HHS Public Access

Author manuscript

Rheum Dis Clin North Am. Author manuscript; available in PMC 2016 August 11.

Published in final edited form as:

Rheum Dis Clin North Am. 2013 November ; 39(4): 833–853. doi:10.1016/j.rdc.2013.05.001.

Biomarkers and Updates on Pediatrics Lupus Nephritis

Michael Bennett, PhD[a] and **Hermine I. Brunner, MD, MSc**[b,*]

[a]Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, University of Cincinnati, MC 7022, 3333 Burnet Avenue, Cincinnati, OH 45229, USA

[b]Division of Rheumatology, Cincinnati Children's Hospital Medical Center, University of Cincinnati, MC 4010, 3333 Burnet Avenue, Cincinnati, OH 45229, USA

Keywords

Biomarkers; Lupus nephritis; Systemic lupus erythematosus; Childhood-onset SLE; Treatment

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multiorgan autoimmune disease with increasing mortality that often targets young women and children of United States minorities. Childhood-onset SLE (cSLE)[1] has manifestations similar to those of SLE in adults, but earlier disease onset is accompanied by more severe multiorgan involvement, including lupus nephritis (LN) in up to 80% of pediatric patients. Treatment of LN in children continues to lack support from large randomized clinical trials. Instead, medication regimens for pediatric LN are deduced from studies in adult SLE and pediatric solid-organ transplants, or are based on consensus reached by associations of health care providers.

The criterion standard for the diagnosis and monitoring of LN remains histologic evidence from a kidney biopsy. Conversely, to reduce cost and avoid invasive procedures, monitoring of LN in clinics is achieved by measures that consider changes in certain blood and urine tests. Because such traditional testing for LN has limited responsiveness to change, it is ill suited to capture worsening or improvement of LN in a timely manner. Recently, promising LN biomarkers have been discovered that accurately reflect LN activity and chronicity as seen on kidney biopsy, and can forecast LN flares. In the future, such biomarkers are expected to facilitate the monitoring of LN in daily clinical care and the conduct of research studies in support of evidence-based therapies for LN in children.

EPIDEMIOLOGY, COURSE, AND ECONOMIC IMPACT

Given the phenotypic differences of cSLE around the world, the prevalence of kidney involvement with cSLE likely also varies with racial background and environmental exposures. The incidence of SLE is thought to have increased 10-fold during the preceding 50 years in industrialized Western countries,[2] which could indicate that cSLE in general, and

*Corresponding author. Hermine.brunner@cchmc.org.

Conflict of Interest: Nil.

LN in children in particular, also are becoming more frequent. Using information available in administrative databases and an algorithm that correctly identifies 80% of LN cases, Hiraki and colleagues[3] report that in the United States 37% of children with cSLE and who are enrolled in Medicaid have renal disease. Based on this study, the risk of developing LN is independent of gender but is higher among teens than younger children. Compared with Caucasians, Asians have almost 5 times and African Americans a nearly 3 times higher risks of developing LN. Overall, the annual incidence of LN is 0.72 cases per 100,000 children in the United States. This figure may be a conservative estimate of the frequency of LN, as higher estimates are reported by population-based studies from tertiary pediatric rheumatology centers and a recent meta-analysis.[4,5]

Recent 5-year renal survival rates in children with cSLE have ranged from 77% to 93%,[6–8] with marked improvement over the preceding decades.[9] Nonetheless, adults with LN have an 8-times higher mortality and children with LN a 19-times higher risk of dying compared with age-matched general populations.[6,7,10] The poor prognosis of children with end-stage renal disease from LN is particularly troublesome. There is 22% mortality during the 5-year period since the initiation of renal replacement therapy, with cardiopulmonary compromise and infections accounting for 47% of all causes of death.[6]

Associated with the higher mortality is the need of more intensive therapy for LN in children. Among the almost 7400 cSLE-related hospitalizations in the United States in 2006, 57% noted the presence of LN[11] with an average charge of $43,100 per admission.[11] Based on this and an earlier study, LN accounts for 11% to 28% of cSLE-associated medical costs in the United States.[12] Taken together, the cost of therapy for LN in children likely exceeds $350 million annually in the United States.[3,11–13]

DIAGNOSIS OF LN AND CLASSIFICATION

Kidney biopsies are required to establish the diagnosis of LN. Despite considerable variation in practice, there is consensus that reproducible daily proteinuria of at least 0.5 g, especially in the setting of an active urinary sediment, warrants a kidney biopsy in a child with cSLE who has not yet been diagnosed with LN.[14,15] Although clinically relevant biopsy findings are more common in the presence of significant proteinuria, the current approach results in at least 50% of newly diagnosed patients already being found to have proliferative LN, rendering them at a higher risk of end-stage renal disease.[16–19] A lower threshold for performing a kidney biopsy arguably is warranted in cSLE patients, including those with persistent isolated glomerular hematuria and new-onset low-grade proteinuria.

When interpreting a kidney biopsy specimen it is important to ensure that an adequate sample with sufficient numbers of glomeruli is available, namely, a minimum of 8 glomeruli that can be examined under light microscopy.[20,21] The International Society of Nephrology/Renal Pathology Society (ISN/RPS) Classification replaced in 2004 the previously used World Health Organization (WHO) Classification for LN.[20] The ISN/RPS Classification is based on light microscopy, rather than electron microscopy, as a tool for interpreting LN histology, even though it has been shown that electron microscopy greatly enhances the interpretation and classification of kidney biopsies.[20]

The ISN/RPS Classification was introduced to standardize and clarify the interpretation of LN histology findings.[20] Six classes of LN are described with focus on changes concerning the renal glomeruli, and the National Institutes of Health (NIH) Histology Score is often used to quantify the degree of LN activity and chronicity (Table 1).[22] The maximum score of the NIH-AI (activity index) and the NIH-CI (chronicity index) is 24 and 12, respectively, because scores from "(fibro)cellular crescents" and "fibrinoid necrosis/karyorrhexis" are given a weight of 2 in the NIH-AI (see Table 1). Pathologic changes of the kidney interstitium, are not well considered in the ISN/RPS Classification, although they are considered critical for the course of LN.[23] However, it is recommended to report the extent, severity, and type of tubulointerstitial (tubular atrophy, interstitial inflammation, and fibrosis) and vascular disease (vascular deposits, thrombi, vasculitis, sclerosis).[20]

RISK FACTORS TO POOR OUTCOME OF LN

Clinical research has identified, albeit inconsistently, several risk factors for poor LN outcome[3,6,16,24–33]; these include male gender, non-Caucasian race, nonadherence to treatment, presence of antiphospholipid or anti-dsDNA antibodies, persistent hypocomplementemia or proteinuria, nephrotic syndrome at presentation, failure to adequately respond to therapy by 3 months,[34] flare of LN,[35] or diagnosis with proliferative LN, especially in the setting of a high degree of histologic activity and damage. Given the multitude of the proposed risk factors for LN, close monitoring of any child patient with LN seems to be warranted in achieving the best possible control of LN.[15,34]

MONITORING OF LN IN CLINICAL CARE

There are no studies that directly compare the clinical features of the various classes of LN between children and adults with SLE. However, the presentation of children with LN varies considerably, ranging from mild abnormalities on urinalysis, to anasarca caused by marked proteinuria, to posterior reversible encephalopathy owing to uncontrolled hypertension with nephritic syndrome.[36]

Proteinuria

Abnormally elevated excretions of albumin and total protein in the urine are highly sensitive indicators of glomerular disease. Albumin is a small-sized molecule, and one of the first proteins able to pass through the kidneys. The value of monitoring microalbuminuria for the early diagnosis of LN has not been well established, and mesangial LN can be present without proteinuria.[37] A prompt and significant decrease in proteinuria after 3 and 6 months of therapy is an important prognostic factor for good long-term renal outcome.[38] Proteinuria furthers the development of tubulointerstitial inflammation and injury, and thereby a decline in renal function in the long term.[39]

Traditionally proteinuria is quantified by a 24-hour urine collection. Conversely, and despite its common use, urine dipstick is poorly suited to quantify the degree of proteinuria.[40] There is now sufficient evidence that the protein-to-creatinine ratio in a random urine specimen, best from first morning urine,[41–43] is adequate to estimate daily proteinuria in cSLE.

Whether 12-hour overnight urine collection is more accurate than estimation of proteinuria using spot urine will need further study.[44]

Urine Sediment

The presence of cellular casts on urine-sediment examinations, for example, the microscopic examination of the cellular components at casts seen in centrifuged urine, is supportive of glomerulonephritis. Accuracy of urine-sediment interpretation requires timely processing of the urine, as lysis of leukocytes and erythrocytes occurs even within the first hour after collection, especially when low specific gravity and high urine pH are present. Presence of mucus in the urine can entrap both cells and casts, and sometimes repeated assessment of urine sediment is necessary to detect cellular casts.[45]

Glomerular Filtration Rate

The reference method for assessing the “true” glomerular filtration rate (GFR) is to measure the renal clearance of inulin, ethylenediaminetetraacetic acid, and iohexol; that is, markers freely filtered through the glomerulus, neither secreted nor reabsorbed by the tubule. Because such techniques are complex and costly to perform, alternative means to estimate the GFR in a clinical setting have been developed.[46] In pediatrics, the 2009 modification of the Schwartz Formula and serum cystatin C–based methods seem reasonably accurate and easy to use in a clinical setting (Table 2).[46] Despite its appeal, the use of serum cystatin C to estimate the GFR of patients with LN will need further evaluation, as levels of cystatin C seem positively correlated with general SLE activity, even in the absence of LN or changes in renal function.[47,48]

SHORTCOMINGS OF TRADITIONAL MEASURES OF LN

Whereas blood urea nitrogen and creatinine often stay in the normal range in cSLE, even if with profound histologic pathology, the urinary sediment and urinalysis are generally abnormal in untreated LN. Conversely, in pretreated patients only minor abnormalities on urinalysis, including mild proteinuria or hematuria, may be present in patients with severely active biopsy-proven LN. This finding is supported by the research of Christopher-Stine and colleagues,[49] who reviewed 25 LN patients undergoing serial kidney biopsies. At diagnosis proteinuria, hematuria, hypoalbuminemia, and hypertension were all associated with a worse LN class. By contrast, none of these parameters correlated with the LN class on follow-up biopsy, raising the possibility that normal urinalyses do not necessarily ensure the absence of active LN.[49]

With LN, there is a balance between complement activation via the classical pathway, which facilitates the removal of immune complexes, and activation of the alternative pathway, which promotes kidney injury.[50] The literature is inconsistent at best as to whether the concentration of complement and anti-dsDNA antibodies can serve as useful markers of concurrent SLE activity or future flares.[51] In 98 patients who experienced 146 flares, Ho and colleagues[52] showed that hypocomplementemia and anti-dsDNA antibodies accompanied SLE relapse in only 54% and 27% of patients, respectively.

Research in adults with LN suggests that less than 25% of LN patients with low C3, C4, or anti-dsDNA levels have a concurrent flare of LN, and only 50% of LN flares are preceded by a drop in the levels of C3 and C4 or an increase in anti-dsDNA antibodies, respectively.[51,53] In other words, these tests are not much better than the flip of a coin in helping clinicians anticipate LN flare. These reports from adults with LN have been confirmed in children with LN.[54]

Like the immunologic markers C3, C4, and anti-dsDNA antibodies that are traditionally used to assess the course of LN, kidney biopsies have their pitfalls. In a recent study, 5 experienced nephropathologists rated 126 renal biopsy specimens of 87 patients with proliferative LN.[55] These experts demonstrated significant variation in agreement when rating the various histologic aspects of biopsy specimens as part of the ISN/RPS Classification. Excellent agreement (>60%) was reached only for the number of glomeruli seen in the biopsy, the overall activity index score, and the presence of proliferative features. Conversely, agreement was less than 40% (interclass correlation coefficient <0.4) for the presence of mesangial proliferation, tubular necrosis, and, notably, the overall ISN-RPS class designation.[55]

The aforementioned shortcomings of kidney biopsies, as well as the limitations of currently available urine and blood laboratory tests, support a need for potent biomarkers to help accurately diagnose LN and to determine the response of LN to therapy in a clinical setting.

BIOMARKERS AND ASSESSMENT OF THEIR QUALITY

In its simplest definition, a biomarker is anything that can be measured to extract information about a biological state or process. The NIH Biomarkers Definitions Working Group has defined a biological marker (biomarker) as “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”[56] Biomarkers are the essential tools for the implementation of personalized medicine. The biomarker development process, also sometimes referred to as biomarker qualification, has typically been divided into 5 phases,[57] as shown in Table 3. In recent years, the ready availability of powerful tools to scan both the genome and the proteome of an organism have revolutionized and greatly accelerated biomarker discovery.

For biomarker discovery, microarrays are used to screen messenger RNA (mRNA) levels. This approach has yielded several biomarkers of kidney disease, such as neutrophil gelatinase-associated lipocalin (NGAL). Microarrays can be combined with other techniques, such as laser-capture microdissection, to target specific areas of diseased tissue to give mechanistic clues not possible just a decade ago. Even with this level of specificity, a daunting amount of biomarker candidates will be identified with these approaches, and the usefulness of such candidates must be sifted through for relevance. Another shortcoming of transcriptomic profiling approaches is that direct measurement in biological fluids is not possible and that mRNA levels do not always correlate with protein levels or enzyme activity. Hence, larger validation studies are necessary that measure protein levels to confirm the biological relevance of mRNA biomarkers.

Focusing on peptides and actual proteins, proteomics allow one to go beyond simple translation of mRNA into protein. Instead protein regulation, posttranslational modifications such as glycosylation and methylation, and even disease-specific fragmentation of proteins are assessed. Proteomic techniques are capable of identifying and quantifying proteins and peptides in exceedingly large numbers.[58] The urinary proteome itself is quite large, with laboratories having identified more than 1500 proteins to date.[59,60] The blood proteome is even larger, with more than 3000 nonredundant proteins identified in the plasma alone.[61–63] Adding the proteome of the cellular component of blood will yield thousands more.[64,65] To this end, we have entered what has been termed an "open loop,"[66] or unbiased, approach to biomarker discovery, in stark contrast to the hypothesis-driven approach of our past. With such a vast pool of potential biomarkers from readily available, noninvasive sources, one must take care to plan and design the proper experimental approach to ensure parsimony.

There are universal characteristics important for any biomarker: (1) they should be noninvasive, easily measured, inexpensive, and produce rapid results; (2) they should be from readily available sources, such as blood or urine; (3) they should have a high sensitivity, allowing early detection, and no overlap in values between diseased patients and healthy controls; (4) they should have a high specificity, being greatly upregulated (or downregulated) specifically in the diseased samples and unaffected by comorbid conditions; (5) their levels should vary rapidly in response to treatment; (6) their levels should aid in risk stratification and possess prognostic value in terms of real outcomes; and (7) they should be biologically plausible and provide insight into the underlying disease mechanism.[56,57]

The most readily available sources of biomarkers are urine and blood. Urine is an excellent source of biomarkers produced in the kidney,[67] and thus may give better mechanistic insight into specific renal abnormalities. Urine is less complex than serum, and thus is easier to screen for potential biomarkers. Urinary biomarker studies typically adjust for urine creatinine to account for differences in urine concentration resulting from hydration status and medications such as diuretics. However, the utility of urine creatinine in biomarker correction has been questioned because of its variable excretion throughout the day and its dependence on normal renal function.

Serum biomarkers are considered more stable, as they are less prone than urine biomarkers to bacterial contamination. However, serum biomarkers are more likely to represent a systemic response to disease, rather than an organ response. There are exceptions, such as the troponins in cardiac disease. The real problem with serum as a source of biomarkers lies in the discovery phase. Serum has a wide range of protein concentrations across several orders of magnitude, with a small number of proteins (such as albumin) accounting for a large percentage of the volume; this can be akin to trying to spot a single strand of cotton in a large tapestry. Although assays do exist to remove these high-abundance proteins from serum, many potential biomarkers have been shown to bind to albumin.[68] Thus, albumin depletion to help identify relevant biomarkers risks erroneous removal of proteins relevant to LN.

The sensitivity and specificity of a biomarker go hand in hand. The receiver-operating characteristic (ROC) curve is a binary classification test, based on the sensitivity and

specificity of a biomarker at certain cutoff points. ROC curves are often used to determine the clinical diagnostic value of a biomarker.[57,69] The area under the ROC curve (AUC_{ROC}) is a common statistic derived from ROC curves. An AUC_{ROC} of 1.0 represents a perfect biomarker, whereas an AUC_{ROC} of 0.5 is a result that is no better than expected by chance. An AUC_{ROC} of 0.75 or greater is generally considered a good biomarker while an AUC_{ROC} of 0.90 is considered an excellent biomarker.[57] However, even a sensitive biomarker with what experimentally would be considered an excellent specificity of 90% would still yield a false-positive rate of 10%, which may be unacceptably high for clinical use as a stand-alone marker.[66] As a result, the best approach clinically may be to find multiple biomarkers that can be combined as part of a panel to achieve even higher specificity.

TYPES OF LN BIOMARKERS

Traditional measures of LN have limited responsiveness to change, and are unsuited to capture worsening or improvement of LN in a timely manner. This lack of early response measures to verify the effectiveness of LN therapies hinders clinical care, requires clinical trials of new medications for LN to study large populations and follow them over several years, and increases the risk of negative trials. In addition, traditional measures of kidney function, such as creatinine clearance or protein-to-creatinine ratio, reflect significant loss of kidney function such that major renal damage can occur before it is detected by these traditional methods. Thus, novel biomarkers that can rapidly detect lupus renal involvement and severity, predict flares, and monitor treatment response and disease progression are greatly needed, and have been the subject of intense research.

The advent of new technologies to rapidly screen the genome and proteome over the last few decades has led to an explosion in the identification of novel biomarkers for many disease states. Ann immense number of biomarkers has been investigated in recent years, far too many to discuss in this article. The authors therefore focus the discussion on the most promising investigational biomarkers for LN discovered over the last several years.

Urine MCP-1

Monocyte chemoattractant protein-1 (MCP-1) is a leukocyte chemotactic protein involved in the mediation of inflammation and renal injury in LN.[70] Animal models of LN have demonstrated direct involvement of MCP-1 in renal abnormality, as blockade of MCP-1 through the use of an antagonist or an RNA oligonucleotide specifically designed to bind to and sequester MCP-1 (also known as a spiegelmer) led to marked improvement in LN and lupus-like inflammatory skin lesions.[71,72] Several cross-sectional studies have demonstrated that urine MCP-1 levels are concurrently higher in those patients with active LN than with nonactive LN.[73–75] The AUC_{ROC} of MCP-1 for distinguishing active LN from inactive LN[76] or nonrenal flares is 0.76.[77] Urine MCP-1 also seems to have promise in helping to distinguish certain classes of LN. Urine MCP-1 levels are significantly higher with ISN/RPS Classes III and IV than with other classes of LN ($P = .01$).[78,79] Both children and adults with Class IV LN have the highest glomerular expression of MCP-1.[46] There are some differing findings regarding the potential of urine MCP-1 to predict renal flares. A study by Rovin and colleagues[73] reported increases in urine MCP-1 as early as 2 to 4 months before the clinical

diagnosis of a renal flare. However, a similar study by Tian and colleagues,[80] while demonstrating elevated MCP-1 during renal flares, did not find MCP-1 levels to be an independent predictor of flare.

Similar results were found by Chan and colleagues[81] when examining chemokine mRNA from urine sediment of LN patients. MCP-1 mRNA levels were elevated during active LN in comparison with inactive LN and healthy controls. However, in this study urine MCP-1 mRNA levels were found not to be useful predictors of LN flares. It should be noted that the best use for MCP-1 as it relates to SLE is as part of a broader panel of markers, as elevated urine MCP-1 can also signal chronic fibrosis[82,83] and has presented in other glomerular disorders.[84] Thus a combinatorial approach may lead to additional specificity for LN.

Urine NGAL

NGAL is expressed in several cell types, including neutrophils, specific epithelia, and renal tubular cells. NGAL is markedly upregulated in the distal tubules in response to many types of kidney injury. It has garnered significant attention as a promising early marker for acute kidney injury,[85–91] but recent studies have also shed light on NGAL's potential as a biomarker for chronic kidney disease, such as diabetic nephropathy[92,93] and focal segmental glomerulosclerosis,[94] as well as LN.[95,96] Two cross-sectional studies investigated NGAL as a biomarker for LN in pediatric patients[95] and adults.[97] In children, elevated urine NGAL levels had a high sensitivity and specificity for active biopsy-proven LN (AUC_{ROC} 0.94). In adults the specificity was still high (91%), but sensitivity was lower (50%) for LN. This thread is a common one in biomarker studies, as adults typically have more concurrent confounding physiologic conditions, which leads to higher variability in biomarker measurements. NGAL was not correlated with extrarenal SLE disease activity in either population. More recent longitudinal studies in the pediatric population have shown that urine NGAL as well as plasma NGAL levels are significantly higher in SLE patients than those with juvenile idiopathic arthritis (JIA) or healthy controls, unrelated to physiologic factors such as height, weight, and age.[98] Levels of urine NGAL, but not plasma NGAL, correlated well with LN activity scores.[96,98] Urine NGAL rose 3 to 6 months before worsening renal disease activity, demonstrating value in predicting flares.[96,98] One study demonstrated a lesser, though significant, increase in plasma NGAL as early as 3 months before flare.[96] In addition, in patients with a biopsy, urine NGAL levels were greater in patients with diffuse proliferative than membranous nephropathy, indicating, along with MCP-1, the possible use of NGAL in a panel to distinguish LN classes.[98] Similar to MCP-1, urine NGAL is not specific to LN and thus must be used in a context-specific setting.

Hepcidin

Hepcidin is a small peptide hormone mainly produced in the liver, and has a role in iron homeostastis. Hepcidin is upregulated in response to high iron levels and inflammation, and decreases during anemia and iron deficiency. Proteomic evaluation by surface-enhanced laser desorption-ionization time-of-flight mass spectrometry (SELDI) revealed the 25- and 20-amino-acid isoforms of hepcidin as potential biomarkers for LN.[79] Zhang and colleagues[79] prospectively analyzed 24 LN flare cycles in 19 patients, and demonstrated an increase in hepcidin-20 4 months before flare, which then decreased to baseline levels by 4

months after flare. An opposing pattern was discovered for hepcidin-25, which decreased during renal activity then returned to baseline along with hepcidin-20 after flare. It will be interesting in future studies to evaluate the physiologic role of hepcidin in LN because it is regulated in part by inflammatory cytokines, such as interferon-α and interleukin (IL)-6, which are known to play a role in modulating tissue damage in SLE,[99,100] and have been shown experimentally to induce monocyte expression of hepcidin in vitro.[101] It has been speculated that monocyte infiltration of the kidney may be the source of urine hepcidin in LN.

Urine Protein Signature

Also using SELDI, Suzuki and colleagues[102] discovered and subsequently validated[54] a protein signature that identified active LN in children. After removal of 4 albumin fragments from the signature, the panel included transferrin (Tf), orosomucoid (or α-1 acid glycoprotein [AGP]), ceruloplasmin (CP), and lipocalin-type prostaglandin D synthase (L-PDGS, or β-trace protein). Using enzyme-linked immunosorbent assay or immunonephelometry, all 4 proteins were found to be significantly higher in patients with active LN than in those with nonrenal SLE or JIA controls. Urine L-PDGS, AGP, and Tf all increased as early as 3 months before renal flare, but Tf did so most consistently, demonstrating increased sensitivity to renal changes in SLE in comparison with L-PDGS or AGP. Urine CP did not demonstrate the ability to predict flares. Combining this panel with other markers such as NGAL and MCP-1 may demonstrate enhanced predictive and diagnostic value in comparison with individual markers alone.

Complement Component C4d

C4d is a breakdown product of the activated complement factor C4b, a critical component of the C5 convertase. In a controversial pilot study using an alternative approach, Batal and colleagues[103] evaluated cellular deposition of the immune complex C4d on circulating erythrocytes, reticulocytes, and platelets as a potential biomarker for LN activity. Previous studies had linked peritubular capillary and glomerular staining of C4d with severity of LN and development of renal thrombotic microangiopathy, respectively.[104,105] The investigators found higher circulating levels of erythrocyte-bound C4d (EC4d) and reticulocyte-bound C4d (RC4d) in LN patients than in both nonrenal SLE patients and patients with renal disease without SLE. Moreover, EC4d levels correlated with the NIH renal activity index. There has been some level of skepticism[106] regarding the ability of these markers to distinguish renal from nonrenal SLE, as higher levels can also observed in SLE patients without LN,[107,108] and there have been no scientific findings to date that dispute the results. An additional study lends credence to the finding in this study, indicating higher levels of certain C4d-positive circulating T cells in LN patients than in those without LN.[109] Further prospective investigations of circulating C4d are needed for it to rise to the levels of the previously discussed biomarkers for LN, but the novel approach warranted mention in this review.

TWEAK

Tumor necrosis factor–like weak inducer of apoptosis (TWEAK) is a member of the tumor necrosis factor (TNF) superfamily, and is involved in modulating cell survival and induction

of several proinflammatory chemokines through its receptor fibroblast growth factor–inducible protein 14 (Fn14).[110] In human kidney, TWEAK acts on multiple Fn14-expressing cells types, including podocytes, tubular cells, and mesangial cells, and is responsible for induction of several mediators of inflammation, including MCP-1, interferon-γ–inducible protein 10 (IP-10), intercellular cell adhesion molecule 1, vascular cell adhesion molecule 1 (VCAM-1), matrix metalloproteinases 1 and 9, and macrophage inflammatory protein α.[111,112] During periods of inflammation, Fn14 expression is upregulated, which lends itself to enhancing a positive feedback loop. The major source of TWEAK in LN is thought to be infiltrating monocytes and macrophages. Cross-sectionally, urinary TWEAK levels are significantly higher in active LN; levels are significantly higher in patients with LN flare than in those with stable disease.[113,114] In a multicenter longitudinal analysis, Schwartz and colleagues[115] discovered that whereas urinary TWEAK levels peaked at the height of renal flare, urinary TWEAK was significantly elevated 4 to 6 months before and following renal flare. Performance of urinary TWEAK in distinguishing LN patients from SLE patients without kidney involvement was better than that of anti-dsDNA levels and complement C3 or C4 levels. The study also demonstrated a strong association between urinary TWEAK levels and LN activity over time. Conversely, serum levels of TWEAK were not associated with LN activity. TWEAK is intriguing as a biomarker for LN, and has a biologically plausible role in LN pathology.

Other Chemokines, Receptors, and Adhesion Molecules

Space does not permit in-depth discussion of all biomarkers under investigation for LN, but several cytokines, chemokines, and their receptors deserve some mention. Chemokine C-X-C motif ligand 10 (CXCL10, also known as IP-10) and its receptor CXCR3 promote T-cell migration to areas of inflammation and are upregulated in SLE.[116,117] CXCL10 and CXCR3 mRNA levels collected from urine sediment were highly specific for identifying Class IV LN (AUC_{ROC} 0.89 for CXCL10 and 0.79 for CXCR3), and also demonstrated reduction in response to successful treatment signified by clinical remission.[118] FOXP3 (forkhead box P3) mRNA collected from urine sediment of LN patients has been found to be significantly higher in LN patients,[119] despite FOXP3 levels in regulatory T cells having been found to be lower in patients with active lupus than in healthy controls.[120] Research has also shown that a reduced number of circulating $FOXP3^+$ T cells and serum transforming growth factor β levels inversely correlated with LN activity as measured by SLE disease activity index renal domain score ($P = .0013$ and 0.0005, respectively).[109] Collection of mRNA from urine sediment presents several technical difficulties, such as stability, which may limit the clinical utility of urine mRNAs as biomarkers. So although there may be a link between FOXP3 and LN, additional study must be completed to solidify its role and usefulness as a biomarker for LN.

VCAM-1 demonstrates reliability as an indicator of renal disease activity in LN. VCAM-1 has been shown to be induced in mice by inflammatory cytokines such as IL-1 and TNF.[121] VCAM-1 plays a role in tethering leukocytes, which are drawn to sites of inflammation, to endothelial cells.[122] Urinary VCAM-1 has been shown in several studies of human disease to be strongly correlated with LN activity and severity[77,123,124] in LN. Serum levels of VCAM have previously been shown to correlate with the severity of LN, being highest in

WHO Class III and IV, versus inactive or mild nephritis (WHO Class I or II),[125] and levels diminished with treatment. Singh and colleagues[126] compared urine levels of VCAM-1, MCP-1, and CXCL16 (another potential LN biomarker) with pathologic features of LN on biopsy collected concurrently with the urine sample. Urine VCAM and MCP-1 were highly predictive of LN when compared with healthy controls (AUC_{ROC} 0.92 and 0.89, respectively). Surprisingly, urine MCP-1 was also significantly higher in African American subjects than in persons of other ethnic origins. Of the 3 markers, urine VCAM-1 was most highly correlated with LN activity, with none shown by CXCL16. CXCL16 and urine VCAM-1 were significantly higher in patients with WHO Class IV LN compared with other Classes, as determined by concurrent biopsy analysis. It should be noted that this association with Class IV proliferative nephritis may not be specific to pathology, but a may be a result of these patients having a high degree of renal disease activity. These findings provide a great deal of support for urine VCAM-1 as a biomarker for LN, but these studies have all been cross-sectional. Longitudinal studies are needed to determine the utility of VCAM-1 in monitoring disease progression and detecting flares. It should also be noted that, like NGAL and MCP-1, elevated VCAM-1 is not exclusive to LN. Increased levels of VCAM-1 have been found in other glomerular diseases such as membranous nephropathy and focal segmental glomerulosclerosis.[126]

CURRENT TREATMENT OF LUPUS NEPHRITIS IN CHILDREN

The novel biomarkers introduced in the preceding sections are not used to support efficacy in clinical trials at present, although validation studies are ongoing to achieve biomarker qualification by regulatory bodies. Qualification would allow for the use of biomarkers in clinical care and research.[127,128] In addition, there is no known biomarker at present that a priori would support the choice of therapeutics for the treatment of LN. However, it seems reasonable to assume that novel biomarkers will become available for clinical use within the next 5 to 7 years.

No medication has likely improved the prognosis of LN more than systemic glucocorticoids (GC), especially if combined with immunosuppressive medications. Nonetheless, use of GC is a concern, given the often devastating short-term and long-term side effects. There is a lack of systematic studies in support of the most appropriate dose of GC in patients with LN. Based on consensus among pediatric rheumatologists in the United States, three GC dosing regimens for the treatment of proliferative LN in children have been proposed,[129] but data are lacking to determine which regimen is the most appropriate for a given patient. Of note, the Joint European League Against Rheumatism and the American College of Rheumatology consider much lower GC exposure sufficient for mainly adults with LN.[14,130]

Unless commanded by cSLE activity in other organ systems, hydroxychloroquine and GC are considered sufficient for the treatment of ISN/RPS Class I and, often, Class II LN.[14,131] For proliferative LN Class III or IV with or without membranous features, treatment with cyclophosphamide or mycophenolate mofetil (MMF) for induction therapy, and maintenance therapy using MMF or azathioprine are proposed.[14,129] Based on a Cochrane review of studies of adults with LN,[132,133] compared with intravenous cyclophosphamide, MMF was as effective in achieving stable kidney function and complete remission of proteinuria. No

differences in mortality or major infections were observed. In maintenance therapy, the risk of LN flare was significantly higher with azathioprine or cyclophosphamide compared with MMF. Based on small studies, children and adolescents have a response to MMF and cyclophosphamide similar to that of adults with LN.[134] Whether MMF is as effective in children as it is in adults[135] or whether cyclophosphamide might have a better risk/benefit profile in children than in adults owing to lower frequency of clinically relevant ovarian injury and lower risk of nonadherence is not supported by high-level scientific evidence.[129] In addition, the pediatric correlate of the "Euro Lupus Regimen" for the dosing of intravenous cyclophosphamide has not been developed or systematically studied.[14]

There is mounting evidence that individualized dosing of MMF based on pharmacokinetic profiling will increase the likelihood of achieving remission of LN.[136,137] Target exposure between 60 and 90 mg/h/L is more often associated with LN improvement, with the highest exposures being reserved for the most severe cases because of the increased frequency of adverse effects.[136] Given high interindividual differences, weight-based or body-surface–based dosing of MMF does not suffice to reliably achieve such a target exposure.[138]

Pure membranous lupus glomerulonephritis (ISN/RPS Class V) seems rarely the initially diagnosed type of LN, and typically the other forms of LN develop into Class V over time. Treatment of Class V probably should not differ from that of idiopathic membranous nephropathy. Depending on the degree of proteinuria, only angiotensin-inhibiting medications, or GC with MMF or other immunosuppressives are the preferred initial therapy.[139]

Despite favorable reports mostly from observational studies,[140–144] the clinical trial of the anti-CD20 antibody rituximab (Rituxan, Mabthera) failed to show clinically relevant improvement of LN.[145] The anti–B-lymphocyte stimulator antibody belimumab (Benlysta) has recently been approved for the treatment of active SLE,[146,147] but its benefit or detrimental effects on LN will require further study.

There are currently several ongoing studies of LN, some including younger patients, which explore the efficacy of various combination therapies of GC with regimens including various combinations of cyclophosphamide, cyclosporin, azathioprine, tacrolimus, MMF, fludarabine, azathioprine, rituximab, abatacept, etanercept, and leflunomide, as well as mesenchymal stem cells. It is hoped that these studies consider the genetic differences of patients and include potent LN biomarkers when assessing the benefits of these therapies under investigation.

It is plausible to assume that the use of novel biomarkers will yield better stratification of patient populations for the purpose of clinical trials, and enable researchers to determine the response to LN therapy earlier and more accurately. This approach would necessitate smaller sample sizes for clinical trials, and ultimately make possible adequately powered studies in children with LN.

Acknowledgments

Funding Sources: H.I.B. is supported by research grants from the National Institutes of Health, including 2P60AR047784, U01AR059509 and UL1 TR000077-04; M.B. is supported by a research grant from the National Institutes of Health, P50 DK096418 and two translational research grants from the Cincinnati Children's Hospital Medical Center/University of Cincinnati Joint Center for Clinical and Translational Science and Training.

References

1. Silva CA, Avcin T, Brunner HI. Taxonomy for systemic lupus erythematosus with onset before adulthood. Arthritis Care Res (Hoboken). 2012; 64(12):1787–93. [PubMed: 22730317]
2. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. Lupus. 2006; 15(5):308–18. [PubMed: 16761508]
3. Hiraki LT, Feldman CH, Liu J, et al. Prevalence, incidence, and demographics of systemic lupus erythematosus and lupus nephritis from 2000 to 2004 among children in the US Medicaid beneficiary population. Arthritis Rheum. 2012; 64(8):2669–76. [PubMed: 22847366]
4. Aletaha D, Landewe R, Karonitsch T, et al. Reporting disease activity in clinical trials of patients with rheumatoid arthritis: EULAR/ACR collaborative recommendations. Arthritis Rheum. 2008; 59(10):1371–7. [PubMed: 18821648]
5. Livingston B, Bonner A, Pope J. Differences in clinical manifestations between childhood-onset lupus and adult-onset lupus: a meta-analysis. Lupus. 2011; 20(13):1345–55. [PubMed: 21951943]
6. Hiraki LT, Lu B, Alexander SR, et al. End-stage renal disease due to lupus nephritis among children in the US, 1995–2006. Arthritis Rheum. 2011; 63(7):1988–97. [PubMed: 21445963]
7. Bernatsky S, Boivin JF, Joseph L, et al. Mortality in systemic lupus erythematosus. Arthritis Rheum. 2006; 54(8):2550–7. [PubMed: 16868977]
8. Lionaki S, Kapitsinou PP, Iniotaki A, et al. Kidney transplantation in lupus patients: a case-control study from a single centre. Lupus. 2008; 17(7):670–5. [PubMed: 18625640]
9. Pereira T, Abitbol CL, Seeherunvong W, et al. Three decades of progress in treating childhood-onset lupus nephritis. Clin J Am Soc Nephrol. 2011; 6(9):2192–9. [PubMed: 21799148]
10. Costenbader KH, Desai A, Alarcon GS, et al. Trends in the incidence, demographics, and outcomes of end-stage renal disease due to lupus nephritis in the US from 1995 to 2006. Arthritis Rheum. 2011; 63(6):1681–8. [PubMed: 21445962]
11. Tanzer M, Tran C, Messer KL, et al. Inpatient health care utilization by children and adolescents with systemic lupus erythematosus and kidney involvement. Arthritis Care Res (Hoboken). 2013; 65(3):382–90. [PubMed: 22899662]
12. Brunner HI, Sherrard TM, Klein-Gitelman MS. Cost of treatment of childhood-onset systemic lupus erythematosus. Arthritis Rheum. 2006; 55(2):184–8. [PubMed: 16583393]
13. NAPRTCS. North American Pediatric Renal Trials and Collaborative Studies Annual Report. 2011. Available at: https://web.emmes.com/study/ped/annlrept/annualrept2011.pdf
14. Bertsias GK, Tektonidou M, Amoura Z, et al. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. Ann Rheum Dis. 2012; 71(11):1771–82. [PubMed: 22851469]
15. Hollander MC, Sage JM, Greenler AJ, et al. International consensus for provisions of quality-driven care in childhood-onset systemic lupus erythematosus. Arthritis Care Res (Hoboken). 2013 [Epub ahead of print].
16. Marks SD, Sebire NJ, Pilkington C, et al. Clinicopathological correlations of paediatric lupus nephritis. Pediatr Nephrol. 2007; 22(1):77–83. [PubMed: 17106692]
17. Brunner HI, Gladman DD, Ibanez D, et al. Difference in disease features between childhood-onset and adult-onset systemic lupus erythematosus. Arthritis Rheum. 2008; 58(2):556–62. [PubMed: 18240232]
18. Hiraki LT, Benseler SM, Tyrrell PN, et al. Clinical and laboratory characteristics and long-term outcome of pediatric systemic lupus erythematosus: a longitudinal study. J Pediatr. 2008; 152(4): 550–6. [PubMed: 18346514]

19. Ruggiero B, Vivarelli M, Gianviti A, et al. Lupus nephritis in children and adolescents: results of the Italian Collaborative Study. Nephrol Dial Transplant. 2013; 28(6):1487–96. [PubMed: 23345627]
20. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol. 2004; 15(2):241–50. [PubMed: 14747370]
21. Corwin HL, Schwartz MM, Lewis EJ. The importance of sample size in the interpretation of the renal biopsy. Am J Nephrol. 1988; 8(2):85–9. [PubMed: 3394725]
22. Austin HA 3rd, Muenz LR, Joyce KM, et al. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. Kidney Int. 1984; 25(4):689–95. [PubMed: 6482173]
23. Hsieh C, Chang A, Brandt D, et al. Predicting outcomes of lupus nephritis with tubulointerstitial inflammation and scarring. Arthritis Care Res (Hoboken). 2011; 63(6):865–74. [PubMed: 21309006]
24. Cortes-Hernandez J, Ordi-Ros J, Labrador M, et al. Predictors of poor renal outcome in patients with lupus nephritis treated with combined pulses of cyclophosphamide and methylprednisolone. Lupus. 2003; 12(4):287–96. [PubMed: 12729052]
25. Zappitelli M, Duffy C, Bernard C, et al. Clinicopathological study of the WHO classification in childhood lupus nephritis. Pediatr Nephrol. 2004; 19(5):503–10. [PubMed: 15022106]
26. Lee BS, Cho HY, Kim EJ, et al. Clinical outcomes of childhood lupus nephritis: a single center's experience. Pediatr Nephrol. 2007; 22(2):222–31. [PubMed: 17131162]
27. Demircin G, Oner A, Erdogan O, et al. Long-term efficacy and safety of quadruple therapy in childhood diffuse proliferative lupus nephritis. Ren Fail. 2008; 30(6):603–9. [PubMed: 18661410]
28. Hagelberg S, Lee Y, Bargman J, et al. Longterm followup of childhood lupus nephritis. J Rheumatol. 2002; 29(12):2635–42. [PubMed: 12465165]
29. Vachvanichsanong P, Dissaneewate P, McNeil E. Diffuse proliferative glomerulonephritis does not determine the worst outcome in childhood onset lupus nephritis: a 23-year experience in a single centre. Nephrol Dial Transplant. 2009; 24(9):2729–34. [PubMed: 19395731]
30. Hersh AO, von Scheven E, Yazdany J, et al. Differences in long-term disease activity and treatment of adult patients with childhood- and adult-onset systemic lupus erythematosus. Arthritis Rheum. 2009; 61(1):13–20. [PubMed: 19116979]
31. Zappitelli M, Duffy CM, Bernard C, et al. Evaluation of activity, chronicity and tubulointerstitial indices for childhood lupus nephritis. Pediatr Nephrol. 2008; 23(1):83–91. [PubMed: 17957388]
32. Chrysochou C, Randhawa H, Reeve R, et al. Determinants of renal functional outcome in lupus nephritis: a single centre retrospective study. QJM. 2008; 101(4):313–6. [PubMed: 18270227]
33. Contreras G, Pardo V, Cely C, et al. Factors associated with poor outcomes in patients with lupus nephritis. Lupus. 2005; 14(11):890–5. [PubMed: 16335581]
34. Houssiau FA. Therapy of lupus nephritis: lessons learned from clinical research and daily care of patients. Arthritis Res Ther. 2012; 14(1):202. [PubMed: 22293173]
35. Mok CC, Ying KY, Tang S, et al. Predictors and outcome of renal flares after successful cyclophosphamide treatment for diffuse proliferative lupus glomerulonephritis. Arthritis Rheum. 2004; 50(8):2559–68. [PubMed: 15334470]
36. Punaro M, Abou-Jaoude P, Cimaz R, et al. Unusual neurologic manifestations (II): posterior reversible encephalopathy syndrome (PRES) in the context of juvenile systemic lupus erythematosus. Lupus. 2007; 16(8):576–9. [PubMed: 17711891]
37. Valente de Almeida R, Rocha de Carvalho JG, de Azevedo VF, et al. Microalbuminuria and renal morphology in the evaluation of subclinical lupus nephritis. Clin Nephrol. 1999; 52(4):218–29. [PubMed: 10543324]
38. Houssiau FA, Vasconcelos C, D'Cruz D, et al. Early response to immunosuppressive therapy predicts good renal outcome in lupus nephritis: lessons from long-term followup of patients in the Euro-Lupus Nephritis Trial. Arthritis Rheum. 2004; 50(12):3934–40. [PubMed: 15593207]
39. Eddy AA, Giachelli CM. Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria. Kidney Int. 1995; 47(6):1546–57. [PubMed: 7643523]

40. Siedner MJ, Gelber AC, Rovin BH, et al. Diagnostic accuracy study of urine dipstick in relation to 24-hour measurement as a screening tool for proteinuria in lupus nephritis. J Rheumatol. 2008; 35(1):84–90. [PubMed: 18085740]
41. Hebert LA, Birmingham DJ, Shidham G, et al. Random spot urine protein/creatinine ratio is unreliable for estimating 24-hour proteinuria in individual systemic lupus erythematosus nephritis patients. Nephron Clin Pract. 2009; 113(3):c177–82. [PubMed: 19672116]
42. KDOQI. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. Am J Kidney Dis. 2007; 49(2 Suppl 2):S12–154. [PubMed: 17276798]
43. Renal Disease Subcommittee of the American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria. The American College of Rheumatology response criteria for proliferative and membranous renal disease in systemic lupus erythematosus clinical trials. Arthritis Rheum. 2006; 54(2):421–32. [PubMed: 16453282]
44. Fine DM, Ziegenbein M, Petri M, et al. A prospective study of protein excretion using short-interval timed urine collections in patients with lupus nephritis. Kidney Int. 2009; 76(12):1284–8. [PubMed: 19759526]
45. Fogazzi GB, Garigali G. The clinical art and science of urine microscopy. Curr Opin Nephrol Hypertens. 2003; 12(6):625–32. [PubMed: 14564200]
46. Bacchetta J, Cochat P, Rognant N, et al. Which creatinine and cystatin C equations can be reliably used in children? Clin J Am Soc Nephrol. 2011; 6(3):552–60. [PubMed: 21115623]
47. Lertnawapan R, Bian A, Rho YH, et al. Cystatin C is associated with inflammation but not atherosclerosis in systemic lupus erythematosus. Lupus. 2012; 21(3):279–87. [PubMed: 22072023]
48. Chew C, Pemberton PW, Husain AA, et al. Serum cystatin C is independently associated with renal impairment and high sensitivity C-reactive protein in systemic lupus erythematosus. Clin Exp Rheumatol. 2013; 31(2):251–5. [PubMed: 23306163]
49. Christopher-Stine L, Siedner M, Lin J, et al. Renal biopsy in lupus patients with low levels of proteinuria. J Rheumatol. 2007; 34(2):332–5. [PubMed: 17183619]
50. Vernon KA, Cook HT. Complement in glomerular disease. Adv Chronic Kidney Dis. 2012; 19(2): 84–92. [PubMed: 22449345]
51. Rovin BH, Birmingham DJ, Nagaraja HN, et al. Biomarker discovery in human SLE nephritis. Bull N Y U Hosp Jt Dis. 2007; 65(3):187–93.
52. Ho A, Barr SG, Magder LS, et al. A decrease in complement is associated with increased renal and hematologic activity in patients with systemic lupus erythematosus. Arthritis Rheum. 2001; 44(10):2350–7. [PubMed: 11665976]
53. Esdaile JM, Abrahamowicz M, Joseph L, et al. Laboratory tests as predictors of disease exacerbations in systemic lupus erythematosus. Why some tests fail Arthritis Rheum. 1996; 39(3): 370–8. [PubMed: 8607885]
54. Suzuki M, Wiers K, Brooks EB, et al. Initial validation of a novel protein biomarker panel for active pediatric lupus nephritis. Pediatr Res. 2009; 65(5):530–6. [PubMed: 19218887]
55. Grootscholten C, Bajema IM, Florquin S, et al. Interobserver agreement of scoring of histopathological characteristics and classification of lupus nephritis. Nephrol Dial Transplant. 2008; 23(1):223–30. [PubMed: 17981886]
56. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001; 69(3):89–95. [PubMed: 11240971]
57. Devarajan P. Proteomics for biomarker discovery in acute kidney injury. Semin Nephrol. 2007; 27(6):637–51. [PubMed: 18061846]
58. Knepper MA. Proteomics and the kidney. J Am Soc Nephrol. 2002; 13(5):1398–408. [PubMed: 11961030]
59. Thongboonkerd V, McLeish KR, Arthur JM, et al. Proteomic analysis of normal human urinary proteins isolated by acetone precipitation or ultracentrifugation. Kidney Int. 2002; 62(4):1461–9. [PubMed: 12234320]

60. Adachi J, Kumar C, Zhang Y, et al. The human urinary proteome contains more than 1500 proteins, including a large proportion of membrane proteins. Genome Biol. 2006; 7(9):R80. [PubMed: 16948836]
61. Omenn GS. Exploring the human plasma proteome. Proteomics. 2005; 5(13):3223–5. [PubMed: 16104055]
62. Omenn GS, States DJ, Adamski M, et al. Overview of the HUPO Plasma Proteome Project: results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core dataset of 3020 proteins and a publicly-available database. Proteomics. 2005; 5(13):3226–45. [PubMed: 16104056]
63. States DJ, Omenn GS, Blackwell TW, et al. Challenges in deriving high-confidence protein identifications from data gathered by a HUPO plasma proteome collaborative study. Nat Biotechnol. 2006; 24(3):333–8. [PubMed: 16525410]
64. D'Alessandro A, Righetti PG, Zolla L. The Red Blood Cell proteome and interactome: an update. J Proteome Res. 2010; 9(1):144–63. [PubMed: 19886704]
65. van Gestel RA, van Solinge WW, van der Toorn HW, et al. Quantitative erythrocyte membrane proteome analysis with Blue-Native/SDS PAGE. J Proteomics. 2010; 73(3):456–65. [PubMed: 19778645]
66. Knepper MA. Common sense approaches to urinary biomarker study design. J Am Soc Nephrol. 2009; 20(6):1175–8. [PubMed: 19470673]
67. Hewitt SM, Dear J, Star RA. Discovery of protein biomarkers for renal diseases. J Am Soc Nephrol. 2004; 15(7):1677–89. [PubMed: 15213255]
68. Dos Remedios CG, Liew CC, Allen PD, et al. Genomics, proteomics and bioinformatics of human heart failure. J Muscle Res Cell Motil. 2003; 24(4–6):251–60. [PubMed: 14620738]
69. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993; 39(4):561–77. [PubMed: 8472349]
70. Rovin BH. The chemokine network in systemic lupus erythematous nephritis. Front Biosci. 2008; 13:904–22. [PubMed: 17981599]
71. Hasegawa H, Kohno M, Sasaki M, et al. Antagonist of monocyte chemoattractant protein 1 ameliorates the initiation and progression of lupus nephritis and renal vasculitis in MRL/lpr mice. Arthritis Rheum. 2003; 48(9):2555–66. [PubMed: 13130475]
72. Kulkarni O, Pawar RD, Purschke W, et al. Spiegelmer inhibition of CCL2/MCP-1 ameliorates lupus nephritis in MRL-(Fas)lpr mice. J Am Soc Nephrol. 2007; 18(8):2350–8. [PubMed: 17625118]
73. Rovin BH, Song H, Birmingham DJ, et al. Urine chemokines as biomarkers of human systemic lupus erythematosus activity. J Am Soc Nephrol. 2005; 16(2):467–73. [PubMed: 15601744]
74. Kiani AN, Johnson K, Chen C, et al. Urine osteoprotegerin and monocyte chemoattractant protein-1 in lupus nephritis. J Rheumatol. 2009; 36(10):2224–30. [PubMed: 19648301]
75. Tucci M, Barnes EV, Sobel ES, et al. Strong association of a functional polymorphism in the monocyte chemoattractant protein 1 promoter gene with lupus nephritis. Arthritis Rheum. 2004; 50(6):1842–9. [PubMed: 15188361]
76. Watson L, Midgley A, Pilkington C, et al. Urinary monocyte chemoattractant protein 1 and alpha 1 acid glycoprotein as biomarkers of renal disease activity in juvenile-onset systemic lupus erythematosus. Lupus. 2012; 21(5):496–501. [PubMed: 22147846]
77. Wu T, Xie C, Wang HW, et al. Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. J Immunol. 2007; 179(10):7166–75. [PubMed: 17982109]
78. Graves DT, Alsulaimani F, Ding Y, et al. Developmentally regulated monocyte recruitment and bone resorption are modulated by functional deletion of the monocytic chemoattractant protein-1 gene. Bone. 2002; 31(2):282–7. [PubMed: 12151080]
79. Zhang X, Jin M, Wu H, et al. Biomarkers of lupus nephritis determined by serial urine proteomics. Kidney Int. 2008; 74(6):799–807. [PubMed: 18596723]
80. Tian S, Li J, Wang L, et al. Urinary levels of RANTES and M-CSF are predictors of lupus nephritis flare. Inflamm Res. 2007; 56(7):304–10. [PubMed: 17659436]

81. Chan RW, Lai FM, Li EK, et al. The effect of immunosuppressive therapy on the messenger RNA expression of target genes in the urinary sediment of patients with active lupus nephritis. Nephrol Dial Transplant. 2006; 21(6):1534–40. [PubMed: 16449281]
82. Gharaee-Kermani M, Denholm EM, Phan SH. Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. J Biol Chem. 1996; 271(30):17779–84. [PubMed: 8663511]
83. Sakai N, Wada T, Furuichi K, et al. MCP-1/CCR2-dependent loop for fibrogenesis in human peripheral CD14-positive monocytes. J Leukoc Biol. 2006; 79(3):555–63. [PubMed: 16415174]
84. Rovin BH. Chemokines as therapeutic targets in renal inflammation. Am J Kidney Dis. 1999; 34(4):761–4. [discussion: 765–7]. [PubMed: 10516362]
85. Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. 2005; 365(9466):1231–8. [PubMed: 15811456]
86. Mishra J, Ma Q, Kelly C, et al. Kidney NGAL is a novel early marker of acute injury following transplantation. Pediatr Nephrol (Berlin, Germany). 2006; 21(6):856–63.
87. Mishra J, Mori K, Ma Q, et al. Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol. 2004; 24(3):307–15. [PubMed: 15148457]
88. Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol. 2008; 3(3):665–73. [PubMed: 18337554]
89. Haase M, Bellomo R, Devarajan P, et al. Novel biomarkers early predict the severity of acute kidney injury after cardiac surgery in adults. Ann Thorac Surg. 2009; 88(1):124–30. [PubMed: 19559209]
90. Krawczeski CD, Goldstein SL, Woo JG, et al. Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass. J Am Coll Cardiol. 2011; 58(22):2301–9. [PubMed: 22093507]
91. Krawczeski CD, Woo JG, Wang Y, et al. Neutrophil gelatinase-associated lipocalin concentrations predict development of acute kidney injury in neonates and children after cardiopulmonary bypass. J Pediatr. 2011; 5:5.
92. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin as an early biomarker of nephropathy in diabetic patients. Kidney Blood Press Res. 2009; 32(2):91–8. [PubMed: 19321980]
93. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. 2009; 4(2):337–44. [PubMed: 19176795]
94. Bennett MR, Piyaphanee N, Czech K, et al. NGAL distinguishes steroid sensitivity in idiopathic nephrotic syndrome. Pediatr Nephrol (Berlin, Germany). 2012; 27(5):807–12.
95. Brunner HI, Mueller M, Rutherford C, et al. Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. Arthritis Rheum. 2006; 54(8):2577–84. [PubMed: 16868980]
96. Hinze CH, Suzuki M, Klein-Gitelman M, et al. Neutrophil gelatinase-associated lipocalin is a predictor of the course of global and renal childhood-onset systemic lupus erythematosus disease activity. Arthritis Rheum. 2009; 60(9):2772–81. [PubMed: 19714584]
97. Pitashny M, Schwartz N, Qing X, et al. Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. Arthritis Rheum. 2007; 56(6):1894–903. [PubMed: 17530720]
98. Suzuki M, Wiers KM, Klein-Gitelman MS, et al. Neutrophil gelatinase-associated lipocalin as a biomarker of disease activity in pediatric lupus nephritis. Pediatr Nephrol. 2008; 23(3):403–12. [PubMed: 18202859]
99. Ivashkiv LB. Type I interferon modulation of cellular responses to cytokines and infectious pathogens: potential role in SLE pathogenesis. Autoimmunity. 2003; 36(8):473–9. [PubMed: 14984024]
100. Tackey E, Lipsky PE, Illei GG. Rationale for interleukin-6 blockade in systemic lupus erythematosus. Lupus. 2004; 13(5):339–43. [PubMed: 15230289]

101. Zhang X, Rovin BH. Hepcidin expression by human monocytes in response to adhesion and pro-inflammatory cytokines. Biochim Biophys Acta. 2010; 1800(12):1262–7. [PubMed: 20801192]
102. Suzuki M, Ross GF, Wiers K, et al. Identification of a urinary proteomic signature for lupus nephritis in children. Pediatr Nephrol. 2007; 22(12):2047–57. [PubMed: 17901988]
103. Batal I, Liang K, Bastacky S, et al. Prospective assessment of C4d deposits on circulating cells and renal tissues in lupus nephritis: a pilot study. Lupus. 2012; 21(1):13–26. [PubMed: 21959138]
104. Li SJ, Liu ZH, Zen CH, et al. Peritubular capillary C4d deposition in lupus nephritis different from antibody-mediated renal rejection. Lupus. 2007; 16(11):875–80. [PubMed: 17971360]
105. Cohen D, Koopmans M, Kremer Hovinga IC, et al. Potential for glomerular C4d as an indicator of thrombotic microangiopathy in lupus nephritis. Arthritis Rheum. 2008; 58(8):2460–9. [PubMed: 18668574]
106. Dhir V. Is cellular C4d a good biomarker for SLE nephritis? Lupus. 2012; 21(9):1036. [PubMed: 22472701]
107. Liu CC, Manzi S, Kao AH, et al. Reticulocytes bearing C4d as biomarkers of disease activity for systemic lupus erythematosus. Arthritis Rheum. 2005; 52(10):3087–99. [PubMed: 16200588]
108. Navratil JS, Manzi S, Kao AH, et al. Platelet C4d is highly specific for systemic lupus erythematosus. Arthritis Rheum. 2006; 54(2):670–4. [PubMed: 16447243]
109. Edelbauer M, Kshirsagar S, Riedl M, et al. Activity of childhood lupus nephritis is linked to altered T cell and cytokine homeostasis. J Clin Immunol. 2012; 32(3):477–87. [PubMed: 22228566]
110. Campbell S, Michaelson J, Burkly L, et al. The role of TWEAK/Fn14 in the pathogenesis of inflammation and systemic autoimmunity. Front Biosci. 2004; 9:2273–84. [PubMed: 15353286]
111. Campbell S, Burkly LC, Gao HX, et al. Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. J Immunol. 2006; 176(3):1889–98. [PubMed: 16424220]
112. Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. Clin Rev Allergy Immunol. 2011; 40(3):138–50. [PubMed: 20127204]
113. Schwartz N, Michaelson JS, Putterman C. Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis. Ann N Y Acad Sci. 2007; 1109:265–74. [PubMed: 17785315]
114. Schwartz N, Su L, Burkly LC, et al. Urinary TWEAK and the activity of lupus nephritis. J Autoimmun. 2006; 27(4):242–50. [PubMed: 17257812]
115. Schwartz N, Rubinstein T, Burkly LC, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther. 2009; 11(5):R143. [PubMed: 19785730]
116. Luster AD. Chemokines—chemotactic cytokines that mediate inflammation. N Engl J Med. 1998; 338(7):436–45. [PubMed: 9459648]
117. Bauer JW, Baechler EC, Petri M, et al. Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. PLoS Med. 2006; 3(12):e491. [PubMed: 17177599]
118. Avihingsanon Y, Phumesin P, Benjachat T, et al. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. Kidney Int. 2006; 69(4): 747–53. [PubMed: 16518330]
119. Wang G, Lai FM, Tam LS, et al. Urinary FOXP3 mRNA in patients with lupus nephritis—relation with disease activity and treatment response. Rheumatology (Oxford). 2009; 48(7):755–60. [PubMed: 19458162]
120. Valencia X, Yarboro C, Illei G, et al. Deficient CD4+CD25 high T regulatory cell function in patients with active systemic lupus erythematosus. J Immunol. 2007; 178(4):2579–88. [PubMed: 17277168]
121. McHale JF, Harari OA, Marshall D, et al. TNF-alpha and IL-1 sequentially induce endothelial ICAM-1 and VCAM-1 expression in MRL/lpr lupus-prone mice. J Immunol. 1999; 163(7):3993–4000. [PubMed: 10491002]
122. Alon R, Kassner PD, Carr MW, et al. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. J Cell Biol. 1995; 128(6):1243–53. [PubMed: 7534768]

123. Kiani AN, Wu T, Fang H, et al. Urinary vascular cell adhesion molecule, but not neutrophil gelatinase-associated lipocalin, is associated with lupus nephritis. J Rheumatol. 2012; 39(6): 1231–7. [PubMed: 22505707]

124. Abd-Elkareem MI, Al Tamimy HM, Khamis OA, et al. Increased urinary levels of the leukocyte adhesion molecules ICAM-1 and VCAM-1 in human lupus nephritis with advanced renal histological changes: preliminary findings. Clin Exp Nephrol. 2010; 14(6):548–57. [PubMed: 20714774]

125. Ikeda Y, Fujimoto T, Ameno M, et al. Relationship between lupus nephritis activity and the serum level of soluble VCAM-1. Lupus. 1998; 7(5):347–54. [PubMed: 9696139]

126. Singh S, Wu T, Xie C, et al. Urine VCAM-1 as a marker of renal pathology activity index in lupus nephritis. Arthritis Res Ther. 2012; 14(4):R164. [PubMed: 22788914]

127. Goodsaid F, Frueh F. Biomarker qualification pilot process at the US Food and Drug Administration. AAPS J. 2007; 9(1):E105–8. [PubMed: 17408233]

128. Goodsaid FM, Frueh FW, Mattes W. Strategic paths for biomarker qualification. Toxicology. 2008; 245(3):219–23. [PubMed: 18280028]

129. Mina R, von Scheven E, Ardoin SP, et al. Consensus treatment plans for induction therapy of newly diagnosed proliferative lupus nephritis in juvenile systemic lupus erythematosus. Arthritis Care Res (Hoboken). 2012; 64(3):375–83. [PubMed: 22162255]

130. Ad Hoc Working Group on Steroid-Sparing Criteria in Lupus. Criteria for steroid-sparing ability of interventions in systemic lupus erythematosus: report of a consensus meeting. Arthritis Rheum. 2004; 50(11):3427–31. [PubMed: 15529380]

131. Pons-Estel GJ, Alarcon GS, McGwin G Jr, et al. Protective effect of hydroxychloroquine on renal damage in patients with lupus nephritis: LXV, data from a multiethnic US cohort. Arthritis Rheum. 2009; 61(6):830–9. [PubMed: 19479701]

132. Henderson L, Masson P, Craig JC, et al. Treatment for lupus nephritis. Cochrane Database Syst Rev. 2012; (12):CD002922. [PubMed: 23235592]

133. Henderson LK, Masson P, Craig JC, et al. Induction and maintenance treatment of proliferative lupus nephritis: a meta-analysis of randomized controlled trials. Am J Kidney Dis. 2013; 61(1): 74–87. [PubMed: 23182601]

134. Sundel R, Solomons N, Lisk L. Efficacy of mycophenolate mofetil in adolescent patients with lupus nephritis: evidence from a two-phase, prospective randomized trial. Lupus. 2012; 21(13): 1433–43. [PubMed: 22922564]

135. Lehman TJ, Sherry DD, Wagner-Weiner L, et al. Intermittent intravenous cyclophosphamide therapy for lupus nephritis. J Pediatr. 1989; 114(6):1055–60. [PubMed: 2656961]

136. Daleboudt GM, Reinders ME, den Hartigh J, et al. Concentration-controlled treatment of lupus nephritis with mycophenolate mofetil. Lupus. 2013; 22(2):171–9. [PubMed: 23257398]

137. Sagcal-Gironella AC, Fukuda T, Wiers K, et al. Pharmacokinetics and pharmacodynamics of mycophenolic acid and their relation to response to therapy of childhood-onset systemic lupus erythematosus. Semin Arthritis Rheum. 2011; 40(4):307–13. [PubMed: 20655577]

138. Sherwin CM, Fukuda T, Brunner HI, et al. The evolution of population pharmaco-kinetic models to describe the enterohepatic recycling of mycophenolic acid in solid organ transplantation and autoimmune disease. Clin Pharmacokinet. 2011; 50(1):1–24. [PubMed: 21142265]

139. Swan JT, Riche DM, Riche KD, et al. Systematic review and meta-analysis of immunosuppressant therapy clinical trials in membranous lupus nephritis. J Investig Med. 2011; 59(2):246–58.

140. Gunnarsson I, Sundelin B, Jonsdottir T, et al. Histopathologic and clinical outcome of rituximab treatment in patients with cyclophosphamide-resistant proliferative lupus nephritis. Arthritis Rheum. 2007; 56(4):1263–72. [PubMed: 17393458]

141. Vigna-Perez M, Hernandez-Castro B, Paredes-Saharopulos O, et al. Clinical and immunological effects of rituximab in patients with lupus nephritis refractory to conventional therapy: a pilot study. Arthritis Res Ther. 2006; 8(3):R83. [PubMed: 16677395]

142. van Vollenhoven RF, Gunnarsson I, Welin-Henriksson E, et al. Biopsy-verified response of severe lupus nephritis to treatment with rituximab (anti-CD20 monoclonal antibody) plus

cyclophosphamide after biopsy-documented failure to respond to cyclophosphamide alone. Scand J Rheumatol. 2004; 33(6):423–7. [PubMed: 15794203]

143. Fra GP, Avanzi GC, Bartoli E. Remission of refractory lupus nephritis with a protocol including rituximab. Lupus. 2003; 12(10):783–7. [PubMed: 14596429]
144. Jonsdottir T, Zickert A, Sundelin B, et al. Long-term follow-up in lupus nephritis patients treated with rituximab—clinical and histopathological response. Rheumatology (Oxford). 2013; 52(5): 847–55. [PubMed: 23287364]
145. Rovin BH, Furie R, Latinis K, et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. Arthritis Rheum. 2012; 64(4):1215–26. [PubMed: 22231479]
146. Manzi S, Sanchez-Guerrero J, Merrill JT, et al. Effects of belimumab, a B lymphocyte stimulator-specific inhibitor, on disease activity across multiple organ domains in patients with systemic lupus erythematosus: combined results from two phase III trials. Ann Rheum Dis. 2012; 71(11): 1833–8. [PubMed: 22550315]
147. Dhaun N, Kluth DC. Belimumab for systemic lupus erythematosus. Lancet. 2011; 377(9783): 2079–80. [author reply: 2080–1]. [PubMed: 21684372]

KEY POINTS

- Lupus nephritis is frequently diagnosed in children with systemic lupus erythematosus and warrants close medical attention to avoid progression to end-stage renal disease.
- Diagnosis of lupus nephritis requires at present a kidney biopsy.
- Current laboratory tests used to monitor lupus nephritis lack accuracy, making appropriate management difficult.
- Novel urine biomarkers hold promise for improving the approach to the surveillance of lupus nephritis and interpretation of patient response to therapy.
- Despite the lack of adequately powered clinical trials, standardized approaches to the therapy for children and adolescents with lupus nephritis are now available.

Table 1

Classification and interpretation of lupus nephritis biopsy findings

ISN/RPS Lupus Nephritis Classification Criteria	
Class I	Minimal mesangial lupus nephritis
Class II	Mesangial proliferative lupus nephritis
Class III	Focal lupus nephritis[a]
Class IV	Diffuse segmental (IV-S) or global (IV-G) lupus nephritis[b]
Class V	Membranous lupus nephritis[c]
Class VI	Advanced sclerosing lupus nephritis

NIH Activity and Chronicity Index[d]	
Active Lesions	**Chronic Lesions**
1. Endocapillary hypercellularity, with or without leukocyte infiltration and with substantial luminal reduction	1. Glomerular sclerosis (segmental, global)
2. Karyorrhexis (fibrinoid necrosis)[e]	2. Fibrous adhesions
3. Rupture of glomerular basement membrane	3. Fibrous crescents
4. Crescents (cellular or fibrocellular)[e]	4. Tubular atrophy
5. Subendothelial deposits identifiable by light microscopy (wireloops)	
6. Intraluminal immune aggregates (hyaline thrombi)	
NIH Activity Index 0–24	NIH Chronicity Index 0–12

[a] Indicates the proportion of glomeruli with active and with sclerotic lesions.

[b] Indicates the proportion of glomeruli with fibrinoid necrosis and cellular crescents.

[c] Class V may occur in combination with class III or IV, in which case both will be diagnosed.

[d] Each item scored from 0 to 3 depending on degree of involvement: 0 = no lesions; 1 = <25% of glomeruli; 2 = 25%–50% of glomeruli; 3 = >50% of glomeruli.

[e] These items scores have a weight of 2.

Table 2

Estimation of GFR (eGFR) in children in comparison with the reference standard of inulin clearance (iGFR)

Comparators	**Modified Schwartz Formula**[a]	**Le Bricon**[b]
	eGFR = 36.5 × Height (cm)/Cr	eGFR 5 (78/Cys) + 4
eGFR means ± SD (mL/min per 1.73 m^2)	109 ± 44[c]	99 ± 26
iGFR-eGFR means ± SD (mL/min per 1.73 m^2)	−8 ± 29	2 ± 19
Accuracy 10% (%)[d]	38	46
Accuracy 30% (%)	84	90
Accuracy 50% (%)	96	98
Correlation between eGFR and iGFR	0.779[e]	0.784[e]

[a] Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009;20:629–37.

[b] Le Bricon T, Thervet E, Froissart M, et al. Plasma cystatin C is superior to 24-h creatinine clearance and plasma creatinine for estimation of glomerular filtration rate 3 months after kidney transplantation. *Clin Chem* 2000;46;1206–7.

[c] $P<.05$, Wilcoxon paired test, in comparison with inulin clearance.

[d] Interpretation: 38% of the patient's eGFR is within 10% of the reference standard, ie, inulin clearance.

[e] $P<.001$; Spearman correlation coefficient.

Table 3

Phases of biomarker discovery, translation, and validation

Phase	Terminology	Action Steps
Phase 1	Preclinical discovery	Discover biomarkers in tissues or body fluids Confirm and prioritize promising candidates
Phase 2	Assay development	Develop and optimize clinically useful assay Test on existing samples of established disease
Phase 3	Retrospective study	Test biomarker in completed clinical trial Test if biomarker detects the disease early Evaluate sensitivity, specificity, receiver-operating characteristic
Phase 4	Prospective screening	Use biomarker to screen population Identify extent and characteristics of disease Identify false referral rate
Phase 5	Disease control	Determine impact of screening on reducing disease burden

From Devarajan P. Proteomics for biomarker discovery in acute kidney injury. Semin Nephrol 2007;27(6):637–51; with permission.