

# Relationship of the Neutrophil–Lymphocyte Ratio with All-Cause and Cardiovascular Mortality in Patients with Diabetic Kidney Disease: A Prospective Cohort Study of NHANES Study

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**Background:** To investigate the association between the NLR and the risk of all-cause and cardiovascular mortality in US adults with diabetic kidney disease (DKD).

**Methods:** The data utilized for this analysis were sourced from ten National Health and Nutrition Examination Survey cycles (1999–2018) with mortality data (up to 31 December 2019) via linkage to the National Death Index. The optimum NLR threshold for predicting survival outcomes was determined through the maximally selected rank statistics. Restricted cubic spline (RCS), weighted Cox proportional hazard regression, stratified analyses, and time-dependent receiver-operating characteristic curve (ROC) were employed to delineate the prospective correlations of the NLR with both all-cause and cardiovascular mortality.

**Results:** In this investigation, a cohort comprising 2581 patients diagnosed with DKD was examined, encompassing 624 individuals with a higher NLR ( $\geq 3.07$ ) and 1957 subjects with a lower NLR ( $< 3.07$ ). Over a median follow-up of 79 months (interquartile range, 44–128 months), 1103 deaths occurred, including 397 from cardiovascular causes and 706 from non-cardiovascular causes. The RCS analysis elucidated the positive linear correlation (both nonlinear  $P > 0.05$ ). In the multivariable analyses, each one-unit increase in the NLR value was correlated with a 51% increased risk of all-cause mortality (1.51(1.28, 1.77)) and a 71% increased risk of cardiovascular mortality (1.71(1.32, 2.21)). The results were largely consistent across stratified analyses encompassing variables such as age, sex, race/ethnicity, marital status, family income, education levels, BMI, drinking status, smoking status, hypertension, CVD, and anti-infective drugs ( $P$  for interaction  $> 0.05$  for all). Time-dependent ROC analyses underscored the NLR's credible predictive efficacy for both short-term and extended durations in forecasting both all-cause and cardiovascular mortality.

**Conclusion:** The findings emphasize the promising use of the NLR in stratifying and prognosticating the risk of mortality in DKD in clinical practice.

**Keywords:** neutrophil–lymphocyte ratio, diabetic kidney disease, predictor, mortality, cohort study

## Introduction

Diabetic kidney disease (DKD) is increasingly recognized as the foremost cause of end-stage kidney disease (ESKD) on a global scale.<sup>1–3</sup> This is primarily attributable to the escalating incidence of diabetes, which is fueled by the unprecedented increase in the obesity epidemic. Notably, approximately 30–50% of subjects with diabetes ultimately develop DKD.<sup>1</sup> Current evidence indicates that subjects with both diabetes and chronic kidney disease (CKD) were found to be related with higher rates of myocardial infarction and 10-year cumulative mortality compared to those with diabetes alone.<sup>4,5</sup> Thus, it is imperative to promptly identify more risk factors to mitigate the progression of DKD and DKD-related mortality.

There is increasing evidence delineating the pivotal role of inflammation in both the initiation and progression of DKD.<sup>6–8</sup> Several immune infiltrating cells are apparent in glomerular and tubular cells and their activation is closely correlated with sustained kidney inflammation.<sup>6,9</sup> The neutrophil–lymphocyte ratio (NLR), a cheap and readily accessible measure of systemic inflammation and immune system activation in clinical use and comprehensive reflection of both innate (neutrophils) and adaptive (lymphocytes) immunological responses,<sup>10</sup> is regarded as a factor associated with adverse outcomes across a spectrum of diseases.<sup>9–13</sup> Association of the NLR and kidney impairment in diabetes is well established.<sup>14,15</sup>

Inflammation is also recognized as playing a pivotal role associated with the etiology of cardiometabolic diseases.<sup>16,17</sup> Recent researchers have identified the NLR as the strongest predictor of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke in two randomized clinical trials, which comprised 6480 participants with diabetes.<sup>18</sup> The NLR also has been found to be correlated with all-cause and cardiovascular mortality in diabetic individuals.<sup>19</sup> However, data are scarce on the linkage of the NLR with all-cause and cardiovascular mortality in DKD.

Given the high prevalence of DKD, understanding whether the NLR has a prognostic value for the risk of mortality in DKD is an important research area. To fill these knowledge gaps, this study aimed to prospectively examine the relationship of the NLR with all-cause and cardiovascular mortality in a nationally representative sample of US adults with DKD.

## Materials and Methods

### Study Population

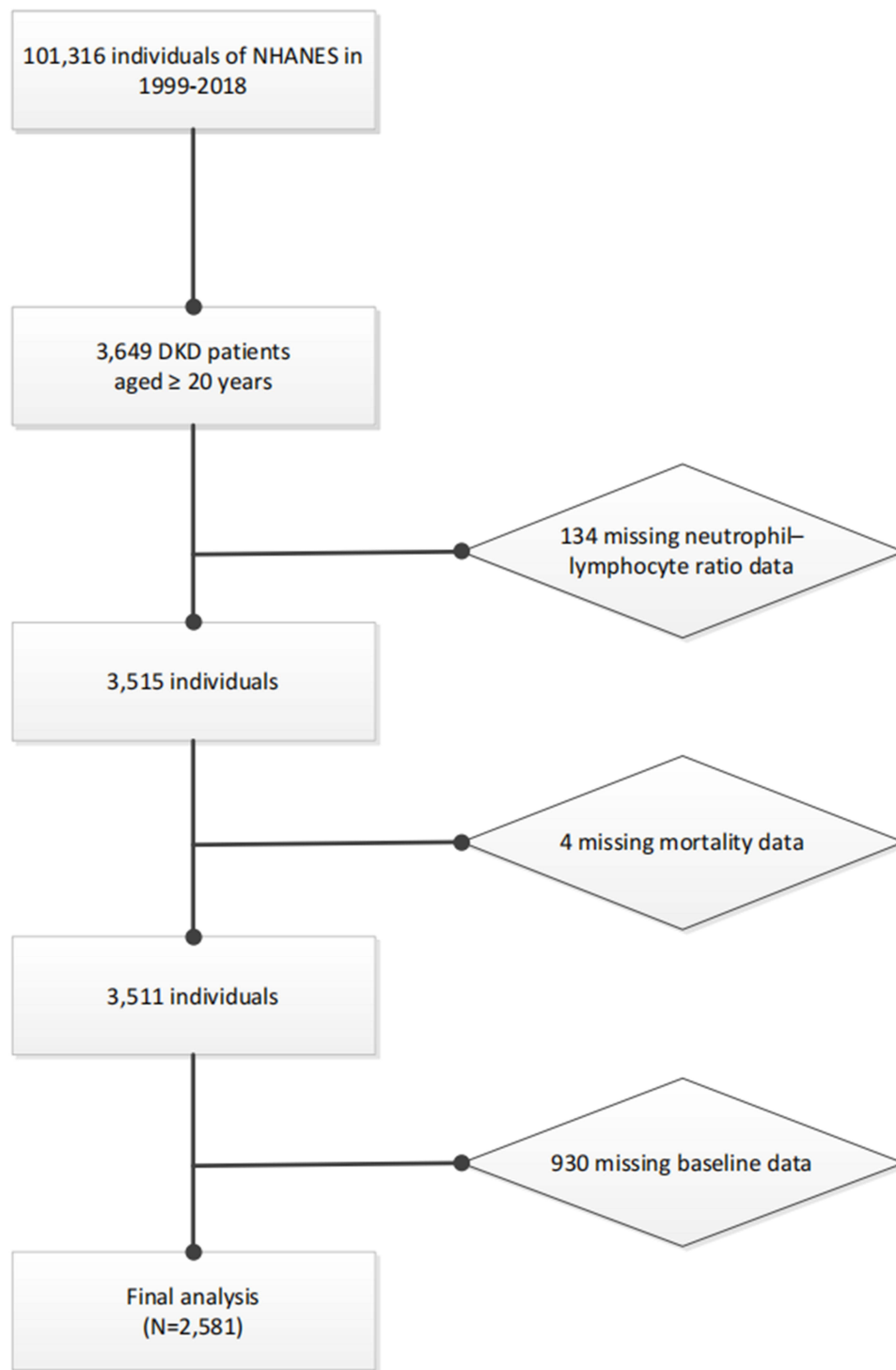
This study is a prospective cohort study utilizing data derived from the National Health and Nutrition Examination Survey (NHANES), which is a continuing, nationally representative, repeated population-based study conducted by the Centers for Disease Control (CDC) and the National Center for Health Statistics (NCHS). The study's design and methodologies have been comprehensively elucidated in previous literature.<sup>20,21</sup> We subsumed ten cycles from 1999 to 2000 through 2017 to 2018. There was a total of 3649 adults aged 20 years or older with DKD. Of this group, we excluded individuals with missing data on NLR ( $n = 134$ ) or mortality status ( $n = 4$ ). A whole number of 930 individuals with missing data on marital status, family income, education levels, body mass index (BMI), glycohemoglobin (HbA1c), serum creatinine (Scr), blood urea nitrogen (BUN), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), urinary albumin creatinine ratio (UACR), estimated glomerular filtration rate (eGFR), drinking status, smoking status, hypertension, CVD, and use of anti-infective drugs were excluded. Subsequent to these exclusions, the final analytic sample size for this investigation comprised 2581 participants (Figure 1). All study procedures were authorized by the National Center for Health Statistics Ethics Review Board prior to data collection, and each subject furnished written informed consent.

### DKD Definition and Blood Cell Counts Measurement

Diabetes was diagnosed according to the criteria of (1) fasting plasma glucose  $\geq 126$  mg/dL, or (2) 2-hour oral glucose tolerance  $\geq 200$  mg/dL, or (3) HbA1c  $\geq 6.5\%$ , or (4) administering antidiabetic medications, or (5) self-reported diabetes. The urine albumin/creatinine ratio was utilized to evaluate the ACR. The Chronic Kidney Disease Epidemiology Collaboration equation was utilized to evaluate eGFR. DKD in participants with diabetes was diagnosed according to the criteria of eGFR  $< 60$  mL/min/1.73 m<sup>2</sup> or UACR  $\geq 30$  mg/g.<sup>22,23</sup> The Beckman Coulter methodology was employed to conduct the complete blood count. The NLR was evaluated by the ratio of the absolute neutrophil count to the absolute lymphocyte count from an identical automated complete blood count specimen.

### Ascertainment of All-Cause and Cardiovascular Mortality

Mortality status and causes of death were ascertained through linkage with the National Death Index (NDI) database by the NCHS based on a probability matching algorithm. The duration of follow-up (in months) spanned from the baseline examination date to the recorded date of death or until 31 December 2019 (the latest available date of the NDI database). The International Classification of Diseases (ICD), 10th Edition, was utilized for ascertaining the primary cause of mortality. Specifically, cardiovascular mortality encompassed fatalities attributed to heart diseases (codes I00 to I09, I11, I13, I20 to I51) and cerebrovascular ailments (codes I60–I69).



**Figure 1** Flow chart of study participants.

## Covariables

Covariate selection was informed by comprehensive evidence from literature.<sup>19,22</sup> The chosen covariates encompassed age, sex, race/ethnicity, marital status, family income, education levels, BMI, HbA1c, Scr, BUN, TC, HDLC, UACR, eGFR, drinking status, smoking status, hypertension, CVD, and use of anti-infective drugs. Race/ethnicity categorization included White, Black, Mexican, and Other. Marital classifications comprised individuals who were married or living

with partner, those who had never married, and those who were widowed, divorced, or separated. Height and weight were utilized to evaluate BMI in  $\text{kg}/\text{m}^2$ . Smoking habits were delineated as never smoker, former smoker, or current smoker. Education levels were stratified into categories: less than high school, high school or equivalent, and college or above. Family income was categorized into  $<1$  (poor), 1–3 (near poor), and  $\geq 3$  (not poor).<sup>21</sup> Drinking status was characterized as follows: never drinker, former drinker, mild drinker ( $\leq 1$  drink for females or  $\leq 2$  drinks for males daily on average over the past year), moderate drinker (1–3 drinks for females or 2–4 drinks for males daily on average over the past year), and heavy drinker ( $\geq 4$  drinks for females or  $\geq 5$  drinks for males daily on average over the past year).<sup>21</sup> The average of the first three blood pressure readings was utilized to assess systolic and diastolic blood pressure. Hypertension was delineated as systolic blood pressure  $\geq 140$  mmHg and/or diastolic blood pressure  $\geq 90$  mmHg. The history of CVD, encompassing conditions such as coronary heart disease, congestive heart failure, heart attack, stroke, and angina, and use of anti-infective drugs were obtained from the standardized questionnaires. Blood samples were procured and sent to central laboratories to measure HbA1c, Scr, BUN, TC, and HDLC. Standard protocols were followed to assess the aforementioned measurements.

## Statistical Analysis

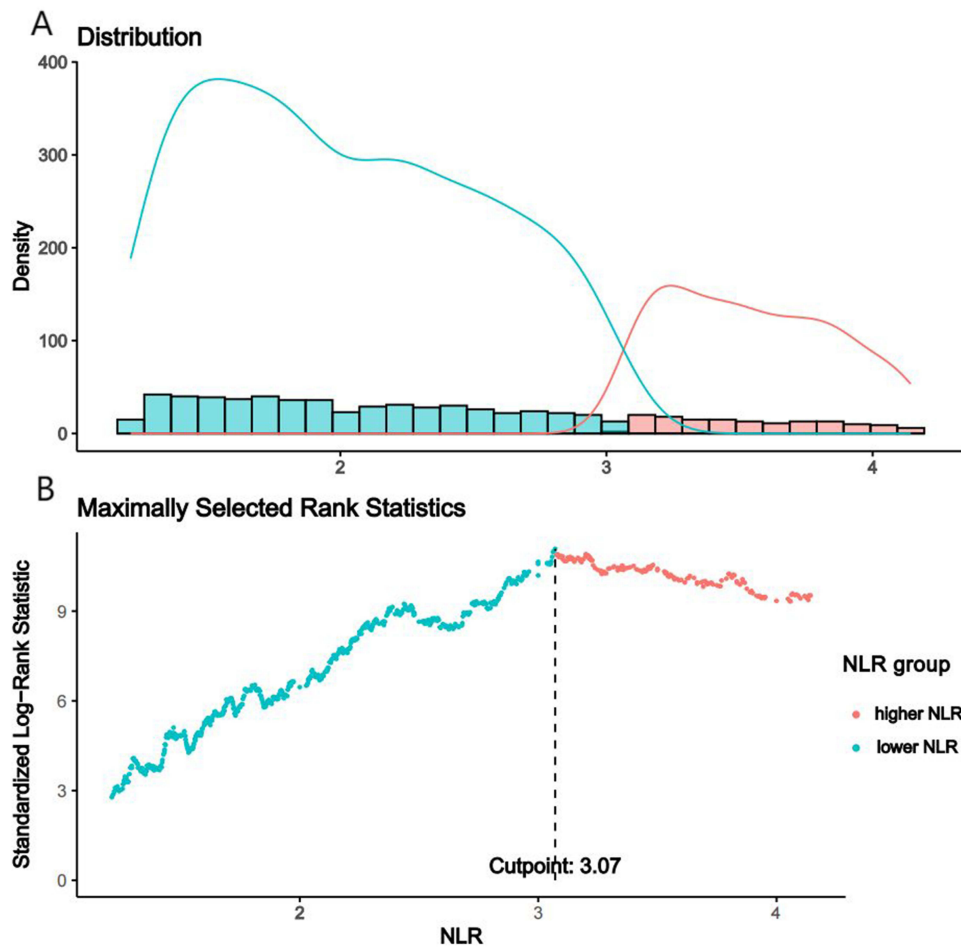
We accounted for the complex sampling design and incorporated mobile examination center sample weights into all statistical evaluations across the study to ensure nationally representative estimates as stipulated by the NHANES analytic and reporting guidelines.<sup>24</sup> Continuous variables were delineated as mean (standard deviation [SD]), while categorical variables were presented as frequency (percentages). To discern disparities between two cohorts concerning continuous variables, we employed either the weighted *t*-test or the Mann–Whitney *U*-test. Concurrently, the weighted chi-square test was engaged for dichotomous variables.

The optimum NLR threshold for predicting survival outcomes was determined through the utilization of the maximally selected rank statistics.<sup>19</sup> Subsequently, participants were stratified into cohorts delineated by low and high NLR values based on this optimal threshold. We also modeled the NLR utilizing restricted cubic spline (RCS) (3 knots at the 5th, 50th, and 95th percentiles) to flexibly explore any potentially nonlinear correlation between the NLR and all-cause mortality and cardiovascular mortality in DKD participants. We utilized weighted Cox proportional hazard regression to model the prospective correlations of the NLR with all-cause mortality and cardiovascular mortality in DKD participants. Four models were applied: a crude model; Model 1 included age, sex, race, marital status, family income, and education levels; Model 2 included all covariables in Model 1 plus drinking status and smoking status; Model 3 included all covariables in Model 2 plus BMI, HbA1c, Scr, BUN, TC, HDLC, UACR, eGFR, hypertension, CVD, and anti-infective drugs. The cumulative Kaplan–Meier (KM) method for the probabilities survival outcomes during follow-up was plotted. Age, sex, race/ethnicity, marital status, family income, education levels, BMI, drinking status, smoking status, hypertension, CVD, and anti-infective drugs were all taken into account in stratified analyses, and *P* for interaction was explored. To ascertain the precision of the NLR at various temporal intervals for predicting survival outcomes, we instituted a time-dependent receiver-operating characteristic curve (ROC) leveraging the “timeROC” package.<sup>25</sup> *P* < 0.05 denoted statistical significance. Statistical analyses were performed using R version 4.0.4 (<http://www.R-project.org>, The R Foundation).

## Results

### Subject Characteristics

The final analysis utilized data from 2581 subjects within the NHANES cohort, representing 7,514,268 subjects with DKD of the USA (Figure 1). The cohort study encompassed 624 individuals with a higher NLR ( $\geq 3.07$ ) and 1957 subjects with a lower NLR ( $< 3.07$ ), through the utilization of the optimum NLR threshold for predicting survival outcomes (Figure 2). A comprehensive characterization of the cohort is elucidated in Table 1. The mean age of 2581 individuals was 63.80 years, more than half of the subjects were male, and 65.07% were White. The subjects in the higher NLR group were more likely to be correlated with CVD, be older, male, White, former smokers, and married or living with partner, be without the use of anti-infective drugs, to have a higher white blood cell count, BUN, UACR,



**Figure 2 (A)** Depicts the distribution of lower and higher NLR and the cutoff point was determined by the maximally selected rank statistics **(B)**.

and HDLC and lower eGFR, HbA1c, and TC, relative to those in the lower NLR group ( $P < 0.05$ ). BMI, drinking status, education levels, family income, platelet count, and hypertension did not show statistical significance ( $P > 0.05$ ).

**Table 1** Characteristic of Participants

Variable	Total (n=2581)	Higher NLR (n=624)	Lower NLR (n=1957)	P value
Age, years, mean(SD)	63.80(0.36)	67.36(0.73)	62.62(0.41)	< 0.001
Sex, n(%)				0.04
Female	1209(48.50)	247(44.11)	962(49.96)	
Male	1372(51.50)	377(55.89)	995(50.04)	
Race/Ethnicity, n(%)				< 0.001
White	1075(65.07)	345(74.75)	730(61.86)	
Black	609(13.87)	90(7.96)	519(15.83)	
Mexican	514(8.96)	113(7.33)	401(9.50)	
Other	383(12.10)	76(9.96)	307(12.81)	
BMI, kg/m <sup>2</sup> , mean(SD)	32.78(0.23)	32.05(0.44)	33.03(0.26)	0.05
BMI category, n(%)				0.11
≥24	2294(90.28)	541(88.48)	1753(90.87)	
<24	287(9.72)	83(11.52)	204(9.13)	

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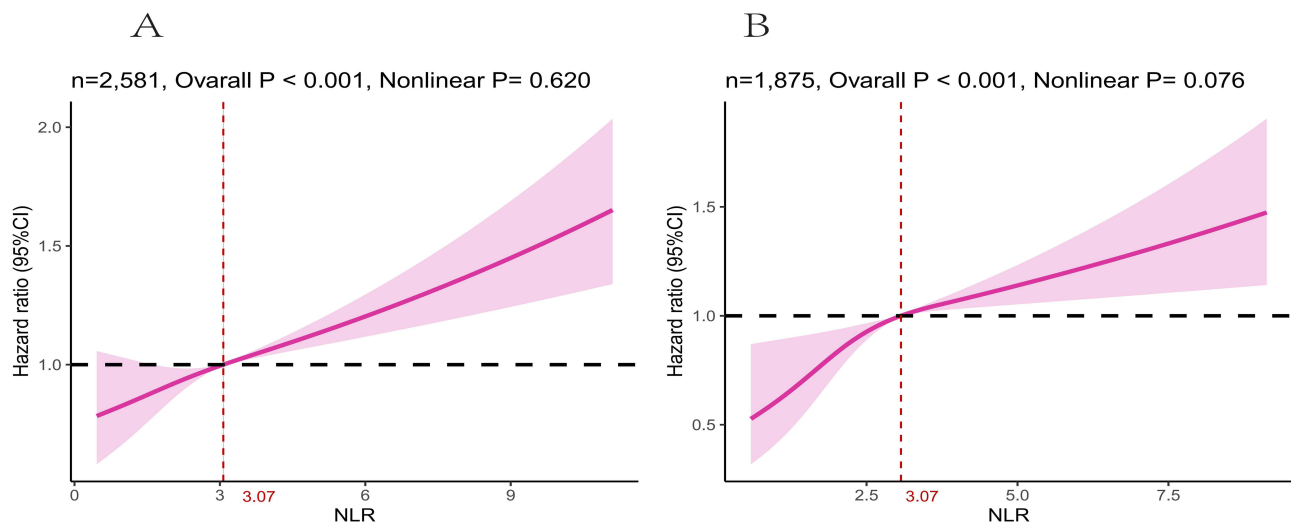
Table 1 (Continued).

Variable	Total (n=2581)	Higher NLR (n=624)	Lower NLR (n=1957)	P value
Smoking status, n(%)				< 0.001
Never	1207(46.20)	253(37.75)	954(49.00)	
Former	977(38.17)	282(48.18)	695(34.84)	
Current	397(15.64)	89(14.07)	308(16.16)	
Drinking status, n(%)				0.12
Never	497(17.68)	101(14.62)	396(18.70)	
Former	835(28.72)	223(32.69)	612(27.40)	
Mild	752(33.33)	196(34.81)	556(32.84)	
Moderate	207(9.20)	50(9.38)	157(9.15)	
Heavy	290(11.07)	54(8.50)	236(11.92)	
Education levels, n(%)				0.14
Less than high school	1032(29.20)	233(26.23)	799(30.18)	
High school or equivalent	584(26.00)	159(29.37)	425(24.87)	
College or above	965(44.81)	232(44.39)	733(44.94)	
Marital status, n(%)				0.002
Never married	180(7.17)	27(3.27)	153(8.47)	
Married or living with partner	1432(59.51)	343(59.93)	1089(59.37)	
Widowed, divorced, or separated	969(33.32)	254(36.80)	715(32.16)	
Family income, n(%)				0.7
Not poor	675(35.84)	163(34.96)	512(36.13)	
Near poor	1286(46.71)	323(48.58)	963(46.09)	
Poor	620(17.45)	138(16.46)	482(17.78)	
NLR	2.64(0.05)	4.62(0.12)	1.99(0.02)	< 0.001
WBC, $\times 10^9/L$ , mean(SD)	7.92(0.07)	8.67(0.14)	7.67(0.09)	< 0.001
Neutrophil, $\times 10^9/L$ , mean(SD)	4.87(0.05)	6.28(0.12)	4.40(0.05)	< 0.001
Lymphocyte, $\times 10^9/L$ , mean(SD)	2.15(0.04)	1.48(0.03)	2.37(0.05)	< 0.001
Platelet, $\times 10^9/L$ , mean(SD)	243.37(2.13)	237.56(4.09)	245.29(2.35)	0.09
Scr, mg/dl, mean(SD)	1.17(0.02)	1.36(0.06)	1.11(0.02)	< 0.001
BUN, mg/dl, mean(SD)	19.36(0.27)	22.02(0.69)	18.47(0.26)	< 0.001
eGFR, mL/min/1.73m <sup>2</sup> , mean(SD)	69.81(0.78)	61.09(1.75)	72.71(0.81)	< 0.001
UACR, g, mean(SD)	309.72(22.49)	430.95(56.59)	269.44(22.86)	0.01
HbA1C, %, mean(SD)	7.38(0.05)	7.15(0.10)	7.46(0.05)	0.01
TC, mg/dl, mean(SD)	190.03(1.21)	180.16(2.54)	193.31(1.27)	< 0.001
HDLC, mg/dl, mean(SD)	47.95(0.51)	50.46(1.69)	47.11(0.48)	0.07
Hypertension, n(%)				0.86
No	472(18.96)	122(19.24)	350(18.86)	
Yes	2109(81.04)	502(80.76)	1607(81.14)	
CVD, n(%)				< 0.001
No	1687(66.66)	348(58.18)	1339(69.48)	
Yes	894(33.34)	276(41.82)	618(30.52)	
Anti-infective drugs				0.004
No	2428(93.49)	579(90.14)	1849(94.60)	
Yes	153(6.51)	45(9.86)	108(5.40)	

Notes: Data are presented as mean (SD) or n (%).

## NLR and All-Cause Mortality

Over a median follow-up of 79 months (interquartile range (IQR), 44–128 months), there were 1103 (42.74%) deaths, including 397 (15.38%) from cardiovascular causes and 706 (27.35%) from non-cardiovascular causes. The NLR exhibited a positive linear association with mortality in DKD subjects according to the RCS analysis (nonlinear  $P = 0.62$ ) (Figure 3A). With respect to the higher-NLR group (Table 2), the NLR led to a 2-fold increased all-cause mortality



**Figure 3** Association between NLR and all-cause (A) and cardiovascular mortality (B) among DKD visualized by restricted cubic spline (NLR breakpoint: 3.07). Adjusted for age, sex, race, marital status, family income, education levels, BMI, HbA1c, Scr, BUN, TC, HDLC, UACR, eGFR, drinking status, smoking status, hypertension, CVD and anti-infective drugs. Both P value for nonlinearity > 0.05.

risk in the crude model (hazard ratio (HR) = 2.10, 95% CI = 1.77, 2.49,  $P < 0.001$ ). In multivariable models, the NLR led to an approximate doubling of the HRs in Model 1 (HR = 1.74, 95% CI = 1.48, 2.05,  $P < 0.001$ ) and Model 2 (HR = 1.72, 95% CI = 1.47, 2.02,  $P < 0.001$ ). In Model 3, participants with higher NLR have an up to 1.51-fold increased relative risk for all-cause mortality (HR = 1.51, 95% CI = 1.28, 1.77,  $P < 0.001$ ), respectively.

### NLR and Cardiovascular Mortality

A total of 1875 DKD patients, incorporating 390 having higher NLR and 1485 having lower NLR, were taken to evaluate the correlation of the NLR with cardiovascular mortality apart from 706 non-cardiovascular deaths. A non-linear correlation was identified between the NLR and the HR of cardiovascular mortality after adjusted for age, sex, race, marital status, family income, education levels, BMI, HbA1c, Scr, BUN, TC, HDLC, UACR, eGFR, drinking status, smoking status, hypertension, CVD, and anti-infective drugs (Figure 3B). The Cox proportional hazard regression model indicated that DKD patients with higher NLR had an increased risk of cardiovascular mortality (HR = 2.34, 95% CI = 1.79, 3.06,  $P < 0.001$ ). The risk of cardiovascular mortality remained significant after adjusting for full adjustment (Model 3, HR = 1.71, 95% CI = 1.32, 2.21,  $P < 0.001$ ), manifesting that each one-unit increase in the NLR value was correlated with a 71% increased risk of cardiovascular mortality (Table 2).

**Table 2** The Relationships Between NLR and Mortality in DKD

Characteristic	Crude model HR(95% CI)	P	Model 1 HR(95% CI)	P	Model 2 HR(95% CI)	P	Model 3 HR(95% CI)	P
All-cause mortality								
Lower NLR(n=1957)	Ref		Ref		Ref		Ref	
Higher NLR(n=624)	2.10(1.77,2.49)	<0.001	1.74(1.48,2.05)	<0.001	1.72(1.47,2.02)	<0.001	1.51(1.28,1.77)	<0.001
Cardiovascular mortality								
Lower NLR(n=1485)	Ref		Ref		Ref		Ref	
Higher NLR(n=390)	2.34(1.79,3.06)	<0.001	1.95(1.51,2.51)	<0.001	1.92(1.50,2.46)	<0.001	1.71(1.32,2.21)	<0.001

**Notes:** Crude model, unadjusted; Model 1, adjusted for age, sex, race, marital status, family income, and education levels; Model 2, adjusted for age, sex, race, marital status, family income, education levels, drinking status, and smoking status; Model 3, adjusted for age, sex, race, marital status, family income, education levels, BMI, HbA1c, Scr, BUN, TC, HDLC, UACR, eGFR, drinking status, smoking status, hypertension, CVD, and anti-infective drugs.

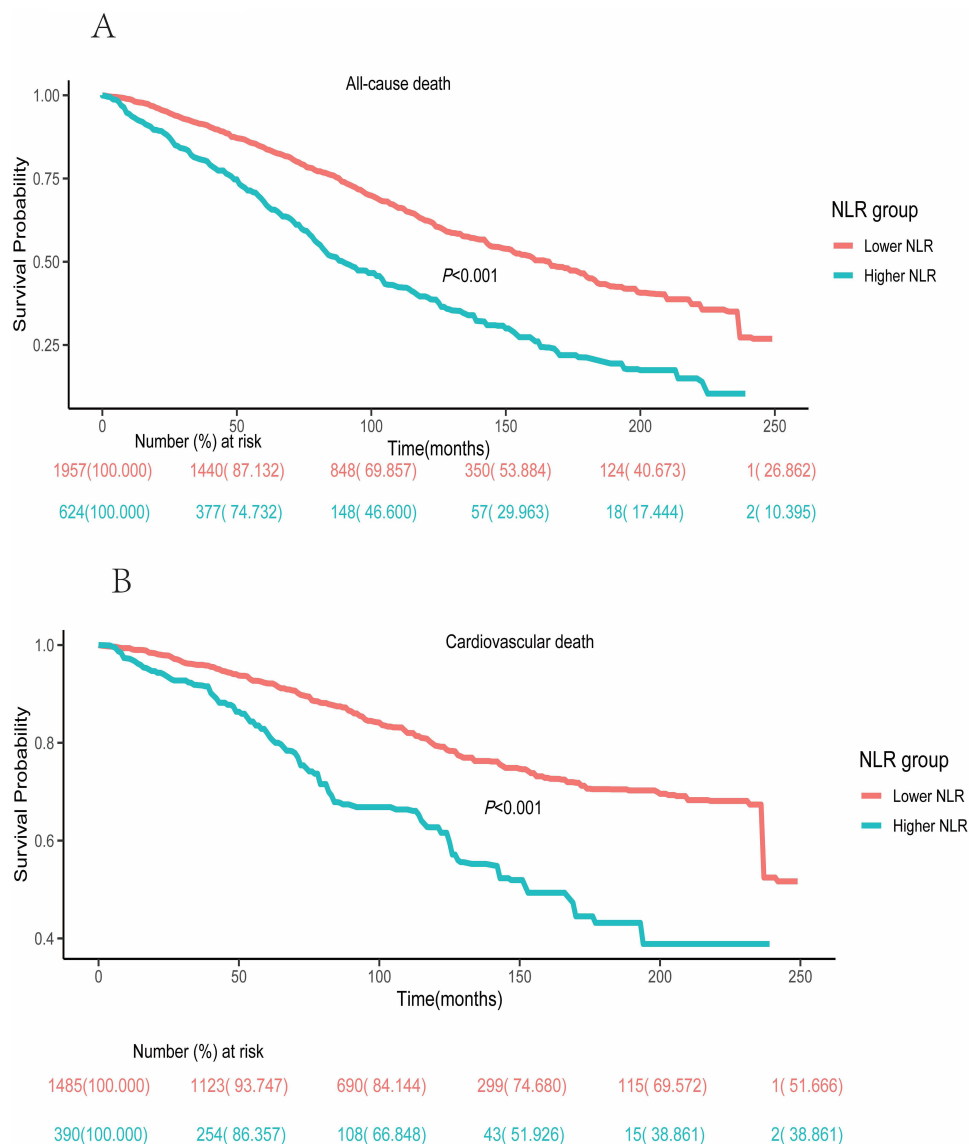


## Survival Analysis

The KM analysis for all-cause mortality underscored a marked disparity between the higher- and lower-NLR groups ( $P < 0.001$ ) (Figure 4A). The results of KM survival rates for cardiovascular mortality indicated that the survival rate was diminished in DKD patients with the higher NLR ( $P < 0.001$ ) (Figure 4B).

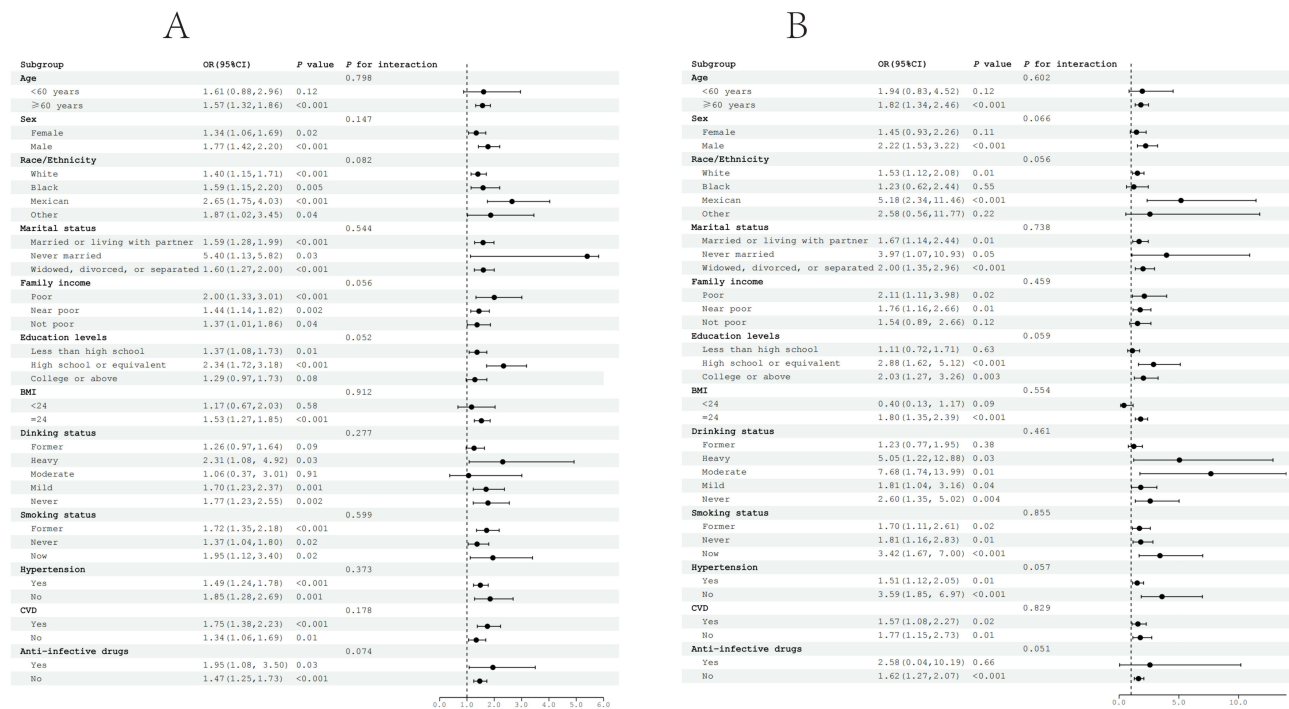
## Stratification Analysis and Ascertainment of the Prognostic Ability

Concurrently, sensitivity analyses were meticulously stratified across a spectrum of variables, including age, sex, race/ethnicity, marital status, family income, education levels, BMI, drinking status, smoking status, hypertension, CVD, and anti-infective drugs, to discern the correlation of the NLR with all-cause (Figure 5A) and cardiovascular mortality (Figure 5B). We investigated the heterogeneity within each subgroup using interaction terms and no significant difference was uncovered ( $P$  for interaction  $> 0.05$  for all), revealing that the results were largely consistent when the individuals were divided into different subgroups. In assessing the predictive performance of the NLR for all-cause and cardiovascular mortality in DKD using time-dependent ROC analysis, it was determined that the NLR exhibited superior



**Figure 4** Kaplan–Meier analysis of the survival rate and the number (%) of at risk DKD patients with higher ( $>3.07$ ) and lower ( $<3.07$ ) NLR values. **(A)** All-cause mortality. **(B)** Cardiovascular mortality.





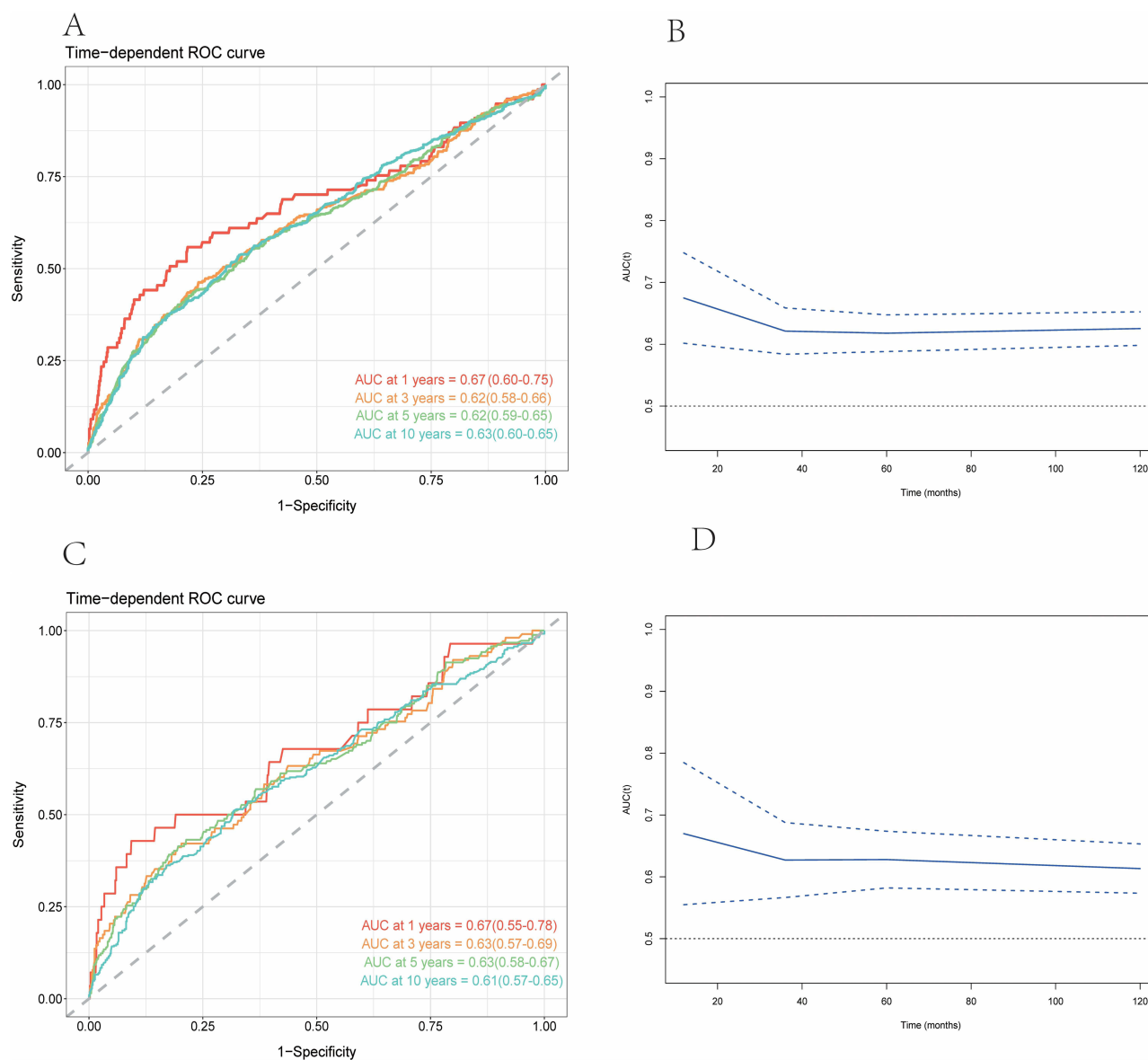
**Figure 5** Subgroup analysis of the associations between NLR and mortality among DKD. Adjusted for age, sex, race, marital status, family income, education levels, BMI, HbA1c, Scr, BUN, TC, HDLC, UACR, eGFR, drinking status, smoking status, hypertension, CVD, and anti-infective drugs. (A) All-cause mortality. (B) Cardiovascular mortality.

predictive value for both all-cause and cardiovascular mortality across short-term and extended durations (1-year, 3-year, 5-year, and 10-year periods) (Figure 6).

## Discussion

In this large-sample nationally representative cohort investigation, we explored the correlation of the NLR with survival outcomes among adults with DKD utilizing the NHANES data collected from 1999 to 2000 through 2017 to 2018. To the best of our knowledge, this study is the first to prospectively examine the relationship of the NLR with mortality in DKD. Our findings underscore that higher NLR levels corresponded with augmented incidences of both all-cause and cardiovascular mortality in a medium follow-up time of 79 months. After adjusting for confounders, the NLR was still significantly correlated to increasing all-cause (HR = 1.51, 95% CI: 1.28, 1.77) and cardiovascular mortality (HR = 1.71, 95% CI: 1.32, 2.21). A variety of stratified analyses and sensitivity analyses demonstrated the robustness of our findings. These results emphasize the promising use of the NLR in stratifying and prognosticating the risk of mortality in DKD in clinical practice.

Emerging scholarly consensus postulates that inflammation significantly mediates the onset and progression of DKD.<sup>6-8</sup> The NLR affords insights into the individual's systemic inflammation and immune system activation. A high neutrophil count signifies persistent inflammatory cascades, while a low lymphocyte count signifies an insufficiency in the regulatory and dormant immune pathways. Preclinical evidence supports that a recruitment of immune infiltrating cells into the kidney, including neutrophils and lymphocytes, releases inflammatory mediators, resulting in formulating a feedback inflammatory loop that enhances chronic inflammation and involves the progression of DKD.<sup>6,26,27</sup> In clinical investigations, the NLR has been widely established as a harbinger for the progression of DKD.<sup>14,28-31</sup> In a longitudinal cohort investigation spanning 3 years, Azab et al reported that increased NLR was correlated with an increased risk of worsening renal function in diabetes.<sup>14</sup> In cross-sectional studies, the NLR has proven to be a prognostic marker for DKD among Indian,<sup>29</sup> Syrian,<sup>28</sup> Chinese,<sup>30</sup> and Turkish populations.<sup>31</sup> With respect to CVD, a systematic review and meta-analysis comprising 76,002 participants reported that persons with higher NLR had a higher risk for CVD,<sup>32</sup> and



**Figure 6** Time-dependent ROC curves and time-dependent AUC values of the NLR for predicting all-cause mortality (**A** and **B**) and cardiovascular mortality (**C** and **D**).

a prospective cohort study comprising 338 diabetic patients in the USA found that participants who had higher NLR had a higher risk for major adverse cardiac events.<sup>33</sup> These results validate our findings of the NLR being an independent factor of adverse outcomes in DKD patients.

Prior investigations have delineated correlations between higher NLR levels and mortality risks across a spectrum of diseases.<sup>19,34–36</sup> In diabetes, after a median follow-up 91 months, Dong and colleagues showed that persons with higher NLR had a 2.03-fold higher risk for all-cause mortality and 2.76-fold higher risk for cardiovascular mortality.<sup>19</sup> In amputated diabetic foot, higher NLR carried a 1.1 times elevated risk of 1-year mortality.<sup>34</sup> In ESKD patients undergoing peritoneal dialysis, the NLR was an independent predictor of all-cause and cardiovascular mortality.<sup>35</sup> In ESKD patients undergoing hemodialysis, higher pre-dialysis NLR was linked with higher risk of short-term all-cause mortality.<sup>36</sup> Nevertheless, the available evidence is scarce on the linkage of the NLR with all-cause and cardiovascular mortality in DKD. Our investigation effectively bridges this lacuna, furnishing novel evidence accentuating the NLR's pivotal role as a standalone predictor of all-cause and cardiovascular mortality in DKD.

Metabolic inflammation is recognized as playing a pivotal role in the mechanisms linking DKD and CVD risk.<sup>37</sup> An increase in neutrophils can exacerbate chronic inflammation,<sup>38</sup> while decreased lymphocyte counts contribute to a weakening of immune defenses, resulting in DKD patients with higher NLR being at increased risk of developing CVD. With respect to the mechanisms, firstly, increased metabolic endotoxemia leads to metabolic inflammation. In the Chronic Renal Insufficiency Cohort (CRIC) study, the accumulation of several uremic solutes in DKD, such as asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and trimethylamine N-oxide (TMAO), resulted in a 1-fold or 2-fold increased risk of cardiovascular mortality.<sup>39</sup> Secondly, lipid accumulation likely serves as a core element in metabolic inflammation. Emerging evidence suggests that lipid metabolism disorders in patients with DKD were linked to inflammation and cardiovascular disease.<sup>40,41</sup> Of note, both innate and adaptive immune responses play a crucial role in initiating metabolic inflammation linked to DKD and cardiovascular disease. Neutrophils representing the innate immune response contribute to inflammatory reaction by releasing inflammatory mediators that lead to dysfunction of the vascular wall, while lymphocytes (representing the adaptive immune response) can either enhance or dampen inflammatory responses.<sup>37</sup> In our study, our findings revealed that a higher NLR was correlated with increased all-cause and cardiovascular mortality in a population of patients with DKD, which holds important suggestions for clinical practice and patient care.

The present study has several strengths. First, this study included a large sample-scale population-based sample with extended follow-up periods (a median follow-up of 79 months) and a variety of stratified analyses and sensitivity analyses, thus providing more robust evidence. Second, this is the first study to explore the role of the NLR in the all-cause and cardiovascular mortality in DKD. Several limitations cannot be ignored. First, we cannot exclude the potentiality that the NLR is influenced by latent confounding variables. Second, the data included in this study were only accessible at baseline, which means that it is uncertain whether changes in NLR levels during the follow-up period still predict all-cause and cardiovascular mortality in DKD. Third, this study was conducted among individuals with DKD in the United States. Therefore, the generalizability of the conclusions to other populations warrants further exploration.

## Conclusions

In sum, our expansive, nationally representative cohort study indicates the NLR is a predictor of all-cause and cardiovascular mortality in DKD. The findings emphasize the promising use of the NLR in stratifying and prognosticating the risk of mortality in DKD in clinical practice.

## Data Sharing Statement

The National Health and Nutrition Examination Survey dataset is publicly available at the National Center for Health Statistics of the Centers for Disease Control and Prevention (<https://www.cdc.gov/nchs/nhanes/index.htm>).

## Ethics Approval and Informed Consent

The NHANES 1999–2018 was approved by the NCHS Research Ethics Review Board (Continuation of Protocol #1999–2018), and each participant signed the written informed consent.

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## Disclosure

The authors declare that they have no competing interests in this work.

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