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The Search for Lupus Biomarkers

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Abstract

Few biomarkers for SLE have been validated and employed for making clinical decisions. The lack of reliable, specific biomarkers for SLE hampers proper clinical management of patients with SLE, and impedes development of new lupus therapeutics. This void has led to renewed enthusiasm for identifying biomarkers that precisely and specifically reflect the pathophysiologic and clinical changes of SLE. Several laboratory markers have shown early promise as biomarkers for lupus susceptibility, diagnosis, and monitoring. These include polymorphisms and copy number variations of complement C4 and Fcγ receptor genes (disease susceptibility), cell-bound complement C4d (diagnosis and/or disease activity), CD27^{high} plasma cells (disease activity), “interferon signature” (disease activity), and anti-C1q and anti-NMDA (disease activity and organ involvement).

Although these and other promising candidate biomarkers have been identified, they still need to be validated through rigorous, large-scale multicenter studies. This chapter reviews briefly the historical aspects of lupus biomarkers and summarizes current efforts to advance the field.

Keywords

biomarkers; diagnosis; disease activity; genetics; lupus; SLE

A. Introduction

Systemic lupus erythematosus (SLE) is arguably the most clinically and serologically diverse autoimmune disease, with more than 100 autoantibodies found in patients and disease spectra ranging from subtle symptoms to life-threatening multi-organ failure^{1–3}. The hallmark characteristics of SLE, including production of autoantibodies, deposition of immune complexes in tissues, and excessive complement activation, are generally thought to be consequences of immune dysregulation^{2, 3}. Owing to its complex etiopathogenesis, heterogeneous presentation, and unpredictable course, SLE remains one of the greatest challenges to both investigators and physicians.

There is an urgent need for SLE biomarkers. This is a critical situation for several reasons including the following. First, SLE is commonly misdiagnosed, even by experienced

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rheumatologists. The diagnosis requires interpretation of complex criteria developed by the American College of Rheumatology, as no single test is sufficiently sensitive and specific to be diagnostic. Second, the course of SLE in a given patient is characterized by unpredictable flares and remissions. Again, there is no laboratory test with reliable capacity to identify or predict the beginning or end of a disease flare. Third, lack of biomarkers has impeded efforts to evaluate new SLE therapeutics in clinical trials. Pharmaceutical companies, with potential therapies in the pipeline and in hand, are reluctant to invest in clinical trials because response to therapy might not be determined with confidence. This past year unfortunately marked the 50th anniversary since the last drug was approved for SLE. Because SLE is a heterogeneous, complex, multisystem disease, a new drug may have subtle but beneficial effects in perhaps only a subset of patients. Currently, it is difficult, if not impossible, to measure such a clinical response.

B. Biomarkers: Definition and Potential Utility in SLE

A biomarker can be defined as a genetic, biological, biochemical, or molecular event whose alterations correlate with disease pathogenesis and/or manifestations and can be evaluated qualitatively and/or quantitatively in laboratories⁴. Several methodologic criteria are required for a laboratory measure to serve as a reliable biomarker for a disease, including: 1) it must be biologically and pathophysiologically relevant; 2) it must be simple for routine practice; and 3) it must accurately and sensitively respond to changes in disease activity (see ^{4, 5} for an extensive review).

Given the complex etiopathogenesis, heterogeneous clinical manifestations, and varying rates of disease progression among individual SLE patients, it is reasonable to predict that a particular SLE biomarker may be informative regarding only one specific aspect, rather than all aspects, of the disease process at any give time. For example, some biomarkers may be used to facilitate accurate and early diagnosis of SLE, some may help identify individuals prone to develop SLE or patients at risk for severe disease and poor prognosis, some may be useful in determining disease severity and/or monitoring disease progression, some may indicate systemic or specific organ involvement, and some may be used to evaluate response to treatment. This “individuality” of biomarkers supports the observation that no single biomarker has been universally accepted as “The Lupus Biomarker”. Ultimately, diagnosis, disease activity assessment, and understanding of the pathogenesis of SLE are likely to require a panel of biomarkers that span the gamut of laboratory and clinical features of SLE.

C. Classification Of Candidate Lupus Biomarkers

Although the etiology of SLE is still incompletely understood, numerous lines of evidence support the conclusion that genetic, hormonal, and environmental factors are clearly involved⁶. Clinical manifestations of SLE are likely the consequence of a multifaceted, patient-unique immune-inflammatory process, which evolves over the course of disease and is characterized by production of autoantibodies, immune complex/complement-mediated tissue injury, and vasculopathy³. Based on these etiopathogenic features of SLE, we will categorize and discuss candidate SLE biomarkers in different classes.

C.1. Biomarkers for Lupus Susceptibility

Genetic factors, either protective or provocative, clearly play a major role in determining whether an individual will develop SLE. The genetic susceptibility is undoubtedly influenced by environmental factors, although these triggers remain poorly identified. Recent searches for “lupus genes,” primarily through candidate gene-association studies and genome-wide microsatellite and single nucleotide polymorphism (SNP) association scans, have clearly demonstrated that SLE is a disease with complex genetic inheritance and no single causative

gene⁷. Multiple genes are involved, perhaps in a hierarchical, interactive manner, to regulate the thresholds of autoreactivity and disease onset^{8, 9}. Initial genetic studies focused on genes that are historically considered to be key components of immune responses, such as major histocompatibility complex (MHC) genes. Specific alleles of MHC (called human leukocyte antigen (HLA) in humans) have been found to be associated with SLE in ethnic groups¹⁰. Recent studies have identified several susceptible loci that harbor many candidate genes with known immune-related functions. In addition to sequence polymorphism, naturally occurring variations in copy number of candidate genes have increasingly been recognized as a source of susceptibility to complex genetic diseases. Recent developments in genetic biomarkers for SLE susceptibility will be succinctly discussed in this section and are reviewed in detail elsewhere^{7, 9, 11-13}.

C.1.1. Complement—Currently, the genes that most warrant being referred to as “lupus genes” are those encoding the components of the classical complement pathway (Table 1)¹⁴. Complete deficiency of one of the early components, C1q, C1r, C1s, C4, or C2, is a strong, albeit rare, risk factor for developing SLE and/or lupus-like diseases¹⁴. Much more common is the partial deficiency of these complement genes, often reflected by varying low levels of circulating complement protein (e.g., C4)¹⁴. Partial C4 deficiency, particularly C4A deficiency, occurs in 30-40% of patients with SLE and has been associated with lupus susceptibility¹⁵. However, it should be noted that partial C4 deficiency and low serum C4 also occur in a significant fraction of the general healthy population.

Human C4 exists in two isotypes, C4A and C4B, which are highly homologous but exhibit markedly different biological activities¹⁶. Two different genes residing within the MHC locus on chromosome 6 encode C4A and C4B; each gene may exist in zero to 4 copies¹⁷. Therefore, significant copy number variation (CNV) of the C4 genes (ranging from zero to 8 copies of C4A and C4B genes combined in a diploid genome) can occur among different individuals, which in turn may account for the varying concentrations of C4 in the sera of these individuals. In a recent study, Yang and colleagues investigated CNV of C4 genes in 1,241 individuals of European ancestry, including 233 SLE patients, 362 first-degree relatives of those patients, and 517 unrelated healthy individuals¹⁸. Of the entire study cohort, the CNV of total C4 genes ranged from 2 to 6 (0 to 5 for C4A and 0 to 4 for C4B), with 4 copies of total C4 consisting 2 each of C4A and C4B being most common. Interestingly, the C4 CNV appeared to shift toward the lower side in the SLE patient population. The risk of SLE susceptibility significantly increased among individuals who have only 2 copies of the C4 genes (odds ratio (OR)=6.514; $p=0.0002$), but decreased in those with 5 or more copies (OR=0.466; $p=0.016$). Specifically, zero and 1 copy of the C4A gene appeared to confer an individual significant risk for SLE (OR=5.267 for zero copy and OR=1.613 for 1 copy; both $p<0.05$), whereas 3 or more copies of C4A seemed to protect from SLE development (OR=0.574; $p=0.012$). These results suggest that low CNV of the C4 genes, particularly C4A, is associated with the susceptibility to SLE.

C.1.2. Fcγ receptors—Recent genetic studies of SLE have focused on correlating SLE (susceptibility, disease spectrum, and severity) with polymorphisms of hypothetical candidate genes^{7, 11-13} (Table 1). These include genes coding for mannose-binding lectin (MBL), cytokines (e.g., IL-6, IL-10, IL-21, TNF- α , and osteopontin), chemokines (e.g., MCP-1), cytokine receptors/antagonists (e.g., type II TNF- α receptor and IL-1 receptor antagonist), Fcγ receptors (e.g., FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb), and other cell surface receptors (e.g., cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death protein-1 (PD-1; also known as PDCD-1). Additionally, CNV of some candidate genes have been investigated as susceptibility factors. For example, Fanciulli, Atiman and colleagues have recently investigated the CNV of the FcγRIIIb gene using patients from the United Kingdom (501 SLE patients with or without nephritis; 90 Wegener's granulomatosis (WG); 76 microscopic polyangiitis (MPA); compared with 862 healthy controls) and France (77 WG versus 181

healthy controls)¹⁹. They reported that low CNV of the FcγRIIIb gene (< 2 copies) was significantly associated with increased susceptibility to SLE (OR=2.43; $p=0.001$), MPA (OR=2.56; $p=0.013$), or WG (OR=2.46; $p=0.015$)¹⁹. Furthermore, the frequency of complete deficiency of FcγRIIIb (zero copy) was significantly increased in patients with these autoimmune diseases (2%; 21 patients with SLE, one with WG, and one with MPA) as compared to healthy controls (0.1%). This finding suggests that complete FcγRIIIb deficiency renders an individual at considerably increased risk for autoimmune diseases, particularly SLE.

C.1.3. Protein tyrosine phosphatase N22 (PTPN22)—Aberrant T and B cell functions are hallmark immune abnormalities in SLE. It is thus not surprising that recent breakthroughs in SLE genetics have occurred primarily with immune-related genes/molecules. In 2004, a missense SNP (1858C→T; corresponding to the R620W amino acid substitution) in protein tyrosine phosphatase N22 (PTPN22) – a lymphoid-specific protein tyrosine phosphatase regulating TCR signaling in memory/effector T cells – was found to be strongly associated with SLE in Caucasian patients²⁰. In that original study, the PTPN22 C1858T SNP (rs2476601) was investigated in 521 SLE patients from North America and 1,961 healthy controls. It was found that the risk for developing SLE is significantly increased if an individual carries a single copy of the minor 1858T allele (OR=1.37; $p=0.0009$) and is dramatically increased with 2 copies of the minor allele (OR=4.37; $p=0.0009$). Subsequent studies by other investigators have confirmed this PTPN22 SNP as a risk factor for SLE, especially in familial SLE of North American patients with European ancestry and in patients with SLE and concurrent autoimmune thyroid disease^{21, 22}. Additionally, numerous studies have demonstrated significant associations of the PTPN22 C1858T polymorphism with other autoimmune diseases including rheumatoid arthritis (RA), type 1 diabetes mellitus (DM), and Grave's disease^{23, 24}. These findings, taken together, suggest that TPTN22 may play a role in certain common pathogenic mechanisms of autoimmune disorders²⁴.

C.1.4. Interferon regulatory factor 5 (IRF5)—High serum levels of interferon (IFN)-α have long been detected in lupus patients²⁵. Recent studies have provided further evidence supporting an important role for IFNα in the pathogenesis of SLE (see further discussion below). Molecules involved in the regulation or execution of the IFNα-pathway have naturally attracted attention in the search for lupus biomarkers. Among them, interferon regulatory factor 5 (IRF5), a transcription factor that controls transactivation of IFNα-related genes, has emerged as the leading contender²⁶. Initially, a Swedish research group screened SNPs in 13 genes from the IFNα pathway in 679 Northern European (Swedish, Finnish, and Icelandic) patients with SLE, 798 unaffected family members, and 438 healthy controls²⁷. They discovered that several SNPs in the tyrosine kinase 2 (TYK2) and IRF5 genes displayed strong linkage and association with SLE. This team and others have subsequently confirmed that a common IRF5 haplotype, which is characterized by a SNP (rs2004640) that creates an alternative spliced isoform of IRF5 and another independent SNP that leads to increased expression of IRF5, is an important genetic risk factor for SLE in different ethnic groups²⁸⁻³⁰. Additional IRF5 SNPs/haplotypes have recently been identified and shown to be either risk or protective genetic factors for SLE^{31, 32}. Like TPTN22, genetic variants of IRF5 have also been reported to confer risk for non-SLE autoimmune diseases, including rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and multiple sclerosis (MS)^{32, 33}. Notably, however, the PTPN22 C1858T variant has been shown to associate with SLE, RA, Grave's disease, and type 1 DM, but not with IBD and MS^{23, 24}.

C.1.5. Signal transduction and activator of transcription 4 (STAT4)—Another immune function-related gene that has recently generated considerable enthusiasm is the signal transduction and activator of transcription 4 (STAT4) gene. The STAT 4 gene encodes a transcription factor involved in the signaling pathways of several cytokines, including IL-12,

IL-23, and type 1 IFN (such as IFN α)³⁴. By screening SNPs in and around 13 candidate genes within a region that has previously been linked to susceptibility to RA, an international team of investigators has identified a haplotype marked by SNP rs7574865 within the third intron of the STAT4 gene³⁵. Verified by using two independent cohorts of RA patients from North America and Sweden and three cohorts of SLE patients, the haplotype marked by rs7574865 was found to be significantly associated with increased risk for both RA (OR=1.32) and SLE (OR=1.55)³⁵. Within a short time since the original discovery, several investigators have reported similar associations between rs7574865 and susceptibility to SLE and RA in other ethnic groups^{36, 37}, and associations of rs7574865 with other autoimmune diseases including IBD and type 1 DM³⁸.

It is of interest to note that the above-mentioned studies showed associations of genetic variants of PTPN22, IRF5, and STAT4 with susceptibility to unique groups of autoimmune diseases (e.g., PTPN22 with SLE, RA, and type 1 DM, but not MS and IBD; STAT4 with SLE, RA, type 1 DM, and IBD, but not MS; IRF5 with SLE, IBD, and MS)^{23, 32, 38}. These recent studies lend additional support to the hypothesis that a common genetic basis may be involved in autoimmunity. However, these studies also suggest that a combination of certain genetic factors and disease-specific mechanisms may play a predominant role in a particular cluster of autoimmune diseases but not in another.

C.1.6. Other candidate genes—In addition to the immune function-related genes discussed above, genome-wide linkage analyses actively ongoing around the world have identified numerous SLE susceptibility loci and positional candidate genes. These include, but are not limited to, genes encoding C-reactive protein (CRP), pre-B cell leukemia transcription factor (PBX1), polyADP-ribose polymerase (PARP), B lymphoid tyrosine kinase (BLK), integrin alphaM (ITGAM; CD11b), and integrin alpha X (ITGAX)³⁹⁻⁴². Most of these susceptibility loci/genes still await further verification.

In conclusion, the field of SLE genetics holds great promise. In some cases, however, reports have not been consistent among different laboratories, possibly reflecting heterogeneous genetic susceptibility influenced by ethnicity and environment. As such, practical and universal genetic biomarkers for SLE susceptibility still await further search and validation. It is likely that distinct sets of genetic biomarkers customized for different ethnic groups will be required for appropriately identifying lupus-susceptible individuals of any given ethnic background. With the unprecedented advance in the complex genetics of SLE worldwide, there is now more than ever a need for accurate phenotyping/subsetting of SLE patients according to patient-unique conditions such as ethnic origins, clinical manifestations, and living environment. This approach is likely to help elucidate the specific roles of individual lupus genes that have been identified in some but not other studies involving widely heterogeneous study cohorts.

C.2. Biomarkers for Lupus Diagnosis

Once clinical symptoms have developed, prompt diagnosis and proper management of SLE remain great challenges to physicians. The diagnosis of SLE relies on revised ACR criteria published in 1982 and 1997, but this approach is problematic in routine clinical practice. For example, development of 4 of 11 criteria for a definite SLE diagnosis may take years to decades to evolve in some patients. Laboratory tests or biomarkers that facilitate early and accurate diagnosis of SLE are essential. More sensitive and specific biomarkers for lupus diagnosis would also improve efficiency and accuracy of clinical trials by assuring that enrolled patients presumed to have SLE actually have the disease.

Traditionally, determination of autoantibodies such as antinuclear antibodies (ANA), anti-extractable nuclear antigen antibodies (e.g., anti-Ro/SSA, anti-La/SSB, anti-snRNP, and anti-Sm), and anti-double stranded DNA (anti-dsDNA) is used in diagnosing and monitoring SLE.

However, there are considerable drawbacks to the use of these immunologic markers (see^{2, 43} for further discussion). In search of biomarkers with better specificity and sensitivity for SLE diagnosis, Manzi et al. have suggested abnormal levels of erythrocyte-bound complement activation product C4d (E-C4d) and complement receptor 1 (E-CR1) as candidates⁴⁴. Using flow cytometric analysis, these investigators showed that patients with SLE had significantly higher E-C4d and lower E-CR1 levels than did patients with other diseases or healthy controls⁴⁴. The E-C4d/E-CR1 test was shown to be 81% sensitive and 91% specific for SLE versus healthy controls and 72% sensitive and 79% specific for SLE versus other diseases, with an overall negative predictive value of 92%. It was also estimated that 86% of the patients with SLE had abnormal E-C4d/E-CR1 at the time of the study visit, compared to 47% who had a positive anti-dsDNA test at the same visit⁴⁴. These data suggest that simultaneous determination of E-C4d and E-CR1 by flow cytometry may have significant impact on the accuracy and timing of lupus diagnosis.

Subsequently, the same group extended the paradigm of cell-bound complement activation products (CB-CAPs) to platelets in a cross-sectional study of platelet bound-C4d (P-C4d) in 105 SLE patients, 106 patients with other diseases, and 100 healthy controls⁴⁵. P-C4d was detected on platelets from 27% of SLE patients, 2% of patients with other diseases, and 0% of healthy controls. Thus, detection of C4d on platelet surfaces is 100% specific in distinguishing SLE patients from healthy controls, and 98% specific in distinguishing SLE patients from patients with other diseases. These results demonstrate great potential for P-C4d measurement as a biomarker for lupus diagnosis.

C.3. Biomarkers for Lupus Disease Activity

Currently, disease activity in SLE is often assessed using composite disease activity indices, such as SLE Disease Activity Index (SLEDAI), Systemic Lupus Activity Measure (SLAM), European Consensus Lupus Activity Measure (ECLAM), and British Isles Lupus Assessment Group Index (BILAG), which comprise a variety of clinical and laboratory parameters⁴⁶⁻⁴⁸. These instruments require appropriate training to interpret and complete accurately, making universal application more challenging. However, this is an effort that may be well worth it in order to standardize measurement of disease activity in SLE.

Autoantibody production and complement activation are considered two of the hallmark features of SLE, and laboratory measures of complement and autoantibodies are components of most disease indices. Numerous studies have been conducted to identify the associations of various autoantibodies (particularly anti-dsDNA) and complement proteins (including native molecules and activation products) with disease activity/severity in SLE. The results, however, are inconsistent, and such ambiguity may also confound the assessment of disease activity with the widely used disease indices. Consequently, the value of conventional tests measuring serum complement and autoantibodies as markers of SLE disease activity is being revisited. Several recent reviews have summarized the controversial issues concerning traditional markers and provided insightful perspective^{49, 50}. This section will focus on a number of potential biomarkers for SLE disease activity that have recently emerged.

C.3.1. CD27^{High} plasma cells—Recent studies of murine lupus models and human lupus have suggested that B cells play a central role in the pathogenesis of SLE⁵¹. A cardinal change reflecting B cell abnormalities is alteration of peripheral B cell homeostasis. In adult SLE patients, the frequency and absolute numbers of CD19⁺CD20⁺CD27⁻ naïve B cells decreased significantly, whereas the frequency and absolute number of CD19⁺CD20⁺CD27^{high} plasma cells (distinguished from CD19⁺CD27^{low-moderate} memory B cells) increased remarkably in patients with active disease⁵². Similarly, in pediatric SLE patients, especially those with active disease, a subset of B cells resembling plasma cell precursors has been detected in the peripheral

circulation⁵³. These observations support a role for autoantibody-producing plasma cells in the pathogenesis of SLE. Recently, Jacobi and colleagues conducted a study to investigate rigorously whether B cell abnormalities, as reflected by altered homeostasis and phenotypes of peripheral blood B cells detected using flow cytometry, may correlate with SLE disease activity⁵⁴. These investigators reported that the number and frequency of CD27^{high} plasma cells were significantly correlated with disease activity scored by SLEDAI, ECLAM and the titer of anti-dsDNA antibodies. They also observed that the expansion of the CD27^{high} plasma cell population increased with duration of disease and decreased after effective treatment with immunosuppressive agents. Moreover, using a nonparametric data-sieving algorithm, the B cell abnormalities provided predictive values for nonactive and active disease of 78.0% and 78.9%, respectively. The positive predictive value (78.9%) of B cell abnormalities for active disease was greater than that of the humoral/clinical data pattern (71.4%). These results suggest that alteration of the B cell subpopulation (namely the increase in CD27^{high} plasma cells) may be a valuable biomarker for monitoring lupus disease activity.

C.3.2. Reticulocyte-bound C4d—Identification of erythrocyte-bound C4d as a potential biomarker for lupus diagnosis led to subsequent studies of reticulocytes⁵⁵. These studies were based on the hypothesis that reticulocytes, the youngest and short-lived erythrocytes with a 24-48 hours lifespan, upon emerging from the bone marrow, are immediately exposed to and acquire C4d at levels proportionate to the extent of complement activation at that time, thereby reflecting lupus disease activity. To verify this hypothesis, it was determined if abnormal levels of C4d are specifically present on the surface of reticulocytes of lupus patients and if reticulocyte-bound C4d (R-C4d) levels correlate with lupus disease activity⁵⁵. Using flow cytometric analysis, a wide range of increased R-C4d levels was specifically detected in lupus patients, and R-C4d levels fluctuated and correlated with clinical disease activity as measured by SLEDAI and SLAM. Specifically, in cross-sectional analysis, patients with R-C4d in the highest quartile compared to those in the lowest quartile had significantly higher SLEDAI ($p=0.00002$) and SLAM ($p=0.02$) scores. These results support the potential of R-C4d as a biomarker for monitoring lupus disease activity.

C.3.3. IFN α and IFN-inducible gene profiles—The role of soluble and genetic biomarkers for lupus disease activity has increasingly been investigated. Prominent among these candidate biomarkers is the type 1 IFN system (e.g., IFN α and related genes)⁵⁶⁻⁵⁹. In a pioneering study, Baechler and colleagues used DNA microarray techniques to study gene expression profiles in peripheral blood mononuclear cells of SLE patients and found a striking pattern of upregulated IFN-inducible genes (subsequently termed “IFN signature”) in a subset of SLE patients⁵⁶. They further observed that the IFN signature predicts more severe disease, such as cerebritis, nephritis, and hematological involvement, in those patients. Other investigators have subsequently reported similar findings, including significant associations of enhanced expression of IFN-inducible genes and/or serum levels of IFN-inducible chemokines with increased disease activity, hypocomplementemia, and the presence of autoantibodies specific for dsDNA and RNA-binding proteins (Ro, U1-RNP, and Sm), in both adult and pediatric SLE patients⁶⁰⁻⁶⁶. A recent study using quantitative real-time polymerase chain reaction analysis further confirmed that IFN α is primarily responsible for inducing the observed IFN signature by peripheral blood mononuclear cells from SLE patients⁶⁷. To further determine whether changes in the IFN signature expression correlate with clinical outcomes such as disease flares, new organ involvement, and response to treatment in SLE patients, a Canadian research group recently investigated the expression levels of selected IFN α -inducible genes, serological variables, and clinical disease activity (as measured by SLEDAI) in 94 SLE patients over a period of 3-12 months⁶⁸. When analyzed cross-sectionally at a single timepoint, expression levels of IFN α -inducible genes were found to be significantly elevated and associated with high SLEDAI scores, active renal disease, decreased C3 levels, and positive

anti-dsDNA and anti-RNA-binding protein autoantibodies. However, when followed over time, no significant correlation between changes in IFN α -inducible gene expression and changes in disease activity, C3 levels, or autoantibody levels was observed. In contrast, preliminary reports from two other laboratories have suggested that the IFN-inducible gene profile of peripheral blood cells was not only associated with current disease activity, but also capable of predicting future disease activity in SLE patients^{69, 70}. Therefore, the candidacy of the IFN signature/IFN-inducible proteins as biomarkers for monitoring and/or predicting SLE disease activity needs to be further investigated. Large-scale, longitudinal studies ongoing in several laboratories are highly anticipated.

It is worth mentioning that recent studies have provided molecular and mechanistic bases linking genetic susceptibility and disease pathogenesis in SLE. Two laboratories have independently discovered that SLE patients carrying the risk allele of PTPN22 C1858T variant (rs2776601)⁷¹ or IRF5 variant (rs2004640)⁷² have higher serum IFN α activity than do patients lacking these risk alleles. Moreover, recent studies have shown that binding of immune complexes containing DNA, RNA, or RNA-binding proteins to plasmacytoid dendritic cells triggers overproduction of IFN α in SLE patients⁷³, and high levels of IFN α and IFN-inducible chemokines may in turn lead to myriad activities contributing to SLE pathogenesis including activation of autoreactive lymphocytes, dysfunction of regulatory T cells, and dysregulation of endothelial cells and vasculogenesis⁷⁴⁻⁷⁶.

C.3.4. B lymphocyte simulator (BLyS)—B lymphocyte stimulator (BLyS; trademark of Human Genome Sciences, Rockville, MD; also known as BAFF, TALI-1, THANK, or TNFSF13B) is expressed as a transmembrane protein on monocytes, macrophages, and monocyte-derived dendritic cells⁷⁷. A soluble form of BLyS, cleaved from the cell surface by a furin protease, is biologically active and critical for B cell growth and survival⁷⁸. The relationship between BLyS overexpression and SLE has been demonstrated convincingly in animal studies^{79, 80}. In humans, cross-sectional studies showed that approximately 30% of SLE patients had significantly elevated circulating levels of BLyS^{81, 82}. Elevated BLyS levels appeared to correlate with increased total IgG and autoantibody (particularly anti-dsDNA) levels⁸¹⁻⁸³, and, in some studies, with increased disease activity (as measured by SLEDAI)⁸⁴. A recent study has shown that excessive productions of IFN γ by activated T cells may be responsible for the induction of BLyS production by monocytes and macrophages in lupus patients⁸⁵.

To delineate the role of BLyS in the long-term immune dysregulation in SLE, Stohl and colleagues recently conducted a longitudinal observational study in which 68 SLE patients were followed regularly for disease activity (measured using SLEDAI) and serum BLyS levels over a period of 147-420 days (median 369 days)⁸⁶. They found that SLE patients exhibited considerable variability in serum BLyS levels with 50% of patients having persistently or intermittently elevated serum BLyS levels over the follow-up period. However, changes in serum BLyS levels did not correlate with changes in disease activity and/or specific organ involvement in individual patients. In another study, Becker-Merok et al. studied clinical disease activity, serological variables, and serum BLyS levels in 60 patients with RA and 42 patients with SLE, 19 of whom were followed prospectively over a period of approximately 16 months⁸⁴. These investigators found that considerably more SLE patients had significantly higher serum BLyS levels (57%; 2.7 ng/ml) than did RA patients (10%; 1.4 ng/ml; both $p < 0.01$). They found that serum BLyS levels generally correlated with SLEDAI scores in SLE patients in cross-sectional comparison, but did not correlate with changes in disease activity over time.

Since BLyS is not known to have direct or immediate proinflammatory activities, changes in serum BLyS levels are unlikely to trigger acute inflammatory reactions and disease

manifestations. Therefore, the reported lack of correlations between changes in BLYS levels and changes in disease activity in SLE patients might not be surprising. However, it is possible that an increase in disease activity may lag behind increases in circulating BLYS levels due to indirect or “delayed” effects of BLYS in the systemic immune-inflammatory reactions of SLE. Indeed, Petri and other investigators have recently conducted a prospective multicenter study that evaluated 254 SLE patients every 3-6 months over a 2-year period⁸⁷. The results showed that plasma BLYS levels were associated with anti-dsDNA levels ($p=0.0465$) and disease activity (measured using SELENA-SLEDAI) ($p=0.0002$). Interestingly, multivariate analyses showed that a greater increase in the SELENA-SLEDAI score in the current visit was significantly associated with higher BLYS levels at the previous visit ($p=0.0042$). Similarly, a greater increase in the BLYS level from the previous visit was associated with a greater SELENA-SLEDAI score in the subsequent follow-up visit ($p=0.0007$). These results suggest a “delayed” causative relationship between circulating BLYS levels and SLE disease activity.

C.3.5. Other soluble biomarkers—In recent years, a growing list of humoral factors, including cytokines (e.g., IL-6, IL-10, IL-16, and IL-18), soluble cytokine receptors (e.g., sIL-2 receptor), soluble adhesion molecules (e.g., sICAM and sVCAM), acute phase proteins (e.g., CRP and ferritin), autoantibodies (e.g. anti-nucleosome and anti-CRP), and soluble thrombomodulin, has been investigated for their associations with the activity and severity of SLE⁸⁸⁻⁹³ (Table 1). Hopefully, this promising array of candidates will ultimately yield a number of validated lupus biomarkers.

C.4. 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. Lupus patient care and lupus clinical trials would both benefit immensely from biomarkers that could determine and/or predict organ-specific disease.

C.4.1. Biomarkers for renal involvement – anti-nucleosome and anti-C1q—Of the myriad manifestations of SLE, nephritis is a common cause of significant morbidity and mortality. It occurs in 25-50% of patients with SLE⁹⁴. Anti-dsDNA has traditionally been used as a serologic indicator to follow the development and severity of lupus nephritis, although there are mixed opinions regarding its utility. Recently, investigators have explored the use of other autoantibodies in monitoring and preferably predicting renal disease in patients with SLE. Among those autoantibodies, anti-chromatin/nucleosome antibodies^{95, 96} and anti-C1q antibodies^{97, 98} have been extensively studied and have shown promise as biomarkers of renal involvement.

Chromatin, the DNA-histone complex found in the nucleus of eukaryotic cells, is organized into a repeating series of nucleosomes. Recent studies have demonstrated that the nucleosome is a major autoantigen targeted by T and B cells in SLE⁹⁹. Anti-nucleosome antibodies are reportedly present in 70-100% of patients with SLE and have a high specificity (up to 97%) for SLE^{43, 95, 100}. Among SLE patients, anti-nucleosome antibodies are more likely to be detected in patients with nephritis¹⁰¹ and may serve a useful biomarker in the diagnosis of active lupus nephritis^{96, 102}. Moreover, some investigators reported that anti-nucleosome antibodies could be found in patients who consistently tested negative for anti-dsDNA antibodies. In one study¹⁰³, 60% of the patients who tested positive for anti-nucleosome antibodies but negative for anti-dsDNA antibodies were shown to have renal disease, suggesting that anti-nucleosome antibodies may serve as a sensitive marker for renal involvement in the absence of anti-dsDNA. In addition, several studies have shown a strong correlation between anti-nucleosome antibodies and SLE disease activity measured by SLEDAI or ECLAM^{95, 103}. However, it should be cautioned that a recent study showed that

anti-nucleosome antibodies are highly prevalent in both SLE patients with (89%) or without (80%) active proliferative nephritis and have limited value in distinguishing these two subgroups of patients¹⁰⁴. Taken together, these studies provide substantial, but not definitive, evidence that anti-nucleosome antibodies may be more sensitive and have greater diagnostic efficiency than anti-dsDNA for active disease, especially nephritis, in SLE patients.

Although anti-C1q antibodies can be detected in a small proportion of healthy individuals (2-8%), they are more common in patients with autoimmune disorders such as hypocomplementemic urticarial vasculities and SLE⁹⁸. These antibodies are mostly of the IgG subtype with IgG1 and IgG2 being predominant. Anti-C1q antibodies have been found in 30% to 60% of patients with SLE^{97, 105}, and a strong correlation between the presence of anti-C1q antibodies and renal involvement in SLE has been reported^{106, 107}. For example, Marto et al. conducted a cross-sectional study to investigate the correlation between anti-C1q titers and renal disease activity in 151 patients with SLE¹⁰⁷. They found a higher prevalence of anti-C1q antibodies in patients with active nephritis than in those with no renal disease (74% vs. 32%); the anti-C1q levels were higher in patients with nephritis than in those without nephritis. Interestingly, it was also shown that anti-C1q antibodies could be detected in 33 out of 83 SLE patients without history of renal disease and that 9 (27%) of those patients developed lupus nephritis within a median interval of 9 months.

The absence of anti-C1q antibodies has been reported to exclude a diagnosis of lupus nephritis¹⁰⁸, and an increase in anti-C1q antibodies has been suggested to predict renal flares¹⁰⁶. Positive predictive value and negative predictive value of anti-C1q antibodies for lupus nephritis have been reported to be 58% and 100%, respectively¹⁰⁶. In several studies, anti-C1q antibodies were shown to correlate with active lupus nephritis with a sensitivity of 44-100% and a specificity of 70-92%^{106, 108}. Recently, Trendelenburg et al. conducted a prospective study including 38 SLE patients who underwent renal biopsy at the time of serum anti-C1q testing¹⁰⁹. They found that 97% of SLE patients with biopsy-proven proliferative nephritis were positive for anti-C1q, whereas only 35% of SLE patients with inactive nephritis and 25% of SLE patients without nephritis were positive for anti-C1q. Thus, the negative predictive value of anti-C1q antibodies for the development of active proliferative nephritis was 97%. Moreover, they noted that levels of anti-C1q antibodies decreased after successful treatment of lupus nephritis.

Taken together, these recent studies demonstrate a strong correlation between the presence of anti-C1q antibodies and lupus nephritis, and suggest that anti-C1q determination may serve as a biomarker to monitor renal involvement and/or predict renal flares.

C.4.2. Biomarkers for central nervous system involvement—Many patients with SLE experience a wide range of neuropsychiatric (NP) events that result predominantly from immunopathogenic injuries of the central nervous system (CNS)¹¹⁰. NP-SLE is arguably the most common yet least measurable manifestation of the disease. Reports of the prevalence of NP-SLE varies widely between 37% and 95% in different studies¹¹⁰, in part due to different criteria defining CNS involvement and the lack of reliable biomarkers. Because autoantibodies are clearly involved in tissue damages in other organs (e.g., the kidney), autoantibodies reactive to CNS antigens naturally become the focus of the investigation of NP-SLE pathogenesis and the quest for NP-SLE biomarkers. Earlier studies led to the discovery of the so-called anti-neuronal antibodies in the cerebrospinal fluid (CSF), but in general have not resulted in useful diagnostic tests or definitive identification of antigenic specificity¹¹¹.

A seminal study published in 2001 has sparked tremendous interest and initiated extensive studies on the role of anti-N-methyl-D-aspartate (NMDA) receptor (anti-NR2) antibodies in NP-SLE. In this study, Diamond and colleagues discovered that a subset of the anti-dsDNA

antibodies cross-reacted with a pentapeptide consensus sequence that is present in the extracellular domain of the NR2a and NR2b subunits of the NMDA receptor¹¹². NMDA receptors bind the excitatory amino acid neurotransmitter glutamate and are expressed by neurons throughout the forebrain, with the highest levels expressed at the hippocampus. A mouse monoclonal antibody specific for this consensus sequence and the cross-reactive anti-dsDNA antibodies derived from the serum and CSF of SLE patients were capable of inducing apoptotic neuronal death *in vitro* and *in vivo* after injection into the hippocampal region of the mouse brain¹¹². In a series of elegant studies, these investigators went on to show that mice could produce anti-NMDA/anti-dsDNA antibodies after immunization with the NMDA consensus peptide, and if the blood brain barrier was breached to allow the entrance of antibodies into the brain, these mice suffered from neuronal damage in the hippocampus and the basolateral amygdala and exhibited significant memory and cognitive dysfunction^{113, 114}.

Subsequent studies by these investigators and others showed that anti-dsDNA antibodies cross-reactive with NMDA are present in the sera, CSF, and brains of SLE patients with progressive decline in cognitive performance¹¹⁵⁻¹¹⁷. Furthermore, human antibodies, injected into the mouse brain or into the circulation of a mouse and allowed to cross a compromised blood brain barrier, could induce neuron damage in the hippocampus and behavioral changes reflecting memory impairment¹¹⁵. Collectively, these studies provide convincing evidence in support of a novel pathogenic mechanism whereby circulating anti-neuronal (specific or cross-reactive) autoantibodies enter the brain upon transient breach of the blood brain barrier, bind to antigens expressed in different regions of the brain, induce non-inflammatory neuronal injury, and induce various neurologic and psychologic changes.

To substantiate the role of anti-NMDA antibodies in human disease, several investigators have begun to examine the association between these antibodies and NP-SLE^{116, 118-121}. Overall, anti-NMDA antibodies (measured by enzyme-linked immunosorbent assays) are reported to be detected the circulation of approximately 30% of SLE patients. Omdal et al. measured anti-NMDA antibodies in the plasma of 57 SLE patients who were subjected to comprehensive psychological and cognitive testing¹²¹. They found an association between anti-NMDA positivity and depressed mood and decreased short-term memory. Similarly, Lapteva and colleagues studied 60 SLE patients and reported an association of serum anti-NMDA antibodies with depressive mood but not with cognitive dysfunction¹¹⁸. In contrast, Harrison et al. found no significant association between serum anti-NMDA positivity and cognitive dysfunction, depressive symptoms, or anxiety in a cross-sectional study of 93 SLE patients¹¹⁹. In another study, Hanly et al. also reported no association between cognitive impairment and serum anti-NMDA antibodies¹²⁰. Moreover, Hanly and colleagues studied these patients longitudinally and found no significant association between persistent elevation or recent rise of anti-NMDA antibodies and changes in cognitive performance or the occurrence of new NP-SLE events during the 5-year follow-up period¹²⁰. These conflicting results warrant additional cross-sectional and prospective studies of larger cohorts of lupus patients.

In a recent study of 80 SLE patients (53 with NP-SLE and 27 without NP-SLE), Yoshio et al. found that anti-NMDA antibody levels in the CSF of patients with NP-SLE were significantly higher than those of patients without NP-SLE ($p=0.0004$), but the serum anti-NMDA levels were only slightly higher in patients with NP-SLE than in patients without NP-SLE ($p=0.04$)¹²². The association of CSF anti-NMDA antibodies and NP-SLE was most significant in patients with both neurologic and psychologic symptoms. Recently, Arinuma et al. independently conducted a study using 56 patients with NP-SLE (38 with diffuse neuropsychological and psychiatric syndromes and 18 with focal neurologic symptoms) and 20 control patients with other noninflammatory neurologic diseases¹¹⁷. Similarly, they found that varying but significantly elevated levels of anti-NMDA antibodies were present in CSF

of patients with diffuse NP-SLE compared with patients with focal NP-SLE or control patients. Taken together, these results suggest that measurement of anti-NMDA antibodies in CSF may be more useful for the diagnosis of NP-SLE than measurement of these antibodies in serum.

D. Perspective

The emerging concept of “Systems Biology” recognizes that to truly understand the biology of a physiological or pathological situation in patients, it is necessary to investigate the interactions between different elements/systems¹²³. SLE is undoubtedly one of the most fitting case studies for systems biology¹²⁴. The greatest challenge in identifying and developing specific biomarkers for SLE is its complex etiopathogenesis and clinical heterogeneity. Reliable SLE biomarkers may be informative at different time points in the disease process, such as at diagnosis, during a flare, in assessment of end-organ damage, or in evaluation of response to treatment. Moreover, owing to the multifactorial nature of SLE, no unique biomarker is likely to emerge. The rapid progress we are witnessing in this field will most likely lead to a validated “Lupus Biomarker Panel” that will include assays for individual molecules as well as for “molecular biosignatures”¹²⁵.

The attempt to discover useful biomarkers for SLE has traditionally been conducted based on hypothesis-driven approaches, i.e., to investigate a single or a small number of factors (e.g., genes, autoantibodies, and cytokines) that are thought to be important in the underlying pathophysiologic mechanisms. Although these approaches have yielded many putative biomarkers, no biomarkers have been validated to date. Advances in high throughput technology during the past decade have opened new avenues to study the complex dynamic disease process in SLE and discover efficient biomarkers for SLE. In this new research arena, several experimental approaches are likely to be the most productive “mines” for lupus biomarkers. These approaches include: 1) genome-wide association scans in conjunction with genome scan meta-analysis, 2) transcriptional profiling at a genome-wide scale with RNA extracted from blood/tissues of patients using a combination of microarray and bioinformatics approaches, 3) autoantibody profiling using autoantigen arrays, and 4) signaling pathway and cytokine/chemokine expression profiling using DNA and antibody microarrays, flow cytometry, and proteomics techniques¹²⁶⁻¹²⁹. Recent and ongoing studies from numerous national and international groups have generated promising results, and point to substantive reasons for optimism in the SLE biomarker arena.

Practice Points

- Many SLE biomarkers in the pipeline are currently “for research use only.”
- Measurement of autoantibodies and complement are currently assays of choice in daily clinical practice. The results of autoantibody/complement tests are likely most informative if interpreted in a “personalized” manner, i.e., reading the results of each test in the context of the long-term disease course/manifestations in a given patient.
- Physicians and patients should be educated and encouraged to actively participate in exploratory or validating studies of potential biomarkers.

Research Agenda

- Discovery and development of biomarkers can be achieved through two distinct, yet, complementary, approaches. In one approach, attempts to identify potential biomarkers are made based on established cellular and molecular mechanisms of SLE. In the other approach, the “survey” or “scan” search of potential biomarkers is driven by novel, high-throughput technology (e.g., genomics and proteomics).

- Large-scale multicenter and longitudinal studies are essential for successful validation of promising biomarkers.
- SLE patients should not be considered equal due to individual heterogeneity in clinical manifestations, disease progression rate, and perhaps pathogenic mechanisms.
- Validation of each biomarker in specific subgroups of SLE patients (selected based on scientific rationale such as the biological basis of the biomarker and relevance to particular clinical manifestations) may yield more useful insight than validation performed using the population of all SLE patients.
- Validated biomarkers might identify potential therapeutic targets and provide direction for drug development.

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Table 1**Potential Lupus Biomarkers[#]**

Category	Marker
Disease Susceptibility	Complement (C1q, C2, C4) deficiency Low copy number of C4 gene FcγRIIa, FcγRIIb, FcγRIIIa polymorphism Low copy number of FcγRIIIb gene MBL polymorphism MHC alleles (DRB1, A1, B8) IL-10, IL-6, TNFα polymorphism TNFR, IL-1Ra polymorphism PD-1 polymorphism CTLA-4 polymorphism PTPN22 polymorphism IRF5 polymorphism STAT4 polymorphism
Disease Diagnosis	Anti-dsDNA Erythrocyte-bound C4d/Erythrocyte-CR1 Platelet-bound C4d
Disease Activity	Anti-dsDNA Serum complement and activation product levels C3, C4, C3a, C5a, C3d, C4d, Ba, Bb, sC5b-9 Serum cytokine levels IL-6, IL-10, IL-12, IL-15, IL-18, IFNα, IFNγ, TNFα Soluble cytokine receptors IL-2R, TNFR, IL-1Ra Soluble cell surface molecules BLyS (BAFF), CD27, CD154 Endothelial activation markers sICAM, sVCAM, thrombomodulin, circulating endothelial cells Acute phase proteins CRP, ferritin Other autoantibodies Anti-nucleosome, anti-C1q Cellular markers CD27 ^{High} plasma cells Erythrocyte/Reticulocyte-bound C4d
Specific organ involvement	Anti-dsDNA (renal) Anti-C1q (renal) Anti-nucleosome (renal) Urinary sVCAM and MCP-1 levels (renal) Anti-NMDA/anti-NR2 (NP-SLE)

[#]This table is not meant to be exclusive. Some of the less-studied markers are not listed.