Article

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Association of Serum Cystatin C with Creatinine-Defined Mildly Reduced eGFR: A Cross-Sectional Analysis of eGFR G2 Versus G1 Categories

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Background: Mild reductions in renal function within the estimated glomerular filtration rate (eGFR) range of 60–89 mL/min/1.73 m^2 may not be detected by routine creatinine-based screening, yet early recognition of subclinical nephropathy at this stage is essential for preventing irreversible renal damage. This study examined the association of serum cystatin C (CysC) with creatinine-defined mildly reduced eGFR and evaluated whether CysC improved discrimination between creatinine-based eGFR G2 and G1 categories.

Methods: We analyzed 422 participants in a cross-sectional study. Participants were classified into an occult renal function decline group (eGFR 60–89 mL/min/1.73 m^2, n = 218) and a healthy control group (eGFR ≥90 mL/min/1.73 m^2, n = 204). A multivariable discrimination model was built by integrating CysC with conventional risk factors including age, sex, glucose, low-density lipoprotein cholesterol (LDL-C), and high-sensitivity C-reactive protein (hs-CRP). Restricted Cubic Splines (RCS), Net Reclassification Improvement (NRI), and Decision Curve Analysis were employed to assess the shape of the association and the added clinical value of CysC.

Results: The Joint Model incorporating standardized CysC demonstrated superior discrimination (Area Under the Curve (AUC) 0.892, 95% CI 0.862–0.922) compared to the base model relying on traditional risk factors (AUC 0.725, 95% CI 0.677–0.773). RCS analysis suggested a non-linear inflection point at 1.03 mg/L. The addition of CysC yielded a substantial continuous NRI of 1.0691 ($p < 0.001$), improving reclassification between the two creatinine-defined eGFR categories. A nomogram was constructed to facilitate clinical translation.

Conclusion: Serum CysC was strongly associated with creatinine-defined mildly reduced eGFR and improved discrimination between creatinine-based eGFR G2 and G1 categories, in this cross-sectional cohort. These findings should be interpreted as evidence of association and classification performance rather than proof that CysC independently predicts true occult renal function decline. Prospective studies using measured GFR and longitudinal renal outcomes are needed for confirmation.

Keywords: serum cystatin C; occult renal function decline; nomogram; restricted cubic spline; creatinine-based eGFR; glomerular filtration rate

Introduction

Chronic kidney disease (CKD) has emerged as an escalating public health burden globally. According to the Global Burden of Disease Study 2023, an estimated 788 million adults worldwide were affected by CKD, representing a marked increase from 378 million in 1990 [1]. CKD is now the ninth leading cause of death globally and is linked to 1.48 million deaths annually [1]. The burden of CKD extends beyond direct mortality. Impaired kidney function contributes to 11.5% of all cardiovascular deaths [1]. This close cardio-renal relationship underscores the critical importance of early detection and timely intervention.

CKD usually develops slowly, with glomerular filtration rate (GFR) declining over years before symptoms become evident. The stage characterized by an estimated GFR (eGFR) of 60–89 mL/min/1.73 m^2, classified as G2 by kidney disease: improving global outcomes (KDIGO), represents a critical yet frequently overlooked window [2]. Individuals within this range may already have subclinical renal vulnerability while exhibiting no clinical manifestations. A 2023 cohort study demonstrated that even mild renal dysfunction was associated with higher all-cause mortality (adjusted hazard ratio (HR) 1.36, 95% CI 1.07–1.70) and more major adverse cardiovascular events in patients with coronary artery disease [3]. A recent JACC publication also confirmed that slight renal dysfunction within this range carries major prognostic implications for comprehensive cardiovascular outcomes, including cardiovascular death [4]. These findings indicate that delaying action until eGFR falls below 60 mL/min/1.73 m^2 may miss an earlier opportunity for intervention.

In routine clinical practice, serum creatinine continues to serve as the principal biomarker for GFR estimation owing to its wide availability and low cost [5,6]. Nevertheless, this marker has well-recognized biological limitations [6]. Creatinine generation depends heavily on muscle mass, dietary protein intake, age, and sex [7,8]. Specifically, a young muscular male may present with elevated creatinine despite normal kidney function, whereas an elderly woman with low muscle mass may exhibit near-normal creatinine despite substantially reduced GFR. This phenomenon, termed the creatinine-blind GFR range, is particularly relevant in mildly reduced kidney function, approximately GFR 60–90 mL/min/1.73 m^2 [9,10,11]. A 2021 study quantified this limitation and showed that creatinine-based eGFR had a sensitivity of only 68.4% for detecting CKD stage 3 in cachectic patients, and a specificity of merely 47.4% in highly muscular individuals [12]. Only 24–38% of eGFR met the clinically relevant P10 accuracy criterion [12]. The 2024 KDIGO Clinical Practice Guideline explicitly acknowledges that creatinine-based eGFR may be unreliable in individuals with extremes of body size, reduced muscle mass, or specific comorbidities [2].

Cystatin C (CysC) presents an attractive alternative. As a 13.3-kDa cysteine protease inhibitor, CysC is generated at a comparatively constant rate by all nucleated cells [6,13]. Unlike creatinine, CysC production is independent of muscle mass, age, sex, and dietary intake [14,15,16]. It undergoes free glomerular filtration and is then almost entirely reabsorbed and degraded by proximal tubular cells, with negligible tubular secretion [17,18]. These properties render CysC a reliable endogenous indicator of glomerular filtration. A landmark meta-analysis by the CKD Prognosis Consortium, in which over 90,000 participants from 11 general-population cohorts were encompassed, demonstrated that CysC-based eGFR exhibited stronger and more linear associations with mortality and cardiovascular events than creatinine-based eGFR [19]. Among participants with creatinine-based eGFR of 60–89 mL/min/1.73 m^2, 14% were reclassified to CysC-based eGFR below 60 mL/min/1.73 m^2, and these individuals experienced a 57% higher adjusted mortality risk [19]. For all-cause mortality, the net reclassification improvement reached 0.23 (95% CI 0.18–0.28) [19]. A 2022 UK Biobank study corroborated these findings, concluding that CysC-based eGFR was more sensitive and specific for cardiovascular disease and mortality risks in mild CKD [20]. The LIPID study, with 16 years of follow-up, established that baseline CysC independently predicted cardiovascular events, CKD development, and long-term mortality even after adjustment for the combined creatinine-cystatin C eGFR equation [21].

Despite this evidence, the clinical utility of CysC for identifying occult renal function decline remains incompletely characterized. While CysC has been validated extensively for risk prediction in established CKD and general populations, its performance specifically in individuals with eGFR 60–89 mL/min/1.73 m^2 range requires further investigation. The dose-response relationship between CysC concentration and the probability of occult renal decline also remains uncertain. Moreover, practical risk-stratification tools, such as nomograms incorporating CysC with routine metabolic variables, are still limited for the population with early-stage renal dysfunction.

To address these issues, we evaluated the predictive performance of serum CysC for occult renal function decline defined as eGFR 60–89 mL/min/1.73 m^2, and compared the performance with traditional markers. We then developed a multivariable model that incorporated CysC with routine risk factors. We also visualized the non-linear dose-response relationship using restricted cubic spline analysis to identify clinically actionable thresholds, and quantified reclassification improvement through net reclassification improvement (NRI) and integrated discrimination improvement (IDI). The model was converted into a practical nomogram and assessed by bootstrap resampling and decision curve analysis. This investigation was to clarify whether CysC can support earlier, more active screening in the creatinine-blind range.

Methods

Study Design and Setting

We performed a retrospective analysis using pre-existing clinical records from patients who visited the outpatient clinic or were admitted to the Department of Nephrology at Zhoushan Hospital between January 2022 and December 2024. The objective was to evaluate the association with creatinine-defined mildly reduced eGFR in a hospital-based cohort.

Study Population

Participants were recruited from both outpatient clinics and inpatient wards. A total of 1048 individuals were initially assessed for eligibility during the study period. Of these, 626 were excluded; among these, 594 had an eGFR <60 mL/min/1.73 m^2 (established CKD stage 3–5), 8 were younger than 18 years, and 24 met one or more of the other predefined exclusion criteria. The remaining 422 participants constituted the final analytical cohort, comprising 204 controls (eGFR ≥90 mL/min/1.73 m^2) and 218 individuals with occult renal function decline (eGFR 60–89 mL/min/1.73 m^2). The participant selection process, summarized in Fig. 1, was reported in accordance with the STROBE guidelines for observational studies.

Participants were included if they met all of the following criteria:

- Complete clinical and laboratory data;
- Age ≥18 years;
- Available serum creatinine and cystatin C measurements obtained on the same day.

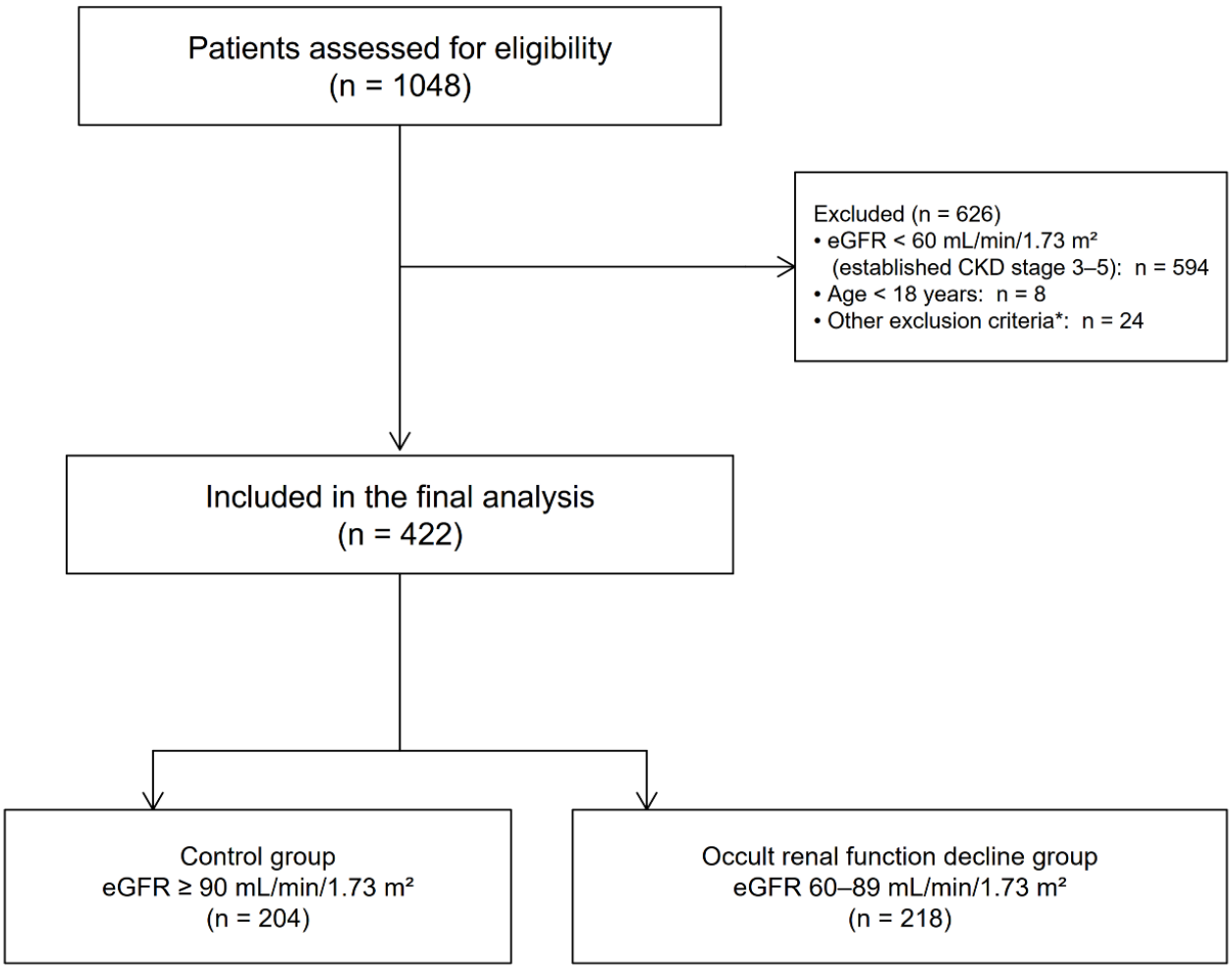

Fig. 1. Flow diagram of participant selection. Of 1048 individuals, the remaining 422 participants formed the final analytical cohort and were classified according to the CKD-EPI 2009 creatinine-based eGFR. The diagram is reported in accordance with the STROBE guideline for observational studies. CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

Eligibility was defined as eGFR ≥60 mL/min/1.73 m^2, calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 creatinine-based equation. This threshold defined the study cohort, which was subsequently divided into the two groups. Participants were excluded if they met any of the following criteria:

● Acute kidney injury (defined as an increase in serum creatinine ≥0.3 mg/dL within 48 hours or ≥1.5 times baseline within 7 days);

● History of kidney transplantation or renal replacement therapy;

● Active malignancy or receiving chemotherapy;

● Pregnancy or lactation;

● Thyroid dysfunction (hyperthyroidism or hypothyroidism), as thyroid hormones may affect serum cystatin C levels.

Laboratory Measurements

Morning fasting venous blood samples were drawn and handled in line with routine laboratory procedures. Serum cystatin C measurement was performed on an automated biochemistry analyzer (Roche Cobas c701, Roche, Mannheim, Germany) using a particle-enhanced immunoturbidimetric assay (PETIA). The assay was calibrated with the manufacturer-matched calibrator, with calibration traceable to the international certified reference material

ERM-DA471/IFCC. The limit of detection was 0.1 mg/L, and the analytical measuring range was 0.4–8.0 mg/L. The intra-assay and inter-assay coefficients of variation (CVs) were 3% and 5%, respectively. Serum creatinine was measured by the enzymatic method on the same platform, with calibration traceable to isotope-dilution mass spectrometry (IDMS). High-sensitivity C-reactive protein was measured using latex-enhanced immunoturbidimetry, fasting glucose by the hexokinase method, and the lipid panel by enzymatic methods, all on the same analytical platform. All assays were performed in the centralized clinical laboratory of Zhoushan Hospital under routine internal quality control.

Definition of Outcomes and Groups

Estimation of Glomerular Filtration Rate

The eGFR was calculated using the 2009 CKD-EPI creatinine-based equation [22] (1 mg/dL = 88.4 µmol/L):

- For females with Scr ≤0.7 mg/dL: eGFR = 144 × (Scr/0.7)^−0.329 × 0.993^Age
- For females with Scr >0.7 mg/dL: eGFR = 144 × (Scr/0.7)^−1.209 × 0.993^Age
- For males with Scr ≤0.9 mg/dL: eGFR = 141 × (Scr/0.9)^−0.411 × 0.993^Age
- For males with Scr >0.9 mg/dL: eGFR = 141 × (Scr/0.9)^−1.209 × 0.993^Age

where Scr is serum creatinine in mg/dL.

The 2009 creatinine-based equation was used for the primary analysis to define the study groups, because the research question specifically concerns the limitations of creatinine within its blind range (eGFR 60–89 mL/min/1.73 m^2). Defining the outcome with a cystatin C-based equation would render cystatin C both an exposure and a determinant of the outcome. To ensure that the conclusions were not dependent on the choice of equation, prespecified sensitivity analyses were performed using the race-free 2021 CKD-EPI creatinine and creatinine-cystatin C equations [23], both of which yielded concordant results.

Definition of Creatinine-Defined eGFR Categories

Eligible participants (eGFR ≥60 mL/min/1.73 m^2) were classified into two groups according to the same CKD-EPI 2009 creatinine-based eGFR:

- Control group: eGFR ≥90 mL/min/1.73 m^2 (n = 204).
- Occult renal function decline group: 60 ≤ eGFR < 90 mL/min/1.73 m^2 (n = 218).

Thus, the eligible range (eGFR ≥60) corresponds exactly to the union of the two groups. Participants with eGFR <60 mL/min/1.73 m^2 (established CKD G3–G5) were not eligible.

Although an eGFR of 60–89 mL/min/1.73 m^2 does not by itself meet the eGFR-based diagnostic threshold for CKD according to KDIGO guidelines [2] in the absence of kidney damage markers, accumulating evidence suggests that individuals within this range already exhibit subclinical renal vulnerability and are at increased risk for future CKD progression and cardiovascular events. Therefore, we refer to this stage as occult renal function decline rather than overt CKD.

Statistical Analysis

Data management and statistical analyses were performed using R software (version 4.5.2; R Foundation for Statistical Computing, Vienna, Austria). The analysis was conducted using the R packages pROC, rms, rmda, tableone, and ggplot2. Statistical significance was set at a two-sided p-value < 0.05.

Descriptive Statistics

Normality of continuous variables was tested using the Kolmogorov-Smirnov and Shapiro-Wilk test, together with visual inspection of histograms and Q-Q plots. Variables conforming to a normal distribution (skewness <0.5 and non-significant normality tests) were expressed as mean ± standard deviation (SD) and compared using the independent Student's t-test. Skewed variables (skewness ≥0.5 or significant normality tests) were expressed as median with interquartile range (IQR), and between-group differences were tested using the Mann-Whitney U test. Categorical data, expressed as counts and percentages, were compared with the chi-square test.

Data Transformation and Handling

Skewed variables, including hs-CRP, systemic immune-inflammation index (SII), and albumin-to-creatinine ratio (ACR), were log-transformed as ln(x + 0.1) before modelling to reduce skewness and better meet regression assumptions. Serum CysC levels were standardized to Z-scores (mean = 0, SD = 1) to allow odds ratios (ORs) to be interpreted per 1-SD increase. The Neutrophil-to-Lymphocyte Ratio (NLR) was categorized into quartiles. A complete-case analysis was performed. Participants with missing data on any of the covariates included in the multivariable models were excluded from the final analytical cohort.

Model Development

Multivariable logistic regression analysis was conducted to estimate the ORs and 95% confidence intervals (CIs) for the association between predictors and occult renal function decline. We constructed two primary models:

- Base Model: Age, Sex, Fasting Glucose (GLU), and LDL Cholesterol (LDL-C).
- Joint Model: The base-model variables plus log-transformed hs-CRP and standardized cystatin C (CysC_z).

Multicollinearity among predictors was assessed using the Variance Inflation Factor (VIF), with a cutoff value of 5 indicating significant collinearity. To evaluate the incremental predictive value of serum cystatin C beyond traditional risk factors, we calculated the NRI and IDI. The

shape of the association between serum cystatin C and the risk of occult renal function decline was visualized using restricted cubic splines (RCS) with three knots.

The predicted probability of occult renal function decline was estimated using the following logistic regression model:

$p = 1 / (1 + e^{(-L)})$,

where $L = -0.5928 + 0.0527 \times \text{Age} - 0.8696 \times \text{Sex(Male)} - 0.0624 \times \text{Glucose} - 0.2804 \times \text{LDL-C} - 0.2178 \times \ln(\text{hs-CRP} + 0.1) + 2.4268 \times [(\text{cystatin C} - 1.0715)/0.2548]$, with age in years, glucose and LDL-C in mmol/L, hs-CRP and cystatin C in mg/L, and Sex(Male) = 1 for men, 0 for women.

Because the primary outcome was defined using the CKD-EPI 2009 creatinine-based equation, which incorporates age and sex, the coefficients for age and sex in the prediction model should not be interpreted as fully independent biological predictors of renal function decline. These variables were retained to reflect routine demographic adjustment and to evaluate whether serum cystatin C provided additional discriminatory information beyond the demographic and metabolic information available in standard creatinine-based assessment. The prediction formula and nomogram should be interpreted as tools for identifying creatinine-defined occult renal function decline, rather than as evidence that all included predictors independently determine true measured GFR or kidney injury.

Among the inflammatory and hematologic markers examined, log-transformed high-sensitivity C-reactive protein (hs-CRP) was prespecified as the inflammatory term in the primary joint model, as it is a standardized, single-analyte biomarker of systemic inflammation consistent with the study's metabolic-inflammatory framework. The SII and the NLR are derived from overlapping leukocyte counts (SII additionally incorporating platelets) and strongly collinear with each other (Spearman $\rho = 0.89$). They were therefore evaluated only as alternative inflammatory terms in supplementary analyses rather than entered jointly. The urinary albumin-to-creatinine ratio (ACR), which reflects kidney damage rather than systemic inflammation, did not differ significantly between the two groups ($p = 0.319$, Table 1) and therefore was not included as a model covariate due to its limited discriminatory performance. None of these markers materially improved discrimination when added to the base model ($\Delta\text{AUC} \leq 0.003$, Table 2 and **Supplementary Fig. 1**). The choice of inflammatory marker therefore did not affect the conclusions.

Predictors were selected a priori rather than chosen by univariate significance. The base model used established and routinely available factors as the reference model for testing the incremental value of CysC. Serum creatinine and creatinine-based eGFR were deliberately excluded because they define the outcome. Uric acid was also excluded from the base model because it is renally excreted and may partly reflect the outcome in a cross-sectional analysis. For a sensitivity check, we fitted a 10-fold cross-validated LASSO-penalized logistic regression model using the full candidate set and compared the discrimination of the base, joint, and full models.

Assessment of Model Discriminative Performance

The discriminative performance of the models was evaluated using Receiver Operating Characteristic (ROC) curve analysis. The Area Under the Curve (AUC) values were compared using the DeLong test. We calculated the continuous NRI and the IDI to assess the incremental predictive value of adding cystatin C to the base model.

Validation and Calibration

Internal validation used 500 bootstrap resamples to estimate potential optimism. We calculated the optimism-corrected AUC to assess the model stability. Model calibration was evaluated using calibration curves with 500 bootstrap resamples to compare predicted probabilities against observed event rates.

Clinical Utility and Visualization

The clinical utility of each model was examined through Decision Curve Analysis (DCA), which quantifies the net benefit across a range of threshold probabilities. A nomogram was constructed based on the coefficients from the Joint Model to provide a visual tool for probability estimation in clinical practice. Restricted Cubic Splines (RCS), with knots placed at the 10th, 50th, and 90th percentiles, were used to depict the potential non-linear dose-response between serum CysC levels and the risk of occult renal function decline.

Sensitivity Analyses

eGFR was re-estimated with the race-free 2021 CKD-EPI creatinine equation, and group assignment and the joint model were repeated. The 2021 CKD-EPI creatinine-cystatin C equation was used as a reference standard to quantify reclassification across the 60 and 90 mL/min/1.73 m^2 boundaries, rather than to redefine the primary outcome.

Subgroup and Effect-Modification Analyses

Potential effect modification of the cystatin C association by diabetes status (diagnosed diabetes or fasting glucose ≥ 7.0 mmol/L), age, and sex was assessed by adding multiplicative interaction terms (standardized cystatin C × modifier) to the joint model and testing them with likelihood-ratio tests. Subgroup-specific odds ratios for cystatin C were estimated within strata. Given that three interactions were tested, a Bonferroni-corrected threshold was applied.

Sample Size and Power

We conducted a sensitivity power analysis based on the achieved sample. The study included 422 participants,

Table 1. Baseline characteristics of the study population stratified by renal function status.

Variable	Overall	Control	Occult_CKD	*p*	Test statistic
n	422	204	218		
Sex = Male (%)	255 (60.4)	117 (57.4)	138 (63.3)	0.212	$\chi^2 = 1.56$
Age (mean (SD))	56.05 (14.60)	50.36 (14.49)	61.38 (12.57)	<0.001	t = –8.36
GLU (median [IQR])	5.06 [4.56, 5.84]	5.11 [4.59, 5.81]	5.01 [4.50, 5.84]	0.323	Z = 0.99
LDL-C (median [IQR])	3.04 [2.34, 4.09]	3.27 [2.57, 4.26]	2.94 [2.20, 3.90]	0.010	Z = 2.58
CysC (median [IQR])	1.03 [0.91, 1.18]	0.91 [0.83, 1.01]	1.14 [1.04, 1.31]	<0.001	Z = –12.68
hs-CRP (median [IQR])	1.30 [1.30, 2.60]	1.30 [1.30, 2.50]	1.30 [1.30, 3.48]	0.888	Z = –0.13
TG (median [IQR])	1.44 [1.01, 2.05]	1.50 [1.04, 2.19]	1.42 [0.99, 1.95]	0.353	Z = 0.93
TC (median [IQR])	5.14 [4.16, 6.48]	5.37 [4.40, 6.69]	4.95 [3.97, 6.15]	0.004	Z = 2.91
HDL (median [IQR])	1.18 [0.96, 1.45]	1.21 [1.02, 1.54]	1.17 [0.92, 1.41]	0.062	Z = 1.87
UA (mean (SD))	343.27 (100.70)	320.40 (96.92)	364.68 (99.66)	<0.001	t = –4.62
Scr (mean (SD))	76.62 (18.56)	64.85 (12.77)	87.63 (16.25)	<0.001	t = –15.94
PLT (mean (SD))	243.20 (70.62)	251.17 (68.88)	235.74 (71.55)	0.025	t = 2.25
NE (median [IQR])	3.77 [2.82, 5.10]	3.88 [2.86, 5.10]	3.70 [2.79, 5.11]	0.430	Z = 0.79
LY (mean (SD))	1.71 (0.63)	1.81 (0.69)	1.62 (0.56)	0.003	t = 3.00
ACR (median [IQR])	1.17 [0.27, 3.12]	1.35 [0.33, 3.12]	1.04 [0.22, 3.10]	0.319	Z = 1.00

Values are presented as mean ± standard deviation (SD) for normally distributed continuous variables, median [interquartile range, IQR] for non-normally distributed variables, and numbers (percentages) for categorical variables. Comparisons between groups were performed using the independent-samples *t* test or Mann-Whitney U test for continuous variables, as appropriate, and the chi-square test for categorical variables. Occult renal function decline (Occult_CKD) was defined as an estimated glomerular filtration rate (eGFR) of 60–89 mL/min/1.73 m^2, while the control group was defined as eGFR ≥90 mL/min/1.73 m^2. All statistical tests were performed using the original unrounded data; displayed summary statistics were rounded for presentation.

Table 2. Discrimination performance of different models discriminating eGFR G2 from G1.

Model	AUC	95% CI	DeLong_P_Base_vs_Joint
Base	0.725	0.677–0.773	
Base + CRP_log	0.725	0.677–0.773	
Base + NLR_q	0.728	0.681–0.776	
Base + SII_log	0.724	0.677–0.772	
Base + ACR_log	0.725	0.677–0.773	
Base + CRP_log + CysC_z	0.892	0.862–0.922	1.55×10^{-13}

Model performance was assessed using receiver operating characteristic (ROC) curve analysis. The area under the ROC curve (AUC) is expressed with 95% confidence intervals (95% CIs). The base model included age, sex, fasting glucose, and LDL-C. Each candidate inflammatory or kidney-damage marker was added individually to the base model. The DeLong test compared the AUC of the joint model (Base + CRP_log + CysC_z) with the base model. LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; NLR, Neutrophil-to-Lymphocyte Ratio; SII, systemic immune-inflammation index; ACR, albumin-to-creatinine ratio.

with 218 events and 204 controls, giving an event fraction of 0.52. With six covariates, the model had 34 events per variable, exceeding the usual minimum of 10 and supporting stable estimation. For a two-sided α of 0.05, the study had ≥80% power to detect an odds ratio of approximately 1.34 (or ≤0.75) per 1-SD change in a continuous predictor, and ≥90% power to detect an odds ratio of approximately 1.40 (≤0.71) per 1-SD change. The observed cystatin C effect (OR 11.3 per 1-SD; 95% CI 6.6–19.4) far exceeded this threshold, indicating adequate power and precision for the primary aim. Statistical power to detect small effects, and particularly interaction or subgroup effects, which require severalfold larger sample sizes, was limited.

Results

Baseline Characteristics

A total of 422 participants were included in the final analysis, comprising 204 controls (eGFR ≥90 mL/min/1.73 m^2) and 218 individuals with occult renal function decline

(Occult_CKD) ($60 \leq$ eGFR < 90 mL/min/1.73 m^2). The baseline characteristics of the study population are shown in Table 1.

Compared with the control group, participants in the Occult_CKD group were significantly older (61.38 ± 12.57 vs. 50.36 ± 14.49 years, $p < 0.001$) and had higher levels of serum cystatin C (1.14 [1.04, 1.31] vs. 0.91 [0.83, 1.01] mg/L, $p < 0.001$), uric acid (364.68 ± 99.66 vs. 320.40 ± 96.92 μmol/L, $p < 0.001$), and serum creatinine (87.63 ± 16.25 vs. 64.85 ± 12.77 μmol/L, $p < 0.001$). Platelet counts were lower in the Occult_CKD group than in controls (235.74 ± 71.55 vs. 251.17 ± 68.88 × 10^9/L, $p = 0.025$). LDL cholesterol and total cholesterol levels were significantly lower in the Occult_CKD group (both $p < 0.05$), whereas HDL cholesterol levels did not differ significantly between groups ($p = 0.062$). Fasting glucose levels were comparable between the two groups ($p = 0.323$).

hs-CRP and triglycerides (TG) showed no significant differences between groups ($p = 0.888$ and $p = 0.353$, respectively) and were analyzed using nonparametric methods. Absolute neutrophil counts were similar between groups ($p = 0.430$), whereas lymphocyte counts were significantly lower in the Occult_CKD group (1.62 ± 0.56 vs. 1.81 ± 0.69 × 10^9/L, $p = 0.003$). No significant difference was observed in the urine ACR between the two groups ($p = 0.319$).

Discrimination Performance of Models Discriminating eGFR G2 from G1

The discrimination performance of the models discriminating eGFR G2 from G1 was evaluated using ROC curve analysis (Table 2 and Fig. 2). The base model, which included age, sex, fasting glucose, and LDL cholesterol, demonstrated moderate discrimination for occult renal function decline, with an AUC of 0.725 (95% CI 0.677–0.773).

Adding log-transformed hs-CRP (CRP_log) to the base model resulted in no meaningful improvement in discrimination (AUC 0.725, 95% CI 0.677–0.773). Incorporation of NLR quartiles (NLR_q) led to a slight increase in discrimination (AUC 0.728, 95% CI 0.681–0.776), whereas inclusion of log-transformed SII (SII_log) did not improve model performance (AUC 0.724, 95% CI 0.677–0.772) (**Supplementary Fig. 1**).

Notably, the joint model combining CRP_log and standardized serum cystatin C (CysC_z) on top of the base predictors achieved markedly improved discrimination, with an AUC of 0.892 (95% CI 0.862–0.922) (Fig. 2). The improvement in AUC compared with the base model was statistically significant according to the DeLong test ($p = 1.55 \times 10^{-13}$), indicating substantial incremental predictive value of serum cystatin C for identifying early occult renal function decline.

Multivariable Logistic Regression Analysis of the Joint Model

Multivariable logistic regression was performed to identify factors associated with Occult_CKD in the joint model (Table 3). After adjustment for covariates, CysC_z was strongly associated with Occult_CKD (OR 11.322, 95% CI 6.817–20.117; $p = 1.24 \times 10^{-18}$). Age remained an independent factor (OR 1.054 per 1-year increase, 95% CI 1.034–1.076; $p = 2.74 \times 10^{-7}$). Male sex was associated with a lower likelihood of Occult_CKD (OR 0.419, 95% CI 0.23–0.745; $p = 0.00368$). In the joint model, age and sex were associated with creatinine-defined Occult_CKD group membership. However, because both age and sex are components of the CKD-EPI creatinine equation used to define the outcome, these associations should be interpreted cautiously as part of the demographic structure of the outcome definition rather than as fully independent pathophysiological predictors. Standardized serum cystatin C remained strongly associated with Occult_CKD after adjustment for these demographic and metabolic covariates. LDL-C was also significantly associated with Occult_CKD (OR 0.755, 95% CI 0.619–0.913; $p = 0.00456$). The association of CRP_log with Occult_CKD showed borderline statistical significance (OR 0.804, 95% CI 0.622–1.028; $p = 0.0869$).

Internal Validation by Bootstrap Resampling

Internal validation was performed using bootstrap resampling with 500 repetitions to assess potential overfitting of the models discriminating eGFR G2 from G1. As shown in Table 4, the apparent AUC of the base model was 0.731, which decreased to 0.707 after optimism correction (optimism = 0.024).

The joint model, which included CRP_log and standardized CysC, demonstrated an apparent AUC of 0.896 and an improved corrected AUC of 0.879 (optimism = 0.017). These results indicate good internal stability, particularly for the joint model.

Calibration Performance of the Joint Model

Calibration of the joint prediction model was evaluated using bootstrap-corrected calibration curves with 500 resamples (Fig. 3). The apparent and bias-corrected calibration curves closely followed the ideal 45-degree line across the range of predicted probabilities, indicating agreement between predicted and observed risks. The mean absolute error was 0.015, suggesting excellent calibration performance of the joint model.

Analysis of the Dose-Response Relationship

Restricted cubic spline analysis identified a non-linear association between serum CysC and the risk of occult renal function decline (Fig. 4). At serum CysC concentrations below the reference value of 1.03 mg/L, the risk of occult CKD remained relatively low. Once levels exceeded

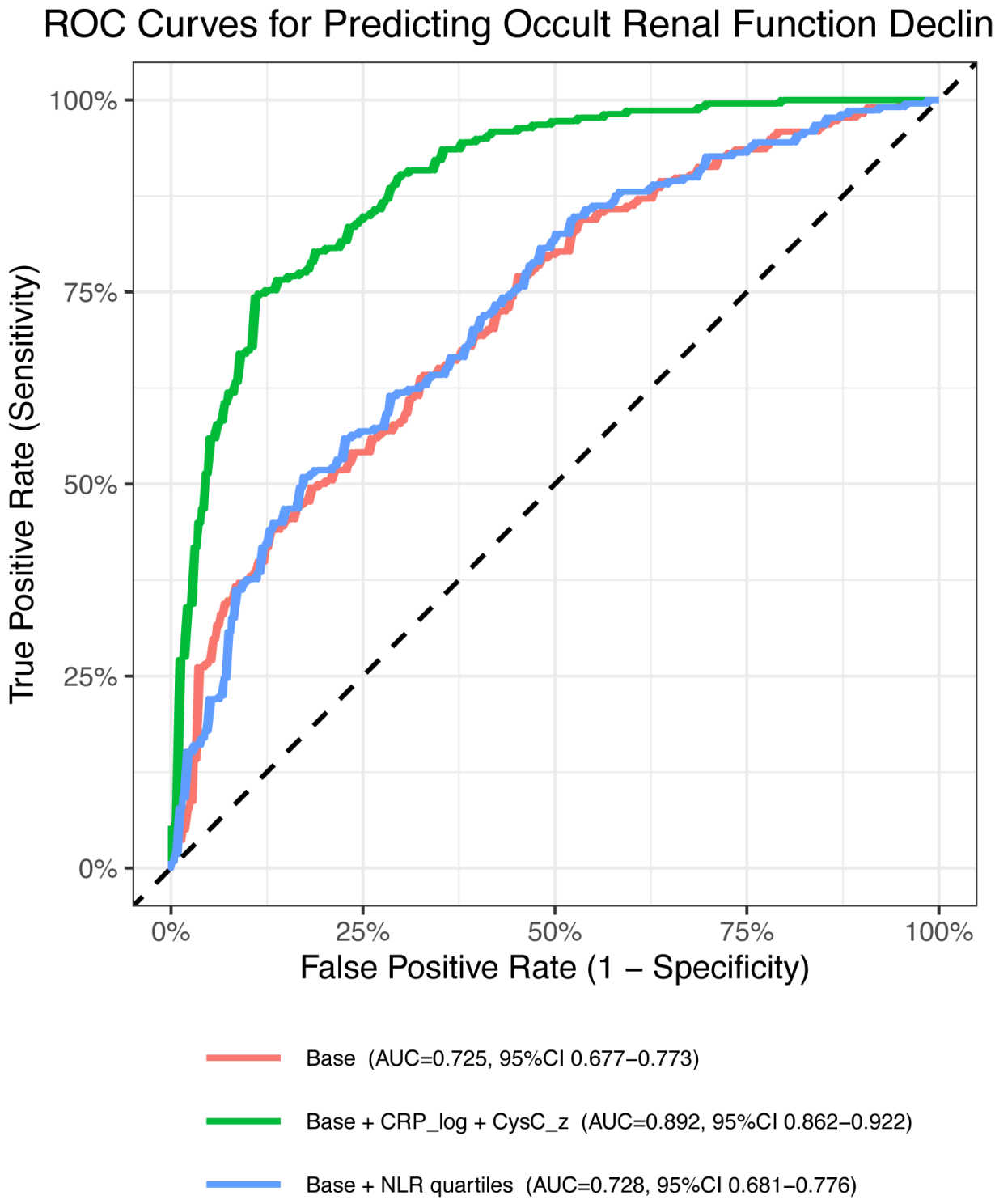

Fig. 2. Receiver operating characteristic (ROC) curves for models identifying occult renal function decline (Occult_CKD). ROC curves of the main models for identifying occult renal function decline (Occult_CKD). The base model included age, sex, fasting glucose, and LDL cholesterol. The joint model additionally incorporated log-transformed hs-CRP (CRP_log) and standardized serum cystatin C (CysC_z). The dashed diagonal line indicates no discriminative ability.

1.03 mg/L, the adjusted odds ratio exhibited a significant increase, reaching an OR of >5.0 at roughly 1.25 mg/L. This pattern indicates that even modest rises in CysC beyond the threshold are associated with a disproportionately elevated risk of renal dysfunction.

Table 3. Multivariable logistic regression analysis for Occult_CKD in the joint model.

Model	Variable	β (coefficient)	OR	95% CI_low	95% CI_high	*p*_value
Base + CRP_log + CysC_z	(Intercept)	–0.5928	0.553	0.112	2.684	0.463
Base + CRP_log + CysC_z	Age	+0.0527	1.054	1.034	1.076	2.74×10^{-7}
Base + CRP_log + CysC_z	Sex (Male)	–0.8696	0.419	0.230	0.745	0.00368
Base + CRP_log + CysC_z	GLU	–0.0624	0.939	0.819	1.078	0.365
Base + CRP_log + CysC_z	LDL-C	–0.2804	0.755	0.619	0.913	0.00456
Base + CRP_log + CysC_z	CRP_log	–0.2178	0.804	0.622	1.028	0.0869
Base + CRP_log + CysC_z	CysC_z	+2.4268	11.322	6.817	20.117	1.24×10^{-18}

The joint model included age, sex, fasting glucose (GLU), LDL cholesterol (LDL-C), log-transformed hs-CRP (CRP_log), and standardized serum cystatin C (CysC_z). Odds ratios (ORs) are presented with 95% confidence intervals (95% CIs). β denotes the logistic regression coefficient (natural-log odds). CysC_z is standardized serum cystatin C, calculated as (CysC - 1.0715)/0.2548 mg/L. CRP_log = ln(hs-CRP + 0.1). Sex is coded as 1 for male and 0 for female. These parameters fully specify the model and allow independent calculation of predicted probabilities.

Table 4. Bootstrap interval validation.

Model	AUC_apparent	Optimism	AUC_corrected
Base	0.731	0.024	0.707
Base + CRP_log + CysC_z	0.896	0.017	0.879

Internal validation was based on 500 bootstrap resamples. Apparent AUC, optimism, and optimism-corrected AUC are reported for the base and the joint models. The high corrected AUC and limited optimism of the joint model suggest stable performance with little evidence of overfitting.

Evaluation of Multicollinearity and Incremental Predictive Value

Collinearity diagnostics revealed no significant multicollinearity among the covariates included in the joint model. The VIFs for all predictors ranged from 1.03 to 1.15 (**Supplementary Table 1**), well below the threshold of 5, confirming the independence of the predictors. The addition of standardized serum cystatin C to the base model (including age, sex, GLU, LDL, and hs-CRP) resulted in substantial improvement in risk reclassification. The continuous NRI was 1.0691 ($p < 0.001$), and the IDI was 0.3067 ($p < 0.001$) (Table 5), indicating that the joint model significantly improved the accuracy of predicted probabilities for both events and non-events compared to the model without cystatin C.

Table 5. Incremental predictive performance of adding serum cystatin C to traditional risk factors and inflammation markers.

Metric	Value	*p*_value	*p*_Formatted
NRI (Continuous)	1.0691	<0.001	<0.001
IDI	0.3067	<0.001	<0.001

Model 1 (Reference Model) included age, sex, fasting glucose, low-density lipoprotein cholesterol (LDL-C), and log-transformed high-sensitivity C-reactive protein (hs-CRP). Model 2 (Joint Model) additionally incorporated standardized serum cystatin C. Discrimination was assessed using the AUC with DeLong's test. Reclassification improvement was evaluated using continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI). All estimates are expressed with 95% confidence intervals. Note: NRI and IDI indicate the improvement in risk prediction accuracy provided by adding cystatin C to the reference model.

Construction of a Clinical Nomogram

We constructed a nomogram based on the joint logistic regression model (Fig. 5). For illustration, a 65-year-old man with glucose 5.5 mmol/L, LDL-C 3.0 mmol/L, hs-CRP 2.0 mg/L, and cystatin C 1.15 mg/L has a standardized cystatin C of (1.15–1.0715)/0.2548 = 0.31, giving L = –0.5928 + 0.0527 × 65 – 0.8696 – 0.0624 × 5.5 – 0.2804 × 3.0 – 0.2178 × ln(2.1) + 2.4268 × 0.31 ≈ 1.36, and $p = 1/(1 + e^{(-1.36)}) \approx 0.797$, corresponding to an estimated probability of occult renal function decline of approximately 79.7%.

Clinical Usefulness Assessed by Decision Curve Analysis

Decision curve analysis was performed to evaluate the clinical utility of the models by quantifying net benefit across a range of threshold probabilities (Fig. 6). The joint model consistently provided a higher net benefit than the base model, as well as the treat-all and treat-none strategies, over a wide range of threshold probabilities (approximately 0.10–0.60). These findings suggest that incorporating serum CysC into the prediction model could improve

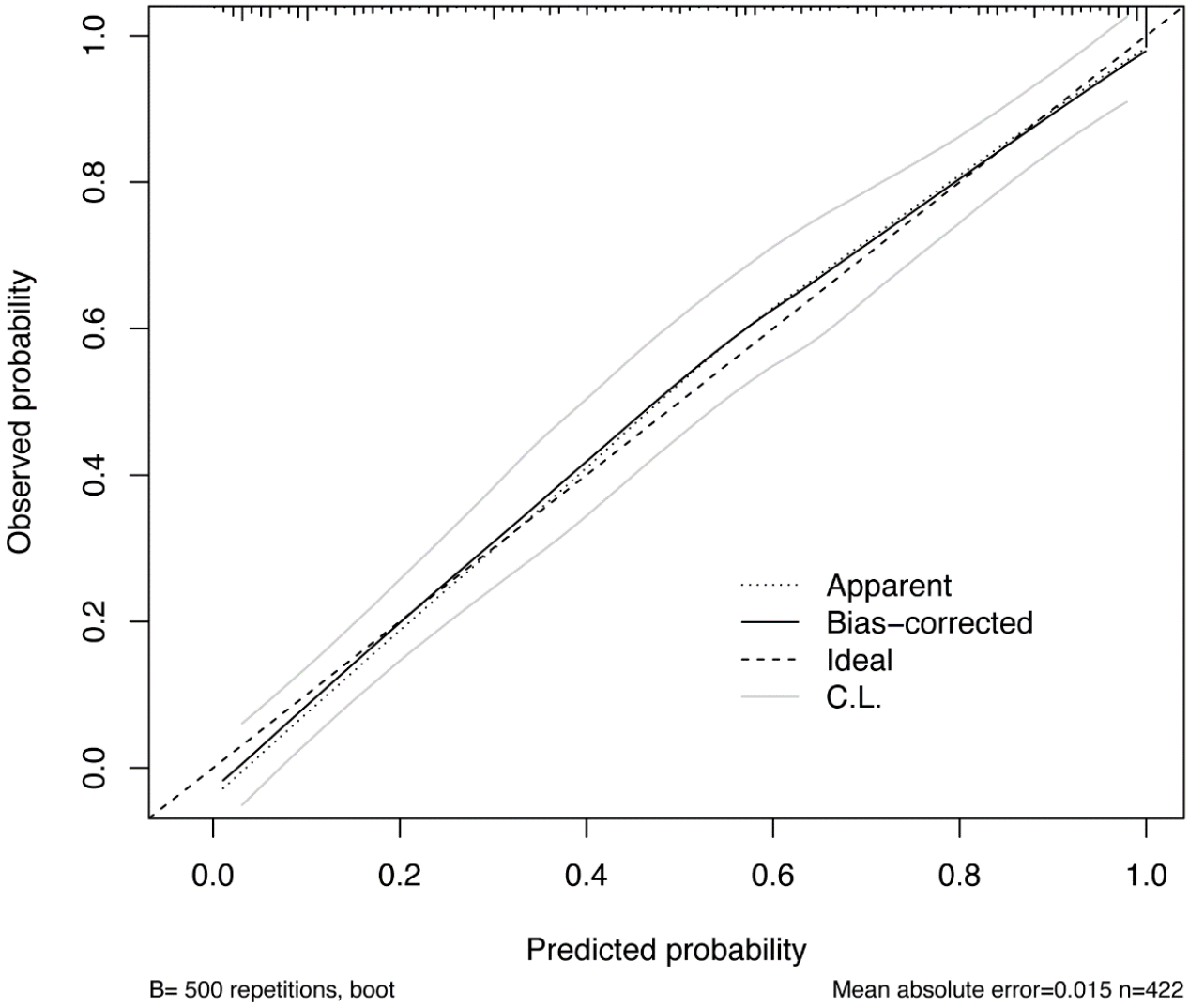

Fig. 3. Calibration curve of the joint prediction model for occult renal function decline. The dotted line shows the apparent calibration, the solid line represents the bias-corrected calibration after 500 bootstrap resamples, and the dashed line indicates the ideal calibration. The light gray lines denote the 95% confidence intervals.

clinical decision-making by early identification of individuals at risk of occult renal function decline.

Sensitivity Analyses

In sensitivity analyses, the 2021 CKD-EPI creatinine equation was almost perfectly correlated with the 2009-based estimate (r = 0.998, median difference 3.5 mL/min/1.73 m^2). Group classification was concordant in 89.3% of participants, with all discordance occurring at the 90 mL/min/1.73 m^2 threshold and none reclassified below 60 mL/min/1.73 m^2. The joint model retained excellent discrimination under the 2021 creatinine-based grouping (AUC 0.860 vs 0.892), with standardized cystatin C remaining a strong independent predictor. When the 2021 creatinine-cystatin C equation was applied as a reference, 45 of 218 participants (20.6%) in the creatinine-defined occult decline group had a combined-equation eGFR <60 mL/min/1.73 m^2, indicating that a substantial fraction already harbored filtration loss undetected by creatinine (**Supplementary Table 2**).

Serum cystatin C and creatinine were only moderately correlated (Pearson r = 0.60), indicating that the two markers are not redundant. Even when continuous serum creatinine, which by construction near-deterministically predicts the creatinine-based outcome, was forced into the joint model, standardized cystatin C retained an independent association with occult decline (OR ≈ 3.09, p = 0.03) (see paragraph above and **Supplementary Table 2**).

In LASSO-penalized logistic regression including all candidate variables (creatinine excluded), standardized cystatin C was retained as the dominant predictor, with by far the largest absolute standardized coefficient. A model containing all candidate variables discriminated only marginally better than the parsimonious joint model (AUC

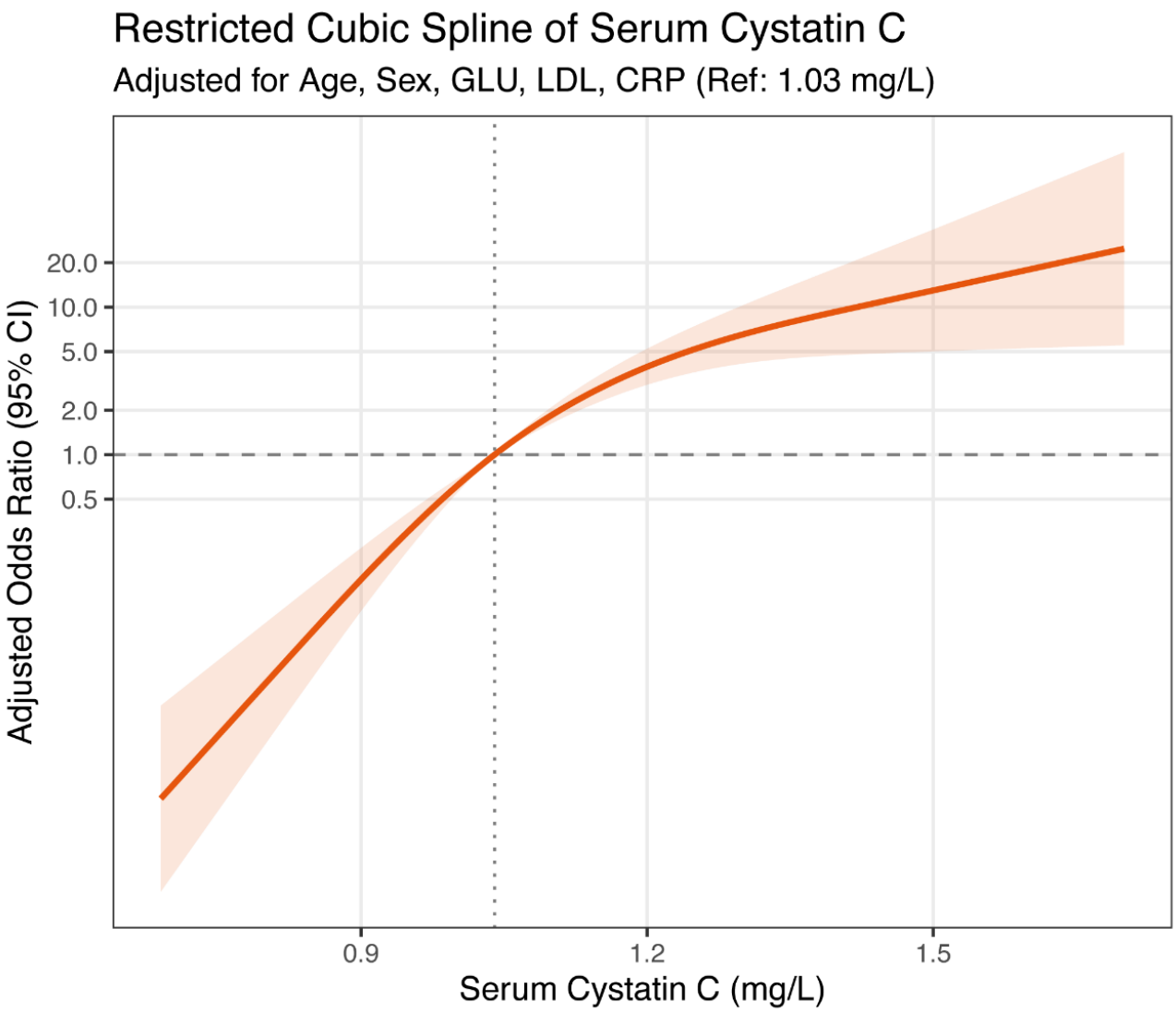

Fig. 4. Restricted cubic spline visualization of the association between serum cystatin C and the risk of occult renal function decline. The solid orange line represents the multivariable-adjusted odds ratio (OR), and the shaded area indicates the 95% confidence intervals.

0.904 vs 0.892), and adding the additional variables showing significant univariate differences (uric acid, platelets, lymphocytes) produced a negligible change (AUC 0.899). These findings indicate that the joint model captures all available predictive information and that the predominance of cystatin C is independent of the variable-selection strategy (**Supplementary Table 3**).

Subgroup and Effect-Modification Analyses

Cystatin C's association with occult renal function decline was consistent across subgroups. No significant interaction was observed with age (p_interaction = 0.450) or sex (p_interaction = 0.234). The adjusted odds ratio per 1-SD increase in cystatin C was comparable in men and women and across age strata. A nominal interaction with diabetes was detected (p_interaction = 0.036). However, the diabetic subgroup was relatively small (n = 69), resulting in a highly imprecise estimate (OR 334, 95% CI 9.6–11697). This indicates sparse-data instability rather than a credible biological interaction, and the association was no longer statistically significant after correction for multiple comparisons (Bonferroni-adjusted p = 0.11). Overall, the predictive value of cystatin C was robust across the metabolic and demographic subgroups examined (**Supplementary Table 4**).

Discussion

This study identifies serum CysC as a strong predictor of occult renal function decline, defined as eGFR 60–89 mL/min/1.73 m^2, in a stage that can be overlooked by creatinine-based screening. The developed Joint Model, in-

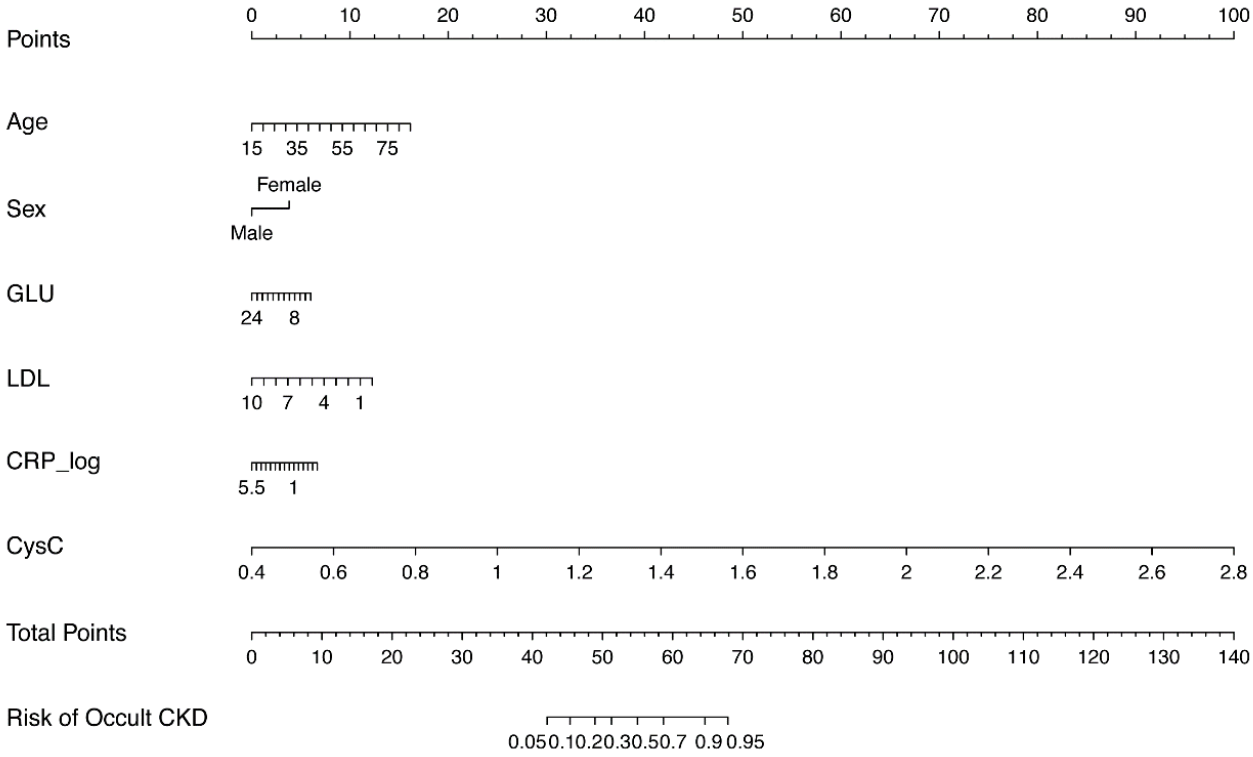

Fig. 5. Nomogram for predicting the probability of occult renal function decline. To apply the nomogram, identify the patient's value on each predictor axis and project upward to read the corresponding score on the "Points" axis. Sum the scores to obtain the "Total Points" value, which is then projected downward from the total-points axis to the "Risk of Occult CKD" axis to estimate the probability of occult renal function decline.

corporating standardized CysC with routine metabolic and demographic factors, demonstrated superior discrimination compared to the base model (AUC 0.892 vs 0.725). Beyond mere association, CysC improved risk stratification with NRI of 1.0691 and IDI of 0.3067. These findings support the integration of CysC into creatinine-based eGFR for subclinical renal dysfunction.

The insensitivity of serum creatinine for capturing modest reductions in GFR is well-recognized, reflecting the nonlinear relationship between serum creatinine and GFR and the so-called creatinine-blind range [24,25]. This limitation is also influenced by muscle mass, age, sex, and dietary protein intake [25,26,27]. The 2024 KDIGO guidelines recommend the use of CysC when creatinine-based eGFR may be inaccurate, particularly in individuals with extremes of body size, reduced muscle mass, or specific comorbidities [2]. Within our cohort, those with occult renal decline showed creatinine values within the normal range yet had markedly increased CysC (1.14 [1.04, 1.31] vs. 0.91 [0.83, 1.01] mg/L, $p < 0.001$), underscoring this clinical blind spot.

Unlike creatinine, CysC is produced at a relatively constant rate by all nucleated cells, freely filtered by the glomerulus, and then almost completely reabsorbed and catabolized in proximal tubular cells, with no appreciable tubular secretion [10,28,29]. This physiological stability makes CysC well suited for the early detection of renal dysfunction before clinically meaningful increases in creatinine. A landmark meta-analysis by the CDK Prognosis Consortium demonstrated that CysC-based eGFR captures early risk trajectories for mortality and end-stage renal disease even within the eGFR range of 60–89 mL/min/1.73 m^2, which was previously considered normal [19]. Specifically, among participants with creatinine-based eGFR of 60–89 mL/min/1.73 m^2, 14% were reclassified to a CysC-based eGFR of less than 60 mL/min/1.73 m^2 and had a relative increase in the adjusted risk of death [19]. Our findings align with this observation that the substantial odds ratio (OR >10) in our multivariable analysis underscores that CysC is not merely a confirmatory marker but an independent driver of risk prediction in this specific subpopulation.

A recent 2022 UK Biobank study by Lees et al. [20] further demonstrated that eGFR based on creatinine alone (45–59 mL/min/1.73 m^2) includes a substantial proportion of individuals at low risk while failing to capture many high-risk individuals for cardiovascular disease (CVD) and mortality. The authors concluded that eGFRcys appears to be more sensitive and specific for CVD and mortality risks in mild CKD. Similarly, the LIPID study with 16 years of follow-up showed that baseline cystatin C indepen-

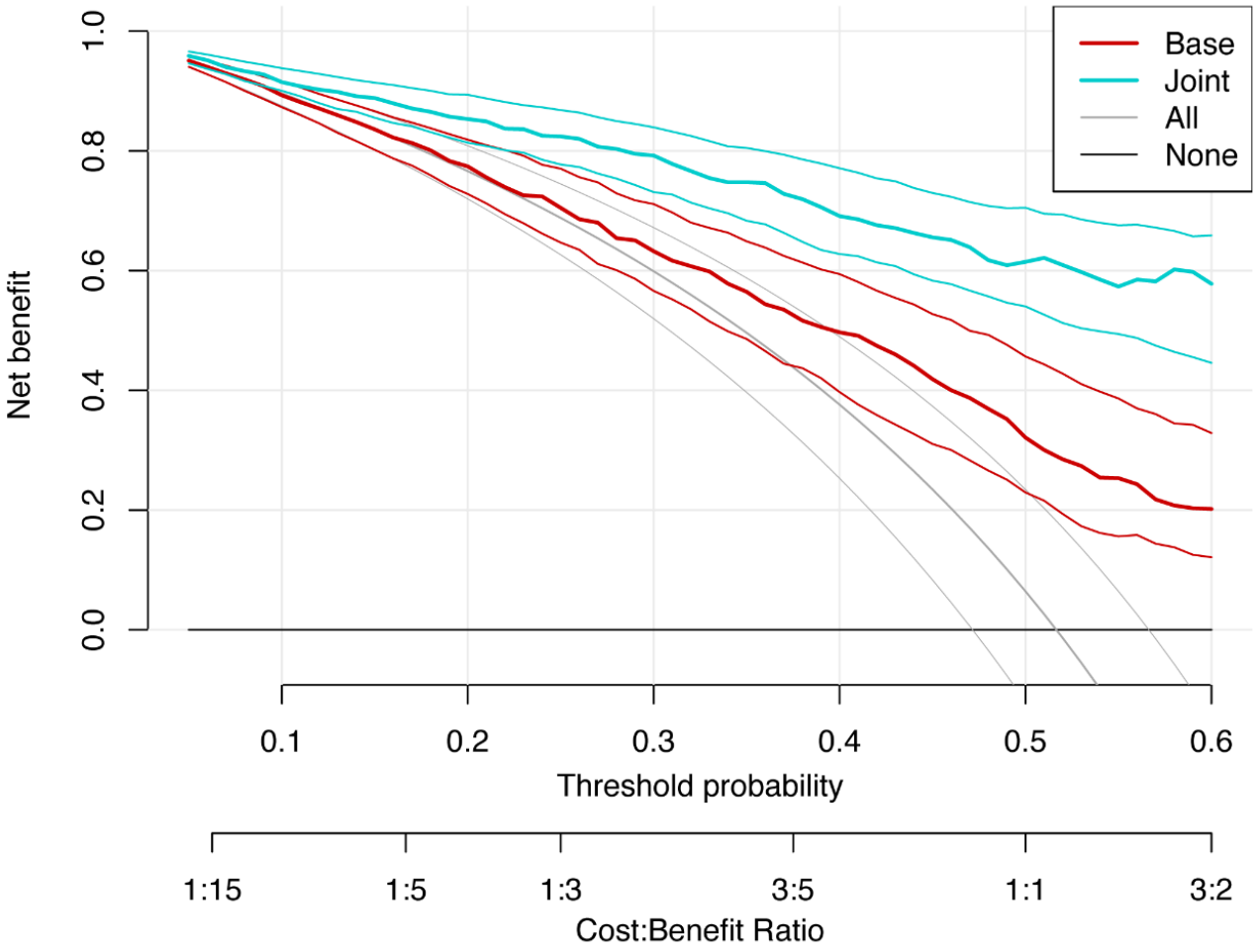

Fig. 6. Decision curve analysis. The net benefit is plotted against a range of threshold probabilities. The horizontal black line represents the strategy of treating no patients. The gray line represents the strategy of treating all patients.

dently predicted major cardiovascular events, development of CKD, and cardiovascular and all-cause mortality, even after adjustment for improved estimation of GFR using the CKD-EPI creatinine-cystatin C equation [21]. These findings collectively support the interpretation that CysC provides unique prognostic information not fully captured by creatinine.

One important contribution of this study is the depiction of the dose-response between CysC and risk, achieved using RCS. We identified an inflection point near 1.03 mg/L, corresponding to the median in our study population. Below this threshold, the risk of occult renal dysfunction remained largely unchanged, whereas the risk increased noticeably above the threshold, reaching an OR of >5.0 at approximately 1.25 mg/L. This indicates that small increases in CysC above this physiological threshold are associated with a disproportionately high risk of renal dysfunction.

Previous large-scale studies have reported more linear relationships between CysC and cardiovascular outcomes [30]. Our data specifically highlight a threshold effect in the setting of early functional decline. This finding identified a clinically relevant warning range in which CysC values above 1.03 mg/L may justify closer surveillance, rather than waiting for values to exceed the arbitrary clinical upper limits such as >1.20 mg/L. The LIPID study grouped baseline cystatin C levels by quartiles with cut points of ≤0.72, >0.72 to ≤0.81, >0.81 to ≤0.93, and >0.93 mg/L [21], suggesting that even values in the upper-normal range warrant attention.

A 2023 Brigham Health retrospective cohort study demonstrated that cystatin C-based eGFR is often lower than creatinine-based eGFR, and many patients would qualify for a later stage of CKD when cystatin C is tested, especially if they are elderly or have more comorbid conditions [31]. 1.03 mg/L threshold in our study provides a practical reference point for clinicians to identify individuals who may benefit from enhanced monitoring or earlier nephrology referral.

The NRI and IDI provide additional evidence of clinical utility beyond simple AUC comparison. An AUC increase can sometimes be insensitive to the addition of new markers in strong baseline models [32,33]. The remark-

ably high continuous NRI (1.0691) in this study indicates that adding CysC correctly reclassified a substantial proportion of individuals, moving those with occult disease into higher-risk categories and healthy controls into lower-risk categories. The IDI of 0.3067 further confirms a meaningful improvement in the separation of predicted probabilities between cases and controls. Although study groups were defined according to creatinine-based eGFR, CysC is not merely a redundant marker. The high NRI (1.0691) and the distinct threshold (1.03 mg/L) suggest that CysC provides an early warning that captures risk before creatinine-based eGFR declines further.

This improvement is consistent with prior literature. Shlipak et al. [19] reported an NRI of 0.23 for death and 0.10 for end-stage renal disease when CysC was added to creatinine-based eGFR. The higher NRI observed in our study may reflect the specific focus on occult renal function decline (eGFR 60–89 mL/min/1.73 m^2), a population in which the limitations of creatinine-based eGFR are most pronounced. These findings confirm that CysC captures incremental pathophysiological information beyond traditional metabolic factors such as age, glucose, and lipid profiles, effectively reducing false-negative classifications in primary care.

Our multivariable analysis indicated an inverse association between LDL-C and occult renal function decline (OR 0.755, 95% CI: 0.619–0.913), and total cholesterol was similarly lower in the occult decline group. Similar inverse lipid–outcome relationships have been reported under the terms lipid paradox or reverse epidemiology in dialysis patients, advanced kidney disease, and older populations [34,35,36]; this pattern may partly reflect malnutrition-inflammation complex syndrome, a framework in which systemic inflammation and undernutrition are linked to lower circulating cholesterol in dialysis patients [37,38]. The mechanisms proposed to underlie such lipid paradoxes in CKD, including inflammation-related suppression of cholesterol synthesis, have recently been reviewed [39]. Our data, however, do not permit a mechanistic attribution, and this finding should be interpreted cautiously. Within our cohort, the inflammation-mediated explanation is not supported. hs-CRP did not differ between groups and LDL-C was essentially uncorrelated with hs-CRP. More parsimonious possibilities include confounding by unmeasured statin use, and the occult decline group was substantially older (mean 61.4 vs 50.4 years), in whom lipid-lowering therapy is more frequent, together with residual confounding, reverse causation, or chance. Because the interpretation of lipid concentrations in CKD requires consideration of inflammatory and nutritional status [40,41], and data on lipid-lowering therapy, nutritional status, and inflammatory cytokines were unavailable, the basis of this association cannot be determined and should be regarded as hypothesis-generating.

We translated the complex regression coefficients into a visual nomogram. This tool allows clinicians to integrate CysC with routinely measured age, sex, LDL-C, fasting glucose, and hs-CRP to generate a personalized probability of occult renal decline without requiring complex calculations. For example, a 65-year-old male patient with a serum CysC level of 1.15 mg/L would have an estimated probability of occult renal function decline of approximately 79.7%.

DCA further validated the model's clinical usefulness across threshold probabilities of 10%–60%. This finding supports the potential clinical use of the Joint Model in diverse settings, from routine health check-ups to targeted CKD screening in high-risk populations such as those with hypertension or diabetes mellitus [42,43,44]. The 2024 KDIGO guidelines emphasize the importance of CysC testing for confirmation of CKD and for clinical decision-making, particularly in circumstances where creatinine-based eGFR may be less accurate [2]. Our nomogram provides a practical implementation tool aligned with these recommendations.

We employed a comprehensive analytical framework including multivariable logistic regression, RCS visualization, NRI/IDI reclassification metrics, bootstrap internal validation, calibration assessment, and decision curve analysis, providing robust evidence from multiple perspectives. The identification of a specific CysC threshold (1.03 mg/L) has direct clinical applicability, and the construction of a nomogram facilitates translation of research findings into clinical practice.

However, several limitations warrant consideration. The cross-sectional design precludes causal inference regarding the progression from occult decline to overt CKD. Longitudinal studies are needed to confirm whether CysC elevation precedes and predicts future CKD development. While eGFR was calculated using the validated CKD-EPI 2009 creatinine equation, the lack of measured GFR (mGFR) using exogenous tracers means some misclassification remains possible. The 2021 CKD-EPI equations, incorporating both creatinine and cystatin C, have been shown to provide the least biased, most accurate GFR estimation compared with measured GFR [23].

A methodological consideration inherent to this design is that the outcome was defined by creatinine-based eGFR, whereas cystatin C, itself a filtration marker, is the principal predictor, increasing the possibility of incorporation bias. Accordingly, our findings most directly demonstrate that cystatin C correlates with, and adds discriminative information about, filtration status within the creatinine normal range rather than definitively proving independent predictive value for occult renal injury per se. Several lines of evidence nonetheless argue that this circularity is partial. The two markers were only moderately correlated ($r = 0.60$). Standardized cystatin C remained independently associated with the outcomes after conditioning on serum creatinine, and one-fifth of the creatinine-defined occult de-

cline group already had an eGFR <60 mL/min/1.73 m^2 by the 2021 creatinine-cystatin C equation. However, the only auxiliary kidney-damage marker available in this dataset, ACR, did not differ significantly between groups and provided limited contrast, and data on hematuria, quantitative proteinuria, and measured GFR were lacking. Residual incorporation bias therefore cannot be excluded. Definitive confirmation that cystatin C independently predicts incident kidney injury will require prospective studies with measured GFR, a broader panel of injury markers, and hard clinical endpoints.

However, our study design specifically aimed to evaluate whether CysC can identify individuals at risk within the "normal" creatinine-based eGFR range, which is a clinically relevant question given creatinine's known limitations. The study population was recruited from a single center in China. External validation in multi-ethnic cohorts is required to generalize the threshold values identified in our RCS analysis. The relatively modest sample size (n = 422) and the lack of data on statin usage, dietary habits, and physical activity may introduce unmeasured confounding. As detailed in Section Model Development, the base-model covariates were prespecified rather than chosen by univariate significance. Serum creatinine, eGFR, and uric acid were excluded for the reasons given there. A formal LASSO sensitivity analysis (**Supplementary Table 3**) confirmed that adding the variables showing significant univariate differences (uric acid, platelets, lymphocytes) or all candidate variables improved discrimination only marginally (AUC 0.892 vs 0.899–0.904), so the predominance of cystatin C did not depend on the variable-selection strategy. Nonetheless, several clinically relevant covariates, including body mass index, blood pressure, and a systematic record of hypertension, diabetes, smoking, and concurrent medication, were not available in this retrospective laboratory-based dataset and therefore could not be incorporated. Residual confounding by these factors cannot be excluded, and prospective studies with comprehensive covariate ascertainment are needed to confirm the independence of the cystatin C association. Prospective cohort studies with longitudinal follow-up are needed to establish whether CysC-based risk stratification can predict progression to established CKD (eGFR <60 mL/min/1.73 m^2) and hard clinical endpoints such as cardiovascular events and mortality. Cost-effectiveness analyses comparing routine CysC screening versus targeted testing in high-risk populations would inform healthcare policy decisions. The integration of CysC with other emerging biomarkers of kidney injury may further improve early detection algorithms. Because absolute cystatin C concentrations depend on the assay and its calibration, the 1.03 mg/L threshold should be interpreted in the context of an ERM-DA471-traceable measurement and re-validated against local calibration before being applied in other laboratories. Although the sample afforded ample power and precision for the primary cystatin C association, statistical power was limited to confirm small effects such as the borderline hs-CRP association and to robustly detect potential effect modification in subgroup analyses. Another important limitation is the possibility of residual confounding due to incomplete clinical covariate ascertainment. Participants in the occult renal function decline group were significantly older than controls and differed in several baseline laboratory indicators. Although the primary model adjusted for age, sex, fasting glucose, LDL-C, hs-CRP, and cystatin C, and sensitivity analyses showed that adding available candidate variables produced only marginal improvement in discrimination, several clinically relevant factors were not available in this retrospective laboratory-based dataset. These included body mass index, direct or indirect measures of muscle mass, blood pressure, history of hypertension and diabetes, smoking status, nutritional status, physical activity, and concurrent medications such as statins, antihypertensive agents, renin-angiotensin system inhibitors, and diuretics. These factors may influence serum creatinine, cystatin C, metabolic profiles, and renal risk, and therefore may partly confound the observed associations. Thus, the present model should be regarded as a hypothesis-generating risk stratification tool that requires prospective validation in cohorts with comprehensive clinical covariate collection, measured GFR, and longitudinal renal outcomes. Finally, validation in diverse populations, including different age groups and ethnic backgrounds, is warranted to enhance the generalizability of our findings.

Conclusion

In conclusion, serum cystatin C was strongly associated with creatinine-defined mildly reduced eGFR and substantially improved discrimination between creatinine-based eGFR G2 and G1 categories in this cross-sectional cohort. The identification of a distinct risk threshold at 1.03 mg/L and the substantial reclassification improvement provided by the Joint Model support a paradigm shift of moving from passive creatinine monitoring to active CysC-based screening. This strategy enables the detection of "silent" renal impairment, providing a critical window for therapeutic intervention before the onset of irreversible pathology. Our findings are consistent with the 2024 KDIGO guideline recommendations and add to the growing body of evidence supporting broader utilization of cystatin C in clinical practice. Prospective studies incorporating measured GFR, kidney damage markers, and follow-up outcomes are required to confirm the clinical significance of these findings.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

JX and XBC designed the research study. JX performed the research. XBC analyzed the data. JX drafted the article. Both authors contributed to important editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by Ethics Committee of Zhoushan Hospital (Zhoushan, Zhejiang, China; Approval No. 2026-Y-054-01). The study was conducted in accordance with the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement for written informed consent was waived by the Ethics Committee. All data were anonymized before analysis to protect patient privacy.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638210.180.

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