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Predicting Decline of Kidney Function in Lupus Nephritis Using Urine Biomarkers

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Abstract

Objective—To evaluate candidate biomarkers to predict future renal function decline (RFD) in children and adults with lupus nephritis (LN).

Methods—At the time of enrollment into prospective observational LN cohort studies liver-type fatty acid binding protein (LFABP), albumin, monocyte chemoattractant protein-1 (MCP-1), uromodulin, transferrin and hepcidin were measured in urine samples of two cohorts of patients with LN, one followed at a pediatric (cohort-1; n=28) and one at an adult institution (cohort-2; n=69). The primary outcome was RFD, defined in cohort-1 as a decrease in eGFR of ≥20% and in cohort-2 as a sustained increase of ≥25% in serum creatinine concentration (SCr), both from baseline.

Results—All patients (n=97) had normal eGFR or SCr at the time of urine collection at baseline. RFD occurred in 29% (8/28) of patients in cohort-1 during a mean follow-up of 6.1 months and in 30% (21/69) of those in cohort-2 during a mean follow-up of 60 months. Individually in cohort-1, levels of MCP-1, transferrin, LFABP and albumin were higher in the RFD group than those who maintained renal function, with statistical significance for LFABP and albumin. In cohort-2 the RFD group also had higher levels of urine MCP-1 and albumin than

others. The combination of LFABP, MCP-1, albumin, and transferrin had good predictive accuracy for RFD in both cohorts (area under the ROC curve = 0.77 – 0.82).

Conclusion—The combinatorial urine biomarker LFABP, MCP-1, albumin, and transferrin shows promise as a predictor of renal functional decline in LN, and warrants further investigation.

Key Indexing Terms

SLE; lupus nephritis; kidney injury; biomarker; renal function decline

Introduction

Lupus nephritis (LN) is a risk factor for poor long-term outcomes in adults and children with systemic lupus erythematosus (SLE), especially if patients develop chronic kidney disease (CKD) or end-stage renal disease (ESRD) (1, 2). Previous studies suggest that timely initiation of appropriate therapy improves the prognosis of LN as there is a strong association between delayed treatment, decline in kidney function and occurrence of ESRD (1). This supports the notion that patients at high-risk for poor LN outcomes may benefit from early aggressive therapy. However, there are no known clinically useful biomarkers to reliably identify such high-risk patients.

Liver-type fatty acid binding protein (LFABP), albumin, monocyte chemoattractant protein-1 (MCP-1), uromodulin, transferrin and hepcidin have previously been proposed as novel biomarkers for LN and other chronic kidney diseases, including diabetic nephropathy (3–5). The urine levels of some of these analytes correlate with LN activity, and change with response to treatment (6–8). However, the usefulness of these urine analytes to help identify patients at risk of future decline in renal function has not been examined.

We investigated whether levels of urinary LFABP, albumin, MCP-1, uromodulin, transferrin and hepcidin either singly or combined are predictive biomarkers that differentiate patients with LN who will develop significant future renal function decline (RFD) from those who will not.

Material and Methods

Patients

The study was approved by the institutional review boards and ethics review committees of the two participating centers. There were two cohorts of patients with LN (9). Cohort-1 was followed at pediatric centers (7, 10), and cohort-2 is part of the Ohio State University SLE Study (4). To be included in this study, patients needed to have LN and an estimated glomerular filtration rate (eGFR) $100 \text{ ml/min/1.73m}^2$, or a normal serum creatinine concentration (SCr) at enrollment. Urine samples from cohort-1 were collected within 60 days of a diagnostic kidney biopsy done for new abnormalities on urinalysis. For cohort-2, urine samples were mostly collected well after the diagnostic kidney biopsy (mean of 26.4 months) during disease quiescence. However, about 22% of cohort-2 urine samples were obtained during a LN flare, as previously defined (4).

Besides patient demographic characteristics, key laboratory measures and concurrent medications were obtained from the medical record at the time of the urine collection. Urine protein excretion in children was estimated by the protein to creatinine (P/C) ratio in a random urine sample, and in adults from a 24-hour urine collection. Histologically, LN was classified according to the International Society of Nephrology/Renal Pathology Society Classification (ISN/RPS) (9).

Definition of renal function decline

There is no generally accepted definition for clinically relevant renal functional decline (RFD). Because SCr increases normally during childhood (11) it is not a good measure of renal function. Hence we employed two different definitions for RFD. In cohort-1 RFD was defined as a sustained decrease in eGFR of at least 20% during follow up. The eGFR was determined by an ageappropriate calculation of creatinine clearance using the modified Schwartz Formula (12). For cohort-2 RFD was defined as a sustained increase of at least 25% in SCr during follow-up after having a normal SCr at baseline (13).

Urinary analyte assays

Urine samples were frozen at -80°C prior to batch processing. We measured urinary concentrations of transferrin by immunonephelometry (Dade Behring BNII Prospect, Marburg, Germany) with intra-assay coefficients of variation (CV_{intra}) of 3.4% and a inter-assay $\text{CV}(\text{CV}_{\text{inter}})$ of 2.5%, respectively. Urine concentrations of MCP1 ($\text{CV}_{\text{intra}}/\text{CV}_{\text{inter}}$: 5.0%/5.1%) were measured by ELISA (R&D Systems, Minneapolis, MN, USA). Urine L-FABP ($\text{CV}_{\text{intra}}/\text{CV}_{\text{inter}}$: 6.1%/10.9%) was measured using the human L-FABP ELISA kit from CMIC Ltd. (Tokyo, Japan) following the manufacturer's protocol. Hepcidin-25 ($\text{CV}_{\text{intra}}/\text{CV}_{\text{inter}}$: 3.5%/3.4%) was measured by EIA kit S-1337 (Peninsula Laboratories, San Carlos, CA) according to manufacturer's instructions. The assay range was 0.02–6.25 ng/ml hepcidin, and was validated in our laboratory (data not shown). Urine albumin ($\text{CV}_{\text{intra}}/\text{CV}_{\text{inter}}$: 5%/3%) was measured using an immunoturbidimetric assay from Roche Diagnostics (COBAS c501) following the manufacturer's protocol. Uromodulin ($\text{CV}_{\text{intra}}/\text{CV}_{\text{inter}}$: 9%/7.5%) was measured by multiple reaction monitoring assays (University of Minnesota) and analyses performed with MultiQuant (2.0.2) software. Urine creatinine measurements ($\text{CV}_{\text{intra}}/\text{CV}_{\text{inter}}$: 2.4%/4.2%) were made using a modified Jaffe reaction, and microalbumin (MALB) was measured by immunoturbidimetry, both on a Dimension Xp HM Clinical Analyzer (Siemens, Munich, Germany). A four-parameter polynomial regression was used for fitting the standard curve.

Concentrations of the urine analytes were measured in ng/ml for MCP1, hepcidin and LFABP, and in mg/dl for transferrin. All urine analytes were normalized to urine creatinine concentration (in mg/mL). Laboratory personnel measuring the analytes were blinded to the clinical status and histological information of the study participants.

Statistical analysis

All urine analyte concentrations were log-transformed prior to statistical analyses to correct the right skewness of their distributions. Fixed-effect models validated for non-parametric data were used to assess the association of each analyte to RFD. Wilcoxon rank sum testing

was used to compare the geometric means of analytes between patients who developed RFD (RFD-group) and those who did not (NoRFD-group). Urine analytes that differed in expression between RFD and NoRFD groups at a p level ≤ 0.1 in cohort-1 or cohort-2 were then tested in multivariate logistic regression models as candidate predictors of future RFD. The ability of single and combined analytes to discriminate between the RFD-group and the noRFD-group was assessed as the areas under the (AUC; range 0–1) receiver operating characteristic (ROC) curves.

The discriminatory power of an analyte or combination of analytes was considered to be outstanding or excellent, good, fair, or poor if the AUC was in the range of >0.9 , $0.81–0.90$, $0.71–0.80$, $0.61–0.70$, or $0.50–0.60$, respectively. A statistically “optimal” cut-off value on the ROC-curve which yields the maximum sum of sensitivity and specificity was determined for each ROC curve. Positive and negative likelihood ratios (LR+ and LR–) were also calculated to evaluate the diagnostic accuracy of a single biomarker and all of the biomarkers combined. We also determined the sensitivity, specificity, the positive (LR+) and the negative likelihood ratios (LR–) for the statistically optimal ROC curve cut-off. Here, LR+ values are interpreted as: > 10 : large, often conclusive increase in the likelihood of “ruling in” the future development of RFD; $5 – 9.9$: moderate increase; and $2 – 4.9$: small increase, respectively. As an example, a LR+ of 2 increases the probability for RFD by 15%, a LR+ of 5 increases it 30%, and a LR+ of 10 increases it 45% (14). LR– are similarly interpreted for “ruling-out” the future development of RFD with LN. Statistical analyses were done using SAS, version 9.4. P values ≤ 0.05 were considered statistically significant.

Results

Patient characteristics

Details on the study cohorts at baseline are shown in Table 1. As expected the mean age in cohort-1 was lower than cohort-2. At the time of the urine collection, 86% (24/28) of cohort-1 patients were being treated with glucocorticoids and seven with immunosuppressive medications (cyclophosphamide [n=4], mycophenolate mofetil [n=3]). In cohort-2, 57 patients were receiving glucocorticoids, 31 were being treated with mycophenolate mofetil, 10 with azathioprine, five with cyclophosphamide, and three with methotrexate. Histological diagnoses included all classes of LN except for class 1. The majority of patients who subsequently developed RFD had class 4 LN on kidney biopsy; 55.6% in cohort-1 and 57.1% in cohort-2. The cohorts differed in the duration of follow-up (mean $\pm$ SD in months): cohort-1, 6.1 ± 2.28 and cohort-2, 60 ± 15.2 . Nonetheless, the incidence of RFD was similar in both groups. In cohort-1, 8 of 29 (27.6%), and in cohort-2 21 of 69 (30.4%) patients developed RFD. Cohort-1 was 34.5% White, 48.3% Black and 17.2% other races/ethnicities, whereas cohort-2 was 53.6% White and 40.5% Black (other 5.9%). The proportion of non-White patients who developed RFD was significantly different between cohorts (cohort-1 vs cohort-2, 25% vs 60.9%; $p<0.05$).

Biomarker levels

Table 2 provides a comparison of the urinary levels of the analytes in both cohorts. In the entire study population (n= 97) the levels of urinary albumin, MCP-1 and transferrin were

significantly higher in the RFD-group than the NoRFD-group. However, these differences were driven primarily by the patients from cohort-1. Notably, the mean values of albumin and LFABP were statistically significantly higher in the RFD-group vs. the noRFD-group of cohort-1. For cohort-2, where RFD was determined using SCr, we did not observe statistically significant differences (at the 0.05 level) in any of the biomarkers between the RFD-group and the NoRFD-group.

Univariate and multivariate logistic modeling to predict RFD

Individual urine analytes showed no more than good accuracy in predicting RFD. AUC values ranged from 0.71–0.79 for cohort-1 and 0.57 to 0.62 for cohort-2, suggesting these urine analytes might have better prediction and discrimination of RFD for children.

LFABP, MCP-1, albumin, and transferrin levels showed relevant differences (p -value <0.1) between the RFD-group and the noRFD-groups in both cohorts. The combination of these four urine analytes (LFABP, MCP-1, albumin, transferrin) improved the prediction of future RFD compared to any of the urine analytes individually. AUC values in cohort-1 and cohort-2 were 0.82 and 0.79, respectively (Table 3 and Figure 1).

The overall sensitivity and specificity of this biomarker panel was modest (range 77–81%), and the LR+ was 3.4 for the cohort-2 and 3.9 for cohort-1. On the other hand, the LR– was 0.3 for both cohorts, a value suggesting the likelihood of RFD is decreased by 25%.

Discussion

Preventing renal functional decline and chronic kidney disease is central to improving health outcomes in LN. To achieve this goal, having predictive biomarkers to identify patients at risk for RFD early in their disease course would be ideal. In this way medical therapies could be optimized. Using a relatively unique collection of urine samples from longitudinal registries and biobanks of LN patients, we found that albumin, LFABP, MCP-1 and transferrin are analyte components of a candidate combinatorial biomarker of children and adults with LN at risk for RFD.

For this investigation, we defined RFD as a sustained decline in eGFR of 20% in cohort-1 and a sustained increase in serum creatinine concentration of 25% in cohort-2. The rationale for using these definitions of RFD was to develop biomarkers of early chronic kidney disease. While these may be considered fairly modest decreases in kidney function, it is becoming increasingly clear that morbidities associated with chronic kidney disease may occur with surprisingly small decrements in kidney function. For example, compared to individuals with no kidney disease, cardiovascular morbidity increases significantly in patients with stage 1–2 chronic kidney disease (GFR 60–90 ml/min/m²) (15). The risk of serious cardiovascular events increases further as renal function continues to fall (15, 16). Patients with SLE are already at increased risk of cardiovascular morbidity, thus we suggest that identification of an early and small decline in kidney function in LN, with a goal of preventing further deterioration, may improve overall outcomes in SLE. One of the limitations of investigating small and early changes in kidney function is that the RFD may be reversible, or due to other factors like medications. Here we required the RFD to be

sustained over time to reduce the likelihood of reversibility. Medications, like inhibitors of the renin-angiotensin system may also cause an increase in serum creatinine concentration or a decrease in eGFR. To avoid confusion, interpretation of these and other biomarkers of kidney function must occur in the context of how the patient is being treated.

Presently, there is little agreement as to the best therapeutic approach for treating LN. Considering the absence of a uniformly accepted treatment regimen it is remarkable that levels of albumin, LFABP, MCP-1 and transferrin form a “RFD combinatorial biomarker” that provides a good prediction of who will develop RFD months to years in the future. It may be considered a strength that the four urine proteins were suited to predict RFD, even in the setting of using different definitions of RFD and across a wide range of patient ages.

In many areas of medicine a single laboratory value in isolation is often insufficient for diagnosis and similarly we did not expect that individual urine analytes measured at the time of active LN would suffice to anticipate RFD.

An ideal biomarker to differentiate between two clinical groups would have an AUC of 1 and concomitantly very high sensitivity and specificity at the cut-off value, both in the range of 80–90%. However, for almost every individual analyte considered and also for the biomarker panel, specificities and positive predictive values were much lower than 80%, but the negative predictive values were high. Tests with high negative predictive values are good in ruling out a disease condition. In our study this type of test could be used to identify a group of patients with LN who would be unlikely develop RFD. Such a biomarker may be clinically useful when discussing prognosis and considering treatment options and efficacy with patients. Patients whose urine biomarkers suggest that they are not “safe” in terms of RFD may be monitored more closely and possibly, treated more aggressively.

Patients of Black race developed RFD more frequently than others, but this was only observed in cohort-2 with its generally older patients. Observed racial differences may be due to genetic factors leading to LN early in life, differences in comorbid condition in young versus older Black patients, including diabetes and chronic hypertension, or may be simply the result of the relatively limited number of patients included in this study. Differences in the accuracy of predictive biomarkers in relationship to race, age and sex will require further investigation.

Several of the biomarkers examined in this study have previously been investigated as biomarkers of LN (17–19). These biomarkers have mainly been studied as markers of LN flare (17–19) and LN histology (19, 20), but not as markers of RFD. The fact that these biomarkers were used to look at other aspects of LN likely accounts for the differences in how a specific biomarker correlated with LN in this study compared to other studies. Interestingly, serum resistin was recently found to correlate with SCr in LN, suggesting it could be a component of a biomarker of RFD (20).

There are a number of limitations of this study. Because sample size was small, and despite differences in the duration of follow-up between cohorts, we conducted a combined analysis to identify combinatorial biomarkers for RFD. However, as this was a pilot study that set out to discover candidate combinatorial biomarkers of RFD, examining the 2 cohorts together

seemed justified. Importantly, in both cohorts the same urine analytes showed similar trends in the RFD vs. noRFD-groups. Nonetheless, future validation studies will need to not only confirm the results of this study but carefully investigate the influence of age, gender, race, medications and comorbid conditions on the performance of the RFD combinatorial biomarker. Another limitation is that the majority of the initial samples from cohort-2 were obtained during a time when their LN was quiescent as compared to the cohort-1, where all the initial urine samples were obtained at time of LN flare. That also makes the two cohorts difficult to combine. Future validation studies will need to take the timing of the sample collection into account. Furthermore, such follow-up studies should evaluate the importance of ISN/RPS histologic severity, immunosuppressive therapies and other concomitant medications, all of which could affect kidney function.

Taken together we propose a RFD combinatorial biomarker consisting of albumin, LFABP, MCP-1 and transferrin that when measured early in the course of LN may help discriminate patients who will develop RFD months to years later from those patients who are likely to maintain their kidney function.

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Figure 1. Receiver operating curves (ROC) using each urine analyte individually or as a composite to predict RFD

ROC analysis of biomarkers of renal functional decline. The ROC curves shown are for individual renal biomarkers, and the larger black ROC curve is a combination of all the biomarkers for predicting renal function decline for both adult (right) and children (left). Dark blue, albumin; light blue, LFABP; green, MCP1; Red, transferrin;

Table 1

Patient characteristics at study entry

<i>Values are n (%) unless stated otherwise</i>	<i>Cohort-2</i>		<i>Cohort-1</i>		<i>P-value</i>	
	No RFD (A)	RFD ¹ (B)	No RFD (C)	RFD (D)	A vs. B	C vs. D
Number of patients	48	21	20	8		
Age in years (mean (range))	36 (19–63)	32 (20–60)	16 (10–23)	18 (14–24)		
Female	91%	91%	87%	63%		
<i>Race</i>						
% White	63.8%	30.4%	29%	50%		
% Black	31.9%	60.9%	58%	25%	0.02	0.06
% Other Race	4.3%	8.7%	13%	25%		
<i>Laboratory features(mean±SD)</i>						
Initial SCr ² (mg/dl)	0.82±0.15	0.83±0.20	0.7±0.20	0.8±0.15	NS	NS
Final SCr ³ (mg/dl)	0.85±0.28	2.26±2.02 ⁶	0.7±0.20	0.9±0.35 ⁶	0.01	NS
Initial eGFR ² (mL/min/1.73m ²)	94.3±27.6	104.7±28.1	109.8±45.9	96.8±13.2	NS	NS
Final eGFR ³ (mL/min/1.73m ²)	95.8±30.8	52.75±25.8 ⁶	112.8±27.3	73.1±14.7 ⁶	0.001	0.001
P/C ⁴ >0.5	35%	89%	35.5%	63%	0.003	NS
<i>Histological ISN/RPS⁵ Class</i>						
Class 2	6.1%	0.0%	0.0%	0.0%	NS	NS
Class 3	21.2%	11.1%	17.2%	14.3%	NS	NS
Class 4	24.2%	55.6%	20.7%	57.1%	NS	NS
Class 5	39.4%	22.2%	55.2%	14.3%	NS	NS
Class 3 or 4 + 5	9.1%	11.1%	6.9%	14.3%	NS	NS

¹ RFD, renal functional decline;

² Renal function at time of urine sample collection; SCr: serum creatinine concentration

³ Renal function at last follow-up; SCr: serum creatinine concentration

⁴ Urine protein to creatinine ratio;

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International Society of Nephrology/Renal Pathology Society Classification of LN;
5
p<0.001, RFL (initial) vs Preserved Renal function (final);
6
NS; not significant P>0.05

Table 2

Mean urine analyte levels at the time of enrollment in patients with and without RFD

<i>Patient type</i>	<i>Analyte</i> [*]	<i>RFD-group</i> ¹	<i>NoRFD-group</i> ^{*2}	<i>p-value</i> ^{**}
Cohort 1 plus 2	<i>N</i>	<i>29</i>	<i>68</i>	-
	Albumin	335.86 (144.81, 778.97)	93.56 (55.13, 158.78)	0.012
	LFABP	6.95 (3.99, 12.11)	3.84 (2.75, 5.35)	0.065
	MCP1	423.28 (277.03, 646.72)	118.30 (63.73, 219.60)	0.014
	Uromodulin	6.07 (3.90, 9.46)	7.58 (5.76, 9.98)	0.396
	Transferrin	10.45 (4.76, 22.92)	4.19 (2.62, 6.70)	0.046
	Hepcidin	30.20 (19.65, 46.40)	32.84 (23.37, 46.15)	0.783
Cohort 2	<i>N</i>	<i>21</i>	<i>48</i>	-
	Albumin	366.02 (146.54, 914.22)	149.71 (84.78, 264.36)	0.100
	LFABP	5.26 (2.93, 9.46)	4.22 (2.84, 6.27)	0.547
	MCP1	395.14 (240.50, 649.23)	121.96 (56.31, 264.14)	0.061
	Uromodulin	3.78 (2.40, 5.95)	5.20 (3.82, 7.06)	0.261
	Transferrin	7.24 (3.41, 15.38)	4.09 (2.35, 7.12)	0.255
	Hepcidin	33.15 (20.48, 53.64)	33.15 (22.10, 49.72)	1.000
Cohort 1	<i>N</i>	<i>8</i>	<i>20</i>	-
	Albumin	267.98 (36.82, 1,950.27)	30.28 (10.76, 85.17)	0.048
	LFABP	14.45 (4.28, 48.70)	3.06 (1.65, 5.66)	0.021
	MCP1	520.27 (221.07, 1,224.42)	110.13 (39.47, 307.29)	0.108
	Uromodulin	21.15 (14.97, 29.89)	18.77 (13.45, 26.19)	0.687
	Transferrin	27.34 (3.70, 201.90)	4.44 (1.79, 11.00)	0.072
	Hepcidin	23.93 (9.39, 61.01)	32.12 (16.90, 61.05)	0.628

* Analytes were log-transformed and normalized to urine creatinine concentration; values in cells are geometric mean (95% CI) in ng/mg;

¹ RFD, renal function decline;

² No RFD, no renal function decline;

^{**} RFD vs No RFD

Table 3

Performance of urine analytes in predicting RFD

	AUC ¹ (95 CI)	Cut off risk score ²	Sensitivity (%)	Specificity (%)	LR ⁺ ²	LR ⁻ ³	PPV ⁴ (%) ‡	NPV ⁵ (%) ‡
Cohort 2								
Multivariate model ^{°€}	0.79 (0.66, 0.91)	0.35	77.3	77.6	3.4	0.3	59.6	88.8
LFABP	0.62 (0.47, 0.76) [*]	0.32	63.6	67.3	1.9	0.5	45.5	81.2
Albumin	0.57 (0.42, 0.72) [*]	0.31	72.7	51	1.5	0.5	38.9	81.4
MCP-1	0.67 (0.53, 0.81)	0.37	63.6	79.6	3.1	0.5	57.2	83.6
Transferrin	0.62 (0.47, 0.76) [*]	0.32	63.6	65.3	1.8	0.6	44	80.7
Uromodulin	0.58 (0.44, 0.72) [*]	0.28	81.8	42.9	1.4	0.4	38	84.6
Hepcidin	0.51 (0.37, 0.65) [*]	0.31	86.4	34.7	1.3	0.4	36.2	85.6
Cohort 1								
Multivariate model ^{°€}	0.82 (0.63, 1.00)	0.34	75	81	3.9	0.3	62.8	88.3
LFABP	0.71 (0.48, 0.95)	0.51	50	90.5	5.3	0.6	69.2	80.9
Albumin	0.74 (0.52, 0.96)	0.18	87.5	52.4	1.8	0.2	44.1	90.7
MCP-1	0.79 (0.56, 1.00)	0.43	62.5	90.5	6.6	0.4	73.8	84.9
Transferrin	0.71 (0.47, 0.94)	0.49	50	90.5	5.3	0.6	69.2	80.9
Uromodulin	0.53 (0.27, 0.79) [*]	0.31	25	90.5	2.6	0.8	52.9	73.8
Hepcidin	0.56 (0.32, 0.80) [*]	0.29	50	71.4	1.8	0.7	42.9	76.9

* Indicates the AUC of individual biomarkers is significantly (p<0.05) lower than that of the multivariate model in adults and children respectively.

[‡] The cutoff point represents a risk score or predicted probability of RFD (range 0–1) from a corresponding logistical model; and it is considered “optimal” as its sum of sensitivity and specificity is the largest on the ROC curve.[°] Included in the multivariate model urine biomarkers with P value <0.1 between RFD vs NoRFD group (albumin, LFABP, MCP1 and transferrin);¹ AUC: area under curve;² LR⁺: positive likelihood ratio;³ LR⁻: negative likelihood ratio;⁴ PPV: positive predictive value;

NPV: negative predictive value

Algorithm of calculating risk scores from the multivariate models:

Step 1. Each patient's observed urine biomarkers, Albumin, LFABP, MCP-1, Transferrin, and Creatinine will be log transformed (for example, if one patient's LFABP level is 15, then it log transformed value $\ln(15)$ or 2.7 will be used in the model).

Step 2. A combined raw scores (CRS) will be calculated using the following formula for each cohort

For cohort 2, $CRS = 0.5 \times Albumin - 0.4 \times LFABP + 0.3 \times MCP_1 - 0.1 \times Transferrin$;

For cohort 1, $CRS = 1.3 \times Albumin + 0.1 \times LFABP + 0.9 \times MCP_1 - 1.2 \times Transferrin$.

Step 3. A logit raw score (LRS) will be calculated in the following:

For cohort 2, $LRS = CRS - 4 + 0.9 \times Creatinine$;

For cohort 1, $LRS = CRS - 9.1 - 0.3 \times Creatinine$.

Step 4. The final risk score (RS) is calculated in the following:

$$RS = \exp(LRS) / [1 + \exp(LRS)], \text{ where RS is ranged } (0, 1).$$