in total and differential leukocyte counts with other CVD subtypes are still lacking.

Therefore, in the present study, we aimed to investigate the independent associations of baseline and 5-year changes in total and differential leukocyte counts with incident CVD and its subtypes. We further explored whether total leukocyte count at the first follow-up and 5-year changes in total leukocyte count were jointly associated with CVD events in middle-aged and elderly Chinese population.

Materials and Methods

Study Population

This study was based on the Dongfeng-Tongji (DFTJ) cohort in Shiyan City, China. As described elsewhere[45], the DFTJ cohort is a prospective cohort to investigate the causes and progression of chronic diseases. The cohort enrolled 27,009 retirees at baseline from the Dongfeng Motor Corporation during September 2008 to June 2010 and then newly recruited 14,120 retirees at the first follow-up in 2013. Finally, questionnaires and blood samples of 41,129 participants at baseline or the first follow-up were collected when they joined the cohort for the first time. Among the 41,129 participants, we excluded participants who were diagnosed with CHD (n=5,468), stroke (n=1,972), cancer (n=2,182), and severely abnormal electrocardiogram (n=674) when they first joined the cohort and who were lost to follow-up (n=709). Since some of the participants may simultaneously have two or more diseases that were described above, we finally excluded 9,378 participants in this step. We further excluded 5,096 participants with missing data of total leukocyte count (n=5,088), with total leukocyte count $>20\times10^9$/L (n=4), and with total leukocyte count $<2\times10^9$/L (n=4). Finally, we enrolled 26,655 participants in the analyses of baseline total leukocyte count (including participants at baseline and the first follow-up when they first joined the cohort). Furthermore, the baseline characteristics were similar between the overall 26,655 participants and the 5,096 participants who were excluded due to missing and extreme values of total leukocyte count **(Supplementary Table 1)**. For the 24,175 individuals who participated in both the baseline and the first follow-up of the DFTJ cohort, we excluded those with self-reported CHD, stroke, cancer, or severely abnormal electrocardiogram at or prior to the first follow-up (n=5,737). Participants with missing information on total leukocyte count, with total leukocyte count $<2\times10^9$/L, and with total leukocyte count $>20\times10^9$/L were also excluded (n=6,844). Finally, 11,594 participants were incorporated into the analyses of the association of the changes in total leukocyte count with incident CVD and its subtypes **(Supplementary Fig. 1)**.

Written informed consent was obtained from all participants, and this study was reviewed and approved by the Ethics and Human Subject Committee of Tongji Medical College, Huazhong University of Science and Technology (2012-10) and Sinopharm Dongfeng General Hospital.

Measurement of Leukocyte Counts

Total and differential leukocyte counts were measured at two time points (baseline survey during 2008–2010 and the first follow-up in 2013) with an average interval of 4.60 years using a fully automated

analyzer CELL-DYN 3700 (Abbott Laboratories. Abbott Park, Illinois, USA) of Sinopharm Dongfeng General Hospital. NLR was calculated as the neutrophil count divided by the lymphocyte count. The 5-year changes in total and differential leukocyte counts were defined as measurements at the first follow-up in 2013 minus that at baseline during 2008–2010. Baseline measurements were categorized into four groups according to the quartiles, and changes in total and differential leukocyte counts were categorized into three groups according to the 25th and 75th percentiles, i.e., the decreased group (< 25%), stable group (25%–75%), and increased group (>75%).

Ascertainment of Outcomes

The outcomes in this study were defined and classified based on the International Classification of Diseases (ICD) codes of the World Health Organization[46]. The outcome of interest was incident CVD, including CHD (ICD-10: I20-I25) and stroke (ICD-10: I60-I61, I63-I64, I69.0-I69.1, and I69.3-I69.4), which firstly occurred from baseline to the end of follow-up (31 Dec 2018). The Dongfeng Motor Corporation healthcare system covered all the retired employees and allowed us to track incident CVD through medical insurance information. We defined incident CHD as the first occurrence of fatal CHD, nonfatal myocardial infarction, stable and unstable angina, or coronary revascularization (coronary artery bypass graft or percutaneous transluminal coronary angioplasty) during follow-up[47]. ACS diagnosis was confirmed according to the diagnostic criteria for acute myocardial infarction and unstable angina[48, 49]. Stroke was defined as sudden or rapid onset of a typical neurological deficit of vascular origin that persisted for more than 24 h or death from stroke[50]. Based on the evidence from computed tomography and/or magnetic resonance imaging, stroke was further classified into IS or HS by expert physicians[51].

Assessment of Covariates

Baseline information (demographic characteristics, lifestyles, family history of CVD, and medication usage) of the DFTJ cohort was obtained using semi-structured questionnaires. Anthropometric indices, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured by trained personnel. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Participants who had been smoking at least one cigarette per day for at least 6 months were defined as current smokers. Participants who had been drinking at least one time per week for more than 6 months were regarded as current drinkers. Physical activity was identified as regular exercise more than five times per week and at least 30 min per time for more than 6 months. Education status was coded as primary school or below, junior high school, senior high school, or higher. Hypertension was defined as a self-reported physician diagnosis of hypertension, SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or intake of anti-hypertensive medications. Hyperlipidemia was defined as total cholesterol ≥ 6.22 mmol/L, triglycerides > 2.26 mmol/L, high-density lipoprotein cholesterol < 1.04 mmol/L, low-density lipoprotein cholesterol ≥ 4.14 mmol/L, intake of lipid-lowering medications, or a self-reported physician diagnosis of hyperlipidemia. Diabetes mellitus was defined as self-reported physician diagnosis of diabetes mellitus, fasting glucose ≥ 7.0 mmol/L, or intake of anti-diabetic medications (oral hypoglycemic medication or insulin). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease equation[52].

Statistical Analysis

Cox proportional-hazards regression models were employed to assess the associations of baseline and 5-year changes in total and differential leukocyte counts with CVD events. The outcomes of interest were followed up until 31 Dec 2018, and the results were expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). In the analyses of the associations between baseline leukocyte counts and incident CVD, potential covariates in the multivariable models were collected at the time when the participants were first recruited (baseline or the first follow-up), including age, sex, BMI, smoking status, drinking status, physical activity, education, hyperlipidemia, hypertension, diabetes mellitus, family history of CVD/CHD/stroke, and medications that may affect leukocyte counts (antibiotics or aspirin), and we additionally adjusted for admission batch (baseline or the first follow-up) in the models. In the analyses of the associations between 5-year changes in leukocyte counts and incident CVD, potential covariates in the multivariable models were collected at the first follow-up in 2013, including age, sex, BMI, smoking status, drinking status, physical activity, education, hyperlipidemia, hypertension, diabetes mellitus, family history of CVD/CHD/stroke, and medications that may affect leukocyte counts (antibiotics or aspirin), and we additionally adjusted for baseline leukocyte counts in the models. Missing data of covariates were filled using imputation methods. For continuous variables, the median values

were used as replacement of the missing values, and we additionally adjusted for a binary variable indicating whether the observation is a missing value, whereas for categorical variables, an extra group was added to replace the missing values. Person-years was calculated from the date of recruitment until the date of the first onset of CVD event, the date of death, or the end of follow-up, whichever came first. Interaction and stratified analyses were separately conducted by age (< 60, ≥ 60 years), sex (men, women), BMI (< 24, ≥ 24 kg/m^2), current smoker (yes, no), current drinker (yes, no), hypertension (yes, no), hyperlipidemia (yes, no), and diabetes mellitus (yes, no). To avoid the effect of baseline inflammatory diseases on leukocyte counts, sensitivity analyses were conducted by excluding participants with baseline diseases, including gout, major rheumatic diseases, and end-stage renal disease (eGFR < 30 mL/min/1.73 m^2). We further calculated intra-class correlation coefficients (ICCs) to assess the consistency between the two measurements of total and differential leukocyte counts. Restricted cubic splines with three knots (5th, 50th, 95th) were used to display the relations of baseline and 5-year changes in total leukocyte count with the risk of CVD, CHD, and stroke.

Furthermore, based on the 11,594 participants in the analyses of changes in total leukocyte count with the risk of CVD, we assessed the joint effects of total leukocyte count at the first follow-up and 5-year changes in total leukocyte count on the risk of CVD events. Total leukocyte count at the first follow-up was categorized into tertiles (low, moderate, and high), together with decreased (< 25%), stable (25%–75%), and increased (> 75%) groups in the analyses of 5-year changes in total leukocyte counts, resulting in nine subcategories. We used low total leukocyte level at the first follow-up and a relatively stable change as the reference group. All statistical analyses were conducted using SAS version 9.4 (SAS institute Inc., Cary, NC). A two-sided *P* value < 0.05 was considered statistically significant.

Results

Basic characteristics of baseline and 5-year changes in total leukocyte count

Among the 26,655 participants in this study, the mean age was 61.5 years (SD = 8.1), and 44.1% were men. Participants in the highest quartile were more likely to be older, men, current smokers and drinkers, with low educational level, and with higher prevalence of hyperlipidemia, hypertension, and diabetes mellitus. Meanwhile, participants who experienced an increase in total leukocyte count were more likely to be men, current smokers and drinkers, and with higher prevalence of diabetes mellitus **(Table 1)**.

Associations of Baseline Total and Differential Leukocyte Counts with CVD Events

During a median follow-up of 10.26 years (interquartile range [IQR], 7.06–10.31 years) for participants who joined the cohort at baseline, and a median follow-up of 5.69 years (IQR, 5.60–5.71 years) for participants who joined the cohort at the first follow-up, we identified 7,285 incident CVD cases, including 5,789 CHD (2,330 ACS) and 1,496 stroke cases (1,170 IS and 326 HS), among 26,655 participants with baseline total leukocyte count. The CVD incidence was slightly higher than that in other Chinese populations[53-55], mainly due to the high mean age among the participants and the relatively comprehensive ascertainment of incident CVD through medical insurance documents, hospital records, and death certificates. As presented in **Table 2**, after multivariate adjustment for potential confounders, total leukocyte count was associated with incident CVD (HR, 1.03; 95% CI, 1.01–1.05), CHD (HR, 1.03; 95% CI, 1.01–1.05), ACS (HR, 1.05; 95% CI, 1.02–1.08), stroke (HR, 1.04; 95% CI, 1.01–1.08), and IS (HR, 1.06; 95% CI, 1.02–1.10) per 10^9/L increase in total leukocyte count; however, the association was not significant for HS (HR, 0.99; 95% CI, 0.92–1.07).

In stratified Cox proportional-hazards regression models, we found that the CVD risk of increased total leukocyte count was significantly higher among men and current smokers (the *P* values for interaction were 0.05 and 0.03, respectively; **Supplementary Fig. 2**). Therefore, we stratified the analyses of the associations between baseline total leukocyte count and the risk of CVD events by sex. High total leukocyte count was associated with incident CVD (HR, 1.04; 95% CI, 1.02–1.06), CHD (HR, 1.03; 95% CI, 1.01–1.06), ACS (HR, 1.05; 95% CI, 1.01–1.08), stroke (HR, 1.05; 95% CI, 1.01–1.09), and IS (HR, 1.07; 95% CI, 1.02–1.12) per 10^9/L increase in total leukocyte count in men, whereas in women, high total leukocyte count was only associated with incident CVD (HR, 1.02; 95% CI, 1.00–1.04) and ACS (HR, 1.06; 95% CI, 1.02–1.10). Restricted cubic spline plots also demonstrated significant linear associations of baseline total leukocyte count with CVD, CHD, and stroke **(Fig. 1)**. Compared with participants in the lowest quartile of total leukocyte count (< 4.71 × 10^9/L), the HRs (95% CIs) of those in the highest quartile (> 6.70 × 10^9/L) were 1.11 (1.03–1.19) (P_{trend} = 0.001) for CVD, 1.10 (1.02–1.19) (P_{trend} = 0.023) for CHD, 1.21 (1.07–1.38) (P_{trend} = 0.002) for ACS, and 1.22 (1.02–

Table 1. Basic characteristics of study participants for baseline total leukocyte count and changes in total leukocyte count

Characteristics	Quartiles of total leukocyte count, $\times 10^9$/L				*P* value	Changes in total leukocyte count, $\times 10^9$/L			*P* value
	<4.71	4.71–5.60	5.61–6.70	>6.70		<−1.18	−1.18 to 0.44	>0.44	
N	6802	6542	6865	6446		2792	5973	2829	
Age (years)	60.1 ± 7.9	61.2 ± 8.0	62.0 ± 8.1	62.8 ± 8.1	<0.001	65.9 ± 7.6	65.7 ± 7.3	65.5 ± 7.3	0.118
Men (%)	2335 (34.3)	2693 (41.2)	3238 (47.2)	3496 (54.2)	<0.001	1436 (51.4)	3511 (58.8)	1702 (60.2)	<0.001
BMI (kg/m^2)	23.4 ± 3.1	24.0 ± 3.2	24.4 ± 3.2	24.9 ± 3.5	<0.001	24.0 ± 3.4	24.1 ± 3.3	24.2 ± 3.4	0.063
Education (%)									
Primary school or below	1272 (18.7)	1432 (21.9)	1569 (22.9)	1584 (24.6)	<0.001	806 (28.9)	1678 (28.1)	872 (30.8)	0.187
Middle school	2382 (35.0)	2355 (36.0)	2560 (37.3)	2373 (36.8)		1038 (37.2)	2237 (37.5)	1052 (37.2)	
High school or beyond	3112 (45.8)	2702 (41.3)	2693 (39.2)	2442 (37.9)		925 (33.1)	2013 (33.7)	883 (31.2)	
Smoking status (%)									
Current smokers	798 (11.7)	1063 (16.2)	1391 (20.3)	1850 (28.7)	<0.001	530 (19.0)	862 (14.4)	395 (14.0)	<0.001
Former smokers	532 (7.8)	648 (9.9)	700 (10.2)	717 (11.1)		344 (12.3)	721 (10.4)	300 (10.6)	
Never smokers	5445 (80.0)	4808 (73.5)	4750 (69.2)	3863 (59.9)		1889 (67.7)	4421 (74.0)	2098 (74.2)	
Drinking status (%)									
Current drinkers	1524 (22.4)	1560 (23.8)	1793 (26.1)	1730 (26.8)	<0.001	761 (27.3)	1481 (24.8)	734 (22.4)	0.001
Former drinkers	241 (3.5)	298 (4.4)	299 (4.4)	357 (5.5)		154 (5.5)	276 (4.6)	136 (4.8)	
Never drinkers	5024 (73.9)	4671 (71.4)	4767 (69.4)	4350 (67.5)		1846 (66.1)	4145 (69.4)	2028 (71.7)	
Physical activity (%)	4810 (70.7)	4698 (71.8)	4903 (71.4)	4528 (70.2)	<0.001	2073 (74.2)	4383 (73.4)	2074 (73.3)	0.605
Antibiotics use (%)	593 (8.7)	496 (7.6)	500 (7.3)	463 (7.2)	0.003	222 (8.0)	459 (7.7)	193 (7.8)	0.050
Aspirin use (%)	573 (8.4)	618 (9.4)	677 (9.9)	713 (11.1)	<0.001	352 (12.6)	761 (11.1)	318 (11.2)	0.076
Family history of CVD (%)	943 (13.9)	752 (11.5)	715 (10.4)	617 (9.6)	<0.001	218 (7.8)	528 (8.8)	217 (7.7)	0.098
Hyperlipidemia (%)	2176 (32.0)	2554 (39.0)	3008 (43.8)	3258 (50.5)	<0.001	1100 (39.4)	2410 (40.3)	1201 (42.5)	0.054
Hypertension (%)	2844 (41.8)	3183 (48.7)	3550 (51.7)	3898 (60.5)	<0.001	1761 (63.1)	3707 (62.1)	1801 (63.7)	0.417
Diabetes mellitus (%)	763 (11.2)	933 (14.3)	1224 (17.8)	1535 (23.8)	<0.001	566 (20.3)	1066 (17.8)	582 (20.6)	0.008
Lymphocyte count, $\times 10^9$/L	1.4 (1.2–1.7)	1.7 (1.5–2.0)	2.0 (1.6–2.3)	2.3 (1.9–2.8)	<0.001	1.6 (1.1–2.2)	1.7 (1.1–2.2)	2.0 (1.2–2.7)	<0.001
Monocyte count, $\times 10^9$/L	0.3 (0.2–0.3)	0.4 (0.3–0.4)	0.4 (0.3–0.5)	0.5 (0.4–0.6)	<0.001	0.3 (0.2–0.5)	0.3 (0.0–0.5)	0.4 (0.2–0.6)	<0.001
Neutrophil count, $\times 10^9$/L	2.3 (2.0–2.6)	3.0 (2.6–3.3)	3.6 (3.2–4.0)	4.7 (4.1–5.4)	<0.001	3.0 (2.1–4.0)	3.1 (2.2–4.1)	4.1 (2.7–5.5)	<0.001
Eosinophil count, $\times 10^9$/L	0.1 (0.1–0.1)	0.1 (0.1–0.2)	0.1 (0.1–0.2)	0.1 (0.1–0.2)	<0.001	0.1 (0.0–0.3)	0.1 (0.0–0.3)	0.1 (0.0–0.3)	<0.001
Basophil count, $\times 10^9$/L	0.1 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.1–0.1)	<0.001	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.2 (0.0–0.3)	<0.001
Neutrophil-to-Lymphocyte Ratio	1.6 (1.2–2.1)	1.7 (1.3–2.2)	1.8 (1.4–2.4)	2.0 (1.5–2.7)	<0.001	2.1 (0.6–3.6)	2.1 (1.1–3.1)	2.4 (1.1–3.7)	<0.001

Abbreviation: BMI, body mass index; CVD, cardiovascular disease.
Continuous variables were described as mean ± SD if normally distributed, or median (interquartile range) if skewed distributed. Categorical variables were described as number (%).
P values were derived from ANOVA tests for continuous variables, and Chi-square test for the category variables.

1.45) (P_{trend}=0.001) for IS. When we restrict the analyses to men, the HRs (95% CIs) of those in the highest quartile were 1.17 (1.06–1.30) (P_{trend}=0.002) for CVD, 1.17 (1.05–1.32) (P_{trend}=0.008) for CHD, 1.26 (1.05–1.52) (P_{trend}=0.013) for ACS, and 1.31 (1.04–1.64) (P_{trend}=0.001) for IS; however, when we restrict the analyses to women, no significant association was observed.

In sensitivity analyses, after excluding participants with gout, major rheumatic diseases, and end-stage renal disease, the associations of total leukocyte count with incident CVD, CHD, and stroke did not materially change **(Supplementary Fig. 2)**. The associations between other differential leukocyte counts and the risk of CVD events are presented in **Supplementary Tables 2–3**.

Associations of Longitudinal Changes in Total and Differential Leukocyte Counts with CVD Events

During a median follow-up of 5.69 years (IQR, 4.81–5.71 years) from the first follow-up in 2013, we identified 2,971 incident CVD cases, including 2,461 CHD (916 ACS) and 510 stroke (404 IS and 106 HS) cases among 11,594 participants. Within a 4.60-year period between baseline and the first follow-up, the participants experienced a decrease of 0.37 × 10^9/L in total leukocyte count on average, and the reproducibility of measurements at baseline and the first follow-up was moderate for total leukocyte count (ICC=0.58), neutrophil count (ICC=0.53),

Table 2. Adjusted HRs (95% CIs) of cardiovascular events according to quartiles of total leukocyte count in men and women

Quartiles of total leukocyte count, $\times 10^9$/L	Q1 (<4.71)	Q2 (4.71–5.60)	Q3 (5.61–6.70)	Q4 (>6.70)	*P* for trend	Per 10^9/L increase
CVD						
All						
Cases/person-years	1464/47376	1673/46946	1953/49467	2195/45921		
HR (95% CI)	1.00 (ref)	1.01 (0.94–1.08)	1.04 (0.97–1.11)	1.11 (1.03–1.19)	0.001	1.03 (1.01–1.05)
Men						
Cases/person-years	597/15396	785/18551	1045/22400	1289/24026		
HR (95% CI)	1.00 (ref)	1.01 (0.91–1.13)	1.08 (0.98–1.20)	1.17 (1.06–1.30)	0.002	1.04 (1.02–1.06)
Woman						
Cases/person-years	867/31980	888/28395	908/27067	906/21895		
HR (95% CI)	1.00 (ref)	1.00 (0.91–1.10)	1.00 (0.91–1.10)	1.04 (0.95–1.15)	0.390	1.02 (1.00–1.04)
CHD						
All						
Cases/person-years	1187/48405	1375/47810	1539/50665	1688/47360		
HR (95% CI)	1.00 (ref)	1.05 (0.97–1.14)	1.05 (0.97–1.13)	1.10 (1.02–1.19)	0.023	1.03 (1.01–1.05)
Men						
Cases/person-years	445/15930	609/19040	779/23166	933/25009		
HR (95% CI)	1.00 (ref)	1.09 (0.96–1.23)	1.12 (0.99–1.26)	1.17 (1.05–1.32)	0.008	1.03 (1.01–1.06)
Woman						
Cases/person-years	742/32475	766/28771	760/27499	755/22351		
HR (95% CI)	1.00 (ref)	1.03 (0.93–1.14)	1.00 (0.90–1.10)	1.04 (0.94–1.16)	0.581	1.02 (0.99–1.04)
ACS						
All						
Cases/person-years	409/43460	528/42562	654/44985	739/40991		
HR (95% CI)	1.00 (ref)	1.10 (0.96–1.25)	1.16 (1.02–1.31)	1.21 (1.07–1.38)	0.002	1.05 (1.02–1.08)
Men						
Cases/person-years	169/14063	258/16903	349/20374	429/21508		
HR (95% CI)	1.00 (ref)	1.13 (0.93–1.37)	1.19 (0.99–1.44)	1.26 (1.05–1.52)	0.013	1.05 (1.01–1.08)
Woman						
Cases/person-years	240/29396	270/25659	305/24610	310/19483		
HR (95% CI)	1.00 (ref)	1.07 (0.90–1.27)	1.12 (0.94–1.33)	1.16 (0.98–1.39)	0.084	1.06 (1.02–1.10)
Stroke						
All						
Cases/person-years	277/51317	298/51547	414/54683	507/51577		
HR (95% CI)	1.00 (ref)	0.88 (0.75–1.04)	1.04 (0.89–1.22)	1.15 (0.99–1.34)	0.006	1.04 (1.01–1.08)
Men						
Cases/person-years	152/16822	176/20574	266/24977	356/27032		
HR (95% CI)	1.00 (ref)	0.86 (0.69–1.07)	1.03 (0.84–1.26)	1.17 (0.96–1.43)	0.007	1.05 (1.01–1.09)
Woman						
Cases/person-years	125/34495	122/30974	148/29706	151/24545		
HR (95% CI)	1.00 (ref)	0.93 (0.72–1.20)	1.08 (0.85–1.37)	1.08 (0.84–1.39)	0.338	1.03 (0.97–1.09)
IS						
All						
Cases/person-years	203/51003	224/51201	337/54311	406/51110		
HR (95% CI)	1.00 (ref)	0.90 (0.74–1.09)	1.14 (0.95–1.36)	1.22 (1.02–1.45)	0.001	1.06 (1.02–1.10)
Men						
Cases/person-years	108/16645	136/20387	214/24746	291/26734		
HR (95% CI)	1.00 (ref)	0.92 (0.71–1.19)	1.14 (0.90–1.43)	1.31 (1.04–1.64)	0.001	1.07 (1.02–1.12)
Woman						
Cases/person-years	95/34358	88/30814	123/29565	115/24376		
HR (95% CI)	1.00 (ref)	0.88 (0.66–1.18)	1.17 (0.89–1.54)	1.07 (0.81–1.43)	0.319	1.04 (0.97–1.10)
HS						
All						
Cases/person-years	74/50433	74/50490	77/53097	101/49743		
HR (95% CI)	1.00 (ref)	0.85 (0.62–1.18)	0.78 (0.57–1.08)	0.96 (0.70–1.31)	0.938	0.99 (0.92–1.07)
Men						
Cases/person-years	44/16351	40/19912	52/23987	65/25733		
HR (95% CI)	1.00 (ref)	0.71 (0.46–1.09)	0.77 (0.51–1.15)	0.85 (0.57–1.27)	0.764	0.99 (0.90–1.09)
Woman						
Cases/person-years	30/34082	34/30578	25/29110	36/24009		
HR (95% CI)	1.00 (ref)	1.09 (0.66–1.78)	0.78 (0.46–1.34)	1.12 (0.67–1.86)	0.840	1.00 (0.88–1.12)

Abbreviation: CVD, cardiovascular disease; CHD, coronary heart disease; ACS, acute coronary syndrome; IS, ischemic stroke; HS, hemorrhagic stroke.
P for trend was calculated when we assigned the median value to each quartile and entered this as a continuous variable in Cox regression models to test its linear effect.
Models were adjusted for age, sex, admission batch, BMI, smoking status, drinking status, education, physical activity, hypertension, diabetes mellitus, hyperlipidemia, family history of CVD/CHD/stroke, and intake of antibiotics or aspirin. Sex-stratified analyses were conducted without adjusting for sex.

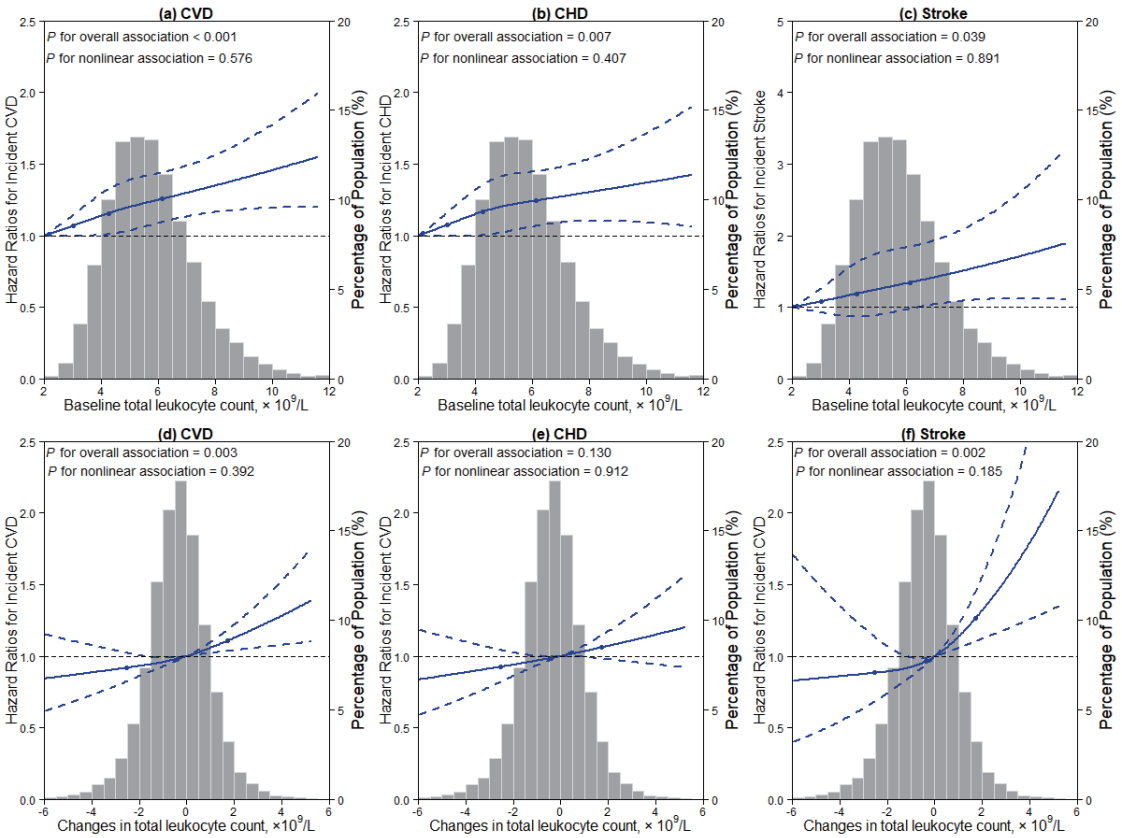

Fig. 1. Restricted cubic splines for the associations of baseline and 5-year changes in total leukocyte count with the risk of CVD, CHD, and stroke

The associations of baseline total leukocyte count with incident CVD (a), CHD (b), and stroke (c) were quantified by Cox proportional-hazards regression, adjusted for age, sex, BMI, smoking status, drinking status, physical activity, education, hyperlipidemia, hypertension, diabetes mellitus, family history of CVD/CHD/stroke, intake of antibiotics or aspirin, and admission batch (baseline or the first follow-up). The associations of changes in total leukocyte count with incident CVD (d), CHD (e), and (f) stroke were adjusted for age, sex, BMI, smoking status, drinking status, physical activity, education, hyperlipidemia, hypertension, diabetes mellitus, family history of CVD/CHD/stroke, intake of antibiotics or aspirin at the first follow-up and additionally adjusted for baseline total leukocyte count. Abbreviation: CVD, cardiovascular disease; CHD, coronary heart disease.

lymphocyte count (ICC=0.53), monocyte count (ICC=0.42), and eosinophil count (ICC=0.61), but low for basophil count (ICC=0.10) and NLR (ICC=0.35). Therefore, the associations of changes in basophil count and NLR with CVD events were not further analyzed.

Compared with the stable group (−1.18 to 0.44×10^9/L), the adjusted HRs (95% CIs) of an increase of $>0.44 \times 10^9$/L in total leukocyte count were 1.14 (1.04–1.24) for incident CVD, 1.11 (1.01–1.22) for CHD, and 1.26 (1.03–1.55) for stroke **(Table 3)**, and the association between 5-year changes in total leukocyte count and incident CVD was consistent in men (HR, 1.15; 95% CI, 1.01–1.31) and women (HR, 1.14; 95% CI, 1.01–1.28). Restricted cubic spline plots demonstrated a J-shaped association between 5-year changes in total leukocyte count and incident CVD and stroke but not CHD **(Fig. 1)**. The associations of changes in differential leukocyte counts with CVD events are presented in **Supplementary Tables 4–5**.

Joint Effects of Total Leukocyte Count at the First Follow-Up and 5-Year Changes in Total Leukocyte Count on the Risk of CVD Events

We examined the joint effects of total leukocyte count at the first follow-up and 5-year changes in total leukocyte count on incident CVD and its subtypes. As can be seen from **Fig. 2**, compared with participants with low total leukocyte count at the first follow-up ($<4.83 \times 10^9$/L) and who experienced stable changes (−1.18 to 0.44×10^9/L) in total leukocyte count, participants with high levels at the first follow-up ($>6.00 \times 10^9$/L) and experienced increased changes ($>$

Table 3. Adjusted HRs (95% CIs) of cardiovascular events according to total leukocyte count changes in men and women

	$<-1.18\times10^9$/L	-1.18 to 0.44×10^9/L	$>0.44\times10^9$/L
CVD			
All			
Cases/person-years	711/13629	1481/29488	779/13624
HR (95% CI)	0.94 (0.85–1.04)	1.00 (ref)	1.14 (1.04–1.24)
Men			
Cases/person-years	366/6521	701/11831	347/5248
HR (95% CI)	0.87 (0.76–1.01)	1.00 (ref)	1.15 (1.01–1.31)
Woman			
Cases/person-years	345/7108	780/17658	432/8376
HR (95% CI)	1.01 (0.88–1.16)	1.00 (ref)	1.14 (1.01–1.28)
CHD			
All			
Cases/person-years	592/13910	1235/30033	634/13981
HR (95% CI)	0.95 (0.85–1.06)	1.00 (ref)	1.11 (1.01–1.22)
Men			
Cases/person-years	293/6702	551/12109	264/5444
HR (95% CI)	0.90 (0.76–1.05)	1.00 (ref)	1.10 (0.95–1.28)
Woman			
Cases/person-years	299/7209	684/17924	370/8538
HR (95% CI)	1.01 (0.87–1.17)	1.00 (ref)	1.12 (0.99–1.27)
ACS			
All			
Cases/person-years	233/12607	467/27110	216/12436
HR (95% CI)	0.91 (0.77–1.08)	1.00 (ref)	1.02 (0.87–1.20)
Men			
Cases/person-years	124/6042	245/10896	101/4786
HR (95% CI)	0.81 (0.63–1.03)	1.00 (ref)	0.96 (0.76–1.21)
Woman			
Cases/person-years	109/6566	222/16214	115/7650
HR (95% CI)	1.05 (0.82–1.35)	1.00 (ref)	1.09 (0.86–1.37)
Stroke			
All			
Cases/person-years	119/15113	246/32396	145/15201
HR (95% CI)	0.88 (0.69–1.12)	1.00 (ref)	1.26 (1.03–1.55)
Men			
Cases/person-years	73/7204	150/13046	83/5905
HR (95% CI)	0.77 (0.56–1.05)	1.00 (ref)	1.27 (0.97–1.67)
Woman			
Cases/person-years	46/7909	96/19350	62/9296
HR (95% CI)	1.10 (0.75–1.61)	1.00 (ref)	1.25 (0.90–1.73)
IS			
All			
Cases/person-years	93/15044	197/32234	114/15110
HR (95% CI)	0.84 (0.64–1.10)	1.00 (ref)	1.24 (0.98–1.57)
Men			
Cases/person-years	60/7168	121/12956	65/5851
HR (95% CI)	0.76 (0.54–1.07)	1.00 (ref)	1.24 (0.91–1.68)
Woman			
Cases/person-years	33/7875	76/19277	49/9260
HR (95% CI)	0.99 (0.63–1.54)	1.00 (ref)	1.22 (0.85–1.77)
HS			
All			
Cases/person-years	26/14835	49/31808	31/14896
HR (95% CI)	1.07 (0.64–1.82)	1.00 (ref)	1.37 (0.87–2.15)
Men			
Cases/person-years	13/7034	29/12686	18/5711
HR (95% CI)	0.80 (0.39–1.66)	1.00 (ref)	1.45 (0.80–2.63)
Woman			
Cases/person-years	13/7801	20/19122	13/9185
HR (95% CI)	1.54 (0.71–3.36)	1.00 (ref)	1.32 (0.65–2.67)

Abbreviation: CVD, cardiovascular disease; CHD, coronary heart disease; ACS, acute coronary syndrome; IS, ischemic stroke; HS, hemorrhagic stroke. Models were adjusted for age, sex, BMI, education, smoking status, drinking status, physical activity, hypertension, diabetes mellitus, hyperlipidemia, family history of CVD/CHD/stroke, intake of antibiotics or aspirin and baseline total leukocyte count. Sex-stratified analyses were conducted without adjusting for sex.

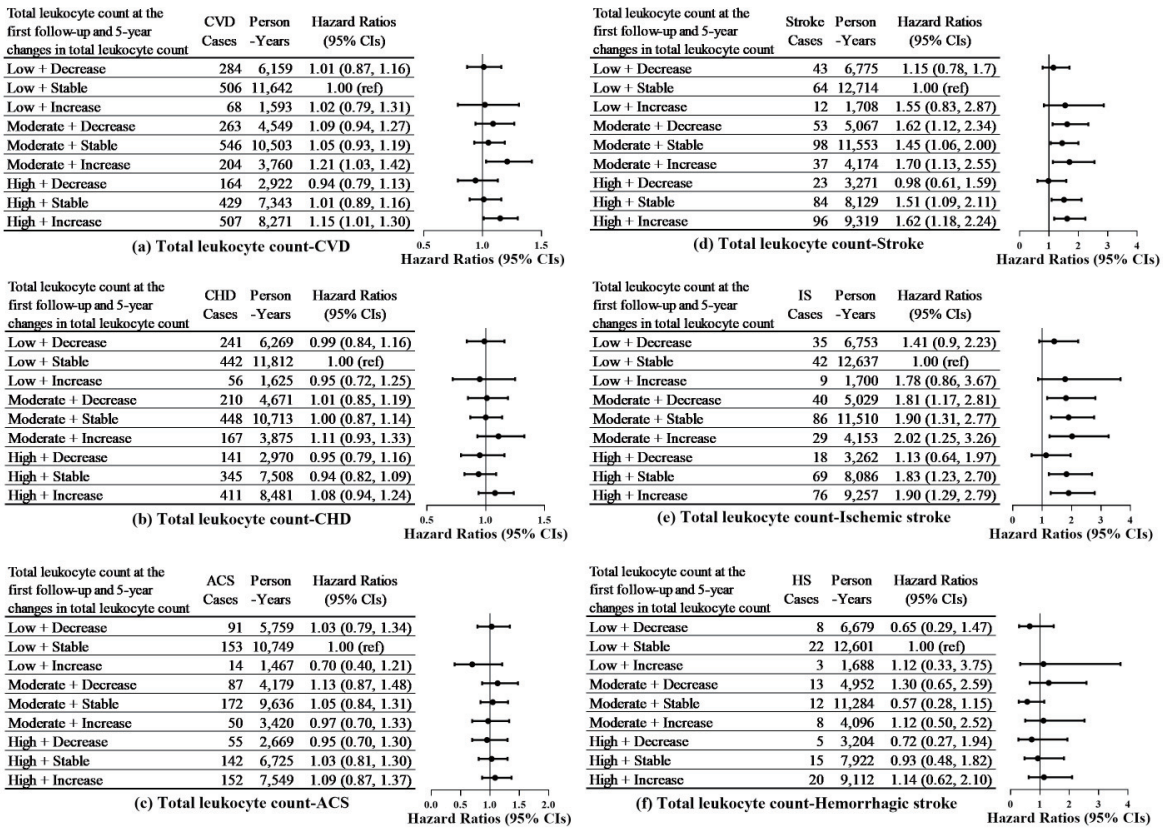

Fig. 2. Joint effect of total leukocyte count at the first follow-up and 5-year changes in total leukocyte count on the risk of incident CVD events

Joint effect of total leukocyte count at the first follow-up and 5-year changes in total leukocyte count on the risk of incident CVD (a), CHD (b), ACS (c), stroke (d), IS (e), and HS (f). Hazard ratios and 95% CIs were obtained by using Cox proportional-hazards regression model, adjusting for age, sex, BMI, smoking status, drinking status, physical activity, education, hyperlipidemia, hypertension, diabetes mellitus, family history of CVD/CHD/stroke, intake of antibiotics or aspirin at the first follow-up of the DFTJ cohort. Total leukocyte count at the first follow-up was categorized into tertiles (low, moderate, and high). Five-year changes in leukocyte count were categorized into three groups, i.e., the decreased group (<25%), stable group (25%–75%), and increased group (>75%). Abbreviation: CVD, cardiovascular disease; CHD, coronary heart disease; ACS, acute coronary syndrome; IS, ischemic stroke; HS, hemorrhagic stroke.

0.44×10^9/L) had a higher risk of incident CVD (HR 1.15; 95% CI, 1.01–1.30), stroke (HR 1.62; 95% CI, 1.18–2.24), and IS (HR 1.90; 95% CI, 1.29–2.79) but not of CHD, ACS, and HS. Notably, participants in the moderate and high total leukocyte count group at the first follow-up with a 5-year increase in total leukocyte count had 102% and 90% higher risk of IS, respectively, and participants in the moderate and high total leukocyte count group at the first follow-up with stable changes also had 90% and 83% higher risk of IS, respectively.

Discussion

In this large prospective cohort comprised of middle-aged and elderly Chinese individuals, we found that high total and differential leukocyte counts as well as their 5-year changes were associated with higher risk of CVD events. In addition, high total leukocyte count at the first follow-up with a 5-year increase in total leukocyte count was associated with higher risk of CVD, stroke, and IS.

Consistent with our findings, the positive associations of increased total leukocyte count with elevated risk of ischemic CVD have been previously reported in different ethnic, age, and sex groups[15, 16, 19, 34, 56]. Our results indicated that the association of total leukocyte count with CVD was mostly explained by its relations with ACS and IS, and this study additionally suggested that participants with high total leukocyte count had a greater risk of ACS than CHD. ACS is considered to be the most serious clinical type of CHD, with serious thrombotic complications on the basis of atherosclerosis and activated immune cells contributing to plaque rupture and endothelial erosion of this process[57]. Friedman *et al.*[58] measured the total

leukocyte count 16.8 months before the onset of myocardial infarction and demonstrated that the total leukocyte count was a predictor of myocardial infarction. Furthermore, the Women's Health Initiative study, in which 72,242 participants (701 cases) were enrolled, suggested that women in the upper quartile of total leukocyte count had a 40% higher risk of nonfatal myocardial infarction[16]. Similarly, this present study measured the leukocyte counts several years before the onset of ACS and suggested that the upper quartile of total leukocyte count had a 21% higher risk of ACS.

In line with previous studies[15, 20, 59], we found significant associations of total leukocyte count with incident IS. The Honolulu Heart Program reported that increased total leukocyte count was an independent predictor of stroke and IS, but not HS, among 3,342 elderly Japanese-American men[20, 59], a finding that is in agreement with the results of this study. Moreover, the non-significant association between total leukocyte count and incident HS may be attributed to the fact that there were only 326 cases of HS. Nonetheless, data from the Malmö Diet and Cancer Study indicated that the total leukocyte count was inversely related to the risk of HS, at marginal significance (P for trend = 0.046). However, high NLR was found to be independently associated with greater risk of stroke, IS, and HS in the present study, and the prospective relation between NLR and risk of HS has not been reported before[33, 34, 59].

In the sex-stratified analyses of baseline and 5-year changes in total leukocyte count, the main results were consistent among men. However, the baseline total leukocyte count among women only indicated a linear correlation with incident CVD and ACS. This can be explained by different lifestyles, estrogen bioactivity, and medication usage, such as hormone replacement therapy or oral contraceptives, in different sexes[18, 40].

The design of this cohort study enabled us to investigate the 5-year changes in total leukocyte count with the risk of CVD events. A previous study found that change in total leukocyte count was an independent predictor of CHD[42], a finding consistent with the results of this study. In addition, we reported that longitudinal change in total leukocyte count was related to increased risk of CVD and stroke in the general population. However, no significant associations were observed between 5-year changes in total leukocyte count and subtypes of CHD or stroke. The risk may be underestimated in these analyses as most severe cases had higher baseline total leukocyte count but lower total leukocyte count during the first follow-up. This is also why we conducted joint analyses to further investigate the joint associations of total leukocyte count at the first follow-up and 5-year changes in total leukocyte count with CVD risk. The Metabolic, Lifestyle and Nutrition Assessment in Young Adults study among Israeli army young adults found that a persistently high total leukocyte count was significantly associated with CHD incidence[44]. Furthermore, we reported that high total leukocyte count at the first follow-up with a 5-year increase in total leukocyte count was related to higher risk of CVD. The findings in these analyses indicate that serial measurements of total leukocyte count might help monitor the health status of middle-aged and elderly individuals; therefore, we could prevent CVD in time.

Different leukocyte subtypes play a role in inflammation and immune response, and atherosclerosis is an immune-mediated inflammatory disease that involves both innate and adaptive immunities[60]. Monocyte-derived macrophage is the main innate immunity cell in the atherosclerotic plague. The plasma lipoproteins beneath the endothelial cell recruit monocytes and trigger monocytes, which differentiate into macrophages or foam cells and further form atherosclerotic plaques[5]. Neutrophils and eosinophils might contribute to the emergence of atherosclerosis and thrombosis through an interplay with platelets and overactivity of extracellular traps[61, 62]. Neutrophils can also release myeloperoxidase and matrix metalloproteinase, which leads to endothelial dysfunction and atherosclerotic plaque instability[63]. Eosinophil degranulation and basophil activation are involved in the progression and rupture of coronary plaque[64]. Moreover, different lymphocyte subtypes play both pro-atherogenic and anti-atherosclerotic roles in the process of atherosclerosis[57, 65]. This study suggested a significant association between high levels of baseline NLR and the risk of CVD, ACS, and the two stroke subtypes, especially HS. In addition, we found that decreased lymphocyte count was related to higher risk of HS, which indicates that lymphocyte-mediated adaptive immunity, together with innate immunity, is involved in the pathogenesis of HS. A potential explanation might be that regulatory T-cell-mediated immunosuppression could balance the adverse effects of excessive inflammation[3] and plays a positive role in the pathogenesis of HS.

This study was mainly strengthened by its large sample size, prospective design, and inclusion of a wide range of established risk factors of CVD as covariates. The cohort study design enabled us to evaluate the relations of total and differential leukocyte counts with the subtypes of CHD and stroke in one

general population. In addition, two measurements over time allowed us to investigate the longitudinal changes in total and differential leukocyte counts with incident CVD and its subtypes, and serial monitoring of total and differential leukocyte counts could better reflect the long-term inflammatory state and help in the prevention of CVD. Furthermore, the results in this study confirmed the important role of innate and adaptive immunities in the pathogenesis of different CVD subtypes. This suggests that controlling and balancing the innate and adaptive immune responses might be preventive measures for different CVD subtypes. However, this study has several limitations. Although we carefully adjusted for a wide range of potential confounding factors, residual confounding may still be present. As the study population was comprised of middle-aged and elderly Chinese individuals without CVD or cancer, caution needs to be taken when applying findings in this study to populations of other age, ethnicity, or health condition groups. Finally, medication usage and baseline inflammatory diseases may have an impact on leukocyte counts. However, we adjusted for medications that may affect leukocyte counts (antibiotics or aspirin) and conducted sensitivity analyses by excluding baseline inflammatory diseases, and the results were materially unchanged.

In conclusion, high total and differential leukocyte counts as well as their changes were associated with elevated risk of CVD events in middle-aged and elderly Chinese population. Our findings further confirm that monitoring longitudinal changes in leukocyte markers may help provide an avenue for the primary prevention of future cardiovascular events.

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Competing Interests

The authors declare no competing interests.

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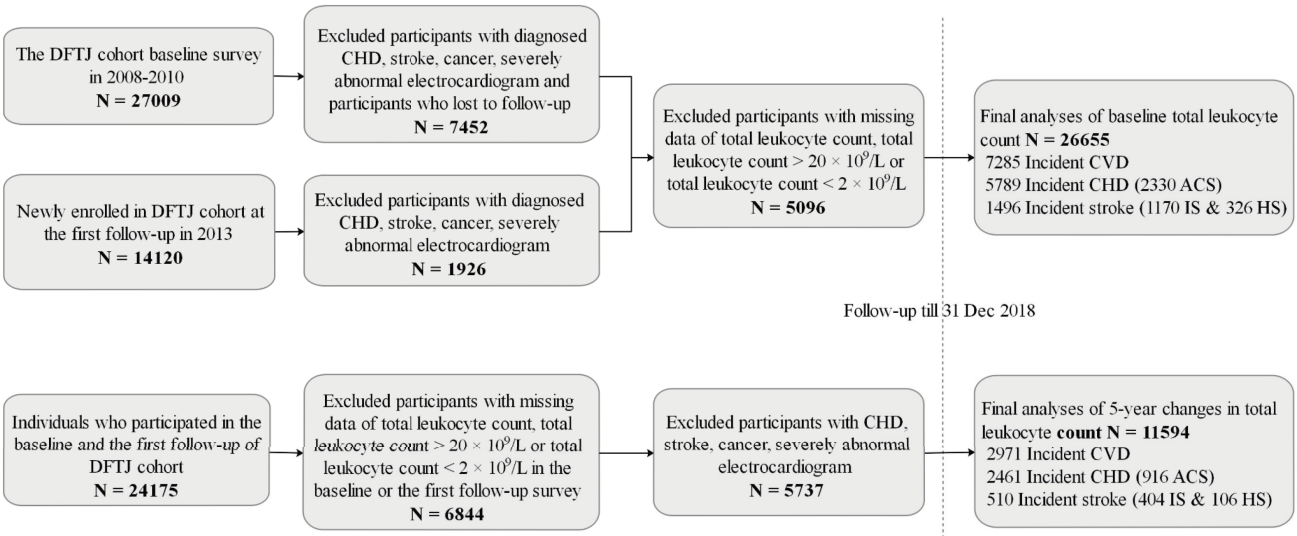
Supplementary Table 1. A comparison of the basic characteristics between the included participants and participants who were excluded due to missing and extreme values of the total leukocyte count

Characteristics	Included	Excluded
N	26655	5096
Age (years)	61.5 ± 8.1	62.6 ± 7.7
Men (%)	11762 (44.1)	2216 (43.5)
BMI (kg/m^2)[§]	24.2 ± 3.3	24.5 ± 3.4
Education (%)[§]		
Primary school or below	5857 (22.1)	1084 (21.4)
Middle school	9670 (36.5)	1773 (35.0)
High school or beyond	10949 (41.4)	2202 (43.5)
Smoking status (%)[§]		
Current smokers	5102 (19.2)	931 (18.5)
Former smokers	2597 (9.8)	480 (9.5)
Never smokers	18866 (71.0)	3629 (72.0)
Drinking status (%)[§]		
Current drinkers	6607 (24.8)	1088 (21.4)
Former drinkers	1195 (4.5)	232 (4.6)
Never drinkers	18812 (70.7)	3764 (74.0)
Physical activity (%)	18939 (71.1)	3344 (65.6)
Antibiotics use (%)	2052 (7.7)	427 (8.4)
Aspirin use (%)	2581 (9.7)	459 (9.0)
Family history of CVD (%)	2849 (10.7)	509 (10.0)
Hyperlipidemia (%)	10996 (41.3)	1433 (28.1)
Hypertension (%)	13475 (50.6)	2409 (47.3)
Diabetes mellitus (%)	4455 (16.7)	562 (11.0)

Abbreviation: BMI, body mass index; CVD, cardiovascular disease.

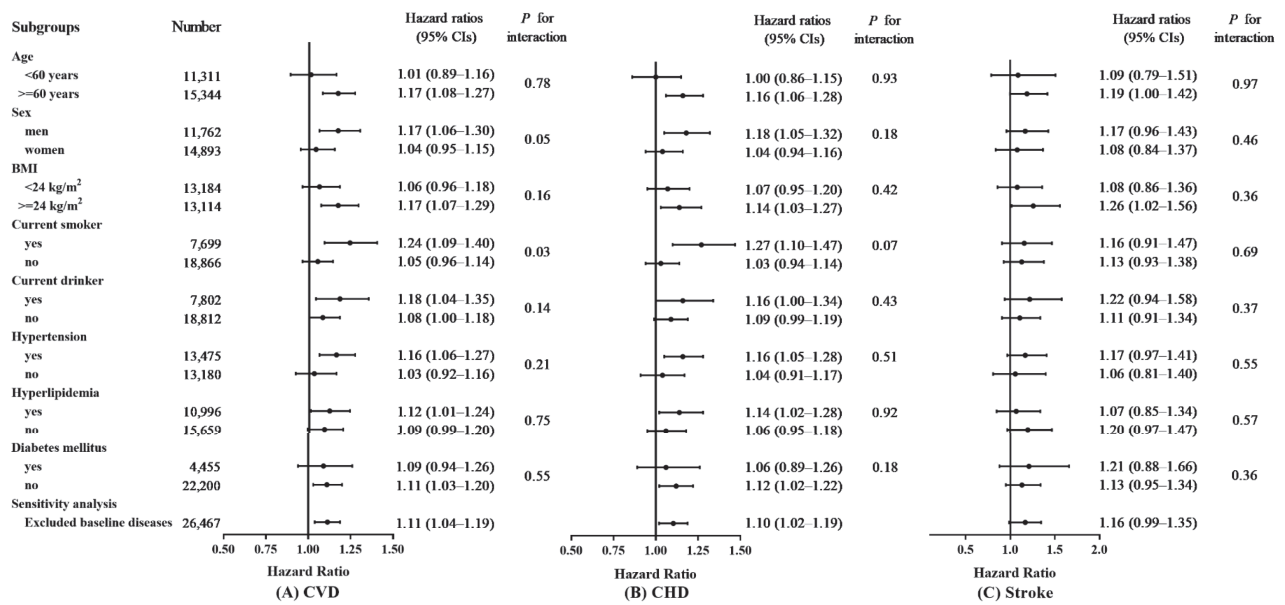
Continuous variables were described as mea ± SD if normally distributed, or median (interquartile range) if skewed distributed. Categorical variables were described as number (%).

[§]Data were incomplete for these variables. For totally 26655 participants included in the analysis, 357 (1.3%), 179 (0.7%), 90 (0.3%) and 41 (0.2%) of participants had missing data for BMI, education, smoking status and drinking status, respectively. The other variables included in the analyses did not have missing data. For the 5096 participants who were excluded due to missing and extreme values of total leukocyte count, 1585 (31.1%), 37 (0.7%), 56 (1.1%), and12 (2.4%) of participants had missing data for BMI, education, smoking status and drinking status, respectively.



Supplementary Fig. 1. Flow chart of participants ultimately included in this study

Abbreviation: DFTJ, Dongfeng-Tongji cohort; CHD, coronary heart disease; CVD, cardiovascular disease; ACS, acute coronary syndrome; IS, ischemic stroke; HS, hemorrhagic stroke.
Severely abnormal electrocardiogram included myocardial infarction, atrial fibrillation/flutter, frequent premature ventricular contractions, pacemaker rhythm and pre-excitation syndrome.



Supplementary Fig. 2. Adjusted HRs (95% CIs) of incident CVD, CHD and stroke according to total leukocyte count in subgroups

Adjusted HRs (95% CIs) for incident CVD/CHD/Stroke in the highest compared with the lowest total leukocyte count quartiles in subgroups stratified by age, sex and other cardiovascular risk factors; The models in Table 2 were used in these analyses. *P* value was tested by including the respective multiplicative interaction terms between these characteristics and total leukocyte count on incident CVD/CHD/stroke; Because of missing values for BMI (n=357), smoking status (n=90) and drinking status (n=41) hence not the same total number for each stratification characteristics. The sensitivity analysis excluded baseline diseases including gout, major rheumatic diseases and end-stage renal disease (estimated glomerular filtration rate $<$30 ml/min).

Supplementary Table 2. Adjusted HRs (95% CIs) of incident CVD, CHD and ACS according to quartiles of differential leukocyte counts and neutrophil-to-lymphocyte ratio

	CVD		CHD		ACS	
	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)
Neutrophil count						
Q1 (<2.601, ×10^9/L)	1425/43934	1.00 (ref)	1167/44840	1.00 (ref)	427/40058	1.00 (ref)
Q2 (2.601–3.230, ×10^9/L)	1508/41275	0.99 (0.92–1.07)	1232/41275	1.01 (0.94–1.10)	473/37238	1.03 (0.91–1.18)
Q3 (3.231–4.030, ×10^9/L)	1743/42235	1.03 (0.96–1.11)	1381/42235	1.03 (0.95–1.12)	584/38228	1.10 (0.97–1.25)
Q4 (>4.030, ×10^9/L)	1988/41800	1.09 (1.01–1.16)	1523/43061	1.05 (0.97–1.14)	679/37493	1.15 (1.01–1.30)
P for trend[§]		0.013		0.221		0.022
Per 10^9/L increase		1.03 (1.01–1.05)		1.01 (0.99–1.04)		1.05 (1.01–1.09)
Lymphocyte count						
Q1 (<1.461, ×10^9/L)	1449/39467	1.00 (ref)	1152/40411	1.00 (ref)	436/36270	1.00 (ref)
Q2 (1.461–1.810, ×10^9/L)	1588/42569	0.98 (0.92–1.06)	1279/43484	1.00 (0.92–1.08)	527/38553	1.03 (0.91–1.17)
Q3 (1.811–2.250, ×10^9/L)	1697/43708	0.99 (0.92–1.06)	1333/44773	0.99 (0.91–1.07)	550/39547	1.00 (0.88–1.13)
Q4 (>2.250, ×10^9/L)	1932/43612	1.02 (0.95–1.10)	1540/44779	1.05 (0.97–1.14)	650/38756	1.05 (0.92–1.19)
P for trend[§]		0.448		0.208		0.538
Per 10^9/L increase		1.02 (0.98–1.05)		1.03 (0.99–1.08)		1.03 (0.96–1.09)
Monocyte count						
Q1 (<0.281, ×10^9/L)	921/28218	1.00 (ref)	787/28650	1.00 (ref)	267/26129	1.00 (ref)
Q2 (0.281–0.374, ×10^9/L)	1171/32663	1.01 (0.92–1.10)	961/33327	0.99 (0.90–1.09)	389/29874	0.99 (0.84–1.16)
Q3 (0.375–0.480, ×10^9/L)	1366/33357	1.06 (0.97–1.16)	1084/34157	1.02 (0.93–1.13)	449/30005	1.01 (0.86–1.18)
Q4 (>0.480, ×10^9/L)	1425/30878	1.05 (0.96–1.14)	1118/31699	1.03 (0.93–1.13)	503/27899	1.05 (0.90–1.24)
P for trend[§]		0.261		0.449		0.399
Per 10^9/L increase		1.10 (0.92–1.32)		1.09 (0.89–1.33)		1.14 (0.85–1.54)
Eosinophil count						
Q1 (<0.061, ×10^9/L)	1038/31636	1.00 (ref)	844/32226	1.00 (ref)	324/29221	1.00 (ref)
Q2 (0.061–0.100, ×10^9/L)	1222/32338	1.07 (0.98–1.16)	997/33012	1.09 (0.99–1.19)	406/29496	1.13 (0.97–1.31)
Q3 (0.101–0.164, ×10^9/L)	1265/30022	1.09 (1.01–1.19)	1016/30775	1.10 (1.00–1.20)	429/27211	1.15 (0.99–1.33)
Q4 (>0.164, ×10^9/L)	1362/31126	1.09 (1.00–1.18)	1097/31826	1.12 (1.02–1.23)	450/27963	1.12 (0.97–1.30)
P for trend[§]		0.128		0.049		0.295
Per 10^9/L increase		1.18 (0.99–1.41)		1.27 (1.05–1.53)		1.08 (0.78–1.51)
Basophil count						
Q1 (<0.047, ×10^9/L)	996/28409	1.00 (ref)	813/28897	1.00 (ref)	282/26334	1.00 (ref)
Q2 (0.047–0.072, ×10^9/L)	1331/34933	0.99 (0.91–1.08)	1080/35779	1.01 (0.92–1.11)	462/31534	1.02 (0.87–1.19)
Q3 (0.073–0.109, ×10^9/L)	1392/33830	1.03 (0.95–1.13)	1106/34662	1.04 (0.94–1.14)	478/30383	1.02 (0.87–1.19)
Q4 (>0.109, ×10^9/L)	1159/27790	1.03 (0.95–1.13)	946/28342	1.06 (0.97–1.17)	386/25519	1.13 (0.97–1.32)
P for trend[§]		0.365		0.175		0.071
Per 10^9/L increase		1.08 (0.88–1.32)		1.19 (0.96–1.48)		1.00 (0.66–1.54)
Neutrophil-to-lymphocyte ratio						
Q1 (<1.34)	1588/43431	1.00 (ref)	1306/44362	1.00 (ref)	511/39074	1.00 (ref)
Q2 (1.34–1.79)	1743/45363	1.02 (0.95–1.09)	1389/46440	0.99 (0.92–1.07)	545/40879	1.00 (0.89–1.13)
Q3 (1.80–2.37)	1686/42043	1.01 (0.95–1.09)	1350/43059	1.00 (0.92–1.07)	573/38042	1.06 (0.94–1.20)
Q4 (>2.37)	1651/38557	1.06 (0.98–1.13)	1260/39617	0.99 (0.91–1.07)	533/35152	1.07 (0.94–1.20)
P for trend[§]		0.148		0.836		0.224
Per one unit increase		1.03 (1.00–1.06)		1.02 (0.99–1.05)		1.07 (1.02–1.11)

Abbreviation: CVD, cardiovascular disease; CHD, coronary heart disease; ACS, acute coronary syndrome; CI, confidence interval; HR, hazard ratio.

HRs were adjusted for age, sex, admission batch, BMI, smoking status, drinking status, education, physical activity, hypertension, diabetes mellitus, hyperlipidemia, family history of CVD/CHD, and intake of antibiotics or aspirin.

[§] *P* for trend was calculated when we assigned the median value to each quartile and entered this as a continuous variable in the model to test its linear effect.

Supplementary Table 3. Adjusted HRs (95% CIs) of incident stroke, IS and HS according to quartiles of differential leukocyte counts and neutrophil-to-lymphocyte ratio

	Stroke		Ischemic stroke		Hemorrhagic stroke	
	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)
Neutrophil count						
Q1 (<2.601, ×10^9/L)	258/47903	1.00 (ref)	202/47671	1.00 (ref)	56/47034	1.00 (ref)
Q2 (2.601–3.230, ×10^9/L)	276/45389	0.93 (0.79–1.11)	213/45070	0.91 (0.75–1.11)	63/44374	1.01 (0.70–1.45)
Q3 (3.231–4.030, ×10^9/L)	362/46948	1.06 (0.90–1.24)	279/46536	1.03 (0.86–1.24)	83/45676	1.17 (0.83–1.65)
Q4 (>4.030, ×10^9/L)	465/46781	1.20 (1.02–1.40)	377/46401	1.22 (1.02–1.46)	88/45039	1.13 (0.80–1.60)
P for trend[§]		0.003		0.003		0.387
Per 10^9/L increase		1.08 (1.03–1.13)		1.08 (1.03–1.14)		1.08 (0.98–1.19)
Lymphocyte count						
Q1 (<1.461, ×10^9/L)	297/43197	1.00 (ref)	207/42812	1.00 (ref)	90/42337	1.00 (ref)
Q2 (1.461–1.810, ×10^9/L)	309/46884	0.94 (0.80–1.10)	256/46646	1.11 (0.92–1.34)	53/45692	0.56 (0.40–0.79)
Q3 (1.811–2.250, ×10^9/L)	364/48177	1.03 (0.88–1.21)	297/47841	1.21 (1.01–1.45)	67/46798	0.63 (0.45–0.86)
Q4 (>2.250, ×10^9/L)	392/48897	0.96 (0.82–1.12)	312/48514	1.08 (0.90–1.30)	80/47427	0.67 (0.49–0.92)
P for trend[§]		0.792		0.515		0.066
Per 10^9/L increase		0.96 (0.89–1.05)		0.99 (0.91–1.09)		0.85 (0.71–1.02)
Monocyte count						
Q1 (<0.281, ×10^9/L)	134/30663	1.00 (ref)	99/30521	1.00 (ref)	35/30301	1.00 (ref)
Q2 (0.281–0.374, ×10^9/L)	210/36003	1.14 (0.91–1.42)	159/35789	1.15 (0.89–1.49)	51/35269	1.12 (0.72–1.74)
Q3 (0.375–0.480, ×10^9/L)	282/37039	1.25 (1.01–1.56)	222/36748	1.30 (1.02–1.67)	60/36054	1.12 (0.73–1.74)
Q4 (>0.480, ×10^9/L)	307/34614	1.17 (0.94–1.45)	249/34346	1.23 (0.96–1.59)	58/33515	0.97 (0.62–1.53)
P for trend[§]		0.263		0.139		0.720
Per 10^9/L increase		1.20 (0.84–1.71)		1.26 (0.87–1.84)		0.96 (0.40–2.31)
Eosinophil count						
Q1 (<0.061, ×10^9/L)	194/34377	1.00 (ref)	142/34139	1.00 (ref)	52/33738	1.00 (ref)
Q2 (0.061–0.100, ×10^9/L)	225/35778	0.99 (0.81–1.20)	169/35568	1.00 (0.80–1.26)	56/35014	0.94 (0.64–1.37)
Q3 (0.101–0.164, ×10^9/L)	249/33479	1.06 (0.87–1.28)	206/33242	1.18 (0.95–1.46)	43/32644	0.73 (0.49–1.10)
Q4 (>0.164, ×10^9/L)	265/34701	0.96 (0.80–1.17)	212/34470	1.03 (0.83–1.28)	53/33759	0.78 (0.52–1.15)
P for trend[§]		0.678		0.833		0.181
Per 10^9/L increase		0.86 (0.54–1.37)		1.01 (0.62–1.64)		0.37 (0.10–1.32)
Basophil count						
Q1 (<0.047, ×10^9/L)	183/31037	1.00 (ref)	138/30876	1.00 (ref)	45/30536	1.00 (ref)
Q2 (0.047–0.072, ×10^9/L)	251/38543	0.92 (0.76–1.13)	197/38269	0.95 (0.76–1.20)	54/37640	0.84 (0.55–1.28)
Q3 (0.073–0.109, ×10^9/L)	286/37786	0.98 (0.81–1.20)	224/37482	1.00 (0.79–1.26)	62/36737	0.94 (0.62–1.41)
Q4 (>0.109, ×10^9/L)	213/30789	0.92 (0.76–1.13)	170/30613	0.95 (0.76–1.20)	43/30063	0.84 (0.55–1.28)
P for trend[§]		0.579		0.762		0.554
Per 10^9/L increase		0.67 (0.38–1.16)		0.69 (0.37–1.28)		0.61 (0.18–2.02)
Neutrophil-to-lymphocyte ratio						
Q1 (<1.34)	282/47980	1.00 (ref)	232/47747	1.00 (ref)	50/46887	1.00 (ref)
Q2 (1.34–1.79)	354/50088	1.16 (0.99–1.35)	281/49728	1.12 (0.94–1.33)	73/48856	1.35 (0.94–1.93)
Q3 (1.80–2.37)	336/46393	1.11 (0.95–1.30)	267/46083	1.07 (0.90–1.28)	69/45096	1.32 (0.92–1.90)
Q4 (>2.37)	391/42734	1.30 (1.12–1.52)	293/42295	1.19 (1.00–1.42)	98/41442	1.89 (1.34–2.67)
P for trend [§]		0.002		0.085		<0.001
Per one unit increase		1.06 (1.01–1.12)		1.05 (0.99–1.11)		1.11 (1.01–1.23)

Abbreviation: CI, confidence interval; HR, hazard ratio.

HRs were adjusted for age, sex, admission batch, BMI, smoking status, drinking status, education, physical activity, hypertension, diabetes mellitus, hyperlipidemia, family history of stroke, and intake of antibiotics or aspirin.

[§] *P* for trend was calculated when we assigned the median value to each quartile and entered this as a continuous variable in the model to test its linear effect.

Supplementary Table 4. Adjusted HRs (95% CIs) of incident CVD, CHD and ACS according to groups of 5-year changes in differential leukocyte counts

Changes ($\times 10^9$/L)	CVD		CHD		ACS	
	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)
Neutrophil count change						
Q1 (<–0.65)	648/12900	0.96 (0.86–1.06)	539/13134	0.97 (0.86–1.08)	213/11946	0.98 (0.82–1.18)
Q2–Q3 (–0.65 to 0.49)	1349/26274	1.00 (ref)	1131/26789	1.00 (ref)	413/24074	1.00 (ref)
Q4 (>0.49)	730/12561	1.10 (1.00–1.20)	596/12884	1.06 (0.96–1.18)	221/11497	1.08 (0.91–1.27)
Lymphocyte count change						
Q1 (<–0.56)	788/13870	0.98 (0.89–1.09)	662/14145	0.98 (0.88–1.09)	276/12730	1.13 (0.95–1.34)
Q2–Q3 (–0.56 to 0.07)	1275/24836	1.00 (ref)	1072/25320	1.00 (ref)	390/22819	1.00 (ref)
Q4 (>0.07)	664/13073	0.99 (0.90–1.09)	532/13387	0.93 (0.84–1.04)	181/12015	0.88 (0.74–1.05)
Monocyte count change						
Q1 (<–0.26)	317/5550	1.02 (0.88–1.17)	263/5684	0.98 (0.84–1.15)	118/5145	1.01 (0.80–1.27)
Q2–Q3 (–0.26 to 0)	1142/22947	1.00 (ref)	975/23336	1.00 (ref)	393/21206	1.00 (ref)
Q4 (>0)	332/5796	1.16 (1.03–1.32)	273/5931	1.12 (0.98–1.28)	96/5311	0.99 (0.79–1.24)
Eosinophil count change						
Q1 (<–0.08)	216/4298	0.89 (0.75–1.05)	184/4376	0.90 (0.75–1.08)	72/3986	0.87 (0.65–1.16)
Q2–Q3 (–0.08 to 0.06)	1358/26305	1.00 (ref)	1146/26806	1.00 (ref)	447/24264	1.00 (ref)
Q4 (>0.06)	218/3676	1.03 (0.89–1.19)	182/3757	1.03 (0.88–1.21)	88/3394	1.26 (1.00–1.59)

Abbreviation: CVD, cardiovascular disease; CHD, coronary heart disease; ACS, acute coronary syndrome; CI, confidence interval; HR, hazard ratio.
HRs were adjusted for age, sex, BMI, education, smoking status, drinking status, physical activity, hypertension, diabetes mellitus, hyperlipidemia, family history of CVD/CHD, intake of antibiotics or aspirin, and baseline differential leukocyte counts.

Supplementary Table 5. Adjusted HRs (95% CIs) of incident stroke, IS and HS according to groups of 5-year changes in differential leukocyte counts

Changes ($\times 10^9$/L)	stroke		Ischemic stroke		Hemorrhagic stroke	
	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)	Events/ Person-years	HR (95% CI)
Neutrophil count change						
Q1 (<–0.65)	109/14215	0.91 (0.71–1.18)	90/14156	0.99 (0.74–1.32)	19/13929	0.64 (0.35–1.18)
Q2–Q3 (–0.65 to 0.49)	218/28969	1.00 (ref)	171/28823	1.00 (ref)	47/28468	1.00 (ref)
Q4 (>0.49)	134/14048	1.23 (0.99–1.53)	106/13971	1.24 (0.97–1.59)	28/13755	1.20 (0.75–1.92)
Lymphocyte count change						
Q1 (<–0.56)	126/15457	1.03 (0.81–1.32)	97/15373	0.93 (0.70–1.23)	29/15173	1.59 (0.92–2.76)
Q2–Q3 (–0.56 to 0.07)	250/27512	1.00 (ref)	169/27414	1.00 (ref)	34/26994	1.00 (ref)
Q4 (>0.07)	132/14312	1.30 (1.04–1.62)	101/14211	1.21 (0.94–1.55)	31/14035	1.75 (1.07–2.86)
Monocyte count change						
Q1 (<–0.26)	54/6190	1.12 (0.79–1.59)	42/6155	1.08 (0.73–1.61)	12/6066	1.27 (0.60–2.70)
Q2–Q3 (–0.26 to 0)	167/25313	1.00 (ref)	127/25205	1.00 (ref)	40/24925	1.00 (ref)
Q4 (>0)	59/6477	1.37 (1.01–1.86)	50/6450	1.54 (1.10–2.16)	9/6327	0.85 (0.41–1.76)
Eosinophil count change						
Q1 (<–0.08)	32/4705	0.85 (0.55–1.31)	24/4680	0.78 (0.47–1.27)	8/4640	1.20 (0.49–2.97)
Q2–Q3 (–0.08 to 0.06)	212/29170	1.00 (ref)	167/29047	1.00 (ref)	45/28658	1.00 (ref)
Q4 (>0.06)	36/4093	1.03 (0.72–1.49)	28/4071	0.99 (0.65–1.50)	8/4009	1.24 (0.58–2.67)

Abbreviation: CI, confidence interval; HR, hazard ratio.
HRs were adjusted for age, sex, BMI, education, smoking status, drinking status, physical activity, hypertension, diabetes mellitus, hyperlipidemia, family history of stroke, intake of antibiotics or aspirin, and baseline differential leukocyte counts.