

Search for useful tools: biomarkers in lupus nephritis



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'For lupus nephritis, the critical issues at stake include identifying those patients at risk for flare, progressive nephritis and development of end-stage renal disease'

Significant advances in our understanding of the cellular and humoral components leading to renal disease in systemic lupus erythematosus (SLE) have been made; however, so far, this has had limited impact in guiding clinicians in the management of patients with lupus nephritis (LN). Over 50% of SLE patients develop nephritis, and despite aggressive therapy, 65–90% of patients with class III and class IV lupus do not achieve remission within 6 months [1]. Quite often, treatments are extended in an effort to delay progression to end-stage renal disease (ESRD). However, adverse events and serious toxicities are often associated with long-duration therapies, and approximately a third of SLE patients will have an inadequate response [2]. Furthermore, relapses are common, and distinguishing overt LN from noninflammatory conditions may be difficult using clinical criteria alone.

These distinctions are especially relevant to evaluation of newer therapies (e.g., biologics with potentially less toxicity), where long-term outcomes remain unknown [3]. Diagnostic markers, such as anti-dsDNA and complement levels, are not widely applicable as determinants of disease activity. In the study of lupus patients, generally they fluctuate with disease activity; however, they may either remain abnormal with remission or not reliably predict disease flares. Traditional determinants of renal involvement, such as proteinuria, hematuria and decreased glomerular filtration rate, may either persist after adequate therapy or only be detected after significant damage has occurred. Furthermore, distinguishing active inflammation from residual scarring utilizing these measures may be especially difficult.

The situation is further complicated by structure–function correlations of disease activity using pathologic evaluation of tissue. New-onset severe LN is easily classified by evaluation of renal pathology, and near end-stage disease is usually clearly recognizable. However, assessing the extent of disease activity from residual scarring is often difficult, and the situation is compounded by prior therapy. With regard to the latter situation, the typical dilemma is: does the patient need more immunosuppression? Clearly other factors play a role in this decision, but assessing disease activity is very relevant to making this judgement.

The presence of diagnostic biomarkers such as anti-dsDNA antibodies and complement levels often reflects an abnormally regulated immune activity leading to the pathogenic autoimmune responses present in LN [4]. However, it has been demonstrated that these traditional markers cannot be utilized in a practical manner to indicate the degree of disease activity, response to treatment or, even, presence of nephritis.

Will any test be able to substitute for pathologic evaluation of tissue? The renal biopsy remains the standard in diagnosing LN, but differences in renal outcomes are not well correlated with WHO classes, and serial biopsies to monitor responses and detect flares are impractical [5]. Furthermore, there are important parameters that are not detected at the level of routine histopathology, and the value of morphological indices may lay even deeper still, at the molecular level. Ultimately, identifying patients who will either develop LN prior to the onset of evident renal pathology or who are at higher risk to develop progressive renal failure are ideal goals. Furthermore, it is likely that different patients may have different pathogenic profiles, and the ability to distinguish them using biomarkers, for more tailored therapy, would be ideal. Therefore, we look to the future and continue our search for useful tools that will inform us clinically and provide a basis for treatment and management decisions in patients with LN. There are many potential candidates on the horizon.

Goals of biomarkers

The search for biomarkers in SLE in general is motivated in part by the heterogeneity of disease manifestations and unpredictable course of SLE. So what should the goals be? Specifically for LN, the critical issues at stake include identifying those patients at risk for flare, progressive nephritis and development of ESRD. Biomarkers should be informative at different time points in the disease process, such as: diagnosis, monitoring the degree of immunological/inflammatory activity; prompt identification of flares and risk for flares; evaluation of response to treatment; and defining end-stage organ damage, where therapy will be ineffective.

Biomarkers should attempt to fulfil the following criteria to be usable and valid: they should be biologically relevant; sensitive and specific to disease; reproducible; and easy to measure in routine practice [6]. More difficult still is validating a biomarker that will stand up to the stringent test of becoming a surrogate end point. In this case, it is defined as a marker whose presence or change predicts a significant clinical end point, such as probability of developing ESRD. Some key difficulties and limitations in validating biomarkers of SLE arise from studies that frequently test SLE populations that are inherently and/or clinically different and are at various stages of their disease. For example, some studies have included patients with various organ manifestations, whereas in others, patients with specific manifestations have been selected. Studies also vary in the inclusion of patients with different therapies prior to entry. Comparative groups have also varied. In this regard, when evaluating for disease activity, SLE patients with nonactive disease should be utilized as controls instead of healthy individuals. Finally, ethnicity will be a particularly important source of difference in studies assessing biologic and genetic markers, as they will likely vary among groups [6].

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Relevant biomarkers may be identified using a variety of tissues sources, and urine and blood are being aggressively studied, since sampling is noninvasive, and standardization across different laboratories is more feasible. Nevertheless, tissue

sampling (e.g., from kidney biopsy specimens) should also have utility, although repetitive testing will be somewhat limited.

Genetic markers

The overall goals are to define patients at highest risk for nephritis and those with nephritis who are most likely to progress to ESRD. A large number of studies have focused on searches for candidate genes, and most have examined susceptibility to SLE. So far, a practical or universal genetic biomarker has not been demonstrated. As one would predict, no single gene is responsible for SLE and multiple genes contribute to susceptibility to, and severity of, nephritis. Initial studies focused on key components of the immune response, such as *MHC* genes and complement deficiencies. Certain class *MHC II* alleles were found to confer risk for SLE, particularly among certain ethnic groups [7]. Despite being present in only a minority of patients, certain complement deficiencies (*C4*, *C2* and *C1q*) have been strongly associated with the development of SLE [8]. Polymorphisms of genes encoding cytokines, cytokine receptors and costimulatory molecules, and those associated with immune complex clearance and apoptosis, have also been studied, since they are involved in the development and maintenance of an auto-reactivity. Thus far, polymorphisms in genes encoding various cytokine and cytokine receptor genes, overall, have not been strongly linked with SLE [9]. For example, polymorphisms of *IL-10* and *IL-6*, from SNPs to microsatellite repeats in the promoter region, failed to yield consistent findings across various ethnic groups, despite initial promise [10,11]. Similarly, evaluation of proinflammatory cytokines that promote murine lupus (e.g., *TNF- α* and *IFN- γ*) have not panned out in human disease [12,13]. And despite prominent roles for costimulatory molecules in the pathogenesis of SLE (e.g., *CD28/B7* family), polymorphisms of the *CD28*, *CD80* and *CD86* genes have not been shown to be associated with increased risk for SLE [14]. On the other hand, a SNP of *PD-1* has been associated with increased risk for SLE in one large study [15].

Genes associated with regulating cell death, survival and apoptosis (*Fas*, *Fas* ligand and *Bcl-2*), as well as those associated with immune regulation and immune complex clearance, such as Fc receptors for IgG (*Fc γ R*), have also been studied. For apoptosis-related genes, several polymorphisms have been described and linked to SLE; however, many of these were only relevant

to specific ethnic populations and are unlikely to be useful as biomarkers of susceptibility [6,16–18]. Studies of *FcγR* polymorphisms have been more promising, with a recent meta-analysis demonstrating increased odds of having SLE with a polymorphism in the *FcγRIIIa* gene, and increased risk, specifically of LN, with allelic variants in the *FcγRIIIa* gene [19,20].

Gene-expression microarrays and transcriptional profiling of renal biopsy tissue and peripheral blood cells in patients with SLE have also yielded promising results. Utilizing peripheral blood cells, several investigators have demonstrated significant upregulation of type I IFN-inducible genes, with IFN- α primarily responsible for the observed IFN signature gene expression in SLE patients [21,22]. The IFN signature was also found to be predictive of more severe disease and LN. A longitudinal study is underway to evaluate whether changes in the IFN signature predict flares and response to treatment [23]. In other studies, investigators have performed transcriptional phenotyping of laser-captured glomeruli from clinical biopsies of patients with LN [24]. Although technically challenging, decreased expression of well-known fibrosis-related growth factors, such as TGF- β 1 in sclerotic glomeruli, was observed. Nevertheless, fibrotic gene expression was found in both glomeruli with morphological evidence of sclerosis and glomeruli with minimal or no involvement at the morphological level, consistent with the conclusion that levels may vary in tissue and/or other factors are operative. Importantly, expression of type I IFN-inducible transcripts was associated with reduced expression of fibrosis-related genes and milder pathological features. This extensive study illustrates the promise of adding tissue biomarkers to histopathology and morphological indices in more reliably predicting damage or outcome in LN.

Serum markers

The approach to diagnosing SLE in routine practice is at times problematic. Although the standard approach relies on American College of Rheumatology criteria, significant organ pathology can occur without fulfilment of the criteria [25]. Most importantly, with regards to LN, many studies support that time to treatment has prognostic significance, and that treatment delays are linked to worse outcomes [26]. Historically, autoantibodies such as antinuclear antibodies (anti-Ro/SSA, anti-La/SSB, anti-snRNP, anti-SM and anti-dsDNA) are used in diagnosing

SLE, with anti-dsDNA and complement used to monitor severity of LN. Numerous studies have investigated whether these markers can predict disease activity and/or severity with inconsistent results. Moreover, anti-dsDNA antibodies are not specific for active renal disease [4]. This has led to investigation of new biomarkers, including erythrocyte-bound complement activation product C4d and complement receptor 1. One study found, via flow cytometric analysis, that patients with SLE had higher C4d and lower complement receptor 1 levels than healthy controls and patients with other autoimmune diseases [27].

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Potential new biomarkers signaling evidence of acute disease or specific organ involvement may surpass current standardized methods of disease detection and diagnosis, allowing for timely treatment and action. Antichromatin/antinucleosome and anti-C1q antibodies have shown promise as new measures for renal involvement [28,29]. Antinucleosome antibodies have been found in patients who were negative for anti-dsDNA antibodies, suggesting that this specificity may be a more sensitive marker of renal involvement [30]. Anti-C1q antibodies can be detected in the kidneys of patients with LN, and some have found a higher prevalence of anti-C1q antibodies in patients with active LN [31]. Others have demonstrated that the absence of anti-C1q antibodies in serum excludes a diagnosis of LN, whereas increases in levels may predict renal flares [32].

Alterations in B-cell populations in SLE, as determined by cell-surface phenotyping, may also be predictive. The number and frequency of CD27^{high} plasma cells correlates with disease activity scales and titers of anti-dsDNA antibodies [33]. CD27^{high} plasma cell levels also had a greater positive predictive value than standard humoral/clinical indices, and a decrease in CD27^{high} plasma population with treatment was observed. Several investigators have focused on regulatory T cells in patients with LN to examine whether changes in this population of immunoregulatory cells reflect disease remission and response to treatment. By flow cytometry, enhanced numbers of different T regulatory cell (Treg) subsets, and enhanced function of these

cells post-rituximab therapy in patients with LN who responded favorably to treatment, was observed [34]. Similar findings were reported using real-time mRNA expression of genes defining Tregs from the peripheral blood of patients with LN: mRNA expression of genes associated with Tregs, such as *CD25*, *CTLA-4*, *GITR* and *FOXP3*, were increased in those patients achieving clinical remission with rituximab [35]. Clearly, mechanisms underlying breakdown of tolerance and emergence of autoimmunity in SLE will most likely involve Tregs. Further studies should examine how modulation of Tregs during disease course and/or treatment contributes to or predicts outcomes in LN.

‘Proteinuria and hematuria can signal renal injury, but are relatively poor indicators of ongoing inflammatory activity.’

Urinary biomarkers

Given the ease and noninvasive approach of sampling urine from patients, examination of the urine via microscopy and dipstick analysis has always been an initial step in assessing renal damage. Proteinuria and hematuria can signal renal injury, but are relatively poor indicators of ongoing inflammatory activity. Researchers have explored whether other urinary markers would better serve the current diagnostic tests utilized for assessing disease activity of LN in SLE.

Much of the focus on urinary biomarkers has been on cytokines and chemokines [36,37]. Increased levels of IL-6 and IL-10 have been found in the serum of SLE patients compared with controls [38,39]. Although initial studies have linked urinary IL-6 with disease activity in LN, larger, more recent studies demonstrate no significant distinction [40]. On the other hand, urinary IL-10 levels were significantly higher in patients with LN compared with SLE patients without LN. However, there was no association between proteinuria and urinary IL-10, and steroid treatment did not influence IL-10 levels [40].

Two major chemokines implicated in both human and animal models of LN include MCP-1 and IP-10 [37]. Studies demonstrate that serum IP-10 levels are increased in active as opposed to nonactive SLE patients [41]. In

addition, IP-10 mRNA from urinary cells distinguished diffuse proliferative glomerulonephritis (WHO class IV) from other classes of LN, suggesting that IP-10 may be useful in identifying those at greatest risk for ESRD progression [42]. MCP-1 did not seem useful in predicting renal histology, but appears to have the best correlation with renal disease activity. MCP-1 levels were predictive of flares during maintenance therapy for LN, with levels increasing 2–4 months prior to flare. Furthermore, patients who responded to therapy had a decline in MCP-1 levels over several months, whereas nonresponders maintained persistently high levels [43]. Another, new urinary marker, TWEAK, a cytokine whose receptor (Fn14) is expressed by mesangial cells and podocytes, has also shown promise. Binding of TWEAK to Fn14 induces secretion of proinflammatory chemokines [44]. Urinary TWEAK levels were found to predict flares, severity of disease activity and response to treatment in LN. Levels also correlated with urinary MCP-1 [45].

Urinary proteomic profiling holds promise in identifying candidate biomarkers. SELDI-TOF mass spectrometry is a new technique that has been able to identify protein patterns in the urine that are associated with different renal conditions from allograft rejection to urolithiasis [46]. In one study, researchers were able to identify two proteins that distinguished active from nonactive LN. Serial urine measurements predicted relapses and remissions, suggesting its potential utility in guiding treatment decisions [47]. Urinary proteomics also has potential in the isolation and identification of novel biomarkers, and the results from ongoing investigations are promising.

Conclusion

Despite significant progress in understanding the molecular and pathophysiologic mechanisms underlying LN, the clinical course of LN remains unpredictable, making its management challenging. There exists an urgent need for identifying biomarkers that will aid in early diagnosis, detecting remissions and flares and identifying those at risk for rapid progression of their renal disease. Over the years, many research techniques have become available to the researcher. These include gene-transcription profiling using DNA microarray and PCR techniques, flow cytometry, autoantigen arrays, ELISA and proteomics to test various sources of

tissue (urinary cells, peripheral blood cells and renal tissue) from LN patients. Many putative biomarkers with great promise have emerged, but none, as yet, have been widely accepted for use in clinical practice. This will require further validation in larger, longitudinal studies in the future. However, initial results have been encouraging, and they provide optimism for identification of biomarkers that will be useful for patients with LN. Based on the early results, it would appear that serologic and/or urinary profiles will emerge that are predictive of disease activity, probability of disease progression

and/or response to various therapeutic regimens. These profiles will be useful in tailoring specific therapies to individual patients.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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