Biomarkers for Lupus Nephritis: The Quest Continues

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Current treatment of severe lupus nephritis is unsatisfactory in terms of both outcome and toxicity. To improve the efficacy and decrease the adverse effects of immunosuppression, it would be ideal to be able to predict the course and pathology of lupus nephritis and adjust therapy appropriately. This will require biomarkers that reflect disease activity. Recently, significant effort has been put into identifying biomarkers that can anticipate impending lupus renal flare, forecast development of chronic kidney disease, or reflect kidney histology at the time of flare. Although these biomarkers are potentially useful, to date none has been clinically validated in a large, prospective cohort of patients with SLE. This article reviews the current status of lupus nephritis biomarker investigation and articulates a perspective of how future efforts should be focused.


Despite aggressive immunosuppression, our ability to control severe SLE GN remains unsatisfactory. Treatment with intravenous or oral cyclophosphamide followed by maintenance immunosuppression with azathioprine or mycophenolate mofetil (MMF) yields complete and partial remissions in less than 50% of patients after 6 mo (1–5). Similar outcomes are seen when MMF is used for both induction and maintenance therapy (2). To make matters worse, these regimens are highly toxic and often poorly tolerated.

The ideal approach to improve the outcome of SLE nephritis is to develop new drugs that are more disease specific, better tolerated, and less toxic. Although this has been the goal of several recent large, randomized, prospective clinical trials, none of these trials was successful in demonstrating a better way to treat lupus nephritis. Thus, for the foreseeable future, currently available drugs need to be used more effectively. It is conceivable that complete remission rates could be improved, and less cytotoxic therapy would be needed if flares could be anticipated and preemptive treatment started. Additionally, modification of drug dose and duration of therapy based on the predicted outcome of a flare (e.g., development of chronic kidney disease [CKD]) would be expected to improve treatment efficacy and reduce toxicity. Finally, because kidney biopsies are not repeated at every flare, a noninvasive predictor of renal pathology would be very useful in choosing therapy. This approach to treatment represents a fundamental change from a reactive to a proactive paradigm and will require biomarkers that accurately predict SLE nephritis activity, pathology, and prognosis to guide therapeutic decisions.

Many lupus nephritis biomarker studies have used cross-sectional cohorts to identify novel markers of disease activity. However, several recent studies examined lupus biomarker expression prospectively, to identify biomarkers that can predict the future course of lupus nephritis. In an effort to optimize current and future therapies, it is relevant to review these studies and their clinical implications. For reference, the biomarkers discussed here are summarized in Table 1.

Biomarkers that Anticipate Impending Renal Flare

A biomarker that can forecast lupus nephritis flare well before thresholds of proteinuria, renal function, and urine sediment that signal clinical flare are reached would be a valuable tool. Such a predictive biomarker could be followed serially, and based on biomarker levels, treatment could be initiated before the development of significant inflammatory injury in the kidney. This is likely to result in more complete remissions and less chronic kidney damage (6,7) than waiting to treat until the flare is fully developed. Early treatment may also permit a decrease in the total exposure to immunosuppressive medications, thereby reducing treatment toxicity. The traditional clinical biomarkers for SLE, including complement components 3 and 4 (C3, C4) and anti-double-stranded DNA antibodies (ADNA) have low sensitivity (49 to 79%) and specificity (51 to 74%) for concurrent renal flare and do not reliably predict impending flare when measured serially, with sensitivities and specificities around 50 and 70%, respectively (8–14). In our SLE cohort, the positive predictive values for C3 and C4 to forecast impending flare were 7.4 and 5.5%, respectively (8). However, there are several potential candidate biomarkers that do seem to forecast flare:

Chemokines

Monocyte chemoattractant protein-1 (MCP-1) is a leukocyte chemotactic factor that is involved in the pathogenesis of renal injury in SLE (15). We postulated that because MCP-1 mediates renal inflammation in SLE nephritis, urine levels of MCP-1 (uMCP-1) may reflect nephritis activity. This was indeed the case; uMCP-1 was significantly greater in patients undergoing a renal flare than in healthy controls, patients with stable renal
Table 1. Summary of candidate biomarkers for SLE nephritis

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Biomarker type</th>
<th>Putative biomarker use</th>
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<tbody>
<tr>
<td>MCP-1</td>
<td>Urine protein/mRNA</td>
<td>Predict impending nephritis flare</td>
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<tr>
<td>NGAL</td>
<td>Urine protein</td>
<td>Predict impending nephritis flare</td>
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<tr>
<td>Transferrin</td>
<td>Urine protein</td>
<td>Predict impending nephritis flare</td>
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<tr>
<td>Hepcidin</td>
<td>Urine protein</td>
<td>Predict impending nephritis flare</td>
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<tr>
<td>Anti-C1Q</td>
<td>Serum protein</td>
<td>Predict impending nephritis flare</td>
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<tr>
<td>LFABP</td>
<td>Urine protein</td>
<td>Predict CKD</td>
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<tr>
<td>mEPCR</td>
<td>Urine mRNA</td>
<td>Predict CKD</td>
</tr>
<tr>
<td>FOXP3</td>
<td>Predict renal pathology</td>
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<tr>
<td>Glycoprotein panel</td>
<td>Urine protein</td>
<td>Predict renal pathology</td>
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<tr>
<td>CXCL10</td>
<td>Urine mRNA</td>
<td>Predict renal pathology</td>
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<tr>
<td>CD29</td>
<td>T cell surface marker</td>
<td>Predict renal pathology</td>
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Neutrophil Gelatinase-Associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a low-molecular-weight anti-bacterial protein that functions by binding bacterial siderophores and sequestering iron (26). Its role in human physiology or pathophysiology is not entirely clear, but it does seem to act as a growth and differentiation factor for epithelia, possibly through its ability to transport iron to and from cells. Interestingly, in acute kidney injury, NGAL mRNA expression increases significantly in the loops of Henle and collecting ducts, and NGAL levels increase in the urine well before serum creatinine rises. This observation has been exploited to develop a urine NGAL (uNGAL) assay for the early detection of ischemic kidney injury in children undergoing heart surgery (27). In experimental models of acute kidney injury (AKI), NGAL was shown to have a protective effect on the renal tubules, and therefore, it may function to attenuate or improve human AKI (26).

Because of these AKI data, NGAL was also tested as a candidate biomarker for SLE nephritis in both children and adults (28,29). In a pediatric cohort, uNGAL values above a threshold level had a sensitivity of 90% and a specificity of 100% for identifying patients with concurrent, biopsy-proven lupus nephritis. The area under the corresponding ROC curve was 0.94 (28). The diagnostic performance of uNGAL in an adult population was less impressive, with a sensitivity of 50%, a specificity of 91%, and an area under the ROC curve of 0.74, but this may be partly explained by the fact that some of the adult patients did not have urine samples before the kidney biopsy establishing lupus nephritis (29). NGAL was not associated with extrarenal disease activity.

More importantly, NGAL has been measured prospectively in a pediatric SLE cohort to determine whether levels change before clinical nephritis flare. This study showed that uNGAL increased significantly between 3 and 6 mo before lupus ne-
phritis worsened to the extent that a flare was declared (30). Using the British Isle Lupus Assessment Group instrument to measure renal lupus activity, uNGAL showed sensitivity and specificity of 82%, a positive predictive value of 61%, and a negative predictive value of 93% for predicting future renal flare. Plasma NGAL also increased before worsening of the kidney disease. Like uMCP-1, uNGAL is increased in several types of kidney injury and is thus not specific for lupus nephritis.

Transferrin, α1-Acid-Glycoprotein, Ceruloplasmin, and Lipocalin-Type Prostaglandin D-Synthetase

Application of the proteomic discovery technique surface-enhanced laser desorption/ionization time-of-flight mass spectrometry to the urine of pediatric SLE patients yielded a protein signature for active lupus nephritis that consisted of eight proteins/peptides ranging in molecular mass from 2.7 to 133 kD (31). Four of these were albumin fragments and the other four were transferrin (TF), α1-acid-glycoprotein (AGP), ceruloplasmin (CP), and lipocalin-type prostaglandin d-synthetase (L-PGDS) (32). These proteins were not increased in nonrenal SLE.

TF, AGP, CP, and L-PGDS were measured prospectively in children with SLE nephritis, and several were found to significantly increase 3 mo before renal flare was declared based on the Systemic Lupus Erythematosus Disease Activity Index. Of these candidate biomarkers, TF was most consistent with a forecaster of impending renal flare, in that it increased only in the group of patients who flared. AGP increased over time not only in patients who flared, but also in patients who had active but stable nephritis and in patients with improving nephritis, whereas L-PGDS increased in flaring patients and patients with active, stable nephritis. CP had no consistent relationship to the renal flare cycle. The predictive performance of this group of proteins as a panel was not analyzed in this study. Interestingly, TF regulates iron homeostasis and is itself regulated by cytokines thought to be involved in the pathogenesis of SLE nephritis (33–35).

Hepcidin

Using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry, we evaluated the urinary proteome of serial lupus urine samples at 2-mo intervals during renal flare cycles (36). This allowed comparison of urine protein expression before, during, and after flare. Differentially expressed proteins were considered potential biomarkers for specific phases of the flare cycle. Several biomarker candidates were identified, including the 20 and 25 amino acid isoforms of hepcidin. Of interest, urine hepcidin-20 increased significantly 4 mo before flare and within 4 mo of treatment returned to baseline (36). Hepcidin-25 showed a different pattern of expression in relation to flare cycle, decreasing at flare and returning to baseline as the flare resolved. Hepcidin is an iron regulatory hormone made mainly in the liver and seems to be involved in the anemia of chronic inflammation through its ability to inhibit iron release from macrophages and gut epithelium (37,38). With respect to SLE, hepcidin is regulated by IL-6 and TNF-α, cytokines involved in the pathogenesis of lupus nephritis (39–42). Furthermore, we found hepcidin expression by infiltrating renal interstitial leukocytes in lupus kidney biopsies (36).

Antibodies to Complement Component C1q

The serum level of antibodies to complement component C1q (anti-C1q) autoantibodies has been reported to be a biomarker for lupus nephritis. The clinical performance of anti-C1q for detecting renal flare was similar to that of C3, C4, and ADNA (43–47). Unlike C3 and C4, prospective studies found that anti-C1q levels began to increase 4 to 6 mo before renal flare (12,43) and became significantly different than baseline 2.3 mo before flare (12). Positive predictive values of 50 to 71% for impending flare were calculated.

Comments

Several points regarding these candidate flare forecasters should be considered. (1) All of the candidates have been derived from relatively small patient cohorts and need to be verified in large, independent populations. (2) None of the candidates seems to have sufficient sensitivity and specificity to be developed into a stand-alone clinical test. It is likely that these and/or other forecasters will need to be combined to create diagnostic biomarker panels. (3) In patients with SLE, the candidate biomarkers are specific for renal involvement; however, when considering all types of glomerular disease, none of the candidates are specific for lupus nephritis. This is not unexpected, because glomerulonephritides share common pathways of injury. Nonetheless, it will be desirable to identify biomarkers specific for lupus nephritis. If not, it will be important to take disease context into consideration when interpreting biomarker data. (4) Several candidates are involved in iron metabolism and homeostasis. This is intriguing and may provide new insight into the pathogenesis of lupus nephritis.

Biomarkers that Anticipate Development of CKD

Liver-Type Fatty Acid Binding Protein

Liver-type fatty acid binding protein (L-FABP) is made by human proximal tubular cells. Recent data from several small studies showed that measurement of urine L-FABP (uL-FABP) could predict progression of nondiabetic CKD (48–50). To determine whether uL-FABP is a potential forecaster of the development of CKD in patients with acute SLE nephritis, it was measured at the time of diagnostic kidney biopsy in 49 patients, who were divided into those who progressed to CKD and those who did not (51). Serum creatine in progressors increased from 1.95 ± 0.47 to 4.95 ± 1.58 mg/dl during a median follow-up of 25 mo, whereas it fell in nonprogressors from 1.3 ± 0.16 to 1.02 ± 0.08 mg/dl during a median follow-up of 17 mo. Progressors had a higher uL-FABP than nonprogressors (89.4 ± 26.7 versus 48.8 ± 13.3 ng/mg Cr). No patient with an uL-FABP ≤30 ng/mg Cr progressed. Thus, the uL-FABP level at the time of kidney biopsy may help forecast the development of CKD in
patients with SLE nephritis. The mechanistic relationship of L-FABP to CKD is not known; however, it is interesting to speculate that because L-FABP is produced by the proximal tubule, it may be a marker of how robust tubular cytokine production is in response to glomerular injury. Because tubules can produce factors that contribute to interstitial scarring, high responders may, over time tend to irreversibly damage the interstitium (leading to CKD), whereas low-moderate responders may experience resolution without significant interstitial fibrosis.

Membrane Endothelial Protein C Receptor
Membrane endothelial protein C receptor (mEPCR) is an integral endothelial membrane protein that facilitates the conversion of protein C to activated protein C and is considered anti-thrombotic and anti-inflammatory (52). The receptor may be shed in patients with active lupus (53). Unexpectedly, mEPCR was found to be upregulated in the renal peritubular capillaries of patients with lupus nephritis compared with peritubular capillaries from normal kidneys (54). Those patients whose biopsies showed that more than 25% of the cortical peritubular capillaries expressed mEPCR responded poorly to therapy at 6 and 12 mo and tended to develop CKD (54). Interestingly, mEPCR as a biomarker of response to treatment was independent of biopsy class, activity and chronicity indices, GFR, and tubulointerstitial damage (54). The functional role of mEPCR in the pathogenesis of lupus nephritis remains to be determined, but these data suggest that intrarenal endothelial activation may select for treatment-resistance.

Urine FOXP3 mRNA Expression
The forkhead transcription factor FOXP3 is important in the development of regulatory T cells (55) and seems to be low in T regulatory cells from patients with active lupus, possibly contributing to the functional defect observed in SLE T regulatory cells (56). FOXP3 mRNA was measured in urine sediment of patients with active lupus nephritis and, interestingly, was found to be significantly higher than in quiescent lupus nephritis or normal controls (57). Furthermore, the level of FOXP3 mRNA seemed to stratify patients into treatment responders and nonresponders. Patients who did not respond to therapy had significantly higher levels of urine FOXP3 mRNA than patients with a complete response (57). It should be pointed out that, in this study, response was examined 6 and 12 wk after initiation of therapy, which is a very short time frame for lupus nephritis to show resolution. Thus, FOXP3 mRNA as a marker of renal prognosis will require further study using a longer time course.

Biomarkers that Predict Renal Pathology
A noninvasive test that accurately reflects kidney histology would be useful when deciding on therapy because it will provide significantly more information on what is happening within the kidney at flare than proteinuria, urine sediment, and creatinine. Ideally, these histologic biomarkers would differentiate active inflammation, necrosis, and crescents from fibrosis and chronic changes or from an etiology other than lupus, such as nephrotoxic acute tubular necrosis. As discussed below, the current putative biomarkers of kidney pathology do not achieve this level of discrimination but instead reflect global pathology.

Urine Protein Signatures
A proteomic approach was used to identify a urine protein signature that could noninvasively distinguish lupus nephritis from other types of proteinuric kidney diseases such as diabetic nephropathy and FSGS (58) and among patients with SLE that could differentiate between classes of lupus nephritis (59). These studies postulated that, in different diseases, as well as different WHO classes of SLE nephritis, the pathologic alterations in glomerular basement membrane size and charge selectivity are disease and class specific. Therefore, the pattern of plasma proteins excreted into the urine should be disease and class specific. To differentiate between diseases with sensitivity and accuracy, a minimum of 50 urine proteins or protein isoforms had to be examined simultaneously. A single biomarker was not sufficient, similar to the situation with flare predictors. Multiple biomarkers were examined with an artificial neural network that was initially trained on a portion of the cohort (58). Using 120 proteins or protein isoforms, lupus nephritis was separated from FSGS, membranous nephropathy, and diabetic nephropathy with a sensitivity of 86% and a specificity of 89%. To differentiate between WHO nephritis classes in a cohort of patients with SLE, a panel of 10 proteins provided the most sensitivity for classification, again using an artificial neural network (59). The proteins in this panel were primarily plasma glycoproteins such as α-1 acid glycoprotein, α1 microglobulin, and zinc α-2 glycoprotein.

Chemokine CXCL10
CXCL10 is a chemokine that mediates Th-1 cell migration and is upregulated in human lupus (60–62). It was thus studied as a potential noninvasive biomarker for monitoring the pathology of lupus nephritis (20). Urine sediment mRNA for CXCL10 and its receptor CXCR3 was found to be higher 2 wk before renal biopsy in patients with class IV nephritis compared with patients with class II, III, and V nephritis. After treatment, CXCL10/CXCR3 urine mRNA was measured prospectively every month. In class IV patients who responded to treatment, chemokine mRNA levels declined, whereas the nonresponders showed a tendency for levels to increase. The nonresponders were re-biopsied, and all showed a worsening of their glomerulonephritis. ROC analysis was used to calculate the sensitivity and specificity of CXCL10 and CXCR3 mRNA to distinguish class IV from other lupus nephritis classes. CXCL10 had a sensitivity and specificity of 73 and 94% and CXCR3 of 65 and 83%, respectively (20). In contrast to patterns of plasma protein excretion, prediction of specific pathologic lesions will likely require biomarkers produced within the kidney during flare.

T Cell Signal Transduction Molecule β1 Integrin
T cell signal transduction molecule β1 integrin (CD29) is an adhesion molecule important for cell–cell and cell–matrix in-
cross-linking CD29 and CD3 on T cells can induce T cell proliferation and upregulation of cell surface CD40 ligand (63). Using T cells from patients with active SLE, stimulation of CD29 alone was sufficient to mediate these events, apparently through a focal adhesion kinase signaling cascade (63). This was related to significantly increased expression of CD29 on the lupus T cells. Importantly, increased expression of CD29 was mainly seen in patients with active class IV nephritis and not other types of SLE GN. This relationship needs to be tested prospectively in a larger population of lupus patients with and without renal involvement, but it is interesting to speculate that CD29 levels could be used to differentiate class IV nephritis from other types of glomerular injury or predict who is likely to develop class IV nephritis. If this relationship holds, it may also provide clues to the pathogenesis of proliferative lupus nephritis.

**Perspective**

It is clear from the preceding discussion that biomarker development for lupus nephritis is still in an early stage. The majority of studies have focused on identifying biomarkers that reflect concurrent disease activity. If disease activity is defined by standard methods, such as proteinuria, urine sediment, and serum creatinine, or instruments that depend on these variables such as British Isles Lupus Assessment Group or Systemic Lupus Erythematosus Disease Activity Index, the novel biomarkers can be no better than these measurements. To improve on standard methods, novel biomarkers must be judged against an activity measurement that is superior to the status quo, such as the kidney biopsy.

More importantly, these putative biomarkers are now being tested in prospective trials to determine whether they predict the course of lupus nephritis, such as impending flare, or future outcome. The clinical utility of such biomarkers will be very high with respect to therapeutic decision making. Biomarkers that reflect renal pathology will be crucial to the proper use of future therapies that are expected to be limited in their scope of immunosuppression.

Although the majority of biomarkers discussed here are urine biomarkers, some serum biomarkers seem to have clinical potential. Because no lupus nephritis biomarker has yet been validated, it is difficult to say whether urine or serum biomarkers will be more useful. However, urine biomarkers, insofar as they are produced in the kidney and may participate in the pathogenesis of renal injury, are particularly appealing as candidates, because they may also be therapeutic targets specific for lupus nephritis. This raises the desirable possibility of developing kidney-focused treatment without systemic morbidity.

The current challenge in biomarker development is the validation of any of the candidate biomarkers discussed here or future candidates. To date this has not been done. As opposed to biomarker discovery, which can be done in a relatively small cohort, validation will require prospective testing in a large population that is ethnically diverse. A scheme for biomarker identification and development is summarized in Figure 1. It is unlikely that a single biomarker will be sufficient for a clinical test, and therefore biomarker panels will need to be developed. Finally, biomarker panels for lupus nephritis may show overlap with other types of GN, necessitating careful, context-specific application.

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None.
References


