RHEUMATOLOGY

Rheumatology 2020;59:v12–v18
doi:10.1093/rheumatology/keaa484

New biomarkers in SLE: from bench to bedside

Riccardo Capecchi[1], Ilaria Puxeddu[1], Federico Pratesi[1] and Paola Migliorini [1]

Abstract

Biomarkers may have a diagnostic or monitoring value, or may predict response to therapy or disease course. The aim of this review is to discuss new serum and urinary biomarkers recently proposed for the diagnosis and management of SLE patients. Novel sensitive and specific assays have been proposed to evaluate complement proteins, 'old' biomarkers that are still a cornerstone in the management of this disease. Chemokines and lectins have been evaluated as surrogate biomarkers of IFN signature. Other cytokines like the B cell activating factor (BAFF) family cytokines are directly related to perturbations of the B cell compartment as key pathogenetic mechanism of the disease. A large number of urine biomarkers have been proposed, either related to the migration and homing of leukocytes to the kidney or to the local regulation of inflammatory circuits and the survival of renal intrinsic cells. The combination of traditional disease-specific biomarkers and novel serum or urine biomarkers may represent the best choice to correctly classify, stage and treat patients with SLE.

Key words: systemic lupus, biomarker, complement, cytokine, chemokine

Rheumatology key messages

- Novel serum and urine biomarkers have been proposed and validated.
- Combination of novel and traditional disease-specific biomarkers may improve diagnosis and management of SLE.

SUPPLEMENT

Introduction

Biomarkers are critical in research and clinical practice [1]. The steadily increasing availability of biological measurements has created the need to correctly define biomarkers and their use. In the document elaborated by a task force from the Food and Drug Administration and the National Institutes of Health, a biomarker is termed as 'a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention' [2].

Thus, biomarkers may have a diagnostic or monitoring value, or may predict response to therapy or disease course.

In a complex disorder such as SLE, a number of biomarkers have been proposed that fall into each of the above-described categories; many of them are well validated and still used in clinical practice. However, the search for and definition of novel biomarkers is of utmost importance, as many unmet needs still exist in the diagnosis and management of the disease.

Novel biomarkers are helpful in early diagnosis and in differential diagnosis from clinically overlapped disorders, in correct evaluation and staging of organ involvement, and in predicting and monitoring response to therapies.

In this review, we will analyse 'traditional' biomarkers whose relevance in SLE has been recently corroborated by new data, and novel biomarkers that have been recently discovered and proposed.

Complement

Complement is a double-edged sword in SLE, protecting from the disease on one hand but mediating organ damage on the other [3]. A huge amount of data support the clinical value of complement measurement in the disease. The detection of complement components in tissues is routinely employed to evaluate immune complex deposition in target organs. Decreased concentration of complement components in blood is strongly associated with active disease and is predictive of flares

[1]Clinical Immunology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

Submitted 24 May 2020; accepted 23 July 2020

Correspondence to: Paola Migliorini, Department of Clinical and Experimental Medicine, Via Roma 67, 56126 Pisa, Italy. E-mail: paola.migliorini@med.unipi.it

[4]. Low levels of serum complement 3 (C3) and C4 are included in the classification criteria developed by EULAR and ACR [5].

However, the detection of C3 and C4 levels suffers from well-known limitations. Complement activation is increased in active disease, but the diminished levels of C3 and C4 are partially compensated by acute phase production of the two proteins. Moreover, the variability in C4 gene copy number in population leads to a wide interval of 'normal' C4 levels. Applying the analysis of C4 gene copy number to SLE patients, it has been reported that 3 out of 11 SLE patients with persistently low C4 levels in spite of low disease activity are carriers of only two copies of the C4 gene [6].

Thus, measurement of C3 and C4 levels is not a sensitive tool to study SLE patients and does not accurately reflect ongoing complement activation in SLE sera. More information can be gained using functional assays that explore the whole complement cascade such as the capacity of erythrocyte lysis (haemolytic potential) or the levels of solid phase or soluble membrane attack complex. On the other hand, it is widely accepted that measurement of complement split products represents a powerful tool to get a full picture of complement status in SLE. The rapid decay of these products has been the main obstacle to their detection, but novel approaches, including evaluation of cell-bound complement products, have been proposed to overcome it.

Detection by cytometry of C4d levels on erythrocytes and B cells (cell-bound complement activation products, CB-CAP) is a sensitive assay for the diagnosis and follow up of SLE patients [7]. Moreover, CB-CAP can predict evolution to classifiable SLE in patients in which the disease is suspected but ACR criteria are not yet fulfilled [8].

iC3b is the breakdown product of C3b, thus reflecting complement activation via the classical, lectin and alternative pathway. Its half-life in serum (90 min) is long enough to allow detection but short enough to reflect ongoing complement activation. iC3b detection by lateral flow assay minimizes serum handling and *in vitro* activation of complement [9]. Its evaluation, expressed as ratio of blood iC3b to serum C3 levels, has been proposed as a useful tool to analyse complement activation in sera. In fact, this ratio identifies active disease and is strongly associated with nephritis [10].

Serum biomarkers

Recently, genome-wide expression studies have indicated that the majority of SLE patients are characterized by an increased expression of type I IFN-regulated genes, indicated as an IFN gene signature [11]. A high number of genes, up to 10%, are under type I IFN control; moreover, genes regulated by type I and type II IFN widely overlap, leading to the current view that all IFNs, including type III, contribute to regulate gene expression in SLE [12].

Direct measurement of type I IFN has always been elusive, because it is present in minute amounts either as protein in serum or as mRNA transcript in peripheral blood mononuclear cells. Recently, a novel assay has been proposed based on a new digital ELISA that allows a 5000-fold increase in sensitivity [13]. By this assay, high circulating levels of IFN-α were detected in SLE sera. However, most data on SLE have been obtained by transcriptomic analysis, the tool to analyse gene expression, that has been performed in whole blood and tissues from SLE patients. These studies led to the identification of gene modules hyperexpressed in SLE patients [14]. However, gene expression analysis is not yet applicable to routine patient evaluation, suggesting the utility of searching for surrogate markers of IFN signature: C-X-C motif chemokine ligand 10 (CXCL10), Galectin-9 and sialic acid binding Ig-like lectin 1 (SIGLEC-1) have been proposed as easy-to-detect biomarkers of IFN signature.

CXCL10, Galectin-9 and SIGLEC-1

The chemokine CXCL10 is present at increased levels in SLE sera and in affected tissue, where it plays a role in the recruitment of CXCR3+ effector and memory T cells, NK cells and plasma cells to inflammatory sites. Among IFN-regulated chemokines, CXCL10 has the highest correlation with disease activity and the best predictive ability for disease flares. Renal flares were accurately predicted by CXCL10 serum levels ($P = 0.001$) but not by 'traditional' biomarkers such as anti-dsDNA antibodies or complement levels [15].

van den Hoogen *et al.* [16] reported a higher correlation with disease activity of Galectin-9 compared with CXCL10 levels ($P = 0.003$ *vs* $P = 0.21$). At variance with CXCL10, serum levels of Galectin-9, a beta-galactoside-binding lectin, reflect organ damage and not only disease activity [17]. Galectin-9 is detectable also in cerebrospinal fluid, suggesting a potential use of the lectin in the diagnosis of CNS involvement [17].

The SIGLEC-1 is an IFN-regulated membrane protein involved in cell adhesion to sialylated pathogens; it is expressed on the surface of cells of myeloid origin and is also detectable in serum, where higher concentrations have been suggested to be associated with increased frequency of renal complications, but not with SLE disease activity index [18, 19].

IL-1 family

The major inducer of type II IFN is IL-18, an IL-1 family cytokine that has been extensively investigated in SLE and proposed as a biomarker of disease activity [20, 21]. In fact, despite the overproduction of the soluble inhibitor IL-18 binding protein (IL-18BP), IL-18 and free IL-18 levels are increased and correlate with disease activity indexes, as well as with other serological markers (anti-dsDNA and anti-C1q antibody titers, complement levels). Active nephritis is the main disease manifestation associated with high IL-18 levels and local production of

the cytokine in the kidney may partly explain this finding [22].

Among IL-1 family cytokines and receptors, the soluble form of ST2/IL-1 receptor 4 (IL-1R4) has been recently proposed as new biomarker in SLE [23]. ST2/IL-1R4, upon recruitment of the accessory chain IL-1R3, mediates IL-33 signalling; the soluble form of the receptor prevents the interaction of IL-33 with membrane receptor, thus behaving as a decoy receptor [24]. Soluble IL (sIL)-1R4 levels are increased in active SLE patients, and highly correlated with disease activity index and with anti-dsDNA and anti-C1q antibody levels. Moreover, in patients with active nephritis, sIL-1R4 is correlated with urinary proteins. When the diagnostic value of sIL-1R4 is directly assessed by means of multivariate analysis, sIL-1R4 contributes to a similar extent as anti-dsDNA and IL-18BP to the identification of patients with active nephritis, while is the most relevant variable in discriminating active from inactive patients. Before now, sIL-1R4 had been considered a biomarker only in cardiovascular diseases, being associated with adverse outcome in myocardial infarction, heart failure and pulmonary diseases and predicting mortality in acute or chronic heart failure [25].

BAFF family

Type I and type II IFNs also regulate the expression and secretion of cytokines and receptors of the B cell activating factor (BAFF) family, which play a fundamental role in B cell development, maturation and survival [26]. The BAFF family, a member of the TNF superfamily, includes two ligands, BAFF and a proliferation-inducing ligand (APRIL), and three receptors, BAFF receptor (BAFF-R), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA). BAFF interacts with BAFF-R, TACI and BCMA with decreasing affinity; APRIL can only interact with TACI and BCMA. The three receptors are expressed in a subset-specific manner starting with BAFF-R in transitional B cells followed by TACI in marginal zone and switched memory B cells, and finally by BCMA in plasma cells. Naïve B cells express only BAFF-R, marginal zone and switched memory B cells express BAFF-R and TACI, and plasma cells express TACI and BCMA.

BAFF levels have been extensively investigated in SLE: increased serum levels have been reported, associated with serological activity more than with global disease activity [27–30]. Higher levels of BAFF may characterize subsets of SLE patients, such as those affected by subclinical atherosclerosis [31]. On the whole, BAFF transcript level in peripheral blood seems a better biomarker of disease activity and predictive of disease flares than serum levels of BAFF protein [27].

Recently, three members of the BAFF family (BAFF, APRIL and soluble BCMA) have been assessed in the same cohort of SLE patients to evaluate their potential use as biomarkers for diagnosis and follow up [32]. Despite the underexpression of membrane BCMA on lupus B cells, the soluble form of the receptor is detectable in higher amounts in SLE sera. Soluble BCMA may counteract BAFF and APRIL, negatively affecting their signalling. Thus, its increase, which probably represents the activation of a regulatory circuit, closely reflects the levels of BAFF and APRIL. The three cytokines are all present in higher amounts in sera from active patients and decrease in remission. They display a similar ability to distinguish normal subjects from SLE patients, with APRIL and soluble BCMA showing a higher specificity and sensitivity than BAFF [32].

Urine biomarkers

Renal involvement has a great impact on morbidity and mortality in SLE: thus, a precise assessment of kidney inflammation is of utmost importance. In this respect, renal biopsy still represents the gold standard, but non-invasive tools to measure disease activity in the kidney are actively searched. Urinary biomarkers are instruments of great relevance, as they may directly reflect the disease process in the affected organ.

Cytokines and chemokines are locally produced by infiltrating inflammatory cells and can be easily detected in urine. Among inflammatory chemokines that mediate leucocyte infiltration and play a significant role in the progression of nephritis, chemokine (C-C motif) ligand 2 (CCL2)/monocyte chemoattractant protein-1 (MCP-1), CXCL10, CXCL4 and CXCL16 are very interesting candidates.

Urinary levels of CCL2 represent a sensitive indicator of renal flare, predicting its severity and response to treatment [33–35], and are also related to active tubulointerstitial lesions [36]. CCR2, receptor of the chemokine, is expressed at higher density in circulating monocytes than in kidney infiltrating monocytes that are excreted in urine. Since the chemokine/receptor complex is rapidly internalized, low receptor expression is probably due to high CCL2 concentration. Thus, available data suggest that CCR2 is the main receptor involved in monocyte recruitment to the kidney [37].

Urinary levels of CXCL10 and CXCL16 are highly increased in lupus nephritis [37]. Corresponding receptors, CXCR3 and CXCR6, are overexpressed on urine CD4 T cells; in particular, a clear correlation is detected between urinary CXCL10 levels and the number of CXCR3-positive CD4 T cells in urine. Urinary CD4 T cells are in fact a very sensitive and specific marker of proliferative glomerulonephritis: a number of CD4 T cells higher than 800/100 ml is detected exclusively in active lupus nephritis; moreover, urinary CD4 T cells may be an indicator of treatment response [38].

Although available data clearly indicate the critical role of CXCL10/CXCR3 in mediating CD4 T cell infiltration of the kidney, a marked intra-patient variability is observed and no single chemokine can be considered a universal biomarker for lupus nephritis [37].

In SLE patients with active renal disease that underwent renal biopsy, CXCL4 urinary levels are correlated

with histological activity index but do not differ in proliferative *vs* non proliferative types of nephritis [39]. CXCL4 is a chemokine constitutively expressed in the kidney that upregulates pro-fibrotic cytokines like IL-4 and IL-13. The contribution of plasmocytoid dendritic cells infiltrating the kidney to local production of the cytokine is presently unclear [39].

Other important players in the recruitment of inflammatory cell to affected organs are cell adhesion molecules, which are expressed on vascular endothelium and interact with leucocyte integrins, allowing their extravasation into inflamed tissues.

In lupus nephritis, vascular cell adhesion molecule 1 (VCAM-1) expression is upregulated in glomerular epithelial cells, mesangium, proximal tubular cells; activated leucocyte CAM (ALCAM) is also hyperexpressed on macrophages and glomerular endothelia. Urinary VCAM-1 and ALCAM are elevated in active *vs* quiescent lupus nephritis and can distinguish active renal involvement from active non-renal disease [39–41]. VCAM-1 levels are also highly correlated with histological activity index [41]. Other adhesion molecules that are emerging as biomarkers in lupus nephritis are E-selectin [40] and ICAM, that is also detectable in urine by a sensitive and specific rapid assay [42].

In a comparative evaluation of urinary biomarkers, a similar discrimination of active renal *vs* active non renal SLE patients was achieved with VCAM-1, CXCL4 and angiostatin [39]. Angiostatin is a plasminogen fragment with a strong anti-angiogenic activity. Its role in lupus nephritis is not fully understood, but it may be protective and related to the inhibition of neutrophil migration and vascular endothelial cell proliferation [39, 43].

Urinary cytokines of confirmed role in the evaluation of SLE nephritis include BAFF, APRIL and tumor necrosis factor-like weak inducer of apoptosis (TWEAK). Given the role of the BAFF family in SLE, it is not surprising that BAFF and APRIL are both detected at increased levels in active nephritis [44, 45].

TWEAK is a proinflammatory cytokine, a member of the TNF family, endowed with multiple activities on renal intrinsic cells. On cultured tubular and mesangial cells, TWEAK activates NF-κB signalling, induces production of proinflammatory cytokines, is mitogenic, and in the presence of sensitizing agents promotes apoptosis [46]. TWEAK appears to be a marker of activity in lupus nephritis [35, 47], not related to renal histopathology [47] but able to predict response to therapy [48].

Initially proposed as marker of renal involvement in childhood-onset SLE [49], urinary neutrophil gelatinase-associated lipocalin (NGAL) has been validated as biomarker of active nephritis in adult disease [36, 50]. NGAL, which is upregulated in intrinsic renal cells in response to acute injury, enhances local inflammation and induces apoptosis of mesangial and tubular cells [51]. In an ample cohort of SLE patients, urinary NGAL levels were measured, and serum traditional biomarkers of lupus activity were also evaluated; the results were validated in a second independent cohort [50]. NGAL is not related to proteinuria; its sensitivity and specificity in detecting active renal disease are similar to those obtained with C3, C4 or anti-DNA antibodies evaluation. However, in the subgroup of patients with biopsy-proven nephritis, urine NGAL levels at preceding visit are a better predictor of renal flare than complement or anti-DNA antibody levels. This predictive ability can be lost in a longer follow-up [52], probably reflecting the acute response of NGAL to renal damage.

Lymphocyte populations

The phenotype of lymphocytes has been proposed as a possible biomarker of active disease, specific organ involvement or response to therapy. B cell compartment is primarily perturbed in SLE, and an increased number of plasmablasts ($CD19^{lo}$ $CD20^{-}$ $CD27^{hi}$ $CD38^{hi}$) and transitional B cells ($CD24^{hi}$ $CD38^{hi}$) are found in peripheral blood [53]. Infection or vaccination normally induce an increase of these two subpopulations, which in SLE is probably expression of the autoantigen-driven B cell expansion.

Regulatory T cells are also altered in SLE. A number of $CD4^{+}$ $Foxp3^{+}$ cells, mainly thymic, with variable CD25 expression, have been reported in active patients by several authors [54–57]. An increase in proinflammatory Th17 cells is also observed in peripheral blood and the Treg/Th17 ratio seems to distinguish SLE patients from those affected by other systemic autoimmune disorders like primary APS [58, 59]. Follicular T helpers, associated with the formation of germinal centres, were also claimed to be a relevant subset, since their number in peripheral blood is increased in SLE patients who are more resistant to treatment. The expression on T cells of molecules involved in cell migration and homing in tissues is also altered in SLE. An example is represented by CD44v3 and CD44v6 isoforms, which are increased on $CD4^{+}$ and $CD8^{+}$ T cells from SLE patients and correlate with disease activity [60, 61].

However, although suggestive, most of these results were obtained in small cohorts of patients and not confirmed in independent studies [62]. Moreover, therapy itself can rapidly modify cell phenotype and number (e.g. steroid for plasmablasts). The identification of more stable surrogate biomarkers of cell subpopulations, such as circulating BMCA for plasma cells, may represent an important technical progress. In fact, flow cytometry still suffers from poor reproducibility; thus, standardization of procedures is needed in order to identify reliable biomarkers.

Conclusion and perspectives

Despite the impressive amount of data that have validated novel biomarkers, as summarized in Table 1, experimental work is ongoing in the attempt to discover new biomarkers tools for the diagnosis, staging and management of SLE patients.

Table 1 Diagnostic and prognostic value of serum and urinary biomarkers

Biomarker	Serum	Urinary	Diagnostic	Prognostic
C4	+		+	+
CB-CAP	+		+	+
iC3b	+		+	+
IFN-α	+		+	
CXCL10	+	+	+	+
Galectin-9	+		+	
SIGLEC-1	+		+	
IL-18	+		+	
ST2/IL-1R4	+		+	
BAFF	+	+	+	+
BCMA	+		+	
APRIL	+	+	+	
TWEAK		+	+	+
CCL2		+	+	+
CXCL16		+	+	
CD4+ cells		+	+	+
CXCL4		+	+	
VCAM-1		+	+	
ALCAM		+	+	
Angiostatin		+	+	
NGAL		+	+	+

The table reports the diagnostic and prognostic value of serum and urinary biomarkers presented in the paper. C4: complement 4; CB-CAP: cell-bound complement activation products; CXCL10: C-X-C motif chemokine 10; SIGLEC-1: sialic acid binding Ig-like lectin 1; ST2/IL-1R4: IL-1 receptor 4; BAFF: B cell activating factor; BCMA: B-cell maturation antigen; APRIL: a proliferation-inducing ligand; TWEAK: tumor necrosis factor-like weak inducer of apoptosis; CCL2: chemokine (C-C motif) ligand 2; VCAM-1: vascular cell adhesion molecule 1; ALCAM: activated leucocyte CAM; NGAL: neutrophil gelatinase-associated lipocalin.

Most of the proposed urinary biomarkers are associated with active nephritis, irrespective of the underlying disease, and also several serum biomarkers are shared by different autoimmune disorders. IFN signature, for example, is a feature of interferonopathies, primary APS and SS. Thus, the parallel use of less sensitive but more disease-specific biomarkers is mandatory, at least in the diagnosis stage.

To obtain an accurate classification and staging of the disease in follow-up, a combination of biomarkers may represent the best choice. In this respect, the possibility to accurately predict inflammation and damage by the combined use of several urinary biomarkers is of interest. Brunner *et al.* [63] propose a combination of MCP-1, ceruloplasmin, alpha-1-acid glycoprotein and protein-to-creatinine ratio to estimate histological activity, and of NGAL together with glomerular filtration rate and MCP-1 to estimate chronicity.

As biological systems are complex and multidimensional, an unbiased approach such as proteomics may offer advantages. In fact, it has allowed confirmation of previously identified biomarkers, and also enabled discovery of new ones, whose relevance in lupus nephritis should be confirmed in future studies [64].

Finally, the future of biomarkers in SLE cannot help but look at genetic and epigenetic factors; in particular, there is a growing interest in microRNA, whose role as a pathogenic factor and biomarker in diagnosis, follow-up and therapy monitoring for SLE has been widely suggested [65]. When proteomics and genomics/epigenomics are combined with single cell analysis, a deeper insight into the mechanisms of disease in the individual patient may be obtained, paving the way to a true liquid biopsy as the starting point to an individualized treatment [66].

Funding: No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article. This paper was published as part of a supplement supported by an educational grant from GSK.

Disclosure statement: The authors have declared no conflicts of interest.

References

1 Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood) 2018;243:213–21.

2 FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and Other Tools) Resource. Silver Spring, MD: Food and Drug Administration (US); Bethesda, MD: National Institutes of Health (US). http://www.ncbi.nlm.nih.gov/books/NBK326791/ (22 September 2017, date last accessed).

3 Scott D, Botto M. The paradoxical roles of C1q and C3 in autoimmunity. Immunobiology 2016;221:719–25.

4 Leffler J, Bengtsson AA, Blom AM. The complement system in systemic lupus erythematosus: an update. Ann Rheum Dis 2014;73:1601–6.

5 Aringer M, Costenbader K, Daikh D *et al.* European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Ann Rheum Dis 2019;78:1151–9.

6 Margery-Muir AA, Wetherall JD, Castley AS *et al.* Establishment of gene copy number-specific normal ranges for serum C4 and its utility for interpretation in patients with chronically low serum C4 concentrations. Arthritis Rheumatol 2014;66:2512–20.

7 Kalunian KC, Chatham WW, Massarotti EM *et al.* Measurement of cell-bound complement activation products enhances diagnostic performance in systemic lupus erythematosus. Arthritis Rheum 2012;64:4040–7.

8 Ramsey-Goldman R, Alexander RV, Massarotti EM *et al.* Complement activation in patients with probable systemic lupus erythematosus and ability to predict progression to American College of Rheumatology-classified systemic lupus erythematosus. Arthritis Rheumatol 2020;72:78–88.

9 Schramm EC, Staten NR, Zhang Z *et al.* A quantitative lateral flow assay to detect complement activation in blood. Anal Biochem 2015;477:78–85.

10 Kim AHJ, Strand V, Sen DP *et al.* Association of blood concentrations of complement split product iC3b and serum C3 with systemic lupus erythematosus disease activity. Version 2. Arthritis Rheumatol 2019;71:420–30.

11 Elkon KB, Wiedeman A. Type I IFN system in the development and manifestations of SLE. Curr Opin Rheumatol 2012;24:499–505.

12 Rönnblom L, Leonard D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. Lupus Sci Med 2019;6:e000270.

13 Rodero MP, Decalf J, Bondet V *et al.* Detection of interferon alpha protein reveals differential levels and cellular sources in disease. J Exp Med 2017;214: 1547–55.

14 Catalina MD, Owen KA, Labonte AC, Grammer AC, Lipsky PE. The pathogenesis of systemic lupus erythematosus: harnessing big data to understand the molecular basis of lupus. J Autoimmun 2020;110: 102359.

15 Bauer JW, Petri M, Batliwalla FM *et al.* Interferon-regulated chemokines as biomarkers of systemic lupus erythematosus disease activity: a validation study. Arthritis Rheum 2009;60:3098–107.

16 van den Hoogen LL, van Roon JAG, Mertens JS *et al.* Galectin-9 is an easy to measure biomarker for the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome. Ann Rheum Dis 2018;77: 1810–4.

17 Matsuoka N, Fujita Y, Temmoku J *et al.* Galectin-9 as a biomarker for disease activity in systemic lupus erythematosus. PLoS One 2020;15:e0227069.

18 Oliveira JJ, Karrar S, Rainbow DB *et al.* The plasma biomarker soluble SIGLEC-1 is associated with the type I interferon transcriptional signature, ethnic background and renal disease in systemic lupus erythematosus. Arthritis Res Ther 2018;20:152.

19 Rose T, Grützkau A, Klotsche J *et al.* Are interferon-related biomarkers advantageous for monitoring disease activity in systemic lupus erythematosus? A longitudinal benchmark study. Rheumatology (Oxford) 2017;56: 1618–26.

20 Migliorini P, Anzilotti C, Pratesi F *et al.* Serum and urinary levels of IL-18 and its inhibitor IL-18BP in systemic lupus erythematosus. Eur Cytokine Netw 2010;21: 264–71.

21 Mende R, Vincent FB, Kandane-Rathnayake R *et al.* Analysis of serum interleukin (IL)-1β and IL-18 in systemic lupus erythematosus. Front Immunol 2018;9:1250.

22 Calvani N, Tucci M, Richards HB, Tartaglia P, Silvestris F. Th1 cytokines in the pathogenesis of lupus nephritis: the role of IL-18. Autoimmun Rev 2005;4:542–8.

23 Italiani P, Manca ML, Angelotti F *et al.* IL-1 family cytokines and soluble receptors in systemic lupus erythematosus. Arthritis Res Ther 2018;20:27.

24 Boraschi D, Tagliabue A. The interleukin-1 receptor family. Semin Immunol 2013;25:394–407.

25 Wettersten N, Maisel AS. Biomarkers for heart failure: an update for practitioners of internal medicine. Am J Med 2016;129:560–7.

26 Naradikian MS, Perate AR, Cancro MP. BAFF receptors and ligands create independent homeostatic niches for B cell subsets. Curr Opin Immunol 2015;34:126–9.

27 Zollars E, Bienkowska J, Czerkowicz J *et al.* BAFF (B cell activating factor) transcript level in peripheral blood of patients with SLE is associated with same-day disease activity as well as global activity over the next year. Lupus Sci Med 2015;2:e000063.

28 Pers JO, Daridon C, Devauchelle V *et al.* BAFF overexpression is associated with autoantibody production in autoimmune diseases. Ann N Y Acad Sci 2005;1050:34–9.

29 Becker-Merok A, Nikolaisen C, Nossent HC. B-lymphocyte activating factor in systemic lupus erythematosus and rheumatoid arthritis in relation to autoantibody levels, disease measures and time. Lupus 2006;15:570–6.

30 Eilertsen GØ, Van Ghelue M, Strand H, Nossent JC. Increased levels of BAFF in patients with systemic lupus erythematosus are associated with acute-phase reactants, independent of BAFF genetics: a case-control study. Rheumatology (Oxford) 2011;50:2197–205.

31 Theodorou E, Nezos A, Antypa E *et al.* B-cell activating factor and related genetic variants in lupus related atherosclerosis. J Autoimmun 2018;92:87–92.

32 Salazar-Camarena DC, Palafox-Sánchez CA, Cruz A, Marín-Rosales M, Muñoz-Valle JF. Analysis of the receptor BCMA as a biomarker in systemic lupus erythematosus patients. Sci Rep 2020;10:6236.

33 Rovin BH, Song H, Birmingham DJ *et al.* Urine chemokines as biomarkers of human systemic lupus erythematosus activity. J Am Soc Nephrol 2005;16: 467–73.

34 Lee YH, Song GG. Urinary MCP-1 as a biomarker for lupus nephritis: a meta-analysis. Z Rheumatol 2017;76:357–63.

35 Dong X, Zheng Z, Luo X *et al.* Combined utilization of untimed single urine of MCP-1 and TWEAK as a potential indicator for proteinuria in lupus nephritis: a case-control study. Medicine (Baltimore) 2018;97:e0343.

36 Ding Y, Nie LM, Pang Y *et al.* Composite urinary biomarkers to predict pathological tubulointerstitial lesions in lupus nephritis. Lupus 2018;27:1778–89.

37 Klocke J, Kopetschke K, Grießbach AS *et al.* Mapping urinary chemokines in human lupus nephritis: potentially redundant pathways recruit $CD4^+$ and $CD8^+$ T cells and macrophages. Eur J Immunol 2017;47:180–92.

38 Enghard P, Rieder C, Kopetschke K *et al.* Urinary CD4 T cells identify SLE patients with proliferative lupus nephritis and can be used to monitor treatment response. Ann Rheum Dis 2014;73:277–83.

39 Mok CC, Soliman S, Ho LY *et al.* Urinary angiostatin, CXCL4 and VCAM-1 as biomarkers of lupus nephritis. Arthritis Res Ther 2018;20:6.

40 Stanley S, Vanarsa K, Soliman S *et al.* Comprehensive aptamer-based screening identifies a spectrum of urinary biomarkers of lupus nephritis across ethnicities. Nat Commun 2020;11:2197.

41 Parodis I, Gokaraju S, Zickert A *et al.* ALCAM and VCAM-1 as urine biomarkers of activity and long-term

renal outcome in systemic lupus erythematosus. Rheumatology (Oxford) 2019;kez528.

42 Wang Y, Tao Y, Liu Y *et al.* Rapid detection of urinary soluble intercellular adhesion molecule-1 for determination of lupus nephritis activity. Medicine 2018;97: e11287.

43 Wu T, Du Y, Han J *et al.* Urinary angiostatin–a novel putative marker of renal pathology chronicity in lupus nephritis. Mol Cell Proteomics 2013;12:1170–9.

44 Vincent FB, Kandane-Rathnayake R, Hoi AY *et al.* Urinary B-cell-activating factor of the tumour necrosis factor family (BAFF) in systemic lupus erythematosus. Lupus 2018;27:2029–40.

45 Phatak S, Chaurasia S, Mishra SK *et al.* Urinary B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL): potential biomarkers of active lupus nephritis. Clin Exp Immunol 2017;187:376–82.

46 Sanz AB, Izquierdo MC, Sanchez-Niño MD *et al.* TWEAK and the progression of renal disease: clinical translation. Nephrol Dial Transplant 2014;29:i54–62.

47 Schwartz N, Rubinstein T, Burkly LC *et al.* Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther 2009;11:R143.

48 Suttichet TB, Kittanamongkolchai W, Phromjeen C *et al.* Urine TWEAK level as a biomarker for early response to treatment in active lupus nephritis: a prospective multicentre study. Lupus Sci Med 2019;6:e000298.

49 Brunner HI, Mueller M, Rutherford C *et al.* Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. Arthritis Rheum 2006;54:2577–84.

50 Rubinstein T, Pitashny M, Levine B *et al.* Urinary neutrophil gelatinase-associated lipocalin as a novel biomarker for disease activity in lupus nephritis. Rheumatology (Oxford) 2010;49:960–71.

51 Pawar RD, Pitashny M, Gindea S *et al.* Neutrophil gelatinase-associated lipocalin is instrumental in the pathogenesis of antibody-mediated nephritis in mice. Arthritis Rheum 2012;64:1620–31.

52 Kiani AN, Wu T, Fang H *et al.* Urinary vascular cell adhesion molecule, but not neutrophil gelatinase-associated lipocalin, is associated with lupus nephritis. J Rheumatol 2012;39:1231–7.

53 Carvajal Alegria G, Gazeau P, Hillion S, Daïen CI, Cornec D. Could lymphocyte profiling be useful to diagnose systemic autoimmune diseases? Clin Rev Allergy Immunol 2017;53:219–36.

54 Hanaoka H, Nishimoto T, Okazaki Y, Takeuchi T, Kuwana M. A unique thymus-derived regulatory T cell subset associated with systemic lupus erythematosus. Arthritis Res Ther 2020;22:13.

55 Schmidt A, Rieger CC, Venigalla RK *et al.* Analysis of FOXP3+ regulatory T cell subpopulations in peripheral blood and tissue of patients with systemic lupus erythematosus. Immunol Res 2017;65:551–63.

56 Alexander T, Sattler A, Templin L *et al.* Foxp3+ Helios+ regulatory T cells are expanded in active systemic lupus erythematosus. Ann Rheum Dis 2013; 72:1549–58.

57 Yang X, Wang W, Xu J *et al.* Significant association of CD4+CD25+Foxp3+ regulatory T cells with clinical findings in patients with systemic lupus erythematosus. Ann Transl Med 2019;7:93.

58 Jakiela B, Kosałka J, Plutecka H *et al.* Facilitated expansion of Th17 cells in lupus nephritis patients. Clin Exp Immunol 2018;194:283–94.

59 Álvarez-Rodríguez L, Martínez-Taboada V, Calvo-Alén J *et al.* Altered Th17/Treg ratio in peripheral blood of systemic lupus erythematosus but not primary antiphospholipid syndrome. Front Immunol 2019; 10:391.

60 Crispín JC, Keenan BT, Finnell MD *et al.* Expression of CD44 variant isoforms CD44v3 and CD44v6 is increased on T cells from patients with systemic lupus erythematosus and is correlated with disease activity. Arthritis Rheum 2010;62:1431–7.

61 Novelli L, Barbati C, Ceccarelli F *et al.* CD44v3 and CD44v6 isoforms on T cells are able to discriminate different disease activity degrees and phenotypes in systemic lupus erythematosus patients. Lupus 2019;28: 621–8.

62 Schreiber K, Nocturne G, Cornec D, Daïen CI. Lymphocytes as biomarkers of therapeutic response in rheumatic autoimmune diseases, is it a realistic goal? Clin Rev Allergy Immunol 2017;53:277–90.

63 Brunner HI, Bennett MR, Mina R *et al.* Association of noninvasively measured renal protein biomarkers with histologic features of lupus nephritis. Arthritis Rheum 2012;64:2687–97.

64 Anania VG, Yu K, Pingitore F *et al.* Discovery and qualification of candidate urinary biomarkers of disease activity in lupus nephritis. J Proteome Res 2019;18: 1264–77.

65 Hong SM, Liu C, Yin Z *et al.* MicroRNA in systemic lupus erythematosus: a perspective on the path from biological discoveries to clinical practice. Curr Rheumatol Rep 2020;22:17–25.

66 Fava A, Buyon JP, Mohan C *et al.*; Accelerating Medicines Partnership in SLE network. Integrated urine proteomics and renal single-cell genomics identify an interferon-γ response gradient in lupus nephritis. JCI Insight 2020;5:138345.