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The role of C-reactive protein to lymphocyte ratio in NAFLD and mortality among NAFLD patients

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Abstract

Background Non-alcoholic fatty liver disease (NAFLD) is recognized as the predominant chronic liver disorder globally. Inflammation is integral to the onset and progression of NAFLD. The C-reactive protein to lymphocyte ratio (CLR), a novel inflammatory marker, has yet to be explored in the context of NAFLD.

Method This investigation encompassed 4371 individuals from the National Health and Nutrition Examination Survey (NHANES) conducted between 2015–2018. Weighted logistic regression was employed to examine the correlation between CLR and NAFLD. Weighted Cox proportional hazards models were utilized to evaluate the association between CLR and all-cause and Cardiovascular disease (CVD) mortality in patients with NAFLD. Restricted cubic spline (RCS) curves were employed to assess the dose–response relationship. Threshold effect analysis was used to determine the existence of an inflection point.

Result After adjusting for all included covariates in Model 3**,** a positive correlation between lnCLR and NAFLD was identified (OR = 1.45, 95% CI = 1.16–1.81, $P = 0.010$). However, no significant association was observed between it and all-cause as well as CVD mortality among patients with NAFLD. The RCS curve illustrated a nonlinear association between CLR and NAFLD (P-nonlinear < 0.0001). Threshold effect analysis determined that the inflection point occurs at CLR = 1.667.

Conclusion CLR exhibited a nonlinear positive association with NAFLD. Higher CLR levels may increase the risk of NAFLD. However, CLR does not affect all-cause and CVD mortality in patients with NAFLD.

Keywords NAFLD, CLR, NHANES, All-Cause Mortality, CVD mortality

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Introduction

Non-alcoholic fatty liver disease (NAFLD), marked by excessive hepatic fat accumulation, is the most prevalent chronic liver disease worldwide, with its incidence steadily increasing, posing a significant threat to global public health [1]. NAFLD is closely associated with complex metabolic disturbances and can progress through chronic inflammatory processes, thereby fostering and sustaining a pro-tumorigenic environment that contributes to the development of hepatocellular carcinoma [2]. Moreover, innate immune activation has been identified as a critical factor in triggering and exacerbating hepatic inflammation in NAFLD [3]. Consequently, inflammation and

immune responses play pivotal roles in the pathogenesis of NAFLD. Elucidating the relationship between inflammatory or immune factors and NAFLD is of paramount importance for its prevention and management.

C-reactive protein (CRP) is a well-documented inflammatory biomarker frequently utilized to assess infection and inflammatory states, while lymphocytes serve as a key component of the immune system. The ratio of C-reactive protein to lymphocytes (CLR), as a composite indicator, integrates the effects of both markers and represents a novel inflammatory biomarker with potential utility in predicting disease prognosis and aiding diagnostic evaluations [4]. CLR has demonstrated significant value in conditions such as COVID-19, inflammatory diseases, and various cancers [5–7]. However, there is no studies to date have explored the relationship between CLR and NAFLD.

Recognizing the critical involvement of inflammation and immune responses in the development of NAFLD, we undertook a cross-sectional investigation aimed at elucidating the relationship between CLR and NAFLD in adults residing in the United States. Furthermore, we sought to analyze the correlation between CLR and all-cause mortality as well as cardiovascular disease (CVD) mortality in individuals diagnosed with NAFLD through a prospective cohort study. Ultimately, this study aims to establish the significant value of CLR in NAFLD.

Methods

Study population

The National Health and Nutrition Examination Survey (NHANES) is a comprehensive, long-term epidemiological study focusing on non-institutionalized residents of the United States. This study employs a stratified, multistage probability sampling technique to gather baseline information and evaluate health status (https://wwwn.cdc.gov/nchs/nhanes/default.aspx). Given that the NHANES program has received approval from the Ethics Review Board of the National Center for Health Statistics (NCHS), and the dataset is publicly available, there was no necessity for additional ethical clearance.

This study analyzed publicly available NHANES data from 9,971 participants in the 2015–2016 cycle and 9,254 participants in the 2017–2018 cycle. Participants were excluded sequentially based on the following criteria: 1) incomplete Fatty Liver Index (FLI) data; 2) age below 20 years; 3) positive for hepatitis B surface antigen, hepatitis C antibody, or hepatitis C RNA; 4) autoimmune hepatitis; 5) significant alcohol consumption; 6) incomplete CLR data. Ultimately, a total of 4371 participants were included in the analysis (Fig. 1). Additionally, follow-up and mortality status data were obtained from the National Death Index records until December 31, 2019 (https://www.cdc.gov/nchs/data-linkage/mortality.htm), to evaluate the influence of CLR on all-cause mortality and CVD mortality among individuals with NAFLD.

Measurement and calculation of CLR

In the NHANES study, CRP levels were quantified using a highly sensitive two-reagent immunoturbidimetric approach at the Advanced Research and Diagnostic Laboratory of the University of Minnesota, which employed high-sensitivity near-infrared particle immunoassay rates, (https://wwwn.cdc.gov/nchs/data/nhanes/2017-2018/labmethods/HSCRP-J-MET-508.pdf). Lymphocyte counts were determined using the Beckman Coulter methodology for cell counting and sizing, while the WBC differential analysis employed VCS technology (https://wwwn.cdc.gov/nchs/data/nhanes/public/2017/labmethods/CBC-J-MET-508.pdf). The CLR was computed as the ratio of CRP (mg/L) to lymphocyte number (1000 cells/μL).

Definition of NAFLD

For participants from 2015–2018, FLI was utilized to evaluate the presence of hepatic steatosis. The FLI calculated as follows:

$$\mathrm{FLI} = \frac{e^{L}}{1+e^{L}} \times 100$$

$$L = 0.953 \times 1n(\mathrm{TG}) + 0.139 \times \mathrm{BMI} + 0.718 \times 1n\,(\mathrm{GGT}) + 0.053 \times \text{Waist Circumference} - 15.745$$

In this formula, triglycerides (TG) were measured in mg/dL, body mass index (BMI) in kg/m^2, γ-glutamyltransferase (GGT) in U/L, and waist circumference in centimeters. According to previous studies, an FLI ≥ 60 was used to define hepatic steatosis [8]. Building on this, NAFLD was further defined as FLI ≥ 60 after excluding cases of viral hepatitis, autoimmune hepatitis, and significant alcohol consumption (defined as more than 3 drinks per day for men or 2 drinks per day for women, with one drink equivalent to 14 g of pure alcohol [9]).

Assessment of covariates

Based on existing literature and clinical relevance, we assess the following covariates relevant to NAFLD. This study incorporated demographic variables such as age, gender, race, family income-to-poverty ratio (PIR), and educational level. Age classifications were made into three categories: ≤ 39 years, 40–60 years, and > 60 years. PIR was also divided into three groups: < 1.0, 1.0–3.0, and > 3.0. Educational level was categorized into three groups: below high school, high school and above. Anthropometric and laboratory covariates included BMI, aspartate aminotransferase (AST), alanine

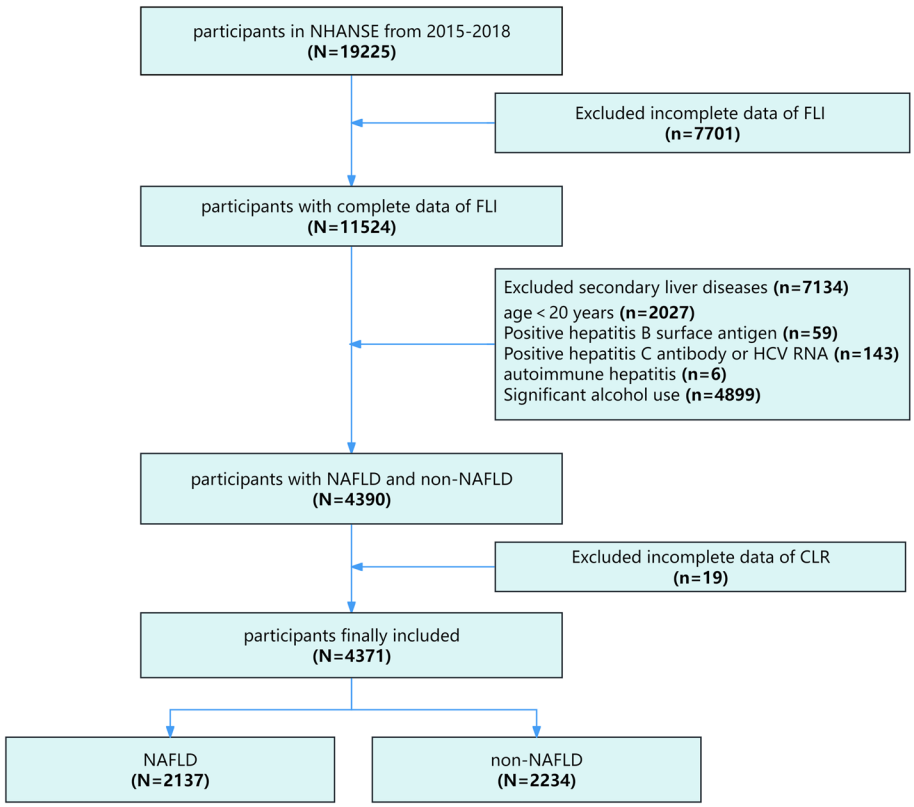

Fig. 1 Flowchart illustrating selection of the study population in NHANES from 2015 to 2018

aminotransferase (ALT), and uric acid (UA). BMI was classified into four groups: < 18.5, 18.5–24.9, 25.0–29.9, and ≥ 30. Medical history covariates included hypertension, diabetes, dyslipidemia, CVD, smoking status and physical activity. Hypertension was determined based on the average of three resting blood pressure measurement, with systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 80 mmHg, incorporating self-reported hypertension from participants or currently receiving antihypertensive medication treatment [10]. Diabetes was defined through a history of prior diabetes (self-reported), HbA1c levels ≥ 6.5%, fasting blood glucose levels ≥ 126 mg/dL, or currently receiving antidiabetic medications or insulin therapy. Dyslipidemia was defined as meeting at least one of the following criteria: a total cholesterol concentration ≥ 200 mg/dL, low-density lipoprotein cholesterol (LDL-C) ≥ 130 mg/dL, triglycerides ≥ 150 mg/dL, or high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL. In addition, self-reported hypercholesterolemia was also considered as dyslipidemia [11]. CVD was defined based on self-reported physician diagnoses obtained through a standardized health status questionnaire administered during personal interviews. Participants were asked: "Has a doctor or other health professional ever told you that you have congestive heart failure, coronary heart disease, angina, myocardial infarction, or stroke?" Individuals who answered "yes" to any of these conditions were classified as having CVD. Specifically, congestive heart failure, myocardial infarction, angina, and coronary heart disease were defined according to the corresponding individual questions [12]. Smoking status was categorized into three groups: never smoked (defined as less than 100 cigarettes in a lifetime), currently smoking (defined as ≥ 100 cigarettes in a lifetime), and previously smoked (defined as ≥ 100 cigarettes and had quit smoking). Physical activity (PA) was assessed using the self-reported physical activity questionnaire from NHANES and categorized based on the recommendations of the Physical Activity Guidelines for Americans. All participants were classified into three levels of PA intensity: inactive (< 600 MET-min/week), moderately active (600–1200 MET-min/week), and highly active (> 1200 MET-min/week) [13, 14].

Statistical analysis

To maximize the sample size, multiple imputation was performed for covariates with missing data using the "mice" R package. Regarding the statistical analysis, the overall population was segregated into NAFLD and non-NAFLD groups, with CLR categorized into quartiles.

Categorical variables were expressed as numbers (percentages) and compared between groups utilizing the weighted χ^2 test with Rao and Scott second-order correction. Continuous variables were presented as means with standard errors (SE) and compared between groups using the weighted Kruskal–Wallis test. Weighted binary logistic regression models were deployed to compute odds ratios (ORs) and 95% confidence intervals (CIs) to access the association between CLR and NAFLD. To investigate the link between CLR and all-cause mortality as well as CVD mortality among the NAFLD population, Weighted Cox proportional hazards regression models were utilized to ascertain hazard ratios (HRs) and 95% CIs. Person-time was quantified from the date of the NHANES interview to either the date of death or the end of follow-up (December 31, 2019), whichever occurred first. Survival rates across groups were compared using Kaplan–Meier curves. Three analytical models were established in this study: Model 1 was unadjusted for any covariates. Model 2 was adjusted for age, gender, and race. And model 3 additionally adjusted for PIR, educational level, BMI, ALT, AST, UC, hypertension, diabetes, dyslipidemia, CVD, smoking status and physical activity.

Additionally, a series of sensitivity analyses were conducted, employing four-node restricted cubic spline (RCS) to investigate the dose–response relationship between CLR and NAFLD, as well as all-cause mortality and CVD mortality in NAFLD. A log-likelihood ratio test was performed by comparing standard logistic regression and two-piecewise logistic regression to examine the presence of threshold effects. Finally, subgroup analyses were performed to ascertain the consistency of the association between CLR and NAFLD, as well as mortality in NAFLD patients across various subgroups. Furthermore, to further validate the robustness of our results, we conducted two additional sets of sensitivity analyses. First, we defined metabolic dysfunction-associated steatotic liver disease (MASLD), a new concept alternative to NAFLD, using the USFLI index and performed a correlation analysis with data from 2015–2018. Second, we conducted a further correlation analysis using VCTE data from 2017–2020.

All statistical analyses carried out in this study were performed using R (version 4.4.1). A two-tailed test was employed, and a p-value of < 0.05 was considered deemed statistically.

Results

Baseline characteristics

In the baseline analysis, a total of 2137 NAFLD patients and 2234 non-NAFLD controls were included, with their baseline characteristics detailed in Table 1. It can be observed that NAFLD patients were generally older, predominantly male, and more likely to be Non-Hispanic White with lower educational level. Additionally, they had higher BMI, ALT, AST,UC, TR, TC, and TG levels, lower HDL-C and physical activity levels, and were more likely to smoke or have comorbidities such as diabetes, hypertension, dyslipidemia, CVD. Nevertheless, no significant difference in PIR was observed between the NAFLD and non-NAFLD groups. Notably, NAFLD patients also exhibited higher CLR levels, and the prevalence of NAFLD increased progressively across CLR quartiles.

Impact of CLR on NAFLD and mortality in NAFLD Patients

The findings from the weighted logistic regression analysis conducted across the three models revealed that CLR is associated with the risk of NAFLD, as detailed in Table 2. After applying a natural logarithmic (ln) transformation to CLR, in Model 1, which did not account for covariates, lnCLR showed a significant association with NAFLD (OR = 2.04, 95% CI = 1.87–2.23, $P < 0.001$). This association was sustained in subsequent models, with Model 2 showing an OR = 2.14 (95% CI = 1.92–2.38, P < 0.001) and Model 3 showing an OR = 1.45 (95% CI = 1.16–1.81, $P = 0.010$). When CLR was analyzed in quartiles, individuals in the second, third, and fourth quartiles exhibited a 2.24, 4.62, and 9.01 times higher risk of NAFLD, respectively, compared to those in the first quartile (Q1) in Model 1 (all $P < 0.001$). Following adjustments for age, gender, and race in Model 2, the NAFLD risk for Q2, Q3, and Q4 participants was 2.30, 4.78, and 10.30 times higher than that of Q1, respectively (all $P < 0.001$). Notably, a significant positive trend was observed across model 1 and model 2, indicating an increasing prevalence of NAFLD with ascending CLR quartiles (both P for trend < 0.001). Upon further adjustment for additional variables including PIR, educational level, BMI, ALT, AST, UC, hypertension, diabetes, dyslipidemia, CVD, smoking status and physical activity in Model 3, although Q2, Q3, and Q4 showed a trend effect compared to Q1 (p for trend = 0.048), no significant association between CLR and NAFLD was observed within Q2, Q3, or Q4 participants.

After a median follow-up of 2.83 years (interquartile range: 1.92 to 3.92 years), further analysis utilizing weighted Cox regression on survival data from 2134 NAFLD participants did not reveal a significant correlation between lnCLR and all-cause mortality as well as CVD mortality across all three modeling approaches. For all-cause mortality, in Model 1, the HR was 1.15

Table 1 Weighted characteristics of the study population based on NAFLD

Characteristic[2]	N	Overall *N* = 111,700,855[1]	Non-NAFLD *N* = 58,533,721[1]	NAFLD *N* = 53,167,134[1]	*p*-value
Age (years)	4371				< 0.001
20–39		1403 (34%)	873 (41%)	530 (26%)	
40–60		1493 (37%)	672 (33%)	821 (42%)	
> 60		1475 (30%)	689 (27%)	786 (33%)	
Gender	4371				< 0.001
Male		2187 (49%)	1013 (42%)	1174 (56%)	
Female		2184 (51%)	1221 (58%)	963 (44%)	
Race and ethnicity	4371				0.002
Mexican American		547 (6.6%)	231 (5.5%)	316 (7.9%)	
Other Hispanic		427 (5.3%)	196 (5.1%)	231 (5.4%)	
Non-Hispanic White		1666 (69%)	834 (69%)	832 (69%)	
Non-Hispanic Black		1002 (10%)	509 (10%)	493 (11%)	
Other Race		729 (8.5%)	464 (9.8%)	265 (7.0%)	
PIR	4371				0.7
< 1.0		616 (9.2%)	323 (9.3%)	293 (9.0%)	
1.0–3.0		1829 (33%)	894 (32%)	935 (33%)	
> 3.0		1926 (58%)	1017 (59%)	909 (58%)	
Educational level	4371				< 0.001
< High school		586 (7.6%)	272 (6.5%)	314 (8.7%)	
= High school		903 (21%)	429 (19%)	474 (23%)	
> High school		2882 (72%)	1533 (75%)	1349 (68%)	
BMI	4371				< 0.001
< 18.5		56 (1.3%)	56 (2.5%)	0 (0%)	
18.5–24.9		1130 (27%)	1105 (50%)	25 (0.9%)	
25.0–29.9		1402 (31%)	907 (40%)	495 (21%)	
≥ 30.0		1783 (41%)	166 (7.0%)	1617 (78%)	
Hypertension	4371				< 0.001
No		2061 (52%)	1337 (65%)	724 (37%)	
Yes		2310 (48%)	897 (35%)	1413 (63%)	
Diabetes	4371				< 0.001
No		3565 (86%)	2018 (94%)	1547 (77%)	
Yes		806 (14%)	216 (5.9%)	590 (23%)	
Smoking status	4371				0.006
Never		2550 (59%)	1378 (62%)	1172 (57%)	
Current		1129 (27%)	486 (24%)	643 (31%)	
Former		692 (13%)	370 (14%)	322 (12%)	
CVD	4371				< 0.001
Yes		428 (7.5%)	154 (5.0%)	274 (10%)	
No		3943 (92%)	2080 (95%)	1863 (90%)	
Dyslipidemia	4371				< 0.001
No		1281 (30%)	913 (42%)	368 (17%)	
Yes		3090 (70%)	1321 (58%)	1769 (83%)	
ALT (U/L)	4371	23 ± (15)	19 ± (10)	28 ± (17)	< 0.001
AST (U/L)	4371	23 ± (10)	22 ± (9)	24 ± (11)	< 0.001
HDL-C (mg/dl)	4371	56 ± (18)	62 ± (18)	48 ± (13)	< 0.001
TG (mg/dl)	4371	146 ± (133)	102 ± (52)	194 ± (172)	< 0.001
TC (mg/dl)	4371	194 ± (42)	189 ± (40)	198 ± (44)	< 0.001
UA (mg/dl)	4371	5.35 ± (1.39)	4.92 ± (1.24)	5.82 ± (1.39)	< 0.001
Physical activity	4371				< 0.001

Table 1 (continued)

Characteristic[2]	N	Overall *N* = 111,700,855[1]	Non-NAFLD *N* = 58,533,721[1]	NAFLD *N* = 53,167,134[1]	*p*-value
Inactive		1485 (30%)	666 (24%)	819 (36%)	
Moderately active		449 (10.0%)	228 (10.0%)	221 (9.9%)	
Highly active		2437 (60%)	1340 (66%)	1097 (54%)	
CLR(mg/cells*10^{-9})	4371	1.79 ± (3.93)	1.28 ± (4.03)	2.36 ± (3.73)	< 0.001
CLR quartile[3]	4371				< 0.001
Q1		1068 (25%)	823 (37%)	245 (12%)	
Q2		1029 (25%)	593 (28%)	436 (21%)	
Q3		1145 (25%)	484 (21%)	661 (30%)	
Q4		1129 (25%)	334 (14%)	795 (38%)	

Abbreviations PIR ratio of family income to poverty, *BMI* body max index, *CVD* cardiovascular disease, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *HDL* cholesterol, high-density lipoprotein cholesterol, *TG* triglyceride, *TC* total cholesterol, *UA* uric acid, *CLR* ratio of C-reactive protein to lymphocytes

[1] N: Weighted population

[2] Mean ± (SD) for continuous variables: *P* value was calculated by design-based KruskalWallis test; % for categorical variables: P value was calculated by Pearson's X^2: Rao & Scott adjustment

[3] Q1–Q4 indicates quartile 1–quartile 4

Table 2 The association between CLR and NAFLD

	NAFLD		
	Model 1 OR (95%CI) *P*-value	**Model 2 OR (95%CI) *P*-value**	**Model 3 OR (95%CI) *P*-value**
lnCLR	2.04 (1.87, 2.23) < 0.001	2.14 (1.92, 2.38) < 0.001	1.45(1.16, 1.81) 0.010
CLR quartile			
Q1 (0.00–0.38)	Ref	Ref	Ref
Q2 (0.38–0.83)	2.24(1.88, 3.15) < 0.001	2.30(1.71, 3.09) < 0.001	0.98(0.40, 2.40) 1.000
Q3 (0.83–1.91)	4.62(3.57, 5.96) < 0.001	4.78(3.62, 6.30) < 0.001	1.50(0.54, 4.17) 0.200
Q4 (1.91–91.31)	9.01(6.87, 11.80) < 0.001	10.30(7.39, 14.30) < 0.001	2.74(0.94, 7.95) 0.056
P for trend	< 0.001	< 0.001	0.048

Model 1 was unadjusted

Model 2 was adjusted for age, gender, race

Model 3 was adjusted for age, gender, race, PIR, educational level, BMI, hypertension, diabetes, smoking status,ALT, AST, UA, dyslipidemia, CVD, physical activity

(95% CI = 0.73–1.83, *P* = 0.547), while Model 2 yielded an HR of 1.25 (95% CI = 0.82–1.92, P = 0.298), and Model 3 provided an HR of 1.06 (95% CI = 0.73–1.54, *P* = 0.757). For CVD mortality, in Model 1, the HR was 1.26 (95% CI = 0.46–3.40, *P* = 0.652), while Model 2 yielded an HR of 1.38 (95% CI = 0.57–3.31, *P* = 0.298), and Model 3 provided an HR of 1.01 (95% CI = 0.67–1.51, *P* = 0.957). Consistently, when CLR was analyzed as quartiles, participants in Q2, Q3, and Q4 did not show significant differences in HR values compared to Q1 across the three models for either all-cause mortality or CVD mortality (all *P* > 0.05, *P* for trend > 0.05) (Supplementary Table 1). When CLR was analyzed in quartiles,, there were no significant differences across quartiles, regardless of whether the outcome was all-cause mortality or CVD mortality, as indicated by the Kaplan–Meier survival curves (Supplemental Fig. 1).

The dose–response relationship and sensitivity analyses

The dose–response relationships between CLR and NAFLD, as well as between CLR and mortality in NAFLD patients, were explored using RCS curves. The outcomes presented in Fig. 2 indicated a significant nonlinear relationship between CLR and NAFLD across all models (P-non-linear < 0.0001 for all). Conversely, no evidence of a nonlinear relationship between CLR and all-cause mortality as well as CVD mortality in NAFLD patients was noted (P-non-linear > 0.05 for all). Additionally, it was observed that within a certain range, the risk of NAFLD increased with rising CLR levels but tended to stabilize once CLR reached a certain threshold. The threshold effect analysis indicated that when CLR was less than 1.667, a significant association with NAFLD risk was established (OR = 1.417, 95% CI = 1.136–1.770, *P* = 0.006). However, this association diminished when

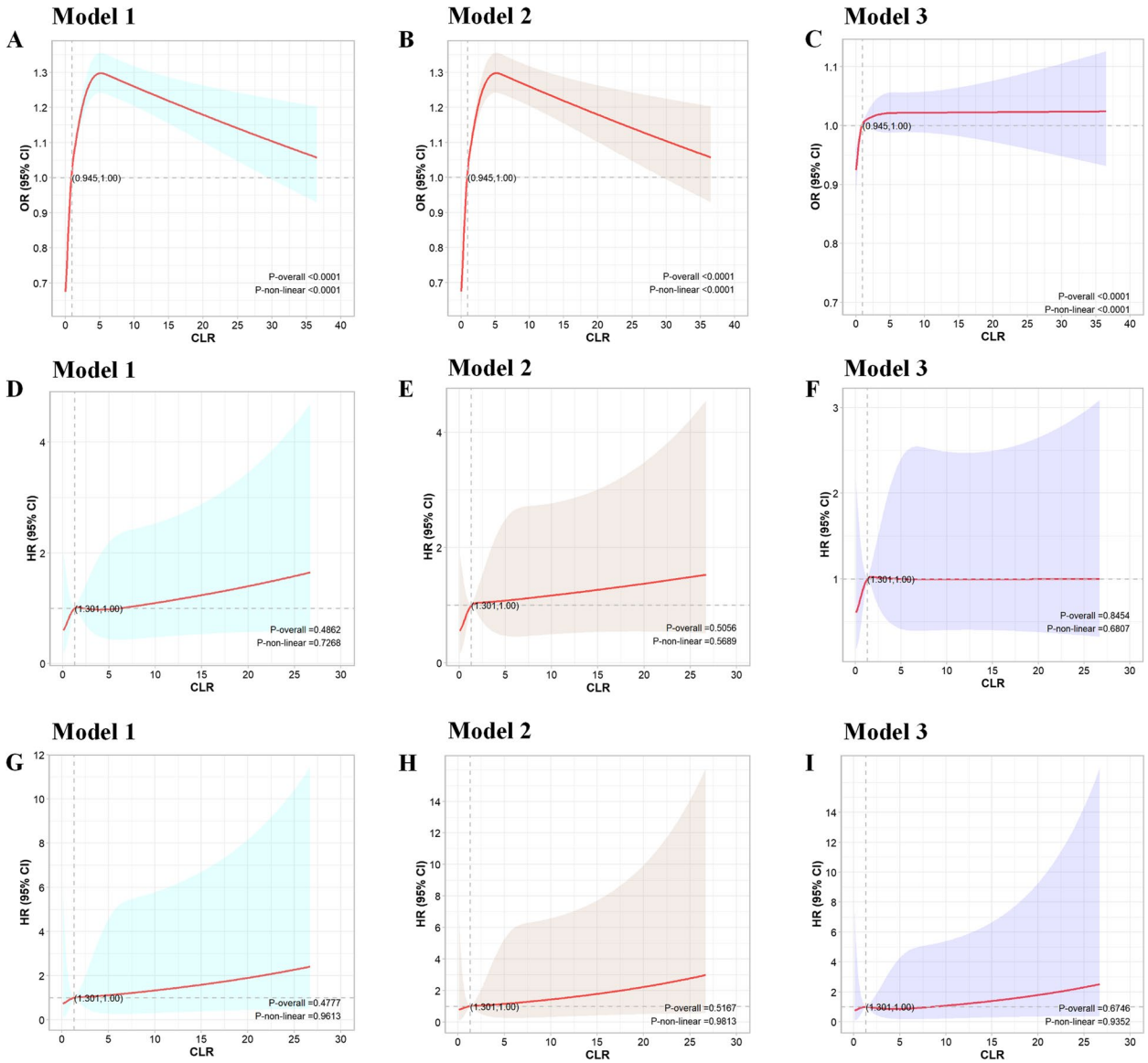

Fig. 2 Association of CLR with NAFLD and mortality among individuals with NAFLD. In Model 1, the OR and HR is presented without adjusting for any variables. In Model 2, the OR and HR are adjusted for age, gender, and race. In Model 3, the OR and HR are additionally adjusted for PIR, educational level, BMI, hypertension, diabetes, smoking status, ALT, AST, UA, dyslipidemia, CVD, physical activity. Shaded areas represent 95% CIs. The CLR value corresponding to an OR of 1 is 0.945, and an HR of 1 is 1.301. **A**-**C** Association of CLR with NAFLD across different models. **D**-**F** Association of CLR with all-cause mortality among individuals with NAFLD across different models. **G**-**I** Association of CLR with CVD mortality among individuals with NAFLD across different models

CLR exceeded 1.667 (OR = 1.021, 95% CI = 0.982–1.055, $P = 0.254$) (Table 3).

Subgroup analyses were performed considering variables such as age, gender, race, PIR, educational level, BMI, physical activity, hypertension, diabetes, dyslipidemia, CVD and smoking status, with the results depicted in Fig. 3. The positive correlation between CLR and NAFLD was notably more pronounced among participants without dyslipidemia. Significant interaction effects were detected between CLR and dyslipidemia. Moreover, whether using the 2015–2018 data to define MASLD through the USFLI index or utilizing the 2017–2020 data to define NAFLD through VCTE data, the association between CLR and NAFLD remained consistently stable (Supplementary Tables 2 and 3). Although the subgroup analysis for all-cause mortality indicated a significant interaction between CLR and PIR, due to the relatively short follow-up period and the low number of

Table 3 Threshold effect analysis of CLR on NAFLD

	OR (95%CI) *P*-value
Model 1	1.041(1.009–1.069) 0.005
Model 2	
Inflection point	1.667
< 1.667	1.417(1.136–1.770) 0.002
> 1.667	1.021(0.982–1.055) 0.254
P for Log-likelihood ratio	0.006

Model 1: Fiting model by standard logistic regression

Model 2: Fitting model by two-piecewise logistic regression

deaths, the results of the subgroup analysis for both all-cause mortality and CVD mortality were inconclusive, and the accuracy of the findings warrants further consideration (Supplementary Tables 4 and 5).

Discussion

This study, based on data from NHANES 2015–2018, explored the association between CLR and NAFLD, as well as CLR and all-cause mortality in NAFLD patients. After adjusting for multiple covariates, this study confirmed a significant negative correlation between CLR and NAFLD. However, this study did not find a significant association between CLR and either all-cause mortality or cardiovascular mortality among individuals with NAFLD. Additionally, a clear nonlinear relationship between CLR and NAFLD was observed. Notably, when CLR was analyzed as quartiles, no significant differences in NAFLD prevalence were observed among Q2, Q3, and Q4 compared to Q1. This may suggest a potential threshold effect of CLR, where extremely elevated CLR values (e.g., > 1.667) may not further increase the risk of NAFLD beyond a certain level. Subsequent exploratory subgroup analyses also supported the robustness of the findings. An intriguing finding from the subgroup analysis was the significant interaction between CLR and dyslipidemia in relation to NAFLD prevalence. Specifically, the association between CLR and NAFLD appeared to be stronger among individuals without dyslipidemia. Numerous studies have demonstrated that dysregulated lipid metabolism and inflammatory responses are two key initiating factors in the pathogenesis of NAFLD [15, 16].The excessive influx of free fatty acids (FFAs) and the accumulation of triglycerides (TAGs) within hepatocytes contribute to the development of a lipotoxic environment, which in turn triggers hepatic inflammation [17]. Furthermore, the "two-hit" hypothesis, which postulates that primary damage caused by triglyceride accumulation, followed by secondary inflammation induced by mitochondrial reactive oxygen species (ROS), also supports the notion of coexisting lipid metabolic dysregulation and inflammatory processes [18]. Thus, it is not difficult to understand that excessive lipid accumulation and a high-fat milieu can inherently induce inflammatory responses. Additionally, it has been shown that adipose tissue, in addition to the liver, is a source of hs-CRP production and can drive systemic inflammation [19]. Therefore, in individuals with dyslipidemia, although CLR levels may be elevated, its role may be attenuated or masked by the dominant influence of lipid metabolism. This could serve as a potential explanation for the interaction effect identified in subgroup analysis.It is plausible that in individuals without underlying lipid abnormalities, the pro-inflammatory state captured by elevated CLR levels may exert a more pronounced influence on the development of NAFLD through mechanisms independent of lipid-driven pathways. In contrast, among those with dyslipidemia, lipid-mediated mechanisms may dominate disease progression. Of course, this remains a hypothesis based on prior studies and requires further validation in future research.

In previous studies, although no research has yet confirmed the correlation between CLR and NAFLD, the association between CRP, lymphocyte count, and other composite inflammatory markers with NAFLD has been widely reported. Hs-CRP, as a widely recognized inflammatory marker, has been shown to be associated with NAFLD in numerous studies. A cohort study indicated that as hs-CRP levels elevated within a healthy demographic, the incidence of NAFLD correspondingly increased, a trend observable even within the normal hs-CRP range. This confirmed that hs-CRP could serve as a predictive marker for NAFLD [20]. Compared to non-NAFLD patients, elevated hs-CRP levels in NAFLD patients were also correlated with liver disease severity. Specifically, hs-CRP concentrations were notably higher in patients diagnosed with NASH compared to those with simple steatosis [21, 22]. Okekunle et al. investigated the correlation between the pro-inflammatory hs-CRP score and the heightened prevalence of NAFLD, reporting an AUC of 0.81 across the overall population and 0.63 for males [23]. Hs-CRP has also been considered a key factor for all-cause mortality in MASLD patients, which is consistent with our hypothesis that inflammation plays a pivotal role in the pathogenesis of NAFLD [24]. Additionally, in NAFLD patients, lymphocyte count is abnormally elevated [25]. A study of the UK Biobank population revealed an L-shaped relationship between lymphocyte count and NAFLD [19]. Another study within the NHANES population similarly confirmed a positive correlation between lymphocyte count and NAFLD [26]. However, other studies suggest that lower lymphocyte counts in NAFLD patients may promote disease progression and increase the risk of HCC [27].

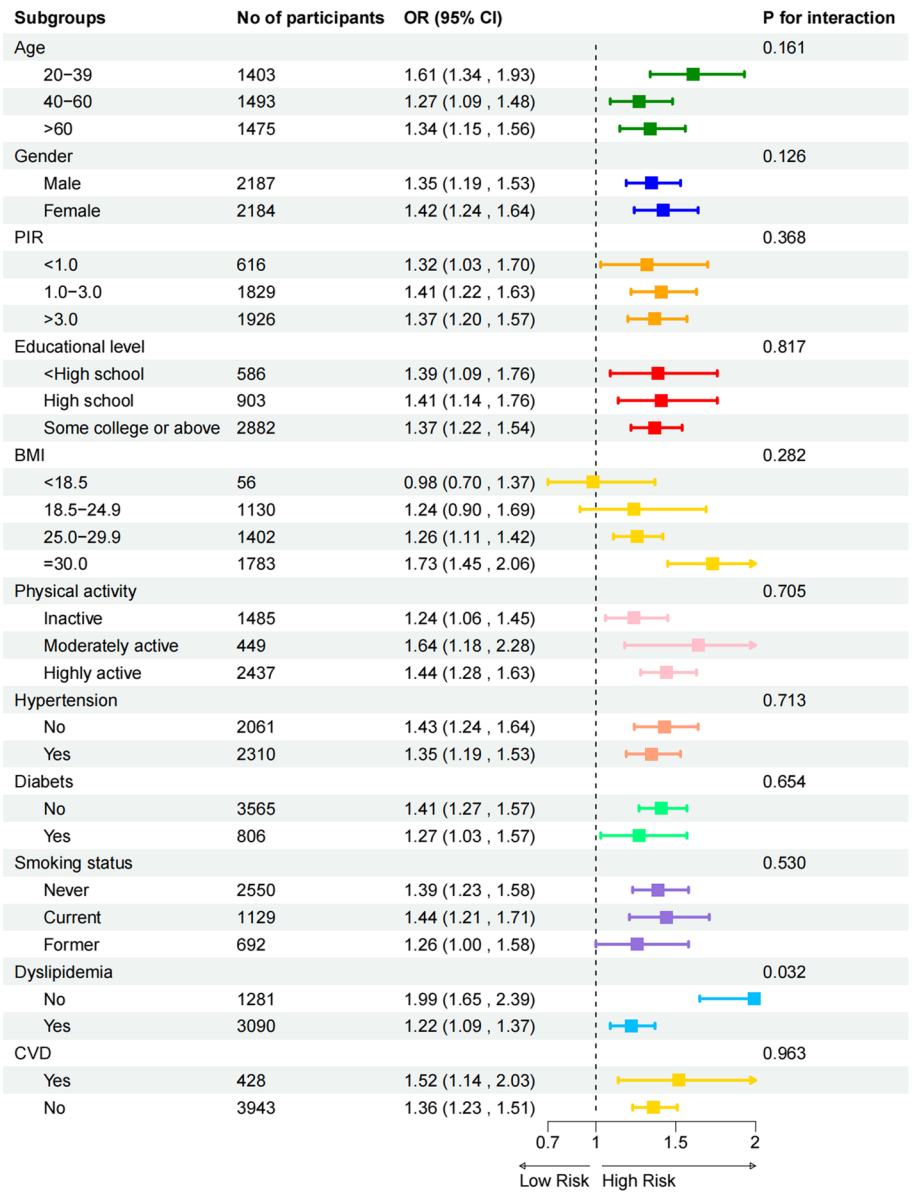

Fig. 3 Subgroup analysis of the association of CLR with NAFLD

Therefore, CLR may also be associated with NAFLD. CLR, as a composite index of hs-CRP and lymphocyte count, reflects the balance of systemic inflammation and immune status, revealing a positive correlation with NAFLD in this study.

Numerous inflammatory biomarkers have been demonstrated to be associated with NAFLD. Liu et al., utilizing data from a large-scale NHANES cohort involving 59,842 participants, revealed significant associations between several systemic immune-inflammatory biomarkers—including the systemic immune-inflammation index (SII), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and lymphocyte to monocyte ratio (LMR) and NAFLD after logarithmic transformation [28]. Furthermore, Zhao et al. provided additional evidence from the NHANES database, showing that elevated SII was positively associated with all-cause mortality among individuals with NAFLD [29]. Beyond the NHANES cohort, similar findings have been reported in other populations. A large-scale cross-sectional study conducted at a community hospital in Beijing, China, recruited 6,306 participants to investigate the association between novel inflammatory markers, such as NLR, PLR, C-reactive protein to albumin ratio (CAR), LMR,

SII, and prognostic nutritional index (PNI) and NAFLD. This study identified significant associations of LMR and PNI with NAFLD, with PNI demonstrating the strongest correlation [30]. Moreover, Gong et al. conducted a prospective cohort study using data from the UK Biobank to explore the relationships between NAFLD and both the individual inflammatory marker CRP and four composite systemic inflammatory markers: LMR, NLR, PLR, and SII. Unlike previous studies, this study focused on severe NAFLD as the primary outcome and found a nonlinear positive association between CRP and NAFLD, with CRP showing the strongest correlation. LMR demonstrated an L-shaped relationship, while NLR, PLR, and SII exhibited U-shaped associations with NAFLD [31]. Other inflammatory biomarkers, such as the neutrophil percentage to albumin ratio (NPAR) and the dietary inflammatory index (DII), have also been increasingly reported in relation to NAFLD [32, 33]. Taken together, despite variations across studies, there is accumulating evidence supporting a link between systemic inflammatory status and NAFLD. In our study, even after adjusting for multiple confounding factors, the inflammation-related marker CLR remained significantly associated with NAFLD (Model 3: OR = 1.45, 95% CI = 1.16–1.81, $P = 0.010$). However, it is noteworthy that we did not observe significant associations between CLR and either all-cause mortality or CVD mortality in NAFLD patients. This might be attributed to the relatively short follow-up period and the limited number of deaths during follow-up, which represents one of the limitations of our study. Moreover, although a growing number of inflammation-related markers have been shown to be associated with NAFLD, there is currently no evidence that CLR outperforms other markers in terms of clinical predictive value. Therefore, at this stage, CLR should be seen as a piece in the broader puzzle of inflammation and NAFLD, rather than as a standalone clinical tool. As research progresses, future studies comparing these inflammatory markers within the same prospective cohort may help clarify their distinct clinical utilities in patients with NAFLD.

It is unquestionable that NAFLD exhibits a higher inflammatory state than non-NAFLD. Inflammatory cytokines play a key role in the onset and progression of NAFLD by activating various inflammatory pathways that interfere with insulin signaling [34]. CRP, a classic non-specific acute-phase protein produced by the liver, can upregulate NF-κB activity and disrupt insulin signaling, thereby promoting the progression of NAFLD [23, 24]. On the other hand, in NAFLD patients, free fatty acids activate TLR4, promoting the production and release of inflammatory cytokines, ultimately leading to the production of hs-CRP [35]. Immune cells also play an indispensable role in the pathogenesis of NAFLD [36]. When liver fat accumulates, lymphocytes are recruited to the liver to address the inflammation it causes [37]. Linoleic acid, a fatty acid that accumulates in NAFLD, can disrupt mitochondrial function, leading to the selective loss of intrahepatic CD4 + T cells and promoting the progression of HCC [38]. Similarly, NKT cells are also preferentially lost in a cholesterol-rich lipid microenvironment [39]. These may be potential mechanisms underlying the association between CLR and NAFLD, which warrant further exploration in future research.

To our knowledge, This study is the first to provide evidence for the association between CLR and NAFLD, and to explore the impact of CLR on mortality in NAFLD patients. Additionally, based on the NHANES stratified, multi-stage probability sampling design, this study performed weight adjustments, making the results nationally representative of the United States. Furthermore, potential confounding variables were accounted for through covariate adjustments, and subgroup analyses were performed to ensure the robustness of the findings. Nevertheless, this study is not without limitations. Being a cross-sectional analysis, it can not establish causal associations between CLR and NAFLD. Although multiple covariates were considered, we could not eliminate the influence of all potential confounders, particularly unmeasured factors such as dietary habits, medication use (e.g., statins or anti-inflammatory drugs), and other underlying inflammatory conditions that were not accounted for due to data limitations. Similarly, owing to limitations inherent in the NHANES dataset, NAFLD was defined using the FLI, rather than direct imaging or histological confirmation. While FLI is a widely accepted surrogate in population-based studies, it remains vulnerable to misclassification. Although sensitivity analyses incorporating imaging-based definitions (e.g., VCTE) were performed to mitigate this, the lack of direct diagnostic measures remains a key limitation. Prospective clinical cohorts with standardized diagnostic protocols are warranted to validate and extend these findings. Moreover, although this study attempted to investigate the correlation between CLR and all-cause mortality as well as CVD mortality in NAFLD patients, the relatively short follow-up period and the low number of deaths during the follow-up mean that the conclusion of no association between CLR and mortality in NAFLD patients, as assessed in this study, should be interpreted with caution and requires further investigation. Moreover, given the chronic and progressive nature of NAFLD, which can ultimately lead to cirrhosis or hepatocellular carcinoma, exploring the association between CLR and liver-related mortality would be of considerable clinical interest. However, the majority

of deaths in our study were unrelated to liver disease, and the NHANES database does not provide specific data on liver-specific causes of death. As such, we were unable to address this question in the present analysis, underscoring the need for future studies specifically designed to investigate this association. Lastly, the findings of this study are specific to U.S. adults, meaning it may not be applicable to other populations. Future research should involve large-scale, prospective, multicenter cohort studies or Mendelian randomization to validate causal relationships.

Conclusion

In conclusion, utilizing data from the NHANES database, we confirmed the positive correlation between CLR and NAFLD, offering valuable assistance in identifying populations with elevated CLR exposure at risk for NAFLD. In individuals with elevated CLR levels, heightened awareness of the risk of NAFLD is crucial to facilitate early interventions and prevent its progression to advanced stages. Moreover, for individuals with NAFLD, CLR will not affect their all-cause mortality or cardiovascular mortality.

Abbreviations

NAFLD	Non-alcoholic fatty liver disease
CLR	The ratio of C-reactive protein to lymphocytes
NHANES	National Health and Nutrition Examination Survey
CRP	C-reactive protein
NCHS	National Center for Health Statistics
BMI	Body mass index
FLI	Fatty liver index
VCTE	Vibration controlled transient elastography
TG	Triglycerides
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	γ-Glutamyltransferase
CAP	Controlled attenuation parameter
HDL-C	High-density lipoprotein-cholesterol
LDL-C	Low-density lipoprotein cholesterol
TC	Cholesterol
UA	Uric acid
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
PIR	The ratio of family income to poverty
PA	Physical activity
SE	Standard errors
ORs	Odds ratios
CIs	Confidence intervals
HRs	Hazard ratios
RCS	Restricted cubic spline
CVD	Cardiovascular disease
MASLD	Metabolic dysfunction-associated steatotic liver disease
USFLI	United States Fatty Liver Index
SII	Systemic immune-inflammation index
NLR	Neutrophil to lymphocyte ratio
PLR	Platelet to lymphocyte ratio
LMR	Lymphocyte to monocyte ratio
CAR	C-reactive protein to albumin ratio
PNI	Prognostic nutritional index
NPAR	Neutrophil percentage to albumin ratio
DII	Dietary inflammatory index
FFAs	Free fatty acids
TAGs	Triglycerides
ROS	Reactive oxygen species

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

Formal analysis & writing: Jianxin Xi and Jason Chi Shing Law; Statistical analysis: Shengnan Wang; Figure production: Jie Chen; Review & editing: Guoyue Lv and Zhongqi Fan.

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Data availability

Data utilized in this investigation are available for public access via the NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm). The datasets analyzed during this study can be found in the NHANES repository, and all pertinent data are accessible to the public in accordance with the guidelines established by the NHANES program.

Declarations

Ethics approval and consent to participate

The present study utilizes publicly accessible data from the National Health and Nutrition Examination Survey (NHANES). Given that the utilized data are anonymized and publicly available, ethical approval was not deemed necessary for this research.

This study used publicly available data from the National Health and Nutrition Examination Survey (NHANES). NHANES is a program conducted by the Centers for Disease Control and Prevention (CDC) that collects health and nutritional data from a representative sample of the U.S. population. The NHANES data used in this research are de-identified and publicly available, so no individual consent was required for this study. The study was conducted in accordance with ethical standards and institutional guidelines for research using publicly available data.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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