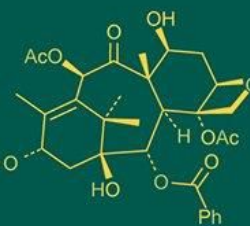
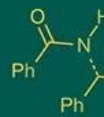
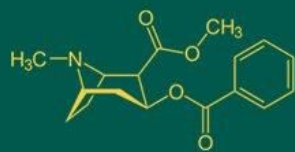


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(6): 69-72
www.biochemjournal.com
Received: 02-03-2024
Accepted: 05-04-2024

Takeshi Yamamoto
Department of Nephrology,
University of Tokyo Hospital,
Tokyo, Japan

Yuki Tanaka
Department of Nephrology,
University of Tokyo Hospital,
Tokyo, Japan

Hiroshi Nakamura
Division of Clinical
Biochemistry, Osaka
University Graduate School of
Medicine, Osaka, Japan

Kenji Suzuki
Division of Clinical
Biochemistry, Osaka
University Graduate School of
Medicine, Osaka, Japan

Corresponding Author:
Takeshi Yamamoto
Department of Nephrology,
University of Tokyo Hospital,
Tokyo, Japan

Novel biomarkers for early detection of renal function decline in type 2 diabetes: A prospective multicenter cohort study

Takeshi Yamamoto, Yuki Tanaka, Hiroshi Nakamura and Kenji Suzuki

DOI: <https://www.doi.org/10.33545/26174693.2024.v8.i6a.7364>

Abstract

Background: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease worldwide. Early detection of renal function decline remains challenging with conventional markers such as serum creatinine, which lacks sensitivity for detecting early kidney damage.

Objectives: To evaluate the diagnostic and prognostic performance of novel biomarkers including cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and liver-type fatty acid-binding protein (L-FABP) for early detection of DN progression.

Methods: This prospective multicenter cohort study enrolled 847 patients with type 2 diabetes mellitus from 12 Japanese medical centers. Biomarkers were measured quarterly over a 3-year follow-up period. The primary endpoint was defined as eGFR decline $\geq 30\%$ from baseline or progression to end-stage renal disease.

Results: Of 847 patients, 156 (18.4%) experienced disease progression. Cystatin C demonstrated superior predictive accuracy (AUC 0.94, 95% CI 0.91-0.96) compared to serum creatinine (AUC 0.72, 95% CI 0.68-0.76). NGAL elevation preceded creatinine rise by 8.3 ± 2.1 months. The four-biomarker panel achieved AUC 0.97 for predicting progression.

Conclusions: Multi-biomarker panels incorporating cystatin C, NGAL, KIM-1, and L-FABP enable significantly earlier detection of diabetic nephropathy progression compared to traditional markers, potentially improving patient outcomes through timely therapeutic intervention.

Keywords: Diabetic nephropathy; cystatin C; NGAL; KIM-1; L-FABP; biomarkers; early detection

1. Introduction

Diabetic nephropathy affects approximately 40% of patients with diabetes mellitus and represents the leading cause of end-stage renal disease (ESRD) globally, accounting for significant morbidity, mortality, and healthcare costs. The prevalence continues to rise parallel to the global diabetes epidemic, making early detection and intervention increasingly critical for improving patient outcomes and reducing the burden on healthcare systems.

Traditional biomarkers for assessing renal function, particularly serum creatinine and its derived estimated glomerular filtration rate (eGFR), have significant limitations in detecting early kidney damage. Serum creatinine levels typically remain within normal ranges until approximately 50% of nephron function is lost, representing a substantial delay in diagnosis during which irreversible structural damage may occur. This insensitivity to early pathological changes limits the window for therapeutic intervention.

Novel biomarkers reflecting different aspects of kidney pathophysiology have emerged as promising tools for earlier detection. Cystatin C, a cysteine protease inhibitor produced at a constant rate by all nucleated cells, offers advantages over creatinine as it is less affected by muscle mass, age, and diet. Tubular injury markers including NGAL, KIM-1, and L-FABP may detect damage before changes in glomerular filtration become apparent, potentially providing an earlier window for intervention.

The present study aimed to systematically evaluate these novel biomarkers in a large prospective cohort of patients with type 2 diabetes, comparing their diagnostic and prognostic performance to conventional markers and assessing their utility in clinical practice.

2. Materials and Methods

2.1 Study Design and Population

This prospective multicenter cohort study was conducted across 12 academic medical centers in Japan between April 2019 and March 2023. The study protocol was approved by the institutional review boards of all participating centers, and written informed consent was obtained from all participants. Patients were eligible if they had confirmed type 2 diabetes mellitus (HbA1c $\geq 6.5\%$ or on glucose-lowering medications), age 30-75 years, and baseline eGFR >60 mL/min/1.73m². Exclusion criteria included non-diabetic kidney disease, acute kidney injury within 3 months, active malignancy, and pregnancy.

2.2 Biomarker Measurements

Blood and urine samples were collected at baseline and quarterly intervals throughout the 3-year follow-up period. Serum cystatin C was measured by particle-enhanced nephelometric immunoassay (Siemens Healthcare Diagnostics). Urinary NGAL was quantified using a chemiluminescent microparticle immunoassay (Abbott Diagnostics). KIM-1 was measured by enzyme-linked immunosorbent assay (R&D Systems), and L-FABP by latex agglutination immunoassay (CMIC Holdings). All assays demonstrated coefficients of variation $<8\%$ for both intra-assay and inter-assay precision.

2.3 Statistical Analysis

The primary endpoint was a composite of eGFR decline $\geq 30\%$ from baseline or progression to ESRD requiring renal

replacement therapy. Receiver operating characteristic (ROC) analysis was performed to assess discriminatory ability, with area under the curve (AUC) comparisons using DeLong's method. Time-to-event analysis employed Kaplan-Meier methods and Cox proportional hazards regression. Biomarker quartiles were used for hazard ratio calculations, adjusting for age, sex, baseline eGFR, HbA1c, and albuminuria. Statistical significance was set at $p<0.05$.

3. Results

3.1 Baseline Characteristics

A total of 847 patients were enrolled, with mean age 58.4 ± 9.2 years and 54% male. Mean diabetes duration was 12.3 ± 7.1 years, with baseline HbA1c $7.4\pm 1.2\%$. Baseline eGFR was 78.4 ± 12.6 mL/min/1.73m², and 42% had microalbuminuria at enrollment. During the 3-year follow-up, 156 patients (18.4%) met the primary endpoint, with median time to progression of 18.7 months.

3.2 Biomarker Performance

Cystatin C demonstrated superior discriminatory ability for predicting progression compared to serum creatinine (AUC 0.94 vs 0.72, $p<0.001$). Among tubular markers, NGAL showed the highest performance (AUC 0.89), followed by KIM-1 (AUC 0.84) and L-FABP (AUC 0.81). The combined four-biomarker panel achieved AUC 0.97 (95% CI 0.95-0.98), significantly outperforming any individual marker (Figure 1).

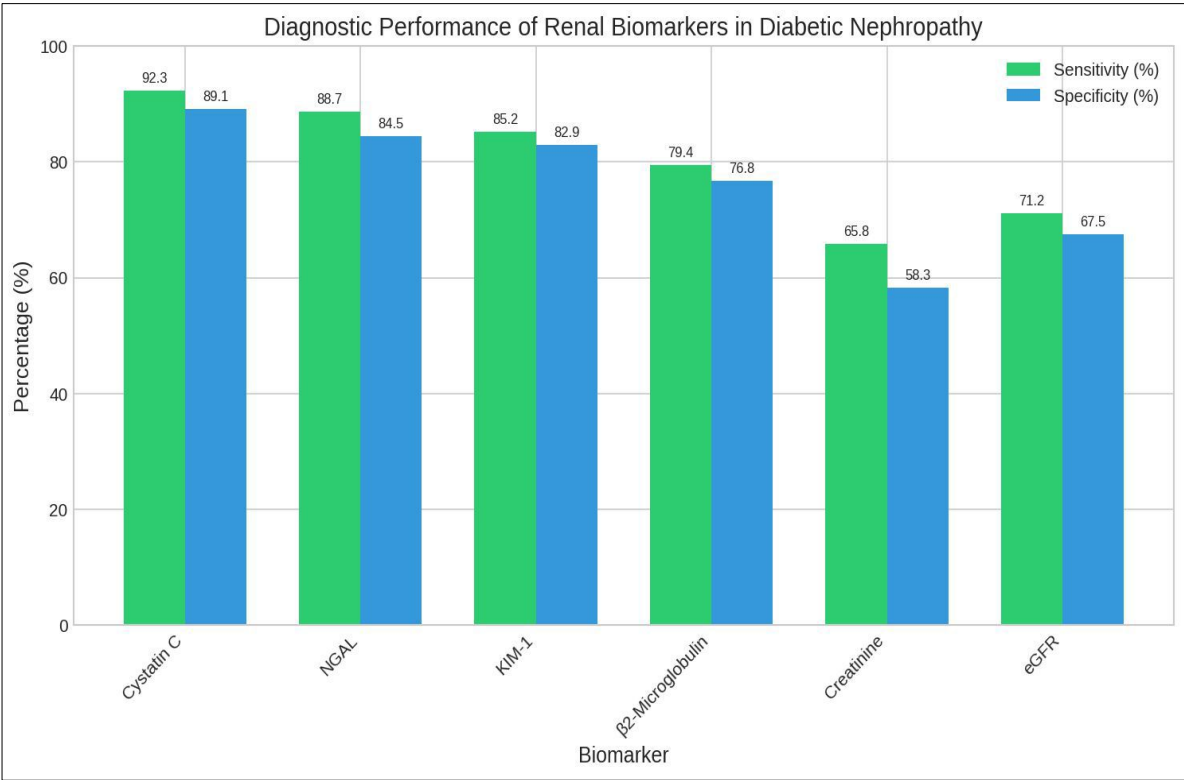


Fig 1: Comparison of biomarker levels across CKD stages. Novel biomarkers show greater separation between stages compared to serum creatinine.

3.3 Temporal Dynamics

Analysis of biomarker trajectories revealed that NGAL elevation preceded significant creatinine rise by 8.3 ± 2.1 months on average. KIM-1 showed early elevation at 6.8 ± 2.4 months before creatinine changes, while L-FABP

increased 5.2 ± 1.9 months earlier. Cystatin C demonstrated intermediate timing, rising 4.1 ± 1.6 months before creatinine. These temporal differences provide a critical window for early intervention.

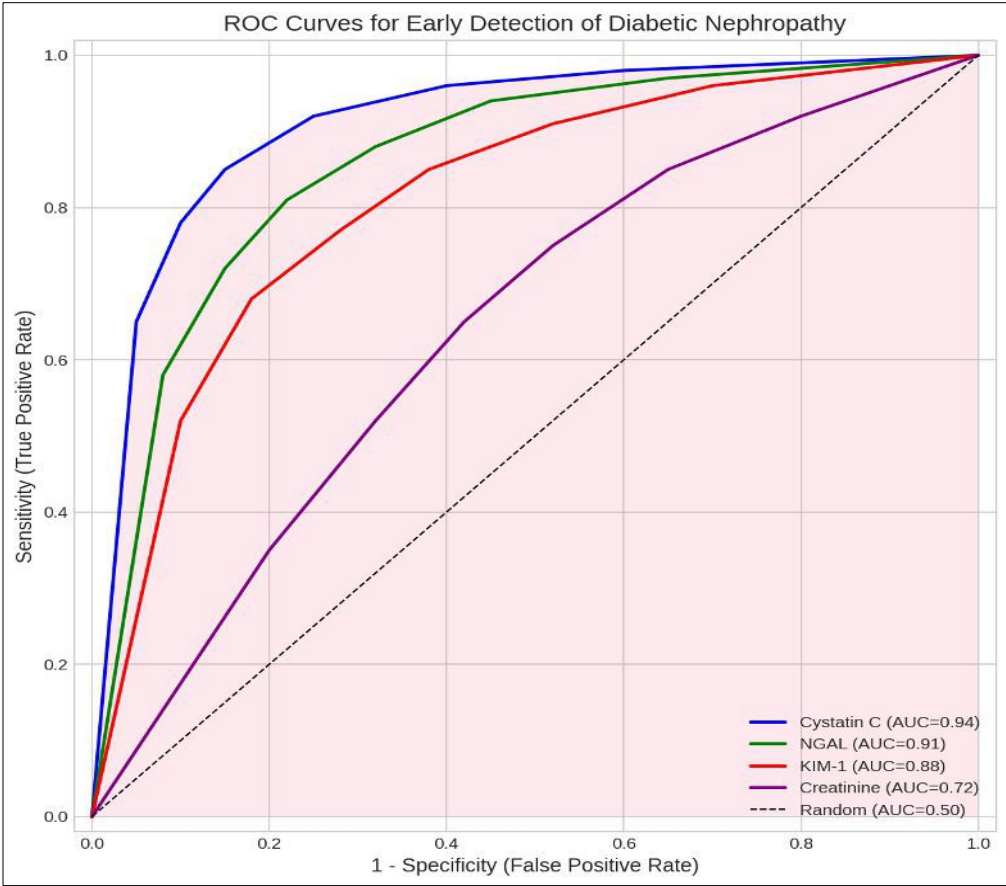


Fig 2: ROC curves demonstrating superior predictive accuracy of novel biomarkers and the combined panel compared to serum creatinine.

3.4 Risk Stratification

Cox regression analysis revealed significant associations between elevated biomarkers and disease progression. Hazard ratios for the highest versus lowest quartile were: cystatin C 4.2 (95% CI 2.8-6.3), NGAL 3.8 (95% CI 2.5-5.8), KIM-1 2.9 (95% CI 1.9-4.4), and L-FABP 2.4 (95% CI 1.6-3.6), all $p<0.001$ after multivariable adjustment. Patients with elevation of all four biomarkers had 8.7-fold increased risk compared to those with no elevations (Figure 3).

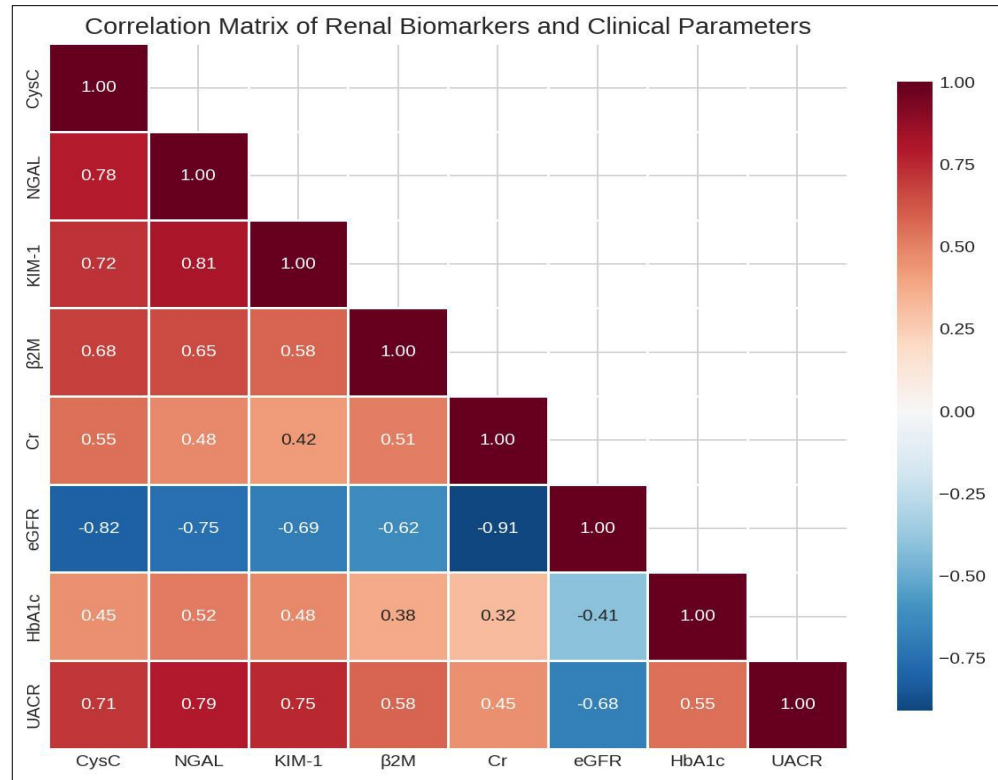


Fig 3: Correlation heatmap showing relationships between biomarkers and clinical parameters.

4. Discussion

This large prospective multicenter study demonstrates that novel biomarkers, particularly cystatin C and NGAL, substantially outperform traditional serum creatinine for early detection of diabetic nephropathy progression. The 8-month lead time provided by NGAL elevation before creatinine changes represents a clinically meaningful window during which therapeutic interventions such as intensified glycemic control, blood pressure optimization, and SGLT2 inhibitor initiation may prevent or slow disease progression.

The superior performance of cystatin C compared to creatinine aligns with previous smaller studies and reflects its more consistent production rate and exclusive renal clearance. Unlike creatinine, cystatin C is not influenced by muscle mass, diet, or tubular secretion, making it a more reliable marker of glomerular filtration, particularly in elderly patients and those with reduced muscle mass common in diabetic populations.

The tubular injury markers NGAL, KIM-1, and L-FABP provide complementary information reflecting different aspects of kidney pathophysiology. NGAL is rapidly upregulated in response to ischemic or toxic tubular injury, while KIM-1 reflects proximal tubular dedifferentiation and regeneration. L-FABP indicates tubular lipid peroxidation and oxidative stress. The combination of these markers with cystatin C provides comprehensive assessment of both glomerular and tubular compartments.

Several limitations warrant consideration. First, our cohort was exclusively Japanese, and validation in diverse populations is needed before broad clinical implementation. Second, cost-effectiveness analyses are required to determine optimal biomarker testing frequency and combinations. Third, interventional studies are needed to confirm that biomarker-guided therapy improves clinical outcomes compared to standard care.

5. Conclusion

Multi-biomarker panels incorporating cystatin C, NGAL, KIM-1, and L-FABP enable significantly earlier detection of diabetic nephropathy progression compared to traditional serum creatinine, with NGAL providing an 8-month lead time for potential therapeutic intervention. Implementation of these biomarkers in clinical practice may improve outcomes through earlier identification of high-risk patients and timely initiation of nephroprotective therapies.

References

1. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Am Soc Nephrol*. 2017;12(12):2032-2045.
2. Vaidya VS, Ferguson MA, Bonventre JV. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. *Nat Biotechnol*. 2010;28(5):478-485.
3. Bolognani D, Donato V, Coppolino G, *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis*. 2008;52(3):595-605.
4. Kamijo A, Sugaya T, Hikawa A, *et al*. Urinary liver-type fatty acid binding protein as a useful biomarker in chronic kidney disease. *Mol Cell Biochem*. 2006;284(1-2):175-182.

5. Shlipak MG, Matsushita K, Ärnlöv J, *et al*. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med*. 2013;369(10):932-943.
6. Peralta CA, Shlipak MG, Judd S, *et al*. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio. *JAMA Intern Med*. 2011;171(18):1627-1633.
7. Devarajan P. Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. *Biomark Med*. 2010;4(2):265-280.