

Neutrophil: lymphocyte ratio is positively associated with subclinical diabetic cardiomyopathy

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Abstract

Background: Subclinical diabetic cardiomyopathy (DCM) occurs frequently in asymptomatic subjects with Type 2 diabetes mellitus (T2DM). Previous studies have shown a direct relationship between the immune system and DCM using reliable biomarkers.

Methods: 507 subjects with T2DM were recruited from April 2018 to October 2019 and divided into T2DM with cardiac dysfunction (DCM) group and T2DM without cardiac dysfunction (non-DCM) group. Adjusted logistic regression models were used to evaluate relationship between the quartiles of Neutrophil: lymphocyte ratio (NLR) and subclinical DCM (covariates: age, sex, BMI, duration of diabetes, and hyperlipidemia).

Results: Blood NLR was significantly upregulated in DCM group compared to non-DCM group ($P = 0.05$). Then the adjusted odds ratio (95% CI) of the highest NLR quartile was 14.32 (2.92-70.31) compared to the lowest quartile of NLR after multiple adjusted ($P < 0.001$). However, neutrophil and lymphocyte counts did not significantly relate to the incidence of DCM in T2DM patients.

Conclusions: The present study demonstrated that NLR was related to the incidence of subclinical DCM, suggesting that NLR may be an efficient and accurate prognostic biomarker for DCM.

Trial registration: Chinese Clinical Trial Registry (ChiCTR1900027080). Registered 30 October 2019. Retrospectively registered: www.medresman.org

Introduction

Diabetic cardiomyopathy (DCM) is defined as a heart muscle-specific dysfunction arising independently of hypertension, coronary artery disease (CAD), or evidence of other structural cardiac diseases[1]. Recent studies showed that over 70% of diabetic patients will develop some form of cardiovascular disease during their lifetime[2]. Importantly, mounting evidence supports the incidence of myocardial dysfunction even in asymptomatic T2DM (a condition also referred as subclinical DCM)[3-5], the incidence of which is estimated to range from 50% to 70%[6, 7]. Obtaining a better understanding of the early-stage risk factors and predictors would enable the design of effective prevention strategies, as strongly recommended in current clinical guidelines, which emphasizes the importance of early diagnosis and intervention in at-risk diabetic patients.

Important pathological processes involved in DCM include cardiac hypertrophy, myocardial and interstitial fibrosis, oxidative stress, apoptosis, and microangiopathy[8, 9]. Activation of inflammatory mediators represents the major driver of all of the indicated pathological processes[8, 10-13]. Prior reports demonstrated increased levels of inflammatory cytokines in diabetic patients, such as c-reactive protein (CRP), interleukin (IL)-1b, IL-6, and tumor necrosis factor (TNF)-a[14, 15]. Furthermore, chronic low-grade inflammation may also lead to cardiometabolic disease by inducing insulin resistance, a major contributor to DCM development[16, 17]. Dysregulation of immune inflammatory factors, including

elevated levels of acute-phase reactants[18], IL-6[18], CRP[19], and cortisol[20], were also well associated with the cardiometabolic-risk profile. Chronic inflammation is therefore hypothesized to underlie the constellation of DCM risk factors[14]. However, current literature on the association between chronic inflammation and DCM in populations remains limited. Whether inflammation is involved in different stages of DCM is also unknown.

Neutrophil: lymphocyte ratio (NLR), which has recently emerged as a novel biomarker of numerous inflammatory diseases[21-23]. NLR is both accessible and affordable, and thus it has become increasingly used in clinical examination and research studies. Currently, several reports have confirmed the relationship between NLR and T2DM[24, 25]; however, no studies have examined the association between NLR and DCM, and whether NLR is associated with different stages of DCM remains uncertain. Therefore, the present study is aimed to explore the possible associations between subclinical DCM and NLR, and to evaluate the predictive power of NLR for DCM.

Materials And Methods

Subject recruitment

This research was conducted as a cross-sectional, hospital-based study. A total of 532 subjects with T2DM were consecutively recruited from the Department of Endocrinology at the Third Affiliated Hospital of Soochow University (Changzhou, China) from April 2018 to October 2019, based on a priori protocol that was independent of the indications found with the echocardiographic examination. Of the 532 subjects with T2DM, 25 were excluded after gated-myocardial perfusion imaging (gated-MPI, including rest and stress) suggested the presence of occult CAD. All protocols were carried out in accordance with the principles outlined in the Declaration of Helsinki and the study was approved by the institutional review committee of the Third Affiliated Hospital of Soochow University. The study was approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University and has been registered in the Chinese Clinical Trial Registry (ChiCTR1900027080).

The inclusion criteria for the subjects were: T2DM patients aged from 40 to 70 years, regardless of the duration of diabetes. The medical treatment consisted of oral anti-hyperglycemic agents and/or insulin therapy. The exclusion criteria were: 1. A history of CAD, hypertension, valvular heart disease, atrial fibrillation, or any other cardiovascular disease. 2. A self-reported history of symptomatic macrovascular or microvascular complications of diabetes, including retinopathy, nephropathy, neuropathy, peripheral vascular disease, and stroke. 3. Pregnancy. 4. Other important comorbidities including infectious diseases, malignancy, thyroid dysfunction, hepatic and renal dysfunction, immune and rheumatic diseases, or significant psychiatric illness. All subjects gave written, informed consent before enrollment in the study.

Trial design

On the same day of admission to the hospital (i.e., the basal day), patients with T2DM provided a complete medical history and were subjected to comprehensive physical and clinical examinations. Peripheral blood samples were collected from each patient after an overnight fast, on the next morning after admission. All patients underwent electrocardiography (ECG), echocardiography, and gated-myocardial perfusion imaging (gated-MPI, including rest and stress). Eligible subjects with evidence of left ventricular diastolic dysfunction (LVDD) and no apparent occult CAD were defined as having subclinical DCM. LVDD was classified according to recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging[26]. To exclude CAD, each patient underwent rest and stress gated-MPI in the presence of a cardiologist. The criteria for defining ischemia based on the gated-MPI results were defined according to the 2016 American Society of Nuclear Cardiology imaging guidelines for single-photon emission computed tomography-based nuclear cardiology procedures[27]. All patients underwent rigorous analysis of clinical variables, metabolic measurements, echocardiography, pre- and post-exercise myocardial function during the baseline-screening phase.

Demographic, clinical, and metabolic data

Demographic data were analyzed regarding the patients' sex, age, basic anthropometry (including body-mass index [BMI] and waist-to-hip circumference ratio), duration of diabetes and concomitant hypoglycemic drug use. The clinical data obtained included the levels of B-type natriuretic peptide (BNP, a marker of heart failure) and myocardial enzymes. Biochemical analysis was performed to determine the levels of alanine aminotransferase (AST), glutamic oxaloacetic transaminase (ALT), creatinine, urea nitrogen (BUN), and creatinine (Cr). Metabolic measurements included fasting-blood glucose (FBG), postprandial-blood glucose (PBG), HbA1c, and lipid profiles. Briefly, HbA1c was measured with boronate affinity high-performance liquid chromatography. HbA1c values were corrected on the basis of the correlation coefficient that was derived from a validation experiment that used sample data that were measured on both analysers. Serum Cr, BUN, myocardial enzymes, BNP, ALT, AST, and lipid profiles including triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol were measured on a Modular P800 analyser (Roche Diagnostics, Mannheim, Germany). The clinical and metabolic data were measured after fasting for at least 8 h and before administering hypoglycemic agents.

Echocardiography and data analysis

The following echocardiography parameters were determined: the left atrial diameter (LAD), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), interventricular septal diameter (IVSD), left ventricular ejection fraction (LVEF), left atrial mass (LA mass), LA mass index, peak early diastolic trans-mitral flow velocity (E), peak late diastolic trans-mitral flow velocity (A), and peak early diastolic mitral annular velocity ($e\dot{c}$). The E/A ratio, E/ $e\dot{c}$ ratio, and LA volume indexes were calculated to evaluate the diastolic function.

Gated-MPI

Gated stress–rest ^{99m}Tc -MIBI MPI was performed for each subject according to a 2-day standard imaging protocol[27]. Images were acquired 60–90 min after ^{99m}Tc -MIBI injection at a dose of 740–925 MBq for both the stress and rest examinations. The exercise stress test was conducted on a bicycle according to the modified Bruce protocol, and the pharmacological stress test was conducted with an intravenous injection of adenosine ($140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) over 6 min. The short-axis, horizontal-axis, and vertical long-axis images were interpreted independently by two experienced nuclear physicians based on qualitative visual interpretation, according to standard guidelines. Both physicians were blinded to the clinical data for each patient. When there was a disagreement, a third expert was recruited to resolve the discrepancies. Myocardial ischemia was defined as reversible sparsity or defects on two different fault planes and two consecutive layers of stress–rest tomography at the same location, whereas myocardial infarction was defined as irreversible sparsity or defects on two different fault planes and two consecutive layers of stress–rest tomography at the same location.

NLR analysis

We determined neutrophil and leukocyte using an automated hematology analyzer. Then, by dividing the neutrophil by the lymphocyte count, we calculated the neutrophil/lymphocyte ratio (NLR). In order to investigate how the NLR, neutrophil, and lymphocyte counts are related to prevalence and incidence of DCM, we divided them into four categories according to quartiles of participants.

Statistical analysis

All clinical data were analyzed using SPSS software (version 23.0). For continuous, normally distributed variables, the results are presented as the mean \pm SD. For categorical variables, the results are presented as percentages. For further analysis, the incidence of DCM was used as a dependent variable, and the quartiles of NLR, neutrophil, and lymphocyte counts were used as independent variables. For baseline characteristics of patients, the differences among quartiles of NLR were analyzed using ANOVA followed by Bonferroni's post-hoc test for continuous variables and logistic regression analysis for proportional variables. Multiple logistic regression analysis was then used to examine the relationship between quartiles of NLR and the incidence of DCM after adjusting for covariates, including sex, age, BMI, duration of diabetes and hyperlipidemia. Odds ratio (OR) (95% CI) were calculated. A receiver–operator characteristic (ROC) curve was generated to evaluate the sensitivity and specificity of NLR for predicting the diagnosis of DCM. A P value <0.05 was deemed to reflect a statistically significant difference.

Results

Patients' characteristics

In the cross-sectional study, 532 subjects were screened from April 2018 to October 2019. Of the 532 subjects with T2DM, 25 were excluded after gated-MPI suggested the presence of occult CAD. We divided the 507 remaining subjects into T2DM with cardiac dysfunction (DCM) group ($n=465$) and T2DM without cardiac dysfunction (non-DCM) group ($n=45$). For the clinical characteristics of subjects, statistically

significant differences were detected in terms of sex, age, BMI, duration of diabetes, FBG, 2-hour PBG, and lipid profiles including TC, HDL and LDL (all P value < 0.05) (Supplemental Table 1) between the two groups. Echocardiographic features of subjects with or without cardiac dysfunction were presented in Supplemental Table 2 and significant difference were observed in cardiac structure (IVSD), EF%, and cardiac diastolic function including A velocity, E/A ratio, e' velocity, and E/e' ratio (all P value < 0.05).

Furthermore, NLR was upregulated in T2DM subjects with cardiac dysfunction (Supplemental Table 1). Then we assessed clinical characteristics across NLR quartiles in Table 1. Compared to subjects in the lowest quartile of NLR, subjects in the upper three quartiles tended to have longer duration of diabetes, to have higher 2-hour FBG, HbA1c%, total cholesterol (TC) and triglyceride (TG) (all P value < 0.05). Other than these results, no significant differences were observed between the subjects in different NLR quartiles.

The cardiac structure and LV function of patients with T2DM across NLR quartiles are presented in Table 2. Compared to subjects in the lowest quartile of NLR, significant differences in IVSD were observed in the upper three quartiles ($P = 0.002$). In terms of LV function, subjects in the upper three quartiles tended to have E/A ratio and e' velocity, but higher A velocity and E/e' ratio (all P value < 0.001). However, E velocity showed no statistical significance across NLR quartiles.

Association of NLR with risk of subclinical DCM in patients with T2DM

Table 3 shows the crude and adjusted relationships between quartiles of NLR and DCM. In the final multivariate models, which adjusted for age, sex, BMI, duration of diabetes and serum lipid, the ORs (95% CI) for DCM across NLR quartiles were 1.00 (reference), 8.02 (1.53, 41.96), 12.55 (2.52, 62.35), and 14.32 (2.92, 70.31) ($P = 0.001$). Furthermore, we performed ROC analysis following AUC to assess the power of NLR (AUC=0.865, 95% CI: 0.818, 0.913), neutrophil (AUC=0.560, 95% CI: 0.473, 0.647) and lymphocyte (AUC=0.399, 95% CI: 0.308, 0.489) to discriminate T2DM patients with or without cardiac dysfunction (Figure 1 and Supplemental Table 3). NLR, but not neutrophil or lymphocyte, was identified as a potential predictor of DCM.

Moreover, we divided neutrophil and lymphocyte counts into four categories according to quartiles of participants as follows (range x 1000 cells/mm³): neutrophil: < 2.68, 2.68-3.34, 3.34-4.18, and > 4.18. Lymphocyte: < 1.54, 1.54-1.90, 1.90-2.30, and > 2.30. After adjusting for multiple variants, the ORs (95% CI) of DCM for increasing quartiles of neutrophil counts were: 1.00, 0.52 (0.18, 1.56), 1.52 (0.59, 3.94), 1.12 (0.43, 2.87). For lymphocyte counts: 1.00, 0.46 (0.17, 1.23), 0.72 (0.29, 1.77), 0.35 (0.13, 0.95) (Figure 2).

Discussion

In this study, NLR, but not neutrophil, and lymphocyte counts, was positively related to the incidence of DCM. This was the first study to relate the risk of subclinical DCM to NLR, an inflammatory biomarker which is used extensively in the medical field.

Recently, NLR has been identified as a useful biomarker in numerous chronic inflammatory diseases including diabetes, which reflects both an increase in the neutrophil counts and a reduction in the lymphocyte counts. For example, a high baseline NLR may serve as a useful indicator for short survival duration in patients with amyotrophic lateral sclerosis (ALS)[28]. Pretreatment NLR and albumin-to-gamma-glutamyl transferase ratio (AGR) predict the diagnosis of prognosis of Grade III oligodendroglial gliomas[22]. In a large-scale cross-sectional cohort study, NLR was significantly increased in diabetic patients and might serve as an efficient and accurate prognostic biomarker for T2DM[24]. Moreover, data from several studies have also established the utility of NLR as a medically relevant biomarker for diabetic complications. In research conducted by Sukhija *et al.*, the NLR was used to identify individuals at risk for sensorineural hearing loss (caused by diabetic vascular complications via inflammation)[29]. In type 2 diabetic nephropathy (T2DN), NLR have been reported to be a prognostic marker for T2DN outcomes[30], including microvascular complications[31] and impaired renal function[32]. In this study, the NLR, but not neutrophil and lymphocyte counts, was independently and significantly related to the incidence of subclinical DCM in T2DM patients. Determining the NLR is both simple and affordable; thus, it could be used as an innovative and effective predictor for DCM.

Previous findings demonstrated that the first manifestation of myocardial involvement in diabetes was preclinical LV diastolic dysfunction (LVDD)[33, 34]. Although myocardial changes can be detected by echocardiography, several cardiac abnormalities can occur even before the onset of structural and hemodynamic dysfunction in T2DM patients without LVDD. A much more focused approach is therefore needed for T2DM patients, involving regularly assessing systemic abnormalities and cardiovascular risk in this population. One such approach would be to employ circulating biomarkers to track diabetes progression and for medication guidance. Increasing evidence suggest that inflammation is involved in the pathophysiology diabetes and heart failure, and inflammation has emerged as a central theme in the pathology of systolic heart disease in recent decades[35, 36]. However, few studies have demonstrated the participation of inflammatory factors in the development of diastolic dysfunction[37], especially in diabetes-induced LVDD. In the present study, NLR positively correlated with impaired LVDD, which provide an independent association with subclinical DCM, and would provide effective prevention strategies, as strongly recommended in clinical guidelines.

Limitations

The main limitation of this study was a deficiency of echocardiographic data and NLR value for patients with DCM at different stages. Echocardiography is the most common and widely used examination to evaluate cardiac structure and function, for asymptomatic DCM patients, it is necessary to have a regular echocardiography to evaluate diastolic and systolic function. However, owing to its relatively high price and a certain error measured by different doctors hamper its largescale application for early detection of asymptomatic DCM patients. NLR is both accessible and affordable, therefore, how to combine echocardiography data and NLR value to early predict DCM is an important clinical task and needs further exploration. Second, although NLR was identified as a significant and independent biomarker for diagnosing DCM, the precise mechanisms underlying the associations between systemic inflammation

and the prevalent conditions remain to be further elucidated. Third, this study was limited by a single center and the findings need to be confirmed in larger and multi-center cohort studies in the future.

Conclusions

To the best of our knowledge, our study provided the first evidence of a relationship between NLR and abnormal diastolic performance in T2DM patients without clinical symptoms. NLR was identified as an independent and early biomarker for the occurrence of DCM] in blood samples of subjects with T2DM. At present, it is not clear which mechanism(s) can explain the association between diastolic abnormalities and NLR. Therefore, the relationship between LVDD and activation of the immunoinflammatory system should be studied more thoroughly in the future, both on experimental and clinical grounds.

List Of Abbreviations

DCM: diabetic cardiomyopathy; T2DM: Type 2 diabetes mellitus; NLR: Neutrophil: lymphocyte ratio; CAD: coronary artery disease; TNF-a: tumor necrosis factor a; Gated-MPI: gated-myocardial perfusion imaging; ECG: electrocardiography; LVDD: left ventricular diastolic dysfunction; BMI: body-mass index; BNP: B-type natriuretic peptide; AST: alanine aminotransferase; ALT: glutamic oxaloacetic transaminase; BUN: urea nitrogen; Cr: creatinine; FBG: fasting-blood glucose; PBG: postprandial-blood glucose; LAD: left atrial diameter; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; IVSD: interventricular septal diameter; LVEF: left ventricular ejection fraction; LA mass: left atrial mass; E: peak early diastolic trans-mitral flow velocity; A: peak late diastolic trans-mitral flow velocity; e': peak early diastolic mitral annular velocity; OR: Odds ratio; ROC: receiver-operator characteristic; ALS: amyotrophic lateral sclerosis; AGR: albumin-to-gamma-glutamyl transferase ratio; T2DN: type 2 diabetic nephropathy

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional review committee of the Third Affiliated Hospital of Soochow University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The present study was approved by the Institutional Review Committee of the Third Affiliated Hospital of Soochow University. All subjects gave written, informed consent before enrollment in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

LT designed the study, analyzed data and drafted the manuscript. FH and XJ participated in the design of the study and coordination of the whole work. XH conducted animal procedures and prepared mouse blood samples. ZQ collected and organized patients' data. MX performed echocardiography of mice and patients. FZ performed Gated-MPI and analyzed the data. All authors have read and approved the manuscript and ensure that this is the case.

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Not applicable.

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Tables

Table 1. Participant characteristics by quartile of neutrophil: lymphocyte ratio (NLR) (n=507).

Participant characteristics	Quartiles of NLR (range)				P value
	Level 1 (<1.35) n=124	Level 2 (≥1.35 and <1.77) n=129	Level 3 (≥1.77 and <2.38) n=127	Level 4 (≥2.38) n=127	
Sex (male, %)	80 (64.52%)	79 (61.24%)	84 (66.14%)	70 (55.12%)	0.894
Age (years)	54.84 ± 8.16	54.84 ± 8.16	54.84 ± 8.16	54.84 ± 8.16	0.083
BMI (kg/m ²)	23.29 ± 3.43	23.64 ± 3.29	23.52 ± 3.87	23.64 ± 3.64	0.935
WHR	1.01 ± 0.91	0.93 ± 0.70	0.94 ± 0.06	0.93 ± 0.06	0.430
Duration of diabetes (years)	5.53 ± 4.68	6.47 ± 6.24	7.24 ± 5.35	7.79 ± 5.29	0.002*
FBG (mmol/L)	9.99 ± 3.03	9.58 ± 3.03	9.27 ± 2.90	9.86 ± 3.26	0.246
2-hour PBG (mmol/L)	13.80 ± 4.40	14.78 ± 5.45	13.57 ± 4.94	15.12 ± 4.96	0.023*
HbA1c (%)	9.41 ± 1.75	9.62 ± 2.23	9.80 ± 2.31	10.28 ± 2.51	0.026*
ALT (U/L)	23.43 ± 14.13	24.98 ± 26.39	21.97 ± 17.08	20.69 ± 13.64	0.268
AST (U/L)	23.35 ± 11.87	24.09 ± 25.71	21.51 ± 12.70	20.27 ± 11.75	0.250
BUN (mmol/L)	5.21 ± 1.30	5.25 ± 1.29	5.39 ± 1.46	5.32 ± 1.53	0.653
Cr (mmol/L)	69.33 ± 14.99	67.66 ± 13.07	69.93 ± 12.48	70.53 ± 13.45	0.506
BNP (ng/L)	33.19 ± 22.25	33.43 ± 27.71	35.52 ± 27.51	39.08 ± 25.71	0.102
CTNI (ng/mL)	0.0017 ± 0.002	0.0025 ± 0.006	0.0025 ± 0.004	0.0016 ± 0.002	0.637
CK (U/L)	74.75 ± 42.10	77.00 ± 43.86	65.44 ± 20.29	72.80 ± 35.44	0.540
CK-MB (U/L)	1.47 ± 0.99	1.48 ± 0.84	1.66 ± 2.33	1.41 ± 0.79	0.488
Myoglobin (ng/mL)	20.16 ± 11.30	19.45 ± 8.68	22.10 ± 11.47	19.47 ± 7.92	0.235
TC (mmol/L)	4.43 ± 1.09	4.63 ± 1.36	4.74 ± 0.94	4.89 ± 2.51	0.018*
TG (mmol/L)	1.84 ± 1.37	1.91 ± 1.37	2.38 ± 2.56	2.85 ± 1.90	0.039*
HDL (mmol/L)	1.10 ± 0.35	1.09 ± 0.36	1.03 ± 0.29	1.09 ± 0.32	0.190
LDL (mmol/L)	2.55 ± 0.80	2.72 ± 0.81	2.78 ± 0.62	2.82 ± 0.77	0.420
Hypoglycemic drug use (%)					
Sulfonylurea	24 (19.35%)	23 (17.83%)	24 (18.90%)	20 (15.75%)	0.741
Metformin	100 (80.65%)	105 (81.40%)	103 (81.10%)	103 (81.10%)	0.500
a-glucosidase inhibitor	104 (83.87%)	110 (85.27%)	112 (88.19%)	109 (85.83%)	0.764
GLP-1	18 (14.52%)	17 (13.18%)	19 (14.96%)	19 (14.96%)	0.636
DPP4 inhibitor	11 (8.87%)	12 (9.30%)	13 (10.24%)	13 (10.24%)	0.473
SGLT2 inhibitor	11 (8.87%)	13 (10.08%)	11 (8.66%)	12 (9.45%)	0.349
Insulin	88 (70.97%)	93 (72.09%)	91 (71.65%)	90 (70.87%)	0.523

The data are summarized as the mean ± SD for continuous variables or as a numerical proportion for categorical variables. BMI: body mass index; WHR: waist-to-hip ratio; FBG: fasting blood glucose; 2-hour PBG: 2-hour postprandial blood glucose; HbA1c: hemoglobin A1c; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BNU: urea nitrogen; Cr: creatinine; BNP: type B natriuretic peptide; CTNI: cardiac troponin I; CK: creatine kinase; TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein.

Table 2. Echocardiographic features of participants by quartile of neutrophil: lymphocyte ratio (NLR) (n=507).

Participant characteristics	Quartiles of NLR (range)				P value
	Level 1 (<1.35) n=124	Level 2 (≥1.35 and <1.77) n=129	Level 3 (≥1.77 and <2.38) n=127	Level 4 (≥2.38) n=127	
LA diameter	(1)	33.88 ± 3.35	33.81 ± 3.09	33.76 ± 3.25	0.918
LVEDD (mm)	45.63 ± 2.07	45.58 ± 2.82	45.99 ± 2.93	46.35 ± 3.48	0.143
LVEDS (mm)	29.71 ± 1.45	29.64 ± 1.55	29.96 ± 1.48	30.41 ± 2.37	0.002*
IVSD (mm)	8.30 ± 0.57	8.10 ± 0.55	8.38 ± 0.64	8.74 ± 1.18	<0.001*
EF (%)	64.58 ± 1.79	64.23 ± 1.55	64.43 ± 1.64	64.27 ± 2.24	0.418
E velocity(cm/s)	76.24 ± 14.66	75.43 ± 11.19	75.42 ± 13.99	76.30 ± 15.60	0.934
A velocity(cm/s)	66.31 ± 13.77	68.02 ± 15.17	75.92 ± 16.69	87.66 ± 12.91	<0.001*
E/A ratio	1.16 ± 0.14	1.13 ± 0.17	1.03 ± 0.23	0.88 ± 0.22	<0.001*
e' velocity(cm/s)	9.92 ± 1.66	9.69 ± 1.78	9.20 ± 1.96	8.32 ± 1.79	<0.001*
E/e' ratio	7.71 ± 0.99	7.86 ± 1.10	8.39 ± 1.56	9.29 ± 1.39	<0.001*

The data are summarized as the mean \pm SD for continuous variables or as a numerical proportion for categorical variables. LA: left atrial; LV: left ventricular; LVEDD: LV diameter in end diastolic; LVESD: LV diameter in end systolic; IVSD: interventricular septal diameter; LVEF: LV ejection fraction; E: the peak early diastolic trans-mitral flow velocity; A: the peak late diastolic trans-mitral flow velocity; e': the peak early diastolic mitral annular velocity.

Table 3. Adjusted relationships of quartiles of neutrophil: lymphocyte ratio (NLR) to diabetic cardiomyopathy (DCM) (n=507).

Adjusted relationships	Quartiles of NLR (range)				P value
	Level 1 (<1.35) n=124	Level 2 (≥ 1.35 and <1.77) n=129	Level 3 (≥ 1.77 and <2.38) n=127	Level 4 (≥ 2.38) n=127	
Number of DCM	2	9	16	18	-
Crude	1.00 (Ref)	4.57 (0.96-21.59)	8.79 (1.98-39.07)	10.01 (2.28-44.47)	$<0.001^*$
Model 1	1.00 (Ref)	5.01 (1.04-24.09)	10.29 (2.26-46.77)	13.43 (2.96-60.95)	$<0.001^*$
Model 2	1.00 (Ref)	5.47 (1.11-27.02)	11.59 (2.48-54.25)	16.16 (3.46-75.36)	$<0.001^*$
Model 3	1.00 (Ref)	8.02 (1.53-41.96)	12.55 (2.52-62.35)	14.32 (2.92-70.31)	$<0.001^*$

Model 1: age-, sex-, and BMI- adjusted;

Model 2: age-, sex-, BMI-, and duration of diabetes- adjusted;

Model 3: multiple adjusted, including age-, sex-, BMI-, duration of diabetes-, and lipid profile.

Figures

Figure 1

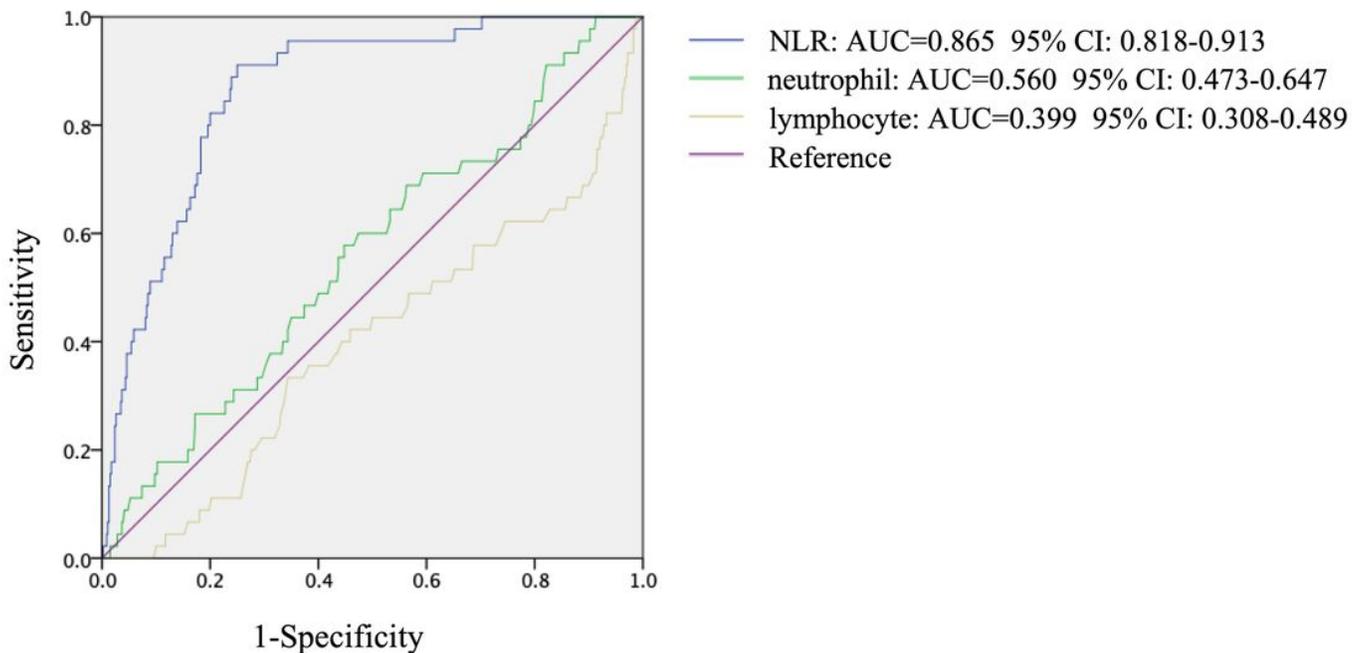


Figure 1

Receiver-operator characteristic (ROC) curve analysis of NLR, neutrophil and lymphocyte in type 2 diabetes with cardiac dysfunction versus type 2 diabetes without cardiac dysfunction.

Figure 2

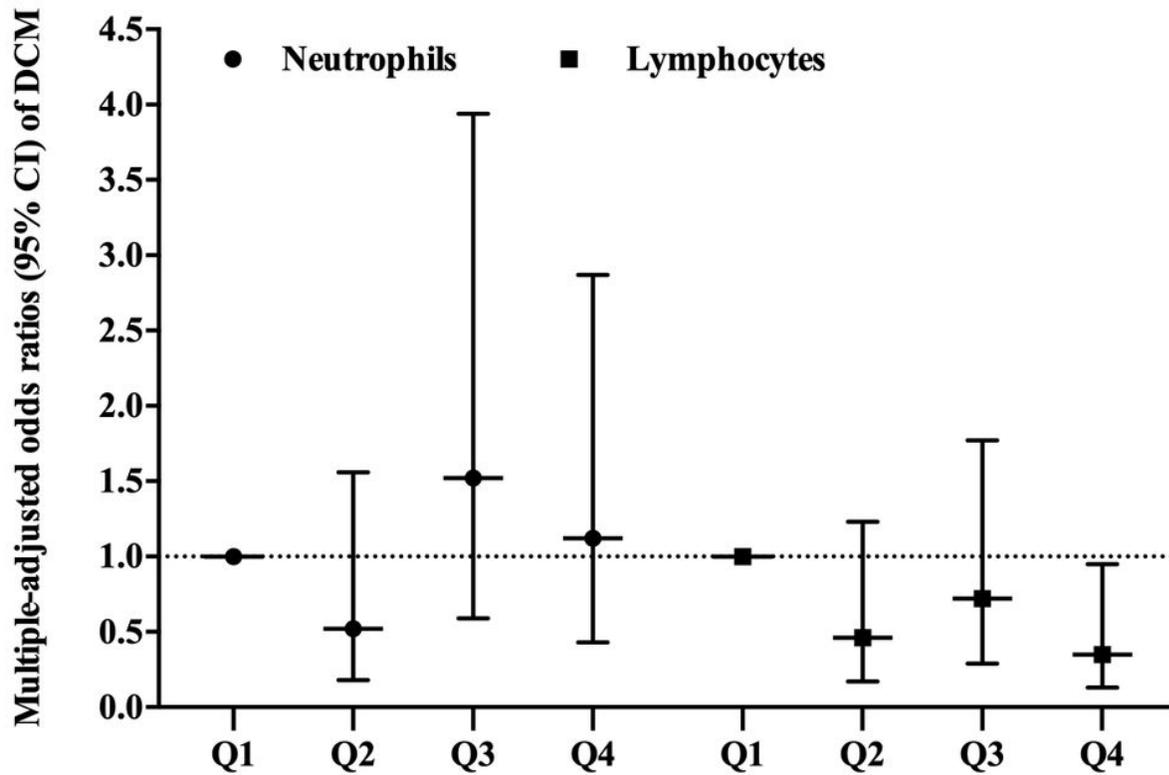


Figure 2

Adjusted Odds Ratio (OR) (95% CI) between the quartiles of neutrophil and lymphocyte counts and the incidence of subclinical DCM. Adjusted for age, sex, BMI, duration of diabetes and hyperlipidemia (total cholesterol ≥ 5.17 mmol/l, triglyceride ≥ 1.7 mmol/l, LDL ≥ 3.37 mmol/l).

Figure 3

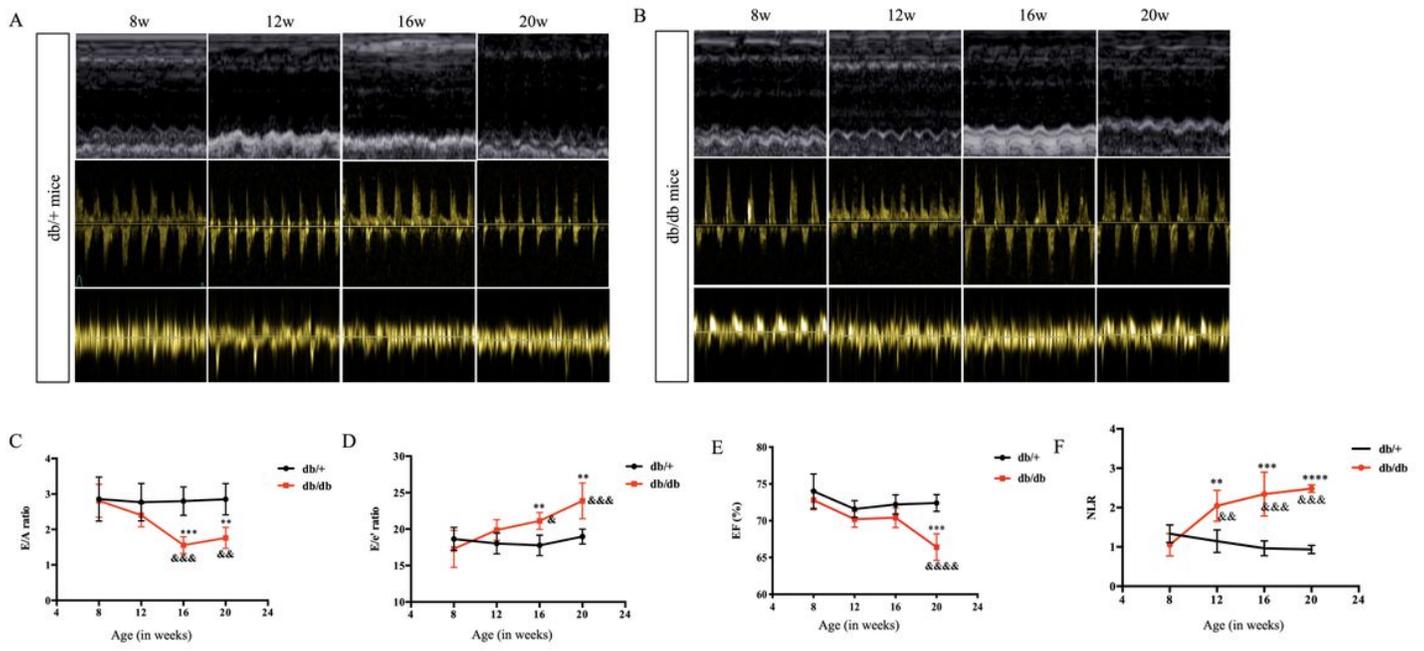


Figure 3

NLR and cardiac function in type 2 diabetic mice model. A-E. Systolic and diastolic function measured by echocardiography. F. NLR analysis in db/db mice versus db/+ mice at different age stages. * (&), $P < 0.05$, ** (&&), $P < 0.01$, *** (&&&), $P < 0.001$, **** (&&&&), $P < 0.0001$. *: db/+ versus db/db, &: db/db (12-weeks, 16-weeks, and 20-weeks of age) versus db/db (8-weeks of age).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalTable.pdf](#)
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