Leukocyte count is associated with incidence of coronary events, but not with stroke

A prospective cohort study

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Abstract

Objective

Elevated leukocyte count is a classic marker of systemic inflammation. This study examined whether the leukocyte count is associated with incidence of coronary events (CE) and stroke during a long follow-up period.

Methods

A total of 17,131 men and 2,932 women, aged 27-61 years, without history of cardiovascular disease (CVD), were enrolled. Incidence of CE and stroke was studied in relation to leukocyte concentrations over a mean follow-up of 24 years.

Results

During the follow-up period, 2,600 CE and 1,333 stroke events occurred. After risk factor adjustments, leukocyte concentrations in the highest quartile (vs. lowest, >7.0 vs. <4.7 x10^9cells/L) was associated with CE in men (HR: 1.31, 95%CI: 1.16-1.48, trend p<0.001), but not significantly in women (HR: 1.46, CI: 0.87-2.46, trend p=0.13). The increased incidence remained significant after adjustments for plasma fibrinogen in a subgroup of 6,018 men (HR: 1.31 CI: 1.08-1.60).

The association between leukocytes and CE was most pronounced in younger men (aged 27-46) and men without hypertension. In younger men, high leukocytes were associated with early CE (within 10 years of follow-up) and late CE (>10 years of follow-up). In older men (46-61 years), leukocytes were not associated with CE after more than 10 years of follow-up.

The leukocyte count was not associated with incidence of stroke.

Conclusion

Elevated leukocyte count in men is associated with incidence of CE, but not with incidence of stroke. The increased risk persisted after more than ten years of follow-up in younger, but not in older men.

Key words

Leukocyte count, coronary events, stroke, cohort study.

Introduction

Inflammation is considered to play an important role in the pathogenesis of atherosclerosis [1]. Leukocyte count is a simple, inexpensive and well-standardized biomarker of acute or chronic inflammation [2]. Some epidemiologic studies [2-5] have demonstrated that high leukocyte count is an independent risk factor for future coronary heart disease (CHD). Only few prospective studies [6-7] examined the association between leukocyte count and incidence of stroke.

In most prospective studies [4-7], the effects of various risk factors are evaluated over a few years follow-up after the baseline examination. Studies of the long-term within-person variation of inflammatory markers have shown that e.g., C-reactive protein (CRP), erythrocyte sedimentation rate and white blood cells are quite stable over time [8,9], and many markers of inflammation, including the leukocyte concentrations, are strongly influenced by genetic factors [10]. This suggests that inflammatory markers could be useful in long-term prediction of cardiovascular disease (CVD). However, studies of CRP have reported that the increased risk associated with high concentrations decline after a few year of follow-up, particularly in the elderly [11, 12]. It is unclear whether the impact of raised leukocyte concentrations on the risk of CVD remains over a very long follow-up period.

The purpose of this population-based study is to examine whether leukocyte count is associated with incidence of coronary events (CE) or stroke in a long-term prospective investigation among Swedish middle-aged men and women.

Methods

Study population

The main purpose of the Malmö Preventive Project (MPP) was to identify high-risk individuals for CVD in an urban-population, Malmö, Sweden. The MPP has been described in detail previously [13]. In short, complete birth cohorts, born between 1921 and 1949, were invited. A total of 22,444 men were examined between 1974 and 1984, and 10,902 women were examined between 1977 and 1992. Participation rate was approximately 71% [13]. The health examination included a physical examination, a panel of laboratory tests and self-administered questionnaire with items relevant for the occurrence of CVD [13].

Leukocyte count was analyzed in participants during the period of 1975-1980 (i.e. in 17,223 men and 2,937 women). In the present study, subjects with a history of myocardial infarction (MI) or stroke (according to questionnaire and/or hospital records, n=72), or with missing value of body mass index (BMI) or blood pressure (BP) (n=17), or with extremely high leukocyte values (i.e.>20.0 $\times 10^9$cells/L, n=8) were excluded from the analysis. Thus, the total study population consisted of 17,131 men (mean age: 44±5 years, range: 27-61) and 2,932 women (mean age: 42±9 years, range: 28-56).

The health service authority of Malmö approved and funded the screening program. All participants gave informed consent.

Baseline characteristics

Information on baseline characteristics, which has been presented previously [9], was collected from self-administered questionnaire and clinical examination. Smoking habits

were categorized into never, former (i.e. smokers who had quit smoking at least 1 year before the examination), and current smokers (i.e. daily smoking with <20 and ≥20 cigarettes/day, respectively) [14]. High alcohol consumption was assessed by means of the modified and shortened version of the Michigan Alcoholism Screening Test [15]. Subjects with more than two (of nine) affirmative answers were considered to have high alcohol consumption. Two categories were used for the classification of leisure-time physical activity, e.g. sedentary or not. Diabetes mellitus was defined as fasting whole blood glucose > 6.1mmol/L and/or self-reported diabetes according to the questionnaire. Information on current use of BP-lowering medication was also included in the questionnaire.

By using mercury sphygmomanometer, BP (mmHg) was measured twice in the right arm after a 10-minutes rest. The average of two measurements was used. Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥ 90 mmHg or use of BP-lowering medication [16]. BMI was calculated as weight/height^2 (kg/m^2) and further categorized into normal weight (BMI<25.0) and overweight/obese (BMI≥25.0) sub-groups.

Laboratory tests

Blood samples were taken after an overnight fast. Leukocyte counts (x10^{9}cells/L) were analysed by using automatic counters in accordance with the standard methods at the laboratory of the Malmö university hospital. In the present analysis, the values of leukocyte count were categorized into sex-specific quartiles.

Serum cholesterol and triglycerides were similarly analysed with standard methods at the laboratory. Hyperlipidemia was defined as total cholesterol ≥5.0 mmol/L (i.e. ≥190 mg/dL) and/or triglyceride >1.7 mmol/L (i.e. >150 mg/dL) [17].

Plasma fibrinogen was available in a subset of 6,018 men (aged 47±4 years) [14, 18]. Electroimmuno assay was used to assess the plasma levels of fibrinogen [19]. The analysis was performed consecutively at the time of study entry.

Retrieval of endpoints

All subjects were followed from the baseline examination until first hospitalization due to incident MI, stroke or death, or December 31st, 2004, whichever came first. The mean follow-up period was 24±6 years for men and 24±5 years for women. An acute coronary event (CE) was defined as fatal or non-fatal MI (ICD-9 codes: 410A-410X; ICD-10: I21) or death from chronic ischemic heart disease (ICD-9 codes: 410-414; ICD-10: I20-I25). The records of patients with CE were retrieved by data linkage with the National Hospital Discharge Register and National Cause of Death Registry [20].

Incident cases of stroke were retrieved by data linkage to the Swedish Hospital Discharge Register and the Stroke Register in Malmö (STROMA) [14, 18, 21]. Stroke was defined as rapidly developing clinical signs of local or global loss of cerebral function that lasted for >24 hours or leading to death within 24 hours. Subtypes of stroke were classified as cerebral infarction (ischemic,ICD-9,code: 434; ICD-10: I63), intra-cerebral hemorrhage (ICD-9,code: 431; ICD-10: I61) and subarachnoid hemorrhage (ICD-9,code: 430; ICD-10: I60) diagnosed by computed tomography (CT), magnetic resonance tomography (MR), lumbar puncture or autopsy. Cases with typical clinical symptoms, but without

verification by CT, MR or autopsy (n=75), were considered as non-specified stroke (ICD-9, code: 436; ICD-10: I64) since these imaging techniques were not available in clinical practice during the initial years of follow-up. This group was collapsed with ischemic stroke as most of non-specified cases assumingly were caused by cerebral ischemia.
In subjects with more than one CE or stroke event, only the first event was used for the analysis.

Statistics

One-way analysis of variance and Chi-square test were used to examine baseline characteristics in relation to leukocyte quartiles. *P* values for the trend analyses were assessed by using general linear or logistic regression model with age-adjustment.
Power calculations were made for CE or stroke in men and women. Assuming a hazard ratio (HR) of 1.3, the statistical power for CE or stroke in men would be 99% and 95%, respectively, and 28% and 21% in women, respectively.
All analyses performed were sex-specific. Kaplan-Meier method was used to assess the rates of endpoints-free survival corresponding to the quartiles of leukocytes. Cox proportional hazards regression was used to compare incidences of endpoints in relation to leukocyte count (in quartiles), and to calculate risk factor adjusted HR (95% confidence interval, CI). The initial model was adjusted for age only. The fully adjusted model included potential confounders, i.e. the baseline characteristics which showed difference (i.e. $p<0.10$) between leukocyte counts in quartiles.
To explore the long-term ability of leukocyte levels to predict CVD risk, the analyses were also performed separately for events that occurred during the first 10 years of

follow-up, and events that occurred after more than 10 years of follow-up. Furthermore, in men the assessments were performed stratified by the median age (i.e. 46 years) to determine whether the association was related to age. No similar analyses were performed in women due to limited number of events.

In men interactions between leukocyte concentrations and other risk factors (i.e. in terms of age groups, smoking habits, high alcohol intake, physical inactivity, overweight, hypertension, hyperlipidemia and diabetes) were explored by interaction terms in the covariate-adjusted regression models.

All comparisons were two-sided and a 5% level of significance was used. The statistical analyses were conducted by the computer software SPSS ^{TM} (version 14.5).

Results

Baseline characteristics

The mean values of leukocyte count at baseline examination were 6.1±2.0 x10^9cells/L (inter-quartile range 4.6-7.1, range 2.0-19.2) in men, and 6.0±2.0 x10^9cells/L (inter-quartile range 4.6–7.0, range 2.0-18.4) in women.

Baseline characteristics by quartiles of leukocytes are described in online Table 1. High leukocyte counts were associated with current smoking, high alcohol intake, presence of diabetes and hyperlipidemia, higher levels of BMI, total cholesterol and triglycerides in both men and women. For systolic BP and physical inactivity, the positive significant association was only observed in men.

Incidence of CE

During the follow-up period, 2,466 men and 134 women, respectively, had a CE. The baseline values of leukocyte count were higher in CE cases, compared to non-cases both in men (6.6±2.2 vs.6.0±2.0x10^9cells/L, p<0.001) and women (6.5±2.4 vs. 5.9±2.0 x10^9cells/L, p=0.001). In men, the age-adjusted HR for CE in the highest compared to the lowest quartile (i.e. >7.0 vs. <4.7 x10^9cells/L) was 2.17 (95% CI: 1.94-2.43), Table 1. The association was weakened, but remained significant (HR: 1.31, 1.16-1.48, p for trend<0.001) after adjustment for other CVD risk factors (Table 1). Although not significantly, there was a similar relationship among women (HR: 1.46, 0.87-2.46, p for trend=0.13).

In a sub-cohort analysis of 6,018 men, aged 47±4 years, plasma fibrinogen was added as a continuous variable in model. The HR for CE in the top quartile of leukocytes

attenuated slightly, from 1.39 (1.14-1.69) to 1.31 (1.08-1.60), after adjustments for fibrinogen.

Long-term relationship with incidence of CE

In all men, the difference in CE-free survival rate between the quartiles of leukocytes increased continuously over three decades of follow-up period (Figure 1). To further explore the risk of CE over time, the analyses were repeated for events that occurred within 10 years and after 10 years of follow-up, respectively (Table 2). For events within 10 years, the HR in the top quartile of leukocytes was 1.42 (1.10-1.83, *p* for trend <0.001) after adjustment for risk factors. For events that occurred after more than 10 years, the HR in the top quartile of leukocyte was 1.28 (1.11-1.47, *p* for trend=0.001). However, when the analyses were performed separately in younger (27-46 years) and older (47-61 years) men, the relationship with incidence of CE after more than 10 years of follow-up was significant in younger men (HR in the top quartile: 1.64, 1.28-2.11, *p* for trend <0.001) but not in older men (HR: 1.12, 0.94-1.34, *p*=0.26). High leukocytes were associated with events within 10 years both in younger (HR: 1.70, 1.02-2.85, *p* for trend=0.003) and older men (HR: 1.31, 0.97-1.77, *p* for trend=0.02).

Leukocyte count and CVD risk factors

Among men, the association between leukocytes and CE was examined in categories of other risk factors (Table 3). The impact of leukocytes on CE seemed to be most pronounced in men aged 27-46 years, in former or current smokers and in men without hypertension. Interaction analyses were also performed in men. Significant interactions

on incidence of CE were observed between leukocyte count (as quartiles or as a continuous variable) and age ($p<0.001$) or hypertension ($p<0.001$).

Incidence of stroke

A total of 1,226 men and 107 women developed a first-ever stroke during follow-up. Of them, 1,098 (82.3%) were classified as ischemic infarction, 162 (12.2%) as cerebral hemorrhage and 73 (5.5%) as subarachnoid hemorrhage (Table 4). In this study, only total stroke and ischemic subtype was examined.

In men, a significant difference in leukocyte count was observed between stroke cases and non-cases (6.3±2.0 vs.6.0±2.0 $\times 10^9$cells/L, $p<0.001$). No significant difference was found in women (6.3±2.4 vs.6.0±2.0 $\times 10^9$cells/L, $p=0.10$). The age-adjusted HR increased moderately across the quartiles of leukocytes in both men and women (Table 4). However, the trends became non-significant in both sexes after adjustments for potential confounders. The results were similar when ischemic stroke was analyzed.

Discussion

Over an average of 24 years follow-up, raised leukocyte concentrations were associated with increased incidence of CE in men. The increased risk was moderate but persistent, in a dose-response manner, independently of other cardiovascular risk factors and fibrinogen. The association was stable over more than ten years of follow-up in younger, but not in older men. Leukocyte count had no notable association with incidence of stroke.

It is still questioned whether leukocytes are involved in the pathogenesis of CVD or are only a risk marker for other risk factors causing the disease. It has been reported that leukocytes participate in the formation of atheromas, even in the earliest stage of the disease process [22]. It is also suggested that leukocytes may affect the cardiovascular system through other pathologic mechanisms, such as mediating inflammation, causing proteolytic and oxidative damage to the endothelial cells, plug the microvasculature, inducing hyper-coagulability, and promotion of infarct expansion [5]. Results from this and other epidemiological studies [4, 5] also indicate that elevated leukocyte count is a risk factor for coronary events, even after adjustments for many established CVD risk factors.

In contrast to incidence of CE, there were no significant relationship between leukocyte count and incidence of stroke in this study. Even though the pathogenesis of CHD and ischemic stroke show many similarities, both CE and ischemic stroke are heterogeneous disorders and there are well-known differences in their relationship with CVD risk factors [23]. For example, cholesterol and male sex seem to be more linked to CHD, while age

and blood pressure seem to be more important for the risk of stroke. Different relationships between leukocytes and incidence of CE and stroke were also reported by Gillum et al [24]. The reason for the differential relationships is not obvious. The statistical power in this study was high, both for the analysis of CE and stroke in men. The results suggest that leukocyte concentration may be another risk factor which is more important for incidence of CE than for stroke in men.

In studies of elderly, it has been reported that inflammatory markers show a time-to-event dependency [11, 12], and that the prognostic value of inflammatory markers on CVD attenuate after a few years of follow-up. In the present study, the leukocyte count was associated with CE even after more than ten years of follow-up. However, this was true only for younger men (aged 27-46 years), and not for the older age group (aged 47-61 years). In our sub-group analyses, the impact of leukocyte count on risk of CE was stronger among men who had lower risk of CVD, i.e. younger men or men who were normotensive. A similar relationship was reported by another study, in which elevated leukocyte count was associated with increased risk of CVD mainly in women without established CVD risk factors [4]. Since the occurrence of atherosclerosis generally is lower in these groups, this suggests that high leukocyte concentrations in plasma may reflect early phases of atherogenesis. It has been suggested that, in younger individuals, inflammatory biomarkers reflect the progression toward vascular lesions and therefore yield both short- and long-term prediction, while in older people inflammatory markers may reflect the degree of complex advanced vascular disease burden, as well as other

morbidities, with the highest levels reflecting severe pathology associated with early risk [11]. Our results are in accordance with that hypothesis.

Few studies [4, 25] have assessed the relationship between leukocyte count and incidence of CVD in women. In the Women's Health Initiative Observational Study [4], women in the fourth quartile of leukocytes had over a 2-fold increased risk for CHD death and more than 40% increased risk for non-fatal CHD and stroke. Similar results have been reported from a large prospective study [25]. In our study, the point estimates were largely similar for men and women. It is likely that the absence of significant relationships among women in the present study could be explained by low statistical power.

The strength of this study lies in the large, prospective and population-based study design with a very long follow up period. Because of the long follow up, a large number of events were recorded, allowing outcome analysis of both the first decade and the complete follow-up period. In addition, information on several potential confounders was also available. Some limitations of this study are worth noting. Lack of repeated measurements of leukocyte count is a limitation, which may result in underestimation of the true association. As to the intra-individual variability of leukocytes, it was shown that the leukocyte count is fairly stable over time [9]. Lack of information on leukocyte differentials is also a limitation. The high risk of CVD associated with total leukocytes has been mainly accounted for by increased granulocyte count [25]. Furthermore, some individuals in the top quartile of leukocytes maybe had an infection, which caused raised leukocyte concentrations at the time of screening. Another weakness is lacking

information on sub fractions of blood lipids, i.e. HDL- and LDL-cholesterol, in the present study.

Conclusion

In this long-term prospective population-based study, elevated leukocyte count was associated with incidence of CE in men. The risks were moderate but independent of multiple cardiovascular risk factors. The increased risk persisted after more than ten years of follow-up in younger men, but not in older age groups. However, there was no significant association between leukocyte count and incidence of stroke.

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Online Table 1. Baseline characteristics in relation to sex-specific quartiles of leukocyte counts.

	Leukocyte Quartiles				
	quartile-1	quartile-2	quartile-3	quartile-4	*p for trend* †
Men					
Number of subjects	4434	4241	4084	4372	
*Leukocyte count (*x10^9cells/L*)*	2.0 - 4.6	4.7 - 5.7	5.8 - 7.0	7.1 - 19.2	
Leukocyte count, median	4.0	5.2	6.3	8.3	
Age, years	43.7 ± 5.6	44.1 ± 5.5	44.0 ± 5.5	44.4 ± 5.2	<0.001
Baseline risk factors					
Smoking habits, %					
Never-smokers	49.4	37.6	25.9	12.1	<0.001
Former smokers	25.1	22.8	16.6	8.0	<0.001
Current-smokers	25.5	39.6	57.5	79.9	<0.001
<20 cigarettes/day	20.9	30.4	42.1	55.7	<0.001
≥20 cigarettes/day	4.6	9.2	15.4	24.2	<0.001
High alcohol intake, %	22.6	24.6	25.8	29.4	<0.001
Physical inactivity, %	50.2	54.8	56.9	59.1	<0.001
Presence of diabetes, %	3.7	4.0	4.4	5.0	0.002
Use of BP-lowering medication,%	3.2	3.5	4.2	4.0	0.07
Physical examination					
Systolic BP, mmHg	127 ± 14	128 ± 15	128 ± 15	128 ± 16	0.03
Diastolic BP, mmHg	86 ± 9	86 ± 10	86 ± 10	86 ± 10	0.45
BMI, kg/m^2	24.5 ± 3.0	24.8 ± 3.3	24.9 ± 3.5	24.7 ± 3.3	<0.001
Laboratory tests					
Total cholesterol, mmol/L	5.54 ±1.05	5.62±1.02	5.66 ± 1.04	5.82 ±1.14	<0.001
Triglycerides, mmol/L ‡	1.36 ±0.86	1.50±0.95	1.59 ±1.02	1.73 ±1.37	<0.001
Hyperlipidemia, % #	71.8	76.0	77.6	81.9	<0.001
Women					
Number of subjects	728	762	716	726	
*Leukocyte count (*x10^9cells/L*)*	2.0 - 4.5	4.6 - 5.6	5.7 - 7.0	7.1 - 18.4	
Leukocyte count, median	3.9	5.1	6.3	8.2	
Age, years	43.5 ± 8.5	42.5 ± 8.6	42.1 ± 8.5	40.9 ± 8.5	<0.001
Baseline risk factors					
Smoking habits, %					
Never-smokers	57.6	43.6	40.1	23.1	<0.001
Former smokers	19.5	18.3	13.2	10.9	<0.001
Current-smokers	22.9	38.1	46.7	66.0	<0.001

<20 cigarettes/day	20.7	34.0	41.9	55.2	<0.001
≥20 cigarettes/day	2.2	4.1	4.8	10.8	<0.001
High alcohol intake, %	7.6	8.0	8.9	11.4	0.04
Physical inactivity, %	52.5	50.3	51.0	53.5	0.25
Presence of diabetes, %	1.1	1.2	2.0	2.6	0.003
Use of BP-lowering medication,%	4.8	3.4	3.5	3.9	0.07
Physical examination					
Systolic BP, mmHg	121 ± 16	120 ± 15	121 ± 15	119 ± 15	0.47
Diastolic BP, mmHg	81 ± 9	80 ± 9	80 ± 10	79 ± 10	0.11
BMI, kg/m^2	22.9 ± 3.4	23.2 ± 3.7	23.7 ± 4.3	23.0 ± 4.0	<0.001
Laboratory tests					
Total cholesterol, mmol/L	5.32 ± 1.01	5.30±1.01	5.36 ± 1.00	5.37 ±1.12	0.002
Triglycerides, mmol/L ‡	1.07 ± 0.51	1.18±0.75	1.19 ± 0.52	1.32 ±0.64	<0.001
Hyperlipidemia, % #	61.1	64.1	62.8	63.1	0.01

BP, blood pressure; BMI, body mass index; SD, standard deviation.
Data are presented as mean (SD) or as proportions.
† Adjusted for age.
‡ Log-transformed in the statistical analyses because of skewed distribution.
# Definition of hyperlipidemia was defined as total cholesterol ≥5.0 mmol/L and/or triglyceride >1.7 mmol/L.

Table 1. Incidence of coronary events in relation to in sex-specific quartiles of leukocyte count.

	Leukocyte Quartiles				
	quartile-1	quartile-2	quartile-3	quartile-4	*p for trend*
Men					
Number of cases	457	521	608	880	
HR, 95%CI					
Age-adjusted	1.0	1.20 (1.06-1.36)	1.51 (1.34-1.70)	2.17 (1.94-2.43)	<0.001
Covariate-adjusted †	1.0	1.02 (0.90-1.16)	1.10 (0.97-1.25)	1.31 (1.16-1.48)	<0.001
Women					
Number of cases	26	30	33	45	
HR, 95%CI					
Age-adjusted	1.0	1.24 (0.73-2.10)	1.62 (0.97-2.71)	2.44 (1.50-3.97)	<0.001
Covariate-adjusted †	1.0	1.05 (0.62-1.79)	1.11 (0.65-1.90)	1.46 (0.87-2.46)	0.13

HR, hazards rate; CI, confidence interval; BP, blood pressure; BMI, body mass index.

† Adjusted for age, smoking habits, high alcohol intake, physical inactivity, BMI, systolic BP, BP-lowering medication, presence of diabetes, total cholesterol and triglycerides (log transformed).

Table 2. Baseline leukocyte counts (in quartiles) in relation to early (≤10 years of follow-up) and late (>10 years of follow-up) incident CE in men, by age-group.

	Leukocyte Quartiles				
	quartile-1	quartile-2	quartile-3	quartile-4	*p for trend*
Follow-up period ≤ 10 years					
Number of cases	94	99	142	253	
HR, 95%CI (covariate-adjusted) †					
All men	1.0	0.85 (0.63-1.13)	1.09 (0.83-1.42)	1.42 (1.10-1.83)	<0.001
Age 27-46 years	1.0	0.72 (0.39-1.33)	1.39 (0.82-2.35)	1.70 (1.02-2.85)	0.003
Age 47-61 years	1.0	0.89 (0.64-1.23)	0.99 (0.72-1.36)	1.31 (0.97-1.77)	0.02
Follow-up period > 10 years					
Number of cases	363	422	466	627	
HR, 95%CI (covariate-adjusted) †					
All men	1.0	1.08 (0.93-1.24)	1.13 (0.97-1.29)	1.28 (1.11-1.47)	0.001
Age 27-46 years	1.0	1.25 (0.97-1.61)	1.57 (1.23-2.01)	1.64 (1.28-2.11)	<0.001
Age 47-61 years	1.0	1.00 (0.84-1.19)	0.92 (0.77-1.10)	1.12 (0.94-1.34)	0.26

HR, hazards rate; CI, confidence interval; BP, blood pressure; BMI, body mass index.

† Adjusted for age, smoking habits, high alcohol intake, physical inactivity, BMI, systolic BP, BP-lowering medication, presence of diabetes, total cholesterol and triglycerides (log transformed).

Table 3. Incidence of coronary events in relation to quartile of leukocytes in men, by exposure to other cardiovascular risk factors.

		Leukocyte quartiles		
		quartile-1	quartile-4	
	No. of cases	HR, 95%CI	HR, 95%CI	*P for trend †*
Age, years				
27-46	809	1.0	1.77 (1.34-2.21)	<0.001
47-61	1655	1.0	1.16 (1.00-1.35)	0.04
Smoking habits				
Never-smokers	483	1.0	1.38 (1.04-1.82)	0.22
Former smokers	379	1.0	1.68 (1.24-2.28)	0.004
Current-smokers	1603	1.0	1.30 (1.09-1.54)	<0.001
High alcohol intake ‡				
No	1883	1.0	1.24 (1.08-1.43)	0.002
Yes	583	1.0	1.56 (1.19-2.04)	<0.001
Physical inactivity ‡				
No	1043	1.0	1.38 (1.14-1.67)	<0.001
Yes	1410	1.0	1.24 (1.05-1.47)	0.003
BMI, kg/m^2				
<25.0	1199	1.0	1.21 (1.01-1.44)	0.02
≥25.0	1267	1.0	1.40 (1.18-1.67)	<0.001
Presence of diabetes ‡				
No	2293	1.0	1.28 (1.12-1.45)	<0.001
Yes	173	1.0	1.75 (1.02-2.99)	0.03
Hypertension ‡				
No	1062	1.0	1.64 (1.35-2.00)	<0.001
Yes	1404	1.0	1.15 (0.97-1.35)	0.08
Hyperlipidemia ‡				
No	285	1.0	1.42 (1.01-2.02)	0.04
Yes	2176	1.0	1.36 (1.19-1.55)	<0.001

HR, hazards rate; CI, confidence interval; BP, blood pressure; BMI, body mass index.

† HRs were adjusted for age, smoking habits, high alcohol intake, physical inactivity, BMI, systolic BP, BP-lowering medication, presence of diabetes, total cholesterol and log transformed triglycerides (when appropriate).

‡ The definitions of risk categories were described in Methods section.

Table 4. Incidence of stroke (and ischemic subtype) in relation to sex-specific quartiles of leukocyte concentrations.

	Leukocyte Quartiles				
	quartile-1	quartile-2	quartile-3	quartile-4	*p for trend*
Men					
All stroke					
Number of cases	267	295	319	363	
HR, 95%CI					
Age-adjusted	1.0	1.16 (0.99-1.37)	1.36 (1.16-1.60)	1.52 (1.30-1.78)	<0.001
Covariate-adjusted †	1.0	1.04 (0.88-1.23)	1.11 (0.94-1.32)	1.11 (0.93-1.33)	0.18
Ischemic stroke					
Number of cases	225	229	255	306	
HR, 95%CI					
Age-adjusted	1.0	1.07 (0.89-1.29)	1.29 (1.08-1.55)	1.52 (1.28-1.81)	<0.001
Covariate-adjusted †	1.0	0.96 (0.79-1.15)	1.04 (0.86-1.25)	1.09 (0.90-1.32)	0.29
Women					
All stroke					
Number of cases	24	23	31	29	
HR, 95%CI					
Age-adjusted	1.0	0.99 (0.56-1.76)	1.58 (0.93-2.70)	1.59 (0.92-2.74)	0.04
Covariate-adjusted †	1.0	0.90 (0.50-1.60)	1.30 (0.75-2.27)	1.12 (0.63-2.02)	0.44
Ischemic stroke					
Number of cases	17	19	27	20	
HR, 95%CI					
Age-adjusted	1.0	1.19 (0.62-2.28)	2.06 (1.12-3.79)	1.69 (0.88-3.23)	0.03
Covariate-adjusted †	1.0	1.06 (0.54-2.05)	1.60 (0.85-3.03)	1.15 (0.57-2.30)	0.45

HR, hazards rate; CI, confidence interval; BP, blood pressure; BMI, body mass index.

† Adjusted for age, smoking habits, high alcohol intake, physical inactivity, BMI, systolic BP, BP-lowering medication, presence of diabetes, total cholesterol and log transformed triglycerides.

Figure legend

Coronary events-free survival in relation to leukocyte count (in quartiles) in 17,131 men during an average of 24 years follow-up (p value for trend < 0.001).

Figure