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The association between inflammatory biomarkers and gallstones in Americans under 50 years old

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Abstract

Background This study aimed to assess the link between inflammatory biomarkers (like the neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammatory index (SII), and systemic inflammation response index (SIRI)) and gallstones in American individuals aged under 50 years.

Methods This study utilized data from the National Health and Nutrition Examination Survey (NHANES) covering 2017 to 2020, centering on individuals under 50 years with comprehensive data on NLR, SII, SIRI, and gallstones. It employed a weighted multiple logistic regression approach to investigate the link between inflammatory biomarkers and gallstones. Furthermore, dose-response relationships and threshold effects were evaluated utilizing restricted cubic spline (RCS) methods and segmented linear regression models. Subgroup examinations and interaction assessments were conducted, too.

Results The investigation encompassed a total of 3,295 individuals. Upon comprehensive adjustment for variables, multivariate logistic regression revealed a positive relationship between inflammatory biomarkers and gallstones: ln-NLR (OR = 1.68, 95% CI: 1.06–2.66, $p = 0.033$), ln-SII (OR = 1.79, 95% CI: 1.08–2.98, $p = 0.032$), and ln-SIRI (OR = 1.46, 95% CI: 1.07–1.99, $p = 0.025$). A non-linear association, shaped like an inverse "U", was noted between ln-SIRI and gallstones. To the left of the inflection point (ln-SIRI = 0.35, SIRI = 1.42), a positive link existed between ln-SIRI and gallstones (OR = 2.45, 95% CI: 1.20–5.03); whereas, to the right of the inflection point, the association was statistically insignificant (OR = 0.60, 95% CI: 0.21–1.73).

Conclusion ln-NLR and ln-SII exhibited a linear and positive relationship with the likelihood of developing gallstones. ln-SIRI demonstrated a nonlinear dose-response relationship with gallstone risk, characterized by an inverted "U" shape.

Keywords Neutrophil-to-lymphocyte ratio, Systemic immune-inflammatory index, Systemic inflammation response index, Gallstone, NHANES

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Introduction

Gallstones are a common digestive disorder with a heterogeneous global prevalence. In some Western populations (e.g., Europe and the United States), up to 20% of people are affected [1–3]. Gallstones are mainly located in the gallbladder or bile ducts. Most often, patients might show no symptoms or endure only slight discomfort [4]. Acute episodes may result in lethal consequences, including acute cholangitis, acute cholecystitis, and biliary pancreatitis [5–7]. Furthermore, those with chronic gallstones are at an elevated risk of developing gallbladder cancer [8]. This situation intensifies the global burden of diseases.

Risk factors for gallstones encompass genetic predispositions, abnormalities in bile acid and cholesterol metabolism, insulin resistance, and dysbiosis of gut microbiota [9–14]. Recent studies have suggested that the inflammatory microenvironment may be involved in the formation and development of gallstones [15, 16]. For example, animal experiments have shown an association between the upregulation of inflammatory factors and increased secretion of gallbladder mucins, which is thought to be a key step in cholesterol crystal aggregation [17–19]; and the inhibition of specific inflammatory pathways in the gallbladder epithelium (e.g., AMPK/SIRT1) reduced stone loading in experimental animals [20], suggesting that inflammatory responses may influence gallstone formation by modulating the local microenvironment. Notably, this association may be bidirectional: clinical studies have observed enhanced inflammatory molecular profiles in the bile of patients with gallstones [21] and elevated levels of inflammatory markers (e.g., interleukins, hs-CRP) in the blood correlated positively with the prevalence of gallstones [22, 23], but it is not possible to differentiate if inflammation is driving the formation of the stones or if the stones are triggering secondary inflammatory responses. Moreover, current studies have mostly focused on single inflammatory pathways or traditional markers, and integrated analyses of systemic immune-inflammatory networks are lacking.

The neutrophil-to-lymphocyte ratio (NLR) is a comprehensive metric to assess the innate and adaptive immune response. It has been thoroughly examined in evaluating the severity and clinical prognosis of many disorders [23–26]. Recent studies have revealed an association between a high NLR and biliary disease risk, such as cholecystitis and gallstone pancreatitis [27, 28]. Based on NLR, the systemic immune-inflammatory index (SII) further integrates platelet counts to constitute a systemic inflammatory biomarker that comprehensively reflects an individual's local immune response and systemic inflammatory state [29, 30]. Meng et al. found an association between SII and gallstones in American individuals under 50 years old [31]. However, the study had limitations and did not include emerging metrics such as the Systemic Inflammatory Response Index (SIRI) (a composite metric combined with monocyte counts, which excels in cancer prognosis) and NLR [32, 33]. In addition, differences in the efficacy of different inflammatory markers were not compared. All three inflammatory biomarkers have served as predictive tools in studies of gallbladder cancer [34–36]; the synergistic role of these indices in gallstones and their specific early warning value in young populations remains unknown.

This study is the first to systematically compare the association of NLR, SII, and SIRI with gallstone risk in a large cross-sectional cohort. Based on nationally representative data from the United States, we focused on people younger than 50 years of age, an age group that is critical for the early occurrence and prevention of gallstones.

Methods

Study design

Conducted by the National Center for Health Statistics (NCHS), the National Health and Nutrition Examination Survey (NHANES) is a countrywide cross-sectional survey focusing on the population to evaluate possible health risks and nutritional conditions in the noninstitutionalized civilian demographic of the United States. The study used stratified multistage random sampling for subject recruitment to guarantee that the sample accurately reflected the demographic characteristics of the United States. Official sanction for the NHANES study protocol was granted by the NCHS Research Ethics Review Board.

This study used NHANES 2017–March 2020 data. The original sample included 15,560 individuals. Given that this study focused on the young and middle-aged population, to ensure the accuracy and reliability of the results, we strictly limited the population to those under 50 years of age, based on previous studies [31, 37]. This design minimized the effect of confounding by age-related comorbidities (e.g., diabetes, NAFLD). Participants over 50 years of age ($n = 4841$) were therefore first excluded before analyses were conducted. Subsequently, people without data required for the calculation of NLR, SII, and SIRI ($n = 2,838$), those with incomplete data on gallstones ($n = 4,034$), and persons missing critical covariate data ($n = 552$) were eliminated. Following the previously mentioned screening process, the study's final sample size consisted of 3,295 participants (Fig. 1). Table S1 presented the baseline characteristics of inclusion and exclusion, and the results showed that the excluded group was older, had a higher prevalence of metabolic diseases such as diabetes and hypertension, and had a lower level of education.

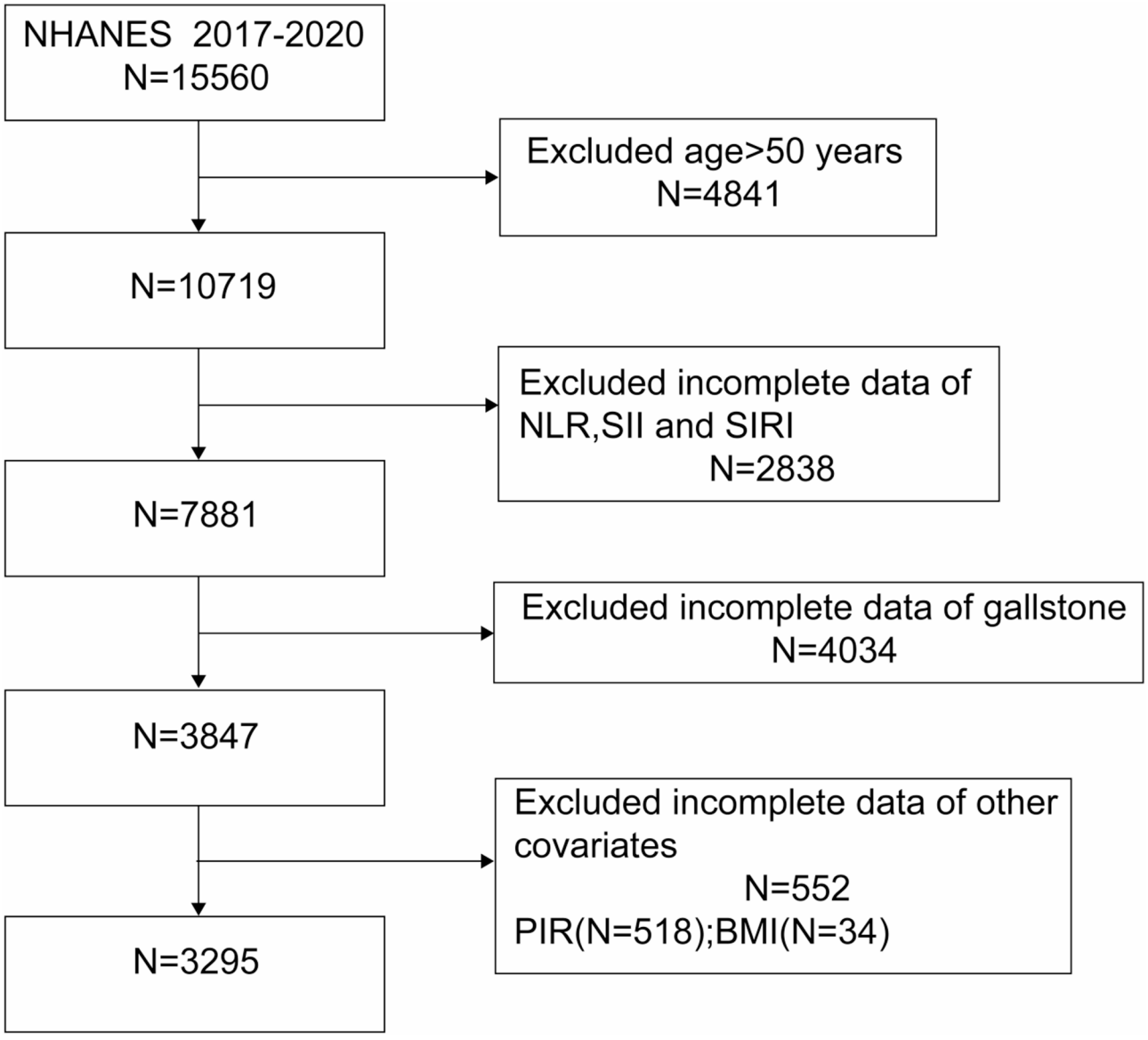

Fig. 1 Flowchart of participant inclusion and exclusion

Definition of gallstones and inflammatory biomarkers

The existence of gallstones was ascertained by participants' responses to the inquiry, "Have you been informed by a doctor that you have gallstones?" on the questionnaire. The Beckman Coulter method was employed to evaluate complete blood counts. NLR = Neutrophil count / Lymphocyte count, SII = Platelet count * Neutrophil count / Lymphocyte count, SIRI = Neutrophil count * Monocyte count / Lymphocyte count.

Covariates

This study included numerous variables that may affect the relationship between blood inflammatory biomarkers and gallstones. These factors comprised demographic data, including gender, age, race, education level, and household income-to-poverty ratio (PIR). Furthermore, the study encompassed laboratory test indicators and physical examination data, such as total cholesterol concentration (mmol/L) and body mass index (BMI, kg/m^2). Physical activity, smoking status, drinking habits, diabetes status, and hypertension were acquired by a specifically prepared questionnaire.

The classification of educational level was bifurcated into two categories: those at or below the high school level and those above it. PIR was categorized based on criteria of 1.3 and 3.5. BMI was categorized into three segments according to the thresholds of 25 kg/m^2 and 30 kg/m^2: Normal weight, Overweight, and Obese. Minutes of sedentary activity ≥ 600 were defined as sedentary. Drinking status was characterized as the intake of

any alcoholic drink at least monthly in the past year. The categorization of smoking status was divided into three groups: current smokers (≥ 100 cumulative cigarettes and actively smoking), former smokers (≥ 100 cumulative cigarettes but have ceased smoking), and never smokers (≤ 100 cumulative cigarettes) [38].

Statistical analysis

This study was based on data from NHANES, which was statistically analyzed using software version R 4.4.1, to explore the relationship between inflammatory biomarkers and gallstones. To ensure that the findings accurately reflected the characteristics of the target population, we created a survey design population using the survey software package, explicitly specified the primary sampling unit (PSU) and stratification (strata), and weighted the analyses with sample weights.

To improve the robustness of the analyses, all three indices were log-transformed (denoted as ln-NLR, ln-SII, and ln-SIRI in the results). Categorical variables were analyzed using chi-square tests, and continuous variables were analyzed using t-tests, with results reported as frequencies (percentages) and means (standard deviations), respectively. Three multivariate logistic regression models were developed to test the association between inflammatory biomarkers and gallstones: model 1 was unadjusted for covariates; model 2 was adjusted for sex, age, and ethnicity; and model 3 was adjusted for various covariates. The results were presented as odds ratios (ORs) and 95% confidence intervals (CIs). Weighted logistic regression analyses were performed using the 'svyglm' function, and variance estimates were automatically adjusted by a Taylor series linearisation method. Sensitivity analyses were also performed on participants based on quartiles of inflammatory biomarkers. Nonlinear associations were analyzed using weighted restricted cubic spline models constructed with the 'svyglm' and 'rms::Glm' functions. In addition, subgroup analyses and interaction tests based on the weighted design of the survey packages were conducted to further validate the robustness of the associations. All analyses were done in the R environment, mainly using the 'survey', 'gtsummary', 'haven', and 'rms' software packages to ensure the accuracy of the analyses and visual presentation of the results. The level of statistical significance was set at a *p-value* less than 0.05.

Results

Baseline characteristics

The study included 3,295 participants. According to participants' self-reported data, 3058 individuals were classified as non-gallstones, while 237 were categorized as gallstone subjects. Subsequent statistical analysis indicated significant disparities in the distribution of age, gender, race, BMI, smoking habits, drinking habits, ln-NLR, ln-SII, ln-SIRI, diabetes, and hypertension between individuals with and without gallstones (all $p < 0.05$). Relative to the non-gallstones cohort, the gallstones cohort exhibited a higher prevalence of females, a tendency towards older age, elevated BMI, a higher percentage of smokers, increased ln-NLR, ln-SII, and ln-SIRI values, a larger proportion of diabetics, a higher incidence of hypertension, and a higher proportion of non-drinkers (all $p < 0.05$), as illustrated in Table 1.

Relationship between inflammatory biomarkers and gallstones

Our findings indicated that elevated inflammatory biomarkers correlated with a heightened likelihood of gallstone formation. Within the comprehensive model (model 3), a consistent positive link remained between the variables and gallstones risk, evidenced by ln-NLR (OR = 1.68, 95% CI: 1.06–2.66, $p = 0.033$), ln-SII (OR = 1.79, 95% CI: 1.08–2.98, $p = 0.032$), and ln-SIRI (OR = 1.46, 95% CI: 1.07–1.99, $p = 0.025$).

To delve deeper into the link between inflammatory biomarkers and the risk of gallstones, a sensitivity analysis was carried out, segmenting continuous inflammatory biomarker variables into four equal parts (Q1 to Q4). In Models 1 and 2, elevated levels of ln-NLR, ln-SII, and ln-SIRI (Q3 and Q4) exhibited a significant positive association (all $p < 0.05$, Table 2) with an increased incidence of gallstones relative to the baseline group (lowest quartile Q1).

Restricted cubic spline regression analysis

We used a restricted cubic spline methodology to evaluate possible dose-response relationships between inflammatory biomarkers and gallstones. As depicted in Fig. 2, after controlling for all covariates, a linear dose-response association exists between ln-NLR, ln-SII, and gallstones (Non-linear $p > 0.05$). The association between ln-SIRI and gallstone risk exhibited an inverted "U" shape (Non-linear $p = 0.034$). Subsequent segmental regression and threshold analyses identified the inflection point as being at ln-SIRI = 0.35 (SIRI = 1.42), the extreme point of the inverted 'U' curve. To the left of the inflection point (ln-SIRI < 0.35), each 1-unit increase in SIRI was associated with a 2.45-fold increase in the risk of developing gallstones. The risk may even decrease when SIRI exceeds this threshold (OR = 0.60), but it is not statistically significant ($p = 0.249$). The p-value for the log-likelihood ratio test was 0.046 (Table 3).

Subgroup analysis

For assessing the uniformity in the link between inflammatory biomarkers and gallstones across various groups, we performed analyses on subgroups and interaction

Table 1 Baseline characteristics of the participants

Characteristic	Non-gallstones, (*N* = 3058)	Gallstones, (*N* = 237)	*p*-value
Age (years)	35.03 ± 8.89	38.92 ± 7.45	< 0.001
Gender			< 0.001
Female	1570 (51.3%)	185 (78.1%)	
Male	1488 (48.7%)	52 (21.9%)	
Race			0.001
Mexican American	434 (14.2%)	36 (15.2%)	
Other Hispanic	285 (9.3%)	27 (11.4%)	
Non-Hispanic White	963 (31.5%)	89 (37.6%)	
Non-Hispanic Black	780 (25.5%)	47 (19.8%)	
Non-Hispanic Asian	420 (13.7%)	15 (6.3%)	
Other Race	176 (5.8%)	23 (9.7%)	
Education level			0.432
High school or less	1143 (37.4%)	82 (34.6%)	
More than high school	1914 (62.6%)	155 (65.4%)	
PIR			0.204
≤ 1.3	938 (30.7%)	85 (35.9%)	
≥ 3.5	965 (31.6%)	65 (27.4%)	
1.3–3.5	1155 (37.8%)	87 (36.7%)	
BMI			< 0.001
Normal weight	923 (30.2%)	28 (11.8%)	
Obese	1255 (41.0%)	169 (71.3%)	
Overweight	880 (28.8%)	40 (16.9%)	
Sedentary (%)			0.765
Yes	496 (16.3%)	40 (17.2%)	
No	2555 (83.7%)	192 (82.8%)	
Smoking status			< 0.001
Current	633 (20.7%)	65 (27.5%)	
Former	465 (15.2%)	52 (22.0%)	
Never	1959 (64.1%)	119 (50.4%)	
Drinking status			0.014
Yes	1579 (58.8%)	103 (49.8%)	
No	1107 (41.2%)	104 (50.2%)	
TC (mmol/l)	4.74 ± 0.99	4.85 ± 0.95	0.081
ln-NLR	0.58 ± 0.45	0.69 ± 0.41	0.001
ln-SII	6.10 ± 0.52	6.30 ± 0.49	< 0.001
ln-SIRI	-0.05 ± 0.61	0.10 ± 0.54	< 0.001
Diabetes			< 0.001
Yes	153 (5.0%)	29 (12.2%)	
No	2904 (95.0%)	208 (87.8%)	
Hypertension			< 0.001
Yes	541 (17.7%)	72 (30.4%)	
No	2513 (82.3%)	165 (69.6%)	

Abbreviations: PIR, household income to poverty ratio; BMI, body mass index; TC, total cholesterol; ln-NLR, natural log-transformed neutrophil-lymphocyte ratio; ln-SII, natural log-transformed systemic immune-inflammatory index; ln-SIRI, natural log-transformed systemic inflammatory response index

tests focusing on variables such as age (categorized into two groups based on 35 years), gender, BMI, diabetes, and hypertension. The study findings indicated that the association was heterogeneous across subgroups defined by age, gender, BMI, etc. The study noted a notable interaction between diabetes status and the link between three inflammatory indicators and gallstones (all p for interaction < 0.05), in contrast to other covariate interactions, which lacked statistical significance (Fig. 3). Further analysis showed that a consistent positive association between inflammatory biomarkers and gallstones remained robust in subjects without hypertension or diabetes. Overall, our results suggested that the association between inflammatory biomarkers and gallstones was modified by diabetes status, with a positive association evident primarily in populations without diabetes.

Table 2 Association of inflammatory biomarkers with gallstones

	Model 1		Model 2		Model 3	
Characteristic	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
ln-NLR						
	2.12 (1.54, 2.93)	< 0.001	1.79 (1.28, 2.52)	0.002	1.68 (1.06, 2.66)	0.033
Categories						
Q1	Ref		Ref		Ref	
Q2	2.53 (1.29, 4.97)	0.009	2.21 (1.07, 4.57)	0.034	2.58 (0.83, 8.02)	0.077
Q3	2.64 (1.66, 4.19)	< 0.001	2.04 (1.27, 3.28)	0.006	2.17 (0.98, 4.81)	0.053
Q4	3.19 (1.80, 5.65)	< 0.001	2.39 (1.37, 4.17)	0.005	2.63 (0.98, 7.06)	0.053
ln-SII						
	2.52 (1.83, 3.48)	< 0.001	1.97 (1.38, 2.81)	< 0.001	1.79 (1.08, 2.98)	0.032
Categories						
Q1	Ref		Ref		Ref	
Q2	1.60 (0.93, 2.73)	0.083	1.34 (0.73, 2.48)	0.323	1.41 (0.55, 3.63)	0.329
Q3	2.74 (1.36, 5.50)	0.007	2.05 (1.00, 4.18)	0.049	2.02 (0.63, 6.47)	0.151
Q4	3.28 (2.17, 4.96)	< 0.001	2.17 (1.34, 3.50)	0.004	1.93 (0.88, 4.21)	0.075
ln-SIRI						
	1.57 (1.28, 1.94)	< 0.001	1.60 (1.27, 2.02)	< 0.001	1.46 (1.07, 1.99)	0.025
Categories						
Q1	Ref		Ref		Ref	
Q2	1.19 (0.73, 1.93)	0.471	1.27 (0.75, 2.16)	0.351	1.15 (0.44, 3.02)	0.670
Q3	2.42 (1.45, 4.02)	0.002	2.29 (1.31, 3.98)	0.006	2.08 (0.63, 6.89)	0.146
Q4	2.08 (1.28, 3.36)	0.005	2.03 (1.19, 3.47)	0.013	1.82 (0.70, 4.73)	0.140

Abbreviations:

OR, Odds ratio; 95% CI, 95% confidence interval

Model 1: No adjustment for covariates

Model 2: Adjusted for gender, age, and race

Model 3: Adjusted for Age, Gender, Race, Education level, PIR, BMI, Sedentary, Smoking status, Drinking status, TC, Diabetes, and Hypertension

Discussion

This study aimed to investigate the relationship between inflammatory biomarkers and gallstones in a US population aged 50 or younger. The cross-sectional analysis involving 3,295 individuals revealed a linear positive relationship between NLR, SII, and gallstones. Meanwhile, a reversed "U"-shaped nonlinear association was identified between SIRI and gallstones, exhibiting different associations on either side of the inflection point (ln-SIRI = 0.35, SIRI = 1.42). There was a positive association between SIRI and gallstones for SIRI values below the inflection point, but no significant association for values above it.

This study marks the inaugural investigation into the link between NLR and gallstones, highlighting a positive association between increased NLR levels and gallstone risk. Earlier studies have established a link between NLR and the likelihood of biliary system disorders. A meta-analysis indicated that NLR was significantly elevated in patients with cholecystitis, irrespective of disease severity, compared to those without cholecystitis. Additionally, NLR levels have been proposed as a predictor of acute cholecystitis [27]. Zhao et al. indicated that NLR may predict the severity of acute pancreatitis (AP), but its applicability is confined to gallstones-induced AP and is not relevant to alcoholic AP [28]. A multicenter retrospective analysis pinpointed NLR ≥ 2.462 [OR = 1.915, 95% CI: 1.099–3.337] as a distinct predictor for recurrent hepatoliths after biliary surgery [39]. Our findings showed a consistent positive association between NLR and gallstones in every model. Sensitivity studies that categorized NLR into quartiles demonstrated that the positive association between elevated levels of ln-NLR

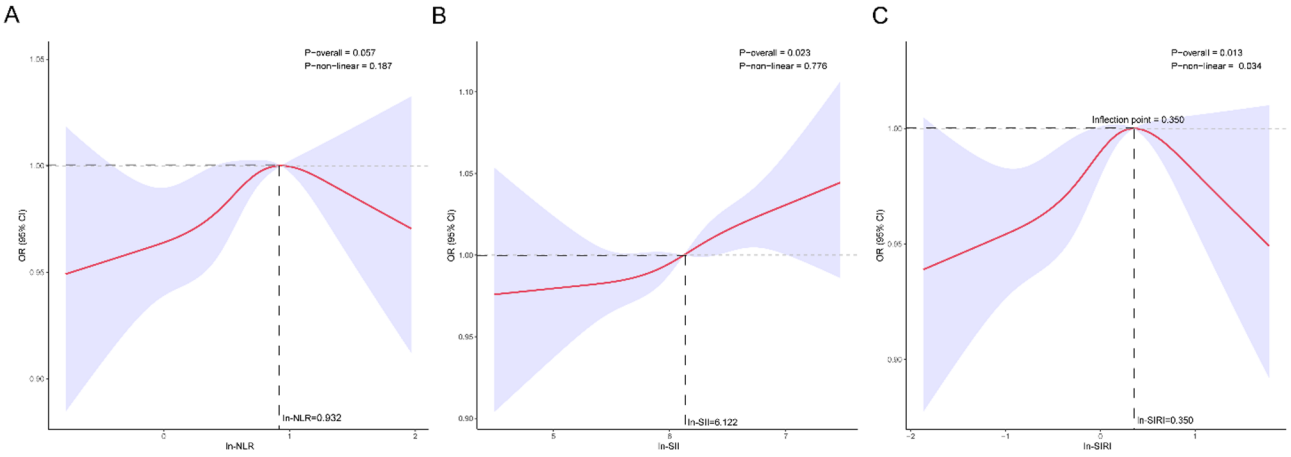

Fig. 2 The dose-response relationship between inflammatory biomarkers (**A**, ln-NLR; **B**, ln-SII; C, ln-SIRI) and gallstones. Adjusted for Age, Gender, Race, Education level, PIR, BMI, Sedentary, Smoking status, Drinking status, TC, Diabetes, and Hypertension. The red curve in the figure represents the odds ratio (OR), and the shaded area represents the 95% confidence interval (CI). *P*-values were used to assess the significance of the overall association and the non-linear relationship

Table 3 Segmented linear regression models to analyze the threshold effect of ln-SIRI on gallstones

	ln-SIRI	
Models	**OR (95% CI)**	***p*-value**
Model I		
	1.46 (1.07, 1.99)	0.025
Model II		
Inflection point(K)	0.35	
< K	2.45 (1.20, 5.03)	0.025
> K	0.60 (0.21, 1.73)	0.249
Log likelihood ratio		0.046

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval

Adjusted for Age, Gender, Race, Education level, PIR, BMI, Sedentary, Smoking status, Drinking status, TC, Diabetes, and Hypertension

(Q3, Q4) and gallstones persisted consistently across all model changes. Consistent with the findings of Meng et al. we found a significant positive association between SII and gallstones (OR = 1.79, 95% CI: 1.08–2.98), which showed a stronger association in our study compared to the study by Meng et al. (OR = 1.001, 95% CI: 1.000-1.001) [31]. And this difference in results may stem from methodological differences: the larger sample size of the present study (3,295 vs. 2,420) improved the ability to detect true effects; and the covariate adjustment strategy was different, with the present study incorporating metabolic variables (TC, diabetes mellitus, hypertension), whereas the Meng study adjusted for nutritional indices (total sugars, fat, etc.). Our findings revealed for the first time an inverted 'U' shaped non-linear relationship between SIRI and gallstones. Specifically, the risk peaked at ln-SIRI = 0.35 (SIRI = 1.42, OR = 2.45, 95% CI: 1.20–5.03) and began to decrease above this threshold. Very high SIRI values no longer increase the risk of gallstones, which may be related to the small sample size in the high-value range or the fact that very high SIRIs occur in different health contexts. To sum up, our study confirmed a positive association between inflammatory indicators and gallstones.

Furthermore, our study revealed a notable interaction among NLR, SII, SIRI, and gallstones within the diabetic subgroup. Inflammatory biomarkers are positively associated with gallstone risk in non-diabetic but not significant in diabetic individuals, consistent with the findings of several cross-sectional studies [37, 40–42]. We delved into the mechanism behind it. Possible explanations are as follows: First, systemic inflammation and metabolic disturbances are commonly present in individuals with diabetes (especially type 2 diabetes) [43–45], which may have led to the inability of NLR/SII/SIRI to significantly differentiate between individuals with gallstones. Second, the number of diabetic patients in the under-50 sample of this study was relatively small ($n = 182$), and the number of gallstones among them was even smaller ($n = 29$). The small sample size may have led to large fluctuations in the results, and the lack of stability of the statistical significance tests makes the non-significant results of the association of inflammatory biomarkers with gallstones among diabetic patients somewhat uncertain. Third, the association between inflammatory markers and gallstone risk may be attenuated in diabetic patients due to good glycaemic control or the use of anti-inflammatory medications (e.g., statins) [38, 46]. Future studies should further explore this finding and validate the relationship between inflammatory markers and gallstones in a larger sample of diabetic patients.

The association of inflammatory markers with gallstones may be achieved through multiple mechanisms. First, experimental studies suggest that activation of adaptive and innate immune systems may promote gallstone formation. For example, transplantation of T

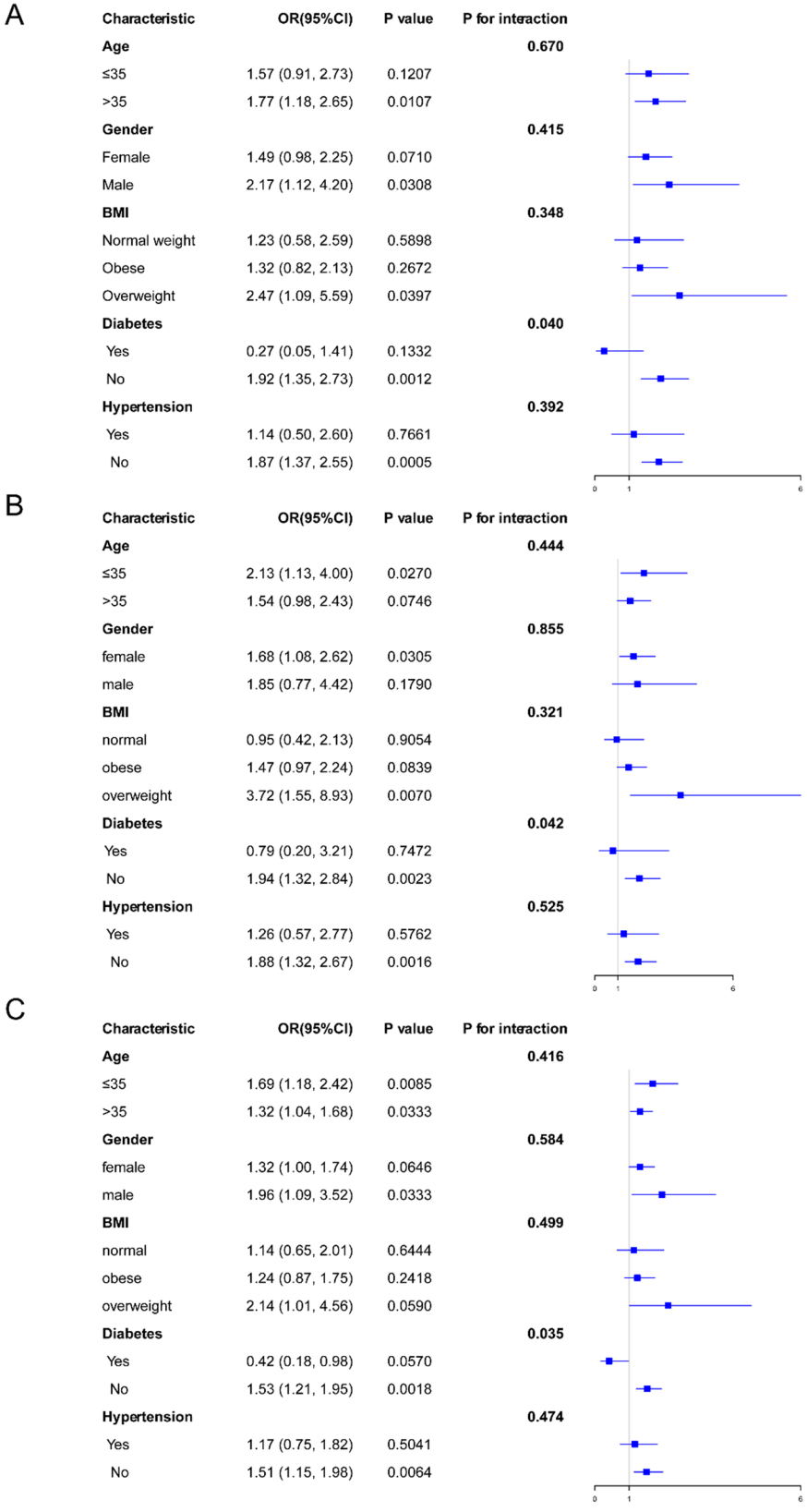

Fig. 3 Subgroup analyses of the association between inflammatory biomarkers and gallstones, including subgroups of age, gender, BMI, diabetes, and hypertension. (**A**) ln-NLR and gallstones; (**B**) ln-SII and gallstones; (**C**) ln-SIRI and gallstones

lymphocytes into Rag2 (-/-) mice significantly increased the incidence of stones [16]. Whereas neutrophil extracellular trap (NET) accelerates cholesterol crystal aggregation by activating innate immune pathways [47, 48]. Several investigations have consistently shown that inflammatory agents, such as tumor necrosis factor-α (TNF-α) and NLRP3 inflammasomes, can stimulate the production of gallbladder mucin [18, 19]. Recognized as an essential nuclear factor [17, 49], gallbladder mucin is pivotal in starting and progressing gallstone formation. Second, high levels of inflammatory markers may be a surrogate for other processes (e.g., chronic stress or subclinical infections) that are the true drivers of gallstone formation. Third, gallstones themselves may induce a local or systemic inflammatory response. Studies have pointed to the presence of subclinical inflammation in individuals with asymptomatic gallstones [50, 51]. A cohort study after cholecystectomy showed a significant decrease in inflammatory factors (IL-6, TNF-α, and c-reactive protein) postoperatively [52], suggesting that gallstones may drive elevated inflammatory markers through mechanical stimulation or intermittent obstruction.

The merits of this investigation are reflected in the following aspects. First, our approach involved utilizing data that represented the national population, meticulously adjusted to ensure a representative sample. Second, to the best of our knowledge, this study is the first exploration of the association between inflammatory markers (NLR, SII, SIRI) and gallstones. Nevertheless, the study's shortcomings should not be overlooked. First, the cross-sectional design of this study could not clarify the temporal relationship between inflammation and gallstones, and future prospective cohort or experimental studies are needed to further validate bidirectional mechanisms. Second, the diagnosis of gallstones in this study, based on self-reported data, would have resulted in causal recall bias due to the lack of evidence of gallstone imaging in the post-2000 NHANES database. In addition, asymptomatic gallstones are prevalent and easily missed in younger age groups. The presence of gross misclassification (omission of asymptomatic cases) may have a bidirectional impact on the results: if their inflammatory marker levels are similar to those of symptomatic cases, the current results underestimate the actual strength of the association, and conversely, if asymptomatic cases have lower inflammation levels, the strength of the association may be overestimated. Therefore, future systematic screening of asymptomatic cases by imaging techniques (e.g., ultrasound or CT) is warranted to clarify the direction of potential bias in this study and the reliability of the association. Third, given that the focus of this study was on early-onset gallstones in U.S. adults under 50 years old, this may limit the generalization of our findings to older adults. In addition, there is a potential selection bias in this study, with analyses of differences in inclusion and exclusion suggesting that the results may be more applicable to younger, more educated groups with a lower burden of metabolic disease, whereas pathomechanisms in older or less educated populations may have been undercaptured. Lower educational attainment may be associated with reduced access to or utilisation of healthcare services, which may affect the availability and completeness of health-related data. Future analyses need to be repeated in older, low-education populations using analyses such as multiple imputation to assess the robustness of the results. Fourth, although the main confounders were carefully considered and adjusted for in this study, the NHANES 2017–2020 data did not include data on women's reproductive history (e.g., number of births, oestrogen therapy) associated with gallstone risk and inflammatory markers. If dietary data were included, new weights would need to be introduced, and the high intra-individual coefficients of variation for single-day dietary recalls could affect the rigour of the analysis and the reliability of the results. Given this, we did not include all covariates in the model, and therefore residual confounders may be present. Future studies should further explore the impact of these unmeasured factors on gallstone risk. Finally, single measurements of inflammatory markers in NHANES introduce potential bias, and multiple repeated measurements in the future will provide a more robust indicator of chronic inflammation.

Conclusion

Our study revealed a positive association between increased inflammatory biomarker levels and the presence of gallstones in the U.S. population aged 50 and under. However, age limitations make it inappropriate to extrapolate the conclusions to older age groups, and broader prospective studies in a wider age range are needed.

Abbreviations

NLR	Neutrophil-to-lymphocyte ratio
SII	Systemic immune-inflammatory index
SIRI	Systemic inflammation response index
NHANES	National Health and Nutrition Examination Survey
CI	Confidence interval
RCS	Restricted cubic spline
OR	Odds ratio
Hs-CRP	High-sensitivity C-reactive protein
NCHS	National Center for Health Statistics
PIR	Income-to-poverty ratio
BMI	Body mass index
AP	Acute pancreatitis
NETs	Neutrophil extracellular traps
TNF-α	Tumor necrosis factor-α

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12876-025-03994-w.

Supplementary Material 1

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Author contributions
FM W conceptualized the study and manuscript. SQ C, YP Z, and L J carried out the data collection and analysis. YP Z, L J, JN L, and RC J interpreted and discussed the results. SQ C drafted the manuscript and FM W then revised it. All authors approved the final version of the manuscript.

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Data availability
This study's data were sourced from databases accessible to the public. https://www.cdc.gov/nchs/nhanes/.

Declarations

Ethics statement
The data for this study were obtained from the publicly available NHANES database, and studies involving humans were approved by the Ethics Review Board of the National Center for Health Statistics. All participants signed informed consent forms. Therefore, no additional ethical approval and informed consent are required for this study, and clinical trial numbers are not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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