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Neutrophil/lymphocyte ratio is correlated with levels of inflammatory markers and is significantly reduced by smoking cessation

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Abstract

Previous studies have reported that the neutrophil to lymphocyte ratio (NLR) is associated with onset and prognosis of cardiovascular disease (CVD). Smoking is a major risk factor for CVD and smoking cessation significantly reduces CVD risk. However, the effects of smoking cessation on the NLR remain unknown. Among smokers visiting our smoking cessation clinics, we examined changes in the NLR and CVD biomarkers before and after smoking cessation. A total of 389 individuals (301 men and 88 women) were enrolled in the study. The median NLR was significantly reduced after successful smoking cessation (before: 1.8, interquartile range [IQR] 1.5, 2.5; after: 1.7, IQR 1.3, 2.4). In a linear regression model adjusted for sex, percent change in NLR comparing before and after smoking cessation was significantly and positively correlated with percent changes in C-reactive protein ($\beta = 0.260$), α1-antitrypsin-low density lipoprotein ($\beta = 0.151$, $p < 0.05$), and serum amyloid A-low density lipoprotein ($\beta = 0.325$). Our study demonstrated for the first time that smoking cessation significantly reduces the NLR in tandem with markers of inflammation and oxidative stress.

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Introduction

Increased neutrophil count is a marker of inflammation, while decreased lymphocyte count is a marker that reflects stress and poor nutritional condition. Several recent studies have reported that the ratio of neutrophil count the lymphocyte count (neutrophil/lymphocyte ratio, NLR) is associated with the onset of cardiovascular disease (CVD) and with prognosis of cancers.[1–4] It has also been reported that NLR is a sensitive inflammatory marker of hypertension, dyslipidemia, diabetes mellitus, and obesity/metabolic syndrome, which are risk factors for arteriosclerosis.[5–8] Inflammation attributable to smoking may destabilize atherosclerotic plaques and play roles in the onset of CVDs such as cerebral infarction and myocardial infarction.

Even in the absence of elevated C-reactive protein (CRP) levels, smokers have increased neutrophil counts.[9] The detailed mechanisms through which smoking contributes to an increase in neutrophil count remain unknown. Cigarette smoke contains more than 250 hazardous substances, including 69 carcinogens; biological reactions to these substances result in increased neutrophil counts.[10] The abundant neutrophils adhere to capillaries, increasing vascular resistance, and produce inflammatory cytokines that induce vascular injury and promote chronic inflammatory conditions in the arterial wall.[11,12]

Despite normal leukocyte counts, an increase in the NLR increases risk for arteriosclerotic CVDs.[13,14] Because the NLR could be predictive of CVD development, greater importance has been attached to leukocyte fractions in recent years.[13,15] Multiple reports have demonstrated that the NLR is significantly higher in smokers than in non-smokers.[16,17] However, the impact of smoking cessation on the NLR has not been clarified. In the present study, we investigated the effects of smoking cessation on leukocyte fractions.

Methods

Participants

Participants were smokers reporting for the first time to a smoking cessation outpatient clinic at the National Hospital Organization Kyoto Medical Centre. Patients with advanced cancer requiring palliative care were excluded. All smokers who fulfilled these criteria were asked to participate in the study. Those who provided written informed consent were included. Cardiovascular markers were measured in these subjects by collecting blood. In addition, medical history and information on smoking behavior was obtained through an interview at the initial visit to our smoking cessation clinic. At this time, body measurements were taken and blood tests were performed.

The present study was conducted in accordance with the principles laid out in the World Medical Association Declaration of Helsinki (revised October

2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects of the Ministry of Health, Labour, and Welfare (amended 22 December 2014). Data were analyzed from two completed and ongoing studies: "Study of the Effects of Smoking Cessation on the Risk of Developing Cardiovascular Diseases (April 2007 to July 2014)" and "Study of Long-Term Changes in Cardiovascular Markers and Prognostic Factors after Smoking Cessation (July 2014–present)." The protocols for both studies were approved by the Ethics Committee of the National Hospital Organization Kyoto Medical Center (approval numbers 14-042 and 06-45).

Measurements

At the first visit to our department, information on participant age, sex, average cigarette consumption (number of cigarettes), duration of smoking, Fagerström Test for Nicotine Dependence (FTND) score, and current medications was obtained. The FTND is a standard test used to assess physical dependence on nicotine.[18–20] The scores ranged from 0 to 10, with higher scores indicating more severe nicotine dependence. The number of cigarettes smoked per day was determined by asking the smoker the following question: "On average, in the past month, how many cigarettes did you smoke per day?"

Body measurements and blood tests were carried out at the start of smoking cessation therapy (0 months, baseline) and at the end of smoking cessation therapy (3 months after the start of therapy). Body measurements included height, weight, body mass index (BMI) abdominal circumference, blood pressure, and exhaled carbon monoxide (CO) concentration. Blood tests included hemoglobin A1C (HbA1c), low density lipoprotein (LDL)-C, high density lipoprotein (HDL)-C, neutral fat level, uric acid level, creatinine level, C-reactive protein (CRP), leukocyte count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, and basophil count. In addition, levels of serum amyloid A-LDL (SAA-LDL), an oxidized form of LDL, and α1-antitrypsin-LDL (AT-LDL) were determined.

Statistical analysis

The Shapiro–Wilk normality test was conducted for individual test items. Normally distributed variables were expressed as means $\pm$ standard deviations (SDs); differences before and after smoking cessation were assessed using the paired t-test. Non-normally distributed variables were expressed as medians (interquartile ranges [IQRs]); differences before and after smoking cessation were assessed using the Wilcoxon signed-rank test.

Linear regression analysis adjusted for sex was carried out to assess correlations between NLR and the other variables before smoking cessation. In addition, correlations between change in NLR following smoking cessation and changes in other variables were assessed. Values of $p<0.05$ were considered statistically significant.

Results

A total of 615 outpatients visiting our smoking cessation department for the first time provided written informed consent, and successfully gave up smoking through a 3-month course of smoking cessation therapy. Among these 615 individuals, leukocyte fraction data before and after smoking cessation were available for 389 (301 men and 88 women). These individuals were included in the analysis.

The mean ($\pm$ SD) age of included smokers was 61.4 ± 11.8 years, the mean number of cigarettes smoked per day was 22.0 ± 10.4, the mean duration of smoking was

39.8 ± 11.5 years, and the mean FTND score was 6.4 ± 2.2 points.

A linear regression analysis adjusted for sex was used to assess correlation between NLR and other variables before smoking cessation therapy. Significant positive correlations were observed between NLR and CRP ($\beta = 0.402$, $p < 0.001$) and between NLR and SAA-LDL ($\beta = 0.162$, $p = 0.006$). Significant negative correlations were observed between NLR and systolic blood pressure ($\beta = -0.114$, $p = 0.029$) and between NLR and diastolic blood pressure ($\beta = -0.110$, $p = 0.039$) (Table 1).

Changes in parameters before and after smoking cessation therapy were investigated. BMI, abdominal circumference, LDL-C, HDL-C, neutral fat, uric acid, and creatinine showed significant increases post-therapy compared with baseline. Systolic blood pressure, diastolic blood pressure, leukocyte count, AT-LDL, and exhaled CO concentration showed significant decreases post-therapy compared with baseline. Among leukocyte fractions, neutrophils count were significantly lower and eosinophil count were significantly higher post-therapy compared with baseline. There were no significant changes in monocyte or basophil counts pre- and post-therapy. The NLR was significantly lower at the end of therapy compared with baseline (Table 2).

Linear regression analysis adjusted for sex was performed to assess correlations between changes in the NLR and changes

Table 1. Sex-adjusted correlation coefficients between NLR and other clinical measurements at study baseline.

	B	SE	β	t	*p*-value
Age	0.006	0.006	0.051	0.988	0.324
Smoking amount	0.003	0.007	0.024	0.468	0.640
Smoking duration	0.006	0.007	0.050	0.940	0.348
Brinkman index	0.000	0.000	0.051	0.970	0.333
FTND score	−0.030	0.033	−0.046	−0.898	0.370
BMI	−0.030	0.019	−0.081	−1.541	0.124
WC	−0.007	0.006	−0.068	−1.224	0.222
SBP	−0.008	0.004	**−0.114**	−2.186	**0.029**
DBP	−0.013	0.006	**−0.110**	−2.072	**0.039**
HbA1c (NGSP)	−0.077	0.070	−0.060	−1.101	0.272
LDL-C	0.000	0.002	0.012	0.224	0.823
HDL-C	0.001	0.004	0.018	0.321	0.748
TG	−0.001	0.001	−0.062	−1.172	0.242
UA	0.026	0.060	0.027	0.437	0.663
Cre	0.301	0.189	0.087	1.590	0.113
CRP	0.427	0.078	**0.402**	5.488	**<0.001**
SAA-LDL	0.011	0.004	**0.162**	2.779	**0.006**
AT-LDL	0.062	0.079	0.046	0.787	0.432
CO	−0.006	0.008	−0.040	−0.774	0.439

B: regression coefficient, β: normalized regression coefficient.

Brinkman index, smoking amount (cigarettes/day) × smoking duration (years); FTND score, Fagerström test for nicotine dependence; BMI, body mass index = weight (kg) / {height (m)}^{2}; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, uric acid; Cre, creatinine; CRP, C-reactive protein; SAA-LDL, serum amyloid A-low density lipoprotein; AT-LDL, α1-antitrypsin-low density lipoprotein: CO, carbon monoxide.

Table 2. Clinical measurements before and after smoking cessation.

	Baseline	3 months	*p*-value	
WBCs ($\times 10^3/mm^3$)	6700 (5500, 8050)	6300 (5300, 7500)	**<0.001**	b
Neutrophils ($\times 10^3/mm^3$)	3872 (3010, 4796)	3415 (2700, 4348)	**<0.001**	b
Lymphocytes ($\times 10^3/mm^3$)	1976 (1551, 2501)	1986 (1560, 2506)	0.192	b
Monocytes ($\times 10^3/mm^3$)	523.8 (408.7, 630.9)	516.0 (400.0, 638.3)	0.744	b
Eosinophils ($\times 10^3/mm^3$)	162.5 (94.8, 253.7)	172.5 (107.5, 295.0)	**0.007**	b
Basophils ($\times 10^3/mm^3$)	45.0 (30.9, 66.3)	47.0 (31.0, 64.9)	0.497	b
Neutrophil/lymphocyte ratio	1.8 (1.5, 2.5)	1.7 (1.3, 2.4)	**<0.001**	b
RBCs ($\times 10^3/mm^3$)	4.5 (4.1, 4.8)	4.4 (4.1, 4.7)	**<0.001**	b
Hb (g/dL)	14.4 (13.3, 15.2)	13.8 (12.9, 14.8)	**<0.001**	b
Ht (%)	43.5 (40.3, 46.5)	42.2 (39.6, 45.1)	**<0.001**	b
MCV (%)	96.0 (93.7, 99.6)	95.6 (92.3, 98.9)	**<0.001**	b
MCH (%)	31.8 (30.7, 33.0)	31.3 (30.5, 32.7)	**<0.001**	b
MCHC (%)	33.0 (32.5, 33.5)	32.8 (32.3, 33.5)	**0.039**	b
BMI (kg/m^2)	23.1 (20.7, 25.3)	23.3 (21.0, 25.8)	**<0.001**	b
WC (cm)	86.3 ± 10.7	87.7 ± 10.7	**<0.001**	a
SBP (mmHg)	131.1 ± 20.0	128.1 ± 18.9	**0.001**	a
DBP (mmHg)	77.7 ± 12.1	76.7 ± 11.9	**0.042**	a
HbA1c (NGSP%)	5.8 (5.5, 6.4)	5.8 (5.5, 6.5)	**<0.001**	b
LDL-C (mg/dL)	111.3 ± 29.6	114.7 ± 31.5	**0.009**	a
HDL-C (mg/dL)	54.0 (45.0, 68.0)	57.0 (46.0, 71.0)	**<0.001**	b
TG (mg/dL)	137.5 (93.8, 208.3)	156.0 (102.8, 240.3)	**<0.001**	b
UA (mg/dL)	5.6 (4.5, 6.4)	5.9 (4.8, 7.0)	**<0.001**	b
Cre (mg/dL)	0.79 (0.69, 0.92)	0.81 (0.70, 0.93)	**<0.001**	b
CRP (mg/dL)	0.07 (0.04, 0.19)	0.08 (0.04, 0.19)	0.879	b
SAA-LDL (μg/dL)	8.5 (5.3, 14.7)	9.0 (5.5, 15.0)	0.949	b
AT-LDL (μg/dL)	1.5 (1.2, 2.2)	1.5 (1.2, 2.1)	**0.044**	b
CO (ppm)	13.0 (9.0, 20.0)	2.0 (1.0, 3.0)	**<0.001**	b

a: mean $\pm$ standard deviation, paired t-test, b: median (interquartile range), Wilcoxon signed-rank test. Values of $p < 0.05$ are shown in bold font.

WBC, white blood cell: RBC, red blood cell: Hb, hemoglobin: Ht, hematocrit: MCV, mean corpuscular volume: MCH, mean corpuscular hemoglobin: MCHC, mean corpuscular hemoglobin concentration; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, uric acid; Cre, creatinine; CRP, C-reactive protein; SAA-LDL, serum amyloid A-low density lipoprotein; AT-LDL, α1-antitrypsin-low density lipoprotein: CO, carbon monoxide.

in other variables pre- and post-therapy. There was a significant positive correlation between change in NLR and changes in HbA1c ($\beta = 0.108$, $p = 0.046$), CRP ($\beta = 0.260$, $p = 0.001$, Figure 1a), SAA-LDL ($\beta = 0.325$, $p < 0.001$, Figure 1b), and AT-LDL ($\beta = 0.151$, $p = 0.01$, Figure 1c). There was a significant negative correlation between change in NLR and change in systolic blood pressure ($\beta = -0.172$, $p = 0.001$) (Table 3).

Discussion

A cross-sectional study conducted pre-smoking cessation therapy revealed a significant positive correlation between NLR and CRP and between NLR and SAA-LDL. A longitudinal study conducted before and after smoking cessation intervention showed a significant positive correlation changes in NLR and levels of CRP, AT-LDL, and SAA-LDL. AT-LDL is an

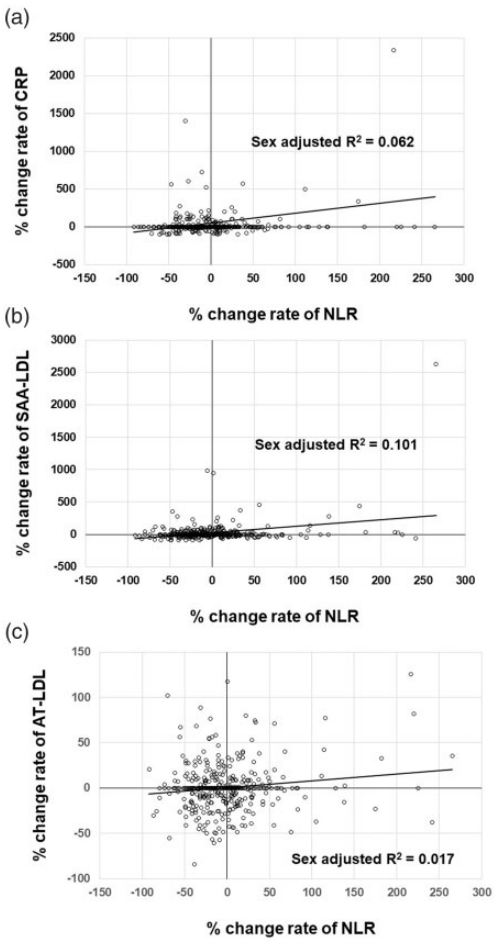

Figure 1. Correlations between change in neutrophil-to-lymphocyte ratio (NLR) and change in C-reactive protein (CRP) (a), serum amyloid A-low density lipoprotein (SAA-LDL) (b), and α1-antitrypsin-low density lipoprotein (AT-LDL) (c).

oxidized form of LDL found in human serum at sites of atherosclerotic lesions. Levels of AT-LDL in the blood reflect the activity of foam cells in atherosclerotic lesions.[21,22] SAA binds to HDL. When reactive oxygen species from inflammatory cells are activated by inflammatory signals in blood vessels, they bind SAA to LDL to form a complex known as SAA-LDL. SAA-LDL is a marker that can potentially be used to predict and prevent the occurrence of coronary events.[23] Based on the above findings, NLR was considered a sensitive marker that reflects reduction of oxidative stress upon smoking cessation. In addition, increased NLR has been suggested to be associated with risk of developing CVDs[2] and a rapid drop in the NLR in tandem with oxidized LDL levels after smoking cessation is likely to indicate decreased risk of cardiovascular injury.

CRP is an inflammatory marker associated with risk of developing CVD.[23] In the present study, NLR significantly decreased at the end of smoking cessation therapy compared with baseline, while CRP did not significantly decrease. NLR had superior diagnostic accuracy for Crohn's disease accompanied by chronic inflammation, while CRP had superior diagnostic accuracy for disease activity.[24] Therefore, CRP and NLR are likely to behave differently depending on symptoms. Inflammatory cytokines such as interleukin (IL)-1β and IL-6 increase CRP levels.[25] IL-1β elevates the NLR by increasing the neutrophil count and decreasing the lymphocyte count; by contrast, IL-6 does not elevate NLR because it increases both neutrophil and lymphocyte counts.[26,27] In addition to IL-1β and IL-6, various cytokines are involved in the induction of inflammation. Differences in the effects of inflammatory cytokines are likely to be associated with the divergent behavior of CRP and NLR.

Because nicotine has an extremely strong vasoconstrictive effect, tonic peripheral vasocontraction is observed in smokers. In the present study, a significant drop in blood pressure was observed after smoking cessation. Moreover, a significant negative correlation was observed between change in NLR and change in systolic blood pressure comparing pre- and post-therapy. Although the NLR reflects inflammation, peripheral vessels are dilated by inflammatory mediators themselves. Therefore, blood pressure

Table 3. Sex-adjusted correlation coefficients between percent change in NLR and other clinical measurements.

	B	SE	β	t	*p*-value
BMI	−0.710	0.540	−0.069	−1.313	0.190
WC	−0.511	0.562	−0.051	−0.910	0.364
SBP	−0.617	0.184	**−0.172**	−3.350	**0.001**
DBP	−0.285	0.186	−0.080	−1.533	0.126
HbA1c (NGSP)	0.591	0.295	**0.108**	2.005	**0.046**
LDL-C	−0.046	0.102	−0.025	−0.456	0.648
HDL-C	−0.015	0.127	−0.006	−0.120	0.905
TG	−0.027	0.031	−0.046	−0.877	0.381
UA	−0.216	0.174	−0.074	−1.243	0.215
Cre	−0.206	0.187	−0.060	−1.104	0.270
CRP	0.015	0.005	**0.260**	3.283	**0.001**
SAA-LDL	0.084	0.014	**0.325**	5.848	**<0.001**
AT-LDL	0.242	0.093	**0.151**	2.599	**0.010**
CO	0.108	0.088	0.064	1.222	0.222

B: regression coefficient, β: normalized regression coefficient. Values of $p < 0.05$ are shown in bold font
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, uric acid; Cre, creatinine; CRP, C-reactive protein; SAA-LDL, serum amyloid A-low density lipoprotein; AT-LDL, α1-antitrypsin-low density lipoprotein: CO, carbon monoxide.

decreases could be minimal among participants whose NLR was greatly reduced. It was reported that NLR was significantly higher in hypertensive patients,[28,29] and vasculitis causes arteriosclerosis gradually over long periods, leading to strong vascular resistance. Thus, it is necessary to assess the relationship change in NLR and change in blood pressure after smoking cessation over longer periods in future studies.

Serum LDL cholesterol levels increased after smoking cessation. This increase was attributable to an increase in body weight. In general, body weight increases peak 3 years after smoking cessation, and then gradually decline. Despite the increase in body weight, epidemiological evidence shows that the risk of CVDs decreases after smoking cessation.[30,31] This decreased risk could result from decreased oxidative stress and inflammation following smoking cessation, resulting in decreased levels of oxidized LDL implicated in atherosclerotic CVDs. Similarly, we previously reported that despite a progressive increase in body weight from baseline to 3 months and 1 year after smoking cessation, oxidized LDL levels progressively decrease.[32]

NLR can be evaluated only by measuring the leukocyte fraction, which can be accomplished in a relatively short time in many medical institutions. The present study revealed for the first time that NLR rapidly drops in a relatively short time (3 months) after smoking cessation. For this reason, NLR may serve as a marker to conveniently evaluate risk of CVD after smoking cessation.

Limitations

The present study had several limitations. First, it was a single-center study and the observation period was only 3 months after smoking cessation. A long-term observational study of NLR is warranted.

Second, we examined two types of oxidized LDLs, AT-LDL and SAA-LDL, that are well-characterized factors associated with CVDs. We demonstrated that the NLR decreased in tandem with AT-LDL and SAA-LDL. Further studies are required to verify whether the decrease in the NLR after smoking cessation is also associated with a reduction in cardiovascular events.

Conclusions

The present study showed that smoking cessation significantly decreases the NLR. NLR is associated with a wide variety of diseases including CVDs, and a decrease in NLR following smoking cessation is suggestive of decreased risk of CVD.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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