New biomarkers in SLE: from bench to bedside

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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

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. 
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-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. Naive 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 leukocyte 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 angiotatin [39]. Angiotatin 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<sup>+</sup> CD20<sup>-</sup> CD27<sup>+</sup> CD38<sup>+</sup>) and transitional B cells (CD24<sup>+</sup> CD38<sup>-</sup>) 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<sup>+</sup> Foxp3<sup>+</sup> 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<sup>+</sup> and CD8<sup>+</sup> 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.
urinary biomarkers of previously identified biomarkers, and also enabled offer advantages. In fact, it has allowed confirmation

NGAL together with glomerular filtration rate and MCP-1 to-creatinine ratio to estimate histological activity, and of est. Brunner et al. combined use of several urinary biomarkers is of inter-

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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-

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 multidimen-
sional, 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.

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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 matur-

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

Most of the proposed urinary biomarkers are associ-

ed with active nephritis, irrespective of the underlying disease, and also several serum biomarkers are shared by different autoimmune disorders. IFN signature, for ex-

ample, 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-

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 multidimen-
sional, 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.
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