REVIEW ARTICLE

Diagnostic and prognostic biomarkers in Systemic lupus erythematosus

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ABSTRACT

One of the principle challenges facing physicians caring for patients with SLE is finding a marker of disease activity that is feasible, affordable and reliably measures the extent and seriousness of disease activity, in order to gauge the aggressiveness of the treatment approach. We provide a review of literature covering new potential biomarkers that help for disease diagnosis, assessment of disease activity and discovery of specific organ involvement.

Systemic Lupus Erythematosus (SLE) is a multisystem autoimmune disease that involves almost all organs in the human body.[1] The great diversity of clinical manifestations in SLE ranges from mild arthritis through pericarditis, nephritis and neuropsychiatric manifestations.[1] The hallmark characteristics of SLE, include autoantibodies production, deposition of immune complexes in tissues and excessive complement activation.[2,3] The clinically heterogonous disorders in different patients showing a wide spectrum of organ involvement, would confound the search for any specific or single biomarker of active disease. Thus, one of the principle challenges facing physicians caring for patients with SLE is finding a marker of disease activity that is feasible, affordable and reliably measures the extent and seriousness of disease activity, in order to gauge the aggressiveness of the treatment approach.[1] Recent researches have provided data concerning new potential biomarkers that help for disease diagnosis, assessment of disease activity and discovery of specific organ involvement.[4]

A biomarker is a measurement including, but not limited to, genetic, biological, biochemical, molecular, or imaging event whose alterations correlate with disease pathogenesis and/or manifestations and can be evaluated qualitatively and/or quantitatively in laboratories.[5] The reliable biomarker must be simple for routine practice, biologically and pathophysiological relevant as well as it must accurately and sensitively respond to changes in disease activity.[5]

NEWLY RECOGNIZED BIOMARKERS FOR LUPUS DIAGNOSIS

Erythrocyte bound complement activation product C4d (E-C4d) and erythrocyte complement receptor1 (E-CR1)

In search for a biomarker with better specificity and sensitivity for SLE diagnosis using flowcytometric analysis, the investigators demonstrated that patients with SLE had significantly higher E-C4d and lower E-CR1 levels than did healthy

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controls.[6] The E-C4d/E-CR1 test was proved to be 81% sensitive and 91% specific for SLE versus healthy controls. It was also estimated that 86% of SLE patients had abnormal E-C4d/E-CR1 at the time of study visit, compared with 47% had a positive anti-dsDNA test at the same visit. These data suggest that simultaneous determination of E-C4d and E-CR1 by flowcytometry may have significant impact on the accuracy and timing of lupus diagnosis.[6]

Moreover, it was recorded that deposition of E-C4d on erythrocytes of SLE patients most probably participate in the pathogenesis of the disease. The deposition of E-C4d on red blood cells leads to calcium dependent cytoskeletal changes that render them less deformable, partially impairing their capacity to flow through capillaries, thus reducing tissue oxygenation.[7]

Cell-bound complement activation product to platelets (P-C4d)

In a study, carried out by Navratil et al, on 105 SLE patients, 106 patients with other diseases and 100 healthy controls, P-C4d was detected on platelets in 27% of SLE patients versus 2% with other diseases and 0% of healthy controls. Thus, detection of P-C4d on platelets surface is 100% specific in distinguishing SLE from healthy controls and 98% specific in distinguishing SLE from other diseases. These findings demonstrate the potential of P-C4d measurement as a biomarker for lupus diagnosis.[8]

Lymphocytes-bound complement activation products (T-C4d & B-C4d)

A cross-sectional study on 224 patients with SLE, 179 patients with other diseases and 114 healthy controls recorded that T-C4d was 56% sensitive/80% specific and B-C4d was 60% sensitive/82% specific in differentiating SLE from other diseases. In addition, compared with measurement of anti-dsDNA (gold standard), serum C3 and/or serum C4, measurement of T-C4d and B-C4d was significantly more sensitive in identification of patients with SLE during a single clinic visit.[9]

Table 1 Newly Recognized Biomarkers For Lupus Diagnosis.[6,7,8,9]

Biomarker	Median Prevalence	
	Sensitivity	Specificity
1. E-C4d (high) 2. E-CR1 (low) • E-C4d/E-CR1	81%	91%
3. P-C4d		• 100% as compared with healthy controls • 98% as compared with other diseases
4. T-C4d	56%	80%
5. B-C4d	60%	82%

TRADITIONALLY ESTIMATED AUTOANTIBODIES IN SLE DIAGNOSIS AND ACTIVITY: DRAWBACKS

Anti-dsDNA antibodies (the gold standard)

The prevalence of anti-dsDNA antibodies in SLE is 40-80%.[10] Hence, anti-dsDNA antibodies were not detected in all patients with SLE. Moreover, the problem of associating anti-ds DNA antibodies with disease activity is that SLE patients can show persistently elevated anti-dsDNA with no evidence of disease activity[11] or persistent clinical activity with normal anti-dsDNA antibody levels.[12]

Anti-ENA antibodies (anti-Ro/SSA, La/SSB, Anti-U1-RNP, Sm)

Autoantibodies against extractable nuclear antigens (Anti-Ro/SSA, La/SSB, anti-U1-RNP and Sm) are frequently ordered for the diagnosis of SLE.[1] However, the prevalence of anti-Ro/SSA and La/SSB in SLE is 24-60% and 6-35% respectively.[10] In addition, the titer of anti-Ro antibodies is not associated with disease activity. Accordingly, its level is of limited value in predicting disease flare.[1] Anti-U1-RNP autoantibodies correlation to disease activity is still a matter of debate. Its prevalence in SLE is 20-40%.[10] Anti-Sm antibodies (anti-smith antibodies) are highly diagnostic markers in SLE diagnosis. However, its prevalence in SLE is 10-55%.[10] Anti-Sm antibodies are sometimes associated with a milder form of nephritis.[1]

Anti-ribosomal P

Anti-ribosomal antibodies are found in about 15% patients with SLE. It aid in the diagnosis of neuropsychiatric involvement in SLE.[13] On the contrary, other researches did not confirm that these autoantibodies are useful.[14]

Table 2 Traditionally estimated Auto-antibodies in SLE Diagnosis

Auto-antibody	Median Prevalence
1- Anti-ds DNA (gold standard)	40 - 80% [(10)]
2- Anti-Sm (unique for SLE)	10 - 55% [(10)]
3- Anti-U1-RNP	20 - 40%
4- Anti-Ro/SSA and La/SSB	24 - 60, 6 - 35% respectively[(10)]
5- Anti-ribosomal P	15% SLE with neuropsychiatric involvement[(10)]

Lupus biomarkers for specific organ involvement

SLE can affect virtually any tissue and organ. However, not all organs are affected simultaneously and involvement of a specific organ will not necessarily be manifested in the same manner in all patients.[4]

Traditionally, determination of autoantibodies (e.g. anti-Ro, anti-La, anti-dsDNA, anti-Sm, anti-U1-RNP), is used in diagnosis and monitoring SLE. However, there are considerable drawbacks to the use of these immunologic markers.[1,2] Lupus patients care and lupus clinical trials would both benefit immensely from biomarkers that could determine and/or predict organ-specific disease.[4]

BIOMARKERS FOR LUPUS NEPHRITIS

Renal involvement is one of the most common complications and it continues to cause significant morbidity and even mortality. Lupus nephritis occurs in 25%-50% of patients with SLE.[13]

Creatinine clearance, proteinuria, urine sediments, serum C3 and C4 as well as anti-dsDNA have been used for decades to follow the onset, course, and severity of lupus nephritis, yet it is generally recognized that these measurements are inadequate.[4] Persistently high level of anti-dsDNA or low level of C3 and C4 can be found in some patients with low SLE disease activity.[1]

Currently, efforts are focused on identifications of more sensitive and specific biomarkers to diagnose and monitor renal disease in lupus with the hope to optimize synchronization of treatment with disease activity, distinguish active inflammation from irreversible damage and to facilitate development of new therapeutics through clinical trials.[4]

1. Antichromatin antibodies

Chromatin is a complex of double stranded DNA with histone and nonhistone proteins. Anti-chromatin antibodies have been described in patients with SLE. Higher level of these antibodies were noted to be correlated with disease activity especially lupus nephritis.[14]

2. Anti-nucleosomes antibodies

Chromosomal DNA is packaged into a complex of histones, each is composed of 145 base pairs of double stranded DNA wound around the core histone octamer. This, in turn, comprises two molecules each of H2A/H2B, H3 and H4. This unit is called nucleosome.[1] Among SLE patients anti-nucleosome antibodies serve as a useful biomarker in the diagnosis of active lupus nephritis.[15] Anti-nucleosomes antibodies are reportedly present in 70-100% of patients with SLE and have a high specificity up to 96%.[16] Moreover, some investigators reported that anti-nucleosome antibodies could be found in patients with SLE who consistently tested negative of anti-dsDNA antibodies. They concluded that anti-nucleosomes antibodies may serve as a sensitive biomarker and have greater diagnostic efficiency for renal involvement in the absence of anti-dsDNA antibodies.[17]

3. Anti-C1q antibodies (C1qAb)

Serum concentration of antibodies to C1q (C1qAb) has been reported to be strongly correlated with SLE activity as well as renal involvement. This correlation is better than anti-dsDNA antibodies in such conditions. Moreover, absence of anti-C1q antibodies has been reported to exclude diagnosis of lupus nephritis. The sensitivity and specificity of anti-C1q antibodies in lupus nephritis are 44-100%, 70-92% respectively.[18] Recent studies suggest that anti-C1q antibodies may serve as a biomarker to monitor renal involvement and/or predict flare.[19]

4. Anti-endothelial cell antibodies (AECA)

Anti-endothelial cell antibodies are frequently found in sera of patients with SLE and nephritis compared with those without nephritis. The highest levels of these antibodies occur in patients with diffuse proliferative glomerulonephritis.[20]

5. Autoantibodies to plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 (PAI-1) molecules are secreted by endothelial cells. PAI-1 regulates the activity of tissue plasminogen activator (t-PA). Tissue plasminogen activator converts plaminogen to plasmin. Plasmin plays an important role in fibrinolysis. Fibrin deposition and intravascular coagulation are important in pathogenesis of lupus nephritis. In one report, autoantibodies to PAI-1 were found to be significantly elevated in 71% of sera from 48 lupus patients compared with normal control subjects.[21]

6. Anti-heparan sulfate antibodies

Heparan sulfate (HS) in the glomerular basement membrane has been implicated as a target antigen or bridging molecule for the binding of autoantibodies or immune complexes to renal tissue. In one study, where disease activity was assessed using BILAG index (British

Isles Lupus Assessment Group disease activity index), higher levels of both anti-dsDNA and anti-heparan sulfate antibodies were found in patients with lupus nephritis. The level of anti-HS antibodies was found to be correlated with the BILAG renal score better than anti-dsDNA.[22]

7. Complement C4d

A strong relationship have been demonstrated histologically between the intensity of glomerular C4d staining and the presence of microthrombi in patients with lupus nephritis.[23]

8. Urinary biomarkers for renal involvement:

a. Chemokines

Monocyte chemo-attractant protein participates in the pathogenesis of lupus nephritis. Several studies have indicated that urinary levels of MCP-1 (uMCP-1) protein and MCP-1 mRNA are promising biomarker candidates due to specificity for renal activity and sensitivity in predicting renal flares.[24]

b. Neutrophil Gelatinase Associated Lipocalin (NGAL)

Cross sectional as well as longitudinal studies have demonstrated the promise of neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker of lupus nephritis in both pediatric and adult patients.[4]

c. uTWEAK

Urinary levels of tumor necrosis-like weak inducer of apoptosis (uTWEAK) were shown to be significantly higher in patients with lupus nephritis (active form) as compared with those with inactive or no nephritis.[25] Moreover, another multicenter longitudinal study demonstrated the potential value of uTWEAK which was superior to current standards anti-dsDNA and serum complement levels in differentiating lupus nephritis from non-renal lupus activity.[26]

Table 3 Lupus nephritis biomarkers

Serum Biomarkers	Urinary Biomarkers
Antinucleosome antibodies (up to 90% specific, positive in SLE patients with negative ds-DNA[16,17]	Chemokines [24]
Antichromatin antibodies [14]	Neutrophil Gelatinase Associated Lipocalin (NGAL)[4]
C1gAB (sensitivity 44-100%, specificity 70-92%)[18]	
Anti-endothelial cell antibodies (AECA)[20]	UTWEAK [26]
Auto-antibodies to plasminogen[21] activator inhibitor-1	
Anti-heparansulfate antibodies[22]	
Complement C4d[23]	

BIOMARKERS FOR CENTRAL NERVOUS SYSTEM INVOLVEMENT

Immunopathogenic injuries of the central nervous system (CNS) can occur in many patients with SLE who experience a wide range of neuropsychiatric (NP) events. The prevalence of NP-SLE varies widely between 37 %- 95 % in different studies.[27] Earlier studies have discovered the so called antineural antibodies in patients with NP-SLE but results are not useful in identification of antigenic specificity.[28]

A seminal study in 2001 described the role of anti-N-methyl-D-aspartate (NMDA) receptor (anti-NR2) antibodies in NP-SLE. However, there are conflicting results about the role of anti-NR2 antibodies in NP-SLE.[29-31]

Omdal et al measured anti-NR2 in plasma of 57 SLE patients who were subjected to comprehensive psychological and cognitive testing. They noticed an association between anti-NR2 positivity, depressed mood and decreased short-term memory.[31]

Similarly, Laptena and colleagues studied 60 SLE patients and reported an association between serum anti-NR2 antibodies positivity with depressive mood but not with cognitive dysfunction.[30] Moreover, Hanly et al, noticed no association between cognitive impairment and serum anti-NR2 antibodies.[32]

Recently, Yoshio et al recorded that anti-NR2 antibody levels in CSF of 80 patients with NP-SLE were significantly higher than without NP-SLE. However the serum anti-NR2 levels were only slightly higher in patients with NP-SLE than in patients without NP-SLE.[33] Moreover, Arinuma et al found significant elevated levels of anti-NR2 antibody in CSF of patients with diffuse NP-SLE compared with patients with focal NP- SLE or control patients with other non-inflammatory neurologic diseases.[34] Results of these recent studies suggest that measurement of anti-NR2 antibody in CSF of NP-SLE patients maybe more useful for diagnosing such disorders than measurement of these antibodies in serum.

Table 4 Biomarkers For CNS and CVS

CNS Biomarker	CVS Biomarker
Anti-NR2 antibody in both serum and CSF[31,33]	Platelet C4d with severe acute ischemic stroke[35]

BIOMARKERS FOR CARDIOVASCULAR DISEASE, STROKE AND MORTALITY

Cardiovascular disease and cerebrovascular accident remain common and partially catastrophic manifestations of SLE. Mehta et al recruited 80 patients hospitalized for acute ischemic stroke. Platelet C4d-positive patients were more likely to have a severe stroke compared to those with negative platelet C4d. They concluded that platelet C4d is associated with severe acute ischemic stroke and that platelet C4d may be a biomarker as well as pathogenic clue that links cerebrovascular inflammation and thrombosis.[35]

Autoantibody panels in monitoring disease activity in SLE

It is likely that a single autoantibody assay will not consistently and accurately monitor disease activity in SLE due to the clinical heterogeneity of SLE flares. Thus, the use of autoantibody panels has been advocated for many years to cover the range of disease activity in SLE.[36]

Conclusion

Autoantibodies in SLE are directed to different cellular components (nuclear, cytoplasmic macromolecules as well as cell membrane). These antibodies differ in their binding characteristics and in their prevalence. The diversity of these autoantibodies may be due to the influence of multigenetic defects that lead to over stimulation of B-cells and support polycolonal B-cell activation as a mechanism of antibodies production. The clinical heterogeneity in SLE plays an important role in discovering a specific biomarker for disease diagnosis, activity and specific organ involvement. Anti-dsDNA is considered as the gold standard autoantibodies that aid in evaluation of disease activity especially if there is renal involvement. However, the predictive value of these autoantibodies for evaluating disease activity is limited. The discovery of newly recognized biomarkers

in diagnosis of SLE activity and specific organ involvement will hopefully substitute the traditional gold standard autoantibody testing for SLE assessment as being more sensitive and specific in predicting disease flare. Ongoing research have generated promising results leading to optimism in SLE biomarker evaluation.

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