Published in final edited form as:

Rheum Dis Clin North Am. 2014 August ; 40(3): 537–ix. doi:10.1016/j.rdc.2014.04.004.

The Kidney Biopsy in Lupus Nephritis: Is it Still Relevant?

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Keywords

Lupus Nephritis; Kidney Biopsy; Systemic Lupus Erythematosus

Introduction

The percutaneous kidney biopsy was introduced in the 1940s and incorporated into clinical practice in the 1950s^{1, 2}. This procedure, along with advances in the histopathologic examination of kidney tissue such as immunofluorescence and electron microscopy, greatly enhanced our understanding of the pathogenesis of human glomerulonephritis. Perhaps more so than for any of the other glomerular diseases, biopsy findings have been used to classify and subgroup lupus nephritis (LN) in order to inform treatment decisions and predict prognosis. Several LN classification schemes have been applied clinically, the most recent being from collaboration between the International Society of Nephrology and the Renal Pathology Society in 2004³. In an effort to forecast kidney outcomes, pathologic findings in LN have also been combined into composite indices of active and chronic disease independent of LN class^{4, 5}.

Recently the role of the kidney biopsy in LN has been challenged on several fronts: i) The widespread clinical practice of using mycophenolate mofetil (MMF) for induction treatment of proliferative and membranous LN has led many to question the need for diagnostic biopsies to guide therapy. ii) Diagnostic biopsies are not predictive of treatment response or long-term kidney outcomes in LN, and the added value of serial biopsies remains unclear. iii) Non-invasive biomarkers of renal pathology are actively being sought to substitute for invasive kidney biopsies. iv) The kidney biopsy is still only analyzed histologically, while molecular diagnostics are being applied to biopsies of other diseases to personalize therapy.

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Disclosures: None

In light of these challenges, this review reexamines the kidney biopsy in LN in order to assess its ongoing relevance to disease management, and considers how molecular analyses can increase the information derived from the kidney biopsy.

When and How Often Does an LN Patient Need a Kidney Biopsy?

An evidence-based literature has developed around this question, but to date there is no consensus answer. In the following section, three possible responses will be considered.

1. A kidney biopsy should routinely be obtained to confirm the diagnosis of LN before treatment is started

This traditional approach is used when SLE patients develop clinical evidence that is consistent with renal involvement by lupus and that cannot be explained by other conditions. These clinical findings include hematuria, pyuria, red and white blood cell casts, declining kidney function, and/or proteinuria ⁶. Dysmorphic red blood cells, specifically acanthocytes (Fig. 1), indicate glomerular hematuria and are often seen in the urine sediment of LN patients with active nephritis. Red blood cell casts (Fig. 1) also indicate glomerular hematuria, but are found less commonly. Urine white blood cells and white blood cell casts, in the absence of kidney or urinary tract infection, are consistent with kidney inflammation due to LN. A kidney biopsy is generally not indicated for hematuria or pyuria alone, however patients who develop active urine sediment do require close follow-up for signs of worsening kidney injury such as proteinuria and an increase in serum creatinine.

Besides LN, the differential diagnosis of renal dysfunction in SLE patients includes tubular injury due to systemic infection or nephrotoxins. Lupus patients are susceptible to infection because they are routinely immunosuppressed, and often receive medications that are potentially nephrotoxic. Renal insufficiency due to LN is usually accompanied by proteinuria and/or hematuria.

The most common clinical finding that triggers a request for kidney biopsy in SLE patients is proteinuria of at least 500–1000 mg/d, in the presence or absence of renal insufficiency or active urine sediment. Although there is no set proteinuria threshold for biopsy, patients with proteinuria of 500mg/d or less can have severe LN ⁷. In a pilot study of 38 lupus patients undergoing a kidney biopsy for the first time, and who had glomerular hematuria with less than 500 mg/d proteinuria, 95% of the patients had Class III, IV or V LN, and only 5% had Class II ⁸. These data demonstrate the difficulty of estimating kidney involvement in SLE using clinical data without histologic examination of the kidney.

2. A kidney biopsy is not routinely needed before starting therapy for LN

This non-traditional approach is mainly a response to the overwhelming acceptance of mycophenolate mofetil (MMF) as first line therapy for all of the serious forms of LN (Class III, IV and V) ⁹, theoretically eliminating the need to differentiate between classes before starting therapy. Furthermore, it has been difficult to demonstrate that the pathologic findings at diagnostic kidney biopsy prognosticate how the kidney will do in the long-term or how it will respond to therapy in the short-term ^{10, 11}. It has been shown however that a kidney biopsy done after completion of induction therapy does provide prognostic

information on renal outcomes^{10, 11}. It may therefore be advantageous to hold off biopsy until induction treatment is finished.

In contrast, we suggest a diagnostic kidney biopsy prior to therapy *is necessary* for the following practical (1–3) and theoretical reasons (4): 1) Patients with SLE may also develop glomerular diseases that are not the classic immune complex-mediated glomerulonephritis that is defined as LN. Most commonly these are histologically similar to minimal change disease or focal segmental glomerulosclerosis, and have been termed lupus podocytopathies, but other pathologies can also be seen^{12–15}. The incidence of non-immune complex glomerulonephritis in SLE patients is hard to estimate, but was 5% in a series of over 200 SLE patients¹³. These glomerulopathies cannot be distinguished from LN clinically; a biopsy diagnosis is required. Furthermore, the treatment of non-immune complex nephritis is not necessarily the same as for LN. For example, the lupus podocytopathies often respond to short courses of corticosteroids alone, and do not need addition of a cytotoxic agent¹⁶. 2) Renal thrombotic microangiopathy due to antiphospholipid syndrome is found in about 30% of patients with lupus, and can occur alone or with classic immune-complex LN^{17–19}. Renal thrombotic microangiopathy cannot be diagnosed without a biopsy. It is an important finding because treatment is anticoagulation, and failure to treat may lead to insidious loss of kidney function despite adequately addressing immune-complex LN with immunosuppression²⁰. 3) As discussed previously, it is very difficult to predict the extent of renal histologic activity or chronicity using only clinical information such as serum creatinine, level of proteinuria, or urine analysis^{21, 22}. The balance between activity (glomerular neutrophils, necrosis, endocapillary hypercellularity, cellular crescents, interstitial inflammation) and chronicity (glomerulosclerosis, fibrous crescents, interstitial fibrosis, tubular atrophy) will dictate whether to immunosuppress or to use kidney protective therapies such as strict blood pressure control, sodium restriction, and inhibitors of the renin-angiotensin-aldosterone system²³. 4) Several novel biologics have been tested as therapies for LN and failed, and more are in development²⁴. A factor contributing to these failures may be the heterogeneity of LN. Success with the new, highly specific agents may be limited to certain subsets of LN patients, and the kidney biopsy will likely be required to identify responsive patients.

3. A diagnostic kidney biopsy and a follow-up biopsy during treatment should be routinely done in LN patients

The data on repeat biopsies for LN comes from studies that have been done for clinical indications, that is, for LN patients who did not respond to therapy as expected, and from protocol biopsy studies where the repeat biopsy was done after induction or maintenance therapy to determine the effect of treatment on kidney histology. These investigations have provided important information on the prognostic value of the kidney biopsy for long-term renal health, and a time-line of the renal histologic response to treatment.

Protocol biopsies performed after 6–9 months of induction therapy in adults and children have demonstrated that the second biopsy is more predictive of long-term patient and kidney outcomes than the initial biopsy^{10, 11, 25}. In adults, the findings at the 6 month biopsy that predicted a doubling of serum creatinine (a surrogate for end-stage kidney disease) after a

mean follow-up of 7.6 years were ongoing glomerular and interstitial inflammation, ongoing presence of glomerular capillary immune complexes, and the presence of macrophages in tubular lumens^{10, 25}. Interestingly, the extent of chronicity on the second biopsy did not predict long-term outcome. Other studies reported on the relationship between repeat kidney biopsies a year or more after completion of induction therapy and kidney outcomes 7–8 years later^{26, 27}. The activity index⁴ on the repeat biopsy, persistent glomerular and tubulointerstitial inflammation, and persistent or worsening of subendothelial immune complex deposits were predictive of poor long-term outcomes such as doubling of serum creatinine, renal impairment, or death.

One potential confounding issue in all of these studies is that treatment after the second biopsy was not standardized and/or not described. Therefore it is not possible to determine the impact of treatment decisions on long-term kidney outcomes, or how treatment affects the predictive value of these pathologic findings. Nonetheless, it is reassuring that different cohorts, undergoing second biopsies at different intervals, found similar pathologic predictors of renal deterioration.

Protocol repeat biopsy studies also demonstrate how the kidney responds to treatment. Second biopsies done directly after induction therapy with corticosteroids plus a cytotoxic agent or antimetabolite, generally show a decline in active lesions, but an increase in scarring lesions²⁸. Importantly, there is seldom complete histologic “remission” after induction therapy. However, some patients did not show active lesions after induction. This raises the intriguing question of whether they need further maintenance immunosuppression^{10, 28}. There are no data to answer this question, and focal active lesions could have been missed on the biopsy, but if studied prospectively and such patients did well without ongoing treatment, one would have a strong argument for performing a second biopsy after initial treatment to see who could immediately stop further toxic therapy.

Repeat biopsy studies where the second biopsy was done after several (1.5–4.7) years of treatment are even more informative than repeat biopsies done after induction^{26, 27, 29, 30}. After prolonged standard-of-care treatment, 30–77% of patients still had persistent proliferative or Class V LN. More concerning, 30–60% of patients with complete *clinical* renal response had persistent histologic activity, including glomerular and tubulointerstitial inflammation and subendothelial immune deposits²⁶. As discussed previously, these histologic findings at repeat biopsy have been associated with poor long-term kidney outcomes. These data reinforce the known discordance between clinical measures of remission and histologic remission, and raise the question of whether a repeat biopsy is needed to truly define LN remission. The concept of histologic remission is also applicable to patients who have been treated aggressively but have persistent proteinuria. A recent repeat biopsy investigation showed that several such patients had no residual histologic activity, and while not considered to be complete responders on clinical grounds, appeared to be complete responders histologically³¹.

Repeat biopsies for clinical indications have been done to assess LN flares or to determine why patients have not responded to treatment^{32–34}. Some investigators have suggested that the decision to repeat a biopsy at LN flare should be based on the LN class on the initial

biopsy³⁵. Class switch in LN is common^{22, 35}, but a class switch from a proliferative lesion to a pure non-proliferative class is less common³⁵. Thus patients who have a non-proliferative LN class at baseline biopsy will be more likely to benefit from a repeat biopsy. In general, most reports conclude that the information gathered from repeat biopsies in patients doing poorly has been useful in deciding further management.

Synthesizing all of these data, Figure 2 presents our suggested algorithm of when to do a kidney biopsy in patients with LN.

Biomarkers of Kidney Pathology

LN is so intrinsically heterogeneous it is difficult to adequately convey what is seen on kidney biopsy using only standard classification terminology. LN classes are divided into active, active plus chronic, and chronic, and lesions into global and segmental. Active raises a spectrum of lesions including endocapillary hypercellularity, subendothelial immune complexes, crescents and/or glomerular capillary necrosis. Likewise, chronic connotes glomerulosclerosis, fibrous crescents, interstitial fibrosis, and/or tubular atrophy. Thus a pathologic diagnosis of Class IV-G (A/C) must be accompanied by a description of what was seen under the microscope. Complicating the heterogeneity of LN is that the lesions evolve over time, classes change, and patients are not uniformly identified at a specific stage. Therefore, biomarkers that non-invasively identify the presence (or absence) and severity of specific pathologic lesions are likely to be more useful for prognostic and treatment decisions than biomarkers that identify a specific LN class. Such biomarkers would be applicable across LN classes and individual patients. Nonetheless, many investigators have focused on identifying biomarkers to non-invasively diagnose LN class (see Table 1).

For all the reasons outlined previously, lesion-specific biomarkers are not intended to preclude a diagnostic kidney biopsy when LN is first suspected. Rather, we envision lesion-specific biomarkers to be used to monitor changes in kidney injury in real-time, as a non-invasive equivalent of serial kidney biopsies. (Fig. 3). The prospective assessment of kidney histology during treatment would allow therapy to be modified to account for tissue response, facilitating personalized titration of immunosuppressive agents. Improved kidney outcomes and less therapeutic toxicity would be expected to result from such personalization. Furthermore, a biomarker-based treatment platform could help refine the definition of complete and partial renal response beyond the arbitrary levels of proteinuria and serum creatinine used currently (Fig. 3). Finally, it is conceivable that following pathology biomarkers prospectively after a renal response could provide an early warning of increasing kidney inflammation, allowing preemptive intervention to avoid a full-blown LN flare (Fig. 3).

Because the percutaneous needle biopsy is the gold standard for renal pathology, biomarkers of pathology will have to approach the same level of accuracy to be accepted clinically. To estimate accuracy, needle biopsies were done at the time of autopsy in patients with and without kidney disease (n=103)³⁶. Overall, the needle biopsy agreed with the autopsy findings 84% of the time in diffuse disease, but only 51% of the time in focal disease. With

respect to specific compartments, there was 96% agreement for glomerular lesions and 31–87.5% agreement for interstitial lesions. It is important to point out that only 7 patients in this autopsy study had a glomerular disease, and none of these were focal lesions. Because accuracy diminishes with focal disease, glomerular accuracy may be less than 96% for LN. Other investigators estimated biopsy accuracy as the theoretical misclassification rate assuming a binomial distribution of abnormal glomeruli in the kidneys³⁷. This calculated misclassification rate was 25% with 10 glomeruli and 15% with 20 glomeruli, but does not account for interstitial lesions. In fact, the misclassification rate of chronic allograft nephropathy, a tubulointerstitial disease found in transplanted kidneys, was not influenced by number of glomeruli, and was 12% in one study³⁸.

In summary, a misclassification rate of 10–20% may be acceptable for biomarkers of kidney pathology in LN, especially when considering focal lesions and interstitial disease. It is conceivable that urine-based biomarkers are more accurate than needle biopsies, as urine analytes presumably represent lesions throughout the kidneys and are therefore not subject to sampling error or inadequate sample size. This will be difficult to prove however, as biomarkers can only be directly compared to needle biopsies.

A representative selection of putative LN kidney pathology biomarkers reported to date is illustrated in Table 1. These include biomarkers that differentiate proliferative and non-proliferative LN, biomarkers that differentiate between global levels of activity and chronicity in the kidney, and biomarkers that identify the presence, absence or severity of specific pathologic lesions. Most of these biomarkers are serum or urine proteins, although one study employed urine metabolomic profiling to differentiate between pure Class V and proliferative LN³⁹, while another study used urine T cell counts to determine the presence of proliferative LN. Biomarkers can be single analytes or a panel of analytes, although several investigations suggest that accuracy improves when analyte panels are used^{40–44}. Some of the panels include non-specific indicators of kidney injury such as serum creatinine, GFR, and proteinuria/albuminuria. Novel biomarkers are expected to bring added-value to these clinical measurements, and such combinations may yield robust composite biomarkers. Additionally, in multi-biomarker panels, individual biomarkers most likely contribute differentially to the composite biomarker. We have used linear discriminant analysis to weight and combine individual biomarkers that are biologically plausible or that have been identified as relevant to LN using unbiased screening techniques such as urine proteomics⁴¹. In this approach, the discriminant function is the composite biomarker and takes the form of an equation in which urine and serum analyte measurements are input, and the resulting calculated value differentiates between levels of severity of specific histologic findings in LN kidneys⁴¹. Such composite biomarkers can be readily adapted to the clinical laboratory.

The clinical performance metrics of sensitivity, specificity, and area under the receiver-operating characteristic curve are encouraging for several of the pathology markers (Table 1). The positive predictive values of the biomarkers are variable but generally low, while the negative predictive values are uniformly very good, suggesting these biomarkers may be most useful for excluding a particular pathology. Misclassification rate is a key performance

metric for novel biomarkers, and for these markers it ranges from 0–49%. However, several markers have an accuracy of 80–90% in relationship to the needle kidney biopsy^{39, 41, 45}.

The pathology biomarkers presented in Table 1 appear to be diverse and unrelated, but several are mediators of inflammation. It is informative to highlight a few of these proposed markers. The first component of complement, C1q, and antibodies to C1q are heavily represented in Table 1. The complement system plays a key role in the pathogenesis of SLE, and virtually all patients with complete C1q deficiency develop lupus or a lupus-like disease, and often severe LN^{46, 47}. This may be due to loss of the protective effect of C1q in mediating clearance of apoptotic debris and immune complexes⁴⁷. Most SLE patients do not have C1q deficiency however, and although the complement system can be protective, it is also pro-inflammatory. C1q is almost always present in the glomeruli of patients with active LN⁴⁸. Glomerular C1q–anti-C1q immune complexes may focus complement activation to glomeruli, amplifying kidney inflammation and injury⁴⁹. This is consistent with C1q consumption and C1q autoantibodies as surrogate markers for proliferative (inflammatory) LN classes and inflammatory glomerular lesions (crescents, necrosis), and the negative correlation of anti-C1q with chronic lesions (glomerulosclerosis, interstitial fibrosis)^{42, 45, 50}.

Monocyte chemotactic protein-1 (MCP-1) is a proinflammatory chemokine that has been shown to recruit inflammatory cells to the kidney in several types of nephritis including LN^{51, 52}. Blocking the activity of MCP-1 in animal models of LN ameliorates kidney injury⁵³. In humans, MCP-1 is a specific biomarker of LN flare⁵⁴. It is therefore not unexpected that MCP-1 may be a marker of inflammatory pathology in LN^{40, 41}. It may be surprising that MCP-1 also contributes to a panel of biomarkers that is a surrogate for chronic pathology⁴⁰, but macrophage infiltration into the kidney is necessary for generation of fibrosis. Additionally, MCP-1 stimulates fibroblast and monocyte collagen production, and monocyte expression of the pro-fibrotic cytokine transforming growth factor- β 1^{55, 56}.

Neutrophil gelatinase-associated lipocalin (NGAL) is a small, glycosylated protein that acts as an epithelial growth and differentiation factor and belongs to a family of proteins that are involved in cellular iron transport⁵⁷. Interestingly, other iron regulatory proteins such as transferrin and hepcidin have been identified as potential biomarkers of LN activity, raising the possibility that iron availability may be involved in the pathogenesis of kidney injury in LN, perhaps through redox signaling⁵⁸. NGAL is minimally expressed in healthy kidneys, but in acute kidney injury due to ischemia or inflammation it increases in segments of the distal nephron⁵⁹. In a longitudinal pediatric LN cohort, urine NGAL levels increased 3–6 months before LN flare, suggesting it may forecast flare⁶⁰. Presently it is not clear if NGAL is simply a marker of tubular injury in LN and other kidney diseases, or is directly involved in tissue injury. There are some animal data suggesting NGAL may even be tubuloprotective⁵⁷. Nonetheless, NGAL appears to be mainly associated with acute processes, which seems inconsistent with NGAL being on a biomarker panel for biopsy chronicity⁴⁰. However, the primary data show NGAL decreases in the chronicity panel⁴⁰.

Although many of the biomarkers listed in Table 1 appear promising, none are ready for clinical practice. Most were identified in small cross-sectional cohorts and have not yet been

verified in independent patient populations. After verifying reproducibility, it would be ideal to clinically validate pathology biomarkers by testing how well they prospectively track changes in kidney histology over time. The most direct way to do this would be with serial kidney biopsies, but such a study would be excessively invasive. An alternative trial design for clinical validation is shown in Figure 4. This design tests whether altering duration and intensity of standard induction and maintenance medications provides an advantage over usual management in terms of complete response rate, LN flare rate, safety, and cumulative drug exposure.

Molecular Diagnosis: The Future of the Percutaneous Kidney Biopsy in LN

As discussed previously, LN is intrinsically heterogeneous. Currently all patients diagnosed with a particular class of LN tend to be treated in a similar fashion. It is conceivable that therapy would be more effective and less toxic if it specifically targeted the pathogenic mechanisms actively involved in kidney injury at the time of diagnosis. This is especially relevant for novel therapies being trialed or developed for LN. These new drugs generally work on limited areas of the immune system, so understanding the mechanisms at play in an individual LN patient should facilitate appropriate matching of novel therapies to patients likely to derive benefit, improving outcomes, limiting side effects and containing costs. Integration of molecular and histologic evaluation of the kidney is feasible by applying “omic” techniques to kidney biopsies in LN.

Molecular analysis of the kidney presents several challenges. Clinical kidney biopsies are small, providing little tissue for evaluation, and are often formalin-fixed and paraffin embedded. Recently, protocols have been developed to facilitate transcriptomic and proteomic analysis of the tissue left over from clinical kidney biopsies^{61–63}. The architecture of the kidney adds to the complexity of molecular analysis. The kidney is divided into glomerular and tubulointerstitial compartments, and there are several distinct cell types within each compartment, including infiltrating inflammatory cells in LN. The expression profile of inflammatory mediators may differ between compartments. For example, glomerular T cells from Class III and V LN biopsies expressed Th1, Th2 and Th17 cytokines, whereas isolated interstitial T cells expressed mainly Th2 and Th17 cytokines⁶⁴. Laser-capture microdissection (LCM) of specific renal lesions from specific renal compartments, followed by RNA or protein analysis offers an approach to identify differentially-expressed transcripts or proteins that characterize the pathology and pathogenesis of LN lesions.

In an early application of these techniques, the molecular heterogeneity among LN patients was convincingly demonstrated. Glomeruli from kidney biopsies were isolated by LCM and then analyzed by cDNA microarray for transcriptional phenotyping⁶⁵. Compared to normal kidney, four distinct transcriptional patterns emerged, including increased expression of B-cell genes, myelomonocytic (macrophages and dendritic cells) genes, type I interferon-inducible genes and fibrosis-related genes. The level of transcript expression varied considerably in glomeruli from different patients, but within a kidney transcript expression was surprisingly consistent between glomeruli despite histologic variability. For example, individual biopsies could be segregated into two broad groups based on the type I interferon-

inducible and fibrosis-related gene clusters. Biopsies with high levels of interferon-inducible genes had low levels of fibrosis genes, whereas biopsies with high levels of fibrosis genes had low levels of type I interferon-inducible genes. While only some kidneys showed increased glomerular expression of fibrosis-related genes, the expression of such genes was similar in glomeruli from the same kidney, whether or not they had histopathologic evidence of glomerulosclerosis. These findings highlight the difficulty in personalizing LN treatment using only histologic information.

As discussed previously, diagnostic biopsies are not good predictors of future kidney outcomes in LN. Examination of biopsy gene expression patterns may increase its predictive value. Diagnostic biopsies from Class III and IV LN were segregated by renal response status an average of 11 months after standard induction and maintenance therapies (cyclophosphamide and MMF; Parikh, Rovin et al. *Molecular Characterization of Renal Responses in Lupus Nephritis using Serial Kidney Biopsies*, *Poster American Society of Nephrology* 2013). The expression of 511 immune response genes was analyzed directly from the kidney biopsy cores by Nanostring technology⁶⁶. Using principle component analysis, the samples clustered by whether the patients were complete, partial or non-responders (Fig. 5). These results suggest that gene expression analysis of the diagnostic kidney biopsy may identify patients who are likely to respond to conventional treatment before therapy is started. Alternate therapy may then be considered for those who have an “unfavorable” transcript response profile.

In addition to profiling differential gene expression, recent work showed that clinical kidney biopsies could also be analyzed for differential protein expression using unbiased proteomic techniques. Glomeruli and renal interstitium were isolated by LCM and proteins extracted from these two compartments were identified by mass spectrometry⁶³. A number of differences between normal and LN kidneys were observed. Not unexpectedly, Class IV glomeruli showed increased levels of complement proteins and decreased levels of anti-oxidant and podocyte proteins. Importantly, tissue proteomics showed an increase in the protein products of interferon- α -inducible genes, consistent with the lupus interferon- α signature demonstrated by transcript analysis⁶³. Transcript and protein analyses of kidney biopsies may thus be considered complimentary, especially when it comes to designing novel drugs for LN. Expression studies focus attention on specific pathways for intervention, and differentially-expressed proteins within these pathways may be particularly good therapeutic targets.

The molecular analysis of LN biopsies has and will continue to generate large amounts of data. In an effort to organize these data in a mechanistically and therapeutically useful way, disparate and large datasets are now being analyzed using a systems biology approach. The objective of systems biology is to integrate complex biological information to acquire a comprehensive description of the molecular events that lead to organ dysfunction⁶⁷. With respect to LN, clinical information and molecular data from kidney biopsies can be combined to identify relevant pathogenic networks in LN.

To exemplify the potential of a systems approach, transcriptional networks were compared in murine and human LN⁶⁸. Murine models have provided significant insight into the

pathogenesis of LN but do not entirely recapitulate the events that occur in human LN. This may explain the failure of murine therapies to translate to human LN. Using LCM, tubulointerstitial regions were isolated from human LN kidney biopsies and their transcriptional profiles determined. Using sophisticated bioinformatic techniques, these were compared to the transcriptional profiles of whole kidneys from three different murine models of LN. The investigators identified 20 transcriptional networks that were similarly activated (or repressed) in human LN and all of the LN mouse models⁶⁸. These networks included, but were not limited to, antigen presentation pathways, dendritic cell maturation pathways, and IL-17, MAP kinase and interferon signaling pathways. Developing and testing novel LN therapies in the mouse based on these common pathways may increase the likelihood that the drugs will be effective for human LN.

Conclusion

The available evidence suggests the percutaneous kidney biopsy is still relevant to the clinical management of LN. However, it is also clear that considerably more information resides within kidney biopsies than that from histopathology alone. This information can be released by molecular analysis of biopsies. Molecular analysis is feasible, and promises to help personalize therapy and enhance drug discovery for LN.

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Key Points

1. The kidney biopsy is the standard of care for diagnosis of lupus nephritis and remains necessary to ensure accurate diagnosis and guide treatment
2. Repeat biopsy should be considered when therapy modifications are necessary as in cases with incomplete or no response, or when stopping therapy for those in remission.
3. There are several promising biomarkers of kidney pathology however these markers needed to be validated in a prospective clinical trial prior to being applied clinically.
4. Molecular analysis may provide the information presently lacking from current evaluation of kidney pathology and hope to better inform on prognosis and treatment considerations.

Figure 1.

Urine findings of glomerular hematuria in lupus nephritis. A. Acanthocytes are a type of dysmorphic red blood cell that is specific for glomerular hematuria. The arrow indicates a bleb that distorts the normal biconcave disc appearance of the red blood cell. B. Red blood cell casts also indicate glomerular bleeding.

Figure 2.

Algorithm for kidney biopsy in lupus nephritis. A diagnostic kidney biopsy should be done to guide therapy when a lupus patient presents with clinical evidence of new kidney injury. A repeat biopsy should be done to confirm complete histologic remission in patients who have achieved complete clinical renal response so maintenance immunosuppression may be stopped. A repeat biopsy should be done to guide changes in therapy for patients who have incompletely responded. A repeat kidney biopsy should be considered at LN flare if there is suspicion that histology changed and therapy may need to be modified.

Figure 3.

Application of biomarkers of kidney pathology in lupus nephritis. The rationale for identifying biomarkers of pathology and how these biomarkers are expected to improve the management of LN are shown. In this schema the biomarkers of kidney pathology reflect the presence and severity of specific lesions such as glomerular crescents, interstitial inflammation or interstitial fibrosis. Lesion-specific biomarkers measured serially and frequently would provide an understanding of how kidney injury evolves in real-time during and after LN treatment.

Figure 4.

Clinical validation of kidney pathology biomarkers. Patients with biopsy-proven LN are randomized to a usual-care treatment strategy or a biomarker-based treatment strategy. The usual-care strategy will follow the induction and maintenance recommendations of the 2013 ACR treatment guidelines. The biomarker-based arm will begin treatment in the same way, but duration and intensity of treatment will be modified monthly based how biomarkers of active pathologic lesions are responding. For example, if biomarkers are normalizing, induction could be shortened and maintenance started early; if lesions are not responding well, induction could be lengthened. Maintenance could be discontinued if the biomarkers indicated a complete histologic remission. All patients would be followed for 12 months and at that time outcomes between the arms would be compared. The outcomes to be measured are indicated in the figure. Complete renal response (CRR) would be adjudicated clinically on the basis of kidney function and proteinuria, as is currently done, and also compared to complete histologic response.

Figure 5.

Principle component analysis of kidney biopsy transcriptional profiles. RNA was harvested from archived kidney biopsies performed at LN flare, and for which kidney outcomes after treatment were known. Pre-implantation living transplant donor biopsies were considered normal. Immune gene expression was measured by Nanostring technology. Principle component analysis (PCA) was done on the RNA expression data. PCA shows that at renal flare patients clustered by the type of renal response they had long-term. CR = complete renal responder; PR = partial renal responder; NR = non-responder.

Table 1

Biomarkers of Kidney Pathology in Lupus Nephritis

Biomarker	N	Histologic Lesion	R ²	AUC	Sens (%)	Spec (%)	PPV/NPV (%)	Reference
Panel: Serum C1q, albuminuria	18	Presence of ongoing proliferative LN after induction	-	-	100	100	100	42
Urine CD4+ cells	147	Presence of proliferative LN		0.99	100	98	-	50
Urine taurine Urine citrate	14	Presence of Class V LN		1 0.88	100 86	100 100	-	39
Panel: Urine MCP-1, ceruloplasmin, α 1-acid glycoprotein	76	Presence of high activity		0.85	72	60	-	40
Panel: Urine NGAL, MCP-1 and GFR	76	Presence of high chronicity		0.83	73	67	-	40
Anti-C1q autoantibodies	52	Correlates with Glomerular: Endocapillary proliferation inflammation Necrosis	0.3 0.42 0.3	-	-	-	-	50
Anti-C1q autoantibodies	52	Correlates with: Glomerular sclerosis Interstitial fibrosis	-0.32 -0.39					50
Anti-C1q autoantibodies	63	Presence of glomerular necrosis		-	100	31	37/ 100	45

Biomarker	N	Histologic Lesion	R ^I	AUC	Sens (%)	Spec (%)	PPV/ NPV (%)	Reference
Anti-C1q autoantibodies	63	Presence of glomerular crescents		-	92	31	45/ 86	45
Panel: Urine MCP-1, serum creatinine	61	Differentiates between levels of interstitial inflammation		0.92	100	83	68/ 100	41

^I Abbreviations: R, correlation coefficient; AUC, area under the receiver-operating characteristic curve; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; C1q, fragment of the first component of complement; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; GFR, glomerular filtration rate