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Evaluation the level of the Neutrophil Gelatinase-Associated Lipocalin, Cystatin C, And Glomerular Filtration Rate and its role in the assessment of the Renal sufficiency: A Case –Control Study

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ABSTRACT

Background: *Neutrophil gelatinase-associated lipocalin (NGAL) reflects tubular injury and stress, whereas cystatin C is an endogenous filtration marker. Evaluating these markers alongside glomerular filtration rate (GFR) may help distinguish kidney injury from impaired filtration.*
Objective*: To compare NGAL, cystatin C, and GFR between patient and control groups and examine their distribution across renal-impairment stage categories.****Methods****: An exploratory comparison was performed using group-level measurements for 89 patients and 89 controls. Results were expressed as mean ± standard deviation. Patient–control mean differences and nominal 95% confidence intervals were calculated using Welch formulas, conditional on independent groups and accurate sample summaries.* ***Results****: Mean NGAL was lower in patients than controls (1.4 ± 3.5 versus 6.13 ± 2.74), whereas cystatin C (17.1 ± 6.33 versus 2.132 ± 1.21) and GFR (40 ± 14 versus 19 ± 2.14) were higher. Conditional mean differences were −4.730 (95% confidence interval, −5.660 to −3.800) for NGAL, 14.968 (13.612 to 16.324) for cystatin C, and 21.000 (18.018 to 23.982) for GFR. Mean NGAL and cystatin C increased across stage categories, but GFR was nonmonotonic. The overall patient NGAL mean was lower than every stage-specific mean.* ***Conclusion****: NGAL, cystatin C, and GFR exhibited contrasting patient–control and stage-related patterns. Inconsistencies between overall and stage-specific measurements, together with incomplete stage definitions and laboratory characterization, limited clinical interpretation.*

Keywords: **Neutrophil gelatinase-associated lipocalin; Cystatin C; Glomerular filtration rate; Renal impairment; Kidney.**

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1.INTRODUCTION

Getting an accurate estimate of how well your kidneys are functioning is crucial for the early identification of kidney impairment, as well as gauging the severity of a disease and determining the appropriate clinical management. Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function that last for at least 3 months and are likely to have an impact on health. The method is based on the cause of the disease, GFR, and albuminuria of CKD classification. So, an isolated abnormal biomarker concentration or filtration estimate is not enough to define CKD or its full risk spectrum [1].

Creatinine-based estimated GFR is the most widely used but can be impacted by factors affecting its nonfiltration determinants of its accuracy. Cystatin C has been studied for its potential to measure filtration, and studies of external validation have found that the equations coupled to either creatinine or cystatin C performed better than the use of a single filtering marker [2] [3]. On the other hand, a relationship between elevated levels of inflammation and the biological mechanisms of neutrophil gelatinase-associated lipocalin (NGAL, also known as lipocalin-2), has also been suggested. Experimental studies have associated NGAL to

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the kidney tubular system's vulnerability to injury, iron handling, in comparison to clinical investigations that have associated serum or urinary NGAL to renal impairment and CKD progression [4] [5] [6]. Hence, NGAL is not a direct filtration marker, but a marker associated with injury. To best describe the kidney dysfunction they complement each other pathophysiologic parameters. In the newly emerging markers, It has been found that Better GFR estimation, and Cystatin C based risk assessment has identified mortality and kidney failure in population and CKD cohorts [5] [8] [9]. Nonetheless, This is however, inferring the diagnostic discrimination, independent prognostic value of these two biomarkers or left further uncertain when compared to the usual measurements that themselves have difference concentrations of biomarkers. The assessment is to be carried out by looking at the clinical population, specimen characteristics, assay calibration, and reference standard.

In this study, we analyzed the distribution of NGAL, cystatin C and GFR in patients and controls and examined how they varied across five categories of renal-impairment stage. The aim was to describe between-group differences and patterns related to stage, distinguishing descriptive findings from evidence of diagnostic or prognostic performance.

2. MATERIALS AND METHODS

2.1 Study design and participants

The study evaluated NGAL, cystatin C, and GFR in a patient group of 89 participants and a control group of 89 participants. The analysis used group-level measurements and additionally examined five renal-impairment stage categories. The prospective design, recruitment setting and dates, sampling strategy, and matching procedures were documented. The available comparison was therefore analyzed with assuming a particular recruitment design. Observational reporting domains were considered according to STROBE recommendations [10].

2.2 Inclusion and exclusion criteria

The patient/control status was the grouping variable. The age criteria and the criteria for the selection of controls. The operational diagnostic criteria for renal impairment were available. In addition inclusion criteria on specimen quality, medication use, dialysis, comorbid disease or missing measurements were documented. Control status was considered indicative of the absence of kidney disease. Renal impairment was considered equivalent to confirmed CKD as data on chronicity and duration of disease were available.

2.3 Sample collection and biomarker measurement

NGAL and cystatin C were analyzed as biomarker outcomes in both groups. The specimen matrix, collection timing, fasting requirements, processing intervals, storage conditions, and sample-handling procedures were documented. Measurements were analyzed on their recorded numerical scales without assigning specimen-specific reference ranges or converting units.

2.4 GFR assessment and stage classification

GFR was analyzed as a renal-function variable. GFR was measured directly. Five stage categories were examined descriptively. The category numbers were therefore treated as confirmed KDIGO CKD stages, and individual classifications were reconstructed from group means.

2.5 Statistical analysis

NGAL, cystatin C and GFR are presented as mean ± SD. Between-group contrasts were calculated by subtracting the patient mean from the control mean. Conditional standard errors were calculated as SE = sort (s P 2 /n P + s C 2 /n C), with n P = n C = 89. Nominal 95%

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confidence intervals and two-sided P values were based on Welch-Satterthwaite degrees of freedom [11]. The three comparisons were exploratory, and the confidence intervals and P values were not adjusted for multiplicity or interpreted as evidence of diagnostic accuracy or clinical validity [12]. Overall and stage-specific means were compared for consistency without pooling categories of uncertain membership. Individual-level regression, correlation, ROC analysis, AUC estimation, sensitivity and specificity were not performed due to lack of independent reference standard and paired measurements. Calculations were performed and descriptive mean ± SD plots produced using Python, NumPy, SciPy and Matplotlib.

3. Results

3.1 Study groups

The patient and control groups each comprised 89 participants. Measurements were also available for five stage categories, although the number of participants in each category was not recorded. baseline comparability and the demographic composition of the stage categories could not be assessed.

3.1 NGAL, cystatin C, and GFR in patients and controls

Mean NGAL was lower in patients than controls, while mean cystatin C and GFR were higher in patients (Table 1). NGAL values were 1.4 ± 3.5 in patients and 6.13 ± 2.74 in controls. Corresponding cystatin C values were 17.1 ± 6.33 and 2.132 ± 1.21, and GFR values were 40 ± 14 and 19 ± 2.14. Figure 1 illustrates the between-group differences and within-group dispersion.

Table 1. NGAL, cystatin C, and GFR in patient and control groups.

Group	n	NGAL, mean ± SD	Cystatin C, mean ± SD	GFR, mean ± SD
Patients	89	1.4 ± 3.5	17.1 ± 6.33	37 ± 14
Controls	89	6.13 ± 2.74	2.132 ± 1.21	75 ± 2.14

Measurement units and specimen matrix were unavailable; values are presented without unit conversion.

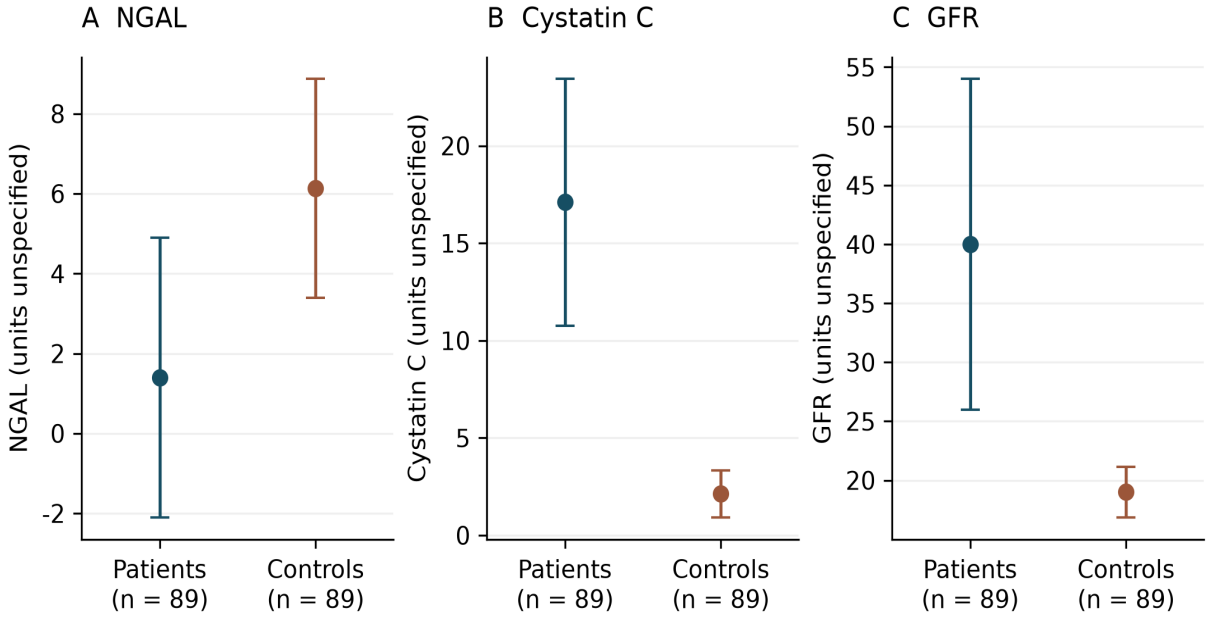


Figure 1. NGAL, cystatin C, and GFR in patient and control groups. Points indicate group means and error bars indicate standard deviations, not confidence intervals. Each group included 89 participants.

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The patient minus control mean difference (estimate) was -4.730 (NGAL), 14.968 (cystatin C), and 21.000 (GFR) (Table 2). All three nominal confidence intervals excluded zero under the assumed independent-sample model.

Table 2. Conditional patient–control mean differences and nominal confidence intervals.

Variable	Patient − control mean difference	Nominal 95% CI	Welch df	Conditional two-sided P
NGAL	−4.730	−5.660 to −3.800	166.41	7.72×10^{-19}
Cystatin C	14.968	13.612 to 16.324	94.42	8.46×10^{-39}
GFR	21.000	18.018 to 23.982	92.11	1.64×10^{-24}

Calculations assume independent, unpaired groups of 89 participants and accurate sample means and SDs. These assumptions could not be fully assessed. Confidence intervals and P values

3.3 Measurements across stage categories

From mean NGAL increased across categories 1–5, from 6.15 to 39, and mean cystatin C increased from 2.18 to 18.124 (Table 3; Figure 2). In contrast, GFR means were 28, 69.021, 53.114, 40.51, and 13.18, respectively.GFR increased between categories 1 and 2 and fell across categories 2–5.

Table 3. NGAL, cystatin C, and GFR across stage categories.

Stage category	NGAL, mean ± SD	Cystatin C, mean ± SD	GFR, mean ± SD
1	6.15 ± 2.132	2.18 ± 1.075	70 ± 18.921
2	10.1002 ± 3.048	3.005 ± 1.805	60.021 ± 13.419
3	12.042 ± 2.286	8.15 ± 3.175	53.114 ± 10.989
4	16.124 ± 7.86	8.621 ± 5.410	38.51 ± 13.151
5	39 ± 134	18.124 ± 4.269	13.18 ± 4.119

Stage definitions and participant counts were unavailable. Categories should not be interpreted as confirmed KDIGO CKD stages.

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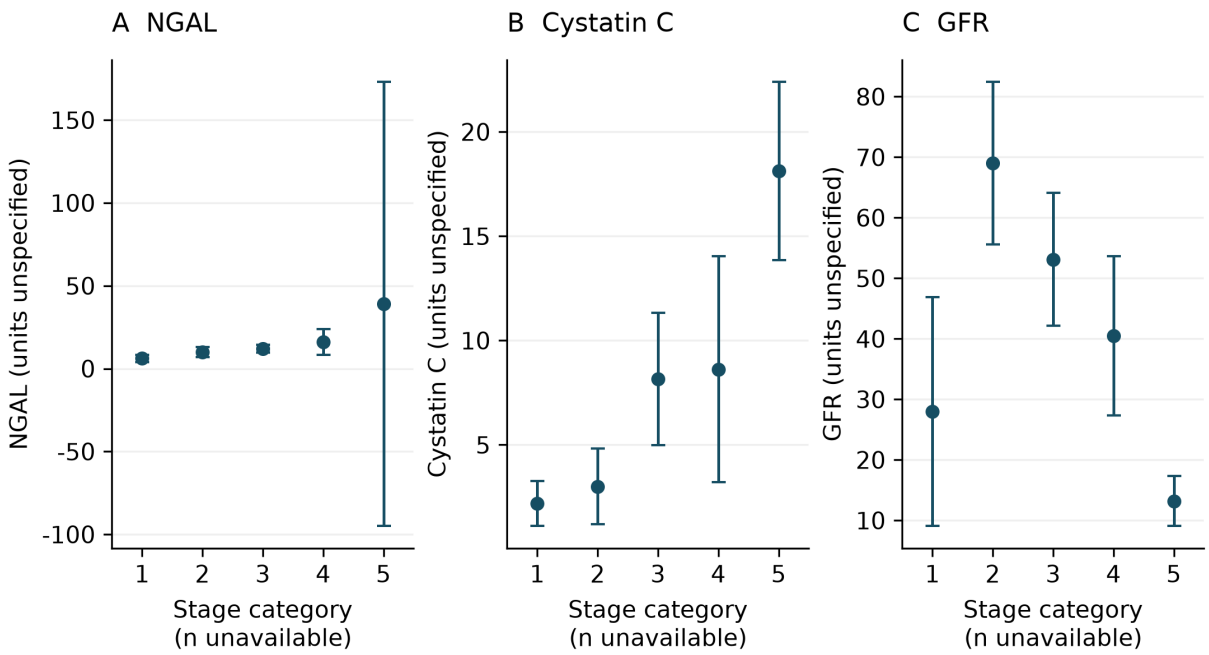


Figure 2. NGAL, cystatin C, and GFR across stage categories. Points indicate means and error bars indicate standard deviations.

4. Discussion

The study identified varying relations in NGAL, cystatin C and GFR. Patients exhibited markedly lower levels of NGAL overall but higher mean levels of cystatin C & GFR. Across stage categories NGAL & cystatin C rose with level number, whereas GFR showed a nonmonotonic trend. Thus, this study further underscores the fact that interpretation of biomarker concentrations in terms of filtration cannot be based on a simple severity gradient.

A major limitation of this study was the disparity between the overall patient NGAL mean and the stage-specific means. If the five categories represented an exhaustive partition of the same patient population on the same measurement scale, the overall mean would lie within the range of the category means. The overall value of 1.4 was lower than the smallest category means of 6.15. This is surely inconsistent and in no way allows us an integrated interpretation of patient–control and stage-related findings. We could not disentangle potential differences in classification, measurement scale or population composition.

Although the nominal confidence intervals for the patient–control contrasts did not include zero, statistical separation is not a substitute for clinical validity. The estimates rest on the accuracy of the group summaries and the presumption of independent observations. Small P values do not validate assay performance, disease classification, or clinically meaningful diagnostic discrimination [12]. Thus, the conditional estimates should certainly be interpreted as exploratory comparisons rather than definitive evidence of biomarker performance.

NGAL is associated with renal tubular damage and long-standing reduction in renal function. Increases in serum and urinary NGAL concentrations and inverse relationships with the remaining GFR have been found in CKD [5] (Bolignano et al.). In a subsequent prospective study of 96 patients with nonterminal CKD, baseline NGAL was associated with risk of progression defined as 2× rise in serum creatinine and/or end stage kidney disease over a median follow-up of 18.5 months [6]. The increasing NGAL values throughout the stages

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match the same trend in earlier findings. Between groups, the averaged lower mean NGAL across all patients versus controls and the difference in group from stage measurements may not allow for a biologically meaningful interpretation of these findings. The independent prognostic value of NGAL is not the same in each patient group. Bourgonje et al. examined 4,660 PREVEND participants without CKD at baseline. They found the relationship between plasma NGAL and incident kidney disease weakens after adjusting for baseline eGFR, as the hazard ratio adjusted from 1.37 to 1.06 per doubling of NGAL [13]. This suggests that the attenuation may be due to the fact that NGAL can reflect existing differences in filtration and also not entirely independently. This study included no longitudinal outcomes or individual covariates as such, it cannot determine whether NGAL adds predictive value to standard renal assessment.

Comparative evidence demonstrates population and reference-standard selection plays a pivotal role. Using ioversol-clearance GFR, serum NGAL, and cystatin C, Mitsnefes and colleagues examined 45 children with CKD with evidence of associations with renal function among children 14]. In 90 patients with type 2 diabetes Bacci and colleagues reported its AUCs as 0.621 for serum NGAL, 0.635 for cystatin C and 0.660 for the albumin-to-creatinine ratio [15]. The association is biological, but usually, strong diagnostic accuracy is not achieved in routine use (in contrast, In these modest discrimination estimates demonstration that biological significance does not always translate into diagnostic relevance as a standalone factor.The present study is unable to determine comparative accuracy because participant-level measurements and a defined reference condition were not available.

NGAL findings in acute kidney injury should not be directly extrapolated to chronic renal staging. Bennett and colleagues reported an AUC of 0.95 for urinary NGAL measured 2 hours after pediatric cardiac surgery, whereas a multicenter pediatric cardiac-surgery cohort reported an AUC of 0.71 [16] [17]. Differences in timing, injury definitions, disease severity, and case mix can substantially influence accuracy. These studies support context-specific evaluation of NGAL rather than the use of a universal concentration threshold for CKD classification.

Higher cystatin C concentrations are often interpreted in relation to reduced filtration. In this study, however, higher mean cystatin C in patients occurred together with higher mean GFR. This discordance requires cautious interpretation because the filtration method, specimen matrix, measurement units, and sampling timing were not characterized. The findings cannot distinguish physiological discordance from differences in measurement or classification.

Evidence supporting cystatin C in clinical filtration assessment is strongest when estimates are validated against an independent GFR reference. Stevens and colleagues evaluated 3,418 people with CKD and reported estimates within 30% of measured GFR in 89% of observations using a combined-marker equation, compared with 81% using cystatin C alone [7]. In external validation, Inker and colleagues subsequently showed improved precision and accuracy with combined creatinine-cystatin C estimation [2]. The 2021 study also showed that race-free equations including both markers were more accurate than race-free single-marker alternatives [3]. In the present study, there is no information on serum creatinine, equation inputs, calibration, and measured reference GFR, so the estimation methods cannot be evaluated.

Other non-GFR determinants also affect cystatin C. Stevens et al. found associations with diabetes and inflammatory characteristics after

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adjusting for measured GFR [18]. While creatinine clearance is not the same as exogenous-marker measured GFR, Knight and colleagues reported associations with demographic characteristics, body size, smoking and inflammation after adjusting for creatinine clearance [19]. These influences were not possible to evaluate in the present study, because demographic and clinical covariates were not available. Therefore, the observed patient-control difference cannot be solely attributed to impaired filtration.

A separate but related rationale for cystatin C evaluation is prognostic associations. Reclassification using cystatin C improved the associations between eGFR and mortality or risk of kidney failure, as shown by Shlipak and colleagues [8]. In addition, Peralta et al. evaluated cystatin C in conjunction with creatinine-based eGFR and albuminuria and provided additional risk information [9]. In the Heart and Soul Study, cystatin C was associated with cardiovascular outcomes and mortality in patients with coronary heart disease [20]. Our findings suggest that cystatin C may be of interest for future risk assessment studies, but a single comparison between groups does not prove prognostic value. The present study was limited to a descriptive evaluation, because there were no albuminuria and follow-up outcomes.

The standard classification of CKD requires an explicit assessment of chronicity and GFR category. KDIGO categories are: G1 ≥90, G2 60-89, G3a 45-59, G3b 30-44, G4 15-29, G5 <15 mL/min/1.73 m2 [1]. If study categories 1 and 4 were intended to approximate KDIGO G1 and G4, respectively, the observed GFR values of 28 and 40.51 do not fall within the expected ranges. Category numbers cannot be interpreted as confirmed CKD stages as classification rules and indexed units were not available. Also group means are not sufficient to classify the individual participants.

As per STARD recommendations, a diagnostic evaluation would need a reproducible independent reference standard, appropriate selection of participants, individual test measurements and a defined target condition [21]. The critical issue in comparing cystatin C with GFR is independence. A GFR estimate based on cystatin C is mathematically dependent on the biomarker and cannot be used as an independent validation standard. Thus, the present study did not identify an optimal cutoff, incremental diagnostic value, sensitivity or specificity for either NGAL or cystatin C.

5. Conclusion

The study did not find any independent biomarker associations, diagnostic discrimination or appropriate CKD staging. It is necessary to confirm participant classification, standardized laboratory techniques and independent filtration references for more extensive assessment. There were differences in NGAL, cystatin C, and GFR between patients and controls and among stage categories.The higher category of GFR did not have an orderly pattern as NGAL and cystatin C. In general, the patient's NGAL mean was contradictory to the stage-specific values when the categories were assumed to be from the same population

Declarations

Ethics approval and consent to participate

The study received ethical approval from Ibn Sina University of Medical and Pharmaceutical Sciences. Ethical conduct was described in accordance with institutional and national requirements and the principles of the Declaration of Helsinki [22]. The approval identifier, approval date, study period, and participant-consent procedure were not available for verification. The applicable version of the Declaration could not be established because the study period was unspecified.

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Availability of data and materials

Data of the study are available from the corresponding author on reasonable request.

Competing interests

The author declares no competing interests.

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Author contributions

Sara Ali Mutashar: conceptualization, methodology, investigation, resources, data curation, formal analysis, validation, visualization, software, supervision, project administration, writing—original draft, and writing—review and editing.

REFERENCES

1. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int. 2024;105(4S):S117-S314. doi:10.1016/j.kint.2023.10.018.
2. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367(1):20-29. doi:10.1056/NEJMoa1114248.
3. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. N Engl J Med. 2021;385(19):1737-1749. doi:10.1056/NEJMoa2102953.
4. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest. 2005;115(3):610-621. doi:10.1172/JCI23056.
5. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. Kidney Blood Press Res. 2008;31(4):255-258. doi:10.1159/000143726.
6. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. 2009;4(2):337-344. doi:10.2215/CJN.03530708.
7. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. Am J Kidney Dis. 2008;51(3):395-406. doi:10.1053/j.ajkd.2007.11.018.
8. Shlipak MG, Matsushita K, Ärnlöv J, Inker LA, Katz R, Polkinghorne KR, et al. Cystatin C versus creatinine in determining risk based on kidney function. N Engl J Med. 2013;369(10):932-943. doi:10.1056/NEJMoa1214234.
9. Peralta CA, Shlipak MG, Judd S, Cushman M, McClellan W, Zakai NA, et al. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. JAMA. 2011;305(15):1545-1552. doi:10.1001/jama.2011.468.
10. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 2007;4(10):e296. doi:10.1371/journal.pmed.0040296.
11. Welch BL. The generalization of 'Student's' problem when several different population variances are involved. Biometrika.

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1947;34(1-2):28-35. doi:10.1093/biomet/34.1-2.28.
12. Wasserstein RL, Lazar NA. The ASA's statement on p-values: context, process, and purpose. Am Stat. 2016;70(2):129-133. doi:10.1080/00031305.2016.1154108.
13. Bourgonje AR, Abdulle AE, Bourgonje MF, Kieneker LM, la Bastide-van Gemert S, Gordijn SJ, et al. Plasma neutrophil gelatinase-associated lipocalin associates with new-onset chronic kidney disease in the general population. Biomolecules. 2023;13(2):338. doi:10.3390/biom13020338.
14. Mitsnefes MM, Kathman TS, Mishra J, Kartal J, Khoury PR, Nickolas TL, et al. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. Pediatr Nephrol. 2007;22(1):101-108. doi:10.1007/s00467-006-0244-x.
15. Bacci MR, Chehter EZ, Azzalis LA, Costa de Aguiar Alves B, Fonseca FLA. Serum NGAL and cystatin C comparison with urinary albumin-to-creatinine ratio and inflammatory biomarkers as early predictors of renal dysfunction in patients with type 2 diabetes. Kidney Int Rep. 2017;2(2):152-158. doi:10.1016/j.ekir.2016.10.001.
16. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol. 2008;3(3):665-673. doi:10.2215/CJN.04010907.
17. Parikh CR, Devarajan P, Zappitelli M, Sint K, Thiessen-Philbrook H, Li S, et al. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. J Am Soc Nephrol. 2011;22(9):1737-1747. doi:10.1681/ASN.2010111163.
18. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney Int. 2009;75(6):652-660. doi:10.1038/ki.2008.638.
19. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int. 2004;65(4):1416-1421. doi:10.1111/j.1523-1755.2004.00517.x.
20. Ix JH, Shlipak MG, Chertow GM, Whooley MA. Association of cystatin C with mortality, cardiovascular events, and incident heart failure among persons with coronary heart disease: data from the Heart and Soul Study. Circulation. 2007;115(2):173-179. doi:10.1161/CIRCULATIONAHA.106.644286.
21. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ. 2015;351:h5527. doi:10.1136/bmj.h5527.
22. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human participants. JAMA. 2025;333(1):71-74. doi:10.1001/jama.2024.21972.

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