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The SLE review series: working for a better standard of care

Systemic lupus erythematosus biomarkers: the challenging quest

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Abstract

SLE, a multisystem heterogeneous disease, is characterized by production of antibodies to cellular components, with activation of both the innate and the adaptive immune system. Decades of investigation of blood biomarkers has resulted in incremental improvements in the understanding of SLE. Owing to the heterogeneity of immune dysregulation, no single biomarker has emerged as a surrogate for disease activity or prediction of disease. Beyond identification of surrogate biomarkers, a multitude of clinical trials have sought to inhibit elevated SLE biomarkers for therapeutic benefit. Armed with new -omics technologies, the necessary yet daunting quest to identify better surrogate biomarkers and successful therapeutics for SLE continues with tenacity.

Key words: systemic lupus erythematosus, autoimmunity, biomarkers, immunosuppressants, lupus nephritis, interleukin, cytokine, chemokine, complement, antibody.

Rheumatology key messages

- Discovery and validation of serum and urine biomarkers continues to advance our understanding of SLE.
- Numerous therapeutics targeting biomarkers have undergone clinical trials in SLE patients with varying success.
- Large-scale proteomic screens yield additional biomarkers of potential clinical relevance that may become future therapeutic targets.

REVIEW

Introduction

SLE is a complex multisystem autoimmune disease commonly characterized by periods of flare and quiescence. Disease manifestations are heterogeneous, ranging from detectable laboratory abnormalities to multi-organ inflammation and failure. The clinical syndrome includes abnormal antibody production to cellular constituents, innate and adaptive immune alterations and dysregulated cytokine production. Several markers have been evaluated to classify diseased individuals and evaluate activity, or to determine treatment responses and predict therapeutic strategies.

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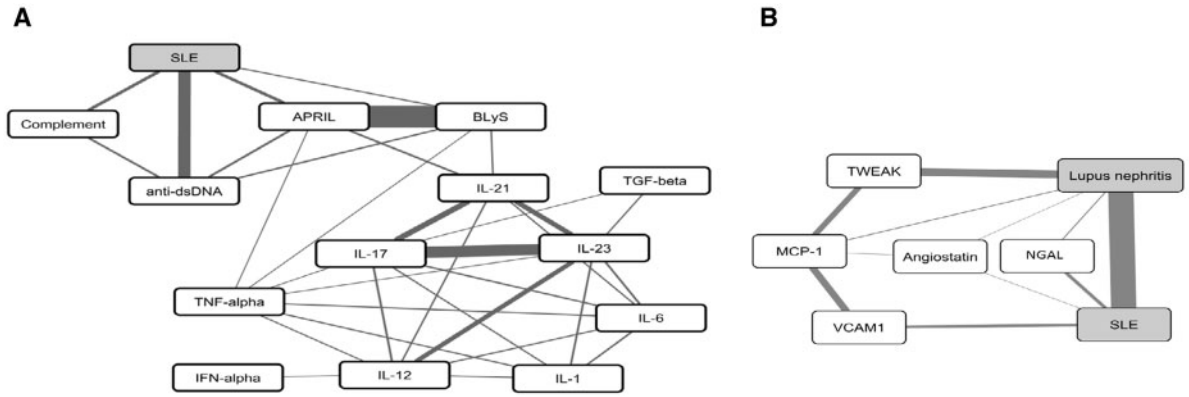
Biomarkers

A biological marker, or biomarker, is ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention’ [1]. Biomarkers can be prognostic, diagnostic, predictive, pharmacodynamic and surrogate. Prognostic biomarkers identify a specific disease manifestation, individuals at risk for disease development or those likely to experience a flare. Diagnostic biomarkers confirm the presence or subtype of disease. Predictive biomarkers use baseline characteristics to predict therapeutic responses. Pharmacodynamic biomarkers assist in determining optimal therapeutic doses. Surrogate biomarkers are intended to substitute for a clinical end point.

Explorations for candidate biomarkers to date have targeted selected immune molecules or used multiple comprehensive screening approaches, examining genomics, transcriptomics, proteomics and metabolomics. Each of these techniques has been applied to SLE and has been reviewed elsewhere [2]. In addition, combining comprehensive and targeted studies reported in the literature with

Fig. 1 SLE biomarkers



(**A**) Serum biomarkers. (**B**) Urine biomarkers. Published relationships between SLE and lupus nephritis (grey fill) and selected biomarkers (white fill) are shown. Lines reflect the degree of mutual information (MI) between nodes (thicker indicates higher MI) as calculated by IRIDESCENT. MI reflects the relative specificity of nodes to each other. For example, there are many papers that mention IFN-α and SLE, but IFN-α is not specific to SLE, so it has a lower MI and therefore no direct connection in this graph. BLyS and APRIL, however, are closely related to each other and often mentioned together, giving them a high MI. As the MI scores are computed solely based on being co-mentioned in the published literature, these links do not necessarily imply a direct mechanistic relationship; the latter needs to be verified experimentally. APRIL: a proliferation-inducing ligand; BlyS: B lymphocyte stimulator; IRIDESCENT: Implicit Relationship IDEntification by in-Silico Construction of an Entity-based Network from Text; MCP-1: monocyte chemoattractant protein 1; TWEAK: TNF-like weak inducer of apoptosis.

disease specific co-citation can potentially identify accepted and emerging biomarkers. A literature-mining program, Implicit Relationship IDEntification by in-Silico Construction of an Entity-based Network from Text (IRIDESCENT) [3–5] was used in this review (August 2015) to identify and rank entities in Medical Literature Analysis and Retrieval System Online, or MEDLARS Online (MEDLINE) related to SLE. Briefly, IRIDESCENT processes all electronically available peer-reviewed findings, as contained in MEDLINE records (>23 million as of this writing). Names and synonyms in the IRIDESCENT thesaurus are obtained from major publicly available databases to be able to recognize concepts and entities within text. The major contributing databases are Online Mendelian Inheritance in Man (OMIM) (diseases and phenotypes), Entrez (genes), CHEMical IDentification (CHEMID), US Food and Drug Administration (FDA; approved drugs) and Gene Ontology concepts (process, component and function). MEDLINE records were searched for concepts that co-occur within titles and abstracts, weighting their relative importance as conceptual pairs by how frequently they co-occur and by the distance between them in each record analysed (e.g. two terms in the same sentence receive higher weight than two terms within only the same abstract). IRIDESCENT identified key SLE biomarkers (followed by literature searches from August 2015 to August 2016) and quantified the strength of association of the biomarkers to each other and to SLE (Fig. 1 and Table 1). IRIDESCENT calculated values for literature strength (Lit. Str.), a measure of co-citation frequency and mutual information (MI), an information-theoretic measure that reflects the probability of seeing one term given the other weighted as an indication of specificity for various serum and urine biomarkers for both SLE and LN.

The goal of this review is to present an overview of blood and urine biomarkers that have been reported in the literature in relationship to SLE disease activity or therapeutics.

Traditional clinical biomarkers

More than half a century of research into SLE blood markers has generated few widely accepted biomarkers for routine clinical care. Although laboratory studies routinely obtained in the general patient population (blood count and urinalysis) can also assist in assessing disease activity and the presence of haematological and renal manifestations, discussion will focus on laboratory parameters from blood and urine that are more specific to SLE. Anti-dsDNA and complement deficiency are included in clinical instruments to assess SLE disease activity, including the SLEDAI [6], the Safety of Estrogens in Lupus Erythematosus National Assessment-SLEDAI [7] and the SLEDAI-2000(2K) [8].

Anti-dsDNA

The ACR [9, 10] and the SLICC [11] include ANA, anti-dsDNA, aPL and the anti-spliceosome antibody anti-Smith in their SLE classification criteria. However, the presence of anti-dsDNA is not unique to SLE. A Saudi Arabian study determined that 58.8% of patients with a positive dsDNA had SLE, yet 41.5% presented with other rheumatological diseases, malignancies, infections, hepatitis or endocrine disorders [12]. Additionally, Chinese patients with ovarian cancer, colon cancer and hepatocellular carcinoma were found to have 30, 40 and 50% dsDNA positivity, respectively [13]. Finally, 7.6% of

Table 1 An example of a data-mining method to identify potential SLE and LN biomarkers

Biomarker	SLE		LN		Years of cited manuscripts
	Lit Str.	MI	Lit Str.	MI	
Traditional clinical					
ANAs	2083.2	7.2	12.5	14.9	Reference
Anti-dsDNA	308.3	20.2	70.8	62.5	1957–2016
Complement	243.6	16.5	35.2	36.5	1953–2015
Non-traditional					
IFNα	296.5	5.8	16.6	0.8	1979–2015
BLyS	254.2	3.9	13.9	3.7	2001–15
APRIL	139.4	1.9	13.2	2.9	2004–15
Emerging					
TNFα	1195.3	0.1	124.9	0.2	1996–2014
IL-6	810.7	0.2	82.1	0.3	1991–2014
IL-12	194.9	0.2	3	0.7	1995–2015
IL-23	66.6	0.5	10.8	1.2	2008–15
IL-1	303.3	0.1	45.5	0.2	1994–2016
TGF-β	8.6	0	79.9	0.2	2006–13
IL-21	142.8	1.1	2.3	0.4	2008–13
IL-17	236.3	0.5	2.5	1.5	2000–15
Ferritin	5.6	0.4	4.9	0	2012–16
IGF binding proteins	3.4	0	26.2	0.1	2016
Urine					
TWEAK	47.4	0.8	42.3	9.1	1997–2016
MCP-1	194.6	0.1	3.3	1.8	1996–2015
NGAL	47.1	0.1	27.8	1.2	2007–15
VCAM-1	82.7	0.2	29.3	0.9	2002–16
Angiostatin	3.7	0	3.7	0.3	2013–15

IRIDESCENT calculated values for literature strength (Lit. Str.), a measure of co-citation frequency, and mutual information (MI), an information-theoretic measure that reflects the probability of seeing one term given the other weighted as an indication of specificity for various serum and urine biomarkers for both SLE and LN. ANA is included for reference, as this biomarker is known to be sensitive for SLE (very high Lit Str.) but not very specific (MI lower than dsDNA and complement). Established biomarkers will have higher values than emerging biomarkers owing to the frequency in medical literature. The years of publication of the manuscripts cited within this review are included in the final column (please note that literature may exist outside of this date range, and the IRIDESCENT values were calculated in August 2015). APRIL: a proliferation-inducing ligand; BlyS: B lymphocyte stimulator; IRIDESCENT: Implicit Relationship IDEntification by in-Silico Construction of an Entity-based Network from Text; MCP-1: monocyte chemoattractant protein 1; NGAL: neutrophil gelatinase-associated lipocalcin; TWEAK: TNF-like weak inducer of apoptosis; VCAM-1: vascular cell adhesion molecule-1.

healthy elderly Italian individuals had positive anti-dsDNA results [14].

Few biomarkers in SLE fulfil multiple biomarker categories; however, anti-dsDNA has been proposed to be a prognostic, predictive and partial surrogate biomarker of end-organ disease, although results are inconsistent. Nearly 60 years ago, antibodies to dsDNA were noted in sera from lupus patients [15–17]. Detectable levels precede clinical diagnosis by ~2.2 years [18, 19]. Furthermore, elevation of anti-dsDNA correlates with the IFN signature, suggesting prolonged immune dysregulation and transition to SLE [20]. Moreover, patients with positive anti-dsDNA have increased antibody levels preceding disease exacerbation [21], and anti-dsDNA correlates with acute illness [22], disease activity [23] and complement levels in SLE patients [24]. Furthermore, anti-dsDNA was predictive of haematological or organ flare [25]. Analysis of patients in the placebo arms of two belimumab (a mAb that binds soluble B lymphocyte stimulator, BLyS) studies determined that high anti-dsDNA levels (>200 IU/ml) predicted SLE flare within 1 year; and anti-dsDNA normalization by 8 weeks was correlated with a reduced risk of severe flare [26]. Additionally, anti-dsDNA positivity predicted therapeutic benefit of belimumab [27], and treatment resulted in significant reduction of anti-dsDNA [28]. In another study, prednisone treatment was associated with reduced anti-dsDNA compared with placebo [29]. However, evaluation of anti-dsDNA levels in serologically active, clinically quiescent patients found no correlation with disease activity or subsequent flare [30].

An association of anti-dsDNA with LN has been described in multiple studies [31–34]. Yung and Chan [35] have previously detailed mechanisms for anti-dsDNA involvement in kidney pathogenesis, describing immune complex formation and renal binding. Anti-

dsDNA levels have also been associated with disease activity and proliferative LN and were predictive of proliferative nephritis [36]. In patients with biopsy-proven proliferative nephritis, anti-dsDNA elevation occurred ~2.7 years before diagnosis [37]. Rituximab, a mAb targeting B lymphocytes [38], resulted in reduction of anti-dsDNA in proliferative LN patients that correlated with reduced proteinuria [39].

More recently, hCDR1 (edratide), a synthesized 19 amino acid peptide of the heavy chain complementary determining region of human anti-DNA antibody, showed some improvements in clinical end points of SLE patients [40].

In summary, dsDNA autoantibodies are predictive of SLE and LN development, correlate with disease activity, are predictive of disease flare, are impacted by immunosuppressive therapeutics and correlate with improvement of proteinuria in LN. Anti-dsDNA is one of few SLE biomarkers measured longitudinally in routine clinical practice for assessment of disease activity.

Complement

The complement system is a key component of the innate immune system. Reduction of complement occurs in congenital complement deficiencies, infections, liver failure, acute pancreatitis, cryoglobulinaemia, thermal burns and SLE [41]. Early studies showed that SLE patients fixed complement, resulting in lower complement levels relative to controls using the total haemolytic complement assay (or CH50) [16, 42]. Complement fixation was present in kidney, liver, spleen and heart tissue of SLE patients and co-localized with antigen–antibody complexes [43]. C1q of the classical complement pathway bound directly to human keratinocytes (skin) [44] and apoptotic human Jurkat cells [45]. Decreases in C1q, C3 and C4 levels can precede a clinically evident flare and correlate with disease activity [24, 46].

Some of the strongest genetic risk factors for SLE result in deficiencies of complement proteins, including C1q, C1r, C4, C2 and C3 [47]. Furthermore, impaired clearance of apoptotic cells owing to complement pathway protein deficiency is a proposed mechanism for the development of SLE [48, 49]. C1q and mannose binding mediate apoptotic cell clearance by dendritic cells (DCs) and macrophages, with resultant increases in IL-6, TNF-α and IL-10 [50]. A recent review highlights multiple pathways for C1q in tolerance and autoimmunity [51]. Antibodies to C1q predicted renal flare in proliferative LN [52], and levels were correlated with renal disease activity [53]. Cell-bound complement activation products on erythrocytes and B cells were recently found to have higher diagnostic sensitivity for SLE than assessment of the standard complement components, C3 and C4 [54].

Eculizumab is a monoclonal antibody to C5 that is FDA approved for use in atypical haemolytic uraemic syndrome. In a small phase I clinical trial on SLE patients, it resulted in >80% reduction of CH50 in the highest dose, with no adverse effects [55]. In addition, SLE patient case reports have noted dramatic clinical improvements in thrombotic microangiopathy and proliferative LN [56, 57]. Likewise, patients with APS have been treated successfully with eculizumab [58–60]. Further evaluation of eculizumab in SLE may be warranted, potentially targeting subgroups with aPL and thrombotic microangiopathy.

Complement and the remaining biomarkers are categorized as prognostic biomarkers. Although most biomarkers are indicative of severity or flare or disease, some are predictive of flares, as noted in their respective section.

Non-traditional and emerging biomarkers

The majority of the non-traditional and emerging biomarkers are not routinely assessed in SLE patients, but are proteins involved in cellular signalling. Communication between various cells in the body, particularly immune cells, is fundamental for SLE pathogenesis. Cytokines, chemokines, growth factors and acute phase reactants allow adjacent and long-distance communication within the body.

IFNα

In the 1980s, studies noted that IFNα was elevated and correlated positively with clinical disease activity in SLE patients [61–64]. A variety of methods have been used to quantify serum (s) IFN, including WISH cells, immunoradiometric assay and ELISAs. IFN levels in serum or plasma can be transient because of localized expression and uptake; therefore, evaluation of IFN-regulated genes provides an additional method of quantification. Peripheral blood mononuclear cells (PBMCs) from SLE patients overexpress genes that are IFN regulated, and this is referred to as the IFN signature [65–67]. PBMCs of SLE patients and controls were compared for expression of type-I IFN-inducible genes and found to have higher levels in SLE that were correlated with disease activity levels, but not longitudinally [68, 69]. Interferon regulatory factor 5 (IRF5) [70–73] and *IRF5*-risk haplotype are associated with higher IFNα activity in SLE patients [74]. Autoimmunity, including lupus-like disease, has developed in patients treated with IFN for hepatitis C [75] and cancer [76–78]. DCs, key actors in SLE pathogenesis, differentiate from monocytes following IFNα induction [79]. DNA, RNA or immune complex binding to Toll-like receptor 7 or 9 can result in plasmacytoid DCs producing IFNα [80], and this has been proposed to occur locally in cutaneous lupus tissue [81, 82]. A further study confirmed the association of IFNα with SLE disease activity and noted that DC maturation is IFN dependent [83]. IFNα activity was correlated with the presence of autoantibodies rather than clinical features or ethnicity [84, 85]. In summary, IFNα plays a role in the pathogenesis of lupus through genetic susceptibility, a self-directed immune response resembling an antiviral response and chronic immune dysregulation [86].

Therefore, IFNα-directed pharmaceuticals are being developed and evaluated for therapeutic benefit in SLE. In a phase II trial, rontalizumab, a mAb targeting IFNα, reduced

disease activity in a subgroup with low IFN; however, it did not meet the therapeutic target in the full group of patients [87].

Sifalimumab, another anti-IFNα monoclonal antibody, reduced the whole-blood IFNα gene signature in phase I studies [88, 89]. In phase II studies, anifrolumab, a type I IFN receptor antagonist, resulted in reduced disease activity in moderate to severe SLE as presented by R. Furie at the 2015 ACR Annual Meeting [90]. Two phase III trials are currently enrolling moderate to severe SLE patients for further evaluation, and a phase II study has begun enrolment of LN patients.

BLyS and APRIL

BLyS is a member of the TNF family which promotes B cell survival and antibody production. BLyS binds to three receptors (TACI, BCMA and BR3/BAFFR) present on the surface of B cells. A similar molecule, a proliferation-inducing ligand (APRIL), binds TACI and BCMA [91]. Elevated BLyS levels were found in SLE patients compared with normal controls, and BLyS was correlated with serum immunoglobulin and anti-dsDNA levels [92, 93]. Additional longitudinal studies from SLE patients confirmed these findings and showed a positive association with disease activity and a negative correlation between CS dosage and BLyS levels [94–97]. Classification criteria of discoid rash, renal disease, serositis and lymphopenia were associated with elevated BLyS levels, but levels failed to predict disease flare [96].

A phase II study of belimumab, a mAb that binds to soluble BLyS, found tolerability and efficacy in serologically active patients [98]. Two large phase III trials, BLISS-52 and BLISS-76, demonstrated sufficient efficacy for belimumab, leading to successful FDA approval for treatment of SLE [99, 100]. Phase I trials of blisibimod, a fusion polypeptide protein that binds both soluble and membrane-bound BLyS, in SLE patients noted a dose-dependent reduction in total B cells and naïve B cells and a relative increase in memory B cells, with favourable safety and tolerability [101]. The subsequent phase II study noted reduced disease activity, proteinuria and anti-dsDNA levels [102]. Furthermore, a phase III study has completed patient enrolment, with a second trial nearing initiation.

Although APRIL has many similarities to BLyS, reports noted a negative correlation of APRIL levels with disease activity and anti-dsDNA levels [103]. Furthermore, APRIL levels were inversely correlated with BLyS, potentially indicating a regulatory relationship or the result of ligand–receptor promiscuity [104]. Assessment of pre-flare and non-flare SLE patients found no significant difference in BLyS or APRIL levels, despite a difference between healthy controls (HCs) and disease activity state in SLE patients [105].

Atacicept is a fusion protein that includes the BLyS/APRIL binding site of the TACI receptor, potentially blocking BLyS and APRIL. TACI is involved in plasma cell survival, B cell proliferation and immunoglobulin production. A phase II/III study tested both a 75 and a 150 mg dose of atacicept against placebo. Concerns of infection risk, including two fatal infections, resulted in premature discontinuation of the 150 mg arm, whereas 75 mg lacked significant impact on disease flare [106]. However, additional phase II/III studies are in progress in SLE. Ultimately, BLyS and APRIL may prove beneficial in a subset of SLE patients.

Additional cytokine/chemokine biomarkers

TNFα

TNFα contributes to activation, differentiation, proliferation and antibody production in B cells and co-stimulates T cells. TNFα levels and soluble TNF receptors, TNFRI and TNFRII, are cleaved in response to inflammation [107, 108]. Levels were higher in SLE than in RA and spondyloarthritis [109]. TNFα was also correlated with disease activity in longitudinal specimens, with significantly higher levels in pre-flare *vs* non-flare patients [105, 110]. Contrary to this, blockade of TNFα can result in production of autoantibodies and drug-induced SLE [111]. An open-label study in six SLE patients on infliximab, a monoclonal antibody that binds TNFα, reported reduced arthritis and proteinuria, but higher levels of dsDNA and cardiolipin autoantibodies [112]. Anti-TNFα therapy, which has been extremely effective for RA, has shown some efficacy in SLE according to case reports. A more recent open-label study of 27 SLE patients randomized to infliximab or control reported improvement in disease activity and reduction in glucocorticoid use [113].

IL-6

IL-6, a pro-inflammatory cytokine produced by antigen-presenting cells, facilitates Th2 and Th17-type adaptive responses, and B cell activation, differentiation and antibody production. Both serum protein levels and PBMC *IL-6* gene expression were significantly elevated in a study comparing SLE patients with HCs [114]. Furthermore, sIL-6 levels are correlated with disease activity, ESR and CRP in SLE patients [115, 116]. The IL-6 levels of pre-flare SLE patients are higher than those of non-flare SLE patients [105]. A phase I open-label, non-placebo-controlled study of 16 SLE patients noted that tocilizumab, an mAb directed at the IL-6 receptor, reduced autoantibody production and disease activity [117].

IL-12 and IL-23

IL-12, produced by antigen-presenting cells, facilitates the Th1 adaptive response by directly stimulating production of IFNγ and Th1 differentiation. sIL-12 levels were found to be higher in SLE patients than in HCs and to increase prior to flare in some patients [118]. In addition to elevated serum and urinary (u) IL-12, LN patients also exhibit IL-12 accumulation in renal glomerular mononuclear cells [119]. IL-12 levels were lower in patients treated with glucocorticoids or other immunosuppressants [120, 121]. IL-12p70 includes two subunits, p35 (also present in IL-23) and p40 (similar to IL-6 receptor α-chain), which is specific to IL-12. The p40 subunit can form a homodimer (IL-12p40/p40) that binds the IL-12 receptor, blocking

signalling activity [122]. A study in 28 SLE patients found that the IL-12p40 monomer was positively correlated with SLE disease activity; however, they failed to detect the IL-12p70 heterodimer [123], contradicting other studies [116, 118]. The IL-12p70 heterodimer was significantly higher in pre-flare SLE patients compared with non-flare SLE patients [105]. Ustekinumab, an IL-12/IL-23 antagonist, is FDA approved for the treatment of moderate to severe psoriasis [124, 125], and a phase IIa study has been initiated for SLE to evaluate safety and efficacy.

Closely related to IL-12, IL-23 contains the IL-12 p40 subunit, and findings linking IL-12 to inflammation and autoimmune disease may also reflect a potential role of IL-23 in SLE pathogenesis. IL-23 levels are higher in SLE patients than HCs and result in significantly greater release of IL-17 in SLE PBMCs compared with HCs [126]. Longitudinal SLE samples were found to have significantly higher IL-23 in pre-flare compared with self non-flare or quiescent [105]. In LN patients, glomerular expression of IL-23 was much higher in proliferative disease and correlated with renal components of the SLEDAI and the histological activity index [127].

IL-1

A genetic polymorphism in IL-1 receptor antagonist was correlated with disease findings of discoid rash and photosensitivity in SLE patients [128]. Additionally, polymorphisms in IL-1 receptor-associated kinase, *IRAK1*, were associated with both childhood- and adult-onset SLE [129, 130]. IL-1 levels were found to be higher in SLE patients compared with controls and elevated in patients with high disease activity prior to treatment [131].

Anakinra, an IL-1 receptor antagonist, was evaluated in an open-label pilot study of four SLE patients with active arthritis and found to be safe and well tolerated, and resulted in a reduction in tender joint count [132].

TGF-β

TGF-β is a fibrotic cytokine involved in wound healing, angiogenesis and formation of scar tissue. Plasma levels of TGF-β were significantly elevated in both inactive and active SLE patients compared with controls; however, *TGF-β* mRNA from SLE lymphocytes was greatly reduced or absent [133]. A study evaluating microparticle proteins found that TGF-β was reduced in SLE, which the authors surmise is related to reduction of platelets, a key producer of TGF-β [134]. Urinary *TGF-β* mRNA levels were higher in diffuse proliferative LN and reduced in patients responsive to therapy [135]. Fresolimumab, an anti-TGF-β mAb, has been given orphan status by the FDA for treatment of primary focal segmental glomerulosclerosis and is being studied in idiopathic pulmonary fibrosis (NCT00125385) and systemic sclerosis (NCT01284322). All of these diseases are characterized by an increase in fibrosis of various organs, which also occurs in later stages of LN. Pirfenidone, an anti-fibrotic agent whose mechanism is not fully understood, results in reduction of TGF-β and collagen synthesis. It has been approved by the FDA for treatment of idiopathic pulmonary fibrosis, and studies have been planned in focal segmental glomerulosclerosis (NCT00001959) and chronic kidney disease (CKD) (NCT02408744). Use of TGF-β-inhibiting agents to prevent renal sclerosis in patients with LN may be beneficial, probably in combination with immunosuppression.

IL-21

IL-21 is a pro-inflammatory cytokine that plays a variety of roles in the activation of NK cells, DCs, T cells and B cells, where it contributes to the generation of (auto)antibody-secreting plasma cells [136]. There are increased IL-21-producing peripheral $CD4^+$ T cells in SLE patients, correlating with a concurrent increase in memory B cells and Th17 cells and reduced Treg cells [137]. Genetic variants in the *IL-21* gene are noted to be associated with SLE [138, 139]. A recent clinical trial of an IL-21 inhibitor, NNC0114-0006, has been terminated according to ClinicalTrials.Gov. Although there has been interest in the inhibition of IL-21 in SLE, the pleotropic effects of this cytokine might result in detrimental immunosuppressive and immunostimulatory effects.

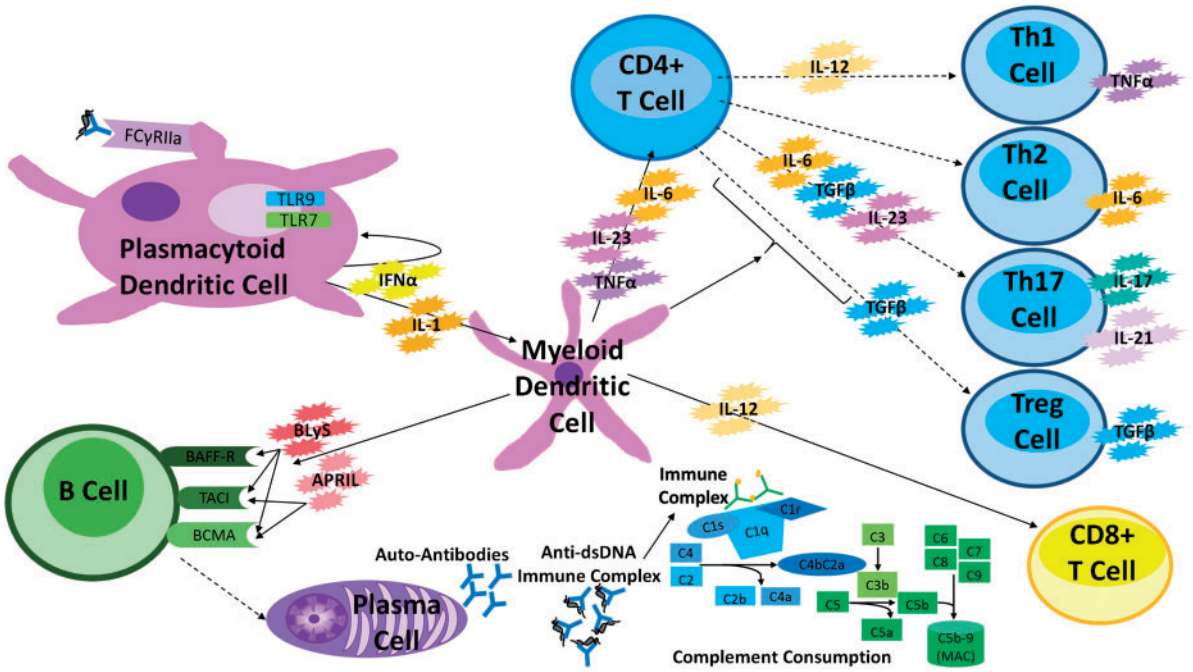
IL-17

IL-17 is a pro-inflammatory cytokine produced by Th cells, γδT cells and Th17 cells [140]. IL-17-secreting $CD4^+$ effector T cells are higher in SLE patients, and plasma IL-17 levels are correlated with disease activity in non-renal SLE patients [116, 126, 141]. IL-17 is significantly higher in pre-flare SLE patients than in non-flare SLE patients [105]. In LN, IL-17 was correlated with proteinuria and dsDNA and was significantly higher in active disease compared with remission [142]. Furthermore, renal glomerular IL-17 expression was elevated in diffuse proliferative disease compared with healthy kidneys, correlating with the histological activity index [127]. Secukinumab, brodalumab and ixekizumab block IL-17, but currently no SLE studies have been undertaken. Testing of these agents in PsA, RA and AS is ongoing, with recent FDA approval of secukinumab for psoriasis.

Other serum biomarkers discovered in proteomic screens

In addition to targeted studies, comprehensive screens of protein panels have uncovered potential disease biomarkers. Increased plasma levels of pro-inflammatory soluble mediators were found using a large panel in pre-flare SLE patients compared with non-flare patients, whereas regulatory mediators were increased during quiescent periods [105]. Axl, Fas, ferritin, intercellular adhesion molecule 1, insulin-like growth factor-binding protein 2 (IGFBP-2), sialic acid-binding Ig-like lectin 5 (Siglec-5) and sTNFRII were found to be promising SLE serum biomarkers from a 274-mediator panel [143]. The higher ferritin levels were confirmed in active compared with inactive SLE patients; a further correlation was identified with the histopathological response in LN patients [144, 145]. Furthermore, assessment of IGFBP-2 showed higher levels in LN, with a correlation with disease activity and clinical and histopathological response [146, 147]. Sera from 94 Chinese SLE patients and 49 HCs evaluated for Axl, sTNFRII, ferritin and IGFBP-2 by ELISA showed the highest levels in active SLE, followed by inactive SLE, then

Fig. 2 A schematic diagram of potential SLE biomarkers



Key biomarkers are included with examples of immune cells that produce them. Continuous lines indicate cytokines acting on a particular immune cell, whereas dashed lines indicate the differentiation of the $CD4^+$ T cell into various subclasses of T cells. A plasmacytoid dendritic cell (pDC) binds an anti-dsDNA immune complex to the FCγRIIa receptor, also shown with an endosome containing Toll-like receptors (TLR) 7 and 9 that can bind DNA, RNA or immune complexes, inducing production of IFNα. Myeloid dendritic cells can produce BLyS and APRIL, which bind BAFF-R, TACI and BCMA or TACI and BCMA, respectively. The B cell differentiates into plasma cells, which produce antibodies, including autoantibodies. Anti-dsDNA binds to dsDNA, forming immune complexes, which bind and activate the complement. Immune complexes are also deposited in various organs and tissues; for example, kidneys. The myeloid dendritic cells also produce IL-6, IL-23 and TNFα, which can activate $CD4^+$ T cells that then differentiate into T cell subclasses, dendritic cells and other cell types. IL-4 (data not shown) results in the differentiation of Th2 cells, which produce IL-6. APRIL: a proliferation-inducing ligand; BlyS: B lymphocyte stimulator; MCP-1: monocyte chemotactic protein 1; TWEAK: TNF-like weak inducer of apoptosis.

HCs; furthermore, all four biomarkers were correlated with disease activity [148]. Analysis of IGFBP-4, a biomarker in diabetic nephropathy, revealed increased levels in LN patients compared with non-lupus CKD and HCs [149]. These protein biomarkers initially discovered from proteomic screens may prove to be clinically informative in SLE and LN. However, some of these biomarkers are elevated in non-specific inflammation, cellular damage or fibrosis. Therefore, additional studies are warranted for further validation.

The traditional serum biomarkers discussed were anti-dsDNA and complement; non-traditional biomarkers discussed were IFNα, BLyS and APRIL. Assessment of the emerging SLE biomarkers found IL-17 and IL-23 to be the most promising prognostic biomarkers. This conclusion is based on the recentness of the biomarkers, the MI and literature strength scores (high despite their youth) and the available studies (e.g. cross-sectional, longitudinal, gene expression).

Urine biomarkers in LN

LN remains a major cause of morbidity and mortality for SLE patients. Urine is routinely evaluated for protein, red blood cells, white blood cells and cellular casts to screen for LN, guide treatment response and assess renal flare. Complete diagnosis of LN relies upon a kidney biopsy to determine classification and guide therapy. The invasiveness of biopsy encouraged investigators to seek serum and urine biomarkers with the ability to predict histopathology, renal flare and treatment response. Urine, easily collected by patients, may allow better assessment of the kidney microenvironment than peripheral blood.

TNF-like weak inducer of apoptosis (TWEAK) is a member of the TNF family capable of inducing IL-8 secretion, apoptosis and cell differentiation [150]. A multicentre longitudinal study found that uTWEAK was correlated with disease activity over time and was not elevated in other autoimmune disease and renal disease control groups [151]. Furthermore, TWEAK was found to activate TGF-β

Table 2 Summary of therapeutics targeting biomarkers

Therapeutic	Target	ClinicalTrials.Gov ID	Highest trial phase
Edratide	Heavy chain of anti-dsDNA	NCT00203151	Phase II
Eculizumab	C5		Phase II
Rontalizumab	IFNα	NCT00541749, NCT00962832	Phase II
Sifalimumab	IFNα	NCT00299819, NCT00482989, NCT00657189, NCT00979654, NCT01031836, NCT01283139	Phase II
Anifrolumab	Type I IFN receptor	NCT01438489, NCT01559090, NCT01753193, NCT02446912, NCT02446899, NCT02547922	Phase III
Belimumab	Soluble BLyS	NCT00071487, NCT00724867, NCT00410384, NCT00424476, NCT00583362, NCT00657007, NCT00712933, NCT00732940, NCT01345253, NCT01484496, NCT01516450, NCT01597492, NCT01597622, NCT01632241, NCT01639339, NCT01649765, NCT01705977, NCT01729455, NCT01858792, NCT01914770, NCT02119156, NCT02260934, NCT02270970, NCT02284984	Phase III
Blisibimod	Soluble and membrane-bound BLyS	NCT01162681, NCT01305746, NCT01395745, NCT02514967	Phase III
Atacicept	TACI receptor (BLyS and APRIL)	NCT00573157, NCT00624338, NCT01369628, NCT01440231, NCT01972568, NCT02070978	Phase II
Infliximab	TNFα	NCT00368264	Phase III
Tocilizumab	IL-6 receptor	NCT00046774	Phase I
Sirukumab	IL-6	NCT01273389, NCT01702740	Phase II
Ustekinumab	IL-12 and IL-23	NCT02349061	Phase II
Anakinra	IL-1		
Fresolimumab	TGF-β		
Pirfenidone	Unknown antifibrotic, reduces TGF-β		
NNC0114-0006	IL-21	NCT01689025	Phase I
Secukinumab	IL-17		
Brodalumab	IL-17		
Ixekizumab	IL-17		
ABN912	MCP-1/CCL-2		
BB11B023	TWEAK	NCT01499355, NCT01930890	Phase II

The table includes names of therapeutics, the target of the drug and available clinical trial identification numbers through ClinicalTrials.gov, and the highest phase of study included. APRIL: a proliferation-inducing ligand; BlyS: B lymphocyte stimulator; MCP-1: monocyte chemoattractant protein 1; TWEAK: TNF-like weak inducer of apoptosis.

in kidney proximal tubule cells [152]. However, a pharmaceutical targeting TWEAK (BBIIB023) did not result in significant improvement in an LN proof-of-concept study [153]; therefore, development was recently terminated by Biogen (NCT01499355).

MCP-1/CCL2 recruits monocytes, memory T lymphocytes and NK cells to inflammatory sites. sMCP-1 and uMCP-1 were higher in LN patients compared with patients without LN and HCs, and MCP-1 was detected in the kidneys of LN patients [154, 155]. Longitudinal assessment of uMCP-1 found that levels increase a few months before renal flare and are correlated with urine protein and treatment response [156]. The anti-MCP-1, ABN912, showed no benefit in RA in a randomized, placebo-controlled trial [157]. It does not appear that additional studies in SLE or LN are planned at this time.

Kidney injury and inflammation results in increased secretion of neutrophil gelatinase-associated lipocalcin from epithelial cells and leucocytes. Levels are higher in SLE patients with LN than in SLE patients without LN and controls, and are correlated with disease activity [155, 158]. Urinary neutrophil gelatinase-associated lipocalcin levels on a preceding visit were correlated with renal flare, with an adjusted odds ratio of 1.7 [159].

Vascular cell adhesion molecule-1 is important for immune cell recruitment into tissues. SLE patients have increased levels compared with HCs, which are correlated with SLE disease activity [160]. Furthermore, patients with active renal disease have higher levels compared with non-renal and inactive SLE [143, 161].

Angiostatin, a proteolytic fragment of plasminogen, inhibits endothelial proliferation and angiogenesis. An initial

array-based screen found urinary angiostatin to be higher in LN. A validation study of 100 SLE patients (~80% with renal activity), 24 CKD patients and 21 HCs noted higher levels in SLE patients compared with HCs, but did not discriminate between SLE and CKD [162]. Additional mechanistic studies in human umbilical vein endothelial cells noted that angiostatin inhibited IL-1β-induced down-regulation of endothelial nitric oxide synthase through the nuclear factor-κB pathway [163]. Urinary angiostatin may be a non-specific marker for kidney damage and may be less likely to translate into beneficial therapeutics. However, urinary angiostatin could potentially serve as a biomarker to differentiate between active disease and prior damage in chronic LN patients.

Assessment of these and other urine biomarkers in clinical care may potentially predict development of LN, predict renal flares in those known to have LN and assist in determining therapeutic response and predicting histopathology.

Conclusion

The events leading to the development and continuation of SLE pathogenesis remain poorly understood. Patients continue to have active inflammation despite currently available treatments. Although the relationship between immune cells and biomarkers is complex, a simplified summary of the discussed blood biomarkers is included (Fig. 2), with a summary table of current therapeutics targeting the discussed biomarkers (Table 2). Combining currently available biomarkers, physical examination and patient history may allow better assessment of disease activity. The continuing growth in -omics technologies will continue to fuel the discovery of novel biomarker candidates, which will potentially guide therapeutic development and assist in assessment of disease activity. Assessment of biomarkers will be likely to provide clues to therapeutic regimen selection in the future. Here, we have summarized bedside to bench to bedside use of biomarkers to design novel therapeutics that will hopefully result in improved patient outcomes in SLE.

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