Journal of Clinical & Translational Endocrinology 1 (2014) 49–53

Contents lists available at ScienceDirect

Journal of Clinical & Translational Endocrinology

journal homepage: www.elsevier.com/locate/jcte

Research Paper

Longitudinal associations between lymphocyte count and LDL cholesterol in a health screening population☆

Eiji Oda, MD *

Medical Check-up Center, Tachikawa Medical Center, Nagachou 2-2-16, Nagaoka, Niigata 940-0053, Japan

ARTICLE INFO

Article history:
Received 19 March 2014
Received in revised form
2 May 2014
Accepted 3 May 2014

Keywords:
Adaptive immune system
Atherosclerosis
Inflammation
LDL cholesterol
Lymphocyte count

ABSTRACT

Background: Longitudinal associations between leukocyte subtype counts and LDL cholesterol have not been reported.
Methods and Results: This is a retrospective observational study in a health screening population. Spearman's correlation coefficients were calculated between leukocyte subtype counts and LDL cholesterol levels at baseline and after four years. Using Cox regression models, hazard ratios (HRs) of hyper-LDL cholesterolemia for leukocyte subtype counts during four years of follow-up were calculated adjusted for age, sex, high-sensitivity C-reactive protein (hs-CRP) and other confounders. Spearman's correlation coefficients (p values) between changes in counts of neutrophil, lymphocyte, monocyte, basophil and eosinophil and changes in LDL cholesterol levels through 4 years were 0.02 (0.494), 0.12 (<0.001), 0.06 (0.016), 0.02 (0.524) and 0.03 (0.257), respectively among 1735 subjects who visited our medical check-up center, did not use anti-hyperlipidemic drugs and revisited after 4 years. Among 1992 followed subjects, 481 developed hyper-LDL cholesterolemia during four years (60.4 per 1000 person-years). The HRs (95% confidence intervals; p values) of hyper-LDL cholesterolemia for each one SD increase in counts of neutrophil, lymphocyte, monocyte, basophil and eosinophil were 1.08 (0.99–1.19; 0.085), 1.14 (1.04–1.25; 0.005), 1.05 (0.95–1.15; 0.339), 1.01 (0.92–1.11; 0.858) and 1.04 (0.95–1.14; 0.397), respectively.
Conclusions: Lymphocyte count and LDL cholesterol were longitudinally positively correlated and lymphocyte count was associated with incidence of hyper-LDL cholesterolemia independently of hs-CRP in a health screening population.

Introduction

While low-density lipoprotein (LDL) cholesterol remains the most important risk factor for atherosclerosis, immune and inflammatory mechanisms of atherosclerosis have gained tremendous interest in the past 20 years [1,2]. In 1979, the adherence of monocytes to the endothelium and the presence of monocytes within the intima of hypercholesterolemic swine arteries were reported [3]. Although, initially, investigators thought that only macrophages are predominantly present within atherosclerotic vessels, several studies reported the presence of most known leukocyte subtypes such as T and B lymphocytes, natural killer cells (NK) and NKT cells, macrophages, dendritic cells and mast cells within atherosclerosis-prone arteries [2,4]. Thus, atherosclerosis is thought to be a chronic inflammatory disease and an increasing body of evidence suggests that the immune system actively participates in the initiation, progression and persistence of atherosclerosis [5]. The presence of inflammatory cells in atherosclerotic lesions depends on the rate of their recruitment and egress and the balance of proliferation, survival, and apoptosis within the arterial wall. Total leukocyte count in peripheral blood is associated with coronary heart disease among patients with diabetes [6] and an independent predictor of cardiovascular disease and all-cause mortality in postmenopausal women [7]. A pro-inflammatory role for neutrophils is also suggested in the development of atherosclerosis [8]. Leukocyte subtype counts in peripheral blood may be associated with complex dynamic processes in atherosclerotic lesions. The previous literature on leukocyte counts has largely focused on total leukocyte and neutrophil counts, and smoking is a key determinant of total leukocyte and neutrophil counts. However, studies on the associations between leukocyte subtype counts and cardiovascular disease or its risk

Abbreviations: LDL, low-density lipoprotein; NK, natural killer; hs-CRP, high-sensitivity C-reactive protein; BMI, body mass index; HR, hazard ratio; HDL, high-density lipoprotein; MetS, metabolic syndrome.

* Corresponding author. Tel.: +81 258 36 6221; fax: +81 258 34 1113.
E-mail address: ijie@venus.sannet.ne.jp.

2214-6237/$ – see front matter © 2014 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcte.2014.05.001

factors are sparse and controversial [9–19] and there has been no report regarding a longitudinal association between leukocyte subtype counts and LDL cholesterol. In the present retrospective observational study, longitudinal associations between leukocyte subtype counts and LDL cholesterol were investigated in a health screening Japanese population. The protocol for this study was approved by the ethics committee of Tachikawa Medical Center.

Subjects and methods

Subjects

Between April 2008 and March 2009, 3866 subjects visited our medical check-up center for annual health screening and gave signed informed consent. The visitors were required to fill out a questionnaire including questions about their history of ischemic heart disease and stroke, smoking and drinking status, level of physical activity and use of antihypertensive, antidiabetic and antihyperlipidemic drugs. Among them, 1735 subjects did not use antihyperlipidemic drugs and revisited our medical check-up center between April 2012 and March 2013, and were included in the longitudinal correlation study. Subtracting 1278 subjects with hyper-LDL cholesterolemia from the 3866 subjects, 2588 subjects became potential candidates for the follow-up study. Among them, 1992 subjects revisited our medical check-up center between April 2009 and March 2013 and were actually followed. Hyper-LDL cholesterolemia was defined as LDL cholesterol $\geq$140 mg/dL according to the Guidelines by Japan Atherosclerosis Society [20] and/or use of antihyperlipidemic drugs.

Measurements

After an overnight fast, blood samples were obtained to measure blood levels of routine medical check-up parameters including LDL cholesterol, high-sensitivity C-reactive protein (hs-CRP) and leukocyte subtype counts. Chemical measurements were performed at BML Nagaoka (Nagaoka, Japan) with routine laboratory methods except for hs-CRP which was measured at BML General Laboratory (Tokyo, Japan) with nephelometry using N-latex CRP-2 (Siemens Healthcare Japan, Tokyo, Japan). The measurement limit of hs-CRP was 0.02 mg/L and the level of hs-CRP less than the measurement limit was considered as 0.01 mg/L. LDL cholesterol was measured with a direct surfactant method using Choletest-LDL (Sekisui Medical Inc., Tokyo, Japan). Leukocyte subtype counts were measured with flow cytometry using XE-2100 (Sysmex Inc., Kobe, Japan) where the subtypes were determined based on FSC/SSC. Average systolic and diastolic blood pressures were calculated from two measurements in a sitting position after each 5 min rest. Body weight was measured with the subjects wearing light clothes provided by our center and the weight of the clothing was subtracted from the measured body weight. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Statistical analysis

Correlation study

Laboratory data at baseline and after 4 years were compared by paired *t*-tests. Spearman's correlation coefficients between leukocyte subtype counts at baseline and after 4 years and their changes through 4 years and LDL cholesterol levels at baseline and after 4 years and their changes through 4 years were calculated.

Follow-up study

Baseline data were compared between potential candidates and actually followed subjects. Baseline data of the actually followed subjects were also compared between subjects who developed hyper-LDL cholesterolemia and those who did not.

Using Cox regression models in which years were used as a unit of the survival variable and drop-out subjects were considered, hazard ratios (HRs) of hyper-LDL cholesterolemia for one SD increase in counts of neutrophil, lymphocyte, monocyte, basophil and eosinophil were calculated adjusted for hs-CRP, sex, age, current smoking, daily drinking, physical activity, history of ischemic heart disease and stroke, and use of antihypertensive and antidiabetic drugs (Model 1), and farther adjusted for LDL cholesterol per se (Model 2). Physical activity was defined as walking for 1 h or longer per day or exercising for 30 min or longer twice or more per week.

The HR calculations were repeated separately in 497 smokers and 1495 nonsmokers and after the redefinition of hyper-LDL choesterolemia as LDL cholesterol $\geq$160 mg/dL and/or use of antihyperlipidemic drugs.

Incidence of hyper-LDL cholesterolemia expressed per 1000 person-years and hazard ratio (HR) of hyper-LDL cholesterolemia adjusted for Model 1 were calculated for each quartile of lymphocyte count. The lowest quartile of lymphocyte count was used as reference group for the calculation of HRs.

Statistical analyses were performed using Dr-SPSS-2 (IBM Japan, Tokyo, Japan). Means were compared with paired or unpaired two-sided *t*-tests and percentages were compared with chi-squared tests. Except for correlation coefficients, p values of lower than 0.05 were considered statistically significant. For correlation coefficients, p values of lower than 0.001 were considered statistically significant.

Results

Correlation study

Laboratory data at baseline and after 4 years of the subjects in the correlation study are presented in Table 1. The neutrophil, lymphocyte and monocyte counts and HDL cholesterol were significantly lower and basophil count, diastolic blood pressure, and fasting glucose were significantly higher after 4 years than at baseline. Spearman's correlation coefficients between leukocyte subtype counts and LDL cholesterol are shown in Table 2. Among the 5 leukocyte subtype counts at baseline, only lymphocyte count was significantly positively correlated with LDL cholesterol both at baseline and after 4 years. Among the 5 leukocyte subtype counts after 4 years, only lymphocyte count was significantly positively

Table 1
Laboratory data of the subjects in the correlation study

n	At baseline	After 4 years	p[a]
n (male %)	1735 (66.1)	1735 (66.1)	
Age (years)	50.7 (9.0)	54.8 (9.0)	
Body mass index (kg/m^2)	22.6 (3.0)	22.7 (3.1)	0.169
Systolic blood pressure (mm Hg)	118.2 (17.5)	118.2 (15.1)	0.988
Diastolic blood pressure (mm Hg)	74.8 (10.9)	78.0 (11.4)	<0.001
Neutrophil count (/L^{-6})	2999 (1056)	2915 (1197)	0.028
Lymphocyte count (/L^{-6})	1808 (515)	1711 (491)	<0.001
Monocyte count (/L^{-6})	277 (94)	270 (95)	0.032
Eosinophil count (/L^{-6})	169 (135)	165 (132)	0.330
Basophil count (/L^{-6})	31 (22)	32 (21)	0.020
High-sensitivity CRP (mg/L)	0.62 (1,79)	0.75 (2.47)	0.074
LDL cholesterol (mmol/L)	3.07 (0.72)	3.09 (0.73)	0.521
HDL cholesterol (mmol/L)	1.60 (0.39)	1.54 (0.39)	<0.001
Triglycerides (mmol/L)	1.18 (0.72)	1.19 (0.78)	0.717
Fasting glucose (mmol/L)	5.15 (0.68)	5.23 (0.81)	0.001
Aspartate aminotransferase (U/L)	23.1 (19.9)	22.9 (9.5)	0.706
Alanine aminotransferase (U/L)	23.6 (18.8)	23.2 (16.5)	0.490

Mean (SD) except for *n* (male%).
[a] paired *t*-tests.

Table 2
Spearman's correlation coefficients (p values) between LDL cholesterol and leukocyte subtype counts

	Neutrophil	Lymphocyte	Monocyte	Eosinophil
Between leukocyte subtype counts at baseline				
LDL cholesterol at baseline	0.04 (0.117)	0.16 (<0.001)	0.02 (0.457)	0.04 (0.120)
LDL cholesterol after 4 years	0.03 (0.230)	0.09 (<0.001)	−0.03 (0.197)	0.01 (0.723)
Changes in LDL cholesterol	−0.02 (0.532)	−0.10 (<0.001)	−0.07 (0.007)	−0.04 (0.062)
Between leukocyte subtype counts after 4 years				
LDL cholesterol at baseline	0.06 (0.019)	0.13 (<0.001)	−0.01 (0.625)	0.05 (0.032)
LDL cholesterol after 4 years	0.04 (0.064)	0.11 (<0.001)	−0.04 (0.123)	0.03 (0.154)
Changes in LDL cholesterol	−0.01 (0.549)	−0.02 (0.358)	−0.02 (0.367)	−0.02 (0.360)
Between changes in leukocyte subtype counts				
LDL cholesterol at baseline	−0.01 (0.825)	−0.06 (0.007)	−0.04 (0.133)	0.02 (0.317)
LDL cholesterol after 4 years	0.00 (0.995)	0.02 (0.337)	−0.01 (0.795)	0.04 (0.121)
Changes in LDL cholesterol	0.02 (0.494)	0.12 (<0.001)	0.06 (0.016)	0.02 (0.524)

correlated with LDL cholesterol after 4 years. Among the changes in leukocyte subtype counts, only changes in lymphocyte count was significantly positively correlated with the changes in LDL cholesterol. Any basophil count was not significantly associated with any LDL cholesterol.

Follow-up study

There were no significant differences in the baseline data between the candidates and followed subjects. Among the 1992 followed subjects, 481 (60.4 per 1000 person-years) developed hyper-LDL cholesterolemia during 4 years (mean of 3.1 years). Baseline data stratified according to the development of hyper-LDL cholesterolemia are shown in Table 3. The means of age, BMI, lymphocyte count, LDL cholesterol, triglycerides and alanine aminotransferase were significantly higher while the mean of HDL cholesterol and the prevalence of daily drinking were significantly lower in subjects who developed hyper-LDL cholesterolemia than those who did not.

Hazard ratios of hyper-LDL cholesterolemia for each one SD increase in leukocyte subtype counts are presented in Table 4. Among the 5 leukocyte subtype counts, only lymphocyte count was significantly associated with the incidence of hyper-LDL cholesterolemia in Model 1 while no leukocyte subtype count was significantly associated with the incidence of hyper-LDL cholesterolemia in Model 2 in all subjects and non-smokers. No leukocyte subtype count was significantly associated with the incidence of hyper-LDL cholesterolemia in smokers.

When hyper-LDL cholesterolemia was redefined as LDL cholesterol ≥160 mg/dL and/or use of antihyperlipidemic drugs, among 3492 candidate subjects at baseline, 2423 subjects (607 smokers and 1816 nonsmokers) revisited between April 2012 and March 2013, and 233 subjects (58 smokers and 175 nonsmokers) developed hyper-LDL cholesterolemia during 4 years. HRs of the redefined hyper-LDL cholesterolemia for each one SD increase in leukocyte subtype counts are presented in Table 5. No leukocyte subtype count was significantly associated with the incidence of hyper-LDL cholesterolemia. However, lymphocyte count was marginally associated with the incidence of hyper-LDL cholesterolemia in Model 1 in all subjects and non-smokers.

Incidence and hazard ratio (HR) of hyper-LDL cholesterolemia for each quartile of lymphocyte count are shown in Table 6. There were significant associations between the quartile of lymphocyte count and the incidence (p for trend = 0.036) as well as the hazard ratio (p for trend = 0.005) of hyper-LDL cholesterolemia.

Table 3
Baseline data stratified according to the development of hyper-LDL cholesterolemia

	Developers	Non-developers	p
n	481	1511	
Male %	62.2	66.1	0.113
Age (years)	52.1 (8.7)	50.7 (9.6)	0.007
Body mass index (kg/m^2)	22.8 (3.0)	22.1 (2.9)	<0.001
Systolic blood pressure (mm Hg)	118.9 (18.1)	117.6 (17.7)	0.149
Diastolic blood pressure (mm Hg)	74.9 (11.6)	74.3 (11.1)	0.321
Neutrophil count (/L^{-6})	3047 (1126)	2962 (1081)	0.137
Lymphocyte count (/L^{-6})	1815 (547)	1757 (490)	0.030
Monocyte count (/L^{-6})	278 (97)	277 (94)	0.821
Eosinophil count (/L^{-6})	168 (132)	171 (140)	0.718
Basophil count (/L^{-6})	30 (20)	30 (21)	0.840
High-sensitivity CRP (mg/L)	0.64 (1.17)	0.56 (1.47)	0.238
LDL cholesterol (mmol/L)	3.20 (0.32)	2.70 (0.52)	<0.001
HDL cholesterol (mmol/L)	1.54 (0.37)	1.65 (0.42)	<0.001
Triglycerides (mmol/L)	1.33 (0.94)	1.08 (0.70)	<0.001
Fasting glucose (mmol/L)	5.21 (0.84)	5.14 (0.69)	0.076
Aspartate aminotransferase (U/L)	22.9 (11.2)	22.7 (20.9)	0.822
Alanine aminotransferase (U/L)	23.8 (19.6)	21.9 (16.7)	0.043
Antihypertensive drugs	15.6	13.7	0.300
Antidiabetic drugs	2.1	1.8	0.679
History of stroke	0.8	1.3	0.446
History of ischemic heart disease	2.3	2.1	0.824
Current smoking	22.5	25.7	0.146
Daily alcohol drinking	34.9	44.3	<0.001
Physical activity[a]	34.3	37.9	0.160

Mean (SD) or %.
[a] Defined as walking for 1 h or longer per day or exercising for 30 min or longer twice or more per week.

Table 4
Hazard ratios of hyper-LDL cholesterolemia for each one SD increase in leukocyte subtype counts

	Model 1[a]		Model 2[b]	
	Hazard ratio (95% CI[c])	p	Hazard ratio (95% CI[c])	p
All subjects				
Neutrophil count	1.08 (0.99–1.19)	0.085	1.04 (0.95–1.14)	0.405
Lymphocyte count	1.14 (1.04–1.25)	0.005	1.03 (0.93–1.13)	0.569
Monocyte count	1.05 (0.95–1.15)	0.339	1.00 (0.91–1.10)	0.999
Eosinophil count	1.01 (0.92–1.11)	0.858	0.96 (0.88–1.06)	0.415
Basophil count	1.04 (0.95–1.14)	0.397	0.99 (0.91–1.09)	0.870
Smokers				
Neutrophil count	1.14 (0.98–1.31)	0.088	1.06 (0.91–1.23)	0.469
Lymphocyte count	1.10 (0.93–1.30)	0.280	1.04 (0.86–1.26)	0.663
Monocyte count	1.00 (0.84–1.19)	0.998	0.92 (0.77–1.10)	0.354
Eosinophil count	0.92 (0.76–1.11)	0.401	0.91 (0.75–1.11)	0.354
Basophil count	1.06 (0.89–1.27)	0.514	1.03 (0.85–1.24)	0.776
Non-smokers				
Neutrophil count	1.05 (0.94–1.18)	0.359	1.04 (0.93–1.16)	0.519
Lymphocyte count	1.15 (1.03–1.27)	0.013	1.02 (0.91–1.14)	0.749
Monocyte count	1.05 (0.94–1.18)	0.359	1.04 (0.93–1.17)	0.498
Eosinophil count	1.04 (0.94–1.16)	0.439	0.97 (0.87–1.08)	0.535
Basophil count	1.04 (0.93–1.15)	0.498	0.98 (0.88–1.09)	0.696

[a] Adjusted for high-sensitivity CRP, sex, age, current smoking, daily drinking, physical activity, histories of ischemic heart disease and stroke, and uses of antihypertensive and antidiabetic drugs.
[b] Adjusted for covariates in Model 1 plus LDL cholesterol.
[c] Confidence interval.

Table 5
Hazard ratios of hyper-LDL cholesterolemia defined as ≥160 mg/dL for each one SD increase in leukocyte subtype counts

	Model 1[a]		Model 2[b]	
	Hazard ratio (95% CI[c])	*p*	Hazard ratio (95% CI[c])	*p*
All subjects				
Neutrophil count	0.99 (0.87–1.13)	0.870	0.91 (0.79–1.04)	0.168
Lymphocyte count	1.13 (1.00–1.28)	0.054	0.98 (0.85–1.11)	0.708
Monocyte count	1.05 (0.92–1.19)	0.457	0.98 (0.85–1.12)	0.726
Eosinophil count	1.02 (0.90–1.15)	0.751	1.00 (0.88–1.14)	0.976
Basophil count	1.03 (0.91–1.16)	0.690	0.96 (0.85–1.09)	0.527
Smokers				
Neutrophil count	1.09 (0.90–1.33)	0.381	0.97 (0.79–1.19)	0.748
Lymphocyte count	1.10 (0.89–1.37)	0.377	0.92 (0.72–1.17)	0.497
Monocyte count	1.11 (0.90–1.37)	0.328	0.94 (0.74–1.19)	0.613
Eosinophil count	1.03 (0.83–1.28)	0.760	0.96 (0.75–1.23)	0.744
Basophil count	1.06 (0.86–1.30)	0.612	0.94 (0.75–1.18)	0.595
Nonsmokers				
Neutrophil count	0.92 (0.77–1.09)	0.347	0.86 (0.71–1.03)	0.095
Lymphocyte count	1.15 (0.99–1.33)	0.073	0.99 (0.84–1.16)	0.892
Monocyte count	1.02 (0.87–1.20)	0.816	0.99 (0.84–1.17)	0.900
Eosinophil count	1.01 (0.87–1.18)	0.890	1.02 (0.87–1.18)	0.846
Basophil count	1.01 (0.87–1.18)	0.868	0.97 (0.83–1.13)	0.708

[a] Adjusted for high-sensitivity CRP, sex, age, current smoking, daily drinking, physical activity, histories of ischemic heart disease and stroke, and uses of antihypertensive and antidiabetic drugs.
[b] Adjusted for covariates in Model 1 plus LDL cholesterol.
[c] Confidence interval.

Discussion

In the present longitudinal study, the baseline lymphocyte count was significantly positively correlated with the LDL cholesterol levels after 4 years, the changes in lymphocyte count were significantly correlated with the changes in LDL cholesterol and the baseline lymphocyte count was associated with the incidence of hyper-LDL cholesterolemia adjusted for hs-CRP, a marker of non-specific inflammation, in a health screening population. However, lymphocyte count was not significantly associated with the incidence of hyper-LDL cholesterolemia after further adjusted for LDL cholesterol itself, and it was not significantly associated with the incidence of hyper-LDL cholesterolemia in smokers. Therefore, leukocyte count may not be a clinically useful predictor of hyper-LDL cholesterolemia and an increase in neutrophil count in smokers might confound the association between lymphocyte count and hyper-LDL cholesterolemia.

Total leukocyte count was associated with obesity, hypertension, hypercholesterolemia, hypo-HDL cholesterolemia, hypertriglyceridemia, hyperglycemia, hyperuricemia and metabolic syndrome (MetS) [21–23]. Total leukocyte count and hs-CRP equally predicted development of MetS, but both are poor predictors of MetS in a health screening Japanese population [24], although hs-CRP is superior to total leukocyte count as an inflammatory component of MetS in a cross-sectional study [25]. Total leukocyte count is also reported to be a predictor of coronary heart disease [6], even after adjusted for hs-CRP [7].

Table 6
Incidence and hazard ratio of hyper-LDL cholesterolemia for each quartile of lymphocyte count

	Q1	Q2	Q3	Q4	*p* for trend
Lymphocyte count	469–1428	1429–1711	1712–2050	2051–4144	
n	499	498	498	497	
Incidence[a]	61.5	49.8	55.8	74.5	0.036
Hazard ratio[b]	1.00	0.84	0.95	1.39	0.005
95% Confidence interval		0.64–1.09	0.73–1.25	1.08–1.81	
p		0.192	0.732	0.012	

[a] Per 1000 person-years.
[b] Compared with Q1 adjusted for high-sensitivity CRP, sex, age, current smoking, daily drinking, physical activity, histories of ischemic heart disease and stroke, and uses of antihypertensive and antidiabetic drugs.

Cross-sectional associations between leukocyte subtype counts and cardiovascular risk factors are controversial [9–14]. Oda et al. previously reported that the lymphocyte count was significantly associated with hyper-LDL cholesterolemia, hypertriglyceridemia, and hypo-HDL cholesterolemia in men and hyper-LDL cholesterolemia in women [15].

Regarding longitudinal associations between leukocyte subtype counts and disease progression in high-risk populations, Nozawa et al. reported that monocyte count, but not lymphocyte or neutrophil count, was associated with coronary plaque progression after acute myocardial infarction [16]. Giugliano et al. reported that neutrophil count, but not lymphocyte or monocyte count, was associated with increased cardiovascular risk in patients with peripheral artery disease [17]. Papa et al. showed that neutrophil to lymphocyte ratio was an independent predictor of cardiac mortality in patients with stable angina [18]. ó Hartaigh et al. reported that neutrophil count and neutrophil to lymphocyte ratio was independent predictors of cardiovascular mortality in a high-risk population [19].

The present study demonstrated that the lymphocyte, not neutrophil or monocyte, count was associated with the incidence of hyper-LDL cholesterolemia in a health screening population after adjusted for hs-CRP, a marker of non-specific inflammation, and that the changes in lymphocyte count were significantly correlated with the changes in LDL cholesterol. Although exact mechanisms underlying the association between baseline lymphocyte count and incidence of hyper-LDL cholesterolemia are unknown, the cross-sectional positive correlation between lymphocyte count and LDL cholesterol levels might play an important role because this association between baseline lymphocyte count and incidence of hyper-LDL cholesterolemia became non-significant after further adjusted for LDL cholesterol levels at baseline. Atherosclerosis starts with an innate immune response involving the recruitment and activation of monocytes that respond to an excessive accumulation of modified lipids within the arterial wall, followed by an adaptive immune response involving antigen-specific T lymphocytes [8]. Thus, the following scenario can be speculated. Increased LDL cholesterol levels result in an excessive accumulation of modified lipids within the arterial wall and subsequent recruitment and activation of monocytes, followed by the recruitment of lymphocytes which respond antigen-presenting activated macrophages, and this recruitment and activation of lymphocytes may stimulates the proliferation and mobilization of lymphocytes from lymphoid tissues, resulting in an increased lymphocyte count in peripheral blood. In contrast to the above reports that monocyte and/or neutrophil counts, not lymphocyte count, were associated with disease progression in high-risk populations with advanced atherosclerotic diseases, the present results suggest that adaptive immune mechanisms mediated by lymphocytes may importantly participate in early stages of atherosclerotic lesion formation in accord with hyper-LDL cholesterolemia beyond innate immune mechanisms mediated by monocytes and neutrophils.

Limitations

The present study was a retrospective observational study and the subjects were not recruited from a general population but recruited from a general health screening population. The number of subjects might be inadequate to find a significant association between lymphocyte count and hyper-LDL cholesterolemia when it was defined as LDL cholesterol ≥160 mg/dL and/or use of

antihyperlipidemic drugs. Detailed information about demographic backgrounds and dietary habits was not available and lymphocyte subtypes were not measured in this study.

Conclusions

The present study demonstrated that lymphocyte, not neutrophil or monocyte, count, was significantly associated with incidence of hyper-LDL cholesterolemia adjusted for hs-CRP, a marker of non-specific inflammation, and lymphocyte count and LDL cholesterol were longitudinally positively correlated with each other. The present results suggest that the adaptive immune system mediated by lymphocytes may importantly participate in early stages of atherosclerotic lesion formation associated with hyper-LDL cholesterolemia beyond innate immune mechanisms mediated by monocytes and neutrophils.

Acknowledgments

The author thanks all subjects who participated in the study, the paramedical staff at our center who assisted with the study, and Dr Shinpei Yoshii and Honorary Prof Yoshifusa Aizawa for their assistance to construct the study environment.

Funding: The author received no grant or financial support.

Disclosure statement: The author has no conflict of interest to disclose.

Author contributions: E. Oda contributed in the design and conduct of the study, data collection and analysis, data interpretation and manuscript writing.

References

[1] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
[2] Weber C, Zernecke A, Libby P. The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models. Nat Rev Immunol 2008;8:802–15.
[3] Gerrity RG, Naito HK, Richardson M, Schwartz CJ. Dietary induced atherogenesis in swine. Morphology of the intima in prelesion stages. Am J Pathol 1979;95:775–92.
[4] Galkina E, Ley K. Leukocyte influx in atherosclerosis. Curr Drug Targets 2007;8:1239–48.
[5] Tuttolomondo A, Di Raimondo D, Pecoraro R, Arnao V, Pinto A, Licata G. Atherosclerosis as an inflammatory disease. Curr Pharm Des 2012;18:4266–88.
[6] Saito I, Folsom AR, Brancati FL, Duncan BB, Chambless LE, McGovern PG. Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Ann Intern Med 2000;133:81–91.
[7] Margolis KL, Manson JE, Greenland P, Rodabough RJ, Bray PF, Safford M, et al. Women's Health Initiative Research Group. Leukocyte count as a predictor of cardiovascular events and mortality in postmenopausal women: the Women's Health Initiative Observational Study. Arch Intern Med 2005;165:500–8.
[8] Zernecke A, Bot I, Djalali-Talab Y, Shagdarsuren E, Bidzhekov K, Meiler S, et al. Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. Circ Res 2008;102:209–17.
[9] Huang ZS, Wang CH, Yip PK, Yang CY, Lee TK. In hypercholesterolemia, lower peripheral monocyte count is unique among the major predictors of atherosclerosis. Arterioscler Thromb Vasc Biol 1996;16:256–61.
[10] Huang ZS, Jeng JS, Wang CH, Yip PK, Wu TH, Lee TK. Correlations between peripheral differential leukocyte counts and carotid atherosclerosis in non-smokers. Atherosclerosis 2001;158:431–6.
[11] Huang ZS, Chien KL, Yang CY, Tsai KS, Wang CH. Peripheral differential leukocyte counts in humans vary with hyperlipidemia, smoking, and body mass index. Lipids 2001;36:237–45.
[12] Tanigawa T, Iso H, Yamagishi K, Muraki I, Kawamura N, Nakata A, et al. Association of lymphocyte sub-populations with clustered features of metabolic syndrome in middle-aged Japanese men. Atherosclerosis 2004;173:295–300.
[13] Lao XQ, Thomas GN, Jiang C, Zhang W, Adab P, Lam TH, et al. White blood cell count and the metabolic syndrome in older Chinese: the Guangzhou Biobank Cohort Study. Atherosclerosis 2008;201:418–24.
[14] Muldoon MF, Marsland A, Flory JD, Rabin BS, Whiteside TL, Manuck SB. Immune system differences in men with hypo- or hypercholesterolemia. Clin Immunol Immunopathol 1997;84:145–9.
[15] Oda E, Kawai R, Aizawa Y. Lymphocyte count was significantly associated with hyper-LDL cholesterolemia independently of high-sensitivity C-reactive protein in apparently healthy Japanese. Heart Vessels 2012;27:377–83.
[16] Nozawa N, Hibi K, Endo M, Sugano T, Ebina T, Kosuge M, et al. Association between circulating monocytes and coronary plaque progression in patients with acute myocardial infarction. Circ J 2010;74:1384–91.
[17] Giugliano G, Brevetti G, Lanero S, Schiano V, Laurenzano E, Chiariello M. Leukocyte count in peripheral arterial disease: a simple, reliable, inexpensive approach to cardiovascular risk prediction. Atherosclerosis 2010;210:288–93.
[18] Papa A, Emdin M, Passino C, Michelassi C, Battaglia D, Cocci F. Predictive value of elevated neutrophil-lymphocyte ratio on cardiac mortality in patients with stable coronary artery disease. Clin Chim Acta 2008;395:27–31.
[19] ó Hartaigh B, Bosch JA, Thomas GN, Lord JM, Pilz S, Loerbroks A, et al. Which leukocyte subsets predict cardiovascular mortality? From the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study. Atherosclerosis 2012;224:161–9.
[20] Japan Atherosclerosis Society. Chapter 3 in Japan Atherosclerosis Society guidelines for prevention of atherosclerotic cardiovascular diseases 2012. Kyorinsha; 2012. pp. 33–6 [in Japanese].
[21] Nakanishi N, Sato M, Shirai K, Nakajima K, Murakami S, Takatorige T, et al. Associations between white blood cell count and features of the metabolic syndrome in Japanese male office workers. Ind Health 2002;40:273–7.
[22] Oda E. Metabolic syndrome: its history, mechanisms, and limitations. Acta Diabetol 2012;49:89–95.
[23] Nagasawa N, Tamakoshi K, Yatsuya H, Hori Y, Ishikawa M, Murata C, et al. Association of white blood cell count and clustered components of metabolic syndrome in Japanese men. Circ J 2004;68:892–7.
[24] Oda E. High-sensitivity C-reactive protein and white blood cell count equally predict development of the metabolic syndrome in a Japanese health screening population. Acta Diabetol 2013;50:633–8.
[25] Oda E, Kawai R. Comparison between high-sensitivity C-reactive protein (hs-CRP) and white blood cell count (WBC) as an inflammatory component of metabolic syndrome in Japanese. Intern Med 2010;49:117–24.