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DEVELOPMENT OF A NOVEL RENAL ACTIVITY INDEX OF LUPUS NEPHRITIS IN CHILDREN & YOUNG ADULTS

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Abstract

Background—Noninvasive estimation of the degree of inflammation seen on kidney biopsy with lupus nephritis (LN) remains difficult. The objective of this study was to develop a *Renal Activity Index for Lupus* (RAIL) that, based solely on laboratory measures, accurately reflects histological LN activity.

Methods—We assayed traditional LN laboratory tests and 16 urine biomarkers (UBMs) in children (n=47) at the time of kidney biopsy. Histological LN activity was measured by the NIH Activity Index (NIH-AI) and the Tubulointerstitial Activity Index (TIAI). High LN-activity status (vs. moderate/low) was defined as NIH-AI scores > 10 (vs. ≤ 10) or TIAI scores >5 (vs. ≤ 5). RAIL algorithms that predicted LN-activity$_{NIH\text{-}AI}$ and LN-activity$_{TIAI}$ status were derived by stepwise multivariate logistical regression, considering traditional biomarkers and UBMs as candidate components. The accuracy of the RAIL for discriminating by LN-activity status was determined.

Results—The differential excretion of six UBMs (NGAL, MCP-1, ceruloplasmin, adiponectin, hemopexin, KIM-1) standardized by urine creatinine was considered in the RAIL. These UBMs predicted LN-activity$_{NIH\text{-}AI}$ status with >92% accuracy and LN-activity$_{TIAI}$ status with >80% accuracy. RAIL accuracy was minimally influenced by concomitant LN damage. Accuracies between 71 and 85% were achieved without standardization of the UBMs. The strength of these UBMs to reflect LN-activity status was confirmed by principal component and linear discriminant analyses.

Conclusion—The RAIL is a robust and highly accurate noninvasive measure of LN-activity. The measurement properties of the RAIL, which reflect the degree of inflammatory changes as seen on kidney biopsy, will require independent validation.

Key Indexing Terms

SLE; lupus nephritis; kidney biopsy; biomarker

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a multi-system inflammatory autoimmune disease, and renal involvement is one of the main determinants of poor prognosis (1). The pathogenesis of lupus nephritis (LN) involves kidney deposition of immune complexes in the setting of impaired apoptosis regulation (2). There are three principal patterns of injury with LN. Firstly, a mesangial pattern which features mesangial hypercellularity and matrix deposits as a response to mesangial immune complex accumulation. Secondly, a proliferative pattern that occurs in response to subendothelial immune complex buildup and is characterized by obliteration of glomerular capillary lumens due to leukocyte accumulation, mesangial proliferation, often capillary wall destruction, and rupture of Bowman's capsule resulting in extra-capillary crescent formation. Lastly, in the membranous pattern there is increased immune complex deposition in the sub-epithelial space with leads to cytotoxic injury to the podocyte, and result in the thickening of the glomerular basement membrane. These three patterns are the basis for the categorization of LN in the International Society of Nephrology/Renal Pathology Society (ISN/PRS) Classification (3).

Recently, novel urine biomarkers (UBM) have been described that can assist with diagnosing active LN and anticipate LN flares (4¯8). Among others, we have provided initial evidence that the urine concentrations of some of the UBMs are associated with distinct histological changes of LN (9). Since our initial studies, additional UBMs have been proposed by other investigators. The 16 most promising of these UBMs were considered in this study: neutrophil gelatinase associated lipocalin (NGAL), monocyte chemotactic protein 1 (MCP-1), ceruloplasmin, adiponectin, hemopexin, kidney injury molecule 1 (KIM-1), alpha-1-acid glycoprotein (AAG), transforming growth factor beta (TGF-β), hepcidin, lipocalin-like prostaglandin synthase (L-PGDS), transferrin, vitamin D binding protein (VDBP), microalbumin, cystatin- C, endothelial protein C receptor (EPCR), and liver type fatty acid-binding protein 1 (L-FABP).

We hypothesized that a selection of these UBMs, alone or in combination with traditional measures of LN, can accurately quantify the degree of histological LN activity. Hence, the objective of this study was to develop in children and young adults a Renal Activity Index for Lupus (RAIL) to non-invasively measure LN activity.

MATERIALS & METHODS

Patients

Patients diagnosed with childhood-onset SLE (10) who required a kidney biopsy as part of standard of care participated in this cross-sectional study. At the time of kidney biopsy, a random urine sample was collected for UBM testing. Prospectively, relevant clinical information and traditional measures of LN were recorded, including the glomerular filtration rate (GFR) (11, 12) and the protein to creatinine ratio (P/C ratio) in a random urine sample. All patients received therapy for childhood-onset SLE at the time of the urine collection and biopsy. There were five patients with repeat biopsies.

The renal domain score of the Systemic Lupus Disease Activity Index (SLEDAI-R; range 0 – 16; 0 = inactive LN) (13) and that of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI-R; range 0– 3; 0= no LN damage) (14) were also completed and served as measures of LN clinical activity and damage, respectively.

Kidney Histology

The histological characteristics of each kidney biopsy were interpreted in a blinded fashion by one expert nephropathologist (DW) as per the ISN/RPS Classification (3, 15). Most studies in LN employ a previously developed scoring system to quantify the amount of overall LN activity, using the *National Institutes of Health Activity Index* (NIH-AI; score range 0–24; 0= inactive) (16). Because the NIH-AI is focused on acute glomerular injury with LN, we also measured the *Tubulointerstitial Activity Index* (TIAI; score range: 0–21; 0=no interstitial activity) (17). The *NIH Chronicity Index* was scored (NIH-CI; score range 0 – 12; 0 = no LN-chronicity) to quantify LN damage as seen on kidney biopsy (16). The ISN/RPS Classification, the NIH-AI, TIAI and the NIH-CI have all been validated for use in children and adults (18, 19).

Urinary Biomarker Assays

The following 16 UBMs were measured: NGAL, MCP-1, ceruloplasmin, adiponectin, hemopexin, KIM-1, AAG, TGF-β, hepcidin, L-PGDS, transferrin, VDBP, microalbumin, EPRC, cystatin-C and L-FABP. Laboratory personnel assaying the UBMs were blinded to clinical and histological information. Spun urine samples were stored at 0°C within 1 hour of collection and frozen at −80°C prior within 24 hours prior to batch processing.

Unless stated otherwise, UBMs were quantified using commercial ELISA kits as per the manufacturers' instructions, and a four parameter logistic curve-fit was used to fit the standard curve. In the following, inter-assay and intra-assay variability of these assays is expressed in percent of the coefficient of variation [CV inter/intra].

NGAL [CV inter/intra: 1.0%/9.1%] was measured by ELISA (Human NGAL ELISA; Bioporto, Grusbakken, Denmark). Ceruloplasmin [CV inter/intra: 4.1% /7.1%] was quantified by ELISA (Assaypro, St.Charles, MO); AAG [CV inter/intra: 5.0%/ 8.5%] by ELISA (R&D Systems, Minneapolis, MN); MCP-1 [CV inter/intra: 5.0%/5.9%] by ELISA

(R&D Systems, Minneapolis, MN); VDBP [CV inter/intra: 5.1%/6.2%] by ELISA (R&D Systems, Minneapolis, MN); and hepcidin-25 [CV inter/intra: 3.5%/3.4%] was measured by ELISA (EIA kit S-1337, Peninsula Laboratories, San Carlos, CA). Adiponectin [CV inter/intra: 4.0%/9.9%] was measured using the Quantikine ELISA Human HMW Adiponectin/Acrp30 (R&D Systems, Minneapolis, MN); hemopexin [CV inter/intra: 4.8%/7.3%] with the AssayMax Human Hemopexin ELISA Kit (Assaypro, St. Charles, MO); EPCR [CV inter/intra: 7.8%/9.0%] with the DuoSet Human EPCR kit (R&D Systems, Minneapolis, MN); and L-FABP [CV inter/intra: 6.1%/10.9%] by ELISA (CMIC Co., Tokyo, Japan), respectively. The KIM-1 assay was constructed using commercially available reagents (Duoset DY1750, R & D Systems, Minneapolis, MN) as described previously (20). Urine creatinine measurements were made using a modified Jaffe reaction, and microalbumin (MALB) was measured by immunoturbidimetry, both on a Dimension Xp and plus HM Clinical Analyzer (Siemens, Munich, Germany). Coefficients of variability for the creatinine measurements were 2.4% (intra) and 4.2% (total), and 2.9% (intra) and 5.9% (inter) for MALB.TGF-β [CV inter/intra: 2.6%/8.3%] was measured by ELISA (R&D Systems, Minneapolis, MN) after acid activation. Briefly, 20 μL of 1N HCl was added to 100 μL of urine sample, mixed by inversion and incubated at room temperature for 10 minutes. Next, the acidified sample was neutralized by adding 20 μL of 1.2 N NaOH/0.5 M HEPES, then the assay was immediately run per manufacturer's instructions [CV inter/intra: 2.0%/7.8%].

Using immunonephelometry (Siemens, BNII, Munich, Germany) we measured cystatin-C [CV inter/intra: 2.5%/2.3%], transferrin [CV inter/intra: 3.4%/2.5%] and L-PGDS [CV inter/intra: 2.3%/6.5%].

Concentrations of the UBMs (in ng/ml: for NGAL, CP, L-FABP, VDBP, adiponectin, EPCR, hemopexin, hepcidin; in pg/ml: for KIM-1 and TGF-β; in pg/ml: MCP-1: in mg/dl: for transferrin and L-PDGS; in mg/L for cystatin-C and microalbumin) were standardized for urine creatinine levels (in mg/mL).

Statistical analysis

The candidate predictors considered for inclusion in the RAIL were the 16 UBMs and the traditional measures of LN. *LN-activity status* served as the dependent variable in the statistical procedures to derive the RAIL and was defined as high versus (vs.) moderate/low based on the scores of the NIH-AI and TIAI, respectively. LN-Activity$_{NIH\text{-}AI}$ was classified as high when NIH-AI scores were >10, and moderate to low when NIH-AI scores were ≤10; LN-Activity$_{TIAI}$ was considered high for TIAI scores >5, and moderate to low for LN-Activity$_{TIAI}$ scores ≤5. The pool of the candidate RAIL-predictors (standardized UBMs, traditional biomarkers) to be considered in the multivariate models was informed by univariate models under a threshold p-value of ≤0.2 for discrimination of high from moderate/low LN-activity (LN-activity$_{NIH\text{-}AI}$; LN-activity$_{TIAI}$). In primary analysis, stepwise selection was used in multiple logistical regression models to identify the components of the RAIL. In secondary analysis, we considered raw amounts of the UBMs rather than standardized UBMs. The appropriateness of the final RAIL predictors to reflect LN-activity status was confirmed by linear discriminant analysis (21) and principal component analysis, adjusted and unadjusted for LN chronicity (NIH-CI score).

The accuracies of RAIL algorithms was considered outstanding, excellent, good, and fair if the area under the receiver operating characteristic curve (AUC) was in the range of 0.9–1.0, 0.81–0.90, 0.71–0.80, and 0.61–0.70, respectively. We also determined sensitivity, specificity, the positive (LR+) and the negative likelihood ratios (LR−) for the statistical optimal receiver operating characteristic (ROC) curve cutoff. Here, LR+ values can be interpreted as: > 10: large, often conclusive increase in the likelihood of "ruling in" the presence of high LN-activity status; 5 – 9.9: moderate increase; and 2 – 4.9: small increase, respectively. In other words, a LR+ of 2 increases the probability for a high LN-activity status by 15%, a LR+ of 5 increases it 30%, and one of 10 increases it even by 45% (22). LR − are interpreted accordingly for "ruling-out" active LN. Statistical analyses were done using SAS version 9.4 software (SAS, Cary, NC, USA). P-values < 0.05 were considered statistically significant. The study was approved by the Institutional Review Boards and Ethics Review Committees of the participating centers. ***Additional details on the statistical analyses are provided online.***

RESULTS

Patient characteristics & features of kidney biopsy

A total of 47 patients with LN were included in this study (Table 1). At the time of the study, on average, their [SD; standard deviation] age was 15.7 [3.01] years, their extrarenal SLEDAI score was 9.7 [8.20], and the time interval between kidney biopsy and urine collection was 0 [3] days. None of the patients had Class 1 or 6 LN. As expected, histological features of LN activity and chronicity were seen often concomitantly in the same biopsy. SDI-R scores were positive in 15 (32%) patients. Given the quality of the biopsy specimens, NIH-AI, TIAI and NIH-CI scores were not assigned to all biopsies, hence were only available for 41, 32 and 40 of the biopsies, respectively. The high and moderate/low NIH-AI consisted of 20 and 21 patients; there were 10 and 22 patients in the high and moderate/low TIAI as well as 21 patients with NIH-CI scores over 0.

Associations of noninvasive measures kidney histology indices

As is summarized in Table 2, proteinuria did not significantly differentiate patients by LN-activity status (NIH-AI, TIAI) or LN-chronicity status (NIH-CI score > 0 vs. 0), irrespective of adjustment for angiotensinogen system blocking medications. The GFR was lower with high LN-activity status and with NIH-CI scores > 0. Seven UBMs significantly differed with LN-activity$_{\text{NIH-AI}}$ status and six UBMs with LN-activity$_{\text{TIAI}}$ status (Table 3). Notably, MCP-1, adiponectin, and TGF-β significantly differed with both LN-activity$_{\text{NIH-AI}}$ and LN-activity$_{\text{TIAI}}$ status.

Associations of noninvasive measures kidney histology features

We then assessed the differential excretion of the UBMs with the presence vs. absence of individual histological findings reflective of active inflammation in LN (Figure 1). NGAL, MCP-1, KIM-1 and L-PGDS were all markedly elevated in the urine of patients whose kidney biopsy showed endocapillary hypercellularity compared to those that did not. Likewise, NGAL, KIM-1 and MCP-1 were found in higher concentrations in patients whose kidney biopsy showed tubular cell flattening and necrosis. There were significantly higher

urine levels of NGAL, but not MCP-1 or KIM-1, in patients with vs. without tubular cell pyknosis and epithelial cells in the tubular lumen. EPCR was not associated with any specific histological feature considered in the TIAI or NIH-AI; while TGF-β, AAG, cystatin-C and L-FABP were only differentially expressed with select histological features scored in the TIAI but with none of the features reflected by the NIH-AI.

Most of the UBMs were weakly (Pearson correlation coefficient r; $|0.2| \leq r < |0.4|$) correlated with the P/C ratio but none was strongly correlated ($r > |0.6|$). The GFR was only moderately correlated with NGAL, weakly correlated only with MCP-1, VDBP, hemopexin and KIM-1, and unrelated to the levels of all other UBMs. Levels of complement C3 and C4 were no more than weakly correlated with any of the UBMs. As expected, the levels of UBMs were differentially associated with each other (see supplemental Figure 2).

UBMs and ISNPRS Class

The mean (95%CI) L-FABP levels were significantly lower with LN Class 3, 4 or 5 vs. LN Class 2 [0.24 (0.16, 0.36) vs. 0.97 (0.26, 3.62); p=0.05]. Similarly, cystatin-C levels significantly differed between LN Class 3, 4 or 5 vs. LN Class 2 [0.72 (0.52, 0.99) vs. 3.27 (1.16, 9.20); p=0.010]. Taken together, urine L-FABP levels of >1.0 decreased the likelihood of presence of Class 3,4 or 5 LN by 30% (LR− 0.2) while cystatin-C levels of >3.3 decreased the likelihood of LN Class 3, 4 or 5 by about 23% (LR− 0.29).

Individual noninvasive LN measures and LN-activity status

Table 3 compares means of LN measures between patients with and without LN activity, after correcting for concomitant LN damage ((NIH-CI score). In addition, each LN marker was used to predict LN activity using a ROC-analysis. The results revealed that KIM-1 was the single best UBM for capturing LN-activity$_{NIH\text{-}AI}$ status [AUC (95% CI): 0.86 (0.74, 0.98)] followed by MCP-1, NGAL and adiponectin which were at least good predictors [all AUCs (95% CI) ≥ 0.70 (0.54, 0.98)]. Irrespective of adjustment for concurrent kidney damage, MCP-1, VDBP, cystatin-C, TGF-β, L-PGDS and hemopexin were all at least good predictors of LN-activity$_{TIAI}$ status. Similarly, GFR was a good predictor of LN-activity status (Table 2).

Development of a combinatorial biomarker of LN-activity status

In stepwise multiple logistical modeling, we identified NGAL, ceruloplasmin, MCP-1, adiponectin, hemopexin and KIM-1 as the best predictors (RAIL-UBMs) of LN-activity$_{TIAI}$ status and LN-activity$_{NIH\text{-}AI}$ status (Figure 2; supplemental Tables 2). Traditional LN measures did not remain in the pool of covariates best suited to predict LN-activity status. The accuracy of the Rail-UBMs in reflecting the LN-activity status was well preserved in models correcting for concurrent LN damage (NIH-CI score) or even when considering simply the raw amounts of the RAIL-UBMs (Supplemental Figure 3).

Besides multiple logistical regression models, other methods such as linear discriminant or principal component analyses have been shown to yield composite scores for measuring complex constructs. The suitability of the RAIL-UBMs for predicting histological correlates

of active LN is supported by congruent results, using linear discriminate and principal component analyses (Table 4).

Proposed RAIL algorithm

Given that the NIH-AI is more commonly used in clinical practice than the TIAI and because one must assume that information on LN chronicity will not readily be available in a clinical setting, we propose the following algorithm that considers log-transformed urine concentrations (creatinine standardized) of the six RAIL-UBMs: RAIL score = − 4.29 – 0.34* NGAL −0.06*ceruloplasmin + 0.89* MCP-1+ 0.18* adiponectin − 0.65 * hemopexin + 0.62 * KIM-1. A RAIL-score of ≥ 0.39 will correctly identify 90% of all cases with high LN-activity status with a false positive rate being controlled at 14% (Figure 2, ***Panel A***).

DISCUSSION

At present, accurate of LN activity requires a kidney biopsy. Based on detailed assessment of the measurement properties of traditional and 16 novel urinary biomarkers of LN, we newly propose a Renal Activity Index for Lupus (RAIL) to noninvasively quantify LN activity. The accuracy of the RAIL is minimally influenced by concurrent LN chronicity and reflects both glomerular and tubulointerstitial inflammation with LN. Further, concurrent use of medications, including those targeting the renin-angiotensin-aldosterone system, do not seem to influence the accuracy of the RAIL to a large degree.

The UBMs included in the RAIL are all involved in putative mechanisms aimed at protecting kidneys from damage due to renal inflammation. Indeed, there is a strong biological rationale for each of the six RAIL UBMs. NGAL is rapidly induced by active inflammation with LN, and promptly declines with therapy (4). In the acute setting, NGAL appears to be a part of a protective anti-apoptotic mechanism that limits tubule cell damage and enhances proliferation (23). MCP-1 is induced by type I interferons and is known to be a predictive biomarker of LN flares and LN severity (5, 24). There is high expression of MCP-1, especially in the tubular epithelial cells (25) with oxidative stress. The antioxidant ceruloplasmin is a copper-containing ferroxidase that can transform ferrous iron, which is highly damaging to kidney tubules, to its nontoxic ferric configuration. High ceruloplasmin levels are associated with renal tissue remodeling as can be observed with LN (4, 26).

The cytokine adiponectin is present on the endothelium of intrarenal vasculature and to a lesser extent in the proximal and distal tubular epithelial cells (27), has anti-inflammatory properties, and urinary concentrations increase with kidney injury, including with LN. Among the UBMs considered, we found only adiponectin to be closely correlated with albuminuria. High levels of adiponectin levels were present in the setting of high glomerular and interstitial inflammation with LN.

Hemopexin, a protease that protects kidney tubules from toxicity of free heme radicals, is produced primarily in the renal cortex in the setting of nephrotoxic insults (28). We found hemopexin levels closely related to glomerular leukocyte infiltrates, subendothelial deposits and interstitial inflammation with LN. The protective role of hemopexin in LN is supported

by the observation that urine levels of hemopexin were highest with Class 2 LN (data not shown).

KIM-1 is responsible for the clearance of debris from damaged renal tubules and assists with the regeneration of the epithelium. Urine KIM-1 levels increase in the setting of proximal tubule injury and interstitial inflammation as can occur with LN (29).

One of our earlier studies suggested that the combination of MCP-1, ceruloplasmin, AAG and the P/C ratio has excellent accuracy in estimating histological LN activity (9). While we confirm the usefulness of ceruloplasmin and MCP-1 in quantifying LN-activity, we believe that the current study has several new strengths. First, different from our earlier study, the majority of the urine samples were collected on the day of kidney biopsy and the interpretation of the kidney biopsies occurred by a single expert nephropathologist. Second, we increased the pool of candidate UBMs and assessed both tubulointerstitial and glomerular features of active inflammation with LN; and third, we considered concomitant LN damage. Nonetheless, previous results are in line with our current findings, given the association of the levels of the various UBMs with each other and also with distinct histological features of LN-activity (4, 7, 9).

The UBMs considered in the RAIL were not well suited to discriminate between the different Classes of LN. Conversely, higher urine levels of two non-RAIL markers (L-FABP levels and cystatin-C) were associated with less severe LN (Class 2 as compared to Class 3, 4 or 5).

Our study must be seen in the light of certain limitations. Given the diverse medication regimens used, the multiplicity of distinct kidney biopsy features and their considerable overlap in a given patient, our study findings will need to be confirmed in a larger cohort. However, rigorous statistical methodology was employed and provided consistent results, irrespective of consideration of potential effect modifiers, supporting the robustness of our findings.

If confirmed in ongoing experiments, the RAIL will allow for more effective and personalized monitoring of LN and its therapy. The availability of standardized clinical platforms for the combined measurement of the urinary biomarkers will enable the testing of this hypothesis in the near future (30). Future research will need to confirm the most appropriate cut-off scores for the RAIL and also investigate how the combinatorial RAIL-UBMs can be used to non-invasively predict response to therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SIGNIFICANCE & INNOVATIONS

- We propose a renal activity index for lupus (RAIL) based on the urine concentrations of six protein biomarkers
- The RAIL quantifies the amount of histological inflammation seen on kidney biopsy tissues as measured by the NIH Activity Index with over 92% accuracy
- Once validated, it is anticipated that the RAIL is used to monitor the degree of inflammation with lupus nephritis non-invasively.

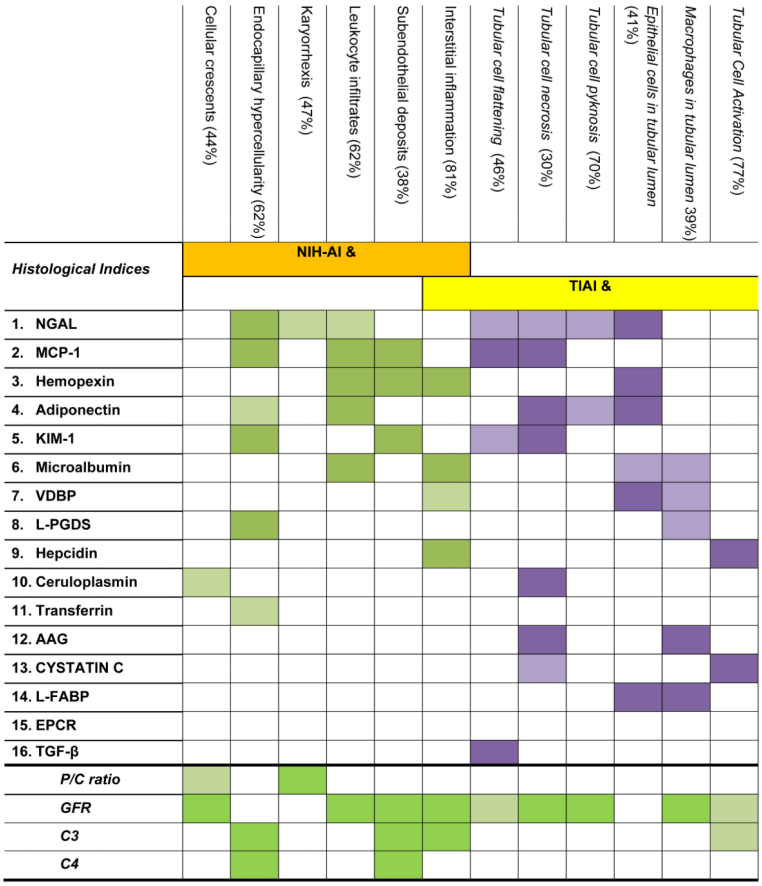


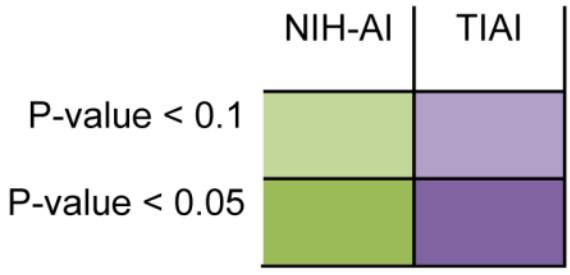
Figure 1. Differences in UBM levels in relationship to histological features of LN group as per the components of the LN-Activity Indices from kidney biopsy

& NIH-AI= NIH Activity Index ; TIAI Tubulointerstitial Activity Index

(% of biopsies with features present)

Neutrophil gelatinase associated lipocalin (NGAL), monocyte chemotactic protein 1 (MCP-1), -PGDS L-prostaglandin synthase, kidney injury molecule 1 (KIM-1), fatty acid-binding protein 1 (L-FABP), vitamin D binding protein ; EPCR is not shown as it was not differentially associated with any of the histological features

P-value from univariate logistic regression to predict presence vs. absence of histological features are color coded as follows:



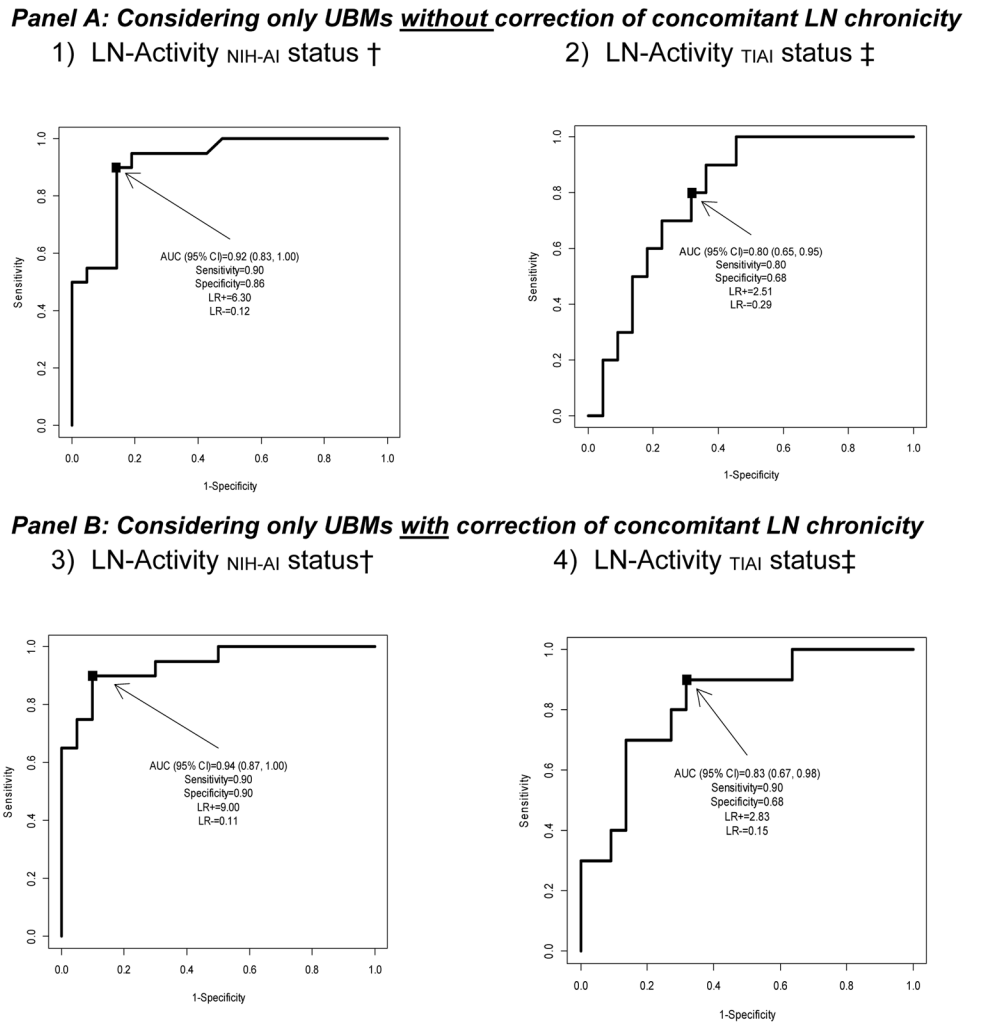


Figure 2. ROC curves for the Candidate RAIL algorithms

Panels A–D feature receiver operating characteristic curves (ROC) of six UBMs (NGAL, ceruloplasmin, MCP-1, adiponectin, hemopexin and KIM-1) considered in the RAIL algorithm for identifying predict low/moderate vs. high LN histological activity. Panels A–B do not consider concurrently observed LN chronicity while Panels C–D show the accuracy of the UBMs after correcting for LN chronicity.

For each of the ROC curves the area under the curve (AUC) is shown. The arrows point towards the statistically optimal cut-off score of the ROC-curve and provides for this point sensitivity, specificity, positive and negative likelihood ratios (LR+, LR−) information. Irrespective of the consideration of LN chronicity (NIH-CI score), the RAIL algorithms. Panel A features the preferred RAIL algorithm: RAIL score = −4.29 –0.34* NGAL −0.06*ceruloplasmin + 0.89* MCP-1 + 0.18* adiponectin − 0.65 * hemopexin + 0.62 * KIM-1.

†LN-activity $_{\text{NIH-AI}}$ high vs. moderate/low is defined based on NIH-AI scores > 10 vs. ≤ 10

‡ LN-activity $_{\text{TIAI}}$ high vs. moderate/low is defined based on TIAI scores > 5 vs. ≤ 5.

Table 1

Demographics and clinical information of the patient at the time of urine collection and time of kidney biopsy

	Features		n of N (%)	Mean (SD)
Females			34 (72.3%)	
Disease duration (in years)				0.16 [2.62]
Race	Black		19 (40.4%)	
	White		19 (40.4%)	
	Asian		1 (2.2%)	
	Native Indian		0 (0%)	
	Mixed racial		8 (17%)	
Medications	Oral prednisone (mg/day)		37 (78.7%)	42 (19.36)
	Pulse methylprednisolone		13 (27.7%)	
	Mycophenolate mofetil		16 (34.0%)	
	Azathioprine		1 (2.1%)	
	Cyclophosphamide		13 (27.7%)	
	Diuretics		7 (14.9%)	
	Angiotensin system blocking drug		19 (40%)	
LN Status	GFR < 60 ml/min/m^2		9 (19.6%)	
	Protein:creatinine ratio > 0.5		42 (93.3%)	
	Renal SDI score[&]			0.26 (0.57)
	Renal SLEDAI score			9.66 (5.18)
	Complement C3 low		30 (66.7%)	
	Complement C4 low		34 (75.6%)	
	Presence of anti-dsDNA antibodies		35 (87.5%)	
Histological Features present	ISNRPS [¶]	Class 2	3 (6.5%)	
		Class 3	8 (17.4%)	
		Class 4	24 (52.2%)	
		Class 5	11 (23.9%)	
	NIH-AI [≠]		42 (89.4%)	9.74 (6.50)
	TIAI [»]		34 (72.3%)	4.94 (2.21)
	NIH-CI [Δ]		42 (89.4%)	1.93 (1.97)

[&]Systemic Lupus International Collaborating Clinics Renal Activity Index, range 0 – 15; 0 = inactive LN

[¶]International Society for Nephrology Renal Pathology Society Class; there was no biopsy consistent with Class 1 or 6

[≠]NIH Activity Index; range 0 – 24; 0 = inactive LN

[»]Tubulointerstitial Activity Index; 0 – 21; 0 = no interstitial changes

[Δ]NIH Chronicity Index; range 0 – 12; 0 = LN without chronic changes

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Table 2

Levels of traditional laboratory measures and their relationship to LN histological indices.

Traditional Biomarker [&]		P/ C ratio[&]	GFR[&]	Presence of active urine sediment**	C3 level[&]	C4 level[&]	Presence of anti- dsDNA antibodies
NIH-AI Score[‡]	≤ ***10***	1.8 (1.2, 2.7)	115 (97.4, 136.0)	64%	65 (53.8, 77.4)	10.1 (8.1, 12.8)	83.78%
	> 10	2.9 (1.7, 4.7)	76 (61.0, 93.5)	89%	42 (33.4, 52.7)	6.4 (4.8, 8.4)	92.00%
	p- value	*0.165/0.297*[#]	*0.003*	*0.023*	*0.005*	*0.015*	*0.353*
TIAI Score[»]	≤ ***5***	2.0 (1.3, 3.0)	109 (94.3, 124.8)	68%	53 (43.5. 65.3)	7.7 (6.1, 9.8)	88.89%
	> 5	2.7 (1.3, 5.5)	70 (54.8, 88.5)	86%	52 (37.3, 73.5)	7.7 (5.2, 11.4)	90.91%
	p- value	*0.489/0.200*[#]	*0.003*	0.206	*0.931*	*0.981*	*0.849*
NIH-CI Score	***0***	1.6 (1.0, 2.5)	115 (96.5, 137.9)	67%	57 (45.7, 70.7)	8.7 (6.7, 11.4)	82.76%
	> 0	2.9 (1.9, 4.4)	80 (66.3, 95.8)	82%	49 (39.7, 61.4)	7.4 (5.7, 9.7)	90.63%
	p- value	*0.060/0.237*[#]	*0.006*	*0.152*	*0.374*	*0.399*	*0.370*
ISNPRS Class[†]	***1+2***	1.9 (0.8, 4.4)	111 (79.7, 155.6)	56%	53 (36.0, 76.6)	6.8 (4.3, 10.8)	100.00%
	3	0.9 (0.5, 1.7)[4,5]	113 (85.9, 148.3)	71%	60 (44.5, 81.5)	9.5 (6.6, 13.7)	76.92%
	4	2.7 (1.8, 4.1)	74 (61.8, 88.3)[3, 5]	89% [1+2,5]	43 (35.4, 52.5)[5]	6.4 (5.1, 8.2)[5]	94.12%[5]
	5	2.9 (1.7, 5.0)	138 (109.2, 173.1)	55%	78 (60.9, 101.1)	12.7 (9.3, 17.3)	63.16%

[&]Values are geometric means (95% confidence intervals) of biomarker concentrations; estimated by modified Schwartz formula, complement levels are reported in mg/dL;

[**]Active urine sediment is defined as present for urine sediments with > 5 white blood cells, or > 5 red blood cells, or cellular casts.

[†]A superscript indicates the Class 4 is significantly different to a coordinated Class.

[‡]NIH Activity Index; range 0 – 24; 0 = inactive LN

[»]Tubulointerstitial Activity Index; 0 – 21; 0 = no interstitial changes

[Δ]NIH Chronicity Index; range 0 – 12; 0 = LN without chronic changes

[#]p-value was obtained after adjusting for angiotensinogen system blocking medications.

Table 3

Differences in UBM levels with LN-activity status as seem on Kidney Biopsy after correction for concomitant kidney damage[†]

LN biomarkers [&]	*LN-Activity$_{NIH-AI}$ Status* [†]			*LN-Activity$_{TIAI}$ Status* [‡]		
	Moderate/low (n=21)	**High (n=20)**	**P-value**	**Moderate/low (n=22)**	**High (n=10)**	**p-value**
1. NGAL	*0.27 (0.17, 0.44)*	*0.66 (0.40, 1.09)*	***0.017***	0.33 (0.20, 0.54)	0.71 (0.35, 1.48)	0.094
2. MCP-1	*6.29 (4.29, 9.21)*	*22.44 (15.17, 33.19)*	***0.000***	9.37 (6.00, 14.63)	23.05 (11.90, 44.65)	***0.035***
3. Ceruloplasmin	*117.38 (64.57, 213.37)*	*355.63 (192.77, 656.05)*	***0.015***	175.57 (96.38, 319.8)	404.19 (166.06, 983.8)	0.138
4. Adiponectin	0.09 (0.03, 0.23)	0.51 (0.17, 1.49)	**0.023**	0.11 (0.04, 0.28)	1.35 (0.22, 8.33)	***0.024***
5. Hemopexin	17.15 (9.01, 32.65)	35.64 (17.97, 70.70)	0.138	17.52 (9.76, 31.45)	109.68 (35.05, 343.16)	***0.010***
6. KIM-1	8.67 (6.18, 12.17)	25.55 (18.05, 36.15)	**0.000**	13.13 (8.82, 19.56)	23.28 (12.50, 43.39)	0.140
7. AAG	0.67 (0.30, 1.49)	1.14 (0.49, 2.68)	0.377	0.59 (0.27, 1.31)	3.16 (0.85, 11.73)	***0.043***
8. TGF-β	*0.16 (0.07, 0.35)*	*0.71 (0.32, 1.55)*	***0.013***	0.38 (0.24, 0.60)	0.99 (0.50, 1.96)	***0.030***
9. Hepcidin	0.55 (0.26, 1.15)	0.66 (0.29, 1.47)	0.753	0.56 (0.26, 1.21)	0.70 (0.15, 3.28)	0.802
10. L-PGDS	0.00 (0.00, 0.01)	0.01 (0.00, 0.01)	0.061	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.081
11. Transferrin	0.06 (0.04, 0.10)	0.14 (0.08, 0.25)	**0.029**	0.09 (0.05, 0.15)	0.12 (0.06, 0.27)	0.478
12. VDBP	5.43 (2.26, 13.07)	6.19 (2.38, 16.14)	0.844	3.95 (1.72, 9.08)	30.18 (6.62, 137.64)	***0.030***
13. EPCR	1.23 (0.89, 1.69)	1.32 (0.93, 1.88)	0.754	1.11 (0.81, 1.52)	1.60 (0.86, 2.98)	0.312
14. L-FABP	0.21 (0.12, 0.36)	0.28 (0.16, 0.50)	0.454	0.20 (0.11, 0.34)	0.45 (0.20, 1.01)	0.099
15. Microalbumin	5.70 (2.81, 11.55)	12.09 (5.76, 25.41)	0.159	5.48 (2.65, 11.35)	20.62 (6.61, 64.33)	0.064
16. Cystatin –C (/1,000)	0.66 (0.48, 0.90)	0.83 (0.59, 1.15)	0.352	0.70 (0.51, 0.96)	1.20 (0.65, 2.18)	0.138

[&] Values are geometric means (95% confidence intervals) of biomarker concentrations after log transformation

[†] LN-activity NIH-AI high vs. moderate/low is defined based on NIH-AI scores > 10 vs. ≤ 10

[‡] LN-activity TIAI high vs. moderate/low is defined based on TIAI scores > 5 vs. ≤ 5

Table 4

Accuracy of the combinatorial UBMs NGAL, ceruloplasmin, MCP-1, adiponectin, hemopexin and KIM-1 to reflect LN-activity study is preserved irrespective of the statistical approach used to derive the RAIL algorithm

Measurement Properties		Multiple logistical regression		Principal component analysis		Linear discriminant analysis	
LN-status high vs. moderate/low measured by		**Unadjusted for NIH-CI scores**	**Adjusted for NIH-CI scores**	**Unadjusted for NIH-CI scores**	**Adjusted for NIH-CI scores**	**Unadjusted for NIH-CI scores**	**Adjusted for NIH-CI scores**
NIH-AI scores	***AUC (95% CI)***	0.92 (0.83, 1.0)	0.94 (0.87, 1.0)	0.86 (0.74, 0.98)	0.87 (0.75, 0.99)	0.98 (0.95, 1.0)	0.99 (0.95, 1.0)
	Sensitivity	0.90	0.90	0.80	0.85	0.95	0.95
	Specificity	0.86	0.90	0.81	0.85	1.00	1.00
	LR+	6.30	9.00	4.20	5.67	>20	>20
	LR−	0.12	0.11	0.25	0.18	0.05	0.05
TIAI score	***AUC (95% CI)***	0.80 (0.65, 0.95)	0.83 (0.67, 0.98)	0.80 (0.63, 0.96)	0.82 (0.64, 1.0)	0.93 (0.85, 1.00)	0.94 (0.87, 1.0)
	Sensitivity	0.80	0.90	0.80	0.80	0.90	1.00
	Specificity	0.68	0.68	0.68	0.82	0.82	0.82
	LR+	2.51	2.83	2.51	4.40	4.95	5.50
	LR−	0.29	0.15	0.29	0.24	0.12	0.00