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Leukocyte Count and Risks of Stroke and Coronary Heart Disease: The Circulatory Risk in Communities Study (CIRCS)

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Aim: This study aimed to investigate the associations of leukocyte count with the risks of stroke and coronary heart disease among the general Japanese population.

Methods: A total of 5,242 residents aged 40–69 years living in two Japanese communities underwent leukocyte count measurements between 1991 and 2000, and the data were updated using 5- or 10-year follow-ups or both. Participants who had histories of stroke, coronary heart disease, or high values of leukocyte count ($>130\times10^2$ cells/mm^3) were excluded. Hazard ratios with 95% confidence intervals (CIs) were calculated according to quartiles of cumulative average leukocyte count.

Results: During follow-up of 21 years, 327 stroke and 130 coronary heart disease cases were determined. After adjustments for age, sex, community, and updated cardiovascular risk factors, the multivariable hazard ratio (95% CI) for the highest versus lowest quartile of leukocyte count was 1.50 (1.08–2.08) for ischemic stroke, 1.59 (1.00–2.51) for lacunar infarction, 1.42 (0.90–2.26) for non-lacunar infarction, 2.17 (1.33–3.55) for coronary heart disease, and 1.40 (1.11–1.76) for total cardiovascular disease. In smoking status-stratified analyses, the corresponding multivariable hazard ratio (95% CI) was 2.45 (1.11–5.38) for ischemic stroke, 2.73 (1.37–5.44) for coronary heart disease in current smokers, 2.42 (1.07–5.46), 1.55 (0.58–4.15) in former smokers, and 1.17 (0.75–1.82), 1.78 (0.83–3.82) in never smokers.

Conclusion: Leukocyte count was positively associated with the risks of ischemic stroke and coronary heart disease among the general Japanese population, especially in current smokers.

Key words: Leukocyte count, Coronary heart disease, Ischemic stroke, Lacunar infarction, Cohort study

Introduction

Leukocytes are major immune system cells responsible for protecting the body from infection and foreign materials. Increased leukocyte count reflects the presence of inflammation, which promotes the pathogenesis of atherosclerosis[1]. Previous prospective cohort studies have reported the positive associations of leukocyte count with the risks of ischemic stroke and coronary heart disease[2-7]. However, two studies reported no association between leukocyte count and the risk of ischemic stroke[8, 9]. Leukocyte count was positively correlated with several cardiovascular risk factors, especially for cigarette smoking[10]. The

positive association of leukocyte count with the risk of ischemic stroke was reported in current smokers[2, 3], former smokers[3], but not in never smokers, whereas the positive association with the risk of coronary heart disease was reported in both current, former, and never smokers[2, 8, 9].

In this study, we aimed to investigate the associations of leukocyte count with the risks of stroke and coronary heart disease among the general Japanese population. We hypothesized that leukocyte count is positively associated with the risks of ischemic stroke and coronary heart disease, and the associations could be observed in both current, former, and never smokers.

Methods

Study Population

This study is a part of the Circulatory Risk in Communities Study (CIRCS), an ongoing dynamic community cohort study of cardiovascular disease among the general Japanese population since 1963[11, 12]. Residents from Ikawa town, a rural community in Akita Prefecture in northwestern Japan, and Kyowa district of Chikusei city, a rural community in Ibaraki Prefecture in mid-eastern Japan were invited to participate in the annual health checkups. A total of 5,360 residents aged 40–69 years were tested for leukocyte count at the baseline from 1991 to 2000. After the exclusion of 104 participants who had histories of stroke or coronary heart disease, and 14 who had high values of leukocyte count ($>130\times10^2$ cells/mm^3), 5,242 participants were available in the analyses. Among them, 1,861 (36%) participants continued to participate in the annual health checkups at the 5-year follow-up, and 2,763 (53%) at the 10-year follow-up, so that the baseline examination data could be updated using the 5-year follow-up or 10-year follow-up or both. The informed consent was obtained from the representatives of the community according to the guidelines of the Council for International Organizations of Medicine of Sciences. This study obtained ethical approval from the Ethics Committees of the Osaka Center for Cancer and Cardiovascular Disease Prevention and Osaka University.

Baseline Examination

Blood was collected into a serum separating tube from participants who are in a seated position and separated within 30 min to obtain the serum. The serum sample of leukocyte count was collected in a tube containing EDTA-2K and measured using automated hematology analyzer (model S-Plus VI, STKR, or Gen-S; Beckman Coulter Inc., Brea, CA). Serum total cholesterol and triglycerides were measured using the enzymatic method during 1991–1993, and the enzymatic method for free glycerol during 1994–2000. Serum high-density lipoprotein levels after heparin-manganese precipitation were measured using the direct Liebermann–Burchard method during 1991–1993, and the dextran sulfate–phosphotungstate–$MgCl_2$ precipitation method during 1994–2000. Serum glucose was measured using the hexokinase method during 1991–1993, and the glucokinase method during 1994–2000. Blood measurements were performed at the Osaka Medical Central for Cancer and Cardiovascular Disease, an international member of the US National Cholesterol Reference Method Laboratory Network[13, 14].

In the annual health checkups, height (in stockinged feet) and weight (in light clothing) were measured, and body mass index was calculated. Information on smoking status, number of cigarettes per day, usual weekly intake of alcohol evaluated by units of "go" (a traditional Japanese unit of volume corresponding to 23-g ethanol), and medication uses were obtained via interviews. Blood pressure was measured on the right arm of participants who are in a seated position using standard mercury sphygmomanometers and unified epidemiological methods[15]. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, and use of antihypertensive medication. Diabetes mellitus was defined as a fasting glucose level of ≥ 7.0 mmol/L, non-fasting glucose level of ≥ 11.1 mmol/L, and use of medication for diabetes mellitus.

Follow-Up and Ascertainment of Cases

The participants were followed up to the end of 2017 for Ikawa and 2014 for Kyowa to determine incident stroke and coronary heart disease. The median follow-up period was 21 years. As described previously[11, 12], the CIRCS researchers ascertained the information of candidate cases of stroke and coronary heart disease from death certificates, national insurance claims, annual household questionnaires, annual cardiovascular risk surveys, and reports by

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either local physicians, public health nurses, or healthy volunteers. All living suspected cases were telephoned, visited, or invited to take part in a questionnaire survey, and medical records were obtained from the local clinics and hospitals to confirm the diagnosis. For deaths, medical histories were obtained from families or attending physicians, and related medical records were surveyed.

Stroke was defined as a focal neurological disorder with rapid onset and persisted at least 24 h or until death. Stroke subtypes, including hemorrhagic stroke and ischemic stroke (lacunar infarction or non-lacunar infarction), were mainly diagnosed using computed tomography (CT) or magnetic resonance imaging (MRI)[16], which were available for 94% of stroke cases. When CT or MRI data were unavailable, stroke subtypes were classified according to clinical criteria as hemorrhagic stroke, ischemic stroke, and unclassified stroke. The modified coronary heart disease criteria proposed by the World Health Organization Expert Committee[17] were used to confirm the coronary heart disease cases in the medical records. Definite myocardial infarction was diagnosed by typical chest pain, lasting ≥ 30 min without definite nonischemic causes, and the appearance of abnormal and persistent Q or QS waves on the electrocardiogram and consistent changes in cardiac enzyme activity or both. These patients who had typical chest pain but whose electrocardiographic and enzyme levels were non-diagnostic or unavailable were diagnosed as possible myocardial infarction. Angina pectoris was diagnosed as repeated episodes of chest pain when exerting effort, especially when walking, usually disappearing rapidly after the cessation of effort or with the use of sublingual nitroglycerin. Sudden cardiac death was diagnosed as death ≤ 1 h after onset, a witnessed cardiac arrest, or abrupt collapse not preceded by >1 h of symptoms. The initial case of definite or probable myocardial infarction, angina pectoris, or sudden cardiac death was indicated as coronary heart disease. The final diagnoses were based on a panel of experienced physician–epidemiologists using the same diagnostic criteria and blinded to the data from the risk factors survey.

Statistical Analyses

Participants were classified according to quartiles of leukocyte count. To reduce misclassification, cumulative averages of leukocyte count at the baseline, 5- or 10-year follow-up surveys or both were used to rank the quartiles instead of the single measurements at baseline. Continuous confounding variables (body mass index, ethanol intake, blood pressure, and lipids) were also updated using the same method. For categorical variables (smoking status, antihypertensive medication use, and diabetes mellitus), the last observations were used. Among participants, 64% had no data on the 5-year follow-up survey, and 47% had no data on the 10-year follow-up survey. For participants who had no data on the 5- and 10-year follow-up surveys, only the baseline data were used. For those who had data on the 5-year follow-up survey but no data on the 10-year follow-up survey, averages of the baseline and 5-year follow-up survey for continuous variables or the observations at the 5-year follow-up survey for categorical variables were used. Moreover, for those who had data on the 10-year follow-up survey but no data on the 5-year follow-up survey, averages of the baseline and 10-year follow-up survey or the observations at the 10-year follow-up survey were used. When participants died, moved out from communities, or had a stroke/coronary heart disease before the 5- or 10-year follow-up surveys, the baseline or updated data before those events were used for analyses.

The analyses of covariance were used to calculate age-, sex-, and community-adjusted mean values or prevalence of updated cardiovascular risk factors. Cox proportional hazard models with updated leukocyte count and confounding variables at the baseline and 5- and 10-year follow-up surveys were used to calculate hazard ratios with 95% confidence intervals (CIs). The hazard ratios were performed according to quartiles of cumulative average leukocyte count referenced to the lowest quartiles, and 1 standard deviation (SD) increment of cumulative average leukocyte count (16.4×10^2 cells/mm^3). The trends in the associations were tested using the median values of the pertinent leukocyte count quartiles.

The initial hazard ratio models were adjusted for age, sex, and community, and these multivariable models were further adjusted for updated body mass index (kg/m^2), cigarette smoking status (never, former, and current 1–19 or 20 cigarettes per day), ethanol intake (g/day), systolic blood pressure (mmHg), antihypertensive medication use (no or yes), tertiles of serum non-HDL cholesterol levels and HDL cholesterol levels (mmol/L), tertiles of serum triglyceride levels (mmol/L), and diabetes mellitus (no or yes). The smoking status-stratified analyses were performed according to never, former, and current smokers. All analyses were conducted using the SAS System for Windows (version 9.4; SAS Inc., Cary, NC).

Table 1. Cardiovascular risk characteristics of participants according to quartiles of cumulative average leukocyte count

	Leukocyte count quartiles ($\times 10^2$ cells/mm^3)				*P* for difference
	Q1 (low)	Q2	Q3	Q4 (high)	
Range of leukocyte count, $\times 10^2$ cells/mm^3	18–52	53–62	63–74	75–130	
Median leukocyte count, $\times 10^2$ cells/mm^3	47	58	68	83	
No. at risk	1,310	1,311	1,308	1,313	
Age at baseline, year	56 (0.2)	55 (0.2)	55 (0.2)	54 (0.2)	<0.001
Men at baseline, %	32	36	41	50	<0.001
Body mass index, kg/m^2	23.3 (0.1)	23.9 (0.1)	24.1 (0.1)	24.3 (0.1)	<0.001
Systolic blood pressure, mmHg	128 (0.4)	130 (0.5)	132 (0.5)	134 (0.5)	<0.001
Diastolic blood pressure, mmHg	76 (0.3)	78 (0.3)	79 (0.3)	81 (0.3)	<0.001
Antihypertensive medication use, %	21	22	27	24	<0.001
Serum non-HDL cholesterol, mmol/L	3.27 (0.02)	3.41 (0.02)	3.46 (0.02)	3.55 (0.02)	<0.001
Serum HDL cholesterol, mmol/L	1.40 (0.01)	1.37 (0.01)	1.33 (0.01)	1.32 (0.01)	<0.001
Serum triglycerides, mmol/L	1.03 (0.02)	1.19 (0.02)	1.32 (0.02)	1.49 (0.02)	<0.001
Diabetes mellitus, %	4	6	7	7	<0.001
Current smokers, %	15	24	32	56	<0.001
Ethanol intake, g/day	8.5 (0.4)	10.2 (0.4)	11.8 (0.4)	14.6 (0.4)	<0.001

Values were presented as means (standard errors) or proportions, adjusted for age, sex, and community.

Results

The cardiovascular risk characteristics of the baseline population according to quartiles of cumulative average leukocyte count are listed in **Table 1**. The range of each leukocyte count quartile was 18–52, 53–62, 63–74, and 75–130 $\times 10^2$ cells/mm^3, and the median value was 47, 58, 68, and 83 $\times 10^2$ cells/mm^3, respectively. Participants who had higher leukocyte count quartiles were younger and had a higher proportion of men than those who had the lowest quartiles. After adjustments for age, sex, and community, leukocyte count was positively associated with body mass index, systolic and diastolic blood pressure, antihypertensive medication use, serum non-HDL cholesterol levels, serum triglycerides, ethanol intake, the prevalence of current smokers and diabetes mellitus, and inversely associated with serum HDL cholesterol levels.

During a median follow-up of 21 years totaling 99,206 person-years, there were 327 incident strokes, including 105 hemorrhagic strokes, 219 ischemic strokes (113 lacunar infarctions, and 106 non-lacunar infarctions), and 3 unclassified strokes, and 130 incident coronary heart diseases.

Table 2 shows the associations of cumulative average leukocyte count with the risks of stroke subtypes, coronary heart disease, and total cardiovascular disease. After adjustments for age, sex, community, and updated conventional cardiovascular risk factors, the multivariable hazard ratio (95% CI) for the highest versus lowest quartile of leukocyte count was 1.50 (1.08–2.08); *P* for trend=0.01 for ischemic stroke, 2.17 (1.33–3.55); *P* for trend <0.001 for coronary heart disease, and 1.40 (1.11–1.76); *P* for trend <0.001 for total cardiovascular disease. The multivariable hazard ratio (95% CI) for 1 SD increment of leukocyte count was 1.14 (1.03–1.27) for ischemic stroke, 1.30 (1.13–1.49) for coronary heart disease, and 1.13 (1.05–1.22) for total cardiovascular disease. There were no significant differences pertaining to sex between leukocyte count and the risks of ischemic stroke (*P* for interaction=0.74) and coronary heart disease (*P* for interaction=0.45) after multivariable adjustments. The positive association with the risk of ischemic stroke was similarly observed for both lacunar infarction and non-lacunar infarction, whereas the trend in non-lacunar infarction was of borderline statistical significance. The multivariable hazard ratio (95% CI) for the highest versus lowest quartile of leukocyte count was 1.59 (1.00–2.51); *P* for trend=0.04 for lacunar infarction, 1.42 (0.90–2.26); *P* for trend=0.09 for non-lacunar infarction. The multivariable hazard ratio (95% CI) for 1 SD increment of leukocyte count was 1.17 (1.01–1.36) for lacunar infarction 1.12 (0.96–1.31) for non-lacunar infarction. A similar association was not observed between leukocyte count and the risk of hemorrhagic stroke.

Table 3 shows the smoking status-stratified associations of cumulative average leukocyte count

Table 2. Hazard ratios (HRs, 95% CIs) of cardiovascular diseases according to quartiles of cumulative average leukocyte count

	Leukocyte count quartiles (× 10^2 cells/mm^3)				*P* for trend	1 SD increment*
	Q1 (low)	Q2	Q3	Q4 (high)		
No. at risk	1,310	1,311	1,308	1,313		
Person-years	24,760	25,191	24,580	24,675		
Total stroke						
No. of events	69	66	98	94		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.12 (0.87, 1.43)	1.41 (1.11, 1.80)	1.45 (1.13, 1.87)	<0.001	1.15 (1.05, 1.25)
Multivariable HR (95% CI)	1.00	1.02 (0.79, 1.31)	1.17 (0.91, 1.50)	1.20 (0.93, 1.56)	0.08	1.06 (0.97, 1.16)
Hemorrhagic stroke						
No. of events	29	21	30	25		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	0.86 (0.57, 1.29)	1.14 (0.77, 1.69)	0.87 (0.55, 1.36)	0.91	0.94 (0.80, 1.10)
Multivariable HR (95% CI)	1.00	0.76 (0.50, 1.15)	0.95 (0.63, 1.43)	0.68 (0.43, 1.08)	0.21	0.86 (0.73, 1.01)
Ischemic stroke						
No. of events	40	45	67	67		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.31 (0.95, 1.79)	1.60 (1.17, 2.18)	1.78 (1.30, 2.44)	<0.001	1.22 (1.10, 1.35)
Multivariable HR (95% CI)	1.00	1.20 (0.87, 1.65)	1.32 (0.96, 1.81)	1.50 (1.08, 2.08)	0.01	1.14 (1.03, 1.27)
Lacunar infarction						
No. of events	19	27	31	36		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.41 (0.91, 2.20)	1.55 (0.99, 2.43)	1.95 (1.25, 3.05)	0.002	1.25 (1.09, 1.44)
Multivariable HR (95% CI)	1.00	1.26 (0.81, 1.98)	1.25 (0.79, 1.99)	1.59 (1.00, 2.51)	0.04	1.17 (1.01, 1.36)
Non-lacunar infarction						
No. of events	21	18	36	31		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.20 (0.76, 1.88)	1.63 (1.06, 2.50)	1.61 (1.03, 2.52)	0.02	1.19 (1.03, 1.37)
Multivariable HR (95% CI)	1.00	1.14 (0.72, 1.79)	1.38 (0.90, 2.15)	1.42 (0.90, 2.26)	0.09	1.12 (0.96, 1.31)
Coronary heart disease						
No. of events	13	22	44	51		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.56 (0.93, 2.61)	2.51 (1.56, 4.05)	2.96 (1.84, 4.76)	<0.001	1.43 (1.26, 1.62)
Multivariable HR (95% CI)	1.00	1.41 (0.84, 2.37)	1.95 (1.19, 3.18)	2.17 (1.33, 3.55)	<0.001	1.30 (1.13, 1.49)
Total cardiovascular disease						
No. of events	79	85	138	139		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.17 (0.94, 1.47)	1.61 (1.30, 2.00)	1.74 (1.40, 2.18)	<0.001	1.23 (1.14, 1.32)
Multivariable HR (95% CI)	1.00	1.07 (0.85, 1.35)	1.32 (1.06, 1.65)	1.40 (1.11, 1.76)	<0.001	1.13 (1.05, 1.22)

*1 SD increment of leukocyte count was 16.4 × 10^2 cells/mm^3.
Q1: 18–52 × 10^2 cells/mm^3, Q2: 53–62 × 10^2 cells/mm^3, Q3: 63–74 × 10^2 cells/mm^3, and Q4: 75–130 × 10^2 cells/mm^3.
Multivariable hazard ratios were further adjusted for updated body mass index, systolic blood pressure, cigarette smoking status, ethanol intake, serum non-HDL and HDL cholesterol levels, serum triglycerides, diabetes mellitus, and antihypertensive medication use.

with the risks of ischemic stroke and coronary heart disease according to updated smoking status. In never smokers, the multivariable hazard ratio (95% CI) for the highest versus lowest quartile of leukocyte count was 1.17 (0.75–1.82); *P* for trend = 0.53 for ischemic stroke, 1.78 (0.83–3.82); *P* for trend = 0.14 for coronary heart disease. In former smokers, the corresponding multivariable hazard ratio (95% CI) was 2.42 (1.07–5.46); *P* for trend = 0.03 for ischemic stroke, 1.55 (0.58–4.15); *P* for trend = 0.35 for coronary heart disease. In current smokers, the corresponding multivariable hazard ratio (95% CI) was 2.45 (1.11–5.38); *P* for trend = 0.05 for ischemic stroke, 2.73 (1.37–5.44); *P* for trend = 0.009 for coronary heart disease. The multivariable hazard ratio (95% CI) for 1 SD increment of leukocyte count was 1.03 (0.88–1.21) for ischemic stroke, 1.23 (0.95–1.59) for coronary heart disease in never smokers, 1.20 (0.97–1.49), 1.13 (0.87–1.47) in former smokers, and 1.26 (1.03–1.54), 1.36 (1.09–1.71) in current smokers.

Discussion

Leukocyte count was positively associated with the risks of ischemic stroke and coronary heart disease among the general Japanese population. The positive association with the risk of ischemic stroke was

Table 3. Hazard ratios (HRs, 95% CIs) of ischemic stroke and coronary heart disease according to quartiles of cumulative average leukocyte count, stratified by updated smoking status

	Leukocyte count quartiles ($\times 10^2$ cells/mm^3)				*P* for trend	1 SD increment*
	Q1 (low)	Q2	Q3	Q4 (high)		
Never smokers						
No. at risk	953	899	812	666		
Person-years	18,466	17,785	16,052	13,177		
Ischemic stroke						
No. of events	28	28	29	25		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.05 (0.71, 1.56)	1.12 (0.74, 1.69)	1.53 (1.00, 2.35)	0.05	1.14 (0.99, 1.33)
Multivariable HR (95% CI)	1.00	0.94 (0.63, 1.40)	0.92 (0.60, 1.41)	1.17 (0.75, 1.82)	0.53	1.03 (0.88, 1.21)
Coronary heart disease						
No. of events	9	13	12	11		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.53 (0.74, 3.13)	1.76 (0.86, 3.61)	2.48 (1.19, 5.17)	0.01	1.37 (1.08, 1.73)
Multivariable HR (95% CI)	1.00	1.49 (0.71, 3.10)	1.42 (0.68, 3.00)	1.78 (0.83, 3.82)	0.14	1.23 (0.95, 1.59)
Former smokers						
No. at risk	172	158	158	130		
Person-years	3,134	2,892	2,797	2,264		
Ischemic stroke						
No. of events	6	8	13	9		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.72 (0.77, 3.81)	2.33 (1.08, 5.00)	2.56 (1.16, 5.63)	0.01	1.28 (1.05, 1.57)
Multivariable HR (95% CI)	1.00	1.71 (0.75, 3.90)	1.99 (0.90, 4.43)	2.42 (1.07, 5.46)	0.03	1.20 (0.97, 1.49)
Coronary heart disease						
No. of events	3	5	8	9		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	1.21 (0.48, 3.08)	1.79 (0.73, 4.36)	1.93 (0.77, 4.83)	0.11	1.22 (0.96, 1.55)
Multivariable HR (95% CI)	1.00	1.19 (0.44, 3.24)	1.61 (0.61, 4.19)	1.55 (0.58, 4.15)	0.35	1.13 (0.87, 1.47)
Current smokers						
No. at risk	185	254	338	517		
Person-years	3,160	4,514	5,731	9,234		
Ischemic stroke						
No. of events	6	9	25	33		
Age-, sex-, and community-adjusted HR (95% CI)	1.00	2.14 (0.95, 4.82)	2.87 (1.32, 6.22)	2.58 (1.20, 5.57)	0.04	1.28 (1.06-1.55)
Multivariable HR (95% CI)	1.00	1.95 (0.86, 4.43)	2.60 (1.18, 5.74)	2.45 (1.11, 5.38)	0.05	1.26 (1.03-1.54)
Coronary heart disease						
No. of events	1	4	24	31		
Age-, sex-, and community-adjusted HR (95% CI)		1.00	3.18 (1.58, 6.37)	3.20 (1.63, 6.26)	0.001	1.43 (1.15, 1.76)
Multivariable HR (95% CI)		1.00	2.75 (1.35, 5.62)	2.73 (1.37, 5.44)	0.009	1.36 (1.09, 1.71)

*1 SD increment of leukocyte count was 16.4×10^2 cells/mm^3.
Q1: $18–52 \times 10^2$ cells/mm^3, Q2: $53–62 \times 10^2$ cells/mm^3, Q3: $63–74 \times 10^2$ cells/mm^3, and Q4: $75–130 \times 10^2$ cells/mm^3.
Multivariable hazard ratios were further adjusted for updated body mass index, systolic blood pressure, ethanol intake, serum non-HDL and HDL cholesterol levels, serum triglyceride, diabetes mellitus, and antihypertensive medication use.

similarly observed in both lacunar and non-lacunar infarctions, although the trend in non-lacunar infarction was of borderline statistical significance. In smoking status-stratified analyses, positive associations with the risks of ischemic stroke and coronary heart disease were more pronounced in current smokers, and similar associations of borderline statistical significance were observed in former and never smokers.

The positive associations of leukocyte count with the risks of ischemic stroke and coronary heart disease in this study are consistent with previous findings. The Atherosclerosis Risk in Communities (ARIC) Study of 13,555 African–Americans and Caucasian aged 45–64 years with a follow-up of 8 years reported that the multivariable hazard ratio (95% CI) of ≥ 70 ×

10^2 cells/mm^3 versus $<48\times10^2$ cells/mm^3 leukocytes was 2.00 (1.29–2.99); *P* for trend=0.002 for ischemic stroke, 1.80 (1.32–2.43); *P* for trend <0.001 for coronary heart disease[2]. Another ARIC study of 14,448 Americans aged 45–64 years with a follow-up of 13.4 years further investigated the associations between 1 SD increment (20×10^2 cells/mm^3) of leukocyte count and the risks of ischemic stroke subtypes; the multivariable hazard ratio (95% CI) was 1.14 (1.02–1.28) for lacunar infarction and 1.07 (0.99–1.15) for non-lacunar infarction[4]. The Honolulu Heart Program of 3,342 Japanese–American men aged 71–93 years with a follow-up of 8 years reported the multivariable hazard ratio (95% CI) of 72–640×10^2 cells/mm^3 versus 9–50×10^2 cells/mm^3 leukocytes was 1.69 (0.99–2.89); *P* for trend=0.04 for ischemic stroke[5]. The Women's Health Initiative of 74,375 American postmenopausal women aged 50–79 years with a follow-up of 16 years reported that the multivariable hazard ratio (95% CI) of the updated leukocyte count at the baseline and 3-years follow-up ($\geq 80\times10^2$ cells/mm^3 versus 53–56.99×10^2 cells/mm^3 [the fifth decile]) was 2.06 (1.60–2.65); *P* for trend <0.001 for coronary heart disease mortality[6]. However, a Japanese prospective cohort study of 4,492 male employees aged 40–59 years with a follow-up of 9 years and the Malmö Preventive Project of 20,063 Sweden men and women aged 27–61 years with a follow-up of 24 years reported no significant association of leukocyte count with the risk of ischemic stroke[8, 9].

Cigarette smoking has a long-term negative impact on leukocyte count[10]. The ARIC study reported that the multivariable hazard ratio (95% CI) of $\geq 70\times10^2$ cells/mm^3 versus $<48\times10^2$ cells/mm^3 leukocytes for ischemic stroke was 1.59 (0.85–2.30); *P* for trend=0.10 in never smokers, 2.13 (0.97–4.69); *P* for trend=0.05 in former smokers, and 3.15 (1.07–9.23); *P* for trend=0.07 in current smokers. The corresponding multivariable hazard ratio (95% CI) for coronary heart disease was 1.73 (1.08–2.79); *P* for trend=0.03, 2.01 (1.16–3.47); *P* for trend=0.02, and 1.44 (0.76–2.73); *P* for trend=0.06, respectively[2]. The Malmö Diet and Cancer Study of 26,972 Sweden men and women aged 45–73 years with a follow-up of 13.6 years reported that the multivariable hazard ratio (95% CI) of $\geq 73\times10^2$ cells/mm^3 versus $<52\times10^2$ cells/mm^3 leukocytes for ischemic stroke was 1.1 (0.8–1.4); *P* for trend=0.94 in never smokers, 1.5 (1.1–1.9); *P* for trend=0.01 in former smokers, 1.7 (1.2–2.5); *P* for trend <0.001 in current smokers[3]. The Japanese prospective cohort study reported that the multivariable hazard ratio (95% CI) of $\geq 60\times10^2$ cells/mm^3 versus $<60\times10^2$ cells/mm^3 leukocytes for myocardial infarction was 3.5 (1.0–1.2) in non-current smokers, 2.6 (1.0–1.2) in current smokers[8]. The Malmö Preventive Project reported that the multivariable hazard ratio (95% CI) of $\geq 70\times10^2$ cells/mm^3 versus $<47\times10^2$ cells/mm^3 leukocytes for coronary heart disease was 1.38 (1.04–1.82); *P* for trend=0.22 in never smokers, 1.68 (1.24–2.28); *P* for trend=0.004 in former smokers, 1.30 (1.09–1.54); *P* for trend <0.001 in current smokers[9].

The potential mechanisms underlying the associations of leukocyte count with the risks of ischemic stroke and coronary heart disease have been explored. Leukocyte count is a marker of systemic inflammation, which is considered to play an important role in the risk of ischemic cardiovascular disease. Leukocytes as a source of thrombogenic stimuli could enhance atherogenesis and lead to atherosclerotic plaque destabilization[18, 19]. Pro-inflammatory leukocytes adhere by adhesion molecules to endothelial cells and enter the vascular intima. Monocytes differentiate into macrophages and concentrate the per-oxidized lipids to become foam cells, which are the prototypical cells in the atherosclerotic plaque[18]. Neutrophils attract molecules to promote foam cell formation and aggravate atherosclerotic lesions[20]. Cigarette smoking is strongly associated with increased leukocyte count and could intermediate the association with the risk of ischemic cardiovascular disease[10, 21]. An early CIRCS report of 557 men aged 60–75 years found that the association of leukocyte count with mean carotid arteries intima-media thickness (IMT) had no significant difference between current and non-current smokers (*P* for interaction=0.65), and parts of lymphocyte subpopulations (CD4+CD45RO+T cells and CD19+CD80+B cells) were positively associated with mean IMT in non-current smokers[22].

The strength of our study lied in the large population-based study design with a 21-year follow-up. Also, we used updated leukocyte count and confounding variables to examine the associations, which could help reduce misclassification of study exposure. As for study limitations, the CIRCS did not measure C-reactive protein at the 1991–2000 baseline, which was another important inflammatory marker and is positively associated with the risks of ischemic stroke, coronary heart disease, and recurrent stroke among the general Japanese population[23-25]. However, subgroup analyses from the Women's Health Initiative Study found that the association of leukocyte count with cardiovascular disease mortality did not change materially after further adjustment for C-reactive protein[6].

In conclusion, leukocyte count was positively

associated with the risks of ischemic stroke and coronary heart disease among the general Japanese population, especially in current smokers.

Appendix
The CIRCS Investigators

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Competing Interest

None declared.

References

1) Libby P. Inflammation in atherosclerosis. Nature, 2002; 420: 868-874
2) Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: atherosclerosis risk in communities study. Am J Epidemiol, 2001; 154: 758-764
3) Zia E, Melander O, Björkbacka H, Hedblad B, Engström G. Total and differential leucocyte counts in relation to incidence of stroke subtypes and mortality: a prospective cohort study. J Intern Med, 2012; 272: 298-304
4) Ohira T, Shahar E, Chambless LE, Rosamond WD, Mosley TH Jr, Folsom AR. Risk factors for ischemic stroke subtypes: the Atherosclerosis Risk in Communities study. Stroke, 2006; 37: 2493-2498
5) Huh JY, Ross GW, Chen R, Abbott RD, Bell C, Willcox B, Launer L, Petrovitch H, Kaya B, Masaki K. Total and differential white blood cell counts in late life predict 8-year incident stroke: the Honolulu Heart Program. J Am Geriatr Soc, 2015; 63: 439-446
6) Kabat GC, Kim MY, Manson JE, Lessin L, Lin J, Wassertheil-Smoller S, Rohan TE. White Blood Cell Count and Total and Cause-Specific Mortality in the Women's Health Initiative. Am J Epidemiol, 2017; 186: 63-72
7) Welsh C, Welsh P, Mark PB, Celis-Morales CA, Lewsey J, Gray SR, Lyall DM, Iliodromiti S, Gill JMR, Pell J, Jhund PS, Sattar N. Association of Total and Differential Leukocyte Counts With Cardiovascular Disease and Mortality in the UK Biobank. Arterioscler Thromb Vasc Biol, 2018; 38: 1415-1423
8) Imano H, Sato S, Kitamura A, Kiyama M, Ohira T, Shimamoto T, Iso H. Leukocyte count is an independent predictor for risk of acute myocardial infarction in middle-aged Japanese men. Atherosclerosis, 2007; 195: 147-152
9) Li C, Engström G, Hedblad B. Leukocyte count is associated with incidence of coronary events, but not with stroke: a prospective cohort study. Atherosclerosis, 2010; 209: 545-550
10) Nieto FJ, Szklo M, Folsom AR, Rock R, Mercuri M. Leukocyte count correlates in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol, 1992; 136: 525-537
11) Imano H, Kitamura A, Sato S, Kiyama M, Ohira T, Yamagishi K, Noda H, Tanigawa T, Iso H, Shimamoto T. Trends for blood pressure and its contribution to stroke incidence in the middle-aged Japanese population: The Circulatory Risk in Communities Study (CIRCS). Stroke, 2009; 40: 1571-1577
12) Yamagishi K, Muraki I, Kubota Y, Hayama-Terada M, Imano H, Cui R, Umesawa M, Shimizu Y, Sankai T, Okada T, Sato S, Kitamura A, Kiyama M, Iso H. The Circulatory Risk in Communities Study (CIRCS): A Long-Term Epidemiological Study for Lifestyle-Related Disease Among Japanese Men and Women Living in Communities. J Epidemiol, 2019; 29: 83-91
13) Nakamura M, Iso H, Kitamura A, Imano H, Kiyama M, Yokoyama S, Kayamori Y, Koyama I, Nishimura K, Nakai M, Dasti M, Vesper HW, Teramoto T, Miyamoto Y. Total cholesterol performance of Abell-Levy-Brodie-Kendall reference measurement procedure: Certification of Japanese in-vitro diagnostic assay manufacturers through CDC's Cholesterol Reference Method Laboratory Network. Clin Chim Acta, 2015; 445: 127-132
14) Nakamura M, Iso H, Kitamura A, Imano H, Noda H, Kiyama M, Sato S, Yamagishi K, Nishimura K, Nakai M, Vesper HW, Teramoto T, Miyamoto Y. Comparison between the triglycerides standardization of routine methods used in Japan and the chromotropic acid reference measurement procedure used by the CDC Lipid Standardization Programme. Ann Clin Biochem, 2016; 53: 632-639

15) Kirkendall WM, Feinlieb M, Freis ED, Mark AL. Recommendations for human blood pressure determination by sphygmomanometers. Subcommittee of the AHA Postgraduate Education Committee. Circulation, 1980; 62: 1146A-1155A
16) Iso H, Rexrode K, Hennekens CH, Manson JE. Application of computer tomography-oriented criteria for stroke subsubtype classification in a prospective study. Ann Epidemiol, 2000; 10: 81-87
17) WHO Expert Committee. Arterial hypertension and ischemic heart disease, preventive aspect: WHO technical report series no. 231. Geneva: World Health Organization, 1962
18) Libby P, Nahrendorf M, Swirski FK. Leukocytes Link Local and Systemic Inflammation in Ischemic Cardiovascular Disease: An Expanded "Cardiovascular Continuum." J Am Coll Cardiol, 2016; 67: 1091-1103
19) Lafont A. Basic aspects of plaque vulnerability. Heart, 2003; 89: 1262-1267
20) Döring Y, Soehnlein O, Weber C. Neutrophil Extracellular Traps in Atherosclerosis and Atherothrombosis. Circ Res, 2017; 120: 736-743
21) SahBandar IN, Ndhlovu LC, Saiki K, Kohorn LB, Peterson MM, D'Antoni ML, Shiramizu B, Shikuma CM, Chow DC. Relationship between Circulating Inflammatory Monocytes and Cardiovascular Disease Measures of Carotid Intimal Thickness. J Atheroscler Thromb, 2020; 27: 441-448
22) Tanigawa T, Kitamura A, Yamagishi K, et al. Relationships of differential leukocyte and lymphocyte subpopulations with carotid atherosclerosis in elderly men. J Clin Immunol, 2003; 23: 469-476
23) Iso H, Noda H, Ikeda A, Yamagishi K, Inoue M, Iwasaki M, Tsugane S. The impact of C-reactive protein on risk of stroke, stroke subtypes, and ischemic heart disease in middle-aged Japanese: the Japan public health center-based study. J Atheroscler Thromb, 2012; 19: 756-766
24) Chei CL, Yamagishi K, Kitamura A, Kiyama M, Imano H, Ohira T, Cui R, Tanigawa T, Sankai T, Ishikawa Y, Sato S, Iso H. C-reactive protein levels and risk of stroke and its subtype in Japanese: The Circulatory Risk in Communities Study (CIRCS). Atherosclerosis, 2011; 217: 187-193
25) Kitagawa K, Hosomi N, Nagai Y, Kagimura T, Ohtsuki T, Maruyama H, Origasa H, Minematsu K, Uchiyama S, Nakamura M, Matsumoto M; J-STARS collaborators. Cumulative Effects of LDL Cholesterol and CRP Levels on Recurrent Stroke and TIA. J Atheroscler Thromb, 2019; 26: 432-441