HHS Public Access

Author manuscript

Transl Res. Author manuscript; available in PMC 2019 November 01.

Published in final edited form as:

Transl Res. 2018 November ; 201: 26–39. doi:10.1016/j.trsl.2018.08.002.

Precision medicine in lupus nephritis: can biomarkers get us there?

Dawn J. Caster[1,2], **Michael L. Merchant**[1], **Jon B. Klein**[1,2], and **David W. Powell**[1]

[1]Department of Medicine, University of Louisville School of Medicine, Louisville, KY

[2]Robley Rex Veterans Affairs Medical Center, Louisville, KY

Abstract

Patients with systemic lupus erythematosus (SLE) frequently develop lupus nephritis (LN), a condition that can lead to end stage kidney disease (ESKD). Multiple serum and urine biomarkers for LN have been proposed in recent years, yet none have become incorporated into clinical use. The majority of studies have been single center with significant variability in cohorts, assays, and sample storage, leading to inconclusive results. It has become clear that no single biomarker is likely to be sufficient to diagnose LN, identify flares, and define the response to therapy and prognosis. A more likely scenario is a panel of urine, serum, tissue, and genetic biomarkers. In this review we summarize traditional and novel biomarkers and discuss how they may be utilized in order to bring precision medicine to clinical practice in LN.

Keywords

biomarkers; lupus nephritis; systemic lupus erythematosus; autoantibodies; precision medicine

Background

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with contribution from genetic, environmental, immunoregulatory, hormonal, and epigenetic factors [1]. The development of SLE occurs when there is a loss of self-tolerance, propagated by poor clearance of apoptotic bodies, and the subsequent development of anti-nuclear antibodies. This, along with immune complex formation, cytokine production, and activation of complement, leads to end organ damage. Kidney involvement, termed lupus nephritis (LN), one of the most critical manifestations of SLE, occurs in approximately 50% of patients with SLE [2]. While clinical outcomes overall have improved for LN, a large number of patients still go on to develop End Stage Kidney Disease (ESKD) and outcomes remain poor among certain ethnic groups, notably African Americans [3, 4].

Corresponding Author: Dawn J. Caster, 570 South Preston Street, Baxter Research Building I, 102D Louisville KY, 40202, dawn.caster@louisville.edu, Phone: 502-852-0014, Fax: 502-852-4384.

The treatment of LN has experienced a slow evolution since the introduction of corticosteroids in the 1950s. Cyclophosphamide therapy was added in the 1970s and then mycophenolate mofetil (MMF) became available in the 2000s. After decades of research, non-specific immunosuppressive regimens remain the core of LN treatments. Newer strategies for therapy, with better efficacy and less toxicity are long overdue. In recent years, several more specific biologic agents have been tested, many of which target specific inflammatory pathways. However, after more than a decade of clinical trials, none have been shown to have added efficacy in LN compared to standard of care [5]. This is likely in part due to poor patient selection due to inadequate current biomarkers. The pathogenesis of LN is complex with genetic and environmental contributions leading to altered immune regulation and local inflammation. For an individual patient, one pathway may be contributing more to the disease process than another. Biomarkers identifying the pathways upregulated in an individual patient may help identify the best therapy for that patient, making precision medicine a reality in the treatment of LN.

What is a Biomarker?

Biomarkers have been defined as biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or response to intervention [6]. Ideal LN biomarkers will: (1) identify those at risk for developing disease, (2) distinguish between active and chronic disease, (3) guide choice and duration of therapy, (4) be easily reproducible in a commercially available assay at a reasonable cost.

Classic Biomarkers

The diagnostic standard for LN is a kidney biopsy. The kidney biopsy, however, is invasive, and only provides a single “snapshot” of an evolving pathogenic process. The International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system for LN was created in 2004 and has replaced the former World Health Organization (WHO) classification system [7]. The classification system is focused on glomerular changes on light microscopy and divides LN into 6 classes (table 1). One of the drawbacks of the ISN/RPS system is that it does not include tubulointerstitial injury or vascular lesions that are both risk factors for disease progression [8–12]. The ISN/RPS classification system also provides limited information on disease activity. Some pathologists continue to report the NIH Activity and Chronicity Index score, but this is not universally used [13]. An international nephropathology working group recently proposed updates to the ISN/RPS classification system to address the limitations with the current system [14]. This will need to be validated in practice and studies to evaluate whether immunosuppression can be tailored to activity index are needed.

Current laboratory values such as serum creatinine, proteinuria, and hematuria remain important clinical and diagnostic biomarkers for lupus nephritis. The presence of proteinuria and hematuria determines referral for initial kidney biopsy. Serum creatinine elevation is often a late finding, occurring only after the accrual of significant renal damage, making it unsatisfactory as an initial biomarker. Patients with a history of LN may have elevations in

creatinine due to progression of fibrosis rather than flare. Repeat biopsy studies demonstrate discordance between clinical renal remission (defined by resolution of proteinuria and hematuria) and histologic remission (defined by low activity index on kidney biopsy) [15, 16]. Malvar et al. demonstrated that nearly 1/3 of patients with clinical renal remission continued to have histologic evidence of lupus activity while a large number of patients with histologic remission (62%) had continued clinical activity [15].

Of the classic biomarkers, proteinuria appears to be the strongest predictor of long term renal outcome. Long term follow up of LN patients in two large European Trials demonstrated proteinuria at one year was the best predictor of long term renal outcome [17, 18]. Further studies are needed to evaluate whether this measure may help stratify patients and guide length of immunosuppressive therapy.

Autoantibodies

Antinuclear antigens

The production of antibodies against nuclear antigens is the hallmark of SLE. ANA is highly sensitive, but less specific, especially at low titers. ANA can also be elevated in other autoimmune conditions and are further characterized by subtypes of Extractable Nuclear Antibodies (ENA). ENA include antibodies to Smith (Sm), Ro (SS-A), La (SS-B), ribonucleoprotein (RNP), histones, and double stranded DNA (dsDNA). Of these, anti-dsDNA is the most specific for SLE and LN.

Anti-dsDNA

Anti-dsDNA antibodies were first identified over fifty years ago and have remained both ubiquitous and contested since their discovery[19]. The predictability of anti-dsDNA in the development of nephritis is complicated by heterogeneity in testing, isotype, and cross reactivity of the antibody to protein antigens. The most commonly available clinical lab tests include immunofluorescence (i.e. *Crithidia lucilae*) and enzyme-linked immunosorbent assays (ELISAs). The Farr assay for anti-dsDNA is a radioimmunoassay in which serum is incubated with radiolabelled dsDNA followed by the addition of ammonium sulfate for immune complex precipitation[20]. Ammonium sulfate precipitation, dissociates immune complexes of low avidity, leaving only high avidity antibodies, making the test highly specific[21].

Several studies have demonstrated increases in anti-dsDNA proceeding SLE and LN flare, anti-dsDNA have been eluted from nephritic kidneys, and animal models have demonstrated pathogenicity of anti-dsDNA [22, 23]. Conversely, not all patients who have anti-dsDNA antibodies develop nephritis and a small percentage of patients with nephritis do not have antibodies to dsDNA [24–26]. Julkunen et al. found the positive predictive value of anti-dsDNA varied between 11–16% while the negative predictive value was 86–91%, in a cohort of 223 patients with SLE [25]. Moroni et al. found the positive and negative predictive value of anti-dsDNA to predict LN flares to be 31% and 91%, respectively, in a prospective longitudinal study of 228 patients with LN [26].

The nephritogenic properties of anti-dsDNA antibodies have been attributed to 3 potential mechanisms: (1) glomerular binding of preformed immune complexes of anti-dsDNA and nucleosomes, (2) binding of anti-dsDNA to chromatin or nucleosomes that have become "planted antigens" glomerular basement membrane (GBM), and (3) binding of anti-dsDNA to native "cross reactive" antigens in the glomerulus [27–29]. To date, there has not been a clinical test that is able to differentiate nephritogenic isoforms of anti-dsDNA from non-nephritogenic forms.

Glomerular Antigens

Several glomerular antigens have been identified as potential targets of autoantibodies in LN, including cross reactive with anti-DSNA or anti-nucleosome antibodies. These include cell specific targets and proteins found on the glomerular basement membrane (table 2). The identification of glomerular antigens provides insight into the pathogenesis of LN. Unfortunately, no assay has been validated in multicenter cohorts.

Alpha-Actinin

The alpha-actinins are a family of four actin binding proteins of which isoforms 1 and 4 are expressed in the glomerulus [30, 31]. Mutations in alpha-actinin-4 lead to podocytopathy and familial FSGS [32]. Glomerular alpha-actinin can be targeted by anti-dsDNA [33, 34]. Lupus prone (MRL/*lpr*) mice have high levels of circulating antibodies to alpha-actinin that can bind to both alpha-actinin-1 and 4 [31]. Non-autoimmune (BALB/c) mice immunized with alpha-actinin develop anti-alpha-actinin antibodies and develop immune complex glomerulonephritis and proteinuria [35]. Antibodies binding alpha-actinin have been identified in patients with LN [36–38].

Annexins

Annexin autoantibodies have been implicated in a number of autoimmune diseases including SLE and antiphospholipid antibody syndrome (APS) and was recently reviewed by Iaccarino and colleagues [39]. The annexin family contains 12 proteins that are calcium dependent phospholipid binding proteins, many of which are expressed in the kidney [39, 40]. They are involved in a number of cell processes including apoptosis, calcium signaling, cell growth, division, and vesicle trafficking [39].

Annexin A1 (ANXA1) is a ubiquitous protein, present in immune cells and resident glomerular cells. In immune cells, ANXA1 helps regulate innate and adaptive immune responses [39, 41]. Circulating IgM and IgG antibodies to ANXA1 were initially identified in patients with SLE and the IgM isotype correlated with activity level [42]. Bruschi et al. identified IgG2 antibodies to ANXA1 in the glomeruli obtained from LN patient biopsy specimens and identified the same isoform in the sera of LN patients [43].

Annexin A2 (ANXA2) is expressed in glomerular mesangial cells, endothelial cells, and epithelial cells [44, 45]. The function of ANXA2 varies with cell type and cellular location. In epithelial cells, such as podocytes, ANXA2 plays a critical role in dynamic remodeling of the actin cytoskeleton [44, 46]. ANXA2 on the cell surface interacts with beta 2-glycoprotein I and toll like receptor 4, leading to proinflammatory and prothrombotic

effects, which may be enhanced by autoantibody binding [47]. AA2 has been identified as an autoantibody in primary and secondary APS [39]. Multiple groups have independently identified ANXA2 as a target for autoantibodies in LN [45, 48, 49]. Cross-reactivity of anti-dsDNA with mesangial ANXA2 has been reported, and ANXA2 co-localizes with glomerular IgG and C3 deposits in LN [45]. Our group demonstrated that patients with class III/IV LN, but not class V LN have elevated autoantibodies to ANXA2 [48].

Alpha Enolase

Alpha Enolase is a multifunctional protein and its location in the cell helps determine its function [50]. Intracellularly, it serves as a glycolytic enzyme while it acts as a plasminogen receptor on the cell surface [50]. Antibodies to alpha-enolase have been identified in multiple immune mediated kidney diseases including LN, mixed cryoglobulinemia, and primary membranous nephropathy [43, 51, 52].

It has been proposed that exposure to alpha-enolase may initially occur in the setting of neutrophil extracellular trap (NET) formation [41]. NET formation, termed NETosis, is a unique form of cell death utilized by neutrophils to entrap microbes. In NETosis, the plasma membrane ruptures releasing strands of chromatin decorated with neutrophil granular proteins as well as other intracellular proteins [53]. A proteomic analysis of isolated human NETs identified nuclear proteins, granule proteins, and other intracellular proteins including alpha enolase [54]. NETosis is a key player in the development of SLE and LN [55–59]. SLE patients with impaired degradation of NETs are more likely to have LN and hypocomplementemia [55, 60].

Glomerular Basement Membrane Proteins

Autoantibodies may bind to proteins within the glomerular basement membrane (GBM) directly or to nucleosomes which have become “planted antigens” bound to GBM proteins [28, 61–63]. Nucleosomes have demonstrated a high affinity for negatively charged laminin, heparan sulfate, and collagen IV [63–65]. A recent study demonstrated that nucleosomes bind avidly to aberrantly expressed laminin β1in human LN [63]. Autoantibodies binding heparan sulfate have been identified in both animal models and human LN [61, 66].

While multiple glomerular antigen targets have been proposed, no clinical test exists for measuring glomerular autoantibodies in LN. While it is reassuring that many of the antigens have been identified by different centers with an ethnically diverse range of patients, no standardized approach has been utilized. While some centers have measured cross reactive anti-dsDNA, others have looked at reactivity alone. Additionally, isotype specificity may be critical for some autoantibodies [41]. In the future, a panel of glomerular autoantibodies might help identify patients with LN.

Complement Pathway

The complement system includes three activation pathways: the classical pathway, alternative pathway, and the lectin pathway. The classical pathway is activated when C1q binds to IgM or IgG in immune complexes. The alternative pathway is spontaneously activated by a conformational change to C3. The lectin pathway is activated when mannose

binding lectin binds to carbohydrate groups which are found on microorganisms. All three pathways have been implicated in LN [67–69]. Activation of each pathway leads to the generation of C5b-9, also known as the membrane attack complex (MAC), which forms pores in the membranes of target cells, causing cell activation and lysis[70].Complement is a “double edged sword” in SLE as genetic complement deficiencies are strongly associated with the development of SLE while complement activation plays a key role in perpetuating the disease [71–73].

Serum C3 and C4 measurements are readily available as clinical tests and can be used alone or within an integrated disease activity score such as the SLE Disease Activity Index (SLEDAI) score [74]. The SLEDAI score is a composite score of serum C3 and C4 measurements along with more than 20 other weighted variables to support the clinical assessment of disease activity within the preceding 10 days[75]. C4 is a component of the classical pathway, which is triggered when C1q binds to immune complexes. C3 is a component of the alternative pathway. Studies to determine how changes in complement levels can serve as SLE or LN flare have yielded variable results [25, 76–78]. Reduced C3 levels are more predictive of active LN flare [77, 79]. A longitudinal study revealed that reduced C4 levels, 2 months prior to clinical manifestations of LN flare were predictive of future flare, whereas reduced levels of C3 were seen at time of the flare [79].

C1q is a component of the classical complement pathway and is critical for opsonization and clearance of apoptotic bodies and immune complexes [80]. Genetic deficiencies of C1q are rare, but are almost universally associated with SLE like autoimmunity, highlighting poor clearance of apoptotic bodies as an important pathogenic mechanism in the development of SLE [80, 81]. Auto-antibodies to C1q are present in a significant number of SLE patients and correlate strongly with renal involvement [24, 82–84]. Several studies demonstrated that the predictive value of was enhanced when combined with anti-dsDNA antibodies and/or hypocomplementemia [26, 82, 83, 85].

Orbai et al. conducted an international cross-sectional study of anti-C1q in patients with SLE vs. rheumatic disease controls from 25 clinical sites to examine the specificity of anti-C1q for SLE and look at the association with nephritis. They found that anti-C1q antibodies were present in 28% of patients with SLE vs. 13% of disease controls. Further, the anti-C1q was associated with proteinuria (OR=3, $p < 0.001$) and red cell casts (OR=2.6, $p=0.015$). Further, they found that the combination of anti-C1q, anti-dsDNA, and low complement was strongly associated with renal involvement (OR=14.9, $p<0.01$) [82].

Moroni et al. examined anti-C1q antibodies in a prospective study of 228 patients with LN to evaluate the role of immunological tests for monitoring LN activity [26]. They found that elevation of anti-C1q antibodies predicted LN flares with 80.5% sensitivity and 71% specificity. This was slightly better than the other individual markers. Anti-C1q combined with C3 and C4 provided the best performance for predicting renal flares at multivariate analysis ($p<0.0005$, $p<0.0005$, $p<0.005$ respectively). Further, the combination of 4 negative tests (negative antibody and normal complement levels) had a high negative predictive value. While their results were promising, they also noted that 46% of non-proliferative (class V) and 20% of proliferative (class III/IV) flares were not associated with high anti-C1q levels

[26]. A recent retrospective study by Bock et al. demonstrated that anti-C1q levels correlated well with global disease activity scores in patients with nephritis, but not in patients without kidney disease. Further they demonstrated a positive correlation between anti-C1q levels and proteinuria and anti-dsDNA, and a negative correlation with complement levels [84].

Terminal complement activation and formation of C5b-9 (MAC) may contribute to the severity of LN. Lupus prone (MRL/*lpr*) mice deficient in the complement regulating protein, factor H, demonstrate increased disease severity [72]. Treatment of lupus prone (NZB/W F1) mice with a monoclonal antibody to C5, prevented formation of C5b-9 (MAC), and attenuated LN [86]. Song et al. demonstrated elevated serum levels of soluble C5b-9 in patients with active LN when compared to patients with LN in remission, active SLE without nephritis, and normal controls [69]. A recent study demonstrated that C5b-9 (MAC) staining on kidney biopsy is associated with poor clinical response to treatment at 6 months [87].

The *combination* of anti-dsDNA, anti-C1q, and C3, C4 levels make up a powerful predictive serum biomarker for LN. Unfortunately, the use of anti-C1q measurements in clinical practice has been limited as it is not widely available in commercial laboratories [88]. Initial assays were technically difficult. Emerging multiplex technologies may finally provide an avenue to help facilitate anti-C1q antibody testing [89]. Whether the use of anti-C1q testing improves management of LN patients remains a matter of future research that requires more widespread availability of the test. Terminal complement activation may contribute to the severity of LN [69, 87]. As new anti-complement therapies emerge, markers of terminal complement activation (increased serum levels of soluble C5b-9 and/or increased tissue expression of C5b-9) may help identify those who would benefit most from anti-complement therapy. At this time, there is limited data on the use of anti-complement therapy in LN. Anticomplement therapy been reported successfully in cases of refractory LN, especially in the setting of concomitant thrombotic microangiopathy [90].

Urinary Biomarkers

From both a clinical and pathogenesis standpoint, urine appears to be the “low hanging fruit” of the LN biomarker world. Urine is easy to obtain and real time changes in the inflammatory milieu of the kidney tissue should translate to measurable differences in urine. These observations are hampered by vast differences in processing, which have variable effects on measurable protein levels, lack of uniformity in assays, and lack of agreement regarding whether there should be a “normalization” step. Additional drawbacks include prevailing use of single center cohorts, the use of tests not commercially available for use in the clinical setting, and unintended longitudinal effects of storage buffers in biorepository samples. Many recent studies have moved away from urinary protein abundance levels of individual markers to multi-marker panels, urinary protein fragments, urine microRNA, protein levels in urine pellets, and urinary cells.

Neutrophil Gelatinase-Associated Lipicalin (NGAL)

NGAL is a small glycosylated protein produced in many tissues, but initially described in neutrophils. Urine and serum NGAL have been studied extensively as a biomarker in acute

kidney injury [91]. In a pediatric lupus cohort, Suzuki et al. found that urinary NGAL, but not serum NGAL levels correlated with renal disease activity [92]. In their analysis they demonstrated that levels increased whether or not they normalized urine NGAL to urine creatinine levels [92]. In a longitudinal study of two ethnically diverse adult SLE cohorts (Bronx, NY; London, UK), urine NGAL level was shown to predict renal activity and was a predictor of flares in patients with known LN [93]. Similarly Satirapoj et al found that with the exception to serum C3 levels urinary NGAL at baseline to be a better predictor of clinical response to treatment of active LN [94].

Monocyte chemoattractant protein 1 (MCP1)

MCP-1 is a leukocyte chemokine that is involved in mediating inflammation in LN. In animal models of LN, blockade of MCP-1 attenuates renal damage [95]. In multiple single center studies, urine MCP-1 level corresponded to LN activity/flare and decreased after disease remission [96–98]. Further, patients who did not respond to treatment appear to have persistently elevated levels [96, 97].

Tumor Necrosis Factor-Like Inducer of Apoptosis (TWEAK)

Tweak is a member of the TNF superfamily and is a multifunctional cytokine involved in inflammatory, apoptotic, and fibrotic pathways [99, 100]. Tweak is thought to play a role in the development of lupus nephritis through increased burden of apoptotic materials and increased inflammation [99, 100]. Urinary tweak was evaluated as a biomarker for lupus nephritis in multicenter cohort study and was found to perform better than anti-dsDNA and complement levels [101]. Xuejing et al. measured urinary TWEAK levels in 46 patients undergoing routine kidney biopsy and found urinary Tweak levels significantly correlated to the activity index ($P<0.05$) [102]. Unfortunately, despite promising pre-clinical studies [103–105], a phase 2 clinical trial utilizing a TWEAK inhibitor was terminated early after failing to meet primary endpoints [5]. It is unclear if stratification of patients based on urinary TWEAK levels would have had any effect on the results and at this time those data are unavailable.

Lessons Learned and Longitudinal Sample Storage Affects

Efficient collection and storage of patient samples is required for the study of current and novel urinary biomarkers. A critical component of study success, beyond maintaining constant and continuous storage at ultra-low temperatures, are sample handling and preservation protocols-aliquot volumes, timed versus spot urine samples, addition of inhibitory compounds such as azide or protease inhibitors. A variety of preservatives are used in urine collections including acetic acid, boric acid, hydrochloric acid, toluene, and thymol, each with different effects on substances measured in the urine (https://www.mayomedicallaboratories.com/it-mmfiles/Urine_Preservatives-Collection_and_Transportation_for_24-Hour_Urine_Specimens.pdf).

A study by Almaani et al. investigated the effects of acetic acid preservative (final concentration 0.5% acetic acid) to the bioreposited samples of patients enrolled in the African American Study of Kidney Disease and Hypertension (AASK) Cohort Study [106]. Prior analysis of AASK patients with overt proteinuria demonstrated high levels of non-

albumin proteinuria, consistent with tubulopathy. Urine biorepositedwith the National Institute of Diabetes and Digestive Kidney Diseases was obtained and tested for seven markers of tubular proteinuria-albumin, ß-2-microglobulin (B2M), cystatin-C (CysC), osteopontin (OPN), epidermal growth factor (EGF), uromodulin (UMOD) and neutrophil gelatinase-associated lipocalin (NGAL). ELISAs for the majority of markers demonstrated poor detectability. The investigators measured these same markers in matched urine samples from patients with lupus nephritis or healthy black controls. The mean values of six of the seven markers were consistently lower in the AASK-N samples. To determine the effect of sample integrity on the ELISA results, the AASK-N samples were studied by denaturing, reducing polyacrylamide gel electrophoresis. The Coomassie stained gels demonstrated the loss of well resolved protein bands and demonstrated severe protein degradation. To determine if the sample collection protocol was the source of the degradation lupus nephritis urine samples were adjusted to 0.5% acetic acid and resolved by gel electrophoresis. These test gels produced similar gel images of protein degradation profile as that of AASK-N urine. These data demonstrate the need for increased rigor in establishing biorepository protocols.

Urinary Biomarker Panels

Urinary biomarkers panels have been shown to help identify interstitial inflammation on kidney biopsy and outperformed individual biomarkers in a single center observational study[107]. One recent prospective observational cohort study of adult and pediatric patients demonstrated that a panel of urinary biomarkers performed better at predicting renal function decline than any single biomarker with the most improvement seen in the adult cohort [108]. They identified that the combination liver-type fatty acid binding protein (LFABP), albumin, MCP-1, and transferrin levels had good predictive accuracy of renal function decline in both pediatric (AUC=0.82) and adult (AUC= 0.77) cohorts [108].

Anti-chymotrypsin (ACT) is a serine proteinase inhibitor and has been associated with a number of chronic diseases such as Alzheimers, Parkinsons and chronic obstructive pulmonary disease but until recently not SLE or LN [109]. Aggrawal et al used a combination of two-dimensional gel electrophoresis and mass spectrometry to compare the urinary proteome of SLE patients [110]. Disease activity was stratified by SLEDAI [111]. The proteomes of patients with active versus inactive disease were compared and 26 gel spots were selected for MS characterization. Three proteins (ACT, haptoglobin, and retinol binding protein) were quantified in all patient samples. ACT and haptoglobin were well correlated with renal SLEDAI ($r > 0.57$ and $r > 0.59$ respectively). Longitudinal follow-up (6- and 12-month) for patients with active disease demonstrated a loss of all three proteins with response to treatment. While haptoglobin and retinol binding protein have previously been associated with LN this is the first report of ACT as a marker of LN.

The KDIGO and ACR guidelines for treatment of LN recommend change in induction therapy in the absence of a response after three to six months, but these recommendations lack consensus on quantitative clinical guidelines for the definition of a failed response. Wolf et al addressed the hypothesis that multivariate analyses incorporating standard clinical measures of renal damage and novel biomarkers of LN would lead to an improved decision

support tool for predicting outcomes in LN treatment [112]. For this study urine from 140 patients with biopsy proven LN were assembled spanning four patient cohorts including (a) the Medical University of South Carolina, (b) the Hopkins Lupus Cohort, (c) the LUNAR study by Genentech and (d) the Abatacept in Lupus Nephritis study by Bristol-Myers Squibb. All patients met the 1997 update of the revised ACR criteria for SLE and patient samples had to be collected from within 2-months of baseline. Patients had to have (1) biopsy proven active class II, III, IV, or V nephritis *or* (2) newly active nephritis (either 500mg increase in urine protein in 24h urine collection or a spot urine protein to creatinine ratio of 0.5). The patient design included 26% (37/140) complete responders (as defined by LUNAR trial parameters[113]) and 74% (103/140) non-responders. Over 50 serum and urinary clinical values were assessed including 46 urinary biomarkers spanning markers of resident and inflammatory cell activation (cytokines), chemokines, growth factors and damage to resident cells were quantified by multiplexed bead assays or enzymatic assays.

Univariate analyses by receiver operating characteristics (ROC) curves were used to assess individual markers for significance to response to therapy. ROC plots are a visual representation of the surrogate biomarker (s) performance as a classifier to define true positive (sensitivity) and false positive (1-specificity). The area under the curve (AUC) for the ROC plot may be interpreted in terms of sensitivity (true positive) and 1-specificity (false positive) for sample classification. A perfect biomarker would have a ROC AUC equal to 1.0 while a biomarker with the same sensitivity as a random coin toss would have a ROC equal to 0.5. In this study the best Five discriminating variables (ROC 0.57–0.67 and p-value <0.05) were: urinary protein to creatinine ratio, estimated GFR, osteoprotegrin (OPN), interleukin-2 receptor-α (IL-2Rα), and interleukin-8 (IL-8). Given the weak classification aspects of these ROC values the authors assess the classification performance of traditional markers (serum C3, C4, creatinine, anti-dsDNA antibody, and 24-hour protein or protein-to-creatinine ratio) with or without the additional novel biomarkers using multivariate (machine learning models based on Random Forest (RF) algorithms) models. The sensitivity of the combined marker group improved from an AUC of 0.32 (poor sensitivity for true positive and better than average sensitivity for false positive classification) to a better performing classifier- 0.76. While a common cutoff for acceptable ROC values for good versus poor models is 0.7, the preponderance of FDA-approved tests for kidney damage have ROCs in excess of 0.8 [114–116]. However, the combination of novel biomarkers to traditional markers did not improve the specificity. The improved sensitivity could be demonstrated with a minimal set of four markers (OPN, IL-2R α, IL-8, and TWEAK) plus the standard markers. OPN has been described as increased in patients with active LN [117]. TWEAK may have a role as both a biomarker and therapeutic target as described earlier in this manuscript.

Urine protein fragments

Progression of LN toward ESRD often proceeds with episodic and punctuated jumps through flare events. Ultimately the kidney suffers from both dysregulated glomerular and tubulointerstitial extracellular matrix remodeling. A window into that remodeling can be opened through direct mass spectrometric analysis of the low molecular weight urine proteome. This approach has been referred to most often as peptidomics as well as

fragmentomics and degradomics. Wei R et al used a capillary electrophoresis (CE)-MS method to analyze the urine of 71 SLE patients including 35 that had renal involvement and 36 that had no renal involvement[118]. These data were compared against the urine peptidome of 58 healthy controls. The abundances of ~300 urinary peptides including 70 collagen fragments were significantly associated with LN. Using a web-tool (Proteasix, proteasix.com) built to compare experimental peptide data against the peptidase motifs within the MEROPS Peptidase database, the authors were able to hypothesize the contributions of 14 specific proteases as being responsible for production of the collagen peptide fragments. Nine proteases differentiated the collagen peptides observed for the SLE +LN versus the SLE-LN samples. Twenty-eight collagen peptides has some value as surrogate diagnostic markers as they correlated to at least on clinical or histological metric of renal damage. These data provide a very promising future for the use of urinary peptidomics as a clinical measure of renal damage in SLE patients. Moreover these results suggest the activity of matrix metalloproteinase may be associated with the development of LN and hence represent targets for drug development.

Urine microRNA

Urine is a biofluid rich in prospective sources of surrogate biomarkers [119]. Mature microRNAs are small single stranded RNA 22–25nt oligomers that function to influence gene expression and regulation [120]. Extra-cellular microRNAs are thought to be stabilized outside cells by either forming a stable RNA:protein complex or residing in the lumen of extracellular vesicles [121]. Cardenas-Gonzales et al examined urinary microRNA as a non-invasive biomarker of kidney pathology using diabetic (n=58) and LN (n=89) CKD cohorts with comparison to healthy controls (n=93 and 119 respectively) [122]. The significant features (using Ct scores <28) for disease state associated microRNA differences included both increased and decreased abundances compared to healthy controls. But when considering both microRNA abundances with CKD (>3-fold for LN and >10-fold diabetes) and receiver operator classification (c-statistics >0.85) it was observed the strongest CKD associating features for diabetic (miR-2681, riR-1915–3p, and miR-4532) and lupus (miR-3201 and miR-1273e) microRNA did not overlap. Correlation analysis for these microRNA with patient suggested the diabetic alterations was most significantly associated with estimate glomerular filtration rate and interstitial fibrosis/atrophy while the LN were associated with endocapillary glomerular inflammation. Validation cohorts were used to verify and confirm expression differences of the diabetic markers between diabetic nephropathy versus diabetics without CKD and between LN and SLE patients. Finally the authors conducted experiments to confirm expression of microRNA in healthy renal tissue. The diabetic microRNAs were observed in the cytoplasm and nucleus in the glomerular and tubular compartments. While the LN-associated miR-3201 was not detected, miR-1273e was detected in the nuclei of the tubular epithelium. These data suggest the progression toward CKD in lupus may be associated with defects in microRNA regulation of gene expression.

Urine cells

Given that cells can be shed into the urine and these cells may reflect the underlying activation status of the kidney, Olivares et al use the cell pellet of an SLE cohort to quantify mRNA (quantitative reverse transcriptase-polymerase chain reaction) and protein

(immunoblot) levels of SIRT1 [123]. SIRT1 is categorized as a histone deactylase (HDAC) that requires NAD+ for enzymatic function (Class III HDAC). SIRT1 plays a variety of roles in gene expression through regulating the transcriptionally permissive state of chromatin and data from mouse studies of SLE suggests a plausible role in human disease [124]. Olivares demonstrated that both SIRT1 mRNA and protein were increased in the cell pellets of patients with active disease as compared to those patients in remission or healthy controls and correlated with anti-dsDNA antibody levels, histologic features of proliferative disease, and renal injury [123].

Ikuma et al demonstrated that the level of urinary podocytes and urinary podocalyxin (with normalization to urine creatinine) strongly correlated with histologic measure of LN in patients with active disease as compared to no renal involvement or health controls [125]. Urinary podocytes to creatinine levels were most strongly associated with the activity index and cellular crescents. Urinary podocalyxin to creatinine was highest in proliferative LN and membranous LN. The combination of both markers was associated with pure class V LN, which is often characterized by nephrotic syndrome and significant podocyte injury. These results show an intriguing association of transcriptionally permissive states with response disease activity/renal damage and likely associated with the podocyte.

Gene Specific Biomarkers

Defining genetic variants that reliably predict susceptibility or risk for development or progress and response to current, standard treatment of LN is invaluable towards improvement of patient care. These non-invasively measurable variants could serve as diagnostic tools and the known molecular and cellular pathways they mediate provide mechanistic targets for development of improved therapeutics.

Investigation of genetic variants contributing to SLE goes back nearly two decades, linking polymorphisms in major histocompatibility complex (MHC) to LN [126]. The completion of the human Genome Project in 2003 launched an era of genome-wide-association studies (GWAS) to identify genetic variants in specific disease cohorts. In the following years a number of GWAS studies of relatively small cohorts of SLE patients from mostly Japanese, Chinese, or European populations reported variants for IRF5, STAT4, ITGAM, BANK1, IRAK1, TNFAIP3, IL-18, Fcgamma Receptors, and Compliment factors [127–132]. Harley et al. published a more comprehensive review of the genes and polymorphisms discovered during this time period in 2009 [133]. They summarized that these genes fall into three general regulatory categories (immune-complex processing, immune signal transduction, and TLR-IFN pathway). That same year, two hallmark, large-scale SLE GWAS studies were reported, one with a Chinese Han population and the other mostly an American cohort that included SLE patients of European, Asian, Hispanic, and African Ancestry [134, 135]. The Chinese Han study genotyped 1,047 cases and 1,205 controls and replicated 78 polymorphisms in two independent cohorts with 3,152 cases and 7,050 controls. The American study included 1,963 cases and 4,329 controls. Together, these studies confirmed most of the previous variants that had been reported for the smaller cohort studies and identified several new genes including PRDM1, JAZF1, VHRF1BP1, Il10, IKZF1, RASGRP3, SLC15A4, and TNIP1. The common new gene identified in both studies was

TNIP1. TNIP1 encodes the protein ABIN1 that functions as a physiological inhibitor of NF-kB and MAPK signaling [136–139]. Our group found that transgenic mice globally expressing an inactivating ABIN1 mutation (ABIN1[D485N]) spontaneously developed systemic autoimmunity and progressive GN [140, 141]. We recently reported that glomerular expression of this inactive ABIN1 leads to increased glomerular inflammation and proteinuria in mice with acute anti-glomerular basement membrane (GBM) GN [142].

In the following years, a number of groups investigated association of some of these genes and different polymorphisms with development of LN. Several of those variants with replicate, confirmed association with LN will be discussed. Our group genotyped five SNPs in TNIP1 (rs4958881, rs2233287, rs7708392, rs999556, rs17728338) that were previous identified in SLE, systemic sclerosis, or psoriasis, in SLE patients with and without nephritis from the Large Lupus Association Study 2 [143] and found a strong associations with LN at rs7708392 in European Americans and rs4958881 in African Americans [141]. Two different groups reported a strong association with the ITGAM allele rs1143679 and LN, a study with 910 SLE Chinese patients living in Hong Kong that was replicated in 278 Thai SLE patients and a study with 2,366 SLE patients of European Ancestry [144, 145]. Large-scale genotyping of 8,329 SLE patients (European-derived, Hispanic, African-American, and Asian) for 16 confirmed SLE susceptibility loci also found that risk alleles in ITGAM associated with LN [146]. Li et al. reported that the Fcgamma receptor type IIIA V/F158 polymorphism was highly associated with LN in Asian and European decent individuals [147]. A meta-analysis of genetic data from 1453 Caucasian SLE patients found that patients with a TNFAIP3 rs5029939 risk allele had a higher risk of developing LN [148]. Lin et al. evaluated 167 variants spanning MYH9 for association with LN in multiethnic American SLE patient samples and found that MYH6 rs2157257 associated with LN in a European-American population [149]. Significant association was reported for two TNFSF4 polymorphisms (rs2205960 and rs10489266) is a study of 814 Chinese SLE patients [150]. A study of 534 African American SLE patients with and 534 without LN showed that the APOL1 nephrology risk alleles G1/G2 strongly impact the risk of LN-ESRD in African Americans [151].

The identified genetic risks for LN and their clinical application were thoroughly reviewed in 2015 by Munroe and James and in 2017 by Iwamoto and Niewold [152, 153]. The 2015 review lists over 50 disease susceptibility genes associated with LN and grouped them into five regulatory systems (program cell death, immune complex clearance, intrarenal pathogenesis, innate immunity, and adaptive immunity). The 2017 review describes 11 confirmed susceptibility loci associated with LN (MHC (HLA-DR), ITGAM, FcgammaIIA and IIIA, IRF5, TNIP1, STAT4, TNFSF4, APOL1, PDGFRA, and HAS2) and focused on their roles in innate and adaptive immunity and intrarenal inflammation. Of note, they describe a T Cell function for TNIP1, but we recently reported involvement of TNIP1 in intrarenal inflammation as well [154]. Perhaps many of these genes have multiple functions in the pathogenesis of LN.

Interestingly, APOL1, PDGFRA, and HAS2, which appear to all function in intrarenal inflammation, are genes with newly discovered association with LN, but have not been associated with SLE. Another gene of this type is PLA2R1. PLA2R1 was identified as the

major target antigen for membranous nephropathy (MN) [155] and a PLA2R1 variant RS4664308 was reported previously to associate with membranous nephropathy [156] in a European population also had a strong association with LN in a cohort of 1,247 Chinese Han SLE patients [157].

In closing, it is clear that genetics plays an important factor in the development of LN. In fact, a recent biostatistics study of large-scale SLE genotyping data estimated the explained heritability for LN is 47% according to a Random Forests Prediction Model [158]. However, how can these susceptibility genes and variants be used for reliable diagnosis of LN? Perhaps this will require the use of a panel of confirmed variants and consideration of the ethnicity of the population for which the association was made and that of the patient being monitored. An important next direction in this process is to determine if progression of disease or response to current standard treatment can be predicted by genetic variants. Lastly, information pertaining to known functions of these associated susceptibility genes could be used to design novel therapies.

Conclusions

Several novel biomarkers for LN have been proposed in recent years, yet none have become incorporated into clinical use[119]. The failure of LN biomarkers to make it to the clinical arena is in part due to the fragmented nature in which they have been studied. The majority of studies have been single center with significant variability in cohorts, assays, and sample storage, leading to inconclusive results. Multicenter longitudinal studies, utilizing standardized storage methods and assays, are needed to develop more robust clinical biomarkers.

It has become clear that no single biomarker is likely to be sufficient to diagnose LN flares, and define the response to therapy and prognosis. A more likely scenario is a panel of urine, serum, tissue, and genetic biomarkers. Each biomarker “panel” will provide unique insight into different clinical questions: a genetics panel may identify whether a patient with SLE is likely to develop nephritis and which inflammatory pathways are likely to be active; a urine biomarker panel may help distinguish between inflammation and fibrosis, eliminating the need for repeat biopsies; a serum biomarker panel may identify nephritogenic autoantibodies that increase in flare and decrease in response to treatment. A more systematic approach involving multiple cohorts needs to be utilized so that precision medicine becomes a reality in LN.

Acknowledgements:

All authors have read the journal’s authorship agreement and have reviewed and approved the manuscript as written.

Disclosures:

DJC participates in clinical trials sponsored by Mallinckcrodt and Aurinia Pharmaceuticals.

Funding Sources:

DJC receives funding from the NIH (K08 DK102542). MLM receives funding from the NIH (R01 AI129959; R01 DK110077). JBK receives funding from the NIH (R01 DK110077; UM1-DK100865).

Abbreviations:

SLE	systemic lupus erythematosus
LN	lupus nephritis
ANA	anti-nuclear antibodies
Anti-dsDNA	anti-double stranded DNA antibodies
ESKD	End Stage Kidney Disease

Literature Cited

[1]. Tsokos GC. Systemic lupus erythematosus. N Engl J Med 2011;365:2110–21. [PubMed: 22129255]

[2]. Bomback AS, Appel GB. Updates on the treatment of lupus nephritis. Journal of the American Society of Nephrology : JASN 2010;21:2028–35. [PubMed: 21051743]

[3]. 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;63:1681–8. [PubMed: 21445962]

[4]. Sexton DJ, Reule S, Solid C, et al. ESRD from lupus nephritis in the United States, 1995–2010. Clin J Am Soc Nephrol 2015;10:251–9. [PubMed: 25534208]

[5]. Parikh SV, Rovin BH. Current and Emerging Therapies for Lupus Nephritis. Journal of the American Society of Nephrology : JASN 2016;27:2929–39. [PubMed: 27283496]

[6]. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89–95. [PubMed: 11240971]

[7]. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. Kidney Int 2004;65:521–30. [PubMed: 14717922]

[8]. Yu F, Wu LH, Tan Y, et al. Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 International Society of Nephrology and Renal Pathology Society system. Kidney Int 2010;77:820–9. [PubMed: 20182417]

[9]. Yu F, Haas M, Glassock R, Zhao MH. Redefining lupus nephritis: clinical implications of pathophysiologic subtypes. Nat Rev Nephrol 2017;13:483–95. [PubMed: 28669995]

[10]. Wu LH, Yu F, Tan Y, et al. Inclusion of renal vascular lesions in the 2003 ISN/RPS system for classifying lupus nephritis improves renal outcome predictions. Kidney Int 2013;83:715–23. [PubMed: 23302713]

[11]. Hsieh C, Chang A, Brandt D, et al. Predicting outcomes of lupus nephritis with tubulointerstitial inflammation and scarring. Arthritis Care Res (Hoboken) 2011;63:865–74. [PubMed: 21309006]

[12]. Mejia-Vilet JM, Cordova-Sanchez BM, Uribe-Uribe NO, Correa-Rotter R, Morales-Buenrostro LE. Prognostic significance of renal vascular pathology in lupus nephritis. Lupus 2017;26:1042–50. [PubMed: 28178879]

[13]. Austin HA, 3rd, Muenz LR, Joyce KM, Antonovych TT, Balow JE. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. Kidney international 1984;25:689–95. [PubMed: 6482173]

[14]. Bajema IM, Wilhelmus S, Alpers CE, et al. Revision of the International Society of Nephrology/ Renal Pathology Society classification for lupus nephritis: clarification of definitions, and modified National Institutes of Health activity and chronicity indices. Kidney Int 2018;93:789–96. [PubMed: 29459092]

[15]. Malvar A, Pirruccio P, Alberton V, et al. Histologic versus clinical remission in proliferative lupus nephritis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2017;32:1338–44.

[16]. De Rosa M, Azzato F, Toblli JE, et al. A prospective observational cohort study highlights kidney biopsy findings of lupus nephritis patients in remission who flare following withdrawal of maintenance therapy. Kidney international 2018.

[17]. Dall'Era M, Cisternas MG, Smilek DE, et al. Predictors of long-term renal outcome in lupus nephritis trials: lessons learned from the Euro-Lupus Nephritis cohort. Arthritis Rheumatol 2015;67:1305–13. [PubMed: 25605554]

[18]. Tamirou F, D'Cruz D, Sangle S, et al. Long-term follow-up of the MAINTAIN Nephritis Trial, comparing azathioprine and mycophenolate mofetil as maintenance therapy of lupus nephritis. Ann Rheum Dis 2016;75:526–31. [PubMed: 25757867]

[19]. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology (Oxford) 2007;46:1052–6. [PubMed: 17500073]

[20]. Pisetsky DS. Anti-DNA antibodies - quintessential biomarkers of SLE. Nature reviews Rheumatology 2015.

[21]. Smeenk R, van der Lelij G, Aarden L. Avidity of antibodies to dsDNA: comparison of IFT on Crithidia luciliae, Farr assay, and PEG assay. J Immunol 1982;128:73–8. [PubMed: 7033379]

[22]. Olson SW, Lee JJ, Prince LK, et al. Elevated subclinical double-stranded DNA antibodies and future proliferative lupus nephritis. Clin J Am Soc Nephrol 2013;8:1702–8. [PubMed: 23833315]

[23]. Mortensen ES, Rekvig OP. Nephritogenic potential of anti-DNA antibodies against necrotic nucleosomes. Journal of the American Society of Nephrology : JASN 2009;20:696–704. [PubMed: 19329762]

[24]. Matrat A, Veysseyre-Balter C, Trolliet P, et al. Simultaneous detection of anti-C1q and anti-double stranded DNA autoantibodies in lupus nephritis: predictive value for renal flares. Lupus 2011;20:28–34. [PubMed: 20943718]

[25]. Julkunen H, Ekblom-Kullberg S, Miettinen A. Nonrenal and renal activity of systemic lupus erythematosus: a comparison of two anti-C1q and five anti-dsDNA assays and complement C3 and C4. Rheumatology international 2012;32:2445–51. [PubMed: 21706294]

[26]. Moroni G, Radice A, Giammarresi G, et al. Are laboratory tests useful for monitoring the activity of lupus nephritis? A 6-year prospective study in a cohort of 228 patients with lupus nephritis. Ann Rheum Dis 2009;68:234–7. [PubMed: 18718989]

[27]. Yung S, Chan TM. Anti-dsDNA antibodies and resident renal cells - Their putative roles in pathogenesis of renal lesions in lupus nephritis. Clin Immunol 2017;185:40–50. [PubMed: 27612436]

[28]. Hanrotel-Saliou C, Segalen I, Le Meur Y, Youinou P, Renaudineau Y. Glomerular antibodies in lupus nephritis. Clinical reviews in allergy & immunology 2011;40:151–8. [PubMed: 20414746]

[29]. van Bavel CC, Fenton KA, Rekvig OP, van der Vlag J, Berden JH. Glomerular targets of nephritogenic autoantibodies in systemic lupus erythematosus. Arthritis Rheum 2008;58:1892–9. [PubMed: 18576314]

[30]. Dong X, Zheng Z, Luo X, et al. Combined utilization of untimed single urine of MCP-1 and TWEAK as a potential indicator for proteinuria in lupus nephritis: A case-control study. Medicine (Baltimore) 2018;97:e0343. [PubMed: 29668584]

[31]. Zhao Z, Deocharan B, Scherer PE, Ozelius LJ, Putterman C. Differential binding of cross-reactive anti-DNA antibodies to mesangial cells: the role of alpha-actinin. J Immunol 2006;176:7704–14. [PubMed: 16751418]

[32]. Feng D, DuMontier C, Pollak MR. The role of alpha-actinin-4 in human kidney disease. Cell & bioscience 2015;5:44. [PubMed: 26301083]

[33]. Mostoslavsky G, Fischel R, Yachimovich N, et al. Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry. European journal of immunology 2001;31:1221–7. [PubMed: 11298348]

[34]. Zou X, Cheng H, Zhang Y, Fang C, Xia Y. The antigen-binding fragment of anti-double-stranded DNA IgG enhances F-actin formation in mesangial cells by binding to alpha-actinin-4. Experimental biology and medicine (Maywood, NJ) 2012;237:1023–31.

[35]. Deocharan B, Zhou Z, Antar K, et al. Alpha-actinin immunization elicits anti-chromatin autoimmunity in nonautoimmune mice. J Immunol 2007;179:1313–21. [PubMed: 17617624]

[36]. Becker-Merok A, Kalaaji M, Haugbro K, et al. Alpha-actinin-binding antibodies in relation to systemic lupus erythematosus and lupus nephritis. Arthritis Res Ther 2006;8:R162. [PubMed: 17062137]

[37]. Zhang WH, Pan HF, Zhao XF, et al. Anti-alpha-actinin antibodies in relation to new-onset systemic lupus erythematosus and lupus nephritis. Molecular biology reports 2010;37:1341–5. [PubMed: 19319662]

[38]. Seret G, Canas F, Pougnet-Di Costanzo L, et al. Anti-alpha-actinin antibodies are part of the anti-cell membrane antibody spectrum that characterize patients with lupus nephritis. Journal of autoimmunity 2015;61:54–61. [PubMed: 26071203]

[39]. Iaccarino L, Ghirardello A, Canova M, et al. Anti-annexins autoantibodies: their role as biomarkers of autoimmune diseases. Autoimmunity reviews 2011;10:553–8. [PubMed: 21527362]

[40]. Markoff A, Gerke V. Expression and functions of annexins in the kidney. Am J Physiol Renal Physiol 2005;289:F949–56. [PubMed: 16210453]

[41]. Bonanni A, Vaglio A, Bruschi M, et al. Multi-antibody composition in lupus nephritis: isotype and antigen specificity make the difference. Autoimmunity reviews 2015;14:692–702. [PubMed: 25888464]

[42]. Goulding NJ, Podgorski MR, Hall ND, et al. Autoantibodies to recombinant lipocortin-1 in rheumatoid arthritis and systemic lupus erythematosus. Ann Rheum Dis 1989;48:843–50. [PubMed: 2554826]

[43]. Bruschi M, Sinico RA, Moroni G, et al. Glomerular Autoimmune Multicomponents of Human Lupus Nephritis In Vivo: alpha-Enolase and Annexin AI. Journal of the American Society of Nephrology : JASN 2014.

[44]. Bharadwaj A, Bydoun M, Holloway R, Waisman D. Annexin A2 heterotetramer: structure and function. International journal of molecular sciences 2013;14:6259–305. [PubMed: 23519104]

[45]. Yung S, Cheung KF, Zhang Q, Chan TM. Anti-dsDNA antibodies bind to mesangial annexin II in lupus nephritis. Journal of the American Society of Nephrology : JASN 2010;21:1912–27. [PubMed: 20847146]

[46]. Rescher U, Ludwig C, Konietzko V, Kharitonenkov A, Gerke V. Tyrosine phosphorylation of annexin A2 regulates Rho-mediated actin rearrangement and cell adhesion. Journal of cell science 2008;121:2177–85. [PubMed: 18565825]

[47]. Canas F, Simonin L, Couturaud F, Renaudineau Y. Annexin A2 autoantibodies in thrombosis and autoimmune diseases. Thrombosis research 2015;135:226–30. [PubMed: 25533130]

[48]. Caster DJ, Korte EA, Merchant ML, et al. Autoantibodies targeting glomerular annexin A2 identify patients with proliferative lupus nephritis. Proteomics Clinical applications 2015.

[49]. Cheung KF, Yung S, Chau MK, et al. Annexin II-binding immunoglobulins in patients with lupus nephritis and their correlation with disease manifestations. Clinical science (London, England : 1979) 2017;131:653–71.

[50]. Diaz-Ramos A, Roig-Borrellas A, Garcia-Melero A, Lopez-Alemany R. alpha-Enolase, a multifunctional protein: its role on pathophysiological situations. Journal of biomedicine & biotechnology 2012;2012:156795. [PubMed: 23118496]

[51]. Pratesi F, Moscato S, Sabbatini A, et al. Autoantibodies specific for alpha-enolase in systemic autoimmune disorders. The Journal of rheumatology 2000;27:109–15. [PubMed: 10648026]

[52]. Bruschi M, Carnevali ML, Murtas C, et al. Direct characterization of target podocyte antigens and auto-antibodies in human membranous glomerulonephritis: Alfa-enolase and borderline antigens. Journal of proteomics 2011;74:2008–17. [PubMed: 21640210]

[53]. Papayannopoulos V, Zychlinsky A. NETs: a new strategy for using old weapons. Trends in immunology 2009;30:513–21. [PubMed: 19699684]

[54]. Urban CF, Ermert D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS pathogens 2009;5:e1000639. [PubMed: 19876394]

[55]. Hakkim A, Furnrohr BG, Amann K, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. Proc Natl Acad Sci U S A 2010;107:9813–8. [PubMed: 20439745]

[56]. Craft JE. Dissecting the immune cell mayhem that drives lupus pathogenesis. Sci Transl Med 2011;3:73ps9.

[57]. Garcia-Romo GS, Caielli S, Vega B, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med 2011;3:73ra20.

[58]. Lande R, Ganguly D, Facchinetti V, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Sci Transl Med 2011;3:73ra19.

[59]. Villanueva E, Yalavarthi S, Berthier CC, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol 2011;187:538–52. [PubMed: 21613614]

[60]. Leffler J, Martin M, Gullstrand B, et al. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. J Immunol 2012;188:3522–31. [PubMed: 22345666]

[61]. Krishnan MR, Wang C, Marion TN. Anti-DNA autoantibodies initiate experimental lupus nephritis by binding directly to the glomerular basement membrane in mice. Kidney international 2012;82:184–92. [PubMed: 22297676]

[62]. Jones B Lupus nephritis: Nucleosomes trapped by aberrantly expressed laminin-beta1. Nature reviews Nephrology 2014;10:4.

[63]. Olin AI, Morgelin M, Truedsson L, Sturfelt G, Bengtsson AA. Pathogenic mechanisms in lupus nephritis: Nucleosomes bind aberrant laminin beta1 with high affinity and colocalize in the electron-dense deposits. Arthritis Rheumatol 2014;66:397–406. [PubMed: 24504812]

[64]. Mjelle JE, Rekvig OP, Fenton KA. Nucleosomes possess a high affinity for glomerular laminin and collagen IV and bind nephritogenic antibodies in murine lupus-like nephritis. Ann Rheum Dis 2007;66:1661–8. [PubMed: 17504842]

[65]. van Bavel CC, van der Vlag J, Berden JH. Glomerular binding of anti-dsDNA autoantibodies: the dispute resolved? Kidney international 2007;71:600–1. [PubMed: 17387307]

[66]. Kim HJ, Hong YH, Kim YJ, et al. Anti-heparan sulfate antibody and functional loss of glomerular heparan sulfate proteoglycans in lupus nephritis. Lupus 2017;26:815–24. [PubMed: 28420046]

[67]. Thanei S, Vanhecke D, Trendelenburg M. Anti-C1q autoantibodies from systemic lupus erythematosus patients activate the complement system via both the classical and lectin pathways. Clin Immunol 2015;160:180–7. [PubMed: 26148903]

[68]. Panda AK, Parida JR, Tripathy R, et al. Mannose binding lectin: a biomarker of systemic lupus erythematosus disease activity. Arthritis Res Ther 2012;14:R218. [PubMed: 23068019]

[69]. Song D, Guo WY, Wang FM, et al. Complement Alternative Pathways Activation in Patients With Lupus Nephritis. Am J Med Sci 2017;353:247–57. [PubMed: 28262211]

[70]. Thurman JM, Nester CM. All Things Complement. Clin J Am Soc Nephrol 2016;11:1856–66. [PubMed: 27340286]

[71]. Bao L, Cunningham PN, Quigg RJ. Complement in Lupus Nephritis: New Perspectives. Kidney diseases (Basel, Switzerland) 2015;1:91–9.

[72]. Bao L, Haas M, Quigg RJ. Complement factor H deficiency accelerates development of lupus nephritis. Journal of the American Society of Nephrology : JASN 2011;22:285–95. [PubMed: 21148254]

[73]. Leffler J, Bengtsson AA, Blom AM. The complement system in systemic lupus erythematosus: an update. Ann Rheum Dis 2014;73:1601–6. [PubMed: 24845390]

[74]. Luijten KM, Tekstra J, Bijlsma JW, Bijl M. The Systemic Lupus Erythematosus Responder Index (SRI); a new SLE disease activity assessment. Autoimmunity reviews 2012;11:326–9. [PubMed: 21958603]

[75]. Mikdashi J, Nived O. Measuring disease activity in adults with systemic lupus erythematosus: the challenges of administrative burden and responsiveness to patient concerns in clinical research. Arthritis Res Ther 2015;17:183. [PubMed: 26189728]

[76]. Esdaile JM, Joseph L, Abrahamowicz M, et al. Routine immunologic tests in systemic lupus erythematosus: is there a need for more studies? J Rheumatol 1996;23:1891–6. [PubMed: 8923362]

[77]. Ricker DM, Hebert LA, Rohde R, et al. Serum C3 levels are diagnostically more sensitive and specific for systemic lupus erythematosus activity than are serum C4 levels. The Lupus Nephritis Collaborative Study Group. Am J Kidney Dis 1991;18:678–85. [PubMed: 1962653]

[78]. Ho A, Barr SG, Magder LS, Petri M. A decrease in complement is associated with increased renal and hematologic activity in patients with systemic lupus erythematosus. Arthritis Rheum 2001;44:2350–7. [PubMed: 11665976]

[79]. Birmingham DJ, Irshaid F, Nagaraja HN, et al. The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. Lupus 2010;19:1272–80. [PubMed: 20605879]

[80]. Thielens NM, Tedesco F, Bohlson SS, Gaboriaud C, Tenner AJ. C1q: A fresh look upon an old molecule. Molecular immunology 2017;89:73–83. [PubMed: 28601358]

[81]. Macedo AC, Isaac L. Systemic Lupus Erythematosus and Deficiencies of Early Components of the Complement Classical Pathway. Frontiers in immunology 2016;7:55. [PubMed: 26941740]

[82]. Orbai AM, Truedsson L, Sturfelt G, et al. Anti-C1q antibodies in systemic lupus erythematosus. Lupus 2015;24:42–9. [PubMed: 25124676]

[83]. Yang XW, Tan Y, Yu F, Zhao MH. Combination of anti-C1q and anti-dsDNA antibodies is associated with higher renal disease activity and predicts renal prognosis of patients with lupus nephritis. Nephrol Dial Transplant 2012;27:3552–9. [PubMed: 22700716]

[84]. Bock M, Heijnen I, Trendelenburg M. Anti-C1q antibodies as a follow-up marker in SLE patients. PLoS One 2015;10:e0123572. [PubMed: 25881125]

[85]. Fatemi A, Samadi G, Sayedbonakdar Z, Smiley A. Anti-C1q antibody in patients with lupus nephritic flare: 18-month follow-up and a nested case-control study. Mod Rheumatol 2016;26:233–9. [PubMed: 26357965]

[86]. Wang Y, Hu Q, Madri JA, et al. Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc Natl Acad Sci U S A 1996;93:8563–8. [PubMed: 8710910]

[87]. Wang S, Wu M, Chiriboga L, Zeck B, Belmont HM. Membrane attack complex (mac) deposition in lupus nephritis is associated with hypertension and poor clinical response to treatment. Seminars in arthritis and rheumatism 2018.

[88]. Mahler M, van Schaarenburg RA, Trouw LA. Anti-C1q autoantibodies, novel tests, and clinical consequences. Frontiers in immunology 2013;4:117. [PubMed: 23717311]

[89]. Papp K, Vegh P, Hobor R, et al. Immune complex signatures of patients with active and inactive SLE revealed by multiplex protein binding analysis on antigen microarrays. PLoS One 2012;7:e44824. [PubMed: 22984570]

[90]. Sciascia S, Radin M, Yazdany J, et al. Expanding the therapeutic options for renal involvement in lupus: eculizumab, available evidence. Rheumatology international 2017;37:1249–55. [PubMed: 28258475]

[91]. Martensson J, Bellomo R. The rise and fall of NGAL in acute kidney injury. Blood purification 2014;37:304–10. [PubMed: 25170751]

[92]. Suzuki M, Wiers KM, Klein-Gitelman MS, et al. Neutrophil gelatinase-associated lipocalin as a biomarker of disease activity in pediatric lupus nephritis. Pediatric nephrology (Berlin, Germany) 2008;23:403–12.

[93]. Rubinstein T, Pitashny M, Levine B, et al. Urinary neutrophil gelatinase-associated lipocalin as a novel biomarker for disease activity in lupus nephritis. Rheumatology (Oxford) 2010;49:960–71. [PubMed: 20144927]

[94]. Satirapoj B, Kitiyakara C, Leelahavanichkul A, Avihingsanon Y, Supasyndh O. Urine neutrophil gelatinase-associated lipocalin to predict renal response after induction therapy in active lupus nephritis. BMC Nephrol 2017;18:263. [PubMed: 28778196]

[95]. Kulkarni O, Pawar RD, Purschke W, et al. Spiegelmer inhibition of CCL2/MCP-1 ameliorates lupus nephritis in MRL-(Fas)lpr mice. Journal of the American Society of Nephrology : JASN 2007;18:2350–8. [PubMed: 17625118]

[96]. Gupta R, Yadav A, Aggarwal A. Longitudinal assessment of monocyte chemoattractant protein-1 in lupus nephritis as a biomarker of disease activity. Clinical rheumatology 2016;35:2707–14. [PubMed: 27624649]

[97]. Rovin BH, Song H, Birmingham DJ, et al. Urine chemokines as biomarkers of human systemic lupus erythematosus activity. Journal of the American Society of Nephrology : JASN 2005;16:467–73. [PubMed: 15601744]

[98]. Singh RG, Usha, Rathore SS, Behura SK, Singh NK. Urinary MCP-1 as diagnostic and prognostic marker in patients with lupus nephritis flare. Lupus 2012;21:1214–8. [PubMed: 22759858]

[99]. Kaplan MJ, Lewis EE, Shelden EA, et al. The apoptotic ligands TRAIL, TWEAK, and Fas ligand mediate monocyte death induced by autologous lupus T cells. J Immunol 2002;169:6020–9. [PubMed: 12421989]

[100]. Michaelson JS, Wisniacki N, Burkly LC, Putterman C. Role of TWEAK in lupus nephritis: a bench-to-bedside review. J Autoimmun 2012;39:130–42. [PubMed: 22727560]

[101]. Schwartz N, Su L, Burkly LC, et al. Urinary TWEAK and the activity of lupus nephritis. J Autoimmun 2006;27:242–50. [PubMed: 17257812]

[102]. Xuejing Z, Jiazhen T, Jun L, et al. Urinary TWEAK level as a marker of lupus nephritis activity in 46 cases. J Biomed Biotechnol 2012;2012:359647. [PubMed: 22719208]

[103]. Zhao Z, Burkly LC, Campbell S, et al. TWEAK/Fn14 interactions are instrumental in the pathogenesis of nephritis in the chronic graft-versus-host model of systemic lupus erythematosus. J Immunol 2007;179:7949–58. [PubMed: 18025243]

[104]. Molano A, Lakhani P, Aran A, et al. TWEAK stimulation of kidney resident cells in the pathogenesis of graft versus host induced lupus nephritis. Immunology letters 2009;125:119–28. [PubMed: 19573558]

[105]. Xia Y, Campbell SR, Broder A, et al. Inhibition of the TWEAK/Fn14 pathway attenuates renal disease in nephrotoxic serum nephritis. Clin Immunol 2012;145:108–21. [PubMed: 22982296]

[106]. Almaani S, Hebert LA, Rovin BH, Birmingham DJ. The Urine Preservative Acetic Acid Degrades Urine Protein: Implications for Urine Biorepositories and the AASK Cohort Study. J Am Soc Nephrol 2017;28:1394–8. [PubMed: 28104821]

[107]. Zhang X, Nagaraja HN, Nadasdy T, et al. A composite urine biomarker reflects interstitial inflammation in lupus nephritis kidney biopsies. Kidney international 2012;81:401–6. [PubMed: 21993584]

[108]. Abulaban KM, Song H, Zhang X, et al. Predicting decline of kidney function in lupus nephritis using urine biomarkers. Lupus 2016;25:1012–8. [PubMed: 26873651]

[109]. Baker C, Belbin O, Kalsheker N, Morgan K. SERPINA3 (aka alpha-1-antichymotrypsin). Front Biosci 2007;12:2821–35. [PubMed: 17485262]

[110]. Aggarwal A, Gupta R, Negi VS, et al. Urinary haptoglobin, alpha-1 anti-chymotrypsin and retinol binding protein identified by proteomics as potential biomarkers for lupus nephritis. Clin Exp Immunol 2017;188:254–62. [PubMed: 28120479]

[111]. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992;35:630–40. [PubMed: 1599520]

[112]. Wolf BJ, Spainhour JC, Arthur JM, et al. Development of Biomarker Models to Predict Outcomes in Lupus Nephritis. Arthritis Rheumatol 2016;68:1955–63. [PubMed: 26867033]

[113]. 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;64:1215–26. [PubMed: 22231479]

[114]. Pencina MJ, D'Agostino RB, Sr., Demler OV. Novel metrics for evaluating improvement in discrimination: net reclassification and integrated discrimination improvement for normal variables and nested models. Stat Med 2012;31:101–13. [PubMed: 22147389]

[115]. Grund B, Sabin C. Analysis of biomarker data: logs, odds ratios, and receiver operating characteristic curves. Current opinion in HIV and AIDS 2010;5:473–9. [PubMed: 20978390]

[116]. Administration USFaD. FDA/CDER Biomarker Qualification Program 2009.

[117]. Kitagori K, Yoshifuji H, Oku T, et al. Cleaved Form of Osteopontin in Urine as a Clinical Marker of Lupus Nephritis. PLoS One 2016;11:e0167141. [PubMed: 27992535]

[118]. Wei R, Gao B, Shih F, et al. Alterations in urinary collagen peptides in lupus nephritis subjects correlate with renal dysfunction and renal histopathology. Nephrol Dial Transplant 2017;32:1468–77. [PubMed: 28339802]

[119]. Birmingham DJ, Merchant M, Waikar SS, et al. Biomarkers of lupus nephritis histology and flare: deciphering the relevant amidst the noise. Nephrol Dial Transplant 2017;32:i71–i9. [PubMed: 28391335]

[120]. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97. [PubMed: 14744438]

[121]. Merchant ML, Rood IM, Deegens JKJ, Klein JB. Isolation and characterization of urinary extracellular vesicles: implications for biomarker discovery. Nat Rev Nephrol 2017;13:731–49. [PubMed: 29081510]

[122]. Cardenas-Gonzalez M, Srivastava A, Pavkovic M, et al. Identification, Confirmation, and Replication of Novel Urinary MicroRNA Biomarkers in Lupus Nephritis and Diabetic Nephropathy. Clinical chemistry 2017;63:1515–26. [PubMed: 28667184]

[123]. Olivares D, Perez-Hernandez J, Forner MJ, et al. Urinary levels of sirtuin-1 associated with disease activity in lupus nephritis. Clin Sci (Lond) 2018;132:569–79. [PubMed: 29440621]

[124]. Hu N, Long H, Zhao M, Yin H, Lu Q. Aberrant expression pattern of histone acetylation modifiers and mitigation of lupus by SIRT1-siRNA in MRL/lpr mice. Scand J Rheumatol 2009;38:464–71. [PubMed: 19922023]

[125]. Ikuma D, Hiromura K, Kajiyama H, et al. The correlation of urinary podocytes and podocalyxin with histological features of lupus nephritis. Lupus 2018;27:484–93. [PubMed: 29050536]

[126]. Lindqvist AK, Alarcon-Riquelme ME. The genetics of systemic lupus erythematosus. Scand J Immunol 1999;50:562–71. [PubMed: 10607304]

[127]. Su K, Wu J, Edberg JC, et al. A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. J Immunol 2004;172:7186–91. [PubMed: 15153543]

[128]. Graham RR, Cotsapas C, Davies L, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. Nat Genet 2008;40:1059–61. [PubMed: 19165918]

[129]. Graham RR, Kyogoku C, Sigurdsson S, et al. Three functional variants of IFN regulatory factor 5 (IRF5) define risk and protective haplotypes for human lupus. Proc Natl Acad Sci U S A 2007;104:6758–63. [PubMed: 17412832]

[130]. Sanchez E, Palomino-Morales RJ, Ortego-Centeno N, et al. Identification of a new putative functional IL18 gene variant through an association study in systemic lupus erythematosus. Hum Mol Genet 2009;18:3739–48. [PubMed: 19584085]

[131]. Han S, Kim-Howard X, Deshmukh H, et al. Evaluation of imputation-based association in and around the integrin-alpha-M (ITGAM) gene and replication of robust association between a non-synonymous functional variant within ITGAM and systemic lupus erythematosus (SLE). Hum Mol Genet 2009;18:1171–80. [PubMed: 19129174]

[132]. Nishimoto K, Kochi Y, Ikari K, et al. Association study of TRAF1-C5 polymorphisms with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in Japanese. Ann Rheum Dis 2010;69:368–73. [PubMed: 19336421]

[133]. Harley IT, Kaufman KM, Langefeld CD, Harley JB, Kelly JA. Genetic susceptibility to SLE: new insights from fine mapping and genome-wide association studies. Nat Rev Genet 2009;10:285–90. [PubMed: 19337289]

[134]. Gateva V, Sandling JK, Hom G, et al. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. Nat Genet 2009;41:1228–33. [PubMed: 19838195]

[135]. Han JW, Zheng HF, Cui Y, et al. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. Nat Genet 2009;41:1234–7. [PubMed: 19838193]

[136]. Heyninck K, Beyaert R. A20 inhibits NF-kappaB activation by dual ubiquitin-editing functions. Trends Biochem Sci 2005;30:1–4. [PubMed: 15653317]

[137]. Mauro C, Pacifico F, Lavorgna A, et al. ABIN-1 binds to NEMO/IKKgamma and co-operates with A20 in inhibiting NF-kappaB. J Biol Chem 2006;281:18482–8. [PubMed: 16684768]

[138]. Verstrepen L, Carpentier I, Verhelst K, Beyaert R. ABINs: A20 binding inhibitors of NF-kappa B and apoptosis signaling. Biochem Pharmacol 2009;78:105–14. [PubMed: 19464428]

[139]. Wagner S, Carpentier I, Rogov V, et al. Ubiquitin binding mediates the NF-kappaB inhibitory potential of ABIN proteins. Oncogene 2008;27:3739–45. [PubMed: 18212736]

[140]. Nanda SK, Venigalla RK, Ordureau A, et al. Polyubiquitin binding to ABIN1 is required to prevent autoimmunity. J Exp Med

[141]. Caster DJ, Korte EA, Nanda SK, et al. ABIN1 Dysfunction as a Genetic Basis for Lupus Nephritis. J Am Soc Nephrol 2013.

[142]. Caster DJ, Korte EA, Tan M, et al. Neutrophil exocytosis induces podocyte cytoskeletal reorganization and proteinuria in experimental glomerulonephritis. Am J Physiol Renal Physiol 2018.

[143]. Adrianto I, Wen F, Templeton A, et al. Association of a functional variant downstream of TNFAIP3 with systemic lupus erythematosus. Nat Genet 2011;43:253–8. [PubMed: 21336280]

[144]. Yang W, Zhao M, Hirankarn N, et al. ITGAM is associated with disease susceptibility and renal nephritis of systemic lupus erythematosus in Hong Kong Chinese and Thai. Human molecular genetics 2009;18:2063–70. [PubMed: 19286673]

[145]. Kim-Howard X, Maiti AK, Anaya JM, et al. ITGAM coding variant (rs1143679) influences the risk of renal disease, discoid rash and immunological manifestations in patients with systemic lupus erythematosus with European ancestry. Ann Rheum Dis 2010;69:1329–32. [PubMed: 19939855]

[146]. Sanchez E, Nadig A, Richardson BC, et al. Phenotypic associations of genetic susceptibility loci in systemic lupus erythematosus. Ann Rheum Dis 2011;70:1752–7. [PubMed: 21719445]

[147]. Li LH, Yuan H, Pan HF, et al. Role of the Fcgamma receptor IIIA-V/F158 polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. Scand J Rheumatol 2010;39:148–54. [PubMed: 20059375]

[148]. Bates JS, Lessard CJ, Leon JM, et al. Meta-analysis and imputation identifies a 109 kb risk haplotype spanning TNFAIP3 associated with lupus nephritis and hematologic manifestations. Genes Immun 2009;10:470–7. [PubMed: 19387456]

[149]. Lin CP, Adrianto I, Lessard CJ, et al. Role of MYH9 and APOL1 in African and non-African populations with lupus nephritis. Genes and immunity 2012;13:232–8. [PubMed: 22189356]

[150]. Zhou XJ, Cheng FJ, Qi YY, Zhao MH, Zhang H. A replication study from Chinese supports association between lupus-risk allele in TNFSF4 and renal disorder. Biomed Res Int 2013;2013:597921. [PubMed: 23936824]

[151]. Freedman BI, Langefeld CD, Andringa KK, et al. End-stage renal disease in African Americans with lupus nephritis is associated with APOL1. Arthritis Rheumatol 2014;66:390–6. [PubMed: 24504811]

[152]. Munroe ME, James JA. Genetics of Lupus Nephritis: Clinical Implications. Semin Nephrol 2015;35:396–409. [PubMed: 26573543]

[153]. Iwamoto T, Niewold TB. Genetics of human lupus nephritis. Clin Immunol 2017;185:32–9. [PubMed: 27693588]

[154]. Korte EA, Caster DJ, Barati MT, et al. ABIN1 Determines Severity of Glomerulonephritis via Activation of Intrinsic Glomerular Inflammation. Am J Pathol 2017;187:2799–810. [PubMed: 28935578]

[155]. Beck LH, Jr., Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. The New England journal of medicine 2009;361:11–21. [PubMed: 19571279]

[156]. Stanescu HC, Arcos-Burgos M, Medlar A, et al. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. The New England journal of medicine 2011;364:616–26. [PubMed: 21323541]

[157]. Li Y, Zhou A, Lv G, et al. Single-nucleotide polymorphisms in the PLA2R1 gene are associated with systemic lupus erythematosus and lupus nephritis in a Chinese Han population. Immunol Res 2016;64:324–8. [PubMed: 26645973]

[158]. Almlof JC, Alexsson A, Imgenberg-Kreuz J, et al. Novel risk genes for systemic lupus erythematosus predicted by random forest classification. Sci Rep 2017;7:6236. [PubMed: 28740209]

Table 1

ISN/RPS Classification of Lupus Nephritis

Class I	Minimal mesangial	Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	Mesangial Proliferative	Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits
Class III	Focal	Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations
Class IV	Diffuse	Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation
Class V	Membranous	Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations
		Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed
Class VI	Advanced sclerosing	≥90% of glomeruli globally sclerosed without residual activity

Adapted from WeeningJJ et al “The Classification of Glomerulonephritis in Systemic Lupus Erythematosus Revisited”[7]

Table 2

Native Glomerular Antigens

Glomerular Antigen	Location	Cross Reactive with anti-dsDNA	Animal Model
Alpha Actinin [36–38]	mesangial cells, podocytes	**Yes**	**Yes**
Alpha-enolase [43]	mesangial cell, podocytes	**No**	**Yes**
Annexin A1 [43]	podocytes	**Not tested**	**Not tested**
Annexin A2 [45, 48, 49]	mesangial cells, podocytes, matrix	**Yes**	**Yes**
Heparan sulfate [61, 66]	glomerular basement membrane	**Yes**	**Yes**
Laminin [63, 64]	glomerular basement membrane	**Yes**	**Yes**