NON-INVASIVE RENAL PROTEIN BIOMARKERS ARE ASSOCIATED WITH HISTOLOGICAL FEATURES OF LUPUS NEPHRITIS

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Abstract

Objective—To investigate the relationship of urinary biomarkers (UBM) and established measures of renal function (EMRF) to the histological findings with lupus nephritis (LN); and to test whether certain combinations of the above mentioned laboratory measures are diagnostic of specific histological features of LN.

Methods—Urine samples of 76 patients were collected within 2 months of a kidney biopsy and assayed for the UBM: lipocalin-like prostaglandin-D synthetase (LPGDS), α1-acid-glycoprotein (AAG), transferrin (TF), ceruloplasmin (CP), neutrophil-gelatinase associated lipocalin (NGAL), and monocyte chemotactic factor 1 (MCP1). Using non-parametric analyses, UBM and EMRF levels were compared to histological features seen with LN: mesangial expansion, capillary proliferation, crescent formation, necrosis, wire loops, fibrosis, tubular atrophy, and epimembranous deposits. The area under the receiver operating characteristic (AUC) curve was calculated to predict LN activity, chronicity or membranous LN.

Results—There was a differential increase of the UBM that formed a pattern reflective of specific histological features seen with active LN. The combination of MCP1, AAG, CP plus protein:creatinine ratio were excellent in predicting LN activity (AUC=0.85). NGAL together with creatinine clearance plus MCP1 was an excellent (AUC=0.83) and MCP1, AAG, creatinine

clearance plus C4 (AUC=0.75) a good diagnostic test of LN chronicity and membranous LN, respectively.

Conclusions—Select UBM are associated with specific tissue changes observed with LN activity and chronicity. Especially in combination with select EMRF, UBM are well-suited to non-invasively quantify LN activity, LN chronicity, and the presence of membranous LN.

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 histological 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 biopsies provide a direct assessment of the presence and severity of acute changes due to active LN and give 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 rates (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 identified previously novel urinary biomarkers (UBM) of LN. These include transferrin (TF), ceruloplasmin (CP), α-1-acid-glycoprotein (AAG; also known as orosomucoid), lipocalin-type prostaglandin-D synthetase (LPGDS), monocyte chemotactic factor 1 (MCP1; also known as chemokine ligand 2), and neutrophil gelatinase associated lipocalin (NGAL) (3–5). We have shown that these UBM correlate with and are responsive to clinical measures of LN activity, and that some UBM are even suited to predict future LN flares (6–8). The relationship of these UBM to specific histological features of LN, however, has not been examined and was the focus of this study.

The objectives were to (1) study the relationship of the UBM and traditional laboratory measures of LN to histological findings seen on kidney biopsy in both children and adults with LN; and (2) test whether certain combinations of the above mentioned laboratory measures are diagnostic for specific histological features of LN.

MATERIALS & METHODS

Patients

Children and adults diagnosed with SLE (9) who required a kidney biopsy as part of standard of care therapy were included in this study, if a random spot urine sample was available that was collected within 60 days of the kidney biopsy. On the day of the urine sample collection, information about patient demographics, medications, and disease activity was collected. Key laboratory measures were obtained, including complement C3 and C4 levels, anti-dsDNA antibodies (present/absent), amount of proteinuria as estimated by the protein to creatinine ratio (P/C ratio) in a random or 24-hour urine sample, serum creatinine, and glomerular filtration rate (GFR) as estimated by age-appropriate calculation of the creatinine clearance (10, 11).

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

Kidney Histology

The histological characteristics of each kidney biopsy, as per report from the local pathologists, were reviewed in a blinded fashion by one expert nephropathologist (DW), as per the International Society of Nephrology/ Renal Pathology (ISN/RPS) Classification (14).

The following histological features reflective of active inflammation with LN were recorded: mesangial proliferation, endocapillary karyorrhexis (also: fibrinoid necrosis); cellular crescents; capillary proliferation, 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, as well as tubular atrophy.

Almost all studies in LN employ a previously developed scoring system to quantify the amount of overall LN activity and overall LN chronicity as is present in the kidney biopsy specimen (15). The features of activity and chronicity listed above were categorized as follows: 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, where higher scores represent 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 six categories. Pronounced predominance of epimembranous deposits is compatible with Class 5 of 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 or higher and BCI scores of 4 or higher (16, 18–25).

Urinary Biomarker Assays

Urine samples were frozen at −80 degree celsius prior to batch processing. We measured urinary concentrations of TF and L-PGDS by immunonephelometry (Dade Behring BNII Prospect, Marburg, Germany). Urinary CP was quantified by ELISA (Human Ceruloplasmin ELISA Quantitation Kit; Assaypro, St.Charles, MO, USA). Intra and inter-assay coefficients of variation of these assays (%CV) were 3.4% and 2.5% for TF, 2.3% and 6.5% for L-PGDS, and 4.1% and 7.1% for CP, respectively. Likewise, urinary AAG (5.0% and 8.5%) was measured using an ELISA kit (Human Orosomucoid ELISA Quantitation Kit; Genway Biotech, Inc., San Diego, CA, USA). MCP1 levels were also measured by ELISA (R&D Systems, Minneapolis, MN, USA). The respective intra-assay and inter-assay CV was 5.0% and 5.1%. We used all these commercial ELISA kits as per manufacturers' instructions, while NGAL was measured as previously reported by our group (6, 7). Intra and inter-assay CV of the NGAL assay were 5.0% and 5.1%, respectively.

Concentrations of the UBM (in ng/ml for AAG, NGAL, CP and MCP1 and in mg/dl for TF and L-PDGS) were standardized for urinary creatinine levels (in mg/mL). Laboratory personnel measuring the UBM were blinded to the clinical and histological information.

Statistical analysis

We inspected the central tendency, dispersion, and skewness of the UBM 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 (IQR) were calculated as measures of central tendency for continuous variables, while categorical variables were summarized by frequency (in percentages). We used Spearman correlation coefficients to examine the strength of the association between numerical variables and Wilcoxon rank sum test to assess for statistically significant differences between types of histological features and UGM and traditional renal markers, respectively.

Because of the skewness, we log-transformed the concentrations of the UBM 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 5 LN) (26).

We also calculated the relative change of the median and IQR of the laboratory measures with the presence vs. absence of a histological feature or a particular LN outcome (ISN/RPS Class 5 LN; BAI score   7; BCI score   4). Hence, values of 100% signify that the UBM (or traditional measure of LN) is present in the same amount with the presence vs. absence of a histological features or a particular LN outcome. Values >100% represent scenarios where the laboratory measure increases, and values < 100% where they decrease with the presence of the histological feature or a particular LN outcomes compared to its absence.

Included in the multivariate logistic models were all candidate biomarkers, and traditional biomarkers measures of LN with p-value of <0.15 on univariate analysis.

As published by our group in the past (5), the diagnostic accuracy of eachbiomarker and biomarker combination was assessed by the receiver operating characteristic (ROC) 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 histology features was considered outstanding, excellent, good, fair, and poor if the AUC was in the range of $0.9 - 1.0, 0.81 - 0.90, 0.71 - 0.80, 0.61 - 0.70$, and $0.50 - 0.60$, respectively.

The sensitivity and specificity to predict LN outcomes (presence of ISN/RPS Class 5 LN; BAI score   7; BCI score   4) were determined for particular cut-off values of each biomarker combination, generally that for sensitivities around 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 conduct was important for the association between UBM and histological features seen with LN.

Statistical analyses were done using SAS version 9.2 software (SAS, Cary, NC, USA). P-values < 0.025 were considered statistically significant. The study was approved by the Institutional Review Boards and Ethics Review Committees of the participating centers.

RESULTS

Patient Characteristics & Features of Kidney Biopsy

A total of 76 patients with a median age of 23 years (range: $9 - 51$ years) was included in the study, and 26 patients were 18 years or younger (Table 1). At the time of urine collection, almost all patients were treated with glucocorticosteroids, many with

immunosuppressive medications, and the median SLEDAI-R score was 8 (range: 0 – 16). Elevated levels of anti-dsDNA antibodies were present in 75% (49/65) of the patients with available information. Only three patients had renal damage as per the SDI-R.

The median time interval between kidney biopsy and urine sample collection was 3.5 days, and for 50% (38/76) of the patients the urine sample was collected prior to or on the day of the kidney biopsy. The histological diagnoses included proliferative LN (Class 3 or 4) in 50% (41/ 76), and ISN/RPS Class 5 LN in 38% (29/76) of the patients. Epimembranous deposits (ISN/RPS Class 5 or together with Class 3 or 4 LN) were observed in 43% (33/76) of the biopsies.

As expected, histological features were seen often concomitantly in the same kidney biopsy specimen. Generally, features reflective of active inflammation were clustered as were those representing LN chronicity. For example, in kidney biopsies with capillary proliferation, there was often (85%) moderate mesangial proliferation. Among patients with tubular atrophy, 95% also had fibrotic changes of the renal tissue (Table 2).

Associations of Clinical and Laboratory Measures with Histological Features

The age of the patients was significantly associated with serum creatinine ($r = 0.27$; $p < 0.017$) and the BCI score ($r = -0.45$; $p < 0.0001$) but not with any of the UBM levels or other traditional measures of LN.

The concentrations of all the UBM were at least weakly correlated ($r \geq |0.2|$) with each other. An exception was TF which was strongly correlated with CP ($r = 0.74$; $p < 0.0001$) and AAG ($r = 0.61$; $p = 0.005$). Among traditional measures of LN, the only strong correlation ($r = 0.79$) was, as expected, between the GFR and serum creatinine levels. Notably, levels of C3, C4 and the P/C-ratio were unrelated ($r < |0.2|$).

This suggests that concentrations of the UBM do not simply increase in the urine due to increased proteinuria and supports the notion that the UBM provide additional information about LN over and above the traditional measures of LN.

Association of SLEDAI Renal Domain Score with LN histology

There were statistically significantly correlations of the SLEDAI-R scores with concentrations of NGAL ($r = -0.39$; $p < 0.0007$), MCP1 ($r = 0.23$; $p < 0.07$), CP ($r = 0.23$; $p < 0.05$), AAG ($r = 0.35$; $p < 0.003$) and L-PGDS ($r = 0.28$; $p < 0.016$), respectively. Serum creatinine ($r = 0.35$; $p < 0.002$), the P/C-ratio ($r = 0.40$; $p < 0.0004$), and C3 levels ($r = -0.34$; $p < 0.0043$) were also correlated with SLEDAI-R scores. Conversely, urine concentrations of TF, C4 and the GFR were unrelated to the SLEDAI-R scores ($r < |0.2|$). BAI scores were weakly correlated with the SLEDAI-R ($r = 0.29$; $p < 0.01$)

Relative changes of the biomarkers with histological features of LN activity

The relative excretion of the UBM and traditional measures of LN with histological features of LN is shown in Figure 1. There was a relative increased excretion of some UBM with mesangial proliferation (Panel A), capillary proliferation (Panel B), cellular crescents (Panel C), and fibrinoid necrosis (Panel D). There was a trend towards more pronounced relative increases of the UBM with the presence of wire-loops but none of these changes reached statistical significance (Figure 1, Panels E). With the exception of the P/C ratio, there were no statistically significant differences in the levels of traditional measure of LN with any of the histological measures of LN activity. The levels of MCP1, CP, TF, AAG, and the P/C ratio all discriminated between low versus high BAI scores (<7 vs. ≥ 7) (Figure 1, Panels F). Of note, urinary L-PGDS and NGAL were not differentially associated with any of the

histological features under consideration. The same was true of the levels of complement C3 and C4.

Relative changes of the laboratory measures with histological features of LN chronicity and epimembranous deposits

The levels of the UBM differed especially with features of active LN but not with features of LN chronicity (Figure 2, Panel A – C). Only the GFR and the serum creatinine levels importantly differed with LN chronicity features and distinguished between high vs. low BCI scores (4 vs. < 4).

None of the UBM or traditional measures of LN differed significantly with the presence vs. absence of epimembranous deposits. However, there was a trend towards the UBM showing larger relative differences in urinary excretion with the presence vs. absence of epimembranous deposits. Only the GFR differed with Class 5 as compared to Class 2 – 4 LN significantly [median (IQR) in ml/min/1.73m^2: 125 (54) vs. 85 (54)].

Univariate and multivariate logistic modeling to predict LN outcomes

In univariate logistic regression, we assessed the diagnostic accuracy (AUC) of each of the biomarkers for *key LN features*, i.e. BAI scores 7, BCI scores 4 and the presence of ISN/RPS Class 5 LN. Good to excellent accuracy to diagnose patients with high BAI-scores were present only for AAG, TF (both AUC = 0.76), CP (AUC = 0.79), MCP1 (AUC = 0.82), and the P/C ratio (AUC= 0.76). Excellent predictors of high BCI scores were the GFR and serum creatinine (both AUC = 0.82). Univariate analysis did not reveal a single UBM or traditional measure of LN that was at least a good diagnostic measure (AUC 0.7) of Class 5 LN. Lastly, the SLEDAI-R scores were a poor proxy measure of BAI scores (AUC = 0.5).

The results of multivariate modeling to predict key LN outcomes (high BAI score, high BCI score, Class 5 LN) were summarized in Table 3. A combination of four different biomarkers each was excellent for diagnosing high BAI scores (AUC = 0.85) as the combination of NGAL, GFR and MCP1 for diagnosing high BCI scores (AUC = 0.83). Combinations of five biomarkers yielded a good diagnostic test for ISN/RPS Class 5 LN (AUC = 0.75).

Exclusion of Urine Samples Collected after the Kidney Biopsy

Although we did not have access to recent changes of medications just prior or after the study visit, we hypothesized that LN therapy was intensified after the results of the kidney biopsy had become available. When only considering patients from whom urine samples were collected no later than the day of the kidney biopsy (n= 38), urinary NGAL concentrations were significantly lower with the presence of cellular crescents [median (IQR) in present vs. absent: 8.74 (44.9) vs. 48.5 (64.3); p< 0.0033], and LPGDS concentrations were significantly lower with the presence of tubular atrophy [median (interquartile range) in present vs. absent: 695 (591) vs. 928 (1021); p< 0.0234]. There were no significant relative changes between LN features and any of the other UBM or traditional measures of LN when only considering this subset of 38 patients. The patterns of relative biomarker excretion in relation to LN histology, including those observed in the subanalysis are summarized in Figure 2, **Panel F**.

DISCUSSION

We examined recently discovered UBM and traditional measures of LN and found especially individual UBM related to specific histological findings representing LN activity. The combination of MCP1, CP, AAG and the P/C was excellent in estimating histological LN activity. NGAL together with GFR and MCP1 were excellent diagnostic tests of LN

chronicity. Combinations of biomarkers provided good markers for the presence of Class 5 LN.

Our previous research supports that the UBM 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 where the UBM were associated with histological features of LN activity. Surprisingly, urinary NGAL and L-PGDS were not differentially associated with features of LN activity in our entire cohort of patients with urine samples collected within 2 months of a kidney biopsy.

We previously reported L-PGDS to be a biomarker of LN activity as measured, among others, by the SLEDAI-R (3). The significance of this protein for inflammatory processes with LN has been confirmed in animal studies (27). Our study found L-PGDS to be weakly associated with clinical measures of LN activity (SLEDAI-R), other UBM and traditional measures of LN but not with a specific histological 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 in histological standard stains of kidney biopsies. Alternatively, as is suggested by the findings of our subanalysis where L-PGDS excretion was 46% 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 which is rapidly induced by active inflammation with LN, and promptly declines with therapy (5). Thus, when we excluded patients whose urine sample was collected after the kidney biopsy, i.e. already on intensive treatment for LN, and instead considered only the remaining patients (n=38), we found NGAL to be much lower in patients with cellular crescents, an important histological feature of active proliferative LN.

The biologic function of NGAL is still under investigation (29). In the acute setting, the biological role of NGAL appears to be a protective anti-apoptotic 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 from structural changes typically associated with active LN. Besides its role in LN activity, NGAL is also associated with LN damage (6). The importance of NGAL as a biomarker of LN chronicity is supported by its role as a predictor of high BCI scores in this study. Urinary NGAL is also elevated in adults with chronic kidney disease, in whom NGAL is inversely correlated with GFR and positively correlated with tubular atrophy (31). The increased production of NGAL in this chronic context likely constitutes a pathophysiological pathway that leads to progressive renal failure (32).

MCP1 has long been known to be a predictive biomarker of LN flares and LN severity (4). Lupus-prone MRL-lpr/lpr and MCP1 knockout mice exhibit significantly lower proteinuria and prolonged survival (33), indicating a role for MCP1 in LN pathogenesis, in addition to its demonstrated capacity as a urinary biomarker for LN (4). The findings of our study are in line with these previous reports because MCP1 was differentially excreted with features of LN activity and high BAI scores. Multivariate models that predict the presence of membranous LN (ISN/RPS Class 5) included MCP1 as an important predictor, supporting previous observations in idiopathic membranous nephropathy of high urinary MCP1 and high expression of MCP1, especially in the tubular epithelial cells (34).

AAG was markedly increased in the urine of patients with mesangial proliferation and crescents. This finding is line with AAG being a known marker of LN activity whose urinary levels are also elevated with 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 the permeability towards macromolecules such as albumin (35).

We found TF to be associated with mesangial and capillary proliferation and cellular crescent formation, an observation that is congruent with previous reports from IgA nephropathy (36). Physiologically, recycled and absorbed iron is delivered to the main iron-transporting protein in blood, transferrin. 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 higher, especially with mesangial or capillary proliferation, crescent formation and fibrinoid necrosis. We reported CP, an oxidative stress-related protein, to be a biomarker of LN activity in the past (3). CP has been associated with tissue remodeling in the kidney after renal tubular injury as can be observed with LN (38).

Individually, neither the UBM nor the traditional measures of LN are suited to determine whether there are epimembranous deposits. Likely because the various histological features of LN often are seen together in the same histological specimen, the GFR was only found to importantly differ with the presence vs. absence of LN Class 5 but not of epimembranous deposits. We speculate that this is also the reason for the differences in trends of the other LN measures in patients with LN Class 5 compared to those patients whose kidney biopsies showed some epimembranous deposits but who did not have LN Class 5.

Although combinations of the biomarkers included in this study yielded excellent diagnostic tests for LN activity and chronicity, 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.

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. Nonetheless, the association of novel as well as traditional biomarkers of LN with specific histological features bears the expectation that accurate longitudinal non-invasive measurement of LN activity and chronicity is feasible. If confirmed this will allow for a more effective and personalized monitoring of LN and its therapy. The availability of standardized clinical platforms for the reliable measurement of the urinary biomarkers will enable the testing of this hypothesis in the near future (39).

Acknowledgments

LITERATURE

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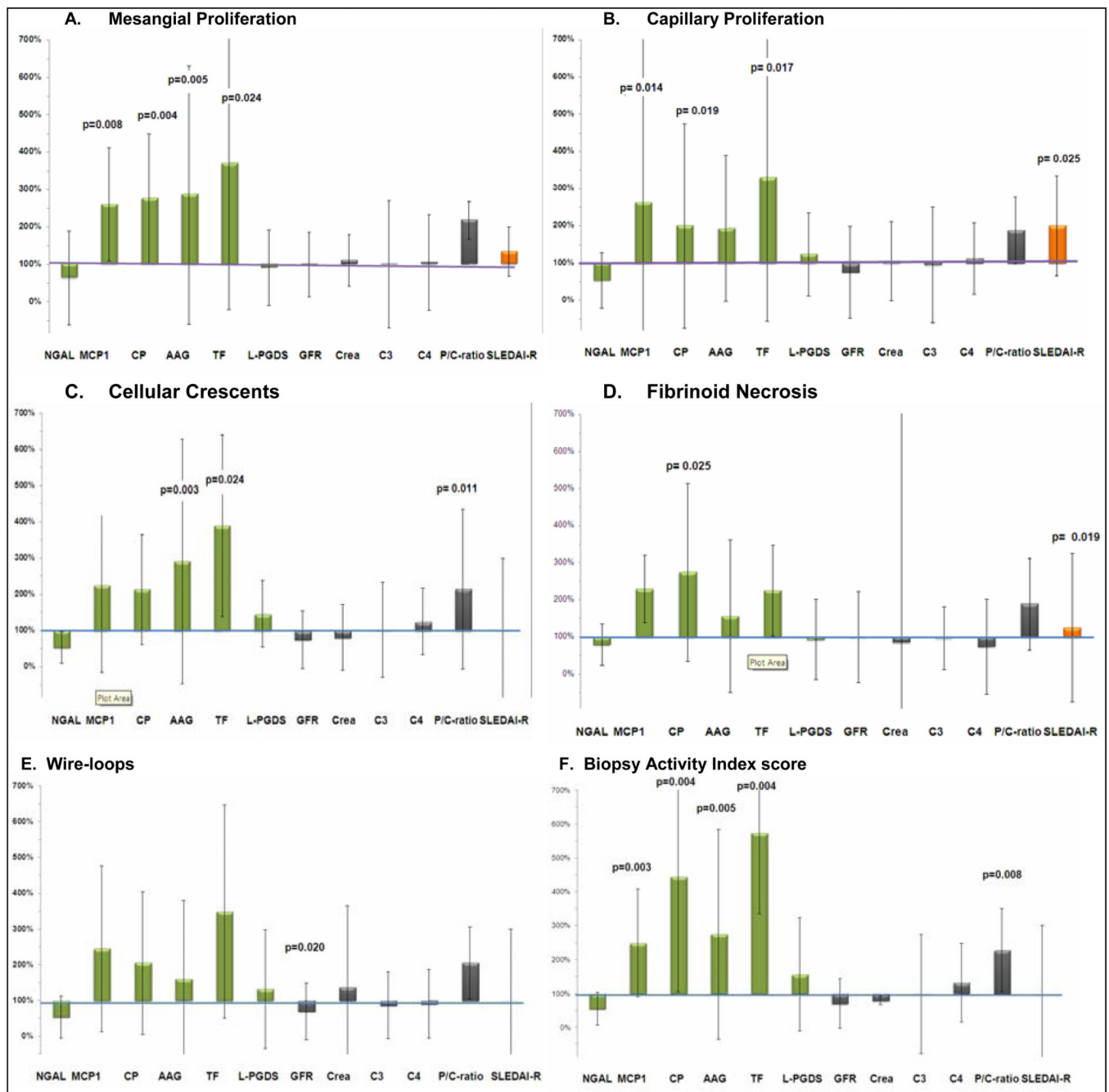
Figure 1.
For the 76 patients considered in the study, relative changes of the median levels with the presence vs. absence of specific histological features associated with LN activity are shown (whiskers are percentage change of the IQR) of the biomarkers: NGAL, MCP1, CP, AAG, TF, L-PGDS; serum creatinine, GFR; complement C3 and C4, P/C ratio; and clinical disease activity as measured by the SLEDAI-R score. _Y-axis:_ Values of 100% signify that there is no difference of the biomarker with the presence vs. absence of the histological feature under consideration, while values of < 100% (> 100%) imply that the levels of the biomarker decrease (increase) with the presence of the histological features.
Panel A: MCP1, CP, AAG and TF significantly increase with mesangial proliferation;

Panel B: MCP1, CP and TF significantly increase with capillary proliferation;

Panel C: AAG and TF and the P/C ratio increase with cellular crescents;

Panel D: CP and the SLEDAI-R significantly increase with fibrinoid necrosis;

Panel E: Especially AAG and TF increase with wire-loops but changes do not reach statistical significance at $p < 0.025$.

Panel F: MCP1, CP, AAP, TF and the P/C ratio significantly increase with high Biopsy Activity Index Scores (BAI).
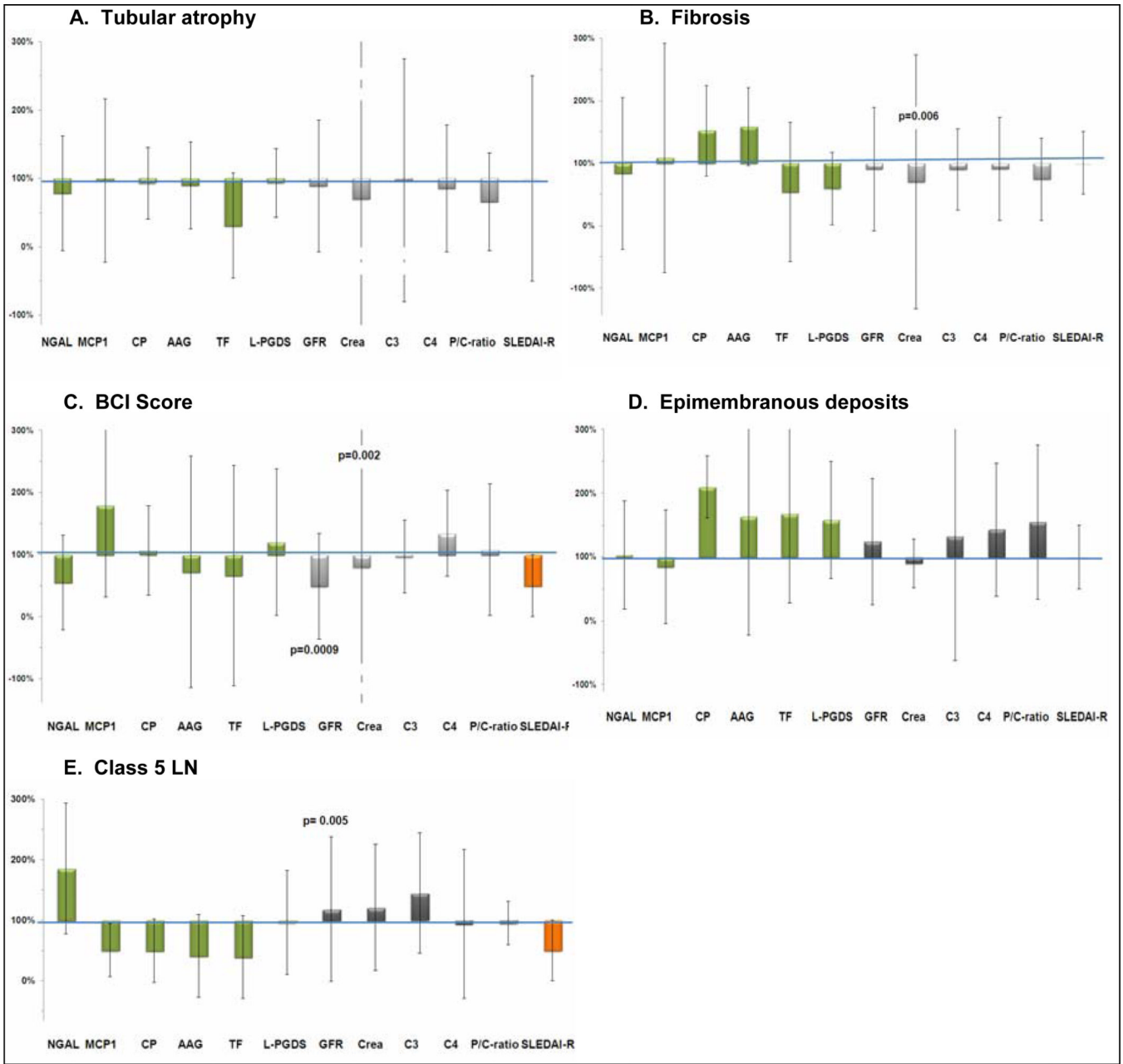
Figure 2.
In **Panels A – C,** relative changes of the median levels (whiskers are percentage change of the inter-quartile ranges) of the biomarkers with the presence versus absence of histological feature or scores associated with LN chronicity are shown. Details on the biomarkers and the interpretation of the values of the y-axis are provided in the legend of figure 1.

Panel A: None of the biomarkers changes significantly with tubular atrophy;

Panel B: Only the serum creatinine increases significantly with fibrosis;

Panel C: Only the serum creatinine and the GFR increase significantly with high Biopsy Chronicity Index Scores (BCI);

Panel D: None of the biomarkers changes significantly with of epimembranous deposits;

Panel E: Only the GFR increases significantly with ISN/RPS Class 5 LN

Biomarkers‡ / Histological features	NGAL	MCP1	CP	AAG	TF	L-PGDS	C3	C4	P/C-ratio	GFR	Serum creatinine
Mesangial proliferation		•	•	•	•						
Capillary proliferation		•	•		•						
Cellular crescents	•			•	•						
Fibrinoid necrosis			•								
Wire-loops										•	
BAI Score		•	•	•	•				•		
Fibrosis											•
Tubular atrophy						•					
BCI Score										•	•
Epimembranous deposits											
Class 5 lupus nephritis										•	

Figure 3.
A summary of significant changes with the presence vs. absence of histological features as are shown in Figure 1 and in Panel A – C of Figure 2. BLUE dots represent changes seen in urine samples that were collected within 2 months of the kidney biopsy (n=76), while GREEN dots represent additional significant differences if only urine samples collected prior to the kidney biopsy are considered (n= 38). The novel urine biomarkers are differentially excreted with histological changes of LN activity but not with membranous changes or LN chronicity. The GFR, serum creatinine and the P/C ratio do not allow for the differentiation between active, chronic or membranous changes of LN.

Table 1

Patient Demographics, medications and renal status at the time of the urine collection

		Number of patients with available information	n of N (%)	Median	Interquartile range
Disease-onset	Childhood-onset LN	76	28 (37%)		
	Adult-onset LN		48 (63%)		
Females			64 (84%)		
Race	Black	76	35 (46%)		
	White		33 (43%)		
	Other+		8 (11)[†]		
	Oral prednisone		73 (96%)		
	Pulse methylprednisolone		33 (43%)		
	Mycophenolate mofetil		23 (30%)		
Medications	Azathioprine	76	3 (4%)		
	Cyclophosphamide		13 (17%)		
	Methotrexate		4 (5%)		
	Angiotensin blocking agent		41 (54%)		
LN Status	GFR < 60 ml/min/m^2	76	14 (18%)		
	Protein:creatinine ratio > 0.5	76	68 (89%)		
	Renal SDI score > 0	22	3 (14%)		0 – 2
	Renal SLEDAI score	76		8	0 – 16
	Presence of double-stranded-dsDNA	65	49 (75%)		
Timing of urine collection	Time interval to biopsy [*]	76		+3.5 days [*]	−60 to +60
	>30 days before biopsy		5 (7%)		
	>30 days after biopsy	76	17 (22%)		
	ISN/RPS class[&] Class 2	76	6 (8%)		
	Class 3		12 (16%)		

	Number of patients with available information	n of N (%)	Median	Interquartile range
Class 4		29 (38%)		
Class 5		29 (38%)		
Mesangial expansion		73		
Capillary proliferation		38		
Cellular crescents		22		
Fibrinoid necrosis	76	22		
Wire -loops		21		
Fibrosis		53		
Tubular atrophy		58		
Epimembranous deposits		33		
BAI score‡	76		3	0 – 15
BCI scoreΔ			2	0 – 9

Histological Features present

*
Positive value indicates that the urine was collected after the kidney biopsy

†
American Indian 1; Asian 3; Mixed racial 5;

&
There were no patients with Class 1 or Class 6 LN

‡
Biopsy Activity Index; range 0 – 24; 0 = inactive LN

Δ
Biopsy Chronicity Index; range 0 – 12; 0 = LN without chronic changes

Table 2

Relationship between Histological Features on Kidney Biopsy[†]

Histological Features		N	Capillary proliferation	Cellular crescents	Fibrinoid necrosis	Wire-loops	Fibrosis	Tubular atrophy	Epimembranous deposits	BAI Score 7	BCI 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-value		<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-value			<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-value				<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-value					0.0005	-	-	0.02	-	-
Wire-loops	Yes	20					80%	65%	20%	60%	15%
	No	55					67%	80%	53%	0	15%
	p-value						-	-	0.02	0.0001	-
Fibrosis	Yes	53						95%	42%	17%	21%
	No	23						39%	48%	13%	-
	p-value							0.0001	-	-	-
Tubular atrophy	Yes	58							47%	12%	19%
	No	18							33%	28%	-
	p-value								-	-	-
Epimembra-nous deposits	Yes	33								9%	12%
	No	43								21%	16%
	p-value									-	-

Histological	Features	N	Capillary proliferation	Cellular crescents	Fibrinoid necrosis	Wire-loops	Fibrosis	Tubular atrophy	Epimembranous deposits	BAI Score 7	BCI Score 4
Biopsy Activity Score	7	12									17%
	<7	64									14%
	p-value										-

Table 3

Prediction of Key Biopsy Features with Lupus Nephritis

Model Outcome Variable	Predictor Variables	Area under the ROC Curve[‡] (95% confidence interval)	Sensitivity[*]	Specificity
Biopsy Activity Score 7	MCP1, CP, AAG, P/C ratio	0.85 (0.69–1.0)	72%	66%
Biopsy Chronicity Score 4	NGAL, GFR, MCP1	0.83 (0.67 – 0.93)	73%	67%
Membranous Lupus Nephritis (Class 5)	MCP1, GFR, AAG, TF, C4	0.75 (0.62–0.86)	75%	48%

[‡]The area under the ROC (receiver operating characteristic curve) ranges between 0 – 1

[*]Clinically relevant point on ROC with sensitivity of at least 70%