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Diagnostic and Prognostic Tests in Systemic Lupus Erythematosus

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Abstract

Systemic lupus erythematous (SLE) is a chronic autoimmune inflammatory disease characterized by autoantibodies directed against numerous self-nuclear antigens. Due to the heterogeneous nature of lupus, it has been challenging to identify markers that are sensitive and specific enough for its diagnosis and monitoring. However, with the sequencing of the human genome, rapid development of high throughput approaches has allowed for a better understanding of the etiopathogenesis of complex diseases, including SLE. Here we will present a review of the latest advancements in biomarker discovery during the 'omics' era, using these novel technologies, for assisting in the diagnosis and prognosis of patients with SLE.

Keywords

Systemic lupus erythematous; Biomarkers; Lupus nephritis; Genomics; Epigenetics; Transcriptomics; Proteomics; Metabolomics

Introduction

SLE is a chronic autoimmune inflammatory disease, characterized by autoantibodies directed against numerous self-nuclear antigens. Due to the heterogeneous nature of lupus, a broad spectrum of clinical manifestations exists. Disease severity also varies depending on the extent of major organ involvement, including most commonly the brain, kidneys, heart, joints, and skin [1].

Several pathophysiologic mechanisms leading to the immune dysregulation seen in SLE have been described, including hyperreactive B and T cells, loss of immune tolerance, and defective clearance of apoptotic cells and/or immune complexes [2]. Nevertheless, despite

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improved understanding of disease pathogenesis, the morbidity and mortality associated with SLE still represent major challenges for patients who face this disease and the clinicians who treat them.

The enhanced focus on identification of pathogenic pathways in SLE has revealed several novel pharmacologic targets (e.g. B lymphocyte stimulator [BLyS]) that have hastened the development of new and promising therapies (e.g. belimumab) [3]. However, SLE continues to have an unpredictable course with remitting and relapsing episodes, not only highlighting that our understanding of lupus remains incomplete, but that current therapies are not curative.

Although originally not developed as diagnostic but rather as classification criteria, patients today are commonly diagnosed based on the American College of Rheumatology (ACR) or the Systemic Lupus International Collaborating Clinics (SLICC) criteria. In a sample of 690 patients, the ACR criteria had a sensitivity of 83% and a specificity of 96%, whereas the SLICC criteria had a sensitivity of 97% and a specificity of 84% [4]. However, evidence from one tertiary care center suggests that only 60% of patients with SLE meet ACR criteria [5]; apparently, patients with early signs or limited disease are excluded by this tool. The development of improved diagnostic biomarkers facilitating the early detection of SLE is of utmost importance, not only because this would allow for rapid treatment and subsequent prevention of organ damage, but also because of the positive economic impact of early diagnosis [6].

Conventional serologic tests currently used for diagnosis and disease monitoring in SLE, such as anti-nuclear antibodies (ANA), anti-double-stranded DNA antibodies (anti-dsDNA), and complement levels, are of limited sensitivity and/or specificity, particularly when used in isolation [7–9]. A more specific and useful test to determine prognosis in patients with renal involvement, kidney biopsy, remains a gold standard; however, this is an invasive procedure that carries additional risk.

The unmet needs described above have urged researchers to search for reliable and non-invasive biomarkers helpful for the diagnosis, classification, prognosis, and treatment of SLE. With the advent of higher throughput and systems biology approaches over the past decade, great advances have been made in this regard. Nevertheless, additional validation is required, and the clinical applicability of such methods needs to be further defined.

This review will focus on novel biomarkers discovered in recent years for the diagnosis and prognosis of SLE, specifically those developed using advanced methodologies which are beginning to enter clinical practice.

The role of biomarkers in SLE diagnosis and prognosis

Before we review these tests, it is important to understand the concept of biomarkers. A biological marker can be defined as a physical sign, or cellular, biochemical, molecular, or gene alteration by which a normal or abnormal biologic process can be recognized and measured [10]. Biomarkers can be diagnostic, prognostic, predictive, pharmacodynamic, or surrogate. Others will serve multiple purposes. Specific for this review, a diagnostic

biomarker refers to one which will confirm the presence or subtype of a disease, whereas a prognostic biomarker will identify a specific disease manifestation, individuals at risk for development of such disease, or those likely to experience a flare [11].

The postgenomic era, after completion of the sequence of the human genome in 2001 [12], has been characterized by the rapid development of highly efficient molecular tools for the study of complex diseases at the functional level of genes. More holistic and comprehensive approaches (as opposed to those only focused on individual mediators) are now a major route for understanding the underlying pathophysiological processes of complex diseases, and can be conducted at any level of the gene expression sequence: genes, messenger RNA (mRNA), proteins, and metabolites [13]. This is what is referred as the “omics” era [14, 15]. Below we will briefly describe each of these technologies and review their roles in biomarker discovery when applied to the study of SLE. Several years ago Mohan *et al.* recognized the contribution of ‘omics’ approaches to biomarker discovery and validation in lupus [14]. In the current review, our focus will be mostly on advances described since then in this rapidly progressing field.

Novel SLE testing

Genomics

Genomics is the branch of molecular biology that centers on the comprehensive study of the structure (structural genomics) and functionality (functional genomics) of the DNA within the cell of a living organism. Functional genomics allows for a better understanding of different patterns of gene expression and, therefore, the pathogenesis of the disease studied. Gene expression profiling involves the analysis of the activity of genes, which when compared between conditions or tissues at different points of time, or between normal and diseased cells, allows for the detection of gene signatures that are specific for that particular disease [16]. Two major technological breakthroughs have been used extensively in the field of rheumatic diseases when trying to understand the underlying pathogenesis. These traditionally included Genome Wide-Association Studies (GWAS), and global profiling of gene expression (microarrays). However, the introduction of high throughput methods such as deep sequencing has allowed for an even more comprehensive study of gene expression in complex and multigenic diseases.

The role of genomics in SLE had initially mainly focused on identifying the genetic risk loci for SLE susceptibility. At least 50 genes have been identified to be associated with SLE [17, 18]. However, individual gene effects on susceptibility are relatively small, with odd ratios (OR) ranging from 1.0 to 2.5 [17]. Only rarely is development of rheumatologic disease confined to a single genetic locus and more commonly is the result of several defective alleles at different loci [19]. However, the cumulative effect size of the identified loci accounts only for 15–20% of the heritability of SLE [20], and the prevalence in monozygotic twins ranges only between 25 and 69% [21, 22].

Micro RNAs

Epigenetics is defined as modifications that regulate gene expression without altering the DNA sequence [23], while epigenomics entails the comprehensive study of those modifications which can include: DNA methylation, chromatin remodeling/histone modification, micro RNAs (miRNAs), and transcription factors. As miRNAs are much easier to detect in laboratory settings than some of the other molecules or processes, a lot of attention has been centered on these as potential biomarkers. Hence, we will focus the discussion pertaining to epigenomics and lupus biomarkers on miRNAs.

miRNAs are small, single-stranded, non-coding RNA molecules that bind target mRNA and regulate gene expression post-transcriptionally. miRNAs function as a guide by base-pairing with target mRNA to negatively regulate its expression, either by directly cleaving the target mRNA with subsequent degradation, or by blocking the subsequent translation [24]. miRNA molecules have been implicated in the regulation of several biologic processes such as cell differentiation, apoptosis, and immune responses, with accumulating evidence suggesting that miRNAs are critical for regulation of the adaptive and innate immune responses [25, 26]. As a result, miRNAs have become an important focus of research in investigating the pathogenesis of SLE and subsequently have been proposed as potential diagnostic and prognostic biomarkers.

With the advent of high throughput technology such as microarrays and chip-based tools, differential expression of miRNAs have been identified in peripheral blood mononuclear cells (PBMC) from patients with SLE [27]. The first panel comprised of 9 up-regulated miRNAs, whereas the second included 7 down-regulated miRNAs. Dai et al. further compared the miRNA profile of kidney biopsies between patients with lupus nephritis (LN) and controls. They found 66 miRNAs in the renal samples, of which 30 miRNAs were down-regulated and 36 miRNAs were up-regulated in LN biopsies. This was the first study to propose a role of miRNA as potential biomarkers for LN. Of note, the 16 miRNAs differentially expressed in lupus PBMC [27] were not found among the 66 miRNAs detected in biopsies from LN patients, indicating that miRNAs could be organ-specific [28].

Another study by Dai *et al.* compared the expression of miRNAs in PBMC and Epstein-Barr Virus (EBV)-transformed cell lines from African American (AA) and European American (EA) patients with LN vs. unaffected controls [29]. As expression patterns of miRNAs can vary over time and between tissues, samples from different cell lines were also tested to look for similarities or differences in expression between them. Of the 850 miRNAs tested, microarray analysis in AA patients with LN revealed 29 differentially expressed miRNAs when compared to controls, whereas 50 were identified in the EA group. Combining both ethnic groups, there were 18 miRNAs that were differentially expressed when compared to unaffected controls, suggesting that certain miRNAs may be SLE-specific. Of these 18, 5 miRNAs were differentially expressed in all specimen types tested, 3 of which weren't previously associated with LN. Dai *et al.* were the first to postulate the unique existence of a miRNA expression signature in patients with LN regardless of race or sample studied, and proposed this as novel biomarker for SLE. However, the lack of disease controls prevents any conclusion regarding the specificity for lupus.

Other groups have studied circulating miRNAs in cell-free plasma by PCR, with different expression profiles found when comparing samples from patients with SLE, rheumatoid arthritis (RA), vasculitis, systemic sclerosis, and healthy controls (HC) [30–32]. Up-regulation of miRNA 142-3p in SLE was found in 2 of the studies [30, 32] and was specifically a strong qualifier when compared to patients with systemic sclerosis. Increased expression of this particular miRNA in patients with lupus was also found by Dai *et al.* [27], highlighting its potential role as a diagnostic biomarker.

A recent study demonstrated the role of dendritic cells (DCs) overexpressing miRNA142-3p in the immune dysregulation of SLE [33]. miRNA 142-3p-overexpressing DCs from lupus patients exhibited increased secretion of IL-6, CCL2, and CCL5, and attracted more CD4+T cells. Therefore, this miRNA was believed to represent a novel therapeutic target.

Chauhan *et al.* further investigated the differences in miRNA expression between 3 subsets of SLE patients with distinct autoantibody profiles: positive for dsDNA antibodies, ENA antibodies, or both [34]. Using TaqMan low density array technology they found different miRNA expression profiles between these groups. The biological pathways that were targeted by those miRNAs were also specific. Dysregulated miRNAs in the anti-dsDNA+ patients targeted molecules implicated in multiple cytokine signaling pathways, whereas in anti-ENA+ patients the prime targets were molecules involved in cell cycle and cytoskeleton remodeling. Common down-regulated miRNAs found in all 3 groups modulated interferon-related pathways. A subgrouping approach, as the one proposed here, may help identify patient subsets within this heterogeneous disease and could serve as a guide for future therapeutic interventions.

Several other miRNAs have been found to correlate with disease activity; one of the first groups to report this association was that of Tang *et al.* Based on the work by Taganov *et al.* which demonstrated the key role of miRNA 146a in innate immunity [35], they further studied its role in SLE. miRNA 146a was found to be under-expressed in PBMC of patients with SLE, with the levels appearing to be independent of medications used to treat the disease. Among SLE patients, those with active disease had lower levels of miRNA 146a expression than patients with inactive disease; similar differences were found in comparing those with proteinuria to those without. Moreover, an inverse correlation was observed between miRNA 146a levels and systemic lupus erythematosus disease activity index (SLEDAI) scores. Other studies have also described the correlation of miRNAs with lupus disease activity [36–38]. Specifically, miRNA 146a correlated with the estimated glomerular filtration rate (eGFR), whereas miRNA 155 correlated with both proteinuria and SLEDAI [36].

Urinary miRNAs

There has been a concerted effort to discover biomarkers that allow for the accurate study of SLE in a less invasive way, in particular for LN in which kidney biopsy is the gold standard but which is a potentially risky procedure. Urine, on the other hand, offers the opportunity for repeated and non-invasive sampling.

RNA is present in the urine either enclosed in detached cells, or in extracellular vesicles (EVs) that are in turn released from epithelial cells facing the urinary space [39, 40]. EVs are classified depending on size, morphology, and biochemical composition, into exosomes, ectosomes, and apoptotic bodies. Exosomes are particularly rich in miRNAs, and have been the focus of study in SLE.

A recent study demonstrated the utility of cell and microvesicle urine miRNA profiling through deep sequencing and its ability to detect biological differences between healthy individuals [41]. Compared to microarray, deep sequencing provides a higher resolution gene expression measurement and is therefore a more advanced tool [42]. This study examined sex differences, the relationship between matched cellular and extracellular miRNA profiles, and the repeatability of miRNA profiles between matched consecutive urine voids. They found that EVs had a higher content of miRNA compared to cells from the urinary sediment. Furthermore, urine from women had higher total RNA content in both cells and EVs compared to men. Unsupervised clustering was maintained regardless of the number of void repeats. They concluded that miRNA profiling of both urine EVs and sediment cells can convey biologically important differences between individuals, and in turn may be extrapolated to the study of organ specific pathology. Based on these results, for miRNAs to be useful as urine biomarkers, careful consideration is needed in biofluid fractionation (cells vs. EVs) and sex-specific analysis, while the time of voiding appears to be less important.

Specific for SLE, and with the use of deep sequencing technology, Goilav *et al.* was the first to describe LN WHO class-specific miRNA expression profiles by analyzing cell-free urine of adult and pediatric patients with biopsy proven renal disease [43]. Samples of 25 adult and 9 pediatric patients with class IV and class V LN were obtained at time of active disease and during remission. Upregulation of 6 miRNAs was found in patients with active class IV LN when compared to class V LN in a paired-sample analysis, and therefore this miRNA profile was suggested as an important prognostic biomarker.

Transcriptomics

The transcriptome is the whole set of mRNAs that is produced by the genome under specific circumstances or in a specific cell, and reflects the genes that are actively expressed. Transcriptomics is therefore the comprehensive study of gene expression from the RNA message that is being transcribed. Comparison of transcriptomes allows the identification of genes that are differentially expressed between individuals, in distinct cell populations, or in response to different treatments [14, 44], and therefore has elucidated important pathophysiologic processes in several complex diseases including SLE.

One important discovery made using lupus transcriptomics was the identification of the IFN signature. As early as the 1980s IFNα was found elevated in the serum of patients with SLE and positively correlated with clinical disease activity [45–47]. However, due to localized expression and uptake, IFN levels in plasma or serum are transient and therefore difficult to measure [11]. As such, with the advent of microarray and more recently with the use of next-generation RNA sequencing, evaluating gene expression instead has provided a more precise reflection of IFN activity in lupus.

The IFN signature was first noted by profiling PBMCs from patients with SLE where differential expression was noted in genes induced by type I IFN (IFNα and IFNβ) [48, 49]. Overexpression of type I IFN-regulated genes was noted in synovial biopsies and platelets as well [50, 51]. Correlation with disease activity in cross-sectional studies was also demonstrated [52, 53], although not longitudinally [54, 55].

The robustness of any gene expression signature identified needs to be validated by independent studies. In an attempt to enhance statistical power, Arasappan *et al.* [56] combined the results of 4 independent studies done in PBMCs of patients with SLE, using pathway-based meta-analysis. Three of the studies included only pediatric patients [49, 57, 58], while the remaining study included only adults [48]. A metasignature of 37 genes was differentially expressed in patients with SLE, 12 of which were involved in IFN signaling and were either IFN induced or IFN regulated.

Given that the study of PBMC expression profiles can yield biased results due to the heterogeneity of the cell populations between patients and controls, some cell-specific studies have been undertaken [14]. This logic has been applied to LN with a recent study demonstrating the advantage of using single cell RNA sequencing (scRNA-seq) as a tool to more precisely analyze some of the heterogeneity seen in LN. This pilot study proved the applicability of this technique to samples obtained during routine care, and proposed the use of scRNA-seq for the discovery of novel prognostic biomarkers [59]. Unbiased scRNA-seq from kidney and skin tissue samples of patients with LN and HC distinctively separated individual cell types based on their gene expression profiles. Principal component analysis (PCA) revealed 6 cell clusters (tubular, endothelial, fibroblast, myeloid, keratinocyte, T-cell). Additional analysis of only tubular cells using PCA was able to further subcluster them into 4 distinct subtypes (proximal tubule, loop of Henle, collecting duct, distal tubule). Furthermore, a correlation was shown between tubular IFN scores, clinical activity, and response to therapy. Independent of LN class type, an IFN signature was detectable in all patients although it varied from patient to patient, with higher IFN scores correlating with chronicity, IgG deposition, and proteinuria. Additionally, IFN scores at baseline predicted response to therapy.

Nonlesional, non-sun exposed skin of patients with LN has previously been suggested as a surrogate for detecting and monitoring renal disease [60]. In the work of Der *et al.* [59], the potential role of skin biopsy as a surrogate biomarker for LN was studied as well using the scRNA-seq approach. Differential expression analysis revealed 28 up-regulated genes and 2 down-regulated genes in the keratinocytes of LN patients compared with HC. Of interest, 4 of the most significantly overexpressed genes were IFN inducible.

The study of transcriptomes in SLE not only has yielded potential biomarkers, but has served as a platform in clinical trials for development of targeted therapies based on the disease-specific IFN signature. Three biologics directed to inhibit the type I IFN response have been tested for their effectiveness and safety in patients with active SLE, with promising results [61–63]. These include rontalizumab, sifalimumab, and anifrolumab; all monoclonal antibodies which target IFNα or its receptor. In all three clinical trials, the clinical response correlated with the degree of the IFN signature. For example, patients with

a “high” IFN signature surprisingly had a poor response to rontalizumab, whereas those with a “low” signature had a better response [61]. With the other two monoclonal antibodies, high baseline IFN signature was associated with greater effect sizes [62, 63]. This is an example of personalized medicine, in which patients can be stratified based on risk of disease or response to therapy using diagnostic tests [64], and provides further support for the concept that understanding disease at the molecular level allows for the development of a more targeted and effective medicine.

Proteomics

The proteome is the entire set of proteins expressed by a genome at a particular time, in a specific cell or tissue, which in turn regulates cell function. Compared to transcriptomics, which reflects the gene activity with ‘intention’ for protein synthesis, proteomics focuses on the comprehensive study of the products themselves [14]. After translation of mRNA into protein, many secondary protein modifications also play an important role in cell regulatory processes and are therefore relevant in understanding aberrant disease-specific pathways. The complexity of these processes, as well as the highly dynamic changes that characterize them, have forced the development of highly advanced techniques for the study of more complete proteomes, as is the case of mass spectrometry (MS), and has shifted away from the use of electrophoresis to study individual proteins. The former technology allows for the identification and quantification of thousands of proteins in complex biological samples in a single run [65].

Proteomic studies are further classified into two broad categories: unbiased approaches attempting to scan the entire proteome, and biased/targeted approaches limited to the study of a predetermined subset of the proteome [14]. The latter method is the protein equivalent of expression profiling and is broadly known as ‘protein profiling’. It also encompasses the term ‘quantitative proteomics’ and uses microarrays or MS in its approach. Conversely, global proteomics (which is also referred as ‘functional proteomics’) is approached by shotgun methods involving the combination of two advanced techniques [66] that are explained below.

Most proteomic-based studies are carried out in two stages, the first of which consists of protein separation by liquid chromatography (LC), or two-dimensional gel electrophoresis (2DE); and the second which consists of protein identification or characterization by mass spectrometry (MS or MS/MS when performed in tandems) [67]. The MS instruments consist of an ion source, a mass analyzer, and a detector, all of which serve to separate and identify different molecules by measuring the mass to charge (m/z) ratio of charged particles. Each MS instrument can use different methodologies [67]; consequently, several platforms have been created by diverse coupling of these different techniques used for separation and protein identification.

Functional Proteomics

Proteomic-based studies have been applied in SLE for discovery of differentially regulated proteins which could then serve as novel biomarkers for early detection, prognosis, and monitoring of the disease. Hundreds of proteins have been identified as potential biomarkers

with this approach [65, 68]; unfortunately, however, many were not confirmed in subsequent studies and/or have yet to be validated clinically. A recent systematic review of published proteomic-based studies in SLE sought to identify those biomarkers with potential disease associations and clinical utility [65]. Based on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, 25 of 775 articles were selected for review. 241 SLE candidate proteomic biomarkers for disease diagnosis, activity, or organ specific involvement were identified with only 13 studies validating their results in an independent cohort, resulting in 28 validated candidate biomarkers. Additionally, 11/28 biomarkers were reproduced in more than one study and 4 of these were also validated (annexin A2 and A5, serotransferrin, and apolipoproteins [A-I, CIII, C-I, A-IV]). The most promising biomarkers that were suitable for SLE diagnosis and disease activity respectively included phosphorylated S100-A9 isoforms [69] and serine-threonine kinase receptor-associated protein (STRAP) [70].

As for LN, 5 biomarkers from kidney biopsy tissue of class IV LN patients were identified: serotransferrin, cytokeratin 18 and 19, albumin, and annexin A5 [71]. Additionally, 5 of the 11 biomarkers that were reproduced in multiple studies were also isolated from kidney tissue as well as from urine, serum, and/or PBMCs, suggesting that these could be used as potential tools for diagnosing LN. For example, alpha-1-antitrypsin (A1AT) which is produced in the kidney in response to injury [72] was found in the urine of during renal flares [71].

Liquid chromatography has been the method of choice for sample preparation in proteomic studies. However, recent advances on this field have led to the discovery of techniques that allow for a faster separation, with higher sensitivity and resolution. Over the past 5 years, capillary electrophoresis (CE) coupled to MS (CE-MS) has been the predominant proteomic technique used in the study of urinary biomarkers for the diagnosis and prognosis of chronic kidney disease (CKD), including LN [73]. Aside from providing higher resolution, this approach termed “peptidomics” is also considered robust, inexpensive, and reproducible [74].

CE-MS led to the discovery of 273 urinary peptide markers, including fragments of various collagens, circulating proteins such as albumin and A1AT, and specific kidney derived proteins like uromodulin. This multipeptide panel, referred to as CKD273, was the first urinary classifier discovered for the diagnosis of CKD irrespective of the underlying mechanism of disease, and has proved to be useful in early prediction of CKD progression when compared to conventional measures such microalbuminuria and eGFR, by detecting earlier decline in renal function [73–76]. CKD273 has been validated in several studies [77–79] and is now being used in clinical trials [80]. However, although characterization of the CKD273 panel is promising, it is still a general classifier for the diagnosis of all CKD types and may not be suitable for the study of certain renal pathologies, specifically LN, where different subclasses within the same disease represent a different prognosis.

Since CE-MS enabled discrimination between patients with and without CKD [77], a subsequent study was conducted using this same technology to assess the value of the urinary proteome for the noninvasive discrimination of various types of CKD from 7

different etiologies, including LN [74]. Using datasets from a large cohort of 1180 individuals with CKD, 287 disease-specific biomarkers were defined. These potential urinary biomarkers were further combined into classifiers specific for each CKD etiology using a support vector machine (SVM) algorithm, and subsequently validated in an independent cohort. The classifiers proved to have a good to excellent discriminatory power, with AUCs ranging from 0.77 to 0.95. Specific for LN, 172 biomarkers were identified, 6 of which showed the most differences when compared to other etiologies of CKD. These included increased collagen, uromodulin, and protein S100-A9 fragments, and decreased clusterin, beta-2-microglobulin, and alpha-2-HS-glycoprotein fragments. Interestingly, a previous study had shown low serum levels of clusterin in SLE patients, especially in those with proteinuria [81], suggesting it may be a more specific biomarker for LN. Furthermore, one of the highly specific peptides that distinguished LN from other types of CKD was a fragment of S100-A9, a finding consistent with the systematic review discussed above which identified S100-A9 as a promising SLE biomarker [65].

Peptidomics was further applied in a recent study for the discovery of biomarkers limited to the diagnosis of SLE [82]. Urine samples were collected from 34 patients with SLE without renal involvement and 58 matched HC. Using CE-MS and SVM algorithms, an SLE-specific panel of 65 peptides was identified which proved to have excellent discriminatory power (AUC of 0.99 when applied to the discovery group). This panel was further validated in an independent multi-center cohort, including LN and non-SLE patients with various renal complications. This analysis resulted in an AUC of 0.80 corresponding to a sensitivity and specificity for LN of 83% and 73%, respectively. The performance of conventional tests such as anti-dsDNA, C3, and C4, has been previously tested in adult SLE and pediatric LN patients [83, 84], with AUC ranging from 0.61–0.79, 0.63–0.73, and 0.48–0.72 respectively. Therefore, compared to conventional tests as historical controls, the panel of 65 peptides was superior for the diagnosis of LN. Finally, using LC and CE coupled to MS/MS, the primary structure of 47/65 peptides was determined. The majority of these fragments originated from different forms of collagen, supporting the theory that these abundant extracellular matrix proteins are major targets of proteolysis in inflamed organs [85].

One of the additional advantages of CE-MS is that proteins are analyzed intact without the need of prior enzymatic treatment with trypsin. This allows for identification and analysis of the end terminal amino acids from which subsequent information can be obtained such as the detection of increased or decreased protease activity, which in turn are responsible for the production of specific peptide fragments [82]. In silico prediction of protease activity identified increased activity of 8 matrix metalloproteinases (MMP) responsible for the peptide fragmentations seen in SLE patients, a finding consistent with the reported role of certain MMP in the pathogenesis of SLE [86, 87].

Quantitative Proteomics

The rapid discovery of autoantibodies in SLE with the use of large scale microarrays coated with various putative autoantigens has been possible largely due to the contribution of targeted proteomic-based studies in this field [14]. We pursued one such application of protein profiling in SLE with the use of iCHIP microarray technology for the development,

verification, and validation of a definitive serologic rule-out diagnostic test: the SLE-Key rule-out test [88].

The SLE-key iCHIP microarray contains 200 antigens (400 features), mostly selected from known SLE-associated pathways; serum samples are tested for their intensity of binding to the array antigens. Sera of 146 SLE patients from varied demographic characteristics was tested and compared to a similar number of HC, and results validated in separate SLE and HC cohorts. Univariate analysis testing revealed a total of 131 autoantigens (including dsDNA, Sm, Ro, and La) and viral antigens (e.g. EBV) which successfully separated SLE patients from HC ($p<0.05$). Further analysis using several multivariate models confirmed the performance and robustness of this test, since it characterized SLE patients regardless of the patient population or activity of the disease. During the validation phase, the linear discrimination analysis (LDA) model showed the best performance of a select number of antigens (sensitivity of 94%, a specificity of 75%, and negative predictive value (NPV) of 93%), and was therefore selected as the informatic model for analyzing the iCHIP results. With these performance characteristics, it is highly unlikely that the SLE-key rule-out test would falsely misdiagnose a SLE patient as not having lupus. Thus, the SLE-Key rule-out test may be useful in shortening the time required for rheumatologic workup by early exclusion of SLE from diagnostic consideration in appropriate patients.

Metabolomics

Different than genomics, transcriptomics and proteomics, in which the comprehensive study of biologic samples is approached in a ‘bottom-up’ manner, metabolomics provides a ‘top-down’ integrated view of the unique chemical fingerprints produced during specific cellular processes, by monitoring the global outcome of all the influencing factors (i.e. gene expression, protein expression, and environmental factors) [17, 89]. The detection of specific small molecule metabolite profiles during different disease states, or in response to therapy, are therefore highly useful biological markers and possible pharmacological targets [89].

Metabolomes are commonly studied by one of 2 techniques: MS (as described above) or by nuclear magnetic resonance (NMR) spectroscopy. The latter is intended to detect the hydrogen containing molecules of a sample; essentially all metabolites. Once reaching detectable levels, each metabolite gives an individual spectrum, which then enables the structure to be determined. The NMR spectrum of a biological sample is the superposition of the spectra of all of the metabolites in the fluid [89]. Using pattern-recognition techniques, differences can be seen when comparing the spectrum of different samples.

One of the first groups that integrated metabolomics for the study of SLE was that of Wu *et al.*, which in 2012 described several disrupted metabolic pathways in affected patients [90]. With the use of LC-MS and gas chromatography coupled with MS (GC-MS) the serum metabolome of 20 SLE patients was compared to HC. Results were validated in an independent cohort of 38 SLE patients. More than 100 metabolites pertaining to several pathways such as glycolysis, fatty acid β oxidation, lipid biosynthesis, eicosanoid biosynthesis, and methyl group metabolism, differed significantly in lupus patients. Findings reflected the high oxidative stress, increased inflammation, reduced energy generation,

altered lipid profile, and prothrombotic state seen in SLE, processes implicated in immune dysregulation, and which also characterize immunometabolism in lupus [91].

In a recent study, a larger sample size was used to examine the metabolomic profile in the serum of SLE patients by the use of GC-MS, and correlated these to SLEDAI and conventional serologic markers [92]. Compared to HC, patients with SLE had significantly lower levels of most amino acids, carbohydrates, fatty acids, and other endogenous metabolites, with the data indicating that the most likely affected pathway in SLE is acid turnover and protein biosynthesis. Receiver operating characteristic (ROC) analysis showed that 6 metabolites associated with oxidative stress, 7 with energy metabolism, and 6 with lipid metabolism were potential sensitive and specific diagnostic biomarkers of SLE, with AUC of 0.94, 0.90, and 0.87 respectively. Other specific endogenous metabolites such as valine, tryptophan, and glycerol positively correlated with serum C3 levels, whereas glycerol and palmitic acid correlated with C4 levels.

Further comparison between patients with active and inactive SLE revealed 6 metabolites during the active phase. Two were found elevated in the serum (glutamate and 2-hydroxyisobutyrate), while 4 had decreased concentrations (citrate, glycerol, linoleic acid, and propylparaben). ROC analysis was performed between SLE patients and HC, as well as between SLE patients with and without active disease. The combination of these 6 metabolites showed good results, with AUC of 0.82 and 0.6 respectively for each comparison group. When these metabolites were further combined with C3 and C4, AUC significantly improved with values of 0.92 and 0.92 respectively, suggesting that these may be excellent prognostic biomarkers. Lastly, some metabolites such as amino acids, organic acids, fatty acids, and saccharides also correlated with specific manifestations such as skin, mucosal, musculoskeletal, hematological, or renal disease, with amino acids having the strongest association especially with hematological involvement.

Another NMR spectroscopy-based metabolomic study applied to SLE also demonstrated dysregulation in protein and lipid biosynthesis pathways by the detection of a specific metabolite phenotype in the serum of patients with lupus. This phenotype was also able to discriminate between HC and patients with RA [93]. A parallel study using the same technique but analyzing the urine metabolome in patients with LN demonstrated differences in the concentration of certain metabolites, such as citrate and taurine, and could discriminate between class III/IV LN patients from those with class V LN [94].

A more recent study specifically analyzed the metabolic profile in LN for the discovery of new serologic biomarkers [95]. Ultra-high-performance liquid chromatography coupled with high-resolution MS (UPLC-HRMS) was used to detect differential metabolic signatures in patients with LN compared to those of healthy and disease controls (idiopathic nephrotic syndrome). Due to its enhanced separation efficiency, as well as its higher sensitivity and resolution, UPLC-HRMS is considered a suitable technique for large scale untargeted metabolic profiling [67, 95]. In this study, 18 metabolites were found to have distinct expression between renal disease groups and HC. Five were significantly different in LN compared to the other 2 groups with increased levels of sorbitol and glycocholic acid metabolites, and decreased levels of cortisol, creatinine, and L-aspartyl-L-phenylalanine.

The diagnostic accuracy of the identified biomarkers was furthered evaluated by ROC analysis. Theophylline, oxidized glutathione, and capric acid had the highest AUC values: 0.77 for the first two and 0.74 for the latter. These 3 combined provided a higher AUC of 0.85, and was therefore suggested as a biomarker panel for the diagnosis of LN with a sensitivity of 88% and a specificity of 68%.

A better understanding of the disrupted metabolic pathways in SLE and their implications for immune dysregulation [91] has led to the identification of specific targets that are now being used in clinical trials [96–99]. One example is the use of metformin targeting the increased glycolysis and mitochondrial oxidative metabolism of hyperreactive CD4+ T cells in SLE, which indirectly regulate the production of IFN-γ and IL-2 by these cells [100]. Metformin may also reduce the production of reactive oxygen species (ROS), which in turn are responsible for the production of neutrophil extracellular traps (NETs) [98]. NETs participate in the immune dysregulation of SLE by potentiating the production of type I IFN [91].

Summary and conclusions

Despite significant progress in understanding basic mechanisms of disease, SLE continues to pose a challenge for clinicians, researchers, and patients due to the continued uncertainty it generates, and its significant morbidity and mortality. Some of the biomarkers which have been described lack sufficient diagnostic and prognostic accuracy, or do not show clear superiority over current conventional serological testing, further reflecting the complexity of SLE. Efforts should be made to focus future SLE biomarker studies on validated and reproducible candidates.

The heterogeneity of lupus has hindered the creation of an objective tool able to be applied to a wide variety of patients. With exponential advances in high throughput technologies, the post genomic era has nevertheless allowed for the development of better strategies to subset patients accurately. However, the comprehensive, richly detailed, and large scale data obtained from these techniques has created another problem, that of data processing and analysis. Therefore, it becomes important for advances in bioinformatics to parallel those in the ‘omics’ era. Perhaps the integration of information coming from several techniques will help provide a more accurate view of the pathophysiologic mechanisms, reduce gaps in understanding the heterogeneity of lupus, and assist in the creation of a comprehensive, non-invasive, and easily applicable panel of tools useful throughout many clinical conditions.

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Conflict of interest

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References

1. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011; 365(22):2110–21. [PubMed: 22129255]

2. Crispin JC, et al. Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med. 2010; 16(2):47–57. [PubMed: 20138006]
3. Bonanni A, et al. Multi-antibody composition in lupus nephritis: isotype and antigen specificity make the difference. Autoimmun Rev. 2015; 14(8):692–702. [PubMed: 25888464]
4. Petri M, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012; 64(8):2677–86. [PubMed: 22553077]
5. Calvo-Alen J, et al. Identification of patient subsets among those presumptively diagnosed with, referred, and/or followed up for systemic lupus erythematosus at a large tertiary care center. Arthritis Rheum. 1995; 38(10):1475–84. [PubMed: 7575697]
6. Oglesby A, et al. Impact of early versus late systemic lupus erythematosus diagnosis on clinical and economic outcomes. Appl Health Econ Health Policy. 2014; 12(2):179–90. [PubMed: 24573911]
7. Liu CC, Ahearn JM, Manzi S. Complement as a source of biomarkers in systemic lupus erythematosus: past, present, and future. Curr Rheumatol Rep. 2004; 6(2):85–8. [PubMed: 15016335]
8. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. Lupus. 2004; 13(5):290–7. [PubMed: 15230281]
9. Isenberg DA, et al. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology (Oxford). 2007; 46(7):1052–6. [PubMed: 17500073]
10. Illei GG, et al. Biomarkers in systemic lupus erythematosus. I. General overview of biomarkers and their applicability. Arthritis Rheum. 2004; 50(6):1709–20. [PubMed: 15188346]
11. Arriens C, et al. Systemic lupus erythematosus biomarkers: the challenging quest. Rheumatology (Oxford). 2017; 56(suppl_1):i32–i45. [PubMed: 28013203]
12. Lander ES, et al. Initial sequencing and analysis of the human genome. Nature. 2001; 409(6822): 860–921. [PubMed: 11237011]
13. Horgan RP, Kenny LC. 'Omic'technologies: genomics, transcriptomics, proteomics and metabolomics. The Obstetrician & Gynaecologist. 2011; 13(3):189–195.
14. Arriens C, Mohan C. Systemic lupus erythematosus diagnostics in the 'omics' era. Int J Clin Rheumtol. 2013; 8(6):671–687. [PubMed: 24860621]
15. Group, N.P. Omics Gateway. Available from: http://www.nature.com/omics/index.html
16. Giannopoulou EG, Elemento O, Ivashkiv LB. Use of RNA sequencing to evaluate rheumatic disease patients. Arthritis Res Ther. 2015; 17:167. [PubMed: 26126608]
17. Teruel M, Chamberlain C, Alarcon-Riquelme ME. Omics studies: their use in diagnosis and reclassification of SLE and other systemic autoimmune diseases. Rheumatology (Oxford). 2017; 56(suppl_1):i78–i87. [PubMed: 28339517]
18. Deng Y, Tsao BP. Genetic susceptibility to systemic lupus erythematosus in the genomic era. Nat Rev Rheumatol. 2010; 6(12):683–92. [PubMed: 21060334]
19. Moser KL, et al. Recent insights into the genetic basis of systemic lupus erythematosus. Genes Immun. 2009; 10(5):373–9. [PubMed: 19440199]
20. Manolio TA, et al. Finding the missing heritability of complex diseases. Nature. 2009; 461(7265): 747–753. [PubMed: 19812666]
21. Deapen D, et al. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum. 1992; 35(3):311–8. [PubMed: 1536669]
22. Gray-McGuire C, et al. Genome scan of human systemic lupus erythematosus by regression modeling: evidence of linkage and epistasis at 4p16–15.2. Am J Hum Genet. 2000; 67(6):1460–9. [PubMed: 11078476]
23. Long H, et al. The critical role of epigenetics in systemic lupus erythematosus and autoimmunity. J Autoimmun. 2016; 74:118–138. [PubMed: 27396525]
24. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics. 2010; 11(7):537–61. [PubMed: 21532838]
25. Baltimore D, et al. MicroRNAs: new regulators of immune cell development and function. Nat Immunol. 2008; 9(8):839–45. [PubMed: 18645592]

26. Dai R, Ahmed SA. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Transl Res. 2011; 157(4):163–79. [PubMed: 21420027]
27. Dai Y, et al. Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. Lupus. 2007; 16(12):939–46. [PubMed: 18042587]
28. Dai Y, et al. Comprehensive analysis of microRNA expression patterns in renal biopsies of lupus nephritis patients. Rheumatol Int. 2009; 29(7):749–54. [PubMed: 18998140]
29. Dai R, et al. Identification of a common lupus disease-associated microRNA expression pattern in three different murine models of lupus. PLoS One. 2010; 5(12):e14302. [PubMed: 21170274]
30. Carlsen AL, et al. Circulating microRNA expression profiles associated with systemic lupus erythematosus. Arthritis Rheum. 2013; 65(5):1324–34. [PubMed: 23401079]
31. Wang H, et al. Circulating microRNAs as candidate biomarkers in patients with systemic lupus erythematosus. Transl Res. 2012; 160(3):198–206. [PubMed: 22683424]
32. Steen SO, et al. The circulating cell-free microRNA profile in systemic sclerosis is distinct from both healthy controls and systemic lupus erythematosus. J Rheumatol. 2015; 42(2):214–21. [PubMed: 25399392]
33. Wang Y, et al. Elevated expression of miR-142-3p is related to the pro-inflammatory function of monocyte-derived dendritic cells in SLE. Arthritis Res Ther. 2016; 18(1):263. [PubMed: 27852285]
34. Chauhan SK, et al. Differential microRNA profile and post-transcriptional regulation exist in systemic lupus erythematosus patients with distinct autoantibody specificities. J Clin Immunol. 2014; 34(4):491–503. [PubMed: 24659206]
35. Taganov KD, et al. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A. 2006; 103(33): 12481–6. [PubMed: 16885212]
36. Wang G, et al. Expression of miR-146a and miR-155 in the urinary sediment of systemic lupus erythematosus. Clinical Rheumatology. 2012; 31(3):435–440. [PubMed: 21987229]
37. Wang G, et al. Serum and urinary free microRNA level in patients with systemic lupus erythematosus. Lupus. 2011; 20(5):493–500. [PubMed: 21372198]
38. Abulaban KM, et al. Relationship of cell-free urine MicroRNA with lupus nephritis in children. Pediatr Rheumatol Online J. 2016; 14(1):4. [PubMed: 26762103]
39. Perez-Hernandez J, Cortes R. Extracellular Vesicles as Biomarkers of Systemic Lupus Erythematosus. Dis Markers. 2015; 2015:613536. [PubMed: 26435565]
40. Perez-Hernandez J, et al. Increased Urinary Exosomal MicroRNAs in Patients with Systemic Lupus Erythematosus. PLoS One. 2015; 10(9):e0138618. [PubMed: 26390437]
41. Ben-Dov IZ, et al. Cell and Microvesicle Urine microRNA Deep Sequencing Profiles from Healthy Individuals: Observations with Potential Impact on Biomarker Studies. PLoS One. 2016; 11(1):e0147249. [PubMed: 26785265]
42. Rai G, et al. Microarray to deep sequencing: transcriptome and miRNA profiling to elucidate molecular pathways in systemic lupus erythematosus. Immunol Res. 2016; 64(1):14–24. [PubMed: 26188428]
43. Goilav B, et al. Deep-sequencing reveals WHO class-specific urinary microRNAs in human lupus nephritis (P3245). The Journal of Immunology. 2013; 190(1 Supplement):192.8–192.8.
44. Kratz A, et al. Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. J Exp Med. 1996; 183(4):1461–72. [PubMed: 8666904]
45. Hooks JJ, et al. Immune interferon in the circulation of patients with autoimmune disease. N Engl J Med. 1979; 301(1):5–8. [PubMed: 449915]
46. Ytterberg SR, Schnitzer TJ. Serum interferon levels in patients with systemic lupus erythematosus. Arthritis Rheum. 1982; 25(4):401–6. [PubMed: 6176248]
47. Kim T, et al. Serum levels of interferons in patients with systemic lupus erythematosus. Clin Exp Immunol. 1987; 70(3):562–9. [PubMed: 2449306]
48. Baechler EC, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A. 2003; 100(5):2610–5. [PubMed: 12604793]

49. Bennett L, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med. 2003; 197(6):711–23. [PubMed: 12642603]
50. Nzeusseu Toukap A, et al. Identification of distinct gene expression profiles in the synovium of patients with systemic lupus erythematosus. Arthritis Rheum. 2007; 56(5):1579–88. [PubMed: 17469140]
51. Lood C, et al. Platelet transcriptional profile and protein expression in patients with systemic lupus erythematosus: up-regulation of the type I interferon system is strongly associated with vascular disease. Blood. 2010; 116(11):1951–7. [PubMed: 20538795]
52. Feng X, et al. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis Rheum. 2006; 54(9): 2951–62. [PubMed: 16947629]
53. Nikpour M, et al. Association of a gene expression profile from whole blood with disease activity in systemic lupus erythaematosus. Ann Rheum Dis. 2008; 67(8):1069–75. [PubMed: 18063674]
54. Petri M, et al. Longitudinal expression of type I interferon responsive genes in systemic lupus erythematosus. Lupus. 2009; 18(11):980–9. [PubMed: 19762399]
55. Landolt-Marticorena C, et al. Lack of association between the interferon-alpha signature and longitudinal changes in disease activity in systemic lupus erythematosus. Ann Rheum Dis. 2009; 68(9):1440–6. [PubMed: 18772188]
56. Verweij CL, Vosslamber S. Combining DNA-microarray data in systemic lupus erythematosus. Genome Med. 2011; 3(5):30. [PubMed: 21639954]
57. Allantaz F, et al. Blood leukocyte microarrays to diagnose systemic onset juvenile idiopathic arthritis and follow the response to IL-1 blockade. J Exp Med. 2007; 204(9):2131–44. [PubMed: 17724127]
58. Chaussabel D, et al. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. Immunity. 2008; 29(1):150–64. [PubMed: 18631455]
59. Der E, et al. Single cell RNA sequencing to dissect the molecular heterogeneity in lupus nephritis. JCI Insight. 2017; 2(9)
60. Izmirly PM, et al. Dysregulation of the microvasculature in nonlesional non-sun-exposed skin of patients with lupus nephritis. J Rheumatol. 2012; 39(3):510–5. [PubMed: 22298906]
61. Kalunian KC, et al. A Phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-alpha) in patients with systemic lupus erythematosus (ROSE). Ann Rheum Dis. 2016; 75(1):196–202. [PubMed: 26038091]
62. Khamashta M, et al. Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. Ann Rheum Dis. 2016; 75:1899–1901. [PubMed: 27329393]
63. Furie R, et al. Anifrolumab, an Anti-Interferon-alpha Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. Arthritis Rheumatol. 2017; 69(2):376–386. [PubMed: 28130918]
64. Stratified, personalised or P4 medicine: a new direction for placing the patient at the centre of healthcare and health education (May 2015). 2015. Available from: https://acmedsci.ac.uk/filedownload/38266-56e6d483e1d21.pdf
65. Nicolaou O, et al. Biomarkers of systemic lupus erythematosus identified using mass spectrometry-based proteomics: a systematic review. J Cell Mol Med. 2017; 21(5):993–1012. [PubMed: 27878954]
66. MacBeath G. Protein microarrays and proteomics. Nat Genet.
67. Xu Q, et al. The use of biophysical proteomic techniques in advancing our understanding of diseases. Biophysical Reviews. 2012; 4(2):125–135. [PubMed: 28510094]
68. Wang L, et al. Comparative proteome analysis of peripheral blood mononuclear cells in systemic lupus erythematosus with iTRAQ quantitative proteomics. Rheumatol Int. 2012; 32(3):585–93. [PubMed: 21120503]
69. Pavon EJ, et al. Increased expression and phosphorylation of the two S100A9 isoforms in mononuclear cells from patients with systemic lupus erythematosus: a proteomic signature for circulating low-density granulocytes. J Proteomics. 2012; 75(6):1778–91. [PubMed: 22230807]

70. Wang L, et al. Differential expression of serine-threonine kinase receptor-associated protein in patients with systemic lupus erythematosus. Lupus. 2011; 20(9):921–7. [PubMed: 21613329]
71. Alaiya A, et al. Proteomic analysis of Class IV lupus nephritis. Nephrol Dial Transplant. 2015; 30(1):62–70. [PubMed: 24914093]
72. Zhang X, et al. Biomarkers of lupus nephritis determined by serial urine proteomics. Kidney Int. 2008; 74(6):799–807. [PubMed: 18596723]
73. Magalhaes P, Mischak H, Zurbig P. Urinary proteomics using capillary electrophoresis coupled to mass spectrometry for diagnosis and prognosis in kidney diseases. Curr Opin Nephrol Hypertens. 2016; 25(6):494–501. [PubMed: 27584928]
74. Siwy J, et al. Noninvasive diagnosis of chronic kidney diseases using urinary proteome analysis. Nephrol Dial Transplant. 2016
75. Gu YM, et al. The urinary proteome as correlate and predictor of renal function in a population study. Nephrol Dial Transplant. 2014; 29(12):2260–8. [PubMed: 24981581]
76. Ovrehus MA, et al. Urinary proteomics in chronic kidney disease: diagnosis and risk of progression beyond albuminuria. Clin Proteomics. 2015; 12(1):21. [PubMed: 26257595]
77. Good DM, et al. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. Mol Cell Proteomics. 2010; 9(11):2424–37. [PubMed: 20616184]
78. Molin L, et al. A comparison between MALDI-MS and CE-MS data for biomarker assessment in chronic kidney diseases. J Proteomics. 2012; 75(18):5888–97. [PubMed: 22842158]
79. Argiles A, et al. CKD273, a new proteomics classifier assessing CKD and its prognosis. PLoS One. 2013; 8(5):e62837. [PubMed: 23690958]
80. Lindhardt M, et al. Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early diabetic nephRopathy in TYpe 2 diabetic patients with normoalbuminuria (PRIORITY): essential study design and rationale of a randomised clinical multicentre trial. BMJ Open. 2016; 6(3):e010310.
81. Newkirk MM, et al. Systemic lupus erythematosus, a disease associated with low levels of clusterin/apoJ, an antiinflammatory protein. J Rheumatol. 1999; 26(3):597–603. [PubMed: 10090169]
82. Pejchinovski M, et al. Urine peptidomic biomarkers for diagnosis of patients with systematic lupus erythematosus. Lupus. 2017:961203317707827.
83. Putterman C, et al. Cell-bound complement activation products in systemic lupus erythematosus: comparison with anti-double-stranded DNA and standard complement measurements. Lupus Sci Med. 2014; 1(1):e000056. [PubMed: 25396070]
84. Smith EM, et al. International validation of a urinary biomarker panel for identification of active lupus nephritis in children. Pediatr Nephrol. 2017; 32(2):283–295. [PubMed: 27590021]
85. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014; 15(12):786–801. [PubMed: 25415508]
86. Faber-Elmann A, et al. Activity of matrix metalloproteinase-9 is elevated in sera of patients with systemic lupus erythematosus. Clin Exp Immunol. 2002; 127(2):393–8. [PubMed: 11876767]
87. Ainiala H, et al. Increased serum matrix metalloproteinase 9 levels in systemic lupus erythematosus patients with neuropsychiatric manifestations and brain magnetic resonance imaging abnormalities. Arthritis Rheum. 2004; 50(3):858–65. [PubMed: 15022328]
88. Putterman C, et al. SLE-key((R)) rule-out serologic test for excluding the diagnosis of systemic lupus erythematosus: Developing the ImmunArray iCHIP((R)). J Immunol Methods. 2016; 429:1–6. [PubMed: 26678162]
89. Nicholson JK, Lindon JC. Systems biology: Metabonomics. Nature. 2008; 455(7216):1054–1056. [PubMed: 18948945]
90. Wu T, et al. Metabolic disturbances associated with systemic lupus erythematosus. PLoS One. 2012; 7(6):e37210. [PubMed: 22723834]
91. Morel L. Immunometabolism in systemic lupus erythematosus. Nat Rev Rheumatol. 2017; 13(5): 280–290. [PubMed: 28360423]
92. Yan B, et al. Serum metabolomic profiling in patients with systemic lupus erythematosus by GC/MS. Mod Rheumatol. 2016; 26(6):914–922. [PubMed: 26915395]

93. Ouyang X, et al. (1)H NMR-based metabolomic study of metabolic profiling for systemic lupus erythematosus. Lupus. 2011; 20(13):1411–20. [PubMed: 21976403]

94. Romick-Rosendale LE, et al. Identification of urinary metabolites that distinguish membranous lupus nephritis from proliferative lupus nephritis and focal segmental glomerulosclerosis. Arthritis Res Ther. 2011; 13(6):R199. [PubMed: 22152586]

95. Li J, et al. Metabolic profiling reveals new serum biomarkers of lupus nephritis. Lupus. 2017:961203317694256.

96. Fernandez D, et al. Rapamycin reduces disease activity and normalizes T cell activation-induced calcium fluxing in patients with systemic lupus erythematosus. Arthritis Rheum. 2006; 54(9): 2983–8. [PubMed: 16947529]

97. Fernandez-Ramos AA, et al. The effect of immunosuppressive molecules on T-cell metabolic reprogramming. Biochimie. 2016; 127:23–36. [PubMed: 27126071]

98. Wang H, et al. Neutrophil Extracellular Trap Mitochondrial DNA and Its Autoantibody in Systemic Lupus Erythematosus and a Proof-of-Concept Trial of Metformin. Arthritis Rheumatol. 2015; 67(12):3190–200. [PubMed: 26245802]

99. Ye, S. A Multicenter, Randomised, Double-blind Placebo Controlled Trial on the Efficacy and Safety of add-on Metformin to Conventional Immunosuppressants in Systemic Lupus Erythematosus. 2016 Jun 22. 2016; Available from: https://clinicaltrials.gov/ct2/show/NCT02741960

100. Yin Y, et al. Normalization of CD4+ T cell metabolism reverses lupus. Sci Transl Med. 2015; 7(274):274ra18.