Biomarkers of systemic lupus erythematosus identified using mass spectrometry-based proteomics: a systematic review

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Introduction

SLE is a chronic autoimmune inflammatory disease with a broad spectrum of clinical manifestations, affecting the majority of organs and tissues. In most cases, vital organs are involved including brain, heart, joints, skin and kidneys [1]. Remarkably, the disease occurs more often in women with a female-to-male ratio of 9:1 and has a higher incidence in non-Caucasian populations [2]. SLE is characterized by the presence of high titres of autoantibodies directed against a broad range of self-nuclear antigens. Accumulation of SLE autoantibodies in the host tissues and formation of immune complexes, activate production of immune system cells that perpetuate a positive

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Abstract

Advances in mass spectrometry technologies have created new opportunities for discovering novel protein biomarkers in systemic lupus erythematosus (SLE). We performed a systematic review of published reports on proteomic biomarkers identified in SLE patients using mass spectrometry-based proteomics and highlight their potential disease association and clinical utility. Two electronic databases, MEDLINE and EMBASE, were systematically searched up to July 2015. The methodological quality of studies included in the review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. Twenty-five studies were included in the review, identifying 241 SLE candidate proteomic biomarkers related to various aspects of the disease including disease diagnosis and activity or pinpointing specific organ involvement. Furthermore, 13 of the 25 studies validated their results for a selected number of biomarkers in an independent cohort, resulting in the validation of 28 candidate biomarkers. It is noteworthy that 11 candidate biomarkers were identified in more than one study. A significant number of potential proteomic biomarkers that are related to a number of aspects of SLE have been identified using mass spectrometry proteomic approaches. However, further studies are required to assess the utility of these biomarkers in routine clinical practice.

Keywords: biomarkers, systemic lupus erythematosus, SLE, mass spectrometry, proteomics, systematic review

Introduction

SLE is a chronic autoimmune inflammatory disease with a broad spectrum of clinical manifestations, affecting the majority of organs and tissues. In most cases, vital organs are involved including brain, heart, joints, skin and kidneys [1]. Remarkably, the disease occurs more often in women with a female-to-male ratio of 9:1 and has a higher incidence in non-Caucasian populations [2]. SLE is characterized by the presence of high titres of autoantibodies directed against a broad range of self-nuclear antigens. Accumulation of SLE autoantibodies in the host tissues and formation of immune complexes, activate production of immune system cells that perpetuate a positive
feedback loop resulting in organ damage [3]. Despite intensive research, the precise aetiology and pathogenic mechanisms underlying SLE are poorly understood. It is believed that SLE results from the interaction between genetic, epigenetic, environmental, hormonal and immunoregulatory factors [1].

The diagnosis of SLE is challenging due to its heterogeneous nature, variable clinical presentation and unpredictable course with periods of remission and flares [1]. Currently, patients need to fulfil at least 4 of the 11 clinical and laboratory criteria outlined by the American College of Rheumatology (ACR), for the formal diagnosis of SLE [4, 5]. However, these criteria were formulated and validated for the classification of patients with established disease and might exclude patients with early signs or limited disease. Evidence from tertiary care centres suggests that only 60% of the patients with SLE meet the ACR criteria [6].

Besides the pressing need to improve the classification of patients with SLE, assessment of disease activity remains another important aspect in the management of patients with SLE. At present, conventional disease assessment methods, including the use of acute phase markers and autoimmune serologic tests (e.g. anti-double-stranded DNA antibodies), are of limited sensitivity and specificity. In lupus nephritis, renal biopsy remains the ‘gold standard’ not only for assessing disease activity, but also for assessing prognosis and monitoring therapy [7]. However, this is an invasive and cumbersome procedure that causes discomfort to the patients. Therefore, there is an urgent need for discovering reliable SLE biomarkers that can be used not only for diagnosis, but also for disease classification, monitoring, identification of organ involvement and better prediction of response to therapy.

A proteomic biomarker is defined as ‘a specific peptide/protein that is associated with a specific condition, such as the onset, the manifestation, or progression of a disease or a response to treatment’ [8]. During the last two decades, advances in mass spectrometry (MS) enable the identification and quantification of thousands of proteins in complex biological samples, in a single run [9]. Application of MS-based proteomics to SLE provides unprecedented opportunities for identifying novel protein biomarkers, which can be used for early diagnosis and contribute to a more effective patient management. The aim of the current systematic review is to summarize and evaluate protein biomarkers identified in patients with SLE, in different biological specimens using MS. In addition, our objective was to provide more comprehensive information about the number and relevant biological function of the proteomic biomarkers detected in SLE, as well as their possible diagnostic and therapeutic utility. This systematic review also outlines the challenges that need to be addressed in future research endeavour related to the discovery of SLE proteomic biomarkers.

**Materials and methods**

**Data sources and searches**

We performed a systematic review of the literature on the discovery of proteomic biomarkers in patients with SLE using MS-based proteomic approaches. Relevant studies were identified by searching two electronic databases, MEDLINE and EMBASE, in July 2015. The search strategy in MEDLINE (1950–July 2015) was developed using the following search terms: (‘Lupus Erythematosus, Systemic’[Mesh] OR ‘Systemic Lupus Erythematosus’ OR ‘SLE’ OR ‘Lupus nephritis’ OR ‘Lupus’) AND (‘Mass Spectrometry’[Mesh] OR ‘Mass Spectrometry’ OR ‘Electrophoresis, Gel, Two-Dimensional’[Mesh] OR ‘Two-Dimensional Electrophoresis’ OR ‘proteome’ OR ‘proteomics’)). The literature search in EMBASE (1988–July 2015) was conducted in a similar way using the terms: (‘Systemic Lupus Erythematosus’ OR ‘SLE’ OR ‘Lupus nephritis’ OR ‘Lupus’) AND (‘Mass Spectrometry’ OR ‘Two-Dimensional Electrophoresis’ OR ‘proteome’ OR ‘proteomics’)). Searching results from the two databases were imported in the bibliographic management software Endnote X8 (Thomson Reuters, PA, USA). Duplicates were automatically removed. The articles were reviewed in a two-stage procedure. In the first stage of the review process, abstracts of all identified articles were screened. Review articles, editorials, case reports, letters to the editor, conference abstracts, notes and news were excluded. In the second stage of the review process, full texts of the remaining studies were evaluated. A checklist of specified inclusion criteria was used to ensure uniformity in the assessment of the identified manuscripts. The final articles that were selected all fulfilled the following eligibility criteria: written in English, used human samples, compared biological fluids from patients with SLE with a control group (healthy or other type of control group), referred to SLE, were informative about the type of biological fluid used, provided details about the mass spectrometry technique used as well as the proteomic biomarkers identified. Articles that did not meet one or more of these inclusion criteria were excluded. This review was performed by two independent reviewers (ON and AK) based on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [10]. In case of any unsolved discrepancies between the two reviewers, a third reviewer (KS) was consulted.

**Results**

The initial literature search identified 1093 records. After duplicate removal, 775 records remained. These were all screened, resulting in a total of 25 selected full-text articles, published between 2007 and 2015, which were included in this systematic review. The detailed review process with the eligibility criteria used is shown in Figure S1. The main findings of each article are summarized in Table 1. The table is divided into two parts. The first part includes targeted and untargeted proteomic studies, whereas the second part lists the validation studies. Information about specimen type, controls, proteomic/validation techniques used, biomarkers identified, disease association and/or possible clinical use of the biomarkers is also included. Additionally, the 25 articles were assessed according to the recommendations proposed for biomarker identification and qualification in clinical proteomics (Table S1) [8]. All selected articles fulfilled at least four of the eight recommendations. It is noted that only the first criterion, ‘justification and description of clinical question, outcome and selection of subjects’, is fulfilled by all the selected articles.

**Types of specimens**

In the 25 selected articles, a variety of specimen types obtained from patients with SLE was used. This can be attributed to the clinical
| Reference      | Specimen type | Sample size (SLE/Control) | Initial phase targeted and untargeted proteomics | Total number of identified proteins / number of differentially expressed proteins | Validation phase | Validation techniques | Biomarker/Protein signature | Disease association and/or possible Clinical Use |
|----------------|---------------|---------------------------|-------------------------------------------------|--------------------------------------------------------------------------------|-----------------|----------------------|----------------------------|---------------------------------|
| Alaiya et al.  | Renal biopsies| 6 LN class IV-Glob. / 5 LN class IV-Segmental/3 ANCA-GN, 3 normal kidneys | 2-DE, MALDI-TOF MS | 28 proteins in total / 9 proteins only if subgroups IV-G and IV-S were classified as one group | Renal biopsies /5 LN class IV-G / ANCA, 3 normal kidneys | Label-free LC/MS/MS | Albumin, annexin A5, cytokeratin 18, cytokeratin 19, serotransferrin | LN diagnosis (class IV molecular subclassification of LN; Subcategories of class IV to IV-G and IV-S not established) |
| Caster et al.  | Serum         | 10 PLN, 15 MLN / 10 non-LN SLE | SDS-PAGE, LC-MS/MS | 102 total common proteins in glomerular and podocytes extracts / 69 of those proteins were membrane-associated | Serum /10 PLN, 10 MLN / 10 non-LN SLE, 10 HC | ELISA analysis | Elevated anti-annexin A2 in PLN patients compared with MLN, non-LN SLE and HC with a P value < 0.01* | Diagnosis of proliferative forms of LN |
| Dai et al.     | PBMC’s        | 9 Female SLE† / 7 age-matched HC | 2-DE, MALDI-TOF/TOF MS | 5 Proteins total: 4 upregulated 1 downregulated | N/A | N/A | N/A | SLE Diagnosis |
| Fang et al.    | Skin biopsies | 10 SLE (affected, untreated skin) / 10 HC (normal skin) | 2-DE, MALDI-TOF MS | 18 proteins total / Focused on keratins; 2 downregulated 6 upregulated | N/A | N/A | N/A | SLE skin lesions |
| Huang et al.   | Serum         | 32 SLE/43 DC† 43 age- and sex-matched HC | MALDI-TOF MS combined with WCX magnetic beads | 60 protein peaks in total; 32 downregulated 28 upregulated / Classification tree analysis resulted in 4 protein peaks: 3 upregulated 2 downregulated | Serum /32 SLE, 42 DC, 40 age- and sex-matched HC | Blinded testing set (WCX magnetic beads, MALDI-TOF MS) | Panel of proteins with m/z ratio 4070.09, 7770.45, 28 045.1, 3376.02 differentiate samples of SLE from other autoimmune diseases and HC with accuracy of 78.1, 85.8 and 90%, respectively | SLE diagnosis |
| Iizuka et al.  | Serum         | 30 CNS-SLE (CSF from 3 patients) / 30 non-CNS-SLE, 5 HC | 2-DE, MALDI-TOF/TOF MS | 4 differentially expressed proteins (autoantigens) | N/A | N/A | N/A | CNS lupus diagnosis |
| Kazemipour et al. | Serum         | 13 SLE / 7 HC | 2-DE, MALDI-TOF/TOF MS | 9 differentially expressed proteins / 3 upregulated 6 downregulated | N/A | N/A | N/A | SLE diagnosis |
| Kimura et al.  | Serum         | 7 NPSLE/12 HC | 2-DE, WB, LC-MS/MS | 6 proteins (antigens) in total / only Rab guanosine | Serum /78 NPSLE /19 SLE without NP symptoms, 45 DC†, 12 HC | 1-DE WB against human anti-aGDI full-length | Anti-aGDI was higher in NPSLE patients with psychosis (80%) | Diagnosis of psychosis in NPSLE |
| Reference | Specimen type | Sample size (SLE/Control) | Initial phase targeted and untargeted proteomics | Validation phase | Biomarker/Protein signature | Disease association and/or possible Clinical Use |
|-----------|---------------|---------------------------|-----------------------------------------------|----------------|---------------------------|-----------------------------------------------|
| Li et al. [20] | CD4+ T cells from PBMCs | 10 Female SLE† / unknown number of age- and sex-matched HC | SDS-PAGE, LC-MS/MS (CD4+ T cells immunoprecipitated with anti-human Gadd45a antibody WB (Conf.) RT-PCR (Conf.) | N/A | N/A | SLE disease activity (positive correlation with SLEDAI) SLE pathogenesis (HMGB1 protein binds to Gadd45a contributing to DNA demethylation in CD4+ T cells) |
| Morgan et al. [16] | Serum | 7 SLE /7 age-, sex- and race-matched HC | 2-DE, LC-MS/MS | 4 downregulated proteins | Serum /1 LN, 24 non-LN SLE /8 HC | ELISA | Apolipoprotein CIII increased in LN compared with non-LN SLE and HC LN diagnosis, increased atherosclerotic risk in LN |
| Mosley et al. [29] | Urine | 26 SLE with active LN /49 SLE with inactive LN, 15 HC | SELDI-TOF MS | 32 protein ions in total / 2 protein ions best discriminate the two groups with 92% specificity and 92% sensitivity P value < 0.01* | Sequential urines /6 SLE with biopsies indicative for active disease | SELDI-TOF MS | m/z 3340 and 3980 predicted a change in disease state prior to clinical classification LN activity diagnosis, LN relapse and remission prediction |
| Nielsen et al. [25] | Platelet-poor plasma | 12 SLE† /12 DC**, 12 HC | LC-MS/MS Label-free, spectral count Flow cytometry (Conf.) | N/A | N/A | SLE disease activity and pathogenesis |
| Nielsen et al. [26] | Platelet-poor plasma | 44 SLE /66 age- and sex-matched HC | LC-MS/MS Label-free, peptide intensities Flow cytometry (Conf.) | Renal biopsies /3 SLE with LN /1 HC | IEM | Anti-G3BP and anti-IgG colocalized in the glomeruli in all 3 biopsies LN pathogenesis (no association with disease activity or clinical manifestations) |
| Østergaard et al. [27] | Platelet-poor plasma | 12 SLE /12 DC**, 12 HC | LC-MS/MS Label-free, peptide intensities | S31 MP-associated proteins in total /248 proteins; 191 upregulated 57 downregulated | N/A | N/A | SLE diagnosis Disease activity |
| Reference          | Specimen type | Sample size (SLE/Control) | Proteomic techniques (confirmation techniques) | Total number of identified proteins / number of differentially expressed proteins | Validation phase | Biomarker/Protein signature | Disease association and/or possible Clinical Use |
|--------------------|---------------|---------------------------|-------------------------------------------------|--------------------------------------------------------------------------------|----------------|-----------------------------|--------------------------------------------------|
| Pavon et al. [21]  | PBMCs         | 6 SLE / 8 age-matched HC  | 2-DE, MALDI-TOF MS MALDI-TOF/TOF MS (Conf.)     | 98 proteins total / ID two S100A9 protein isoforms and their phosphorylated counterparts; S100A9-S, S100A9-1 (phosphorylated at Thr113) | PBMCs /14 SLE | S100A9 increased HSP 90α/β, HSP 70 and PPIase A decreased | S100A9 proteomic signature for the abnormal presence of activated low-density granulocytes in SLE PBMCs |
| Serada et al. [17] | Serum         | 41 SLE (23 LN) / 60 DC, 19 HC | 2D-PAGE, WB, LC-MS/MS Sera immunoblotted with human umbilical vein endothelial cells ELISA (Conf.) | 6 proteins total / one novel autoantigen; Aldolase A | N/A | N/A | LN diagnosis |
| Somparn et al. [30] | Urine        | 5 active LN / 5 age- and sex-matched inactive LN | 2-DE, ESI-Q-TOF MS/MS | 14 proteins in total | Urine /50 active LN /26 inactive, 14 non-LN glomerular diseases, 8 age- and sex-matched HC | ELISA to validate differential levels of Prostaglandin H2-D-isomerase increased in active LN compared with the other 3 groups | LN activity diagnosis (class III or IV) |
| Sui et al. [33]    | Renal biopsies| 10 Female LN (3 class V and 7 not reported) /13 age- and sex-matched HC | LC-MS/MS / ITRAQ-labelled | 512 proteins in total / 16 proteins with difference of more than 1.5-fold; 9 upregulated proteins 7 downregulated Protein (2 unnamed proteins) | N/A | N/A | LN diagnosis |
| Sun et al. [36]    | CSF           | 27 NPSLE before and 2 weeks after treatment and 17 discosis, 10 SLE without NP manifestations | MALDI-TOF MS combined with WAX magnetic beads | 12 protein peaks total; 10 upregulated and 2 downregulated / Decision tree recognizes NPSLE with 92.6% sensitivity and specificity; Panel m/z peaks 8595, 7170, 7981, 7740, 5806 (3 upregulated and 2 downregulated) | CSF / Blind test group: 12 NPSLE before treatment / 12 lumbar disc herniation, 9 with other autoimmune diseases with NP involvement CSF /1 NPSLE incubated with anti-ubiquitin polyclonal Ab for 2 h / same CSF sample incubated with normal rabbit polyclonal Ab (negative control) CSF /16 NPSLE / 7 SLE without NP manifestations | Immunoprecipitation, MALDI-TOF MS combined with WAX magnetic beads | Decision tree recognizes NPSLE with 91.7% sensitivity and 85.7% specificity; Panel of m/z peaks 8595, 7170, 7981, 7740, 5806 8595 peak identified as ubiquitin, downregulated in CSF of NPSLE after treatment Anti-ubiquitin levels higher in NPSLE group | NPSLE diagnostic proteomic model (Panel of m/z peaks) Disease activity (CSF ubiquitin levels) |
| Reference     | Specimen type | Initial phase targeted and untargeted proteomics | Proteomic techniques (confirmation techniques) | Total number of identified proteins / number of differentially expressed proteins | Validation phase | Biomarker/Protein signature | Disease association and/or possible Clinical Use |
|---------------|---------------|--------------------------------------------------|------------------------------------------------|-----------------------------------------------------------------|-----------------|---------------------------|-----------------------------------------------|
| Wang et al. [22] | PBMC          | 6 active SLE /6 stable SLE, 6 age- and sex-matched HC | MALDI-TOF/TOF MS iTRAQ-labelled                  | Unknown number of total proteins/focused on STRAP downregulated in active SLE | PBMC /4 active SLE /1 stable SLE, 11 HC | W6                        | Lower expression of STRAP in active SLE       |
| Wang et al. [23] | PBMC          | 6 active SLE, 6 stable SLE /6 rheumatoid arthritis (RA), 6 age- and sex-matched HC | MALDI-TOF/TOF MS iTRAQ-labelled                  | 452 proteins in total /67 differentially expressed unique proteins; | N/A             | N/A                       | SLE diagnosis SLE disease activity Discrimination of SLE from RA |
| Wu et al. [40]  | Serum         | 7 SLE family cases /63 individual SLE, 83 HC      | MALDI-TOF MS combined with WCX magnetic beads   | 4 discriminative protein peaks; 1 upregulated protein peak 3 downregulated protein peaks | N/A             | N/A                       | SLE risk prediction                           |
| Zhang et al. [31] | Urine         | 19 Female LN† who experienced 25 flares (5 class III /11 class IV /3 class V) Samples taken: pre-flare, flare, treatment and baseline | SELDI-TOF MS Direct on-chip peptide sequencing LC-MS/MS (Cont.) | 176 protein peaks in total; 27 differentially expressed protein ions between flare intervals; / selected peaks resulted in identification of 2 proteins; | Renal biopsies /3 LN class IV /1 HC | IHC (staining with anti-hepcidin polyclonal Ab) | Increased expression of hepcidin in SLE nephritis patients compared with controls |
| Zhou et al. [24] | PBMCs         | 14 Female SLE† /9 age- and sex-matched HC         | 2-DE, LC-MS/MS                                  | 16 proteins in total; 5 upregulated 11 downregulated / Focused on annexin A5 | PBMCs /47 Female SLE† /31 age- and sex-matched HC | W6                        | Upregulation of annexin A5 in SLE            |
|               |               |                                                   |                                                  |                                                                | Serum /123 Female SLE† /113 age- and sex-matched HC | ELISA                      | Downregulation of annexin A5 in SLE sera Anti-annexin A5 levels did not greatly differ between SLE and controls |
|               |               |                                                   |                                                  |                                                                | Platelet-poor plasma /30 Female SLE† /30 age- and sex-matched HC | Coagulation assays | Elevated annexin A5 could shorten prothrombin time, activated partial thromboplastin time, prolonged thrombin time |
|               |               |                                                   |                                                  |                                                                |                                                   |                           |                                               |
| Reference       | Specimen type | Sample size (SLE/Control)       | Proteomic techniques (confirmation techniques) | Total number of identified proteins / number of differentially expressed proteins | Validation phase |
|-----------------|---------------|---------------------------------|-------------------------------------------------|--------------------------------------------------------------------------------|------------------|
| Zhou et al. [18]| Serum         | Group 1: 12 SLE Females† with LN and FH Group 2: 12 SLE Females‡ with LN but no FH Group 3: 12 SLE Females§ without LN or FH Controls: 6 sex-matched HC | MALDI-TOF MS combined with WCX magnetic beads | 6 protein peaks in total / 1 peak upregulated in groups 12 and 3, 1 peak present only in groups 12 and 33 peaks present only in groups 1 and 2, 1 peak present only in group 1 | N/A               |

LN: lupus nephritis; ANCA: antineutrophil cytoplasmic antibody vasculitis; GN: glomerulonephritis; 2-DE: two-dimensional gel electrophoresis; MALDI: matrix-assisted laser desorption/ionization; TOF: time-of-flight mass spectrometry; MS: mass spectrometry; PLN: proliferative LN; MLN: membranous LN; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; LC-MS: liquid chromatography MS; HC: healthy controls; ELISA: enzyme-linked immunosorbent assay; PBMCs: peripheral blood mononuclear cells; N/A: not applicable; without validation; SLE: systemic lupus erythematosus; Conf.: confirmation techniques; WB: Western blot analysis; IHC: immunohistochemistry; DC: disease controls; WCX: weak cation exchange chromatography; CNS: central nervous system; CSF: cerebrospinal fluid; NPSLE: neuropsychiatric SLE; MP: microparticle; IEM: immune electron microscopy; MP: microparticle; IEM: immune electron microscopy; ID: identified; S100A9: S100 calcium-binding protein A9; HSP 90α/β: heat-shock protein 90α/β; HSP 70: Heat-shock protein 70; PPIase A: peptidyl-prolyl cis-trans isomerase A; RT-PCR: reverse transcription–polymerase chain reaction; iTRAQ: isobaric tagging reagent for absolute quantitation; 2D-PAGE: two-dimensional polyacrylamide gel electrophoresis; ESI-Q-TOF MS/MS: electrospray ionization quadrupole time-of-flight mass spectrometry; Ab: antibody; STRAP: serine-threonine kinase receptor-associated protein.

* P value stated only if other than 0.05.
† Gender stated only if study did not use both genders.
‡ Twelve patients with SLE were divided into four groups of three patients each: Group I: biopsy-proven active nephritis and high disease activity (SLEDAI > 12), Group II: biopsy-proven active nephritis, current quiescent disease (low SLEDAI = 0), Group III: No history of nephritis, current active disease other than nephritis (high SLEDAI > 10).
§ State the disease controls categories from this article: 15 rheumatoid arthritis, 15 Sjögren’s syndrome, 13 systemic sclerosis.
¶ State the disease controls categories from this article: 12 multiple sclerosis, 13 infectious meningococcal meningitis, 10 polyneuropathy, 10 psychotic syndromes.
** State the disease controls categories from this article: 6 rheumatoid arthritis, 6 systemic sclerosis.
†† State the disease controls categories from this article: 6 rheumatoid arthritis, 6 systemic sclerosis.
‡‡ State the disease controls categories from this article: 49 rheumatoid arthritis, 11 polymyositis.
§§ LN: lupus nephritis; ANCA: antineutrophil cytoplasmic antibody vasculitis; GN: glomerulonephritis; 2-DE: two-dimensional gel electrophoresis; MALDI: matrix-assisted laser desorption/ionization; TOF: time-of-flight mass spectrometry; MS: mass spectrometry; PLN: proliferative LN; MLN: membranous LN; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; LC-MS: liquid chromatography MS; HC: healthy controls; ELISA: enzyme-linked immunosorbent assay; PBMCs: peripheral blood mononuclear cells; N/A: not applicable; without validation; SLE: systemic lupus erythematosus; Conf.: confirmation techniques; WB: Western blot analysis; IHC: immunohistochemistry; DC: disease controls; WCX: weak cation exchange chromatography; CNS: central nervous system; CSF: cerebrospinal fluid; NPSLE: neuropsychiatric SLE; Gadd45a: growth arrest and DNA damage-inducible-alpha; SLEDAI: SLE disease activity index; SELDI: surface-enhanced laser desorption/ionization; MP: microparticle; IEM: immune electron microscopy; ID: identified; S100A9: S100 calcium-binding protein A9; HSP 90α/β: heat-shock protein 90α/β; HSP 70: Heat-shock protein 70; PPIase A: peptidyl-prolyl cis-trans isomerase A; RT-PCR: reverse transcription–polymerase chain reaction; iTRAQ: isobaric tagging reagent for absolute quantitation; 2D-PAGE: two-dimensional polyacrylamide gel electrophoresis; ESI-Q-TOF MS/MS: electrospray ionization quadrupole time-of-flight mass spectrometry; Ab: antibody; STRAP: serine-threonine kinase receptor-associated protein.

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heterogeneity of the disease, affecting almost all organs and tissues. Serum was the most frequently used biological fluid (9 of the 25 studies) [11–18], followed by the peripheral blood mononuclear cells (PBMCs) (6 of the 25 studies) [19–24], and platelet-poor plasma (3 of 25 studies) [25–27]. Theoretically, blood serum or plasma may be ideal to analyse as they contain specific biomarkers for almost all human diseases, but it is recognized that potential biomarkers in these samples may be present at very low concentrations [28]. Three studies [29–31] examined the urine proteome and two studies [32, 33] analysed the protein extract from kidney biopsies. Potentially, urinary biomarkers may be more meaningful as they reflect more accurately renal disease than their serum counterparts. Indeed, an emerging concept is that urine is potentially a liquid biopsy of the kidney [34]. Nephritis is a common and serious complication of SLE; thus, differentially expressed proteins during renal flare might be potential novel and predictive lupus nephritis (LN) biomarkers. Another study examined the proteome from skin biopsies, as skin is the second most commonly affected organ in SLE after joint involvement [35]. Finally, one study analysed cerebrospinal fluid as all parts of the nervous system can be affected in patients with SLE causing neuropsychiatric syndromes [36].

**Proteomic techniques**

The term proteomics describes the large-scale characterization of the whole protein content of a cell, organ or organism at a given time [37]. Herein, the proteomic studies were classified into two broad categories. The first category includes discovery or unbiased approaches and the second targeted or biased approaches. A typical MS-based workflow involves two main steps: (i) separation of proteins and peptides present in a complex biological sample using gel-based or gel-free techniques, (ii) MS-based protein identification [38], which involves protein digestion prior to analysis [39]. Alternatively, studies using targeted proteomic approaches aim to detect specific proteins, for example autoantigens. The experimental workflow is also different compared with the untargeted approach. In studies included in this systematic review, in which targeted approaches were used, the tissue of interest was separated by either one-dimensional gel electrophoresis (1-DE) or 2-DE, followed by immunoblotting with antisera of patients with SLE. The immune-reactive bands were then subjected to MS analysis for identification.

In the current systematic review, 10 studies used 2-DE, two studies used sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) [11, 20] and one study used 2D-PAGE combined with Western blot [17] for protein separation. Moreover, four studies [12, 18, 36, 40] used weak cation exchange (WCX) magnetic beads to enrich low molecular weight peptides prior to MS analysis. Indeed, the combination of magnetic beads with matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) enables robust, precise and rapid protein profiling of complex samples [41, 42]. Subsequently, protein spots were analysed by MS and proteins were identified by database searches.

A variety of MS approaches was used to obtain proteomic data from different biological samples. These include MALDI-TOF MS, MALDI-TOF/TOF MS, surface-enhanced laser desorption/ionization (SELDI)-TOF MS and liquid chromatography (LC) combined with tandem MS (LC-MS/MS, including the use of triple-quadrupole MS instruments and hybrid quadrupole–TOF instruments: Table 1). Both label-free and labelled approaches were used. Particularly, 3 of the 25 studies used isobaric tags for relative and absolute quantitation, namely isobaric tagging reagent for absolute quantitation (iTRAQ)-labelled approach [22, 23, 33], whereas 3 of 25 used a label-free approach [25–27]. Significantly, 13 of these studies validated the identified candidate biomarkers. For validation, most studies used well-established immune-based methods including Western blot (WB), enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) and four studies used additional proteomic methods.

**Identified proteins**

A total of 241 candidate biomarkers were identified in the 25 studies included in this review (Table 2). In 13 of the 25 studies, validation studies of a selected number of biomarkers were performed in an independent cohort, which resulted in the validation of 28 candidate biomarkers. These include albumin, annexin A5, cytokeratin 18, cytokeratin 19, serotransferrin [32], annexin A2 antibody [11], a panel of proteins with m/z ratio of 4070.09, 7770.45, 28045.1, 3376.02 [12], Rab guanosine diphosphate dissociation inhibitor α (αGDI) antibody [15], apolipoprotein CIII [16], peaks of m/z 3340, m/z 3980 [29], galectin-3-binding protein (G3BP) antibodies, anti-immunoglobulin G (IgG) [26], S100 calcium-binding protein A9 (S100A9), heat-shock protein (HSP) 90 α/β, HSP70, peptidyl-prolyl cis-trans isomerase A (PPiase A) [21], prostaglandin H2D-isomerase [30], panel of proteins with m/z ratio 8595 (ubiquitin), 7170, 7661, 7740, 5806 [36], serine-threonine kinase receptor-associated protein (STRAP) [22], hepcidin [31] and annexin A5 [24].

Due to the systemic, chronic and heterogeneous nature of the disease, the selected studies covered a wide range of applicability of the potential biomarkers relating to different aspects of SLE management including disease diagnosis and activity, or specific organ involvement. Interestingly, 11 potential biomarkers were identified independently in more than one study (Table 3) and further details are presented in the discussion section.

**Discussion**

The aim of this systematic review was to critically review proteomic biomarkers identified in patients with SLE using MS-based proteomics. Although in recent years many SLE protein biomarker reports have been published, this is the first attempt to present a systematic review on this important topic. This review summarizes the candidate proteomic biomarkers identified so far in SLE, as well as their potential disease association and clinical use. This review has revealed 241 potential SLE protein biomarkers that have been identified by MS-based proteomics, using both
| Specimen type | Disease association | Biomarkers | Reference |
|---------------|----------------------|------------|-----------|
| Serum         | SLE                  | m/z ratio 4070.09, 7770.45, 28045.1, 3376.02; Apolipoprotein A-1, prothrombin, keratin type II (cytoskeletal), keratin 1, albumin, type II keratin, transthyretin, haptoglobin 2; m/z ratio 9342.23, 4094.03, 5905.35, 7973.53 | Huang et al. [12], Kazemipour et al. [14], Wu et al. [40] |
| LN            | Annexin A2 antibody, ATP synthase subunit-alpha, mitochondrial, ATP synthase subunit-beta, mitochondrial, alpha-enolase ENO1, Moesin, glyceraldehyde-3-phosphate dehydrogenase, Elongation factor 1-alpha 1, guanine nucleotide-binding protein G(i) subunit-alpha-2, isoform 2 of AP-2 complex subunit mu, isoform 2 of protein disulphide isomerase A6, pyruvate kinase PKM, 60-kDa heat-shock protein mitochondrial, actin-related protein 3, V-type proton ATPase subunit B brain isoform, myosin-9, isoform 3 of heterogeneous nuclear ribonucleoprotein, septin-7, isoform 2 of coronin-1C, tubulin-beta-4A chain, T-complex protein 1 subunit-gamma, isoform 2 of ATP-dependent RNA helicase, isoform 2 of basigin, dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48-kDa subunit, heat-shock protein HSP 90-beta, ezrin, isoform 2 of neutral cholesterol ester hydrolase 1, isoform 2 of heat-shock cognate 71-kDa protein, heterogeneous nuclear ribonucleoprotein U, Rho GTPase-activating protein 1, isoform 2 of fatty aldehyde dehydrogenase, serine palmitoyltransferase 1, isoform 2 of ATP-citrate synthase; IgM heavy chain, apolipoprotein CIII, HSA fragment, haemopexin fragment; glyceraldehyde-3-phosphate dehydrogenase, heterogeneous nuclear ribonucleoprotein A2/B1, annexin A2, aldolase A, elongation factor 1-gamma, lupus La protein (SS-B/La); m/z 4207 Da, 2658 Da, 1465 Da, 5332 Da, 5900 Da, 1943 Da | Caster et al. [11], Morgan et al. [16], Serada et al. [17], Zhou et al. [18] |
| CNS-SLE       | Peroxiredoxin-4, ubiquitin carboxyl-terminal hydrolase isozyme L1, splicing factor arginine/serine-rich 3, histone H2A type 1 | Iizuka et al. [13] |
| NPSLE         | Stress-70 protein, Rab guanosine diphosphate dissociation inhibitor α antibody, Isocitrate dehydrogenase [NAD] subunit-alpha, L-lactate dehydrogenase B chain, F-actin-capping protein subunit-alpha-2, Rab guanosine diphosphate dissociation inhibitor-beta | Kimura et al. [15] |
| PBMCs         | SLE                  | Immunoglobulin J chain, apolipoprotein A-IV precursor, glutathione S-transferase, calpain1 protein L1H, zinc finger protein subfamily 1A; high-mobility group box protein 1 (CD4+ T cells); S100 calcium-binding protein A9, heat-shock protein 90α/p, heat-shock protein 70, peptidyl-prolyl cis-trans isomerase A, serine-threonine kinase receptor-associated protein; cDNA FLJ61039, AF4/FMR2 family member 1, cDNA FLJ5107, structural maintenance of chromosome protein 3, protein S100-A9, protein S100-A8, protein S100-A12, lysozyme C, glutathione S-transferase kappa 1 isoform c, isoform 2 of zinc finger protein 549, 26-kDa protein, histone H2A type 1, myeloblastin, brain acid-soluble protein 1, protein S100-P, neutrophil defensin 1, isoform 2 of metalloendopeptidase OMA1 (mitochondrial), 42-kDa protein, latent transforming growth factor-beta-binding protein 1 isoform, resistin, AF4/FMR2 family member 1, cDNA FLJ61340, HLA class I histocompatibility-alpha chain, histone H1.2, cDNA FLJ51589, putative uncharacterized protein RCA, FGR3B protein, nucleolar protein 5A, serine-threonine kinase receptor-associated protein, myosin regulatory light polypeptide 9, isoform A of bromodomain and WD repeat-containing protein 1, vacuolar protein sorting-associated protein 35, putative uncharacterized protein PTMA, isoform 2 of retinol dehydrogenase 11, ribosomal protein L10 (fragment), cathepsin A isoform a precursor, high-mobility group protein B2, protein disulphide | Dai et al. [19], Li et al. [20], Pavon et al. [21], Wang et al. [22], Wang et al. [23] |
| Specimen type | Disease association | Biomarkers | Reference |
|---------------|--------------------|------------|-----------|
| SLE Thrombophilia | Annexin A5, glyceraldehyde-3-phosphate dehydrogenase, integrin-linked protein kinase, adenylcyclase-associated protein 1, transketolase, proline-serine-threonine phosphatase-interacting protein 2, triosephosphate isomerase, tyrosine-protein kinase CSK, dynamin-1-like protein, elongation factor 1-alpha 1, T-complex protein 1 subunit zeta, heat-shock protein-beta-1, phosphoglycerate kinase 1, alpha-enolase, osteoclast-stimulating factor 1, heat-shock cognate 71 kD protein | | Zhou et al. [24] |
| Platelet-poor plasma | SLE | IgG-MPs, IgM-MPs, C1q-MPs (proteins discussed by the author out of 248 proteins) | Nielsen et al. [25] Ostergaard et al. [27] |
| Urine | LN | MP-G3BP, MP-C1q (3 subunits), MP-lg (most abundant: IgJ, IgM, IgG2), Ig (IgG, IgM, IgA), complement proteins (C1), fibronectin, 14-3-3\(\text{\textgamma}\), desmosomal proteins, ficolin 2, galectin-3-binding protein, \(\beta_2\) -glycoprotein I, \(\beta_\text{gal}\)-tubulin, \(\beta_\text{micro}\)-tubulin, lysosome-associated membrane protein 1, transforming factor \(\beta_1\), \text{m/z ratio 3340, 3980; prostaglandin H}_2\text{D-isomerase, serotransferrin, alpha-1-glucoprotein, alpha-2-HS-glycoprotein, haptoglobin, alpha-1-antitrypsin, albumin, Zn-alpha-2-glycoprotein, Ig kappa chain V-III SLE region, Ig kappa chain V-III HAH region, Ig kappa chain C region, retinol-binding protein 4, beta-2-microglobulin, transthyretin, hepcidin (isoforms 20 and 25), a1-antitrypsin, N-terminal region of albumin} | Nielsen et al. [25] Mosley et al. [29] Somparn et al. [30] Zhang et al. [31] |
| Biopsies | LN | Renal: Ezrin P81, serotransferrin, cytokeratin 18, cytokeratin 19, alpha-1-antitrypsin, albumin, plasma glutathione peroxidase, 1433 protein epsilon, annexin A5, heterogeneous nuclear ribonucleoproteins A2/B1 isoform B1, lamin A protein, mimcecan preproprotein, annexin A1, annexin A2 isoform 2, alpha-1-antitrypsin precursor, glutathione S-transferase-P1c, adenine phosphoribosyltransferase isoform a, collagen type VI alpha-3 (isoform CRA\(\text{\textgamma}\)), formiminotransferase cyclodeaminase form C, aldolase B, aldehyde dehydrogenase, 2-oxoglutarate dehydrogenase (mitochondrial isoform 1 precursor), L-arginine:glycine amidinotransferase, pyrrole-5-carboxylate dehydrogenase, antiquitin | Alaiya et al. [32] Sui et al. [33] |
| SLE Skin lesions | | Skin: keratin 10, keratin 16, keratin 14, Keratin 6, keratin 5, keratin 2e, keratin 1, involucrin | Fang et al. [35] |
| CSF | CNS-SLE | m/z peaks 8595 (ubiquitin), 7170, 7661, 7740, 5806 | Sun et al. [36] |

Only highlighted (bold) proteins in table were validated by the authors.
| Biomarker | Studies                  | Specimen type   | Sample size (SLE /Control) | Proteomic techniques       | Validation | Disease association and/or possible clinical use |
|-----------|--------------------------|----------------|---------------------------|-----------------------------|------------|------------------------------------------------|
| **1**     | Annexin A2               | Serum (autoantibodies) | 10 PLN/ 15 MLN, 10 non-LN SLE | SDS-PAGE, LC-MS/MS         | Yes        | Diagnosis of proliferative forms of LN         |
|           | Caster et al. [11]       |                |                           |                             |            |                                                |
|           | Serada et al. [17]       | Serum (autoantibodies) | 41 SLE (23 LN)/ 60 DC, 19HC | 2D-PAGE, WB, LC-MS/MS ELISA | No         | LN Diagnosis                                   |
|           | Sui et al. [33]          | Renal Biopsies  | 10 LN (3 class V and 7 not reported)/ 3 age- and sex-matched HC | LC-MS/MS iTRAQ-labelled     | No         | LN Diagnosis                                   |
| **2**     | Annexin A5               | Renal Biopsies  | 6 LN class IV-Global 5 LN class IV-Segmental /3 ANCA-GN, 3 normal kidneys | 2-DE, MALDI-TOF MS          | Yes        | LN diagnosis                                   |
|           | Alaiya et al. [32]       |                |                           |                             |            |                                                |
|           | Zhou et al. [24]         | PBMCs          | 14 SLE/ 9 age- and sex-matched HC | 2-DE, LC-MS/MS             | Yes        | SLE-related thrombophilia (increased intracellular and decreased serum annexin A5 levels are protective) |
| **3**     | Alpha-1-antitrypsin      | Urine          | 5 active LN/ 5 age- and sex-matched inactive LN | 2-DE, ESI-Q-TOF MS/MS      | No         | LN activity and diagnosis                       |
|           | Sompam et al. [30]       |                |                           |                             |            |                                                |
|           | Zhang et al. [31]        | Urine          | 19 LN (5 class III /11 class IV/ 3 class V). Samples taken: pre-flare, flare, treatment and baseline | SELDI-TOF MS, Direct on-chip peptide sequencing LC-MS/MS | No         | SLE renal flare prediction                     |
|           | Alaiya et al. [32]       | Renal Biopsies  | 6 LN class IV-Global 5 LN class IV-Segmental /3 ANCA-GN, 3 normal kidneys | 2-DE, MALDI-TOF/MS          | Yes        | LN diagnosis                                   |
| **4**     | Serotransferrin          | Renal Biopsies  | 6 LN class IV-Global 5 LN class IV-Segmental /3 ANCA-GN, 3 normal kidneys | 2-DE, MALDI-TOF MS          | Yes        | LN diagnosis                                   |
|           | Alaiya et al. [32]       |                |                           |                             |            |                                                |
|           | Somparn et al. [30]      | Urine          | 5 active LN/ 5 age- and sex-matched inactive LN | 2-DE, ESI-Q-TOF MS/MS      | No         | LN activity and diagnosis                       |
| **5**     | Ezrin                    | Serum (autoantibodies) | 10 PLN/ 15 MLN, 10 non-LN SLE | SDS-PAGE, LC-MS/MS         | Yes        | Diagnosis of proliferative forms of LN         |
|           | Caster et al. [11]       |                |                           |                             |            |                                                |
|           | Alaiya et al. [32]       | Renal Biopsies  | 6 LN class IV-Global 5 LN class IV-Segmental /3 ANCA-GN, 3 normal kidneys | 2-DE, MALDI-TOF MS          | Yes        | LN diagnosis                                   |
| Biomarker | Studies | Specimen type | Sample size (SLE /Control) | Proteomic techniques | Validation | Disease association and/or possible clinical use |
|-----------|---------|---------------|---------------------------|----------------------|------------|-----------------------------------------------|
| 6 Elongation factor 1-alpha 1 | Caster et al. [11] | Serum (autoantibodies) | 10 PLN/ 15 MLN, 10 non-LN -- SLE | SDS-PAGE, LC-MS/MS | Yes | Diagnosis of proliferative forms of LN |
| Zhou et al. [24] | PBMCs | 14 SLE/ 9 age- and sex-matched HC | 2-DE, LC-MS/MS | Yes | SLE-related thrombophilia (increased intracellular and decreased serum annexin A5 levels are protective) |
| 7 Glyceraldehyde-3-phosphate dehydrogenase (G3PD) | Caster et al. [11] | Serum (autoantibodies) | 10 PLN/ 15 MLN, 10 non-LN SLE | SDS-PAGE, LC-MS/MS | Yes | Diagnosis of proliferative forms of LN |
| Serada et al. [17] | Serum (autoantibodies) | 41 SLE (23 LN)/ 60 DC, 19HC | 2D-PAGE, WB, LC-MS/MS ELISA | No | LN Diagnosis |
| Zhou et al. [24] | PBMCs | 14 SLE/ 9 age- and sex-matched HC | 2-DE, LC-MS/MS | Yes | SLE-related thrombophilia (increased intracellular and decreased serum AnxA5 levels are protective) |
| 8 Alpha-enolase | Caster et al. [11] | Serum (autoantibodies) | 10 PLN/ 15 MLN, 10 non-LN SLE | SDS-PAGE, LC-MS/MS | Yes | Diagnosis of proliferative forms of LN |
| Zhou et al. [24] | PBMCs | 14 SLE/ 9 age- and sex-matched HC | 2-DE, LC-MS/MS | Yes | SLE-related thrombophilia (increased intracellular and decreased serum annexin A5 levels are protective) |
| 9 Haptoglobin | Somparn et al. [30] | Urine | 5 active LN/ 5 age- and sex-matched inactive LN | 2-DE, ESI-Q-TOF MS/MS | No | LN activity and diagnosis |
| Kazemipour et al. [14] | Serum | 13 SLE/ 7 HC | 2-DE, MALDI-TOF/TOF MS | No | SLE diagnosis |
| 10 Transthyretin | Somparn et al. [30] | Urine | 5 active LN/ 5 age- and sex-matched inactive LN | 2-DE, ESI-Q-TOF MS/MS | No | LN activity and diagnosis |
| Kazemipour et al. [14] | Serum | 13 SLE/ 7 HC | 2-DE, MALDI-TOF/TOF MS | No | SLE diagnosis |
| 11 Apolipoprotein A-I | Kazemipour et al. [14] | Serum | 13 SLE/ 7 HC | 2-DE, MALDI-TOF/TOF MS | No | SLE diagnosis |
| Apolipoprotein CIII | Morgan et al. [16] | Serum | 7 SLE/ 7 age-, sex- and race-matched HC | 2-DE, LC-MS/MS | Yes | LN diagnosis Increased atherosclerotic risk in LN |
targeted and untargeted approaches. Due to the large number of proteins detected, herein we are discussing the most promising biomarkers for SLE by focusing on two particular aspects. The first aspect includes studies in which proteins were validated in an independent cohort, using either MS or immunobased techniques, and the second includes the studies that had identified common biomarkers. In order to present a more targeted discussion, we divided the validated protein biomarkers into three main categories, based on their association with different clinical aspects of the disease. These categories are as follows: (i) SLE biomarkers, (ii) LN biomarkers and (iii) biomarkers associated with neuropsychiatric SLE (NPSLE).

### SLE biomarkers

The first category includes the most promising biomarkers that are suitable for SLE diagnosis or activity assessment. An increased expression of phosphorylated S100A9 isoforms was detected in the proteome of SLE PBMCs, suggesting abnormal S100A9 signalling, as well as reflecting the increased numbers of circulating low-density granulocytes in these patients [21]. S100A9 is a pro-inflammatory protein, expressed mainly in the cytosol of neutrophils and monocytes [43], and it was suggested that mature neutrophils recruited to the inflammatory sites may result in local S100A9 release, which induces neutrophil degranulation [44]. These activated neutrophils contribute to SLE pathogenesis via many mechanisms, including their ability to form neutrophil extracellular traps (NETs), and produce increased interferon-α [45, 46].

Serine-threonine kinase receptor-associated protein is another important biomarker that was found to be under-expressed in PBMCs of active SLE [22]. STRAP was inversely correlated to SLEDAI, suggesting an association with a favourable clinical course in patients with SLE and could thus be used as a potential biomarker of clinical SLE activity/severity. The mechanisms of action of STRAP in SLE may be attributed to its interaction, either with transforming growth factor-β receptor [47] or with apoptosis signal-regulating kinase 1 [48].

### LN biomarkers

A significant number of biomarkers were found to be associated with LN diagnosis or clinical activity/severity. Five biomarkers for diagnosing LN in kidney biopsies of class IV LN patients including serotransferrin, cytokeratin 18, cytokeratin 19, albumin and annexin A5 were identified [32].

Serotransferrin, an iron-binding transport protein coregulated by interferon-α, plays a role in iron metabolism and the innate immune system [49, 50]. Plasma levels of serotransferrin were associated with SLE disease activity [49] and high urinary levels were associated with paediatric LN activity and severity, suggesting that it may be used as a predictive LN biomarker [51]. More recently, transferrin in combination with other urine biomarkers predicted the decline of renal function in LN patients [52].
Cytokeratins (CKs) are a family of intermediate filament proteins, comprising 20 known CKs, which are classified into type I keratins (K9–K20) or type II keratins (K1–K8) [53]. CKs, particularly cytokeratins 18 and 19, undergo caspase-mediated degradation during apoptosis, in which organized cell fragmentation prevents initiation of inflammatory responses. Thus, programmed destruction of cytokeratin components may affect the sensitivity of the cell to apoptosis [54–56]. CKs have also been associated with other autoimmune diseases. Particularly, increased levels of anti-CX18 and anti-CX19 antibodies were observed in the sera of patients with autoimmune hepatitis [57]. In the kidney, they were recently shown to represent early markers of tubular injury and stress [58].

Annexins are calcium-dependent, phospholipid-binding proteins [59], involved in several cell functions including vesicle trafficking, calcium signalling, cell growth, division and apoptosis [60]. Some of the annexins have anti-inflammatory actions. Annexins A1 and A2 play a crucial role in the phagocytosis of apoptotic lymphocytes, reducing inflammation through the release of immunosuppressive cytokines [61]. Annexins could be used as potential LN biomarkers; however, their implication in LN pathogenesis is not known. Annexin A5 was found to be elevated in kidney biopsy samples of class IV, LN patients [32] and was associated with SLE-related thrombophilia [24]. Heterogeneous transcellular distribution of annexin A5 in patients with SLE, which is increased in PBMCs and decreased in sera, indicated a protective response to SLE-related thrombophilia [24]. Annexins A2 and A5 have a high affinity for phospholipids, which are involved in the regulation of the coagulation cascade. Antibodies against annexins A5 and A2 were detected in thrombotic-associated diseases and other autoimmune diseases besides SLE, such as primary antiphospholipid syndrome and systemic sclerosis [60]. Besides annexin A5, anti-annexin A2 antibodies were also reported as possible biomarkers for the proliferative form of LN (PLN, class III or IV). Serum anti-annexin A2 antibody levels discriminated PLN patients, not only from patients with other autoimmune diseases and healthy controls, but also from patients with a membranous form of LN [11]. Annexin A2 was also identified as a target of serum antibodies in patients with SLE [17]. In addition, studies in LN patients and SLE-prone mice showed that annexin A2 facilitates the binding of anti-dsDNA antibodies to mesangial cells, contributing to the LN pathogenesis [62].

Serum apolipoprotein CIII (apoCIII) was associated with an increased atherosclerotic risk in LN patients [16]. Apo-CIII involved in the regulation of triglyceride level and elevated apo-CIII production is related with hypertriglyceridemia [63]. In this context, increased apo C-III levels in patients with SLE may result from increased plasma very low-density lipoprotein (VLDL) cholesterol and triglycerides and decreased high-density lipoprotein (HDL) cholesterol [16]. Finally, Apo C-III-containing Apo B lipoprotein subclasses were associated with increased atherosclerosis risk, in patients with rheumatoid arthritis [64].

Urinary prostaglandin H₂,D-isomerase (PGDS) was identified as a candidate biomarker for LN activity [30]. PGDS catalyses the conversion of prostaglandin H₂ (PGH₂) to PGD₂, which is implicated in physiological processes such as sleep regulation, prevention of platelet aggregation, allergy and inflammation [65, 66]. Studies on monkeys demonstrated de novo synthesis of PGDS in podocytes, and Bowman’s capsule of the glomeruli [67]. Interestingly, in paediatric SLE patients urinary PGDS was associated with LN activity. However, no comparison with non-LN glomerular diseases was performed [51]. In murine SLE models, elevated levels of urinary PGDS were associated with LN severity [68] and PGDS correlated with glomerular inflammation in adriamycin-induced nephropathy in mice [69].

Hepcidin is a 25-amino acid peptide hormone mainly produced by hepatocytes and a key regulator of systemic iron homeostasis [70, 71]. It is known to be involved in the pathogenesis of the anaemia of chronic inflammation including that of chronic kidney disease [71]. It appears to be associated with proinflammatory cytokines such as interleukin-6 and tumour necrosis factor-α [72, 73], molecules that are known to be implicated in SLE pathogenesis [74, 75]. Hepcidin isoforms were differentially expressed in the urine of LN patients during renal flares, indicating hepcidin as a promising biomarker for the assessment of renal severity in LN patients. Interestingly, it was suggested that hepcidin may be produced within kidney during renal flare, rather than being only filtered [31]. Additionally, serum pro-hepcidin levels were shown to reflect disease activity, in patients with rheumatoid arthritis [76].

**Biomarkers associated with NPSLE**

SLE affects both the central and the peripheral nervous system. NPSLE is a common manifestation of SLE, with a prevalence of 14–80% in adults and 22–95% in children. It has been suggested that NPSLE pathogenesis may be associated with autoantibody-mediated neuronal dysfunction and vasculopathy [77].

Anti-α-GDI antibody was identified as a potential diagnostic biomarker of psychosis-associated NPSLE [15]. α-GDI is a small GTP-binding protein, which is involved in the regulation of vesicle trafficking [78]. In addition, it is a brain-specific antigen, which is localized in neurons [79, 80]. Mutations in the Gdi1 gene that encodes α-GDI were reported in families with X-linked non-specific mental retardation [81]. Experiments in Gdi1-deficient mice demonstrated damage of associative memory as well as changes in social behaviour without any anatomic abnormality [82]. Kimura and colleagues suggested that the function of α-GDI may be prevented by anti-α-GDI antibodies, affecting the exocytosis of synaptic vesicles during neurotransmitter release that is related with psychosis in patients with NPSLE [15]. More recent studies in mice suggest a key role of α1-GDI by specific Ras-related protein in brain (RAB) GTPases acting specifically in forebrain regions at the pre-synaptic sites involved in memory formation [83].

Ubiquitin is a small regulatory protein, member of a family of structurally conserved proteins, which regulate numerous processes in eukaryotic cells. The most significant function of ubiquitin is targeting proteins for degradation [84]. Ubiquitin is part of the ubiquitin-proteasome system, which is involved in several important cellular processes such as regulation of apoptosis, cell cycle progression, cell division, cell development and differentiation, cell trafficking as well as modulation of immune and inflammatory responses [85]. Imbalances in the ubiquitin-proteasome system may lead to systemic autoimmunity and
neurodegenerative disorders [86] and elevated levels of ubiquitin in cerebrospinal fluid (CSF) of NPSLE are associated with disease activity [36]. Studies in other neurological disorders, including Creutzfeldt–Jakob [87] and Alzheimer’s disease [88], demonstrated also elevated ubiquitin levels in the CSF of patients, indicating a possible role of ubiquitin in neural degradation and apoptosis.

**Biomarkers identified in multiple studies**

This systematic review has also identified 11 biomarkers that were detected in at least two independent studies (Table 3). These biomarkers are as follows: annexins A2 and A5, alpha-1-antitrypsin (A1AT), serotransferrin, ezrin, elongation factor-1 gamma (EF-1γ), glyceraldehyde-3-phosphate dehydrogenase, alpha-enolase, haptoglobin, transferrin and apolipoproteins (A-I, CIII, C-I, A-IV) (Table S1). Four of these, namely annexins A2 and A5, serotransferrin and apolipoproteins, were also validated in independent cohorts and were discussed above. It is noteworthy that 5 of the 11, annexins A2 and A5, A1AT, serotransferrin and ezrin were detected in kidney biopsies and it is of interest that these were also present in serum, urine and/or PBMCs, supporting the notion that these biomarkers can be utilized as potential LN diagnostic tools.

In this content, A1AT was found in the urine of active LN and during SLE renal flares [31], as well as in renal tissue of class IV LN patients [32]. A1AT is an acute phase protein and the most prominent circulating protease inhibitor that also plays an important role in regulating immunity, inflammation and apoptosis [89]. Although it is mainly produced in the liver, it was shown that A1AT is also produced in the kidney in response to injury [90] and confers cytoprotective effects [90, 91]. Moreover, it was found in the urine and serum of patients with GN [92] and in the urine of patients with SLE, distinguishing them from patients suffering from other proteinuric diseases [93].

Ezrin was identified as a candidate antigen for serum autoantibodies in proliferative LN forms [11]; it was also found to be upregulated in class IV nephritis kidney tissue [32]. Ezrin is the prototypic member of the ezrin protein subfamily, which serves as a linker between the plasma membrane and the cytoskeleton. Indeed, these proteins act as intracellular scaffolds [94, 95], regulating B- and T-cell activation [96]. A recent study showed that ezrin plays a role in regulating inflammation, via limiting the B-cell IL-10 reduction [97].

Elongation factor 1-alpha 1 (EF-1α) was also a candidate antigen in proliferative LN and was downregulated in PBMCs from patients with SLE, compared with healthy controls [24]. Moreover, elongation factor 1-gamma (EF-1γ) was identified as a target antigen of serum antibodies in patients with RA, polymysitis and healthy controls [17]. Elongation factor 1 (EF1) is a major translational factor that consists of four different subunits, EF-1α, EF-1γ, EF-1ε. Apart of its canonical function, EF-1 is a multifunctional protein that has been implicated in various important cellular processes such as cell growth, signal transduction, cytoskeletal organization apoptosis and tumorigenesis [98].

Another interesting protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was found to be a candidate antigen for antibodies in patients with proliferative LN [11] and in patients with SLE compared with RA, polymysitis and healthy controls [17]. GAPDH was also downregulated in PBMCs from patients with SLE compared with healthy controls [24]. GAPDH is a classical glycolytic enzyme involved in energy production, but it is also implicated in numerous important cellular pathways including receptor-mediated cell signalling, transcriptional and post-transcriptional gene regulation, maintenance of DNA integrity, oxidative stress response and apoptosis [99]. In patients with SLE, GAPDH was found to interact with proliferating cell nuclear antigen, a known autoantigen targeted by antibodies, indicating its possible role in autoimmune responses induction against proliferating cell nuclear antigen complexes in SLE [100].

In addition, alpha-enolase was identified as a candidate antigen in proliferative LN forms [11] and was upregulated in SLE PBMCs compared with healthy controls [24]. Alpha-enolase is a multifunctional glycolytic enzyme that is involved in various biological and pathophysiological processes [101, 102]. Evidence revealed that α-enolase plays a role in systemic autoimmune diseases. Anti-α-enolase antibodies were present in patients with SLE with active LN and other autoimmune diseases such as systemic sclerosis [103]. In addition, anti-α-enolase IgG2 levels were increased in LN serum, enabling the discrimination between SLE patients with LN, SLE patients without LN and patients with rheumatoid arthritis [104].

Transthyretin and haptoglobin (Hp) were upregulated in urine samples from active LN [30] patients, compared with inactive LN and were also be upregulated in the serum proteome of patients with SLE, compared to healthy controls [14]. Transthyretin is a serum and cerebrospinal fluid transporter of the thyroid hormone thyroxine (T4) and retinol [105]. It has been associated with numerous disorders including, familial amyloid cardiomyopathy and senile systemic amyloidosis [106, 107]. A pilot study showed that transthyretin levels were upregulated in the sera of paediatric SLE patients compared to healthy controls [108]. Additionally, increased serum levels of transthyretin were associated with the severity of rheumatoid arthritis, suggesting a role in disease pathogenesis [109]. The main biological role of Hp is to bind haemoglobin, prevent iron loss and subsequent kidney damage during haemolysis. It is also an acute phase protein with antioxidative and immunomodulatory properties. Hp expression was associated with inflammatory autoimmune diseases, including arthritis and SLE, and as a marker of disease activity [110]. Plasma levels of Hp were associated with disease severity in patients with SLE [111]. In addition, Hp2-2 phenotype was found to be over-represented in patients with SLE, and may be contribute to cardiovascular complications in SLE due to its lower antioxidant capacity [111, 112].

**Genome research of lupus**

Finally, advances in genomic technologies during the past decade have enabled the identification of numerous risk genetic factors associated with susceptibility to SLE. To date, more than 60 SLE susceptibility loci have been identified by genome-wide association studies (GWAS) in different population cohorts, including HLA, STAT4 and IRF5. However, risk variants identified so far explain only a small
fraction of the overall SLE heritability [113]. A number of next-genera-
tion DNA sequencing (NGS) methodologies have been used not only
to validate previously identified susceptibility genes and loci associ-
ated with SLE such as IRF2, IRF5, UBE2L3, IFIHI, TNIP1, TNFAIP3
and BLK, but also enabled the discovery of additional gene variants,
especially rare variants that are not identified by GWAS [114, 115].
Although the genetic aspects of SLE are beyond the scope of this
review, we attempted to correlate the protein biomarkers summarized
in this review with susceptibility genes identified using high-through-
put genomic methodologies such as NGS and GWAS [113–116].
Overall, no direct associations were found linking proteins or
biomarkers as described in this review to their corresponding genes
or loci. This may be partly explained by the fact that most of GWAS
identified susceptibility loci are located within non-coding DNA
regions. Evidently these have no apparent role in encoding proteins,
suggesting a possible regulatory role of these variants in protein dys-
function and consequently in disease pathology [117]. In addition,
epigenomics may explain part of the missing heritability [118].
Recently, the term ‘proteogenomics’ has been introduced, which is an
area of research at the interface of genomics and proteomics,
with great potential towards the discovery of biomarkers for many
diseases and in particular SLE [119, 120].

Study limitations

An ideal SLE biomarker would be biologically and pathophysiologi-
cally relevant, reproducible, simple to apply in routine practice (inexp-
ensive, easy and rapid to quantify) and would have a high degree of
sensitivity and specificity [121]. At present, no SLE biomarker exists
that fulfills all of the above. It is appreciated that some of the above-
described candidate biomarkers are non-specific stress proteins,
linked with a multitude diseases and conditions. One of the main limi-
tations of the majority of the reviewed studies was the small sample
size and lack of disease control groups. Other limitations that
emerged include the absence of details about the ethnic group and
age of the subjects as well as lack of validation of the results. In addi-
tion, although a number of studies validated their results in an inde-
pendent cohort, the size of the cohort used for the validation was not
always sufficient. Finally, bias due to the absence of standardized pro-
tocol for preparing and presenting patient samples constitutes
another important limitation, showed by most of the reviewed
studies.

Future prospects

Although a significant number of SLE biomarker reports have been
published to date, there are still many challenges that need to be
overcome in future proteomic studies that aim to identify clinically
useful SLE biomarkers. Firstly, differences in proteomic results
across different studies can be attributed to differences in the
selection criteria of the samples. A recent published study uncov-
ered the molecular heterogeneity of SLE, providing an explanation
for the failure of the clinical trials [122]. Thus, there is a need to
establish universally accepted sample selection criteria in order to
better streamline phenotype-genotype correlations and make
results across different proteomic studies more comparable. Future
studies need to recruit a larger number of patients. Secondly, MS
identified SLE biomarkers should be validated in multicentre studies
using standardized immunobased proteomic techniques or other
MS methodologies. In addition, future proteomic studies should
focus on biomarkers that have already been identified in multiple
studies and in several, invasive as well as non-invasive specimen
types. Due to the heterogeneous nature of SLE, it is more likely
that a panel of proteomic biomarkers rather than a single protein
will be needed by the physicians, for SLE diagnosis and treatment.
Therefore, studies working with panels of biomarkers that are
involved in biologically relevant pathways may be more meaningful
and more substantial than studies focus on single biomarkers.
Finally, although promising panels of biomarker protein peaks have
been identified, the exact identity of the detected proteins is still
not known.

Conclusions

Advances in high-throughput MS technologies have undoubtedly cre-
ated new avenues for discovering sensitive and specific SLE biomark-
ers. MS-based proteomics have been used to study a plethora of
biological specimens from patients with SLE, leading to the identifica-
tion of biomarkers related to disease diagnosis and activity as well as
to specific organ involvement. There are already a number of valuable
MS-based proteomic studies in the literature that fulfill most of the
requirements for clinical proteomic biomarker reporting. A significant
number of potential biomarkers have been identified to be associated
with many clinical aspects of SLE, including diagnosis, disease acti-
ity and prognosis. It is noteworthy that almost half of these studies
have validated their results in an independent cohort. What is lacking
and should be addressed in future biomarker studies, is the use of
larger patient cohorts, as well as the validation of already identified
biomarkers in independent patient cohorts. Furthermore, protein
biomarkers, which have been already identified in multiple studies,
particularly those that were also detected in non-invasive biological
samples, hold a great promise. Such biomarkers should form the
backbone and be at the forefront of executing larger multicentre stud-
ies in future using well-characterized patient cohorts, in order to
prove their clinical utility.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

The manuscript was written through contributions of all authors.
All authors have given approval to the final version of the manu-
script.
Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Flow diagram showing the systematic literature search and review process. All methods were applied in accordance with the PRISMA guidelines.

Table S1. Requirements for scientific reporting of proteomic biomarker data reported by Mischak et al. 2010 implemented for the 25 articles obtained in the systematic review.

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