REVIEW

The Role of Clinical Features and Serum Biomarkers in Identifying Patients with Incomplete Lupus Erythematosus at Higher Risk of Transitioning to Systemic Lupus Erythematosus: Current Perspectives

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Abstract: Discovery of antinuclear antibodies (ANA) enabled earlier diagnosis of systemic lupus erythematosus (SLE) and other ANA+ connective tissue diseases (CTD). Rheumatologists increasingly encounter high referral volume of ANA+ patients. It has been estimated that only a small percentage of these patients will eventually transition to either SLE or other specified CTD. Incomplete lupus erythematosus (ILE) has been defined as a subset of patients who have some SLE-specific clinical manifestations but do not meet currently accepted classification criteria for SLE. Several studies have been performed with the goal of identifying clinical features, serum and tissue biomarkers that can distinguish those patients with ILE at risk of transitioning to SLE from those who will not. Increased autoantibody diversity, presence of anti-double-stranded DNA (dsDNA) antibodies, high expression of type I and type II interferon (IFN)-gene products, increased serum levels of B-cell-activating factor of the TNF family (BAFF), and certain serum cytokines and complement products have been identified as markers with positive predictive value, particularly when combined together. Once this patient population is better characterized biochemically, clinical trials should be considered with the primary objective to completely halt or slow down the transition from ILE to SLE. Hydroxychloroquine (HCQ) appears to be a promising agent due to its good tolerability and low toxicity profile and open-label studies in ILE patients have already shown its ability to delay the onset of SLE. Other therapeutics, like those targeting abnormal type I and type II IFN-signatures, B-cell specific signaling pathways, complement activation pathways and high BAFF levels should also be evaluated, but the risk to benefit ratio must be carefully determined before they can be considered.

Keywords: incomplete lupus erythematosus, systemic lupus erythematosus, hydroxychloroquine, type I interferon pathway, B cell activating factor of the TNF family

Introduction

SLE is a highly heterogeneous multisystemic polygenic autoimmune disease with unpredictable disease flares and highly variable organ involvement. The spectrum of SLE manifestations ranges from easily recognizable multiorgan disease involvement to cases of isolated central nervous system (CNS) or renal disease wherein a whole spectrum of differentials must be first excluded before a diagnosis of SLE can be entertained.

SLE is characterized by the presence of a myriad of autoantibodies, including those targeting a variety of nuclear antigens. It is estimated that between 100–200 different autoantibodies can be detected in SLE.[1] SLE has a clear predilection for females compared to males (9:1 ratio) and can present in a very diverse way by affecting multiple organ systems. SLE disease activity tends to involve flares followed by periods of relative quiescence of disease/inactivity. At disease onset, the most observed clinical manifestations are those of mucocutaneous involvement, inflammatory poly-arthritis, and hematologic abnormalities.[2] However, some SLE-specific manifestations, such as malar rash or discoid

Released nucleoproteins
(from damaged cells)
(nucleosomes, spliceosomes)
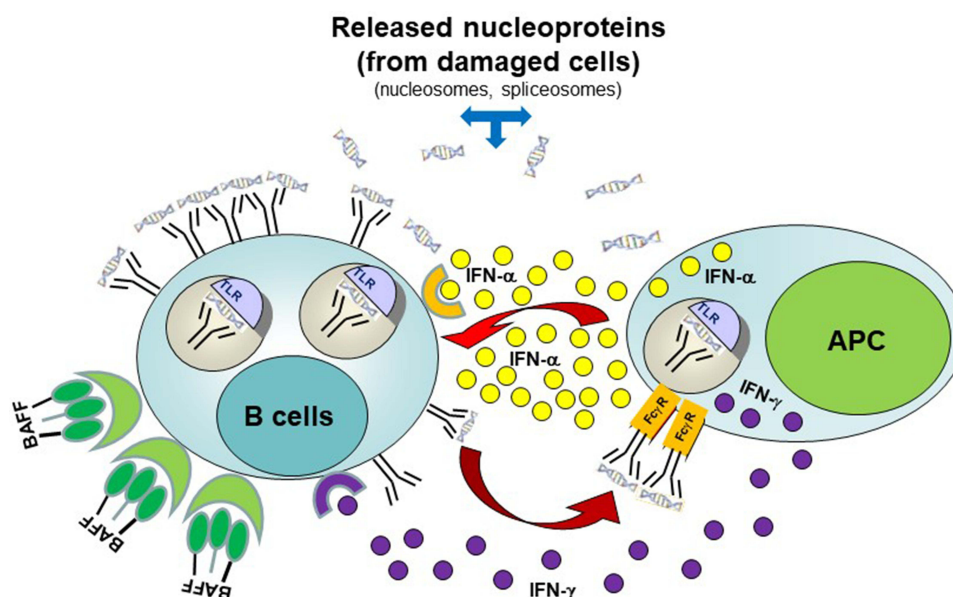
Figure 1 Interaction between autoreactive B-cells and antigen-presenting cells (APC) in ILE. Autoantigen (nucleoprotein)-specific B-cells may survive the selection processes in bone marrow. These cells are constantly exposed to autoantigens derived from either apoptotic cells, necrotic cells, or neutrophil extracellular traps. In genetically predisposed individuals, elevated levels of B-cell survival factors (BAFF or April) or APC (antigen-presenting cells)-derived type I (or type II) IFN, or abnormal intrinsic B-cell receptor signaling involving the Syk/BTK pathway may lead to upregulation of nucleic-acid sensing TLR and other innate receptors resulting in B-cell activation. In turn, this can lead to activation and maturation of autoreactive B-cells into anti-nucleoprotein secreting plasmablasts/plasma cells. Secreted autoantibodies can then combine with self-derived nucleoproteins and signal APCs through their Fc-γ receptors. Subsequent activation of innate receptors in APCs can trigger robust type I and II IFN response, which in turn can further decrease the threshold for B-cell activation by up-regulating IFN-inducible genes ("IFN-signature"). Released type I and type II IFN can also prime other immune cells and ultimately cause tissue inflammation and damage resulting in clinically overt SLE. Adapted from *J Allergy Clin Immunol.* 137(5), Singh N, Kumar B, Aluri V, Lenert P. Interfering with baffled B cells at the lupus tollway: promises, successes, and failed expectations. 1325–1333. Copyright 2016, with permission from Elsevier.[91]

rash, greatly vary from cohort to cohort and are observed only in a small percentage of newly diagnosed SLE patients, which makes the early diagnosis of SLE challenging. While at disease onset a high percentage of SLE patients (95–100%) test positive for anti-nuclear antibodies, early diagnosis of SLE remains a challenge.[2,3]

The exact etiology of SLE remains unknown. Multiple factors have been considered to play a role in susceptibility to SLE including genetic, epigenetic, and environmental risk factors (including but not limited to infections such as with Epstein-Barr virus, smoking, ultraviolet light, certain drugs, vitamin D deficiency, and hormonal factors).[4,5] An abnormally high type I and type II IFN signature can be detected before SLE is diagnosed and this finding correlates with a break in tolerance resulting in emergence of autoreactive B cells[6] (Figure 1).

Genetic Background of SLE

Regarding the possible role of genetics in SLE, it has been estimated that SLE rates range from 24–56% in monozygotic twins, while in dizygotic twins the concordance rate drops down to only 2–4%.[7] Familial linkage studies have identified candidate genes involved in innate and adaptive immunity which were associated with increased risk of SLE.[8] The first published SLE genome-wide association scan (GWAS) was performed on SLE patients of European ancestry[9] and the results were later confirmed in 2 additional studies.[10,11] The strongest associations were found between SLE and the Human leukocyte antigen (HLA) region on chromosome 6p21 and the *IRF*5 on chromosome 7q32. Several single-nucleotide polymorphisms (SNP) on chromosome 8p23.1, 8p21.1 and *Lyn* on 8q12 have also been shown to be associated with SLE. For example, on chromosome 8p23.1, the region upstream of the transcription-initiation site of the gene encoding C8orf13-B lymphocyte kinase (BLK) was associated with increased disease risk in both US and Swedish populations (rs13277113; odds ratio, 1.39; P=1x10^{-10}).[11] The risk allele was in the promoter-region causing reduced expression of *BLK* and increased expression of *C8orf12* in transformed B cell lines.[12]

However, common disease alleles identified by SNP-based GWAS studies only account for a small proportion of SLE heritability. GWAS studies have also frequently missed low-frequency functional risk alleles; for example, the recently identified Death-associated protein 1 regulatory haplotype has been linked to low transcription associated with higher autoantibody levels and enhanced autophagy which allows for the survival of autoreactive B-cells.[13]

Therefore, while inheritance definitively plays a role in susceptibility to SLE, the value of genetic testing in identifying patients who are at risk of developing SLE is limited and currently not recommended for either diagnostic, prognostic, or therapeutic purposes.

Pathophysiology of SLE

Discovery of Lupus Erythematosus cells (LE cells) followed by identification of antinuclear antibodies (ANA) by complement fixation assay or indirect immunofluorescence on cryogenically frozen tissue specimens (liver, kidney) or immortalized cell lines (such as Hep-2 cells) has tremendously helped in recognizing evolving SLE and other systemic rheumatologic diseases.[14–16] Therefore, ANA has been included as the entry criterion for SLE classification in the 2019 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria.

However, ANA positivity is fairly common even in healthy individuals. Many patients are referred to rheumatology to evaluate for SLE in the setting of a positive ANA but lack any other critical (SLE-specific) diagnostic criteria. It has been estimated that despite its high sensitivity (98%), the utility of ANA for diagnosing SLE is limited by its much lower specificity (75–80%). Up to 20–25% of healthy subjects (women in particular) can have a positive ANA test and ANA positivity also increases with age. It has been suggested that the clinical utility of this test highly depends on its positive predictive value (PPV), for example ANA ordered by rheumatologists tends to have a higher PPV than ANA ordered by non-rheumatologists.[17] One study evaluated the utility of ANA in 232 patients referred to Rheumatology for a positive ANA. The PPV for SLE in this cohort was 2.1% and for any ANA-associated rheumatic disease it was 9.1%; in other words, more than 90% of patients in the study who were referred to a tertiary rheumatology clinic for a positive ANA test had no evidence of ANA-associated rheumatic disease.[18] Thus, the utility of the ANA test can be improved by selectively ordering it only in patients with higher PPV.

Early mediators in SLE include IFN-inducible gene expression, IFN-γ induced protein 10 (IP-10) and BAFF. Treatment of mice predisposed to develop a lupus-like disease with type I IFN can accelerate the course of disease.[19] Treatment of humans with type I IFN has been occasionally linked to the induction of a lupus-like disease.[20,21] The question remains: where is IFN-α coming from in patients with SLE? Herpes simplex virus–containing immune complexes stimulated the production of type I IFN in rabbits.[22] Similarly, nucleic acid–containing immune complexes could induce type I IFN secretion in mouse and human dendritic cells. This effect was believed to involve Toll-like receptors (TLR) 7 and 9.[23] Once released, IFN-α could amplify this response by upregulating IFN-responsive genes (and thus creating an "IFN signature"). Ronnblom and his colleagues pioneered this work and observed that nucleic acid-containing immune complexes in sera of patients with SLE could induce type I IFN production in peripheral blood mononuclear cells obtained from healthy individuals.[24,25] Furthermore, type I IFN from sera of patients with SLE could promote differentiation of normal monocytes into dendritic cells (DC).[26] Interestingly, type I IFN further enhanced B-cell activation via BAFF.[27]

Transitioning from ILE/UCTD to SLE

Prompt diagnosis of SLE and timely institution of treatment is imperative to limit disease progression, organ damage and mortality. Failure to recognize early progressive forms of ILE may have potentially dangerous consequences, however, not all patients will progress to SLE. Due to a variety of presenting symptoms and inconsistent presence of biomarkers, early diagnosis of SLE still remains elusive.[28]

The term ILE has been proposed to describe patients who meet some criteria, but do not fulfill the SLE classification criteria. Similarly, the term Undifferentiated CTD (UCTD) was proposed by LeRoy in 1980 to describe patients with positive ANA and non-specific symptoms typically lasting for more than 12 months who do not meet criteria for diagnosis of any specific CTD (including SLE). Many of these patients never develop a definite CTD.[29,30] For the purposes of this paper, we are considering the terms UCTD and ILE to be synonymous.

Patients with ILE may present with a wide spectrum of disease manifestations, ranging from photosensitivity and mild skin disease to severe life-threatening renal involvement. Moreover, the timing of symptom onset and the rate of progression of symptoms is highly variable between patients. For example, the time interval between the first manifestations attributable to SLE and final classification of SLE has been estimated to be anywhere between 24 and 50 months.[2,31,32] Decreased time interval to diagnosis was associated with better quality of life and improved survival.[33,34]

Autoantibody production and other immune abnormalities may precede the development of clinically active disease for months to years, thus creating another layer of complexity when approaching patients referred to Rheumatology for positive ANA. Many of the patients who are referred for evaluation of a positive ANA will have nonspecific symptoms, often widespread myofascial pain, and will not meet the classification criteria for SLE (or other systemic autoimmune disease) diagnosis at initial assessment. Most of these patients will never go on to develop clinically significant rheumatologic disease. Unfortunately, there is a scarcity of studies addressing the transition of patients with high-positive ANA titers into clinically significant CTD. One study showed that only 5 out of 27 patients who responded to a survey and had their blood retested for ANA developed a CTD after 10 years of follow-up.[35] Interestingly, 21 of 27 remained ANA positive. Retrospective studies have shown that 63–88% of SLE patients will have serum autoantibodies years before they can be classified as having SLE.[36,37] Anti-Ro/SS-A has been identified as one of the earliest autoantibodies detectable in ILE patients, followed by anti-dsDNA and anti-spliceosome antibodies.[38] Autoantibody diversity tends to increase closer to the time of SLE diagnosis. Anti-Ro/SS-A antibody particularly targeting the 60 kD antigen is relatively common in SLE and tends to be associated with photosensitivity, cutaneous lupus, and hematologic manifestations.[39] An Italian inception cohort study on SLE patients with disease duration less than 12 months has established that SS-A antibody can be detected in 28% of early SLE patients.[2] However, its sole presence is not sufficient to predict the future transition to SLE.

Multiple studies have attempted to identify symptoms and biologic markers which may be used to stratify risk for progression to SLE. It is estimated that between 5–55% of ILE patients will eventually transition to definite CTD or SLE, however, the majority of these patients will never transition to overt CTD or SLE.[29,40–45] It is therefore extremely important to stratify the risk for disease progression from ILE to SLE. Low-risk patients may require less frequent follow-ups, while those in the high-risk category should be monitored more frequently, and even considered for earlier treatment.

It remains challenging to distinguish patients who will evolve into having a definitive CTD or SLE versus those who will remain with clinically "stable, non-progressive ILE". Non-progressing patients with ILE tend to have absence of severe organ involvement and have a relatively simple serologic profile. In contrast, those who tend to progress to SLE characteristically not only have a positive ANA, but frequently will have multiple other specific autoantibodies (such as anti-dsDNA, anti-proliferating cell nuclear antigen, anti-C1q, anti-cardiolipin, etc.). These patients also tend to have more clinical symptoms of SLE and tend to have higher ANA titers at baseline.[46] Autoreactive IgM autoantibodies are more commonly observed in patients with ILE than in SLE.[46] Disease transition rate is higher in ANA$^+$ African American women compared to ANA$^+$ European American (white) women. It has been estimated that 20–60% of those patients with ILE who will ultimately evolve into SLE will do so within 1–5 years of follow up.[47] Some studies have shown oral ulcers, the presence of anti-dsDNA antibodies and proteinuria are independent predictors of developing future SLE, but unfortunately other studies have failed to identify any disease features that are predictive of eventual progression to SLE.[29,41,48] Among other factors, the diagnosis of ILE vs SLE also depends on which classification criteria is used. The 2012 Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) criteria appear to be more sensitive for diagnosing SLE (and thus more inclusive for clinical trials) than the widely used EULAR/ACR criteria.[49] Regardless of classification criteria used, patients with SLE when compared to ILE have been shown to have higher incidence of malar rash, photosensitivity, oral ulcers, serositis, hematologic disorders, and immunologic disorders. Additionally, SLE patients have higher rates of renal and neurologic dysfunction (Table 1).

Biomarkers of ILE/SLE

It appears that patients with SLE vs ILE display different immunopathogenic patterns. Various biomarkers have been studied, including complement levels, soluble mediators, urinary mediators, and interferons (IFN) to better distinguish

Table 1 Comparison of Features in SLE versus ILE in ACR-1997 Criteria and SLICC Criteria (Olsen NJ et al, 2016[89])

ACR Features More Common in SLE vs ILE	SLICC Features More Common in SLE vs ILE
Malar rash	Acute cutaneous lupus
Oral ulcers	Oral ulcers
Serositis	Serositis
Hematologic disorder	Leukopenia
Photosensitivity	Anti-Sm
Immunologic disorder	Anti-dsDNA

these two categories of patients. One potential biomarker of interest identified is the B-lymphocyte stimulator, also known as B-cell-activating-factor (BAFF), a soluble mediator belonging to the TNF cytokine family, which plays a crucial role in B cell survival and differentiation to plasma cells. Elevated levels of BAFF have been linked to survival of anergic autoreactive B-cells, thus prompting a breach in tolerance. This has been clearly implicated in autoantibody production and subsequent development of autoimmune disease in various mouse models.[50] BAFF has been shown to be elevated at the time of diagnosis of SLE and has been used to predict possible future SLE flares. Specifically, in relation to ILE and SLE, BAFF levels in ILE patients have been shown to be higher than the general population, but lower than in SLE patients.[49] The question remains whether targeting ILE patients expressing higher levels of BAFF with monoclonal anti-BAFF therapeutics, such as belimumab or blisibimod, may be used to prevent their evolution into full-blown SLE (assuming high benefit to risk ratio).[51] Belimumab has been approved in 2011 for treatment of SLE, but at this time no clinical study is planned to address this important question.[52]

Common immune characteristics of SLE patients are increased expression of both type I and II IFN pathways along with evidence of T cell expansion. ANA⁺ healthy African Americans tend to express higher levels of T cell activation markers and plasma IL-6 concentrations compared to ANA⁺ healthy European American (white) counterparts. In contrast, CD11c⁺autoimmunity-associated B-cells were found to be lower in healthy ANA⁺ European Americans.[53]

A study of the healthy blood relatives of patients with SLE (n=409) who were followed for 6.4 years identified 45 patients (11%) who transitioned to classified SLE.[54] Those patients who transitioned to SLE tended to have more SLE-associated antibodies at baseline, higher SLE activity scores, elevated inflammatory cytokines (BAFF, stem cell factor, etc.) and IFN-associated chemokines. Not unexpectedly, regulatory cytokines such as IL-10 and TGF-β tended to be lower over time. Plasma levels of stem cell factor and TGF-β appeared to be significant and independent predictors of SLE transition.

ILE and SLE patients showcase distinct patterns of biomarker positivity. High serum levels of IFN-α and evidence of high expression of type I IFN related genes (IFN signature) are found in approximately 50% of SLE patients.[55–64] ILE patients, on the contrary, tend to display lower levels of type I IFN-inducible genes (specifically, IP-10, MCP-1, and MIP1-alpha) compared to patients with SLE. IFN-γ and BAFF have been found to increase around the time of diagnosis of SLE. Healthy first-degree relatives of patients with SLE, interestingly, also display high type I IFN activity probably related to IFN-regulatory factor (IRF)-5 and IRF-7 expression.[65,66] The odds of progression to well defined autoimmune disease (SLE or primary Sjogren's syndrome) was higher in patients with a family history of autoimmune rheumatic disease and in those with IFN-stimulated gene expression score (IFN-Score B).[67] Others have confirmed that IFN score was elevated in ILE (in 50% of ILE patients compared to 46% of SLE patients) and its expression correlated with the Myxovirus-resistance protein A expression in blood and in unaffected skin (found in 29% of ILE skin biopsies vs 31% of SLE skin samples).[68]

Disease severity in SLE also tends to correlate with high vs low serum levels of IFN-α. A rare haplotype involving the promoter region of EFNA5 gene particularly, and to some degree PPM1H, PTPRM and NRGN regions, has been associated with high IFN-α levels.[69]

Single cell RNA sequencing applied to renal biopsies in patients with SLE nephritis helped identify potential new targets for treatment in SLE further confirming the role of type I IFN-response signatures in tubular cells.[70]

In addition to serum IFN-α and Interferon-γ inducible protein 10 (IP-10) levels, monocyte-specific surface sialic acid binding Ig-like lectin 1 (SIGLEC-1 protein) and its circulating soluble form correlate well with type I interferon-regulated gene expression in patients with SLE[71] and appear to be good biomarkers for monitoring SLE disease activity.[72] Higher concentrations of soluble SIGLEC-1 were particularly seen in patients of European origin with lower serum C3 levels and with higher incidence of renal disease.[73]

Immune Dysregulation Preceding the Development of SLE (Table 2)

Amplified crosstalk between innate and adaptive immunity is felt to cause a break of tolerance leading to survival and subsequent activation of autoreactive B cells.[74,75] Numerous cytokines have been implicated as key mediators of this crosstalk including IFN-γ (Th1), interleukin (IL)-4 and IL-5 (Th2), IL-17 and IL-21 (Th17) which help facilitate formation of germinal centers and pathogenic autoantibody production with help from T-follicular cells.[62]

IFN-γ becomes elevated prior to SLE-associated autoantibodies, and thus may be a nice biomarker of early disease.[62] As already discussed above, BAFF is upregulated in response to type I and type II IFNs and further propagates autoantibody production.[50] Not only do these mediators drive the production of pathogenic autoantibodies, but they also contribute to inflammation preceding a flare of active SLE and contribute to organ damage in SLE.[76,77] Age-associated B-cells (defined as $CD19^+CD21^{low}CD11c^+$) correlated with IgG levels and together with serum BAFF and IFN scores were elevated in patients with ILE compared with healthy controls.[78] However, we still do not fully understand the role of these mediators in transition to SLE.[62]

Lu et al found that patients who went on to develop SLE, exhibited increased inflammatory mediators from multiple immune pathways more than 3.5 years pre-diagnosis. Implicated inflammatory mediators included IL-4, IL-5, IL-6, IL-12p70, IFN-γ, and the IFN-associated chemokine IFN-γ-inducible protein 10.[62] Levels of IL-2 (p=0.008), IL-5 (p=0.001), and IL-21 (p=0.007) increased throughout the pre-classification period in case patients, while healthy controls showed low and stable levels of these mediators.[62] These mediators remained dysregulated throughout the preclinical period with additional immune dysregulation observed as patients approached disease diagnosis. Additionally, investigators found lower levels of the regulatory mediator TGF-β in cases who went on to develop SLE compared to controls at all time periods, with no significant longitudinal changes in either group.[62] Notably, cases who moved toward SLE

Table 2 Biomarkers Associated with Higher Risk of Progression to SLE

	References
Anti-dsDNA	Mosca M et al, 1999[29]
Positive Coombs test	Mosca M et al, 1999[29]
Higher titer ANA (>1:1280)	Aberle T et al, 2017[49]
Anti-C1q	Olsen NJ et al, 2016[89]
Elevated levels of BAFF	Aberle T et al, 2017[49]
IFN pathways: IP-10; MCP-1; MIP-1α; Myxovirus-resistance protein A; IFN-γ	Olsen NJ et al, 2016; Munroe ME et al, 2016; Md Yusof MY et al, 2018; Lambers WM et al, 2019[64,67,68,89]
Reduced levels of regulatory cytokines (IL-1 RA, TGF-β, and IL-10)	Munroe ME et al, 2017[54] Lu R et al, 2016[62]
Complement-activation products	Ramsey-Goldman et al, 2019[80]

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classification gained an average of 0.5 dysregulated mediators per year, compared to 0.06 in controls (p<0.001).[62] IFN activity was particularly notable with cases showing evidence of expanding IFN activity and increasing levels of the IFN-associated mediators IP-10 (p<0.001) and monokine-induced by IFN-γ (MIG) (p<0.001).[62] TNFRI, TNFRII, BAFF, and APRIL were dysregulated only as patients approached SLE classification, suggesting these are later mediators of the evolving pathologic process.[62] Lu et al implicated dysregulated innate and adaptive mediators superimposed on a background of deficient regulatory mechanisms in the pathogenesis of early preclinical SLE.[62] In random forest models, a combination of autoantibody positivity against ANA and SS-A along with elevated levels of IL-5, IL-6 and MIG was able to distinguish future SLE with 92% accuracy (compared to 78% accuracy with ANA alone).[62]

Attempts to stratify SLE patients according to inflammatory pathways and neutrophilic vs lymphocytic gene clusters involved in active disease may help characterize those patients who tend to have more progressive disease. For example, some data suggest that patients in the neutrophil-driven clusters have an increased risk of developing proliferative forms of lupus nephritis.[79] The same strategy may help identify those patients who may benefit from anti-type I IFN receptor therapies versus those who better respond to B cell targeted therapies.

Complement System

Complement activation by in situ formed or circulating immune complexes plays an important role in the immuno-pathogenesis of SLE. In a retrospective study in patients with ILE meeting 1–3 ACR criteria, decreased C3 complement levels were more commonly observed in ILE progressors (25%) than in non-progressors (3%) with the caveat that only 8 patients showed evidence of progression from ILE to SLE.[42] This observation was not confirmed in a different ILE cohort studied by Md Yusof MY.[67]

A recent study by Ramsey-Goldman et al found that cell-bound complement activation products in red blood cells and B-cells aided in diagnosis of SLE when combined with anti-dsDNA and other serum autoantibodies in multianalyte assay scores. In addition, patients with ILE who expressed these cell-bound products tended to more commonly evolve into SLE.[80]

Classification Criteria for Identifying Incomplete and Complete Cases: SLICC Criteria for SLE Classification May Identify a Subset of Patients Transitioning from ILE to SLE

Primary care physicians have an extremely important role in identifying patients not only with full blown SLE but also those with suspected ILE and referring them to practicing rheumatologists, nephrologists, or other specialists (eg, neuroimmunologists) for further work-up that may include organ biopsy when necessary to rule out possible SLE mimickers such as drug-induced lupus-like disease or infections.

The concepts of lupus diagnosis and classification are often used interchangeably in clinical practice and classification criteria are frequently used for making SLE diagnosis even though these two concepts are clearly separate entities. Classification criteria typically combine a very limited number of clinical features and laboratory tests with the primary aim of identifying a relatively homogenous group with a particular disease that can be compared in clinical trials, and thus not meant to be used for diagnostic purposes. In contrast, the diagnosis of SLE is individualized to a particular patient and accounts for a wider spectrum of information which frequently includes items not considered specific enough for classification criteria and thus tends to be more sensitive, but less specific.[81] There are no agreed upon diagnostic criteria for SLE, and thus classification criteria are often used for diagnostic purposes.

Patients with SLE were traditionally classified based on fulfillment of the 1997 American College of Rheumatology (ACR) criteria, which required meeting 4 or more out of 11 clinical and/or serologic criteria.[82,83] SLICC classification criteria were then developed and validated in 2012. SLICC criteria are more sensitive and equally specific for SLE compared to 1997-revised ACR criteria.[84] Therefore, they tend to be more inclusive as they classify more patients with SLE but can still distinguish those with ILE.[49] Of 440 patients meeting 3 ACR criteria, 149 patients met criteria for SLICC-SLE, while 291 did not meet SLICC classification criteria. This additional number was based on complement and individual autoantibody criteria. SLICC also classified more African American patients based on leukopenia/

lymphopenia criterion. Those patients who had 3 ACR criteria (ILE) were further compared with those classified as having SLE based on SLICC criteria. Patients with ILE tended to be slightly older and presented with milder symptoms. They also rarely had ANA titers > 1280, had fewer autoantibody specificities (1.3 vs 2.6) and had lower serum BAFF levels.

Another study by Mosca et al used newly revised criteria for SLE and systemic sclerosis to classify 91 patients diagnosed with UCTD.[47] They found 12 patients could be re-classified with SLE and 3 with systemic sclerosis.

Recently, a new European Alliance of Associations for Rheumatology (EULAR)/ACR classification criteria have been released with the aim to further improve sensitivity and specificity for SLE. In a validation cohort these criteria reached sensitivity of 96% and specificity of 93%. Entry criterion was the presence of ANA at >1:80 titer, while clinical and immunological features were organized in 10 domains and the total score had to be at least 10, with at least one clinical criterion required for classification. It yet remains to be determined whether this new SLE classification can better identify patients with ILE at increased risk of transitioning to SLE.[85]

Potential Treatment of ILE

An interesting study of anti-malarial use in patients with ILE (SMILE) is ongoing. It aims at understanding whether hydroxychloroquine (HCQ) can slow down the progression from ILE to full-blown SLE and will hopefully characterize candidate biomarkers that may help to make earlier treatment decisions in the future (ClinTrial.gov NCT03030118).[86]

HCQ has been used in an open-label study in the past to abrogate autoantibody production and delay progression of ILE into full blown SLE.[87] It is an attractive medication due to its low risk of toxicity associated with long-term use. A recent small sample study in patients with ILE and new-onset SLE showed that HCQ lowered levels of IFN-inducible genes and serum BAFF level.[88] There was also a trend toward lowering IP-10 levels. Another study by Olsen et al showed that treatment of ILE with HCQ resulted in better self-reported health status scores and lower expression profiles of IFN-inducible genes compared to ILE patients not treated with HCQ.[89] This correlates with studies on the in vivo and in vitro mechanism of action of HCQ which has been associated with an overall reduction in IFN-α levels in patients with SLE.[90,91] Patients treated with HCQ additionally had lower levels of anti-C1q antibodies and cytokine IL-9.[89] However, a subset of patients who present with symptoms of ILE will never go on to develop severe disease or full-blown SLE. In these patients, the risks of long-term HCQ treatment may outweigh the benefits and therefore this subgroup remains to be identified and better characterized before the treatment is initiated.

Targeting type I and type II IFN pathways, neutralizing B-cell stimulatory and survival molecules BAFF and a proliferation-inducing ligand (APRIL), targeting B-cell intrinsic signaling pathways (eg, Bruton's tyrosine kinase), blocking TLR 7/9 signaling or neutralizing IL-6 by IL-6 receptor blockade are all attractive targets not only for full-blown SLE, but potentially also for those ILE patients at risk of transitioning. However, one must carefully weigh the risks and benefits of these medications particularly in those ILE patients with non-specific musculoskeletal complaints, as all these agents can cause profound immunosuppression and increase the risk for life-threatening opportunistic infections. Additional concern comes from the ability of these agents to interfere with the post-vaccinal immune response, for example with the induction of neutralizing antibodies against the COVID-19 virus and other emerging pathogens, potentially blunting the response and causing inadequate immunoprotection.

Neutralizing Type I IFN as a Treatment for SLE

Based on evidence of increased type I IFN activity in patients with ILE and particularly in those with full blown SLE it is not a surprise that targeting this pathway with neutralizing antibodies has been attempted in several recent clinical trials.[68] Rontalizumab is an antibody with activity against all type I IFN subtypes. Results of a Phase 1 study in patients with SLE showed good tolerability but a subsequent Phase 2 study (NCT00962832) did not reach the primary end point.[92,93] Genentech discontinued further development of rontalizumab. Sifalimumab is another anti–IFN-α antibody that neutralizes most IFN-α subtypes. Results from a phase 1a study showed a dose-dependent inhibition of type I IFN–related mRNA expression associated with a trend towards improved clinical activity (NCT00299819).[94] There was also a trend towards improvement in disease activity, but despite promising results, development of sifalimumab for SLE was stopped for strategic business reasons in 2015.[95] IFN-α kinoid (IFN-K) is an interesting construct that contains

inactivated IFN-α coupled to a protein keyhole limpet hemocyanin. The safety, immunogenicity, and biologic effects of active immunization with IFN-K in patients with SLE was studied and showed that this approach could result in decreased expression of IFN-inducible genes but no improvement in clinical activity has been observed.[96] It remains to be determined if earlier treatment with any of these agents in patients with ILE showing evidence of increased type I or II IFN signature may prevent the future progression to SLE.

At this time, the only agent targeting the IFN pathway approved for treatment of SLE is anifrolimumab (Saphnelo[R]) which targets IFN alpha receptors (IFNAR1). It is indicated for patients with moderate to severe SLE who are on standard therapy. It has not been studied in SLE patients with severe active SLE nephritis or severe CNS disease. Clinical trials have shown that anifrolimumab can significantly reduce the SLE disease activity (measured with BICLA score) at week 52 (TULIP-2 study, NCT02446899), primarily by improving the mucocutaneous and musculoskeletal symptomatology and decreasing the total corticosteroid dose.[97] It would be of interest to see if the drug-manufacturer plans clinical trials in those ILE patients with high IFN signatures at higher risk of transitioning to SLE.

Conclusions

Multiple facets of SLE can make it difficult to diagnose, especially in cases with a relatively slow evolution. Identification of patients with ILE who are at higher risk of transition to SLE is critical so that these patients can be considered for earlier treatment before organ damage ensues. Over the last few decades, several groups have studied the potential predictive value of numerous immune biomarkers. While ANA has high sensitivity for SLE, its relatively low specificity generates a potential problem of over-diagnosing patients with non-specific musculoskeletal complaints of possibly having SLE. Therefore, a better understanding of lupus specific clinical features together with identification of disease specific biomarkers may help identify patients with ILE at risk of transitioning to SLE. Increased autoantibody diversity, presence of anti-dsDNA and other autoantibodies, increased expression of type I and type II IFN-gene products, BAFF serum levels, and other serum cytokines, together with the evidence of complement activation (including detection of membrane bound complement activation products) have been identified as markers with positive predictive value, particularly when combined. These biomarkers should be studied in a longitudinal manner in larger cohorts of ILE patients who are at increased risk of transitioning towards SLE. As immune biomarkers for transitioning from ILE to SLE are better understood, this will guide risk-benefit decisions about initiating early therapy.

Abbreviations

ACR, American College of Rheumatology; ANA, anti-nuclear antibodies; APC, antigen-presenting cells; APRIL, A proliferation-inducing ligand; BAFF, B cell activating factor of the TNF family; BLK, B lymphocyte kinase; CNS, central nervous system; CTD, connective tissue disease; EULAR, European Alliance of Associations for Rheumatology; GWAS, genome-wide association scan; HCQ, hydroxychloroquine; HLA, human leukocyte antigen; IFN, interferon; IFN-K, IFN-α kinoid; IL, interleukin; ILE, incomplete lupus erythematosus; IRF, interferon-regulatory factor; LE cells, lupus erythematosus cells; MIG, monokine induced by IFN-γ; PPV, positive predictive value; SIGLEC-1, sialic acid binding Ig-like lectin 1; SLE, Systemic Lupus Erythematosus; SLICC, Systemic Lupus Erythematosus International Collaborating Clinics; SNP, single-nucleotide polymorphism; TLR, toll-like receptors; UCTD, undifferentiated connective tissue disease.

Disclosure

The authors report no conflicts of interest in this work.

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