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Inflammation-immune-nutrition biomarkers and PSA variability: A population-based study on the clinical potential of the CALLY index

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Abstract. Prostate cancer (PCa) is the second leading cause of cancer-associated mortalities worldwide. Prostate-specific antigen (PSA) testing is pivotal for screening for PCa, despite its limited specificity due to confounding factors such as inflammation and nutritional status. The present study investigated the association between the C-reactive protein (CRP)-albumin-lymphocyte (CALLY) index and PSA levels in a population without PCa. Using data from 5,320 men aged ≥40 years from the 2003-2010 national health and nutrition examination survey cycles, weighted multivariate linear regression and restricted cubic spline analyses were conducted. The findings revealed a significant inverse linear association: For each unit increase in the CALLY index, the PSA levels decreased by 0.09 ng/ml (β coefficient, -0.09; 95% confidence interval, -0.16 to -0.02). This association persisted across individuals with different ages, smoking habits and comorbidity subgroups. By integrating markers of systemic inflammation (such as the CRP levels), nutritional status (such as the albumin levels) and adaptive immunity (such as the lymphocyte counts), the CALLY index may refine the interpretation of the PSA levels and reduce the number of PCa false positive results, which occur due to subclinical inflammation. The present cross-sectional study highlighted that the CALLY index, as an adjunct biomarker, may have increased the accuracy of the screening for PCa. However, due to the cross-sectional design of the present study, causal associations cannot be established, and clinical applicability should be interpreted with caution. Therefore, further longitudinal and experimental studies are needed to elucidate the causal pathways and underlying mechanisms that link the CALLY index to the PSA levels.

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Key words: C-reactive protein-albumin-lymphocyte index, prostate-specific antigen, national health and nutrition examination survey, cross-sectional study

Introduction

Prostate cancer (PCa) is the second leading cause of cancer-associated mortalities worldwide and the most commonly diagnosed cancer among men in 2025 (1). Despite its high prevalence, prostate-specific antigen (PSA) testing remains the most widely used screening method due to its cost-effectiveness and practicality (2). However, numerous studies demonstrate that PSA levels can be influenced by various non-cancer-associated factors, including benign prostatic hyperplasia (3), prostatitis (4), antibiotic use (5) and body mass index (BMI) (6). These confounding factors can lead to diagnostic inaccuracies, increasing the risk of a false-positive or -negative diagnosis, which may result in unnecessary or inappropriate treatment (7). Therefore, relying solely on PSA levels for the screening of PCa presents notable challenges that warrant further investigation and improvement (8).

Growing evidence suggests that immune inflammation and nutritional status serve critical roles in the development and progression of PCa (9,10). A previous study reveals a potential association between abnormal immune-inflammatory responses, nutritional imbalances and elevated serum PSA levels in men (11). C-reactive protein (CRP), an acute-phase reactant with levels that increase in response to acute inflammation, infection or tissue damage, is linked to poor clinical outcomes in patients with PCa (12). For example, a previous meta-analysis demonstrates that increased CRP levels are notably associated with a reduced overall survival (OS), cancer-specific survival and progression-free survival (PFS) in patients with PCa (13). The systemic immune-inflammation index (SII), which is calculated as: (Platelets x neutrophils)/lymphocytes, serves as a simple yet effective prognostic biomarker. Elevated pre-treatment SII values are associated with reduced OS and PFS outcomes in patients with PCa (14). Compared with normal tissues, the levels of tumor necrosis factor are increased in various tumors and are associated with inflammatory cell infiltration and increased vascularization within the tumor microenvironment (15). Other inflammatory markers, such as the neutrophil-to-lymphocyte ratio (NLR) and

platelet-to-lymphocyte ratio (PLR), may also help differentiate benign prostatic hyperplasia from PCa and serve as potential diagnostic tools (16-18). Serum albumin, a readily available and cost-effective indicator of nutritional status, is also linked to PSA levels. It is reported that lower serum albumin levels in middle-aged men are associated with higher PSA concentrations (19). However, a cross-sectional analysis reveals that a dietary protein intake of >181.8 g/day is positively associated with elevated PSA levels (20).

Previous studies report associations between individual inflammatory or nutritional markers (such as CRP, albumin and NLR) and the PSA levels or prognosis of PCa (21-24). However, to the best of our knowledge, the CRP-albumin-lymphocyte (CALLY) index, a composite score integrating inflammation (such as the CRP levels), nutritional status (such as the albumin levels) and immunity (such as the lymphocyte count), is yet to be evaluated in association with the PSA levels in a general population without PCa (25). Previous retrospective studies demonstrate that the CALLY index is an independent prognostic factor of OS and PFS in various types of cancer, including gastric (26), non-small-cell lung (27), colorectal cancer (28) and hepatocellular carcinoma (29). However, previous research on the association between the CALLY index and PCa is limited. Accurate interpretation of PSA values, accounting for potential confounding effects from subclinical inflammation and nutritional status, is essential to increase the reliability of the screening for PCa.

Therefore, using data from the 2003-2010 national health and nutrition examination survey (NHANES) cycles, the present study investigated the association between the CALLY index and the PSA levels in a population of American men aged ≥40 years without known prostate conditions.

Subjects and methods

Study description and population. A NHANES (https://www.cdc.gov/nchs/nhanes/) is a cross-sectional study designed to assess the health and nutritional status of adults and children in the United States of America and is stipulated by a Code of Federal Regulations (45 CFR 46.101). It is conducted by the National Center for Health Statistics (NCHS), a part of the Centers for Disease Control and Prevention (CDC) (30). All NHANES protocols were approved by the NCHS research ethics review board, and written informed consent was obtained from all participants. The present study received an exemption of ethics approval from the Huai'an No. 1 People's Hospital institutional review board. The present study involved a secondary analysis of NHANES data and adhered to the reporting guidelines outlined in the strengthening the reporting of observational studies in epidemiology statement for cross-sectional studies.

The present study used data from four NHANES cycles between 2003-2010 (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2003; https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2005; https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2007; https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2009), as these were the only cycles that included comprehensive PSA information. Participants were included if there was complete data for PSA and all components of the CALLY index. The following exclusion criteria were applied: i) Age, <40 years; ii) missing data for PSA, CRP, albumin or lymphocyte counts; iii) missing information regarding BMI, comorbidities, lifestyle factors or education level; iv) conditions or treatments known to affect PSA levels, including the use of 5-α reductase inhibitors, diagnosis of benign prostatic hyperplasia or prostatitis, prostate biopsy within the past week, urological surgery within the past month, or the diagnosis of PCa.

Definitions of PSA and the CALLY index. Serum samples were stored at 2-8˚C and analyzed by Collaborative Laboratory Services LLC. Total PSA concentrations were measured using the Hybritech method (31) and validated using a chemiluminescent immunoassay platform with the Beckman Access® Immunoassay System (Beckman Coulter, Inc.). Testing was carried out according to standardized NHANES protocols (https://www.cdc.gov/nchs/nhanes/about/erb.html). Each sample was analyzed once without replication. Rigorous quality control procedures, including internal calibration and commercial controls (bench quality controls; https://wwwn.cdc.gov/nchs/data/nhanes/public/2009/labmethods/PSA_F_met_complex.pdf), were carried out to ensure measurement accuracy. PSA concentrations were reported in ng/ml. A total of 5,320 serum samples from men aged ≥40 years were included in the final analysis.

The CALLY index was calculated using the following formula: CALLY index=[albumin concentration (g/l) x lymphocyte count (10^9/l)]/[CRP concentration (mg/l) x 10]. CRP concentrations were quantified using latex-enhanced nephelometry on a Behring Nephelometer (Siemens Healthineers). Lymphocyte counts were analyzed using the Beckman Coulter MAXM Instrument. Serum albumin levels were measured using the Beckman Synchron LX20 and Beckman UniCel DxC800 Synchron systems. Due to the skewed distribution of the CALLY index, a logarithmic transformation was applied prior to statistical analyses.

Covariates. A range of covariates that could influence the association between the CALLY index and PSA levels were included in the analysis. These covariates included demographic characteristics (such as age, ethnicity, education level, BMI, smoking status and alcohol consumption), laboratory indices [such as the levels of blood urea nitrogen (mmol/l), cholesterol (mg/dl), glucose (mg/dl), serum lactate dehydrogenase (LDH; U/l), total bilirubin (mg/dl), triglycerides (mmol/l), serum uric acid (mg/dl), serum creatinine (mg/dl), aspartate aminotransferase (U/l) and alanine aminotransferase (U/l)] and clinical history (such as the presence or absence of chronic diseases including hypertension, diabetes, coronary artery disease, angina pectoris and history of neoplastic diseases).

Statistical analysis. All statistical analyses were carried out using R version 4.3 (https://www.r-project.org) and EmpowerStats version 2.0 (http://www.empowerstats.net/en/), incorporating the complex sampling design of NHANES in accordance with CDC analytical guidelines. Participants were stratified into quartiles based on their CALLY index values: Q1, <1.91; Q2, 1.91-4.27; Q3, 4.28-9.68; and Q4, >9.68.

Continuous variables are presented as the mean ± standard deviation, while categorical variables are presented as counts and percentages. Differences between groups across CALLY quartiles were analyzed using one-way analysis of variance with Tukey's honestly significant difference post hoc test for continuous variables and Pearson's chi-square test for categorical variables.

To investigate the association between the CALLY index and PSA levels, both weighted univariate and multivariate linear regression models were used, with β coefficients (β) and 95% confidence intervals (CIs) reported. Three models were constructed: i) Model 1 was unadjusted; ii) model 2 was adjusted for age, ethnicity and BMI; and iii) model 3 was fully adjusted for a comprehensive set of covariates selected based on biological plausibility, prior literature and data availability in NHANES (32). These variables may affect the PSA levels and/or components of the CALLY index, including comorbidities (such as the presence or absence of hypertension, diabetes, coronary heart disease, angina, tumor history and BMI), lifestyle factors (such as smoking status, alcohol intake, age, ethnicity and level of education) and laboratory markers [such as the levels of CRP, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, bilirubin, triglycerides, monocytes, neutrophils, platelets, creatinine, LDH, uric acid and glucose]. Variables such as globulin, testosterone and physical activity were not included due to incomplete data, limited availability across NHANES cycles and lack of consistent associations with PSA or CALLY components in the population without cancer.

To investigate potential non-linear associations, restricted cubic spline (RCS) regression was used. Additionally, subgroup analyses were conducted to assess whether the association between the CALLY index and PSA varied by demographic or clinical characteristics. P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics of participants. The present study initially included 41,156 participants. However, a number of participants were excluded due to incomplete information on key variables. Specifically, exclusions were made due to missing data regarding PSA levels (n=35,138), factors known to influence PSA levels (n=232), albumin levels (n=11), CRP levels (n=1), lymphocyte counts (n=29), BMI (n=86), chronic health conditions or tumor history (n=21), education level (n=1) and alcohol consumption history (n=265). After applying these criteria, a total of 5,320 eligible participants were included in the final analysis. The mean age of participants was 59.44±12.51 years. Participants were divided into quartiles based on their CALLY index values (Q1-Q4, Table I). The mean total serum PSA level was 1.82±3.20, with a statistically significant decreasing trend observed across increasing CALLY quartiles. Compared with participants with low CALLY index values, those with increased values tended to be younger and had lower levels of blood glucose, uric acid, LDH, creatinine, globulin and BMI as well as reduced counts of monocytes, neutrophils and platelets. Additionally, compared with participants with low CALLY index values, those with increased values were associated with a reduced prevalence of hypertension, diabetes, coronary heart disease, history of cancer and cigarette smoking. However, no significant differences were observed in the levels of ALT, AST, alcohol consumption or incidence of angina.

Association between the CALLY index and PSA levels. In the unadjusted model (model 1), there was a significant inverse association between the CALLY index and serum total PSA. Each one-unit increase in the CALLY index corresponded to a 0.17 ng/ml decrease in the PSA levels (β=-0.17; 95% CI, -0.24 to -0.10). Sensitivity analysis using CALLY quartiles revealed that participants in Q4 had a 48% reduced PSA level compared with those in Q1 (β=-0.48; 95% CI, -0.73 to -0.24). In model 2, which was adjusted for age, ethnicity and BMI, each one-unit increase in the CALLY index corresponded to a 0.10 ng/ml decrease in the PSA levels (β=-0.10; 95% CI, -0.17 to -0.03). In a fully adjusted model, model 3, controlling for all relevant covariates, the association remained significant in which PSA levels decreased by 0.09 ng/ml for each one-unit increase in the CALLY index (β=-0.09; 95% CI, -0.16 to -0.02) (Table II).

There is not a non-linear association between CALLY index and PSA. Due to the skewed distribution of PSA and CALLY index values, both variables were log-transformed to achieve normality prior to modeling. The RCS regression was then performed using the fully adjusted model (model 3) to assess potential non-linear associations. The results indicated that there was no significant evidence of a non-linear association between the log-transformed CALLY index and PSA levels (Fig. 1).

Subgroup analysis. To assess the consistency of the association across various subpopulations, subgroup analyses were carried out after the population was stratified by age, smoking status, alcohol consumption, and the presence or absence of hypertension, diabetes and coronary heart disease. As shown in Fig. 2, interaction tests did not yield statistically significant results, indicating that the inverse association between the CALLY index and PSA levels was not significantly modified by any of the stratified variables tested. Therefore, this suggested that the observed association was robust across different demographic and clinical subgroups.

Discussion

The present study was, to the best of our knowledge, the first to investigate the association between the CALLY index and PSA levels in a population without PCa in the United States of America. Using cross-sectional data from 5,320 participants in the NHANES database, the present study identified a significant linear association between the CALLY index and PSA concentrations, with PSA levels decreasing as the CALLY index increased. Furthermore, this inverse association was consistent across multiple subgroups, including those stratified by age, smoking status, alcohol consumption, hypertension, diabetes and coronary heart disease. Specifically, each one-unit increase in the CALLY index was associated with a 0.09 ng/ml reduction in the PSA levels. Although the association between increased CALLY index values and reduced PSA levels was statistically significant, the absolute

Table I. Baseline characteristics of participants sorted by CALLY index quartiles (n=5,320).

		CALLY index quartiles					
Variables	Total (n=5,320)	1 (n=1,330)	2 (n=1,330)	3 (n=1,328)	4 (n=1,332)	Statistic	P-value
ALT[a]	28.61±20.66	27.63±29.14	29.15±18.23	29.40±17.38	28.25±14.92	F=2.07	0.102
AST[a]	28.02±15.39	28.19±19.94	28.03±14.49	27.78±12.65	28.10±13.42	F=0.17	0.916
Cholesterol[a]	5.13±1.10	5.00±1.11	5.16±1.13	5.23±1.10	5.12±1.04	F=10.13	<0.001
Glucose[a]	5.98±2.31	6.41±2.78	6.02±2.39	5.79±1.95	5.71±1.94	F=24.86	<0.001
Lactate dehydrogenase[a]	133.87±33.42	139.33±45.50	134.20±31.79	132.10±26.05	129.84±25.73	F=19.68	<0.001
Bilirubin[a]	14.25±5.21	13.65±5.28	14.11±5.04	14.49±5.34	14.76±5.10	F=11.61	<0.001
Triglycerides[a]	1.95±1.49	1.82±1.24	2.06±1.57	2.04±1.64	1.87±1.47	F=8.31	<0.001
Uric acid[a]	362.14±80.04	378.38±91.79	369.99±77.30	356.53±72.61	343.72±72.57	F=49.52	<0.001
Creatinine[a]	94.10±48.59	101.14±65.41	94.58±42.21	90.45±34.83	90.22±45.77	F=14.81	<0.001
Globulin[a]	29.36±4.97	31.29±5.82	29.38±4.64	28.84±4.39	27.92±4.26	F=116.29	<0.001
PSA[a]	1.82±3.20	2.10±3.97	1.93±3.29	1.64±2.64	1.62±2.73	F=7.14	<0.001
Monocyte[a]	0.58±0.20	0.60±0.23	0.58±0.19	0.57±0.18	0.56±0.21	F=13.29	<0.001
Neutrophil[a]	4.30±2.14	4.85±2.87	4.37±2.31	4.14±1.52	3.83±1.38	F=55.64	<0.001
Age, years[a]	59.44±12.51	62.37±12.54	59.98±12.52	58.20±12.29	57.20±12.07	F=44.97	<0.001
Platelet[a]	238.90±63.85	246.41±75.68	238.10±58.52	237.64±61.65	233.48±57.26	F=9.62	<0.001
BMI[a]	28.87±5.68	30.49±7.08	29.69±5.35	28.53±4.92	26.77±4.29	F=114.48	<0.001
Ethnicity, N (%)						χ^2=42.50	<0.001
Non-Hispanic White	935 (17.58)	192 (14.44)	247 (18.57)	255 (19.20)	241 (18.09)		
Non-Hispanic Black	342 (6.43)	67 (5.04)	92 (6.92)	96 (7.23)	87 (6.53)		
Mexican American	2,923 (54.94)	749 (56.32)	724 (54.44)	724 (54.52)	726 (54.50)		
Other ethnicity	949 (17.84)	288 (21.65)	234 (17.59)	207 (15.59)	220 (16.52)		
Other Hispanic ethnicity	171 (3.21)	34 (2.56)	33 (2.48)	46 (3.46)	58 (4.35)		
Education level, N (%)						χ^2=46.63	<0.001
<9th grade	855 (16.07)	231 (17.37)	224 (16.84)	210 (15.81)	190 (14.26)		
9-11th grade	767 (14.42)	214 (16.09)	186 (13.98)	195 (14.68)	172 (12.91)		
High school grade	1,255 (23.59)	329 (24.74)	324 (24.36)	323 (24.32)	279 (20.95)		
University attendance or associate of arts degree	1,307 (24.57)	324 (24.36)	334 (25.11)	318 (23.95)	331 (24.85)		
University graduate or postgraduate education	1,136 (21.35)	232 (17.44)	262 (19.70)	282 (21.23)	360 (27.03)		
Alcohol use, N (%)						χ^2=4.94	0.176
Yes	4,375 (82.24)	1,092 (82.11)	1,070 (80.45)	1,099 (82.76)	1,114 (83.63)		
No	945 (17.76)	238 (17.89)	260 (19.55)	229 (17.24)	218 (16.37)		
Hypertension, N (%)						χ^2=99.76	<0.001
Yes	2,352 (44.21)	715 (53.76)	615 (46.24)	555 (41.79)	467 (35.06)		
No	2,968 (55.79)	615 (46.24)	715 (53.76)	773 (58.21)	865 (64.94)		
Diabetes, N (%)						χ^2=26.44	<0.001
Yes	845 (15.88)	263 (19.77)	202 (15.19)	193 (14.53)	187 (14.04)		
No	4,344 (81.65)	1,025 (77.07)	1,095 (82.33)	1,109 (83.51)	1,115 (83.71)		
Borderline	131 (2.46)	42 (3.16)	33 (2.48)	26 (1.96)	30 (2.25)		
Coronary heart disease, N (%)						χ^2=26.48	<0.001
Yes	471 (8.85)	150 (11.28)	138 (10.38)	94 (7.08)	89 (6.68)		
No	4,849 (91.15)	1,180 (88.72)	1,192 (89.62)	1,234 (92.92)	1,243 (93.32)		

Table I. Continued.

Variables	Total (n=5,320)	CALLY index quartiles				Statistic	P-value
		1 (n=1,330)	2 (n=1,330)	3 (n=1,328)	4 (n=1,332)		
Angina, N (%)						χ^2=5.98	0.113
Yes	245 (4.61)	77 (5.79)	59 (4.44)	56 (4.22)	53 (3.98)		
No	5,075 (95.39)	1,253 (94.21)	1,271 (95.56)	1,272 (95.78)	1,279 (96.02)		
Tumor history, N (%)						χ^2=16.24	0.001
Yes	461 (8.67)	151 (11.35)	105 (7.89)	103 (7.76)	102 (7.66)		
No	4,859 (91.33)	1,179 (88.65)	1,225 (92.11)	1,225 (92.24)	1,230 (92.34)		
Smoking status, N (%)						χ^2=38.25	<0.001
Yes	3,295 (61.94)	903 (67.89)	843 (63.38)	789 (59.41)	760 (57.06)		
No	2,025 (38.06)	427 (32.11)	487 (36.62)	539 (40.59)	572 (42.94)		

[a]Mean ± standard deviation. Statistical significance was assessed using one-way analysis of variance followed by Tukey's post hoc test for continuous variables and the Chi-square test for categorical variables. ALT, alanine aminotransferase; AST, aspartate aminotransferase; PSA, prostate-specific antigen; BMI, body mass index; CALLY, C-reactive protein-albumin-lymphocyte.

Table II. Associations between the CALLY index and serum prostate-specific antigen levels across three regression models.

CALLY index	Models					
	1		2		3	
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
Continuous[a]	-0.17 (-0.24-0.10)	<0.001	-0.10 (-0.17-0.03)	0.006	-0.09 (-0.16-0.02)	0.017
Quartiles						
1	Reference	-	Reference	-	Reference	-
2	-0.18 (-0.42-0.07)	0.156	-0.03 (-0.27-0.20)	0.795	-0.01 (-0.25-0.22)	0.903
3	-0.46 (-0.71-0.22)	<0.001	-0.22 (-0.46-0.01)	0.065	-0.20 (-0.45-0.04)	0.105
4	-0.48 (-0.73-0.24)	<0.001	-0.23 (-0.47-0.01)	0.066	-0.20 (-0.45-0.06)	0.126

[a]CALLY index as a continuous variable. Model 1 was the unadjusted crude model. Model 2 was adjusted for ethnicity, age and BMI. Model 3 was fully adjusted for demographic (such as age, ethnicity, level of education, alcohol intake and smoking status), clinical (such as presence or absence of hypertension, diabetes, coronary heart disease, angina, tumor history and BMI) and laboratory variables (such as the levels of CRP, albumin, alanine aminotransferase, aspartate aminotransferase, cholesterol, glucose, lactate dehydrogenase, bilirubin, triglycerides, uric acid, creatinine, monocytes, neutrophils and platelets). CI, confidence interval; β, regression coefficient; CRP, C-reactive protein; CALLY, CRP-albumin-lymphocyte; BMI, body mass index.

effect size was relatively small and may have limited clinical impact at the individual level. However, in population-level screening, even modest reductions in PSA, particularly when consistently observed, may contribute to increased specificity and help mitigate overdiagnosis due to subclinical inflammation. Sensitivity analyses confirmed the robustness of this association, supporting its reliability and validity. These findings suggest that an increased CALLY index may serve as an independent predictor of reduced PSA levels.

The CALLY index, calculated from levels of CRP, serum albumin and lymphocyte counts, represents a composite indicator of systemic inflammation, nutritional status and immune function (33). It is used as a prognostic biomarker in various malignancies of the digestive system such as gastric and colorectal cancer. Previous studies demonstrate that a reduced CALLY index score is associated with a reduced OS in patients with non-small cell lung cancer (34), gastric cancer (35), esophageal cancer (36), breast cancer (37) and renal cell carcinoma (38). In addition, data from NHANES indicates that, compared with reduced CALLY indices, an increased CALLY index is associated with a reduced risk of all-cause and cause-specific mortality in patients with cancer (39). Specifically, after comprehensive adjustment for variables (such as sex, age, ethnicity, poverty income ratio, smoking status, alcohol consumption, BMI, total cholesterol, ALT, serum creatinine, serum total bilirubin, types of cancer, prevalences of diabetes mellitus, cardiovascular disease, chronic kidney disease and hypertension), each one-unit increase in the natural logarithm of the CALLY index was associated with an 18% reduction in the risk of

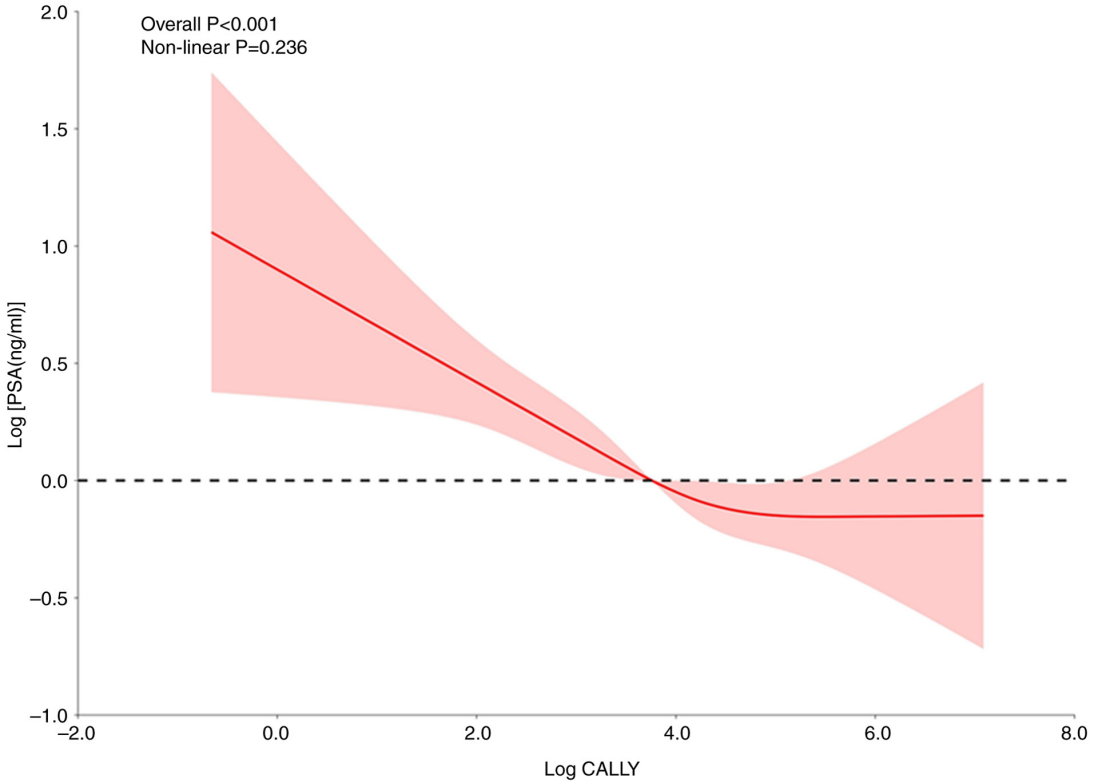

Figure 1. Association between the CALLY index and PSA levels. A fitted curve from restricted cubic spline regression that models the continuous association between the CALLY index and serum PSA levels (red solid line). The shaded red area indicates the 95% confidence interval. Both axes have continuous scales. CALLY, C-reactive protein-albumin-lymphocyte; PSA, prostate-specific antigen.

Variables	n (%)	OR (95% CI)	Interaction P-value
All patients	**5,320 (100.00)**	**-0.17 (-0.24, -0.10)**	
Alcohol use			**0.073**
Yes	4,375 (82.24)	-0.20 (-0.28, -0.12)	
No	945 (17.76)	-0.04 (-0.18, 0.10)	
Hypertension			**0.622**
Yes	2,352 (44.21)	-0.14 (-0.24, -0.04)	
No	2,968 (55.79)	-0.17 (-0.27, -0.08)	
Diabetes			**0.672**
Yes	845 (15.88)	-0.11 (-0.25, 0.03)	
No	4,344 (81.66)	-0.19 (-0.27, -0.11)	
Borderline	131 (2.46)	-0.10 (-0.46, 0.26)	
Coronary heart disease			**0.262**
Yes	471 (8.85)	-0.05 (-0.02, 0.13)	
No	4,849 (91.15)	-0.18 (-0.26, -0.11)	
Smoking status			**0.379**
Yes	3,295 (61.94)	-0.20 (-0.28, -0.11)	
No	2,025 (38.06)	-0.14 (-0.25, -0.02)	
Age, years			**0.500**
40-49	1,429 (26.86)	-0.01 (-0.06, 0.04)	
50-59	1,279 (24.04)	-0.05 (-0.12, 0.01)	
60-69	1,286 (24.17)	-0.13 (-0.24, -0.01)	
70-79	873 (16.41)	-0.02 (-0.29, 0.25)	
>80	453 (8.52)	-0.21 (-0.61, 0.19)	

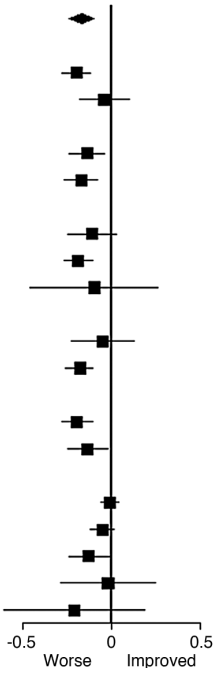

Figure 2. Subgroup analyses of the association between the CALLY index and PSA levels. A forest plot revealed the β coefficients and 95% CIs for the association between the CALLY index and PSA levels across subgroups (such as age, smoking status, alcohol use and comorbidities). CALLY, C-reactive protein-albumin-lymphocyte; PSA, prostate-specific antigen; CI, confidence interval.

all-cause mortality among cancer patients (39). However, the association between the CALLY index and PCa is yet to be elucidated.

The present study identified a negative association between the CALLY index and PSA levels. Although, to the best of our knowledge, this specific association has not been previously

reported, previous studies examine the individual components of the CALLY index in association with PSA levels and PCa. For example, a previous study reveals a non-linear association between serum albumin and PSA levels, highlighting an inverse association when albumin concentrations exceed 41 g/l (21). Furthermore, a previous study by Gao *et al* (32) reveals that among men >40 years of age without prostate diseases, the albumin-globulin ratio (AGR) demonstrates a non-linear association with PSA, with a negative association when the AGR is <1.32. Low levels of serum albumin also act as a prognostic factor in patients with metastatic castration-resistant prostate cancer (mCRPC) (22). Elevated CALLY index values are inversely associated with serum LDH levels, which is a clinically notable finding since increased LDH activity indicates enhanced tumor glycolysis, angiogenesis and cellular proliferation, and is an adverse prognostic biomarker across multiple malignancies, including PCa (40).

Inflammation serves a critical role in the development and progression of cancer, prompting investigations into the prognostic relevance of elevated CRP levels in various malignancies, including PCa (41). A prospective population-based cohort study by Stikbakke *et al* demonstrates that, compared with low serum CRP levels, high serum CRP levels are associated with an increased risk of PCa and have a reduced prognosis (23). Furthermore, two meta-analyses confirm that CRP is a strong predictor of adverse outcomes in PCa, including in patients with mCRPC (42,43). Elevated CRP is also associated with PSA levels in populations with cancer (44). As an acute-phase protein, CRP can suppress albumin synthesis and promote prostatic epithelial cell apoptosis through cytokine-mediated pathways (such as the IL-6 and TNF pathways), which may reduce the secretion of PSA (45). Inflammatory markers demonstrate strong associations with PSA levels and PCa outcomes (46). NLR, a marker of systemic inflammation, has prognostic value in PCa (47). Elevated NLR and total PSA levels are notably associated with increased Gleason scores (≥7) (24). Furthermore, PLR is an independent prognostic indicator for both PFS and OS in patients with PCa (48) and can be used to distinguish benign prostatic hyperplasia from PCa (49). Lymphocyte-mediated mechanisms may also influence PSA expression levels. The secretion of interferon-γ by infiltrating lymphocytes may suppress androgen receptor activity, which reduces the transcription of PSA (50). Although numerous studies demonstrate the prognostic value of systemic inflammatory markers such as NLR and PLR in PCa, it is important to acknowledge their non-specific nature (46-50). These ratios may be influenced by a wide range of conditions that are not associated with malignancy, including infections (such as pancreatitis and pulmonary infection) (51,52), autoimmune diseases (such as systemic lupus erythematosus) (53), trauma (such as traumatic brain injury) (54) and metabolic disorders (such as diabetes) (55). This non-specificity limits their utility as standalone indicators for PCa screening or prognosis. The findings of the present study suggested that the CALLY index, as a composite marker, potentially reflected these complex interactions. One potential mechanism is that the CALLY index captures a systemic immune state that is characterized by a Th1/Th2 balance that is skewed toward an anti-inflammatory profile (56). Although, to the best of our knowledge, there are not any direct studies on the impact of the CALLY index on the Th1/Th2 balance, the findings of the present study suggested that CRP, albumin and lymphocyte levels individually influenced the Th1/Th2 balance. The Th1/Th2 balance may skew toward an anti-inflammatory (Th2-dominant) profile due to chronic infections, allergies, immunosuppressive cytokines (such as IL-10 and TGF-β), hormonal influences, aging, diet or environmental factors, which may suppress the proinflammatory Th1 responses (57). This immune milieu may inhibit the recruitment of tumor-associated macrophages (58), which reduces the passive diffusion of PSA from the prostate stroma into the bloodstream (59). The recruitment of tumor-associated macrophages does not directly induce passive PSA diffusion but creates a permissive microenvironment (via inflammation, ECM breakdown and vascular leakiness) that enhances PSA entry into circulation. This aligns with observations that aggressive tumors often show higher serum PSA levels (60).

However, the present study had a number of limitations. Firstly, due to the cross-sectional design, causal associations between the CALLY index and PSA levels cannot be established. Therefore, prospective cohort studies should be carried out in the future in order to validate the findings of the present study. Secondly, PSA measurements in NHANES were carried out using the Hybritech method, which differs systematically from the World Health Organization (WHO)-standardized methods used in a number of clinical laboratories (61). The Hybritech PSA assay uses proprietary monoclonal antibodies and an in-house calibration standard, which yields values 20-30% higher compared with the WHO-calibrated methods. The WHO methods use polyclonal antibodies and the WHO International Standard (code, 96/670) (62). This discrepancy may introduce variability and limit the generalizability of the present results. Thirdly, a number of laboratory variables, such as globulin and testosterone levels, as well as lifestyle-associated factors including the dietary inflammatory index and physical activity, were not included in the present analysis due to incomplete data, limited availability across NHANES cycles and a lack of consistent associations with PSA or CALLY components in the population without cancer. The omission of these variables may result in an underestimation of the true association between the CALLY index and PSA levels.

In conclusion, PSA, the primary biomarker for PCa screening, is criticized for its limited diagnostic specificity, which contributes to the ongoing clinical challenges. The present study, to the best of our knowledge, was the first to reveal a negative association between PSA levels and the CALLY index, a composite marker reflecting inflammation, immunity and nutritional status. The present findings provided an insight into the potential biological contributors of PSA variability, which may provide guidance to future research regarding strategies for reducing false positives in PSA screening. However, due to the cross-sectional design of the present study, the observed association does not imply a causal association, nor does it support the immediate clinical use of the CALLY index as a diagnostic adjunct. Prospective and mechanistic studies are required to validate the findings of the present study and investigate the implications for PCa screening strategies.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JH contributed to the conception and the design of the present study. JH, NX and MF contributed to the acquisition, analysis and interpretation of the data. JH, NX and MF contributed to drafting the manuscript or revising the manuscript. JH, NX and MF read and approved the final version of the manuscript. JH, NX and MF confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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