ORIGINAL ARTICLE

High neutrophil-to-lymphocyte ratio is associated with increased carotid artery intima-media thickness in type 2 diabetes

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ABSTRACT

Aims/Introduction: Emerging evidence suggests that the neutrophil-to-lymphocyte ratio (NLR) is a novel potential marker of inflammatory responses. The objective was to evaluate the association between NLR and carotid artery intima-media thickness (cIMT) in type 2 diabetes.

Materials and Methods: We carried out a case–control study involving 320 patients with type 2 diabetes, and 250 age-, sex- and body mass index-matched healthy controls who all underwent carotid ultrasonography and took a blood examination. We divided the diabetes patients into two groups according to cIMT: 188 diabetes patients with high cIMT and 132 diabetes patients with low cIMT, and compared baseline characteristics and NLR between the two groups and healthy controls.

Results: The mean NLR was significantly higher in the group of diabetes patients with high cIMT than the group of diabetes patients with low cIMT, who in turn showed a significantly higher NLR compared with control participants. Logistic regression analysis showed that the NLR was an independent risk factor for diabetes patients with high cIMT (odds ratio 140.89, 95% CI 1.71–11615.30, $P = 0.028$). Based on the receiver operating characteristic curve, use of the NLR as an indicator for diabetes patients with high cIMT diagnosis was projected to be 3.16, and yielded a sensitivity and specificity of 36.2% and 93.2%, respectively, with an area under the curve of 0.606 (95% CI 0.544–0.667).

Conclusions: High NLR might be a potential biomarker for the increased cIMT in type 2 diabetes patients. Future studies are required to validate our findings.

INTRODUCTION

Type 2 diabetes has become a major public health problem in the late 20th and 21st centuries. There are 28 million people in the USA who have type 2 diabetes, and more than 350 million people who have type 2 diabetes worldwide[1,2]. The chronic complications of type 2 diabetes, such as diabetic retinopathy, nephropathy, and peripheral and autonomic neuropathies[3], increases with patients' age, disease course and diabetes mellitus severity[3]. In addition, type 2 diabetes also increases the risk of atherosclerosis/cardiovascular disease two- to fivefold[4]. Carotid artery intima-media thickness (cIMT), which serves as a well-established biomarker of subclinical atherosclerosis, is a risk factor for cardiovascular disease and can be used to predict cardiovascular events[5,6]; it is significantly greater in patients with type 2 diabetes than that in non-diabetes subjects[7].

Some studies have pointed out that inflammation plays an important role in diabetes and its chronic complications, and is also associated with cardiovascular events[8–10]. Inflammatory cells contribute to atherosclerotic lesion initiation and lesion disruption, which could cause acute coronary syndrome and other cardiovascular events. Several inflammatory markers (such as fibrinogen, C-reactive protein [CRP], interluekin-18 and tumor necrosis factor-α), which are associated with markers of asymptomatic atherosclerosis in type 2 diabetes[11], are difficult to obtain in common clinical practice. Therefore, simpler and more convenient markers are required.

Mounting evidence has shown that the white blood cell (WBC) count and its subtypes are classic indicators of inflammation[12]. The neutrophil-to-lymphocyte ratio (NLR) was

calculated as a simple ratio of the absolute neutrophil count to the absolute lymphocyte count. It was defined as a potential biomarker of inflammation in tumors[13–15], cardiovascular conditions[16,17], and diabetes and its complications[18,19]. Previous studies have shown that a higher level of NLR was related to a higher severity of coronary artery disease and worse clinical outcome in patients undergoing percutaneous coronary intervention[20–23]. Akbas *et al.*[18] reported that the level of NLR was significantly higher in diabetic nephropathy patients with albuminuria. Ulu *et al.*[24] also reported a positive correlation between the NLR and different diabetic retinopathy grades. The exact mechanisms of the higher level of NLR being associated with diabetes and its complications are still unclear, and the most important mechanism might be inflammation. However, the relationship between diabetic cIMT and NLR has not been investigated to date. The objective of the present study was to investigate the role of NLR in cIMT development among type 2 diabetes patients.

METHODS

Participants

Written approval for the study was obtained from the ethics committee of Huai'an First People's Hospital, Nanjing Medical University. The approval number of the institution review was IRB-PJ2011-009-01. Informed consent was obtained from each participant.

A total of 320 patients with type 2 diabetes, and 250 age-, sex- and body mass index (BMI)-matched healthy controls were recruited from January 2012 to December 2013. Type 2 diabetes patients were recruited from the Department of Gerontology and Endocrinology, Huai'an First People's Hospital, Huai'an, Jiangsu, China – they were all hospitalized patients. Healthy participants were recruited from the Checkup Center, Huai'an First People's Hospital, and were not receiving any medications. All patients with type 2 diabetes met the 2015 American Diabetes Association diabetes standards and classification[25]. Patients were excluded if they were aged younger than 18 years or had type 1 diabetes mellitus, any acute inflammation, active infection, cancer, chronic liver diseases, renal disorders, hypertension, coronary heart disease, cerebrovascular disease, lower extremity arterial disease, or any acute diabetic complications.

Design

Data at the time of admission for demographics (age, sex, body mass index [BMI] and waist circumference), smoking status, duration of type 2 diabetes, family history of diabetes, oral antidiabetic drugs and insulin used, aspirin or statins used, and systolic (SBP) blood pressure and diastolic blood pressure (DBP) were obtained from medical records. Systemic BP was measured as described previously[26]. BMI was calculated as weight in kilograms divided by height in meters squared.

Fasting blood samples were taken for measurement of a routine blood test, fasting plasma glucose (FBG), creatinine (Cr), glycosylated hemoglobin A1c (HbA1c), high-sensitivity CRP

(hs-CRP) and lipid profile (total cholesterol [TC], triglyceride [TG], high-density lipoprotein cholesterol [HDL-C] and low-density lipoprotein cholesterol [LDL-C]). The routine blood test was measured using an automated complete blood cell counter (model XT 2000i; Sysmex, Kobe, Japan), which simultaneously provided values for total white blood cell count, red blood count, hemoglobin, platelet count, absolute neutrophil count and absolute lymphocyte count. NLR was calculated as a simple ratio of the absolute neutrophil count to the absolute lymphocyte count. HbA1c levels were measured by the high-performance liquid chromatography method. The hs-CRP was measured using a latex-enhanced immunoturbidimetric method with lower limits of detection at 0.02 mg/L. Serum lipids, FBG and creatinine were measured by enzymatic methods on a Hitachi 7600 automatic clinical chemistry analyzer (Boehringer Mannheim, Mannheim, Germany) using reagent kits supplied by the manufacturer.

The cIMT of both carotid arteries was measured by using high-resolution ultrasound Doppler system (SSI6000; Kai-Li Ultrasound, Shenzhen, China). Two assessors measured the degree of cIMT based on the ultrasound images respectively (Excel S1). When there was a discrepancy in the measurement of the degree of cIMT, or the cIMT difference was greater than 0.2 mm between the two assessors, a third assessor was asked to carry out the measurements, and the averaged results of the findings were used. All the participants were measured in a supine position. We measured the minimum and maximum cIMT in each carotid artery, and selected the thickest as the maximum cIMT value of the carotid artery. Type 2 diabetes patients were divided into two groups according to the maximum cIMT values: the low cIMT group (maximum cIMT ≤1 mm; DM-Low cIMT group) and the high cIMT group (maximum cIMT >1 mm; DM-High cIMT group).

Statistical analysis

The Kolmogorov–Smirnov normality test was used to evaluate the distribution of all quantitative data. Normally distributed data were expressed as mean ± standard deviation, and analyzed by SPSS software version 20.0 (SPSS, Inc., Chicago, IL, USA). Non-parametric statistics was used if data were not normally distributed. The independent samples *t*-test was used for normally distributed data to compare continuous variables; the chi square-test was used to compare categorical variables.

Logistic regression was used to analyze the cIMT risk factors. Receiver operating characteristic (ROC) curve analysis was used to compare the prognostic powers of the NLR for cIMT. The predictive validities were quantified as areas under the ROC curves. The positive predictive value and negative predictive value were calculated, and $P < 0.05$ was considered statistically significant.

RESULTS

We divided the diabetes patients into two groups according to the maximum cIMT values: DM-high cIMT group and DM-low

cIMT group. The baseline characteristics of the diabetes patients and healthy controls are shown in Table S1 and Table 1.

There were no differences between the diabetes group and control group in age, sex, BMI, waist circumference, smocking status, serum TG, creatinine, WBC, monocyte, and platelet levels (Table S1). The levels of SBP, DBP, FBG, HbA1c, TC and LDL-C were significantly higher, and the level of HDL-C was significantly lower in the diabetes group than in the control group (Table S1). There were still significant differences between the two groups in the percentage of family history of diabetes, duration of diabetes, insulin and oral antidiabetic drug used, and aspirin or statins used (Table S1). The levels of cIMT, hs-CRP, neutrophils and NLR were significantly higher, and the level of lymphocytes was significantly lower in the diabetes group than in the control group (Table S1).

Furthermore, there were also no significant differences between diabetes patients with high cIMT and low cIMT in age, sex, BMI, waist circumference, smoking status, the levels of SBP, DBP, FBG, HbA1c, TG, TC, LDL-C, HDL-C and creatinine, the percentage of family history of diabetes, duration of diabetes, insulin and oral antidiabetic drug used, and aspirin or statins used (Table 1). Diabetes patients with high cIMT had significantly higher levels of NLR, hs-CRP, neutrophils and cIMT, and significantly lower levels of lymphocytes compared with diabetes patients with low cIMT (Table 1). There were also no significant differences between diabetes patients with high cIMT and low cIMT in the levels of WBC, monocyte, and platelet (Table 1).

Duration of diabetes, levels of TG, WBC, lymphocytes, neutrophils, NLR and hs-CRP were regarded as dependent variables, and the status of cIMT (high or low) were regarded as

Table 1 | Description and comparison of clinical and hematological parameters among the three groups

	DM-High cIMT group ($n = 188$)	DM-Low cIMT group ($n = 132$)	Control group ($n = 250$)
Age (years)	64.51 ± 6.93	65.58 ± 6.81	64.33 ± 6.80
Sex (male)	108 (57.44%)	76 (57.58%)	142 (56.80%)
BMI (kg/m^2)	23.97 ± 1.68	23.92 ± 1.10	24.02 ± 1.14
WC (cm)	91.66 ± 6.31	91.07 ± 5.85	91.66 ± 6.34
Duration of DM (years)	3.67 ± 1.56[†††]	3.95 ± 1.70[‡‡‡]	0.00 ± 0.00
Smoking (%)	46 (24.47%)	32 (24.24%)	54 (21.60%)
Diabetes family history (%)	50 (26.60%)[†††]	34 (25.76%)[‡‡‡]	14 (5.60%)
Insulin used (%)	112 (59.57%)[†††]	78 (59.09%)[‡‡‡]	0 (0.00%)
Oral antidiabetic drugs used (%)	76 (40.43%)[†††]	54 (40.91%)[‡‡‡]	0 (0.00%)
Aspirin used (%)	132 (70.21%)[†††]	90 (68.18%)[‡‡‡]	0 (0.00%)
Statins used (%)	156 (82.98%)[†††]	104 (78.79%)[‡‡‡]	0 (0.00%)
cIMT (mm)	1.40 ± 0.17[***,†††]	0.86 ± 0.07[‡‡‡]	0.71 ± 0.13
SBP (mmHg)	132.63 ± 17.40[†††]	134.10 ± 11.96[‡‡‡]	124.16 ± 9.41
DBP (mmHg)	83.76 ± 7.25[†††]	82.85 ± 7.61[‡‡‡]	78.97 ± 5.96
FBG (mmol/L)	5.44 ± 0.67[†††]	5.47 ± 0.68[‡‡‡]	4.89 ± 0.60
HbA1c	6.91 ± 0.45[†††]	6.85 ± 0.46[‡‡‡]	4.92 ± 0.45
TG (mmol/L)	1.39 ± 0.58	1.50 ± 0.59	1.42 ± 0.59
TC (mmol/L)	4.96 ± 0.64[†††]	5.03 ± 0.62[‡‡‡]	4.61 ± 0.51
LDL-C (mmol/L)	2.89 ± 0.38[†]	2.93 ± 0.38[‡‡]	2.80 ± 0.40
HDL-C (mmol/L)	1.23 ± 0.25[††]	1.19 ± 0.25[‡‡‡]	1.31 ± 0.25
Scr (μmol/L)	74.44 ± 11.74	73.34 ± 11.66	73.07 ± 9.88
Hs-CRP (mg/L)	5.80 ± 0.85[***,†††]	4.29 ± 0.87[‡‡‡]	2.35 ± 0.96
WBC (×10^9/L)	6.27 ± 1.03	6.12 ± 0.77	6.11 ± 1.05
Lymphocytes (×10^9/L)	1.40 ± 0.17[*,†††]	1.45 ± 0.19[‡‡‡]	1.70 ± 0.28
Neutrophils (×10^9/L)	3.86 ± 1.02[*,†††]	3.66 ± 0.68[‡‡]	3.42 ± 1.05
Monocyte (×10^9/L)	0.40 ± 0.05	0.41 ± 0.07	0.40 ± 0.05
Platelet (×10^9/L)	208.30 ± 28.68	211.84 ± 47.82	209.40 ± 46.37
NLR	2.79 ± 0.80[***,†††]	2.53 ± 0.47[‡‡‡]	2.08 ± 0.76

All values are expressed as mean ± standard deviation for continuous variables and as the number of patients (percentage) for categorical variables. Continuous variables were analyzed by independent-sample t-tests, two-sided type I error of 0.05. Categorical variables were analyzed by chi square-tests. *$P < 0.05$; ***$P < 0.001$, diabetes patients with high carotid intima-media thickness (DM-High cIMT) group vs diabetes patients with low carotid intima-media thickness (DM-Low cIMT) group. [†]$P < 0.05$; [††]$P < 0.01$; [†††]$P < 0.001$, DM-High cIMT group vs the control group. [‡‡]$P < 0.01$; [‡‡‡]$P < 0.001$, DM-Low cIMT group vs the control group. BMI, body mass index; cIMT, carotid intima-media thickness; DBP, diastolic blood pressure; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NLR, neutrophil-to-lymphocyte ratio; SBP, systolic blood pressure; Scr, serum creatinine; TC, total cholesterol; TG, triglyceride; WBC, white blood cell; WC, waist circumference.

independent variables when we carried out logistic regression analysis. As shown in Table 2, it was shown that independent risk factors for high cIMT were NLR (β = 4.95, odds ratio [OR] 140.89, 95% confidence interval [CI] 1.71–11615.30, *P* = 0.028) and hs-CRP (β = 1.97, OR 7.20, 95% CI 4.66–11.11, *P* < 0.001).

Figure 1 shows that based on the ROC, as an independent risk factor for cIMT, the cut-off value of NLR was 3.16, and the sensitivity and specificity of the NLR for cIMT diagnosis were 36.2% and 93.2%, respectively, with an area under the curve at 0.606 (95% CI 0.544–0.667). The positive predictive value and negative predictive value for NLR for cIMT diagnosis was 88.31% and 50.62%, respectively.

DISCUSSION

Type 2 diabetes is a major public health problem worldwide, and vascular complications still represent the main cause of morbidity and mortality in type 2 diabetes patients. The main pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls and ischemia throughout the body. Mounting evidence has shown that inflammation plays an important role in the pathogenesis of atherosclerosis and its complications[27]. There is a series of inflammatory cytokines that are upregulated in patients with diabetes and its complications[11]. However, some of these biomarkers are difficult to obtain in common clinical practice. NLR is defined as a novel potential biomarker to determine inflammation in diabetes and its complications[18,19]. Calculation of NLR is a very simple method compared with other inflammatory cytokines tested[28]. However, no data to date are available regarding the relationship between NLR and cIMT in type 2 diabetes.

In the present study, we found that the levels of SBP, DBP, FBG, HbA1c, TC and LDL-C, and the percentage of family history of diabetes were significantly higher, and the level of HDL-C was significantly lower in diabetes patients than in control participants, which might be the reason that diabetes

patients have coexistence of multiple cardiovascular disease risk factors. There were no significant differences in the levels of SBP, DBP, FBG, HbA1c, TC, LDL-C and HDL-C, and the percentage of family history of diabetes between diabetes patients with high cIMT and low cIMT. There were also no differences in duration of diabetes, insulin and oral antidiabetic drug used, aspirin or statins used between diabetes patients with high cIMT and low cIMT. These results might be associated with active and effective treatment between the two groups of diabetes patients.

In the present study, we found that diabetes patients with high cIMT had higher levels of NLR compared with diabetes patients with low cIMT, who in turn showed significantly higher levels of NLR compared with healthy participants. Logistic regression analysis showed that the NLR was an independent risk factor for diabetes patients with high cIMT (OR 140.89, 95% CI 1.71–11,615.30, *P* = 0.028). Based on the ROC, use of the NLR as an indicator for diabetes patients with cIMT diagnosis was projected to be 3.16, and yielded a sensitivity and specificity of 36.2% and 93.2%, respectively, with an area under the curve of 0.606 (95% CI 0.544–0.667). It might prove that an increased NLR in peripheral blood could be an independent risk factor for predicting the development of subclinical atherosclerosis in diabetes patients with relatively high sensitivity and specificity.

However, the exact mechanisms of high NLR being related with increased cIMT in diabetes patients are as yet unknown.

Table 2 | Logistic regression analysis showing independent predictors of carotid intima-media thickness

Variables	β	OR	95% CI	*P*-value
Duration of DM (years)	−0.15	0.86	0.71–1.04	0.117
TG (mmol/L)	−0.70	0.93	0.55–1.59	0.798
WBC (×10^9/L)	0.92	2.51	0.02–360.99	0.717
Lymphocytes (×10^9/L)	6.78	876.07	0.11–6896734.68	0.139
Neutrophils (×10^9/L)	−4.07	0.02	0.00–7.92	0.194
NLR	4.95	140.89	1.71–11615.30	0.028
Hs-CRP (mg/L)	1.97	7.20	4.66–11.11	0.000

CI, confidence interval; cIMT, carotid intima-media thickness; DM, diabetes mellitus; hs-CRP, high-sensitivity C-reactive protein; NLR, neutrophil-to-lymphocyte ratio, OR, odds ratio; TG, triglyceride; WBC, white blood cell.
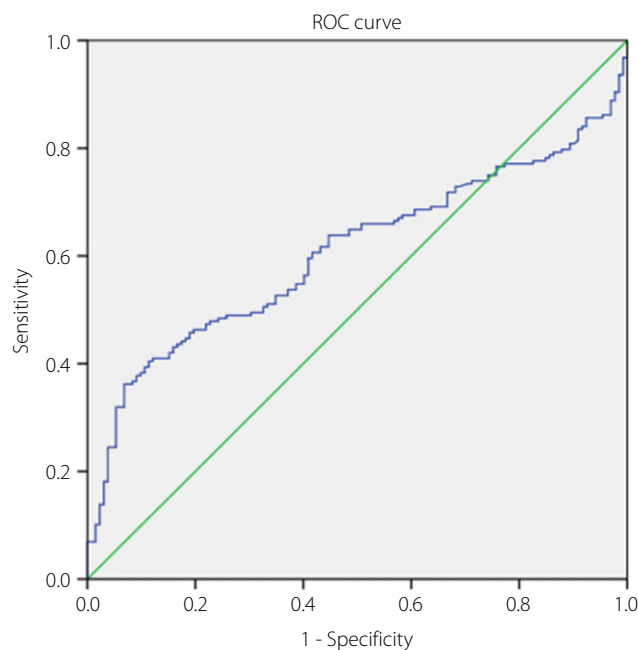

Figure 1 | Receiver operating characteristics (ROC) curve analysis for neutrophil-to-lymphocyte ratio. The ROC curve analysis for neutrophil-to-lymphocyte ratio showed neutrophil-to-lymphocyte ratio as a predictor of the severity of carotid intima media thickness.

Most likely, it can be explained in view of various functions of NLR. As we all know, high NLR could represent not only the increased level of neutrophils, but also the decreased level of lymphocytes. Hyperglycemia, or advanced glycated end-products, leads to persistent activation of neutrophils, as evidenced by the increased activity of neutrophil alkaline phosphatase[29]. Then the neutrophils of diabetes patients show increased necrosis and enhanced production of reactive oxygen species[30], and significantly lower neutrophil chemotactic responses[31]. Previous studies have also shown that activation of leukocytes and their adhesion to the endothelium could cause endothelial injury[32]. These leukocytes secrete cytokines and growth factors that can promote the migration and proliferation of smooth muscle cells, which can induce further vascular damage and cause most complications of atherosclerosis[33,34].

Nevertheless, the presence of lymphopenia might also be an important factor. In the present study, we found that diabetes patients with high cIMT had significantly lower level of lymphocytes compared with diabetes patients with low cIMT, who in turn showed significantly lower levels of lymphocytes compared with control participants. Previous studies have shown that inflammation plays an important role in both diabetes and obesity. Tanaka et al.[35] showed that T lymphocytes and their subsets are characteristically reduced in obese persons. Worsening obesity might increase lymphopenia development, which, in turn, increases NLR. Therefore, we deduced that development of lymphopenia is related with inflammation, which might promote atherosclerosis.

Another possible mechanism is that NLR can reflect an autonomic balance of the vascular bed. Previous studies have shown that the distribution of leukocytes is regulated by the autonomic nervous system in humans and animals. The number and function of granulocytes are stimulated by sympathetic nerves, whereas those of lymphocytes are stimulated by parasympathetic nerves[36,37]. Therefore, a higher level of NLR might indicate a higher ratio of sympathetic-to-parasympathetic activity. An increased sympathetic tone is positively related with higher rates of oxygen consumption and increased production of proinflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α[38,39]. These cytokines might play a key role in regulating the vascular tone by affecting the release of nitric oxide and endothelin-1; they also contribute to the development of proliferative vascular lesions by stimulating smooth muscle and interstitial cell proliferation, which could accelerate atherosclerosis development[40,41].

However, there were also some limitations to the present study. The main limitation was the limited number of study samples. Future studies are required to clarify these relationships of large sample analysis. Second, the patients in the present study were of Chinese origin. Larger studies are required to determine whether the observations in this study are present in individuals of various races and ethnicities. Finally, the present study was an observational study, further studies regarding clinical outcome should be carried out.

In conclusion, the data presented show for the first time that high NLR might be an independent risk factor for increased cIMT in type 2 diabetes. Early screening of patients might help distinguish a high-risk group and guide prophylactic initiatives.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Centers for Disease Control and Prevention. National diabetes statistics report, 2014. Available from http://www.cdc.gov/diabetes/data/statics/2014StatisticsReport.htlm Accessed January 5, 2015.
2. International Diabetes Federation. IDF Diabetes Atlas, 6th edn. Brussels, Belgium: International Diabetes Federation, 2013.
3. Nathan DM. Long-term complications of diabetes mellitus. N Engl J Med 1993; 328: 1676–1685.
4. Preis SR, Hwang SJ, Coady S, et al. Trends in all-cause and cardiovascular disease mortality among women and men with and without diabetes mellitus in the Framingham Heart Study, 1950 to 2005. Circulation 2009; 119: 1728–1735.
5. Bots ML, Hoes AW, Koudstaal PJ, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997; 96: 1432–1437.
6. O'Leary DH, Polak JF, Kronmal RA, et al. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999; 340: 14–22.
7. Taniwaki H, Kawagishi T, Emoto M, et al. Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type diabetes. Vessel wall properties in type 2 diabetes. Diabetes Care 1999; 22: 1851–1857.
8. Schmidt MI, Duncan BB, Sharrett AR, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999; 353: 1649–1652.
9. Fujita T, Hemmi S, Kajiwara M, et al. Complement-mediated chronic inflammation is associated with diabetic microvascular complication. Diabetes Metab Res Rev 2013; 29: 220–226.
10. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340: 115–126.
11. Ray A, Huisman MV, Tamsma JT, et al. The role of inflammation on atherosclerosis, intermediate and clinical cardiovascular endpoints in type 2 diabetes mellitus. Eur J Intern Med 2009; 20: 253–260.

12. Horne BD, Anderson JL, John JM, *et al.* Which white blood cell subtypes predict increased cardiovascular risk? *J Am Coll Cardiol* 2005; 45: 1638–1643.

13. Gunduz S, Mutlu H, Tural D, *et al.* Platelet to lymphocyte ratio as a new prognostic for patients with metastatic renal cell cancer. *Asia Pac J Clin Oncol* 2015; 11: 288–292.

14. Ozaksit G, Tokmak A, Kalkan H, *et al.* Value of the platelet to lymphocyte ratio in the diagnosis of ovarian neoplasms in adolescents. *Asian Pac J Cancer Prev* 2015; 16: 2037–2041.

15. Liu J, Du J, Fan J, *et al.* The neutrophil-to-lymphocyte ratio correlates with age in patients with papillary thyroid carcinoma. *ORL J Otorhinolaryngol Relat Spec* 2015; 77: 109–116.

16. Akyel A, Yayla Ç, Erat M, *et al.* Neutrophil-to-lymphocyte ratio predicts hemodynamic significance of coronary artery stenosis. *Anatol J Cardiol* 2015; 15: 1002–1007.

17. Oylumlu M, Yıldız A, Oylumlu M, *et al.* Platelet-to-lymphocyte ratio is a predictor of in-hospital mortality patients with acute coronary syndrome. *Anatol J Cardiol* 2015; 15: 277–283.

18. Akbas EM, Demirtas L, Ozcicek A, *et al.* Association of epicardial adipose tissue, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio with diabetic nephropathy. *Int J Clin Exp Med* 2014; 7: 1794–1801.

19. Shiny A, Bibin YS, Shanthirani CS, *et al.* Association of neutrophil-lymphocyte ratio with glucose intolerance: an indicator of systemic inflammation in patients with type 2 diabetes. *Diabetes Technol Ther* 2014; 16: 524–530.

20. Poludasu S, Cavusoglu E, Khan W, *et al.* Neutrophil to lymphocyte ratio as a predictor of long-term mortality in African Americans undergoing percutaneous coronary intervention. *Clin Cardiol* 2009; 32: E6–E10.

21. Shen XH, Chen Q, Shi Y, *et al.* Association of neutrophil/lymphocyte ratio with long-term mortality after ST elevation myocardial infarction treated with primary percutaneous coronary intervention. *Chin Med J (Engl)* 2010; 123: 3438–3443.

22. Sen N, Afsar B, Ozcan F, *et al.* The neutrophil to lymphocyte ratio was associated with impaired myocardial perfusion and long term adverse outcome in patients with ST-elevated myocardial infarction undergoing primary coronary intervention. *Atherosclerosis* 2013; 228: 203–210.

23. Park JJ, Jang HJ, Oh IY, *et al.* Prognostic value of neutrophil to lymphocyte ratio in patients presenting with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Am J Cardiol* 2013; 111: 636–642.

24. Ulu SM, Dogan M, Ahsen A, *et al.* Neutrophil-tolymphocyte ratio as a quick and reliable predictive marker to diagnose the severity of diabetic retinopathy. *Diabetes Technol Ther* 2013; 15: 942–947.

25. American Diabetes Association. Approaches to glycemic treatment. Sec. 7. In Standards of Medical Care in Diabetesd 2015. *Diabetes Care* 2015; 38(Suppl. 1): S41–S48.

26. Montalcini T, Gorgone G, Fava A, *et al.* Carotid and brachial arterial enlargement in postmenopausal women with hypertension. *Menopause* 2012; 19: 145–149.

27. Ayhan H, Kasapkara HA, Aslan AN, *et al.* Relationship of neutrophil-to-lymphocyte ratio with aortic stiffness in type 1 diabetes mellitus. *Can J Diabetes* 2015; 39: 317–321.

28. Friedman GD, Tekawa I, Grimm RH, *et al.* The leucocyte count: correlates and relationship to coronary risk factors: the CARDIA study. *Int J Epidemiol* 1990; 19: 889–893.

29. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol Med Microbiol* 1999; 26: 259–265.

30. Shurtz-Swirski R, Sela S, Herskovits AT, *et al.* Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. *Diabetes Care* 2001; 24: 104–110.

31. Delamaire M, Maugendre D, Moreno M, *et al.* Impaired leucocyte functions in diabetic patients. *Diabet Med* 1997; 14: 29–34.

32. van Oostrom AJ, vanWijk JP, Sijmonsma TP, *et al.* Increased expression of activation markers on monocytes and neutrophils in type 2 diabetes. *Neth J Med* 2004; 62: 320–325.

33. Goldin A, Beckman JA, Schmidt AM, *et al.* Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006; 114: 597–605.

34. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002; 105: 1135–1143.

35. Tanaka S, Isoda F, Ishihara Y, *et al.* T lymphopaenia in relation to body mass index and TNF-alpha in human obesity: adequate weight reduction can be corrective. *Clin Endocrinol (Oxf)* 2001; 54: 347–354.

36. Ito BR, Schmid-Schonbein G, Engler RL. Effect of leukocyte activation on myocardial vascular resistance. *Blood Cells* 1990; 16: 145–163.

37. Abo T, Kawamura T. Immunomodulation by the autonomic nervous system: therapeutic approach for cancer, collagen diseases, and inflammatory bowel diseases. *Ther Apher* 2002; 6: 348–357.

38. Das UN. Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? *Prostaglandins Leukot Essent Fatty Acids* 2002; 63: 351–362.

39. Tracey KJ. The inflammatory reflex. *Nature* 2002; 420: 853–859.

40. Kahaleh MB, Fan PS. Effect of cytokines on the production of endothelin by endothelial cells. *Clin Exp Rheumatol* 1997; 15: 163–167.

41. Bhagat K, Vallance P. Effect of cytokines on nitric oxide pathways in human vasculature. *Curr Opin Nephrol Hypertens* 1999; 8: 89–96.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Description and comparison of clinical and hematological parameters between the diabetes group and control group.
Excel S1 | The data of intra- and inter-individual differences between the two assessors.