Relationship of neutrophil-to-lymphocyte ratio, in addition to C-reactive protein, with cardiovascular events in patients with type 2 diabetes

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ABSTRACT

Aim: To quantify the relationship of neutrophil-to-lymphocyte ratio (NLR) with cardiovascular events and all-cause mortality in patients with type 2 diabetes (T2D), independent of C-reactive protein (CRP).
Methods: Patients with T2D from the UCC-SMART-cohort were studied using multivariable-adjusted Cox regression. The relationship of NLR and CRP with vascular events (cerebrovascular events, myocardial infarction and vascular death) and all-cause mortality was quantified.
Results: During 10,833 person-years, 232 vascular events and 302 deaths occurred in 1,239 patients with T2D. Risk of vascular events and all-cause mortality increased per standard deviation (SD) in NLR (hazard ratio (HR) 1.27; 95 % confidence interval (CI):1.11–1.46) and 1.15; 95 % CI:1.02–1.30) after adjustment for CRP. CRP was not associated with vascular events after adjustment for NLR, (HR per SD 1.03; 95 % CI: 0.90–1.19), but was associated with all-cause mortality (HR per SD 1.18; 95 % CI: 1.04–1.33). Notably, NLR was related to vascular events in patients with CRP < 2 mg/L (HR per unit 1.45; 95 % CI: 1.19–1.77).
Conclusion: In patients with T2D, NLR is related to higher risk of CVD and all-cause mortality, independently from CRP. NLR is related to CVD even when CRP is low, indicating that NLR is a marker of CVD-risk in addition to CRP. Both NLR and CRP are independently related to all-cause mortality in T2D patients.

1. Introduction

On average patients with type 2 diabetes (T2D) are at high or very high risk of cardiovascular disease (CVD) and guidelines recommend optimizing glycemic control, low-density lipoprotein cholesterol (LDL-c) and blood pressure in these patients to prevent CVD [1]. In addition to a healthy lifestyle and traditional risk factor management, patients with T2D might also benefit from lowering low-grade inflammation to reduce CVD risk [2].

Low-grade inflammation is often quantified using serum C-reactive protein (CRP) levels, a protein produced by the liver in response to interleukin (IL)-6, which is downstream of the NLRP3 (nucleotide-binding domain and leucine-rich repeat protein-3) inflammasome IL-1β

pathway [3]. Activation of this pathway can be caused by adipose tissue dysfunction, which leads to the release of pro-inflammatory cytokines such as IL-1β and IL-6, and subsequently CRP production by the liver [3]. Inhibiting this pathway reduces future CVD events in patients with established CVD [4]. Another marker of low-grade inflammation is the neutrophil-to-lymphocyte ratio (NLR). NLR reflects the number of neutrophils relative to lymphocytes in a patient's bloodstream and thereby reflects the relationship between the innate and adaptive immune response [5]. NLR was first used as a marker of immune dysfunction in septic patients and has later been associated with CVD [5,6]. In the relationship with CVD, higher neutrophil counts are associated with an increased CVD risk and studies suggest that neutrophils are directly involved in the development of atherosclerosis and CVD [7,8]. In

Table 1
Baseline characteristics of patients with type 2 diabetes stratified by NLR.

	Overall	NLR		
		1st tertile (NLR < 1.7)	2nd tertile (NLR 1.7–2.4)	3rd tertile (NLR > 2.4)
	(N = 1,239)	(N = 413)	(N = 413)	(N = 413)
Age (years)	62 ± 9	60 ± 9	62 ± 9	64 ± 9
Sex (female)	346 (28)	123 (30)	110 (27)	113 (27)
Level of education				
Low	379 (31)	118 (29)	123 (30)	138 (33)
Medium	533 (43)	181 (44)	176 (43)	176 (43)
High	327 (26)	114 (28)	114 (28)	99 (24)
Alcohol consumption (units/week)				
0 – 10	921 (74)	311 (75)	305 (74)	305 (74)
11–20	205 (17)	66 (16)	69 (17)	70 (17)
>20	113 (9)	36 (9)	39 (9)	38 (9)
Smoking Status				
Never	330 (27)	116 (28)	112 (27)	102 (25)
Current	244 (20)	79 (19)	74 (18)	91 (22)
Former	665 (54)	218 (53)	227 (55)	220 (53)
Packyears	12 [0–30]	12 [0–31]	13 [0–29]	12 [0–29]
Physical activity (METh/week)				
1st tertile	413 (33)	137 (33)	136 (33)	140 (34)
2nd tertile	413 (33)	143 (35)	135 (33)	135 (33)
3rd tertile	413 (33)	133 (32)	142 (34)	138 (33)
History of CVD				
Absent	349 (28)	137 (33)	107 (26)	105 (25)
CAD	554 (45)	177 (43)	189 (46)	188 (46)
CeVD	117 (9)	34 (8)	41 (10)	42 (10)
PAD and/or AAA	71 (6)	24 (6)	27 (7)	20 (5)
>1 location	148 (12)	41 (10)	49 (12)	58 (14)
Hypertension	934 (75)	298 (72)	303 (73)	333 (81)
BMI (kg/m^2)	29.3 ± 4.7	29.2 ± 4.6	29.4 ± 4.6	29.2 ± 5.1
SBP (mmHg)	144 ± 20	143 ± 19	144 ± 20	146 ± 21
HbA1c (%, mmol/ mol)	6.7 [6.2–7.4] (50 [44–57])	6.7 [6.2–7.4] (50 [44–57])	6.7 [6.1–7.5] (50 [43–58])	6.6 [6.1–7.3] (49 [43–56])
Non-HDL-c (mmol/L)	3.1 [2.5–3.9]	3.1 [2.5–4.0]	3.1 [2.5–3.9]	3.1 [2.4–3.8]
eGFR (mL/min/ 1.73 m^2)	83 [68–97]	88 [74–99]	82 [68–96]	79 [62–94]
CRP (mg/L)	2.0 [1.0–4.4]	1.5 [0.8–3.2]	1.9 [1.0–4.4]	2.7 [1.4–5.7]

Baseline characteristics of patients included in the main analysis, stratified by NLR tertile. Data are presented as mean (standard deviation), count (percentage) or median [interquartile range]. CVD = (cardio)vascular disease. CeVD = cerebrovascular disease. PAD = peripheral artery Disease. AAA = abdominal aortic aneurysm. BMI = body mass index. SBP = systolic blood pressure. Non-HDL-c = non-high-density lipoprotein cholesterol. eGFR = estimated glomerular filtration rate calculated using the 2021 creatinine-based equations without race[22]. CRP = C-reactive protein.

patients with established vascular disease neutrophil counts are associated with increased CVD risk and in the general population genetically predicted neutrophil counts are causally related to ischemic heart disease [9,10]. Lymphocytes, on the other hand, are associated with a lower CVD risk, explaining the relationship between NLR and increased CVD risk [7,10]. Additionally, exploratory analyses suggest that NLR and neutrophil-associated proteins might be lowered by the anti-inflammatory drug colchicine, further explaining the potential link between NLR and CVD risk in patients with established CVD [11,12].

Inflammation is involved in the development of T2D and the relationship between inflammation and CVD in those with T2D might be different than in those without T2D [13,14]. Studies in patients with T2D have shown that CRP is related to all-cause mortality, CVD mortality, and potentially to (fatal and non-fatal) CVD events in patients with T2D [15,16]. A high NLR was related to CVD incidence in a subgroup of patients with T2D and coronary artery disease patients undergoing percutaneous coronary intervention [17]. Outside the acute setting, NLR is related to all-cause and CVD-mortality in T2D patients,

but this relationship was not adjusted for CRP [17]. CRP is only moderately correlated with NLR, suggesting NLR may reflect other inflammatory pathways leading to CVD than CRP [18]. The role of NLR as a marker of chronic inflammatory CVD risk in T2D patients, and its independent relevance alongside CRP, remains unclear. Understanding this relationship could identify T2D patients at very high CVD risk who might benefit from targeted anti-inflammatory therapies, thereby guiding future clinical trials and clinical practice. The aim of this study therefore is to quantify the relationship of NLR, independently from CRP, with CVD events and all-cause mortality in patients with T2D.

2. Methods

2.1. Study population

Patients with T2D were included from the Utrecht Cardiovascular Cohort – Second Manifestations of ARTerial disease (UCC-SMART) study. Patients with T2D who were enrolled between 2005 and 2020 were included in the study. T2D was defined as a referral diagnosis of T2D, self-reported T2D, usage of glucose-lowering medication upon inclusion or a fasting serum glucose of 7 mmol/L or higher at baseline. Patients with T2D can be included in UCC-SMART in two ways: based on the presence of T2D or based on the presence of manifest CVD and with T2D as a comorbidity. Patients with T2D are primarily included at the department of internal medicine, cardiology, cardiothoracic surgery, neurology and vascular surgery. CVD at baseline was classified as absent, coronary artery disease (CAD), cerebrovascular disease (CeVD), peripheral artery disease (PAD) and/or abdominal aortic aneurysm (AAA), or CVD at more than one location.

2.2. Data collection

Data in UCC-SMART were collected at baseline in an outpatient setting. Medical history was collected using standardized questionnaires on medical health, physical examination, standardized blood pressure measurements and laboratory testing [19]. Neutrophil and lymphocyte counts were retrieved from the Utrecht Patient Orientated Database (UPOD) [20]. Data on red and white blood cell count is automatically measured when hemoglobin is measured using the CELL-DYN Sapphire analyzer and has since 2005 been collected in UPOD. Hemoglobin is a standard baseline measurement in the UCC-SMART cohort and data on hematological parameters could therefore be retrieved for all patients that were included from 2005 onwards. CRP was measured using immunonephelometry (Nephelometer Analyzer BN II, Siemens, The Hague, The Netherlands) until 2013. From 2013 CRP has been determined using turbidimetry (Beckman Coulter, Brea, USA). Both assays measure CRP in the high-sensitivity range and are strongly correlated (r = 0.99) [21]. Measurements could therefore be pooled [19]. Data on events was collected using biannual questionnaires and adjudicated using hospital discharge letters and other medical correspondence by the three physicians of the SMART endpoint committee [19]. The combined vascular endpoint is a composite of cerebrovascular events, myocardial infarction, retinal infarction, and cardiovascular death (fatal myocardial infarction (MI), fatal stroke, sudden death, fatal ruptured aortic aneurysm and fatal heart failure) (Table S1). The other outcome of this study is mortality from any cause.

2.3. Data analyses

Baseline characteristics of normally distributed data are presented as mean (standard deviation), non-normally distributed data as median (interquartile range) and frequency data as absolute count (percentage). Missing data was 3 % or less for each variable and was imputed with single imputation using the aregImpute function from the Hmisc package in R (R Foundation for Statistical Computing, Vienna, Austria). Patients with a neutrophil or lymphocyte count above the 99th percentile
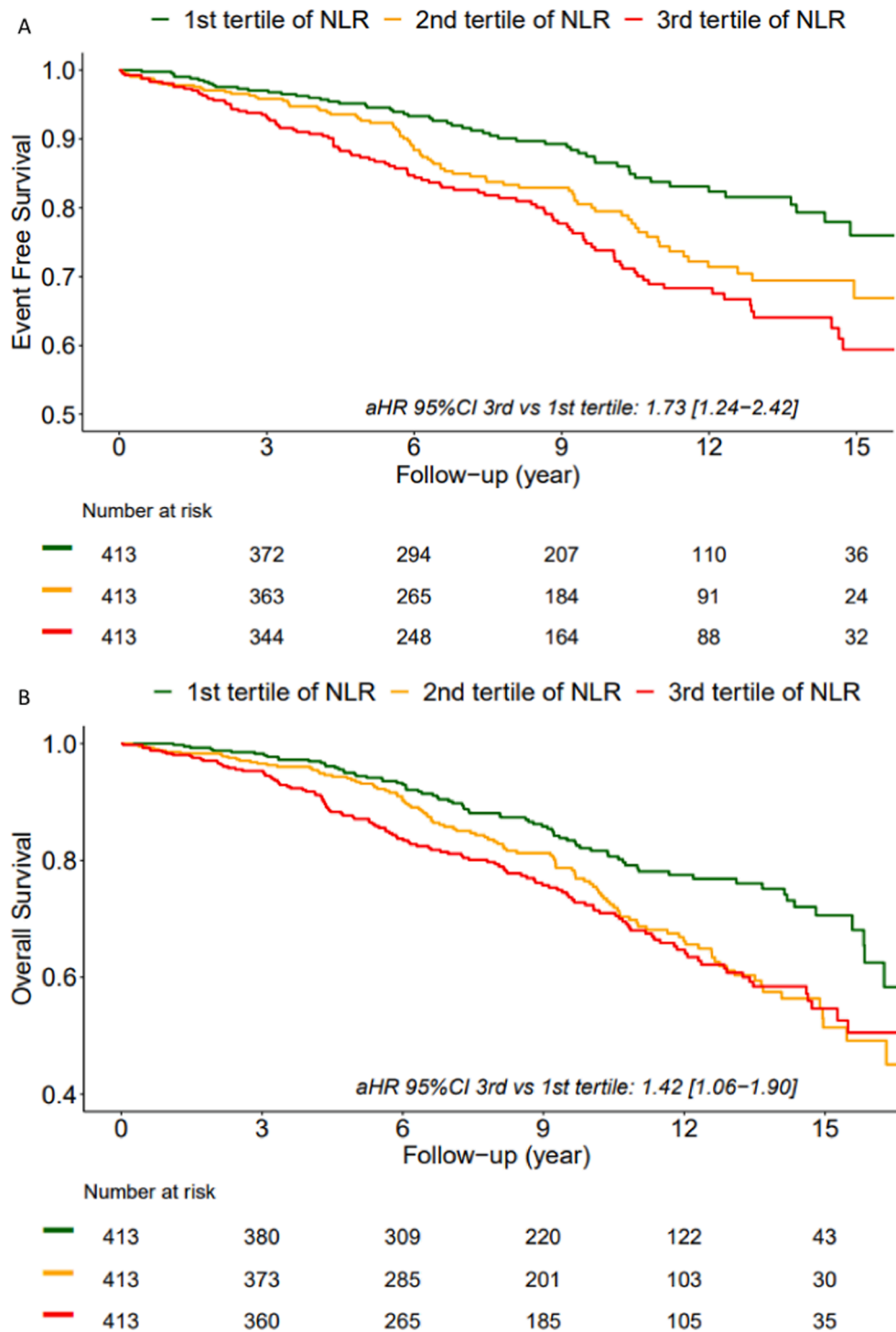
Fig. 1. **Vascular event-free (A) and overall (B) survival according to neutrophil-to-lymphocyte ratio (NLR) tertile (1st tertile < 1.7, 2nd tertile: 1.7–2.4, 3rd tertile > 2.4).** The relationship between NLR and the outcomes was adjusted for age, sex, level of education, units of alcohol, smoking status, packyears, physical activity and body mass index using Cox regression. The adjusted Hazard Ratio (HR) with 95 % confidence interval is displayed in the lower-right corner.

or below the 1st percentile were excluded from the analysis to exclude patients with an abnormal NLR due to acute inflammation or bone marrow disease. Similarly, patients with a CRP > 20 mg/L were excluded from the analyses to exclude patients with acute inflammation. Kaplan-Meier curves were plotted to describe the relationship of NLR in tertiles with the outcomes. The relationship of CRP and NLR (continuous and per SD after log-transformation) with CVD and mortality was assessed using Cox regression analyses. Three separate Cox regression analyses were performed: Model 1 was adjusted for age and sex. Model 2 was adjusted for the confounders in model 1 and for smoking (yes/no), packyears, alcohol use (glasses per week), level of education (low, middle, high) and physical activity level (tertiles). Model 3 (the exploratory model) was adjusted for model 2 and non-high-density-lipoprotein-cholesterol (non-HDL-c), systolic blood pressure (SBP), glycated haemoglobin (HbA1c), estimated glomerular filtration rate (eGFR), statin use and antiplatelets use, which were thought to be either confounder or mediators [22]. The interaction between CRP and NLR in relation with clinical outcomes was assessed with an interaction of the

Table 2

The relationship of NLR with all-cause mortality and cardiovascular events.

	Nr. of events/ person-years	HR [95 % CI]		
		1st tertile NLR (<1.7)	2nd tertile NLR (1.7–2.4)	3rd tertile NLR(>2.4)
Myocardial Infarction	110/9,396			
Model 1		1 [Reference]	1.37 [0.83–2.26]	1.70 [1.05–2.74]
Model 2		1 [Reference]	1.37 [0.83–2.26]	1.61 [0.99–2.60]
Model 3		1 [Reference]	1.28 [0.77–2.11]	1.51 [0.92–2.47]
Cerebrovascular event	67/10,538			
Model 1		1 [Reference]	1.39 [0.76–2.56]	1.28 [0.69–2.37]
Model 2		1 [Reference]	1.35 [0.73–2.48]	1.25 [0.67–2.33]
Model 3		1 [Reference]	1.20 [0.65–2.21]	1.13 [0.60–2.12]
Vascular Death	137/ 10,833			
Model 1		1 [Reference]	1.37 [0.87–2.16]	1.66 [1.07–2.56]
Model 2		1 [Reference]	1.41 [0.89–2.21]	1.65 [1.07–2.56]
Model 3		1 [Reference]	1.38 [0.87–2.17]	1.52 [0.98–2.37]
Combined vascular events	232/ 10,251			
Model 1		1 [Reference]	1.45 [1.03–2.05]	1.76 [1.26–2.46]
Model 2		1 [Reference]	1.44 [1.02–2.03]	1.73 [1.24–2.42]
Model 3		1 [Reference]	1.32 [0.93–1.87]	1.55 [1.10–2.18]
All-cause mortality	302/ 10,833			
Model 1		1 [Reference]	1.38 [1.03–1.86]	1.41 [1.06–1.89]
Model 2		1 [Reference]	1.41 [1.05–1.89]	1.42 [1.06–1.90]
Model 3		1 [Reference]	1.36 [1.01–1.83]	1.35 [1.01–1.82]

The relationship of NLR with vascular events and all-cause mortality was quantified using a Cox model. Model 1 was adjusted for age and sex. Model 2 was adjusted for model 1 + level of education, units of alcohol, smoking status, number of packyears, physical activity and BMI. Model 3 was adjusted for Model 2 + and non-high-density lipoprotein cholesterol, systolic blood pressure, glycated haemoglobin, estimated glomerular filtration rate, statin and antiplatelet use. Results are displayed as hazard ratio [95 % confidence interval].

continuous variables added to model 2 of the Cox regression. The relationship between NLR and CRP in patients with T2D was described by calculating Spearman's correlation coefficient. Finally, the relationship of NLR with CVD and all-cause mortality in strata of high and low CRP and the other way around (i.e. CRP in strata of NLR) was assessed using model 2. High CRP was defined as CRP $\geq$ 2 mg/L. High NLR was defined as NLR $\geq$ median (2.0). The proportional hazard assumption was checked visually by plotting the scaled Schoenfeld residuals against follow-up time. Non-linearity was assessed visually by plotting the Martingale residuals against NLR and CRP and formally by adding NLR and CRP as a restricted cubic spline function to the models. Several sensitivity analyses were performed to assess the robustness of the findings. Reverse causation was assessed by subsequently excluding patients with less than 1, 2 or 5 years of follow-up. Exploratory subgroup analyses were performed using model 2 in the following subgroups: age (<or $\geq$ 62.5 years), sex (male or female), BMI (<or $\geq$ 30 kg/m^2), anti-

platelet usage (yes or no), non-HDL-c (<or $\geq$ 3.4 mmol/L), hypertension (systolic blood pressure (<or $\geq$ 140 mmHg), chronic kidney disease (eGFR < 60 mL/min/1.73 m^2), HbA1c (<or $\geq$ 7 % (53 mmol/mol) and type of CVD at inclusion (CAD, CeVD, PAD and/or AAA). After Bonferroni correction for multiple testing, at 5 % significance levels with 11 subgroups, a P-for-interaction < 0.0045 was considered statistically significant. Lastly, separate Cox regressions were performed using model 2 including only neutrophil count and only lymphocyte count as independent variables.

3. Results

3.1. Baseline characteristics

A total of 1,239 patients with T2D were included. Mean age was 62 $\pm$ 9 years and 28 % of patients were female (Table 1). Patients had a mean BMI of 29.3 $\pm$ 4.7 kg/m^2 and a median HbA1c of 6.7 % (50 mmol/mol) [interquartile range (IQR):6.2–7.4 (44–57 mmol/mol)]. The median NLR was 2.0 [IQR:1.5–2.7] (supplemental Fig. 1A) and the median CRP was 2.1 [IQR:1.0–4.4] mg/L (supplemental Fig. 1B).

3.2. Relation of NLR and CRP with clinical endpoints

During a follow-up of 10,833 person-years, 232 combined vascular events and 302 deaths occurred (Fig. 1). After adjustment for confounders, NLR was related to an increased risk of combined vascular events (hazard ratio (HR) 3rd vs 1st tertile 1.73 [95 % confidence interval (CI) 1.24 – 2.42] and HR per SD increase 1.28 [95 % CI:1.12–1.47] Tables 2 and S4) and all-cause mortality (HR 3rd vs 1st tertile: 1.42 [95 %CI:1.06 – 1.90] and HR per SD increase 1.18 [95 % CI:1.05–1.33], Tables 2 and S3). The hazard ratio of CRP for combined vascular events was (HR 3rd vs 1st tertile: 1.26 [95 %CI:0.90–1.77] and HR per SD increase 1.08 [95 %CI:0.94–1.24], Table S3-S4). CRP was related to all-cause mortality (HR 3rd vs 1st tertile 1.47 [95 % CI:1.08–1.99] and HR 1.21 [95 %CI:1.07–1.36] per SD increase after log-transformation, Table S3-S4).

3.3. Independent relation of NLR and CRP with clinical endpoints

Additional adjustment for log-CRP did not affect the relationship between NLR and combined vascular events or all-cause mortality (HR per SD after log-transformation 1.27 [95 %CI:1.11–1.46] and 1.15 [95 % CI:1.02–1.30] respectively, table S4). After additional adjustment for NLR, the relationship between CRP and combined vascular events was attenuated (HR per SD after log-transformation 1.03 [95 % CI:0.90–1.19]). The relationship of CRP with all-cause mortality did not meaningfully change after adjustment for NLR (HR per SD after log-transformation 1.18 [95 %CI:1.04–1.33], table S4).

3.4. Strata-specific relationships of NLR and CRP with clinical endpoints

Analyses in strata showed that NLR was related to an increased risk of combined vascular events in both patients with a high and a low CRP (HR 1.16 [95 %CI:0.97–1.38] and 1.45 [95 %CI:1.19–1.77] per unit increase, respectively, Table 3). NLR was related to all-cause mortality in patients with a high and low CRP (HR 1.17 [95 %CI:1.01–1.36]; HR 1.17 [95 %CI:0.96–1.44] per unit increase, respectively, Table 3). CRP was not related to the risk of combined vascular events in patients with a high or low NLR (HR 1.02 [95 %CI:0.96–1.08] and 0.99 [95 % CI:0.94–1.03], respectively, Table 3). There was no or little effect of CRP on all-cause mortality in patients with a high or low NLR respectively (HR 1.04 [95 %CI:1.00–1.07] and 1.03 [95 %CI:0.98–1.08], Table 3).

3.5. Relationship of NLR and CRP combined with clinical endpoints

Patients with a low NLR and low CRP were at the lowest risk of

Table 3

The continuous relationship of the inflammatory markers with cardiovascular events and all-cause mortality in patients in strata of inflammatory levels.

Determinant	Stratum	Events (N)	Patients (N)	HR [95 % CI]	P for interaction
Combined vascular events					
NLR	Low CRP	107	605	1.45 [1.19–1.77]	0.15
NLR	High CRP	125	634	1.16 [0.97–1.38]	
CRP	Low NLR	92	628	1.02 [0.96–1.08]	0.52
CRP	High NLR	140	611	0.99 [0.94–1.03]	
All-cause mortality					
NLR	Low CRP	128	605	1.17 [0.96–1.44]	0.73
NLR	High CRP	174	634	1.17 [1.01–1.36]	
CRP	Low NLR	127	628	1.03 [0.98–1.08]	0.47
CRP	High NLR	175	611	1.04 [1.00–1.07]	

The relationship of NLR and CRP with CVD and all-cause mortality was analysed using a Cox model. Results from a Cox regression adjusted for age and sex, level of education, units of alcohol, smoking status, physical activity and BMI (model 2) and displayed as hazard ratio [95 % confidence interval]. Patients with a CRP > 20 mg/L were excluded. Low CRP was defined as CRP < 2 mg/L. High CRP as CRP $\geq$ 2 mg/L. Low NLR was an NLR < 2.0. High NLR was defined as NLR $\geq$ 2.0. CRP = c-reactive protein. NLR = neutrophil-to-lymphocyte ratio.

combined vascular events (Fig. 2). Patients with a high CRP and low NLR had a trend towards a higher risk (HR 1.24 [95 %CI: 0.82–1.89] (Fig. 2). Patients with a high NLR were at the highest risk of combined vascular events regardless of high or low CRP (HR 1.69 [95 %CI:1.16–2.46] and 1.63 [95 %CI:1.11–2.41], respectively, Fig. 2). For mortality, patients with a low NLR and a low CRP were at the lowest risk (Fig. 2). Patients with either a high NLR or a high CRP had a trend towards a higher risk (HR 1.25 [95 %CI:0.88–1.78] and 1.36 [95 %CI:0.95–1.94], respectively, Fig. 2). Patients with both a high NLR and CRP were at highest risk of mortality (HR 1.71 [95 %CI:1.23–2.36], Fig. 2).

3.6. Sensitivity analyses

Excluding patients with less than 1, 2 and 5 years of follow-up did not change the results (Table S8). Similarly, the addition of CVD type as a confounder to models one to three did not change the effect estimates (Table S9). Using a stricter definition of combined vascular events (including MI, stroke and CVD-mortality and without retinal infarction) resulted in 228 instead of 232 events and did not change the results (Table S10). Exploratory subgroup analyses did not reveal any significant differences in the effect of NLR and/or CRP on combined vascular events within subgroups (age, sex, type of CVD, BMI, hypertension, chronic kidney disease, baseline HbA1c or non-HDL-c levels or anti-platelet usage, p > 0.0045 after Bonferroni correction, Tables S11-S14). However, effect estimates suggest that the relationship with vascular events might be stronger in those with CKD than without CKD (HR per unit increase 1.34 (95 %CI:1.14–1.57) and that the relationship with all-cause mortality and vascular events might be stronger in those with Hba1c < 7 % compared to $\geq$ 7 % (HR per unit increase 1.27 (95 % CI: 1.09–1.49). Cox regressions including only neutrophil count or only lymphocyte count, showed that neutrophil count was related to an increased risk of combined vascular events and all-cause mortality (HR 1.12 [95 %CI: 1.02–1.23] and 1.17 [95 %CI: 1.08–1.27] per unit increase respectively, Table S5), while lymphocyte count was associated with a trend towards a lower risk of combined vascular events and was not significantly related to all-cause mortality (HR 0.86 [95 %CI: 0.70–1.07] and 1.09 [95 %CI:0.91–1.30], respectively, Table S5).

4. Discussion

In patients with T2D, NLR is related to an increased risk of CVD, even after adjustment for CRP. Furthermore, NLR is related to CVD in T2D patients with a high CRP and, importantly, a low CRP. Both NLR and CRP are independently related to all-cause mortality in T2D patients. The highest risk of all-cause mortality is observed in patients with a high CRP and a high NLR.

The present study demonstrates that NLR is related to an increased risk of CVD in patients with T2D, independently from CRP. This is the first study that describes and compares the relationship of NLR and CRP with CVD in patients with T2D. A study in patients with CAD also performed stratified analyses in CAD patients with T2D. In that study, a low NLR was related to a lower risk of CVD compared to a high NLR after adjustment for CRP (HR for NLR < 2.85; 0.77 [95 %CI: 0.61–0.97]) [17]. Furthermore, ln(NLR) was associated with a trend towards an increase in CVD events (HR 1.24 [95 %CI: 0.99–1.54]) [17]. This is in line with the present findings, although the present study found stronger associations. These differences might be because in the CAD study, NLR was measured during admission for percutaneous coronary intervention and might therefore represent acute inflammation after an intervention, rather than chronic low-grade inflammation. Data from trial patients with established CVD (primarily MI) and/or residual inflammatory risk, showed that the relation between NLR and CVD was present even after adjustment for CRP in patients with established CVD [18]. Overall, the existing evidence is in line with the finding that both NLR and CRP are associated with CVD in T2D patients, and that the relationship of NLR with CVD might be independent of CRP. Interestingly, the present study shows that NLR was related to CVD even in T2D patients with low CRP (<2 mg/L). This is the first study that shows that NLR is a relevant inflammatory marker for T2D patients, even when CRP is low. Additionally, results from the present study indicate there is only a modest relationship between CRP and CVD risk in patients with T2D. This is in line with a previous study in patients with T2D, which found that each 1 SD increase in CRP was associated with a small, not statistically significant trend towards a higher risk of macrovascular events, consisting of CVD-mortality, MI and stroke (HR 1.09 [95 %CI:0.99–1.19]) [15]. This effect size is comparable to the effect sizes found in the present study for CRP. Furthermore, the present study found that the relationship between CRP and CVD risk in T2D patients was not independent of NLR and adjusting for NLR attenuated the relationship of CRP with CVD. These results suggest that NLR is the inflammatory marker that is more strongly related to CVD risk than CRP in patients with T2D.

In the present study, NLR is also related to all-cause mortality amongst T2D patients. This is consistent with results from two previous studies in patients with CAD, in which the relationship of NLR with mortality for patients with CAD and T2D was described [17,23]. In CAD patients with T2D, high relative to low NLR was related with (a trend towards) increased risk of all-cause mortality [17,23]. Additionally, the present study confirms a significant relationship between CRP and all-cause mortality, with effect sizes consistent with a previous study in T2D patients (HR 1.14 [95 %CI:1.04–1.26] per SD).[15] However, previous studies did not take NLR into account. In the present study, both NLR and CRP are independently associated with mortality and the highest risk was observed in patients with a high CRP and a high NLR. Additionally, T2D patients with a low CRP but a high NLR might still be at increased risk of mortality. The present study therefore adds that NLR is a relevant marker of mortality risk in T2D patients, in addition to CRP
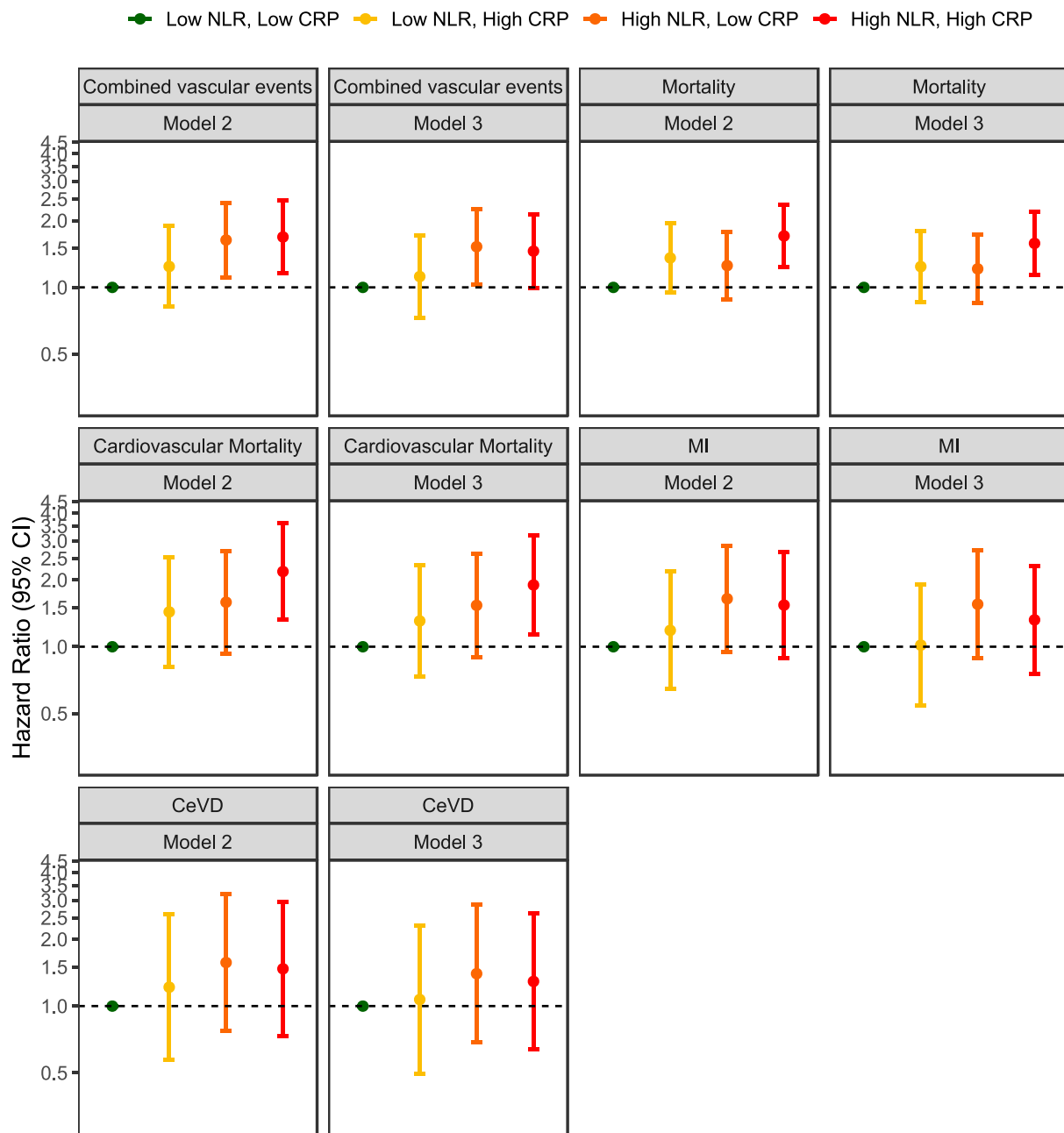
Fig. 2. The relationship of inflammatory status with cardiovascular events and all-cause mortality. Inflammatory status is defined by CRP (low < 2 or high ≥ 2 mg/L) and NLR (low < 2.0 or high > 2.0). Results are adjusted for age and sex, level of education, units of alcohol, smoking status, physical activity and body mass index (model 2) and additionally for systolic blood pressure, glycated haemoglobin, non-high-density lipoprotein cholesterol, estimated glomerular filtration rate, statin and antiplatelet use (Model 3). Hazard ratio's and confidence intervals are provided numerically in Table S7. NLR = neutrophil-to-lymphocyte ratio. CRP = C-reactive protein. MI = myocardial infarction. CeVD = cerebrovascular disease.

and across multiple levels of CRP.

The interesting finding that NLR is related to an increased CVD risk in T2D patients with a CRP below 2 mg/L, suggests that CRP and NLR might only partially reflect the same inflammatory pathway. This may be a plausible explanation since previous studies have described that neutrophils in adipose tissue release a broad range of inflammatory cytokines [8]. Some of these, like interleukin-1 or tumor necrosis factor-alpha, will lead to an increase in serum CRP, but others might for instance attract macrophages and not directly contribute to increased CRP levels [8]. In line with that, studies report that neutrophils are also thought to be directly involved in the formation of atherosclerosis, plaque destabilization and plaque erosion [8]. Furthermore, neutrophils

are recruited to the site of ischemia in case of myocardial infarction or stroke. Here they can have pro-inflammatory functions and lead to tissue infiltration by, amongst others, macrophages after ischemic tissue damage [8]. Similarly, certain lymphocytes might also be involved in the development of CVD via multiple pathways. For instance, the upregulation of regulatory T-lymphocytes is now being investigated to promote tissue healing and outcomes after myocardial infarction [8,24]. NLR therefore represents another set of inflammatory pathways in addition to CRP and can be of similar relevance in the context of inflammatory CVD risk for patients with type 2 diabetes.

The implications of these findings are twofold. First, a higher NLR is related to a higher risk of cardiovascular disease and all-cause mortality

in patients with T2D, which is important given that anti-inflammatory therapy, such as canakinumab and colchicine, can potentially reduce NLR and decrease cardiovascular risk [11,18,25]. A post-hoc analysis of the CANTOS trial, showed that canakinumab (an IL-1 β inhibitor) lowered the incidence of major adverse cardiac events (nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death) in patients with previous MI and a CRP $\geq$ 2 mg/L in a dose-dependent manner. Interestingly, canakinumab simultaneously lowered NLR in a dose-dependent manner [18]. Similarly, colchicine, which reduces incident CVD, also reduced NLR in a trial in 40 patients with metabolic syndrome [11,25]. However, most trials with anti-inflammatory therapy have been conducted in patients with myocardial infarction and not primarily T2D. One meta-analysis that included T2D subgroups from randomized controlled trials with anti-inflammatory therapy (four with colchicine and one with canakinumab) showed that anti-inflammatory therapy is associated with a reduced risk of CVD in patients with T2D [2]. Together, these results suggest that in patients with T2D and a high NLR, CVD risk might be lowered by lowering NLR.

Secondly, results from this study suggest that CRP and NLR could be used in conjunction to quantify inflammatory risk in T2D patients. The present study shows that a high NLR in a low CRP setting is associated with an increased CVD risk. NLR is an affordable and readily available biomarker across multiple settings. Measuring NLR in addition to CRP could therefore be easily implemented in clinical practice. If the efficacy of anti-inflammatory therapy in patients with T2D becomes more clear, both CRP and NLR might be relevant markers for identifying T2D patients with high inflammatory risk. Furthermore, T2D patients could be selected for (future trials with) anti-inflammatory therapies such as colchicine, based on a high NLR and irrespective of CRP status. Lastly, NLR might be an interesting prognostic risk factor in CVD risk prediction models in addition to (or instead of) CRP.

Some strengths and limitations of this study should be considered. Strengths are the use of a prospective cohort design, which establishes a temporal relationship between NLR and CVD occurrence. Furthermore, NLR was measured in an outpatient clinic setting instead of during admission, thus ensuring that the measurement reflects chronic low-grade inflammation instead of acute inflammation during hospitalization. Furthermore, where previous studies primarily included patients with CAD, this study also included patients with type 2 diabetes without established CVD and with other types of CVD such as cerebrovascular disease and peripheral artery disease. Limitations of this study are, first, that residual confounding cannot be entirely excluded. For instance, no data was available on chronic obstructive pulmonary disease (COPD) status, which is related to an increased risk of CVD and might potentially lead to an increased inflammatory state [26]. However, all analyses were adjusted for smoking status, which is closely related to COPD development. A second potential limitation is that NLR was measured only once. Currently, there is limited knowledge on factors influencing NLR and it is unknown if NLR is a stable long-term determinant, although the previously discussed post-hoc analysis of trials in high-risk CVD patients showed that NLR remained relatively stable in control groups during 4 years of follow-up [18].

To conclude, NLR is related to CVD in patients with T2D, independently from CRP. Both NLR and CRP are independently associated with all-cause mortality. NLR is associated with CVD in both T2D patients with high CRP and low CRP. These results indicate that NLR might be a relevant marker of low-grade inflammation, even when CRP is low. Measuring NLR in addition to CRP might aid in identifying T2D patients at high CVD risk who could benefit from anti-inflammatory therapy.

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CRediT authorship contribution statement

Lukas L.F. Hoes: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Niels P. Riksen:** Writing – review & editing, Conceptualization. **Johanna M. Geleijnse:** Writing – review & editing, Funding acquisition. **Mark C.H. de Groot:** Writing – review & editing, Data curation. **Yvonne T. van der Schouw:** Writing – review & editing, Conceptualization. **Frank L.J. Visseren:** Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Charlotte Koopal:** Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2024.111727.

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