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 (<4.71×10^9/L), participants in the fourth quartile (>6.70×10^9/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 (>0.44×10^9/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
studies found that high total leukocyte count was associated with greater risk of coronary heart disease (CHD) or stroke incidence\ref{12,22}, whereas others failed to find any significant association\ref{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\ref{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\ref{27-29}, and monocyte count was also reported as a risk factor for CVD, CHD, and IS\ref{15, 30, 31}. However, eosinophil and lymphocyte counts were reported to be inversely associated with the risk of CVD\ref{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\ref{33}. Elevated NLR was reported to be linked to CHD incidence\ref{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\ref{35-38}, which may change in response to age, sex, obesity, lifestyles such as smoking and drinking, and environmental factors\ref{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\ref{42-44}, and existing studies only reported significant associations of leukocyte count changes with incident CHD and mortality\ref{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\ref{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 \(>20 \times 10^9/L\) \((n=4)\), and with total leukocyte count \(<2 \times 10^9/L\) \((n=4)\). 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 \(<2 \times 10^9/L\), and with total leukocyte count \(>20 \times 10^9/L\) 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
follow-up 47). ACS diagnosis was confirmed according to transluminal coronary angioplasty during coronary artery bypass graft or percutaneous coronary revascularization for unstable angina, or coronary revascularization for CHD, nonfatal myocardial infarction, stable and unstable angina. 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 (m²). 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 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/m²), 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 total leukocyte count. The CVD incidence was slightly higher than that in other Chinese populations, 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 (1.02–1.10) (P_trend=0.001) for IS.
Table 1. Basic characteristics of study participants for baseline total leukocyte count and changes in total leukocyte count

| Characteristics          | Quartiles of total leukocyte count, ×10^9/L | Changes in total leukocyte count, ×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 | P value |
| 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) | 2573 (36.8) | <0.001 | 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) | <0.001 | 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) | <0.001 | 344 (12.3) | 721 (10.4) | 300 (10.6) |         |
| Never smokers            | 5445 (80.0) | 4808 (73.5) | 4750 (69.2) | 3863 (59.9) | <0.001 | 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) | <0.001 | 154 (5.5) | 276 (4.6) | 136 (4.8) |         |
| Never drinkers           | 5024 (73.9) | 4671 (71.4) | 4767 (69.4) | 4350 (67.5) | <0.001 | 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) | 0.050 |
| Anticorruption use (%)   | 593 (8.7) | 496 (7.6) | 500 (7.3) | 463 (7.2) | <0.001 | 222 (8.0) | 459 (7.7) | 193 (7.8) |         |
| 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 |

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.

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), 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.
### 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, × 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 HR (95% CI)          | 1464/47376  | 1673/46946      | 1953/49467      | 2195/45921  | 0.001       | 1.03 (1.01–1.05)  |
| Men Cases/person-years HR (95% CI)          | 597/15396   | 785/18551       | 1045/22400      | 1289/24026  | 0.002       | 1.04 (1.02–1.06)  |
| Woman Cases/person-years HR (95% CI)        | 867/31980   | 888/28395       | 908/27067       | 906/21895   | 0.390       | 1.02 (1.00–1.04)  |
| **CHD**                                    |             |                 |                 |             |             |                   |
| All Cases/person-years HR (95% CI)          | 1187/48405  | 1375/47810      | 1539/50665      | 1688/47360  | 0.023       | 1.03 (1.01–1.05)  |
| Men Cases/person-years HR (95% CI)          | 445/15930   | 609/19040       | 799/23166       | 933/25009   | 0.008       | 1.03 (1.01–1.06)  |
| Woman Cases/person-years HR (95% CI)        | 742/32475   | 766/28771       | 760/27499       | 755/22351   | 0.581       | 1.02 (0.99–1.04)  |
| **ACS**                                    |             |                 |                 |             |             |                   |
| All Cases/person-years HR (95% CI)          | 409/43460   | 528/42562       | 654/44985       | 739/40991   | 0.002       | 1.05 (1.02–1.08)  |
| Men Cases/person-years HR (95% CI)          | 169/14063   | 258/16903       | 349/20374       | 429/21508   | 0.013       | 1.05 (1.01–1.08)  |
| Woman Cases/person-years HR (95% CI)        | 240/29396   | 270/25659       | 305/24610       | 310/19483   | 0.084       | 1.06 (1.02–1.10)  |
| **Stroke**                                 |             |                 |                 |             |             |                   |
| All Cases/person-years HR (95% CI)          | 277/51317   | 298/51547       | 414/54683       | 507/51577   | 0.006       | 1.04 (1.01–1.08)  |
| Men Cases/person-years HR (95% CI)          | 152/16822   | 176/20574       | 266/24977       | 356/27032   | 0.007       | 1.05 (1.01–1.09)  |
| Woman Cases/person-years HR (95% CI)        | 125/34495   | 122/30974       | 148/29706       | 151/24545   | 0.338       | 1.03 (0.97–1.09)  |
| **IS**                                     |             |                 |                 |             |             |                   |
| All Cases/person-years HR (95% CI)          | 203/51003   | 224/51201       | 337/54311       | 406/51110   | 0.001       | 1.06 (1.02–1.10)  |
| Men Cases/person-years HR (95% CI)          | 108/16645   | 136/20387       | 214/24746       | 291/26734   | 0.001       | 1.07 (1.02–1.12)  |
| Woman Cases/person-years HR (95% CI)        | 95/34358    | 88/30814        | 123/29565       | 115/24376   | 0.319       | 1.04 (0.97–1.10)  |
| **HS**                                     |             |                 |                 |             |             |                   |
| All Cases/person-years HR (95% CI)          | 74/50433    | 74/50490        | 77/53097        | 101/49473   | 0.938       | 0.99 (0.92–1.07)  |
| Men Cases/person-years HR (95% CI)          | 44/16351    | 40/19912        | 52/23987        | 65/25733    | 0.764       | 0.99 (0.90–1.09)  |
| Woman Cases/person-years HR (95% CI)        | 30/34082    | 34/30578        | 25/29110        | 36/24009    | 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.
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 × 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 × 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 x 10^9/L | -1.18 to 0.44 x 10^9/L | > 0.44 x 10^9/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               | 62/2926          |
| 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.
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\textsuperscript{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\textsuperscript{57). Friedman \textit{et al.} \textsuperscript{58) measured the 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.

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\textsuperscript{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\textsuperscript{57). Friedman \textit{et al.} \textsuperscript{58) measured the 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.
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. 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, 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, 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.

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.

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, 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. 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. Monocyte-derived macrophage is the main innate immunity cell in the atherosclerotic plaque. The plasma lipoproteins beneath the endothelial cell recruit monocytes and trigger monocytes, which differentiate into macrophages or foam cells and further form atherosclerotic plaques. Neutrophils and eosinophils might contribute to the emergence of atherosclerosis and thrombosis through an interplay with platelets and overactivity of extracellular traps. Neutrophils can also release myeloperoxidase and matrix metalloproteinase, which leads to endothelial dysfunction and atherosclerotic plaque instability. Eosinophil degranulation and basophil activation are involved in the progression and rupture of coronary plaque. Moreover, different lymphocyte subtypes play both pro-atherogenic and anti-atherosclerotic roles in the process of atherosclerosis. 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 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/m²)                 | 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 mean ± 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%), and 12 (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

| Neutrophil count | CVD | CHD | ACS |
|------------------|-----|-----|-----|
| Q1 (<2.601, ×10^9/L) | 14245/43934 1.00 (ref) | 1167/44840 1.00 (ref) | 427/40058 1.00 (ref) |
| 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) | 1152/40111 1.00 (ref) | 436/36270 1.00 (ref) |
| 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) |
| Per 10^9/L increase | 1.10 (0.92–1.32) | 1.09 (0.93–1.13) | 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) |
| 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) |
| 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) |
| 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 Events/ Person-years | HR (95% CI) | Ischemic stroke Events/ Person-years | HR (95% CI) | Hemorrhagic stroke Events/ Person-years | HR (95% CI) |
|----------------------|----------------------------|-------------|--------------------------------------|-------------|----------------------------------------|-------------|
| **Neutrophil count** |                            |             |                                      |             |                                        |             |
| Q1 (<2.601, × 10^9/L)| 258/47903 1.00 (ref)       |             |                                      |             |                                        |             |
| Q2 (2.601–3.230, × 10^9/L)| 276/45389 0.93 (0.79–1.11)|             |                                      |             |                                        |             |
| Q3 (3.231–4.030, × 10^9/L)| 362/46948 1.06 (0.90–1.24)|             |                                      |             |                                        |             |
| Q4 (>4.030, × 10^9/L)| 465/46781 1.20 (1.02–1.40)|             |                                      |             |                                        |             |
| P for trend⁸            | 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)       |             |                                      |             |                                        |             |
| Q2 (1.461–1.810, × 10^9/L)| 309/46884 0.94 (0.80–1.10)|             |                                      |             |                                        |             |
| Q3 (1.811–2.250, × 10^9/L)| 364/48177 1.03 (0.88–1.21)|             |                                      |             |                                        |             |
| Q4 (>2.250, × 10^9/L)| 392/48897 0.96 (0.82–1.12)|             |                                      |             |                                        |             |
| P for trend⁸            | 0.792                                    |             |                                      |             |                                        | 0.515        |
| 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)       |             |                                      |             |                                        |             |
| Q2 (0.281–0.374, × 10^9/L)| 210/36003 1.14 (0.91–1.42)|             |                                      |             |                                        |             |
| Q3 (0.375–0.480, × 10^9/L)| 282/37039 1.25 (1.01–1.56)|             |                                      |             |                                        |             |
| Q4 (>0.480, × 10^9/L)| 307/34614 1.17 (0.94–1.45)|             |                                      |             |                                        |             |
| P for trend⁸            | 0.263                                    |             |                                      |             |                                        | 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)       |             |                                      |             |                                        |             |
| Q2 (0.061–0.100, × 10^9/L)| 225/35778 0.99 (0.81–1.20)|             |                                      |             |                                        |             |
| Q3 (0.101–0.164, × 10^9/L)| 249/33479 1.06 (0.87–1.28)|             |                                      |             |                                        |             |
| Q4 (>0.164, × 10^9/L)| 265/34701 0.96 (0.80–1.17)|             |                                      |             |                                        |             |
| P for trend⁸            | 0.678                                    |             |                                      |             |                                        | 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)       |             |                                      |             |                                        |             |
| Q2 (0.047–0.072, × 10^9/L)| 251/38543 0.92 (0.76–1.13)|             |                                      |             |                                        |             |
| Q3 (0.073–0.109, × 10^9/L)| 286/37786 0.98 (0.81–1.20)|             |                                      |             |                                        |             |
| Q4 (>0.109, × 10^9/L)| 213/30789 0.92 (0.76–1.13)|             |                                      |             |                                        |             |
| P for trend⁸            | 0.579                                    |             |                                      |             |                                        | 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)       |             |                                      |             |                                        |             |
| Q2 (1.34–1.79)        | 354/50088 1.16 (0.99–1.35)|             |                                      |             |                                        |             |
| Q3 (1.80–2.37)        | 336/46939 1.11 (0.95–1.30)|             |                                      |             |                                        |             |
| Q4 (>2.37)            | 391/42734 1.30 (1.12–1.52)|             |                                      |             |                                        |             |
| P for trend⁸            | 0.002                                    |             |                                      |             |                                        | <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.

⁸P 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 (× 10⁹/L) | CVD Events/Person-years | HR (95% CI) | CHD Events/Person-years | HR (95% CI) | ACS 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 (× 10⁹/L) | Stroke Events/Person-years | HR (95% CI) | Ischemic stroke Events/Person-years | HR (95% CI) | Hemorrhagic stroke 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 (0.75–1.86) |
| **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.