Prognostic Significance of Plasma Galectin-9 and Kidney Histopathology in Biopsy-Proven Kidney Disease

Abstract

Background

Galectin-9 (Gal-9) is a multifunctional protein that has emerged as a potential biomarker in various renal diseases. Its role in kidney pathophysiology, particularly in relation to chronic kidney disease (CKD) progression, remains underexplored. This study aims to evaluate the relationship between plasma Gal-9 levels and kidney histopathology, as assessed by the Mayo Clinic Chronicity Score (MCCS), while also investigating their predictive value for major adverse renal events (MARE) in patients with biopsy-proven kidney disease.

Methods

We conducted a prospective cohort study that enrolled individuals undergoing renal biopsy at Taipei Veterans General Hospital between July 2019 and April 2022. Plasma Gal-9 concentrations were measured, and renal biopsies were evaluated using the MCCS. We examined the correlations between the MCCS, plasma Gal-9 levels, estimated glomerular filtration rate (eGFR), and degree of proteinuria. A Cox proportional hazards regression model was used to access the association of MCCS and plasma Gal-9 level on MARE, defined as a composite outcome of a ≥40% decline in eGFR, initiation of dialysis due to end-stage renal disease, or death from renal or cardiovascular causes. The C-index, net

reclassification index (NRI), and integrated discrimination improvement (IDI) were calculated to evaluate the ability of the Gal-9 and MCCS to predict MARE.

Results

A total of 366 individuals were examined in this study. The median follow-up period was 1.8 years (interquartile range, 1.3–2.6) and the MARE was noted in 133 patients. We found that elevated plasma Gal-9 levels significantly correlated with higher MCCS (rho = 0.37, $p < 0.001$), increased proteinuria (rho = 0.23, $p < 0.001$), and lower eGFR (rho = -0.54, $p < 0.001$). Cox proportional hazards analysis revealed that the association of plasma Gal-9 with MARE was attenuated after adjustment for clinical covariates associated with CKD progression (adjusted hazard ratio [aHR], 1.33; 95% confidence interval [CI], 0.90－1.97; $p = 0.154$). In contrast, the MCCS was still significantly associated with the MARE after adjustment for the same covariates (aHR, 1.16; 95% CI, 1.06－1.29; $p = 0.010$). Moreover, adding the MCCS to a model containing known progression indicators improved the predictive ability (continuous NRI by 0.292; 95% CI, 0.087–0.437; and IDI by 0.065; 95% CI, 0.017–0.117).

Conclusion

In patients with biopsy-proven kidney disease, higher plasma Gal-9 levels were associated with a greater risk of MARE. However, this association diminished after adjusting for clinical factors. In contrast, the MCCS remains a robust predictor of adverse outcomes after full

adjustment, enhancing risk stratification when integrated into clinical models.

Introduction

Chronic kidney disease (CKD) is a multifaceted condition characterized by diverse causes, clinical presentations, and histopathological phenotypes, resulting in varying rates of disease progression[1, 2]. Although conventional parameters such as creatinine-based estimated glomerular filtration rate (eGFR) and proteinuria are commonly used for CKD diagnosis and prognosis, they offer limited insight into the histological changes associated with renal injury and have constraints in predicting clinical outcomes[3-7]. As a result, extensive ongoing research is focused on identifying novel biomarkers with high sensitivity and specificity for detecting relevant histopathological abnormalities, aiming to enhance CKD patient management.

Galectin-9 (Gal-9), a multifunctional protein expressed in various cells such as endothelial and epithelial cells, fibroblasts, and immune cells, participates in various biological processes both intra- and extracellularly[8-10]. Recent studies indicate an increase in Gal-9 levels in both peripheral blood and kidneys as kidney function declines[11, 12]. In advanced CKD, macrophages are recognized as the primary interstitial cells responsible for producing Gal-9. Furthermore, elevated plasma levels of Gal-9 are associated with a higher risk of poor prognosis in individuals with biopsy-proven kidney disease[13]. Although Gal-9 holds promise as a potential CKD biomarker, the molecular mechanisms defining its role in renal disease still require clarification.

Previous studies have found renal fibrosis to be a significant prognostic factor for poor kidney outcomes, even after adjusting for baseline eGFR and proteinuria. To assess the severity of chronic histopathological lesions, various grading systems have been developed, among which the Mayo Clinic Chronicity Score (MCCS) can be applied to various kidney diseases and demonstrates good prognostic prediction capabilities[14]. Currently, there remains uncertainty regarding whether kidney biomarkers, such as plasma Gal-9, can accurately indicate chronic renal histological lesions and their association with MCCS. The prognostic values of MCCS and plasma Gal-9 for major adverse renal events (MARE) have not yet been directly compared[15, 16]. At our institution, we collected biofluid samples from individuals undergoing native renal biopsy and followed them longitudinally. Utilizing this database, we conducted a study to explore the relationship between plasma Gal-9 and MCCS. Additionally, we compared the predictive performance of these markers for the occurrence of MARE in this patient population.

Methods

Study participants

We conducted a retrospective analysis using data obtained from the Taipei Renal Transcriptomics and Outcomes Investigation (TRTOI) cohort. In brief, the study enrolled adults (≥20 years old) who underwent native renal biopsies when clinically indicated since

October 2018. Written informed consent was obtained from all patients, and the study protocol was approved by the local ethics committee, ensuring adherence to the principles of the Declaration of Helsinki. The exclusion criteria, including refusal of consent, prior renal replacement therapy, pregnancy, and inadequate biopsy samples, yielded 366 participants. **Supplemental Figure S1** illustrates the enrollment flowchart of the study cohort. Blood and urine samples were collected on the day of biopsy. Kidney tissues were processed according to standard procedures, with interpretations by an experienced nephropathologist.

Examining Gal-9 levels in plasma

Plasma samples were stored at -80°C until analysis. Gal-9 was measured in 50 µl of plasma, diluted four times, using a previously described in-house, multiplex, bead-based immunoassay. The data were obtained using a Bio-Plex 200 instrument (Bio-Rad, Hercules, California) and processed using Bio-Plex Manager 6.1 software. All samples were tested in duplicate to ensure statistically valid results.

Clinical and laboratory data

Baseline patient characteristics, including age, sex, body mass index (BMI), and comorbidities, along with laboratory and outcome data, were extracted from electronic medical records. Kidney function was assessed using the eGFR (simplified MDRD formula),

while proteinuria was determined by the spot urine protein-creatinine ratio (UPCR).

Histopathological evaluation and the MCCS grading system

Histopathological lesions in the kidney were characterized and graded using the method outlined by Srivastava et al., which involved evaluating glomerular, vascular, and tubulointerstitial injuries. The distribution of severity grades for eight histopathological lesions is displayed in **Supplemental Table S1**. Due to limited cases, several categories—endocapillary glomerular inflammation, extracapillary cellular crescents, focal glomerular necrosis, and fibrocellular crescents—were combined into one variable, "glomerular inflammation," for data analysis. The definitive primary clinicopathologic diagnosis was established through a comprehensive review of all participants' medical records in conjunction with histopathologic evaluations. Patients were classified into six diagnostic groups, and their primary clinicopathologic diagnoses are listed in **Supplemental Table S2**.

Furthermore, for a comprehensive assessment of the severity of kidney fibrosis, the MCCS was calculated by summing the scores of global glomerulosclerosis (0–3), interstitial fibrosis (0–3), tubular atrophy (0–3), and arterial sclerosis (0–1). As the original MCCS assesses interstitial fibrosis and tubular atrophy (IFTA) independently, we multiplied our IFTA scores by two. Our semiquantitative scale for arteriosclerosis was also changed to a

binary format, where 0 indicates none/mild and 1 indicates moderate/severe, following the MCCS criteria. Therefore, the severity of kidney fibrosis is categorized into four grades: minimal (0-1), mild (2-4), moderate (5-7), and severe (≥8), based on the total MCCS score.

MARE

The MARE was a composite outcome defined as a decline in eGFR of ≥40% from baseline to the last visit, initiation of kidney replacement therapy due to kidney failure, or death from kidney or cardiovascular causes, whichever occurred first. Participants were observed until they experienced kidney failure, death, were lost to follow-up, or until the study concluded on March 31, 2023. To mitigate bias in assessing outcomes, observations were censored if no subsequent eGFR measurement was available within 1.5 years from the last recorded measurement.

Statistical analyses

All variables contained complete data without any missing values and were presented using appropriate measures such as mean ± standard deviation, median (interquartile range), or number (percentage). Plasma Gal-9 levels underwent natural log transformation to address the skewed distribution and enhance normality. Individuals were grouped according to either tertiles of plasma Gal-9 concentration or into 4 grades of MCCS for the assessment of

relevant parameters. The Kruskal-Wallis H test assessed differences in continuous variables among groups. Spearman correlation analysis was employed to evaluate the correlations among clinical-laboratory parameters, plasma Gal-9 levels, and MCCS. Multivariate logistic regression analyses were performed to evaluate the relationship between plasma Gal-9 and different histopathological lesions, with adjustments made for age, sex, proteinuria, and eGFR. Kaplan-Meier curves and the log-rank test were utilized to assess the cumulative MARE incidence, categorized by plasma Gal-9 tertiles and MCCS grades. Subsequently, both univariate and multivariate Cox proportional hazards regression analyses were conducted to investigate the correlation between plasma Gal-9 and MCCS with adverse kidney outcomes. The multivariate model was adjusted for baseline covariates such as age, sex, hemoglobin, eGFR, and proteinuria, in addition to the primary clinicopathologic diagnosis, distinguishing between diabetic kidney disease (DKD) and other diagnoses. These covariates were chosen as potential confounders due to their biological plausibility. To explore the MCCS's added predictive capability, we compared Harrell's C-index between fully adjusted Cox proportional hazards models with and without MCCS. Additionally, we evaluated improvements in risk discrimination at median follow-up using category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI). The 95% confidence intervals for differences in C-index, category-free NRI, and IDI were based on 10,000 bootstrap samples. All statistical analyses were performed with a two-tailed significance level of $P < 0.05$ using

SAS version 9.4 (SAS Institute, Inc., Cary, NC) and R software version 3.5.2 (R Development Core Team, Vienna, Austria, 2018).

Results

Baseline participant characteristics

Table 1 presents the baseline characteristics of the study cohort. The mean age was 54 ± 17 years, with 42% females. Moreover, 44% had hypertension, and 29% had diabetes. The median values for plasma Gal-9, eGFR, and UPCR were 690 pg/mL (IQR: 488–1029 pg/mL), 36 ml/min per 1.73 m² (IQR: 18–66 ml/min per 1.73 m²), and 2.9 (IQR: 0.9–7.0), respectively. Proliferative glomerulonephritis (30%), nonproliferative glomerulopathies (23%), and DKD (20%) comprised the most frequent clinicopathologic diagnostic categories within the studied population. **Supplemental Table 2** lists clinicopathologic diagnoses by category, while **Supplemental Table 3** shows that DKD patients had the highest levels of plasma Gal-9, MCCS, and UPCR, along with the lowest eGFR compared to others.

Spearman's correlation analyses were conducted to investigate the relationship between MCCS, plasma Gal-9, eGFR, and proteinuria. The findings presented in **Table 2** demonstrate a positive correlation between MCCS and Gal-9, as well as direct associations of both MCCS and plasma Gal-9 with UPCR, and an inverse relationship with eGFR.

Associations between plasma Gal-9 levels and kidney histopathologic lesions

We additionally examined the correlation between plasma Gal-9 and various histopathological lesions. In **Table 3**, we presented multivariable-adjusted odds ratios and 95% confidence intervals for histopathological features, accounting for both continuous and categorical plasma Gal-9 levels. Elevated levels of plasma Gal-9 were found to be associated with the presence of mesangial expansion; however, our results showed no significant correlation between plasma Gal-9 and any of the MCCS criteria, including global glomerulosclerosis, IFTA, or vascular sclerosis.

Relationships between plasma Gal-9, MCCS, and MARE

The Kaplan–Meier curves in **Figure 1** depict MARE stratification by baseline plasma Gal-9 tertiles and MCCS grades, demonstrating significant differences in MARE incidence among these categories. Cox proportional hazards analysis showed that higher plasma Gal-9 tertiles were significantly associated with an increased risk of MARE (**Table 4**). In univariate analysis, the third tertile had a hazard ratio (HR) of 7.16 (95% CI, 3.97–12.92) compared to the first tertile. A per 1-unit increase in Ln-Gal-9 was associated with an HR of 3.45 (95% CI, 2.51–4.75). After adjusting for age, sex, eGFR, urine protein-to-creatinine ratio, and primary clinicopathologic diagnosis in Model 2, the association remained significant, with the third tertile having an HR of 2.22 (95% CI, 1.17–4.19). However, the per 1-unit increase in

Ln-Gal-9 was no longer statistically significant after adjustment.

Regarding MCCS, it still exhibited a significant association with the outcome, even in the fully adjusted model. Compared with those with minimal-to-mild chronic changes, the HRs for the outcomes of individuals with severe chronic changes were 2.58 (95%, 1.22－5.47) Each one-point increase in MCCS was also significantly associated with a higher risk of MARE (aHR, 1.16; 95% CI, 1.06 to 1.29).

Model performance for predicting MARE with the addition of plasma gal-9 and MCCS

The addition of plasma Gal-9 to the model containing clinical covariates (age, sex, eGFR, and urine protein-to-creatinine ratio) slightly increased the C-index from 0.830 (95% CI, 0.797–0.862) to 0.832 (95% CI, 0.800–0.865). The continuous NRI for the Gal-9 model was -0.131 (95% CI, -0.299 to -0.214, p = 0.346), indicating no significant improvement in risk reclassification. The absolute IDI was also negligible at 0.001 (95% CI, -0.003 to 0.017, p = 0.578).

In contrast, including MCCS in the model significantly improved risk reclassification and discrimination ability. The C-index remained at 0.830 (95% CI, 0.799–0.862), but there was a marked improvement in reclassification, with a continuous NRI of 0.292 (95% CI, 0.087–0.437, p = 0.007). The absolute IDI also increased to 0.065 (95% CI, 0.017–0.117, p = 0.007), as shown in **Table 5.**

Discussion

This prospective cohort study provides a comprehensive assessment of the clinical utility of plasma Gal-9 levels and the MCCS for predicting renal outcomes in individuals with CKD. In our study population, we found a significant correlation between elevated plasma Gal-9 levels and the severity of chronic lesions in the kidney, as evaluated by the MCCS. Notably, higher MCCS predicted an increased risk of major adverse renal events, even after adjusting for potential confounders such as age, sex, primary clinicopathologic diagnosis, baseline hemoglobin, proteinuria, and baseline eGFR. While elevated levels of plasma Gal-9 were observed to show a trend towards an increased risk of major adverse renal events, this trend did not achieve statistical significance.

Our findings add to the growing evidence suggesting that plasma Gal-9, a member of the galectin family, may play an important role in the pathogenesis and progression of kidney disease. Mechanistic details regarding the association between plasma Gal-9 levels and kidney function are still under investigation, but several potential pathways have been suggested. Gal-9 has a molecular weight of approximately 36 kDa and can be filtered through glomerular capillaries. A reduction in GFR might be linked to an elevation in serum Gal-9 levels[11]. A prior study has found a significant correlation between plasma galectin-9 levels and intrarenal mRNA expression of the LGALS9 gene in patient with kidney disease, suggesting that injured kidneys might contribute to elevated circulating galectin-9[12]. Furthermore, increased Gal-9 mRNA expression in peripheral blood mononuclear cells has

been reported in CKD patients compared to those without CKD, indicating that Gal-9 may play a role in the immune response associated with kidney disease[17].

Gal-9 is known to mediate numerous biological processes such as neutrophil adhesion, monocyte differentiation, angiogenesis and immune regulation[18-20]. Enhanced expression of galectin-9 is also associated with an increased production of T cell cytokines[21].

Elevated plasma levels of Gal-9 might indicate more advanced disease stages characterized by the excessive inflammation, leading to chronic tissue damage, fibrosis, and inflammation. This could explain the observed association between high plasma Gal-9 levels and the severity of renal fibrosis as measured by the MCCS.

Our results also suggest a role for the MCCS as a useful tool for the prediction of long-term renal outcomes in CKD patients. The MCCS allows for the standardization of grading chronic lesions in native renal biopsy tissues[14]. Our findings align with previous studies that demonstrated the prognostic utility of the MCCS for various kidney diseases. We found that the MCCS was significantly associated with renal outcomes, independent of traditional risk factors such as baseline eGFR and proteinuria. The MCCS, therefore, may serve as a valuable adjunct to these traditional parameters, offering additional insight into the severity of renal pathology and allowing for more accurate risk stratification of CKD patients.

The main strength of our study is the prospective design with the inclusion of 366 patients with biopsy-proven kidney disease. To the best of our knowledge, this is the first study

examining both the relationship between plasma Gal-9 concentrations and long-term renal outcomes as well as its correlation with the grading of chronic changes in kidney samples. We demonstrated that MCCS has a stronger predictive value for renal outcomes than Gal-9, across a variety of kidney disease etiologies. The results suggest that we should pay more attention to MCCS on renal biopsies, irrespective of the clinicopathologic diagnosis. Further research is needed to explore the prognostic value of Gal-9 in renal diseases.

There are some limitations in this study that should be acknowledged. First, the analysis was carried out in a single tertiary hospital and may not be representative of other populations with different clinical characteristics or healthcare settings. Second, we included individuals who were clinically indicated for renal biopsies, with the majority being diagnosed with glomerular diseases. This could limit the applicability of our results to a wider spectrum of kidney diseases. Third, therapy administered after the biopsy may have affected the clinical outcomes of our patients, but data on such factor was not address in this study.

In conclusion, our study identified an association between plasma Gal-9 and histopathologic lesions found in renal biopsy specimens. While the MCCS demonstrated a significant predictive ability for MAREs in patients with biopsy-proven kidney disease, the association of plasma Gal-9 with renal outcomes did not reach statistical significance in our analysis. Further large-scale studies are needed to validate our findings and explore the underlying mechanisms linking Gal-9 with kidney disease.

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Table 1. Baseline Clinical Parameters and Chronic Histopathologic Findings for Participants in the TRTOI Cohort

Baseline Characteristics	***N* = 366**
Plasma galectin-9 concentrations, pg/mL	690 (488 – 1029)
Clinical characteristics	
Age, yr	54 ± 17

Women, *n* (%)	155 (42)
BMI, kg/m^2	25.5 ± 4.5
Comorbid conditions, *n* (%)	
Diabetes mellitus	105 (29)
Hypertension	162 (44)
Prevalent CVDs	50 (14)
SLE	37 (10)
Malignancy	82 (22)
Laboratory test results	
BUN, mg/dL	30 (18－49)
Serum creatinine, mg/dl	1.8 (1.0－3.4)
eGFR, ml/min per 1.73 m^2	36 (18－66)
Proteinuria, mg/mg creatinine	2.9 (0.9－7.0)
Serum albumin, g/dL	3.3 (2.6－3.9)
Hemoglobin, g/dL	11.1 (9.3－13.1)
Leukocytes, 10^3/mm^3	6.7 (5.2－8.2)
Primary clinicopathologic diagnosis, *n* (%)	
Proliferative glomerulonephritis	109 (30)
Nonproliferative glomerulopathies	84 (23)
Diabetic nephropathy	72 (20)
Vascular	34 (9)
Tubulointerstitial	30 (8)
Other	37 (10)
Mayo Clinic Chronicity Score, *n* (%)	
Minimal chronic changes (0－1)	45 (12)
Mild chronic changes (2－4)	134 (37)
Moderate chronic changes (5－7)	111 (30)
Severe chronic changes (≥8)	76 (21)
Medications, *n* (%)	
ACEi/ARB	212 (58)
Beta-blockers	170 (46)
Calcium channel blockers	223 (61)
Glucocorticoids	195 (53)
Immunosuppressants other than glucocorticoids	66 (18)

Data are presented as mean ± SD, median [interquartile range], and number (percentage). Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; SLE, systemic lupus erythematosus.

Table 2. Spearman's rank correlation coefficients among the Mayo Clinic Chronicity Score, plasma galectin-9, renal function, and proteinuria.

	Gal-9	MCCS	eGFR	BUN	SCr	Proteinuria
Gal-9	1	0.37 (<0.001)	-0.54 (<0.001)	0.51 (<0.001)	0.53 (<0.001)	0.23 (<0.001)
MCCS	0.37 (<0.001)	1	-0.73 (<0.001)	0.64 (<0.001)	0.72 (<0.001)	0.17 (0.001)
eGFR	-0.54 (<0.001)	-0.73 (<0.001)	1	-0.87 (<0.001)	-0.99 (<0.001)	-0.14 (0.01)
BUN	0.51 (<0.001)	0.64 (<0.001)	-0.87 (<0.001)	1	0.86 (<0.001)	0.17 (0.001)
SCr	0.53 (<0.001)	0.72 (<0.001)	-0.99 (<0.001)	0.86 (<0.001)	1	0.14 (0.009)
Proteinuria	0.23 (<0.001)	0.17 (0.001)	-0.14 (0.01)	0.17 (0.001)	0.14 (0.009)	1

Abbreviations: BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; Gal-9, galectin-9; MCCS, Mayo Clinic Chronicity Score; SCr, serum creatinine.

P values are shown in parentheses.

Table 3. The association between plasma Gal-9 with each histopathological lesion

	GI[b]	ME[b]	SS[b]	GS[c]	ATI[b]	IFTA[d]	Arteriosclerosis[e]	Arteriolosclerosis[e]
Variables	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)
Plasma Gal-9 (pg/mL)								
Tertile 1	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Tertile 2	1.25 (0.68－2.29)	1.54 (0.90－2.65)	1.78 (1.03－3.10)	1.50 (0.78－2.89)	1.13 (0.63－2.04)	1.77 (0.84－3.75)	1.04 (0.59－1.84)	1.67 (0.85－3.27)
Tertile 3	1.40 (0.71－2.74)	2.73 (1.47－5.07)	1.51 (0.82－2.76)	0.90 (0.45－1.81)	1.17 (0.62－2.22)	1.22 (0.55－2.68)	1.20 (0.65－2.25)	1.03 (0.50－2.13)
Per 1-Ln increase	1.40 (0.83－2.35)	1.81 (1.13－2.92)	1.03 (0.65－1.64)	0.68 (0.39－1.17)	1.31 (0.80－2.15)	0.94 (0.50－1.80)	1.19 (0.73－1.94)	0.79 (0.46－1.35)

[a] Logistic regression models were constructed using each histopathological lesion as the dependent variable and the tertiles and Ln-transformed plasma Gal-9 as the independent variables. Each multivariate model was adjusted for age, sex, proteinuria, and eGFR.

[b] Dependent variable is presence of lesion.

[c] Dependent variable is involvement of >25% of glomeruli.

[d] Dependent variable is involvement of >25% of cortical volume.

[e] Dependent variable is moderate-to-severe degree of lesion severity.

Abbreviations: ATI, acute tubular injury; CI, confidence interval; Gal-9, galectin-9; GI, glomerular inflammation; GS, global glomerulosclerosis; IFTA, interstitial fibrosis and tubular atrophy; Ln, natural logarithm; ME, mesangial expansion; OR, odds ratio; SS, segmental glomerulosclerosis.

Table 4. Univariate and multivariate Cox proportional hazard models incorporating plasma galectin-9 and the Mayo Clinic Chronicity Score

Variable	Major Adverse Renal Events Hazard Ratio (95% Confidence Interval)		
	Univariate	Model 1	Model 2
Plasma galectin-9, pg/mL			
Tertile 1: 411 (324－489)[a]	Reference	Reference	Reference
Tertile 2: 690 (607－773)[a]	3.80 (2.06－7.02)	1.92 (1.03－3.59)	1.80 (0.95－3.40)
Tertile 3: 1190 (1027－1540)[a]	7.16 (3.97－12.92)	2.51 (1.35－4.66)	2.22 (1.17－4.19)
Per 1-unit increase of Ln-Gal-9	3.45 (2.51－4.75)	1.42 (0.99－2.05)	1.33 (0.90－1.97)
MCCS			
Minimal-to-mild chronic changes (0－4)	Reference	Reference	Reference
Moderate chronic changes (5－7)	7.95 (4.56－13.86)	3.64 (1.96－6.74)	1.86 (0.94－3.68)
Severe chronic changes (≥8)	15.93 (9.12－27.85)	6.49 (3.45－12.24)	2.58 (1.22－5.47)
Per 1-unit increase in the MCCS	1.48 (1.38－1.59)	1.31 (1.20－1.42)	1.16 (1.06－1.29)

Model 1 was adjusted for age, sex, eGFR, urine protein-to-creatinine ratio.

Model 2 included the covariates in model 1 and was further adjusted for primary clinicopathologic diagnosis.

[a]Plasma galectin-9 level are presented as median and interquartile range.

Abbreviations: Gal-9, galectin-9; Ln, natural logarithm; MCCS, Mayo Clinic Chronicity Score.

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Variable	MARE Hazard Ratio (95% Confidence Interval)		
	Univariate	Model 1	Model 2
Plasma galectin-9, pg/mL			
Tertile 1: 411 (324－489)[a]	Reference	Reference	Reference
Tertile 2: 690 (607－773)[a]	3.80 (2.05－7.02)	2.36 (1.26－4.43)	1.83 (0.98－3.45)
Tertile 3: 1190 (1027－1540)[a]	7.15 (3.96－12.91)	3.40 (1.80－6.42)	2.20 (1.16－4.16)
Per 1-unit increase of Ln-Gal-9	3.45 (2.51－4.75)	1.78 (1.22－2.61)	1.19 (0.81－1.76)
MCCS			
Minimal-to-mild chronic changes (0－4)	Reference	Reference	Reference
Moderate chronic changes (5－7)	7.94 (4.55－13.85)	5.08 (2.83－9.12)	3.07 (1.64－5.75)
Severe chronic changes (≥8)	15.92 (9.11－27.81)	7.49 (3.98－14.09)	4.09 (2.03－8.22)
Per 1-unit increase in the MCCS	1.48 (1.38－1.59)	1.34 (1.22－1.46)	1.22 (1.10－1.35)

Model 1 is adjusted for the following factors: age, sex, presence of diabetic kidney disease, renin–angiotensin-aldosterone system inhibitors (angiotensin-converting enzyme inhibitors or angiotensin receptor blockers), immunosuppressive medications (glucocorticoids or other immunosuppressants), as well as baseline hemoglobin and proteinuria.

Model 2 is Model 1 and further adjusted for eGFR at baseline.

Patients were stratified into groups based on plasma Gal-9 tertiles or MCCS grades, respectively.

Abbreviations: Gal-9, galectin-9; Ln, natural logarithm; MARE, major adverse renal events; MCCS, Mayo Clinic Chronicity Score.

Table 5. Difference of Harrell concordance index between Cox regression models with or without the galectin-9 level and MCCS and the category–free net reclassification improvement and integrated discrimination improvement for predicting the outcome at median follow-up obtained by adding the galectin-9 level and MCCS.

Model	C-Index (95% CI)	Continuous NRI (95% CI)	P value	Absolute IDI (95% CI)	P value
Clinical covariates	0.830 (0.797 - 0.862)	Reference		Reference	
Clinical covariates + galectin-9	0.832 (0.800 - 0.865)	-0.131 (-0.299－0.214)	0.346	0.001 (-0.003－0.017)	0.578
Clinical covariates + MCCS	0.830 (0.799 - 0.862)	0.292 (0.087－0.437)	0.007	0.065 (0.017－0.117)	0.007

Clinical covariates: age, sex, eGFR, urine protein-to-creatinine ratio

Abbreviations: C-Index, concordance index; 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; MCCS, Mayo clinic chronicity score; NRI, net reclassification improvement; IDI, integrated discrimination improvement

Figure legends

Figure 1. Probability of major adverse renal events (MARE) stratified by plasma galectin-9 (Gal-9) and the Mayo Clinic Chronicity Score (MCCS). (A) Event-free survival curves according to the median plasma Gal-9 concentrations. The estimated 2-year event-free survival rate was 84% in low Gal-9 group and 56% in high Gal-9 group. (B) Event-free survival curves for patients stratified by MCCS (≤4 vs. >4). The estimated event-free survival at 2 years was 93% in the low MCCS group and 48% in the high MCCS group. There were significant differences of the event-free survival between the different Gal-9 and MCCS groups. MARE: ≥40% reduction in estimated glomerular filtration rate from baseline, end-stage renal disease (ESRD) or death before ESRD attributed to cardiovascular and renal complications. Survival rates were compared using the log-rank test.