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Association of Noninvasively Measured Renal Protein Biomarkers With Histologic Features of Lupus Nephritis

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Objective. **To investigate the relationship of urinary biomarkers and established measures of renal function to histologic findings in lupus nephritis (LN), and to test whether certain combinations of the above-mentioned laboratory measures are diagnostic for specific histologic features of LN.**

Methods. **Urine samples from 76 patients were collected within 2 months of kidney biopsy and assayed for the urinary biomarkers lipocalin-like prostaglandin D synthase (L-PGDS), α_1-acid glycoprotein (AAG), transferrin (TF), ceruloplasmin (CP), neutrophil gelatinase–associated lipocalin (NGAL), and monocyte chemotactic protein 1 (MCP-1). Using nonparametric analyses, levels of urinary biomarkers and established markers of renal function were compared with histologic features seen in LN, i.e., mesangial expansion, capillary proliferation, crescent formation, necrosis, wire loops, fibrosis, tubular atrophy, and epimembranous deposits. The area under the receiver operating characteristic curve (AUC) was calculated to predict LN activity, chronicity, or membranous LN.**

Results. **There was a differential increase in levels of urinary biomarkers that formed a pattern reflective of specific histologic features seen in active LN. The combination of MCP-1, AAG, and CP levels plus protein:creatinine ratio was excellent in predicting LN activity (AUC 0.85). NGAL together with creatinine clearance plus MCP-1 was an excellent diagnostic test for LN chronicity (AUC 0.83), and the combination of MCP-1, AAG, TF, and creatinine clearance plus C4 was a good diagnostic test for membranous LN (AUC 0.75).**

Conclusion. **Specific urinary biomarkers are associated with specific tissue changes observed in conjunction with LN activity and chronicity. Especially in combination with select established markers of renal function, urinary biomarkers are well-suited for use in noninvasive measurement of LN activity, LN chronicity, and the presence of membranous LN.**

Systemic lupus erythematosus (SLE) is a multisystem inflammatory autoimmune disease in which renal involvement is one of the main determinants of poor prognosis (1). Histologic features seen on kidney biopsy constitute the current criterion standard for the diagnosis of lupus nephritis (LN) and are used to guide LN treatment. Kidney biopsy enables direct assessment of

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the presence and severity of acute changes due to active LN and provides insight into the chronicity of LN (2). Obtaining kidney biopsies is necessary because traditional measures of LN, such as blood pressure, proteinuria, urine sediment, complement components C3 and C4, and glomerular filtration rate (GFR), are considered too inaccurate to reliably discriminate between the acute inflammatory changes that are amenable to immunosuppressive therapy and the chronic degenerative changes that will not improve despite control of SLE activity.

Using proteomic techniques, we have previously identified novel urinary biomarkers of LN. These include transferrin (TF), ceruloplasmin (CP), α_1-acid glycoprotein (AAG; also known as orosomucoid), lipocalin-type prostaglandin D synthase (L-PGDS), monocyte chemotactic protein 1 (MCP-1; also known as CCL2), and neutrophil gelatinase–associated lipocalin (NGAL) (3–5). We have shown that levels of these urinary biomarkers correlate with and are responsive to clinical measures of LN activity, and that some urinary biomarkers are even suited for predicting future LN flares (6–8). The relationship of these urinary biomarkers to specific histologic features of LN, however, has not been examined and was the focus of the present study. The specific objectives of this study were 1) to investigate the relationship of the urinary biomarkers and traditional laboratory measures of LN to histologic findings seen on kidney biopsy in both children and adults with LN, and 2) to test whether certain combinations of the above-mentioned laboratory measures are diagnostic for specific histologic features of LN.

PATIENTS AND METHODS

Patients. The study was approved by the institutional review boards and ethics review committees of the participating centers. Children and adults diagnosed as having SLE according to the American College of Rheumatology (ACR) criteria (9) in whom kidney biopsy was required as part of standard-of-care therapy were included in this study if a random spot urine sample collected within 60 days of the kidney biopsy was available. On the day of the urine sample collection, information about patient demographic characteristics, medications, and disease activity was recorded. Key laboratory measures were obtained, including complement C3 and C4 levels, anti–double-stranded DNA (anti-dsDNA) (present/absent), amount of proteinuria as estimated by the protein-to-creatinine (P:C) ratio in a random or 24-hour urine sample, serum creatinine level, and GFR as estimated by age-appropriate calculation of creatinine clearance (10,11).

The renal domain score of the Systemic Lupus Disease Activity Index (SLEDAI-R) (range 0–16; 0 = inactive LN) (12) was used as the clinical measure of LN activity (12). The Systemic Lupus International Collaborating Clinics/ACR Damage Index items addressing renal damage (SDI-R) (range 0–3; 0 = no LN damage) (13) were recorded as a clinical measure of kidney damage in patients with LN.

Kidney histology. The histologic features in each kidney biopsy specimen were initially reported by local pathologists. Subsequently, the reports were reviewed, under blinded conditions, by an expert nephropathologist (DW) and classified according to the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) criteria (14). The following histologic features reflective of active inflammation in LN were recorded: mesangial proliferation, endocapillary karyorrhexis (also: fibrinoid necrosis), cellular crescents, capillary proliferation, and subendothelial deposits identifiable by light microscopy (also: wire loops). We also noted features representing LN chronicity or degenerative damage. These included glomerular sclerosis (segmental or global), fibrosis (including fibrous adhesions and fibrous crescents), and tubular atrophy.

Almost all studies in LN use a previously developed scoring system to quantify the amount of overall LN activity and overall LN chronicity based on findings in the kidney biopsy specimen (15). The features of activity and chronicity listed above were categorized as 0 (no lesions), 1 (lesions in up to 25% of glomeruli), 2 (lesions in 25–50% of glomeruli), or 3 (lesions in >50% of glomeruli). Using these numeric values, a biopsy activity index (BAI) score (range 0–24) and a biopsy chronicity index (BCI) score (range 0–12) can be calculated, with higher scores representing higher LN activity or chronicity, respectively.

Epimembranous deposits, although not included in the BAI or the BCI scores, were also recorded. Depending on the findings of active inflammation and chronic changes observed on kidney biopsy, LN is classified in 6 categories. Pronounced predominance of epimembranous deposits is compatible with class V LN.

The ISN/RPS classification, the BAI, and the BCI have all been validated for use in adults and children with LN (16,17). Risk factors for poor LN outcome include BAI scores of ≥7 and BCI scores of ≥4 (16,18–25).

Urinary biomarker assays. Urine samples were frozen at −80°C prior to batch processing. We measured urine concentrations of TF and L-PGDS by immunonephelometry (BNII Prospect; Dade-Behring). Urinary CP was quantified by enzyme-linked immunosorbent assay (ELISA) (Human Ceruloplasmin ELISA Quantitation Kit; Assaypro). Intra- and interassay coefficients of variation (CVs) of these assays were, respectively, 3.4% and 2.5% for TF, 2.3% and 6.5% for L-PGDS, and 4.1% and 7.1% for CP. Likewise, urinary AAG was measured by ELISA (Human Orosomucoid ELISA Quantitation Kit; GenWay Biotech) (intra- and interassay CVs 5.0% and CV 8.5%, respectively). MCP-1 levels were also measured by ELISA (R&D Systems), with intra- and interassay CVs of 5.0% and 5.1%, respectively. All of these commercial ELISAs were performed according to the manufacturers' instructions. NGAL was measured as previously described by our group (6,7). Intra- and interassay CVs of the NGAL assay were 5.0% and 5.1%, respectively.

Concentrations of the urinary biomarkers (in ng/ml for AAG, NGAL, CP, and MCP-1 and in mg/dl for TF and L-PDGS) were standardized to urinary creatinine levels (in mg/ml). Laboratory personnel measuring the urinary biomarkers were blinded with regard to the clinical and histologic information.

Statistical analysis. We inspected the central tendency, dispersion, and skewness of the urinary biomarkers and traditional markers of LN (C3, C4, GFR, P:C ratio) and found them not to fit well into normal distributions. Therefore, medians and interquartile ranges (IQRs) were calculated as measures of central tendency for continuous variables, while categorical variables were summarized by frequency (in percentages). We used Spearman's correlation coefficient to examine the strength of the association between numerical variables, and the Wilcoxon rank sum test to assess for statistically significant associations between types of histologic features and levels of urinary biomarkers or traditional renal markers. Because of the skewness, we log-transformed the concentrations of the urinary biomarkers and traditional measures of LN prior to considering them in univariate and multivariate logistic regression modeling to determine relevant predictors of key LN features that are associated with poor prognosis (BAI score ≥7, BCI score ≥4) or that may require differential therapy (i.e., ISN/RPS class V LN) (26).

We also calculated the relative change in the median and IQR of the laboratory measures with the presence versus absence of a histologic feature or a particular LN outcome (ISN/RPS class V LN, BAI score ≥7, BCI score ≥4). Hence, values of 100% signify that the urinary biomarker (or traditional measure of LN) is present in the same amount in the presence versus the absence of a histologic feature or a particular LN outcome. Values of >100% represent scenarios in which the laboratory measure is increased, and values of <100% represent scenarios in which it is decreased, with the presence of the histologic feature or a particular LN outcome compared to its absence.

All candidate biomarkers were included in the multivariate logistic models. Additionally, traditional biomarker measures of LN with a P value of <0.15 on univariate analysis were included in the multivariate models. As described by our group in the past (5), the diagnostic accuracy of each biomarker and biomarker combination was assessed by receiver operating characteristic curve analysis, and the corresponding area under the curve (AUC; range 0–1) was calculated. The accuracy of the biomarker and biomarker combinations in predicting LN histologic features was considered outstanding, excellent, good, fair, or poor if the AUC was in the range of 0.9–1.0, 0.81–0.90, 0.71–0.80, 0.61–0.70, or 0.50–0.60, respectively. The sensitivity and specificity for predicting LN outcomes (presence of ISN/RPS class V LN, BAI score ≥7, BCI score ≥4) were determined for particular cutoff values of each biomarker combination, generally for sensitivities of ~75%. Furthermore, we tested whether biomarker concentrations and specific kidney biopsy features systematically changed with patient age and explored whether the lag time between urine collection and kidney biopsy was important for the association between urinary biomarkers and LN histologic features.

Statistical analyses were performed using SAS, version 9.2. P values less than or equal to 0.025 were considered significant.

Table 1. Demographic characteristics, treatment, and renal status at the time of urine collection in the 76 patients with LN*

Disease onset	
Childhood-onset LN	28 (37)
Adult-onset LN	48 (63)
Female	64 (84)
Race	
Black	35 (46)
White	33 (43)
Other	8 (11)†
Treatment	
Oral prednisone	73 (96)
Pulse methylprednisolone	33 (43)
Mycophenolate mofetil	23 (30)
Azathioprine	3 (4)
Cyclophosphamide	13 (17)
Methotrexate	4 (5)
Angiotensin-blocking agent	41 (54)
LN status	
GFR <60 ml/minute/1.73m^2	14 (18)
Protein:creatinine ratio >0.5	68 (89)
SDI-R score >0‡	3 (14)
SLEDAI-R score, median (IQR)	8 (0–16)
Anti-dsDNA positive§	49 (75)
Timing of urine collection	
Time interval (days) to biopsy, median (IQR)¶	+3.5 (−60 to +60)
>30 days before biopsy	5 (7)
>30 days after biopsy	17 (22)
ISN/RPS class#	
Class II	6 (8)
Class III	12 (16)
Class IV	29 (38)
Class V	29 (38)
Histologic features present	
Mesangial expansion	73
Capillary proliferation	38
Cellular crescents	22
Fibrinoid necrosis	22
Wire loops	21
Fibrosis	53
Tubular atrophy	58
Epimembranous deposits	33
BAI score, median (IQR)**	3 (0–15)
BCI score, median (IQR)††	2 (0–9)

* Except where indicated otherwise, values are the number (%) of patients. GFR = glomerular filtration rate; SDI-R = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index items addressing renal damage; SLEDAI-R = renal domain of the Systemic Lupus Disease Activity Index; IQR = interquartile range; anti-dsDNA = anti–double-stranded DNA; ISN = International Society of Nephrology; RPS = Renal Pathology Society; BAI = biopsy activity index; BCI = biopsy chronicity index.
† American Indian (n =1), Asian (n = 3), and mixed race (n = 4).
‡ Data available on 22 patients.
§ Data available on 65 patients.
¶ Positive value indicates that the urine was collected after the kidney biopsy.
# There were no patients with class I or class VI lupus nephritis (LN).
** Range 0–24 (0 = inactive LN).
†† Range 0–12 (0 = LN without chronic changes).

RESULTS

Patient characteristics and findings on kidney biopsy. A total of 76 patients were included in the study (Table 1). The median age was 23 years (range 9–51), and 26 of the patients were ≤18 years old. At the time of urine collection, almost all patients were being treated with glucocorticoids, and many were also receiving im-

Table 2. Relationships between histologic features seen on kidney biopsy in the patients with lupus nephritis*

Histologic feature (n)	Capillary proliferation	Cellular crescents	Fibrinoid necrosis	Wire loops	Fibrosis	Tubular atrophy	Epimembranous deposits	Biopsy activity index score ≥7	Biopsy chronicity index score ≥4
Mesangial proliferation									
Moderate (27)	85	44	52	62	78	74	37	41	14
No/mild (49)	31	20	16	8	65	78	47	2	14
P	<0.0001	–	0.0016	<0.0001	–	–	–	<0.0001	–
Capillary proliferation									
Yes (38)		53	39	49	79	76	34	32	13
No (38)		5	18	5	61	76	53	–	16
P		<0.0001	–	<0.0001	–	–	–	<0.0001	–
Cellular crescents									
Yes (22)			68	55	77	73	32	41	9
No (54)			13	15	67	78	48	6	17
P			<0.0001	<0.0001	–	–	–	0.0004	–
Fibrinoid necrosis									
Yes (22)				57	68	64	23	41	9
No (54)				15	71	82	52	6	17
P				0.0005	–	–	0.02	–	–
Wire loops									
Yes (20)					80	65	20	60	15
No (55)					67	80	53	0	15
P					–	–	0.02	0.0001	–
Fibrosis									
Yes (53)						95	42	17	21
No (23)						39	48	13	–
P						0.0001	–	–	–
Tubular atrophy									
Yes (58)							47	12	19
No (18)							33	28	–
P							–	–	–
Epimembranous deposits									
Yes (33)								9	12
No (43)								21	16
P								–	–
Biopsy activity index score									
≥7 (12)									17
<7 (64)									14
P									–

* Values are the percent of patients with both features. Only *P* values less than or equal to 0.025 are shown.

munosuppressive medications. The median SLEDAI-R score at the time of urine collection was 8 (range 0–16), and 75% of the patients with available information had elevated levels of anti-dsDNA antibodies (49 of 65). Only 3 patients had renal damage as assessed according to the SDI-R.

The median time interval between kidney biopsy and urine sample collection was 3.5 days, and in 38 patients (50%) the urine sample was collected prior to or on the day of the kidney biopsy. The histologic diagnoses included proliferative LN (ISN/RPS class III or IV) in 54% of the patients (41 of 76) and class V LN in 38% (29 of 76). Epimembranous deposits (ISN/RPS class V or together with class III or IV) were observed in 43% of the patient biopsy specimens (33 of 76).

As expected, specific histologic features were often seen concomitantly in the same kidney biopsy specimen. Generally, features reflective of active inflammation were clustered, as were those representing LN chronicity. For example, among biopsy samples with capillary proliferation, a large proportion (85%) exhibited moderate mesangial proliferation. Among samples with tubular atrophy, 95% also showed fibrotic changes of the renal tissue (Table 2).

Associations of laboratory measures with LN histologic features. The age of the patients was significantly associated with serum creatinine levels ($r = 0.27$, $P < 0.017$) and BCI scores ($r = -0.45$, $P < 0.0001$), but not with levels of any of the urinary biomarkers or traditional measures of LN. Concentrations of all of the

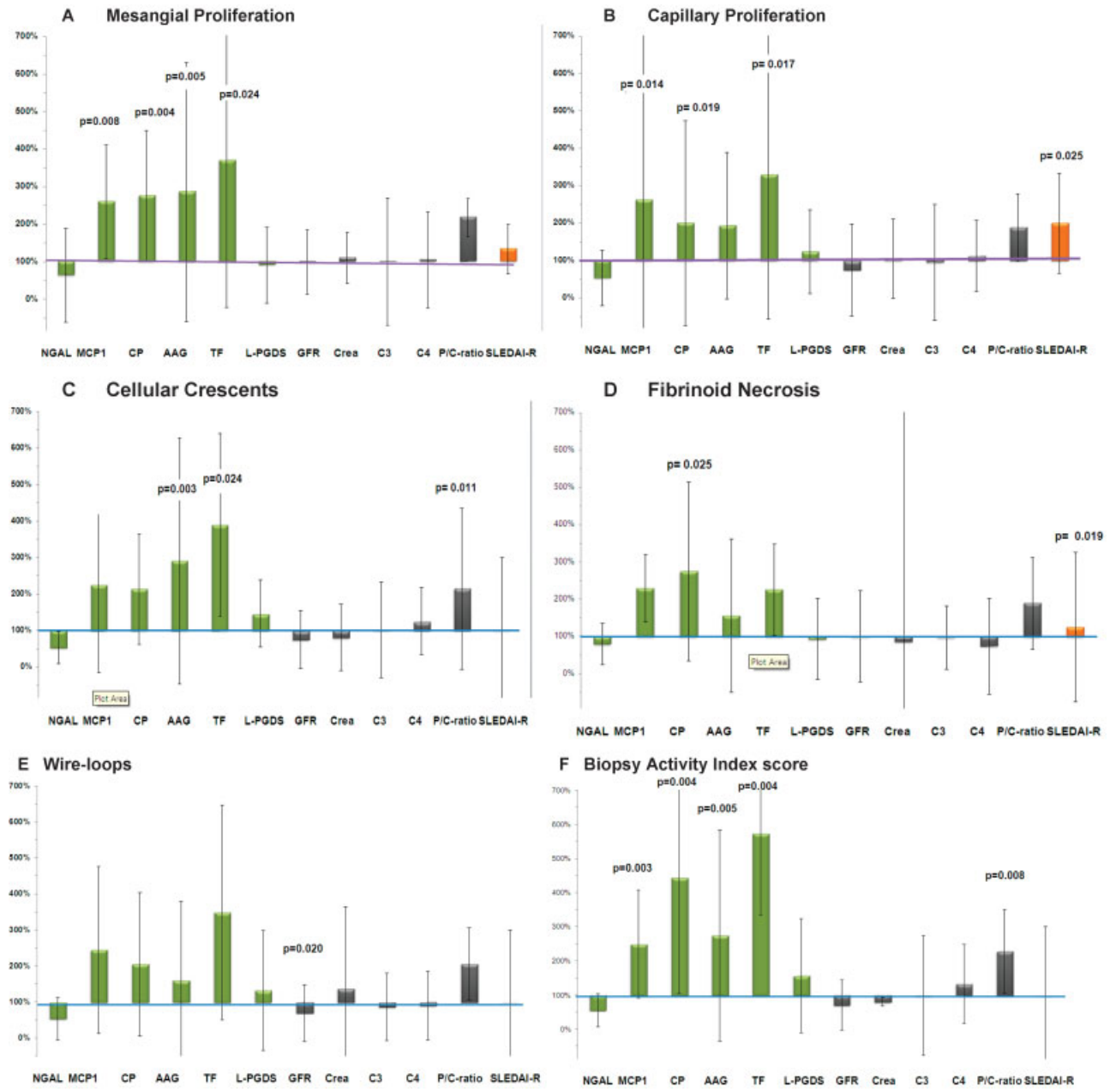

Figure 1. Changes in levels of the biomarkers neutrophil gelatinase–associated lipocalin (NGAL), monocyte chemotactic protein 1 (MCP-1), ceruloplasmin (CP), α_1-acid glycoprotein (AAG), transferrin (TF), and lipocalin-like prostaglandin D synthase (L-PGDS), the glomerular filtration rate (GFR), the serum creatinine (Crea) level, the levels of complement components C3 and C4, the protein-to-creatinine (P:C) ratio, and clinical disease activity as measured by the renal domain of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-R) in relation to the presence versus absence of specific histologic features associated with lupus nephritis activity in 76 patients. Values of 100% signify that the level of the biomarker does not differ in the presence versus the absence of the given histologic feature; values of $<$100% and values of $>$100% signify that levels of the biomarker decrease and increase, respectively, in the presence of the histologic feature. Changes with a *P* value of less than or equal to 0.025 were considered significant. **A,** Significant increases in MCP-1, CP, AAG, and TF in the presence of mesangial proliferation. **B,** Significant increases in MCP-1, CP, TF, and the SLEDAI-R in the presence of capillary proliferation. **C,** Significant increases in AAG, TF, and the P:C ratio in the presence of cellular crescents. **D,** Significant increases in CP and the SLEDAI-R in the presence of fibrinoid necrosis. **E,** Lack of significant increase in levels of any of the urinary biomarkers in the presence of wire loops. However, several of the biomarkers, especially AAG and TF, were increased to a degree that approached statistical significance. In addition, the GFR was significantly decreased in the presence of wire loops. **F,** Significant increases in MCP-1, CP, AAP, TF, and the P:C ratio in the presence of high biopsy activity index scores. Values are the median and interquartile range.

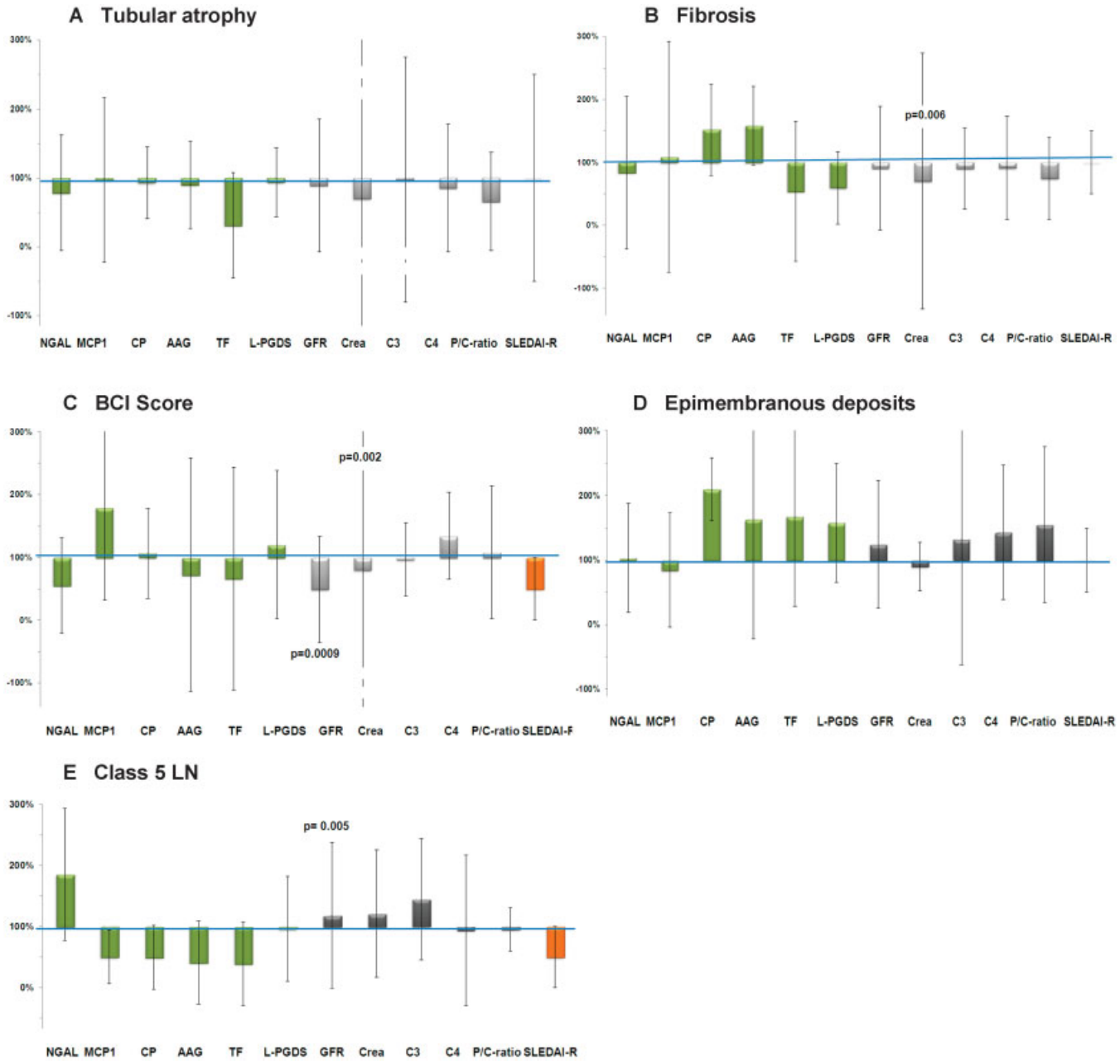

Figure 2. Changes in levels of the biomarkers NGAL, MCP-1, CP, AAG, TF, and L-PGDS, the GFR, the serum creatinine level, the levels of complement components C3 and C4, the P:C ratio, and clinical disease activity as measured by the SLEDAI-R in relation to the presence versus absence of specific histologic features associated with lupus nephritis (LN) chronicity in 76 patients. Values of 100% signify that the level of the biomarker does not differ in the presence versus the absence of the given histologic feature; values of <100% and values of >100% signify that levels of the biomarker decrease and increase, respectively, in the presence of the histologic feature. Changes with a *P* value of less than or equal to 0.025 were considered significant. **A,** Lack of significant change in levels of any of the biomarkers in the presence of tubular atrophy. **B,** Significant increase in serum creatinine in the presence of fibrosis. **C,** Significant increase in serum creatinine and decrease in GFR in the presence of high scores on the biopsy chronicity index (BCI). **D,** Lack of significant change in levels of any of the biomarkers in the presence of epimembranous deposits. **E,** Significant increase in serum creatinine in the presence of class V LN according to the International Society of Nephrology/Renal Pathology Society criteria. Values are the median and interquartile range. See Figure 1 for other definitions.

Table 3. Combinations of markers predicting key biopsy features in LN*

Model outcome variable	Predictor variables	Area under the ROC curve (95% CI)†	Sensitivity, %‡	Specificity, %
Biopsy activity index score ≥7	MCP-1, CP, AAG, P:C ratio	0.85 (0.69–1.0)	72	66
Biopsy chronicity index score ≥4	NGAL, GFR, MCP-1	0.83 (0.67–0.93)	73	67
Membranous LN (class V)	MCP-1, GFR, AAG, TF, C4	0.75 (0.62–0.86)	75	48

* LN = lupus nephritis; 95% CI = 95% confidence interval; MCP-1 = monocyte chemotactic protein 1; CP = ceruloplasmin; AAG = α_1-acid glycoprotein; P:C = protein-to-creatinine; NGAL = neutrophil gelatinase–associated lipocalin; GFR = glomerular filtration rate; TF = transferrin.
† The area under the receiver operating characteristic (ROC) curve ranges from 0 to 1.
‡ Clinically relevant point on ROC, with sensitivity of ≥70%.

urinary biomarkers were at least weakly correlated with one another ($r \geq |0.2|$). In addition, some strong correlations between TF levels and levels of other biomarkers were observed ($r = 0.74$, $P < 0.0001$ for the correlation between TF and CP levels; $r = 0.61$, $P = 0.005$ for the correlation between TF and AAG levels). Among traditional measures of LN, the only strong correlation was, as expected, between the GFR and the serum creatinine level ($r = 0.79$). Notably, levels of C3 and C4 and the P:C ratio were unrelated ($r < |0.2|$). These findings suggest that concentrations of the biomarkers do not simply increase in the urine due to increased proteinuria and support the notion that the urinary biomarkers provide additional information about LN over and above that obtained with the traditional measures of LN.

Association of the SLEDAI-R score with LN histologic features. SLEDAI-R scores were significantly correlated with levels of urinary biomarkers, i.e., NGAL ($r = -0.39$, $P < 0.0007$), MCP-1 ($r = 0.23$, $P < 0.07$), CP ($r = 0.23$, $P < 0.05$), AAG ($r = 0.35$, $P < 0.003$), and L-PGDS ($r = 0.28$, $P < 0.016$). Additionally, SLEDAI-R scores were significantly correlated with serum creatinine levels ($r = 0.35$, $P < 0.002$), P:C ratios ($r = 0.40$, $P < 0.0004$), and C3 levels ($r = -0.34$, $P < 0.0043$). Conversely, urine concentrations of TF and C4 and the GFR were unrelated to the SLEDAI-R scores ($r < |0.2|$). BAI and SLEDAI-R scores were weakly correlated ($r = 0.29$, $P < 0.01$).

Changes in levels of the biomarkers in relation to histologic features of LN activity. The relationship of levels of the urinary biomarkers and traditional measures of LN with histologic features of LN is shown in Figure 1. Excretion of some urinary biomarkers was increased in the presence of mesangial proliferation, capillary proliferation, cellular crescents, and fibrinoid necrosis (Figures 1A–D). There was a trend toward more pronounced increases in levels of the urinary biomarkers in the presence of wire loops, but none of these changes reached statistical significance (Figure 1E). With the exception of the P:C ratio, none of the traditional measures of LN showed statistically significant increases in relation to any of the histologic measures of LN activity. Levels of MCP-1, CP, AAG, and TF and the P:C ratio all discriminated between low versus high BAI scores (<7 versus ≥7) (Figure 1F). Of note, urinary L-PGDS and NGAL levels were not differentially associated with any of the histologic features that were analyzed, nor were the levels of complement components C3 and C4.

Changes in levels of the biomarkers in relation to histologic features of LN chronicity and epimembranous deposits. The levels of the urinary biomarkers differed especially in relation to features of active LN, and not in relation to features of LN chronicity (Figures 2A–C). Only the GFR and the serum creatinine level differed significantly in the presence versus the absence of features of LN chronicity and distinguished between high and low BCI scores (≥4 versus <4).

None of the urinary biomarkers or traditional measures of LN differed significantly in the presence versus the absence of epimembranous deposits. However, there was a trend toward larger relative differences in levels of the urinary biomarkers in the presence of epimembranous deposits versus its absence. Only the GFR differed significantly in the presence of class V LN versus class II–IV LN (median 125 ml/minute/1.73 m^2 [IQR 54] versus 85 ml/minute/1.73m^2 [IQR 54], respectively).

Univariate and multivariate logistic modeling to predict LN outcomes. In a univariate logistic regression analysis, we assessed the diagnostic accuracy (AUC) of each of the biomarkers for key LN features, i.e., BAI scores ≥7, BCI scores ≥4, and presence of ISN/RPS class V LN. Good to excellent accuracy in identifying patients with high BAI scores was demonstrated only for AAG (AUC 0.76), TF (AUC 0.76), CP (AUC 0.79), MCP-1 (AUC 0.82), and the P:C ratio (AUC 0.76). The GFR and serum creatinine were excellent predictors of high BCI scores (both with an AUC of 0.82). Univariate

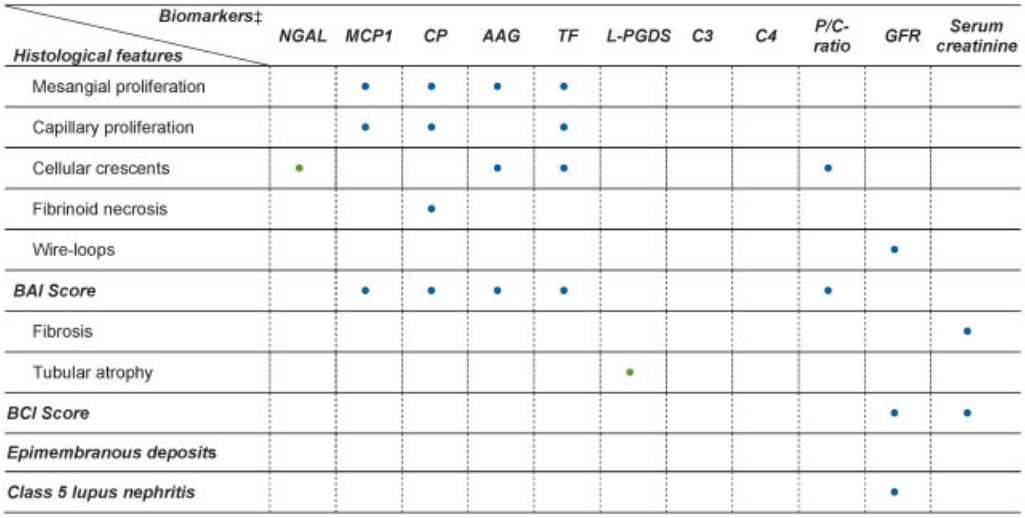

Figure 3. Summary of significant changes in marker levels in relation to the presence versus absence of histologic features of lupus nephritis (LN). Blue dots represent changes seen in urine samples that were collected within 2 months of the kidney biopsy (n = 76); green dots represent additional significant differences observed in the analysis that included only urine samples collected prior to the kidney biopsy (n = 38). The novel urine biomarkers are differentially excreted in the presence of histologic features of LN activity but not in the presence of membranous changes or features of LN chronicity. The P:C ratio, GFR, and serum creatinine level do not allow for differentiation between active, chronic, or membranous changes of LN. Complement levels do not distinguish between any histologic features. BAI = biopsy activity index; BCI = biopsy chronicity index (see Figure 1 for other definitions).

analysis did not reveal any urinary biomarker or traditional measure of LN that was at least a good diagnostic measure (AUC $\geq$0.7) for class 5 LN. Lastly, the SLEDAI-R score was a poor proxy measure of the BAI score (AUC 0.5).

The results of multivariate modeling to predict key LN outcomes (high BAI score, high BCI score, class 5 LN) are summarized in Table 3. A combination of 4 different biomarkers (MCP-1, CP, AAG, and P:C ratio) was excellent for diagnosing high BAI scores (AUC 0.85), as was the combination of NGAL, GFR, and MCP-1 for diagnosing high BCI scores (AUC 0.83). A combination of 5 biomarkers was good for diagnosing ISN/RPS class V LN (AUC 0.75).

Findings after exclusion of urine samples collected after the kidney biopsy. Although we did not have access to data on recent changes in treatment just prior to or after the study visit, we hypothesized that LN therapy was intensified after the results of the kidney biopsy had become available. When only patients from whom urine samples were collected no later than the day of the kidney biopsy (n = 38) were included in the analyses, urinary NGAL concentrations were significantly lower in the presence than in the absence of cellular crescents (median 8.74 ng/ml [IQR 44.9] versus 48.5 ng/ml [IQR 64.3]; $P < 0.0033$], and L-PGDS concentrations were significantly lower in the presence of tubular atrophy than in the absence of this feature (median 695 mg/dl [IQR 591] versus 928 mg/dl [IQR 1,021]; $P < 0.0234$]. There were no significant changes in levels of any of the other urinary biomarkers or traditional measures of LN in relation to presence or absence of LN features, when only this subset of 38 patients was considered. The patterns of relative biomarker excretion in relation to LN histology, including those observed in the subanalysis, are summarized in Figure 3.

DISCUSSION

We examined recently identified urinary biomarkers and traditional measures of LN and found that individual urinary biomarkers were related to specific histologic findings in LN, especially those representing LN activity. The combination of MCP-1, CP, AAG, and the P:C ratio was excellent for estimating histologic LN activity. NGAL together with GFR and MCP-1 was an excellent diagnostic test for LN chronicity. Another combination of biomarkers exhibited good ability for the diagnosis of class V LN.

Our previous research supports the notion that levels of the urinary biomarkers correlate with and are responsive to change in clinical measures of LN activity

(3,7). These observations are confirmed by the findings of this study, in which the urinary biomarkers were associated with histologic features of LN activity. Surprisingly, however, urinary NGAL and L-PGDS were not differentially associated with features of LN activity in our overall cohort of patients with urine samples collected within 2 months of kidney biopsy.

We previously reported that L-PGDS was a biomarker of LN activity as measured by, among other instruments, the SLEDAI-R (3). The significance of this protein for inflammatory processes in LN has been confirmed in animal studies (27). The present study showed L-PGDS to be weakly associated with clinical measures of LN activity (SLEDAI-R), other urinary biomarkers, and traditional measures of LN, but not with a specific histologic feature of LN. This might be due to the observation that elevated levels of L-PGDS are reflective of increased permeability of injured glomerular capillary walls (28), a feature not directly visible on standard histologic stains of kidney biopsy specimens. Alternatively, as is suggested by the findings of our subanalysis in which L-PGDS excretion was 34% higher in patients with tubular atrophy, it may represent an early biomarker or one that rapidly declines with immunosuppressive therapy.

NGAL is an early and predictive urinary biomarker that is rapidly induced by active inflammation in LN, and promptly declines with therapy (5). Thus, when we excluded patients whose urine sample was collected after the kidney biopsy (i.e., already receiving intensive treatment for LN) and instead considered only the remaining patients (n = 38), we found NGAL levels to be much lower in patients with cellular crescents, an important histologic feature of active proliferative LN. The biologic function of NGAL is still under investigation (29). In the acute nephritis setting, NGAL appears to provide a protective antiapoptotic mechanism that limits tubule cell damage and enhances proliferation (30). Hence, the low NGAL levels found in patients with cellular crescents may represent a failure to protect against structural changes typically associated with active LN. Besides its role in LN activity, NGAL is associated with LN damage (6). The importance of NGAL as a biomarker of LN chronicity is supported by its inclusion in the combination of markers that served as a predictor of high BCI scores in this study. Urinary NGAL is also elevated in adults with chronic kidney disease, in whom NGAL levels are inversely correlated with GFR and positively correlated with tubular atrophy (31). The increased production of NGAL in this context of chronic nephritis likely constitutes a pathophysiologic pathway that leads to progressive renal failure (32).

MCP-1 has long been known to be a predictive biomarker of LN flare and LN severity (4). Lupus-prone MRL-lpr/lpr and MCP-1–knockout mice exhibit significantly reduced proteinuria and prolonged survival (33), indicating a role of MCP-1 in LN pathogenesis in addition to its demonstrated capacity as a urinary biomarker for LN (4). The findings of our study are in accordance with these previous results: MCP-1 was differentially excreted in relation to features of LN activity and high BAI scores. In multivariate models to predict the presence of membranous LN (ISN/RPS class V), MCP-1 was included as an important predictor, supporting previous observations of high urinary MCP-1 levels as well as high expression of MCP-1, especially in the tubular epithelial cells, in idiopathic membranous nephropathy (34).

AAG was markedly increased in the urine of patients with mesangial proliferation and crescents. This finding is consistent with the fact that AAG is a known marker of LN activity whose urinary levels are also elevated in other inflammatory kidney diseases. AAG is produced in epithelial cells and is thought to play an important role in regulating the dynamic properties of the glomerular capillary wall by reducing permeability toward macromolecules such as albumin (35).

We found an association of TF levels with mesangial and capillary proliferation and cellular crescent formation, an observation that is consistent with previous findings in IgA nephropathy (36). Physiologically, recycled and absorbed iron is delivered to TF, the main iron-transporting protein in blood. Some TF normally enters the glomerular filtrate, but it is retrieved by specific receptor-mediated uptake in the kidney tubular system (37). Thus, tubular injury will lead to increased urinary TF concentrations.

Similarly, urinary concentrations of CP were increased, especially in the presence of mesangial or capillary proliferation and fibrinoid necrosis. We have previously reported that CP, an oxidative stress-related protein, is a biomarker of LN activity (3). Additionally, CP has been associated with tissue remodeling in the kidney after renal tubular injury, as can be observed in LN (38).

Individually, neither the urinary biomarkers nor the traditional measures of LN were suited to determine whether epimembranous deposits are present. Likely because multiple histologic features of LN often are seen together in the same specimen, the GFR was found to importantly differ only with regard to the presence

versus absence of class V LN, but not in relation to epimembranous deposits. We speculate that this is also the reason for the differences in trends of the other LN measures in patients with class V LN compared to patients whose kidney biopsies showed some epimembranous deposits but who did not have class V LN.

Combinations of the biomarkers included in this study yielded excellent diagnostic abilities for LN activity and chronicity. However, the presented analyses also suggest that additional markers are needed to provide the highly accurate (AUC >0.9) diagnostic tests that are urgently needed by clinicians to help guide LN therapy.

Certain limitations must be taken into account when considering the present results. Given the diverse medication regimens used, the multiplicity of distinct kidney biopsy features, and their considerable overlap in individual patients, our findings will need to be confirmed in a larger cohort. Nonetheless, the association of novel as well as traditional biomarkers of LN with specific histologic features suggests that accurate longitudinal noninvasive assessment of LN activity and chronicity is feasible. If confirmed, this will allow for more effective and personalized monitoring of LN and its therapy. The availability of standardized clinical platforms for reliable measurement of the urinary biomarkers (39) will enable the testing of this hypothesis in the near future.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Brunner had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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