Candidate proteomic biomarkers in systemic sclerosis discovered using mass-spectrometry: an update of a systematic review (2014–2020)

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Background. Systemic sclerosis (Ssc) is an autoimmune disease characterized by graduate cutaneous and tissue fibrosis development and irreversible fibroproliferative vascular changes.

The aim of the current systematic review was to update the list of proteomic candidate biomarkers identified from Ssc samples with mass spectrometry techniques.

Methods. Medline and Scopus databases were searched on 1st September 2020. Relevant articles were searched from March 2014 until September 2020. Two independent reviewers evaluated the retrieved articles.

Results. From a total of 97 articles, 9 articles were included in the final analysis summarizing 539 candidate proteomic biomarkers from various samples from Ssc patients (a larger number compared to the previous systematic review). Most biomarkers were identified from cutaneous biopsies. Only 5 articles included a validation step of the findings with only 13 biomarkers being validated.

Conclusions. Although many candidate biomarkers were additionally identified, independent validation studies are needed in order to evaluate the importance of these biomarkers for Ssc patients.

Key words: systemic sclerosis, biomarker, proteomic, mass spectrometry, validation.

What is new? What is important?

The current work presents an update for candidate proteomic biomarkers for Ssc patients identified using mass spectrometry techniques. 539 biomarkers were identified. Only a small number of biomarkers have been validated on independent cohorts and future confirmation studies are mandatory in order to evaluate their diagnostic/prognostic capacity.

INTRODUCTION

Systemic sclerosis (Ssc) is an autoimmune disease characterized by graduate cutaneous and tissue fibrosis development and irreversible fibroproliferative vascular changes [1]. Scientific community is focusing its efforts in order to discover relevant biomarkers for diagnosis, prognosis and therapeutic response of such patients. However, this task is not easy as it is rather difficult to find a highly specific and sensitive biomarker or biomarker panel due to the great variability of the disease. Biomarkers have several characteristics that should be always taken into account: intra- or inter-individual variability, validity and most importantly they should be practical [2]. Therefore, validation studies of candidate biomarkers that test all these aspects are

crucial. Proteomic biomarkers have the advantage to be easily quantified in routine clinical laboratory setting using immune-based reactions (ELISA) as they do not need expensive equipments, nor intensive personnel training [3]. Candidate proteomic biomarkers can be identified using mass spectrometry techniques. However, this is only the first step, as multiple confirmatory independent studies using antibody based assays are needed in order to confirm and validate the candidate proteomic biomarker in the clinical setting.

The aim of the current systematic review was to update the list of proteomic candidate biomarkers identified with mass spectrometry techniques from Ssc patient's samples. Previous list of candidate biomarkers was published in 2014 and comprised articles indexed in Medline from inception until

February 2014 [4]. Such list could help the scientific community to identify relevant biomarkers that should be confirmed and validated on independent Ssc cohorts.

MATERIAL AND METHODS

A comprehensive research strategy (Table 1) was applied in two medical databases: Medline and Scopus on 1st September 2020. Relevant articles were searched from March 2014 until September 2020. Only articles in English language were included in the analysis. Two independent reviewers (PB and AB) evaluated the title and abstracts of retrieved articles and decided about inclusion/exclusion of articles. Where there were discrepancies between decision of inclusion/exclusion, the two reviewers discussed and reached consensus. Letters to editor, review articles, editorials and case reports were excluded. Also studies performed on animal models were excluded from the analysis. Only articles that

analyzed various biological samples from Ssc patients using mass spectrometry techniques were included. Eligible articles were analyzed full text and data was extracted by the two independent reviewers in a database. The database contained the bibliographic reference of the article, mass spectrometry technique used for analysis, number of Ssc patients and controls, sample type, proteomic biomarkers that were identified, and also fold changes compared to controls (if given). In addition, if the article also comprised a validation cohort for the identified biomarkers, these details were also included in the analysis. In the end, quality of reported data from the studies was evaluated using criteria described previously by Mischak *et al.*, 2010 [5]. They refer to justification of clinical question, assessing of subject description, sampling description, clear methodological description, presentation of statistical approach for the data analysis, validation of findings, limitation acknowledgments and also clearly statement of each author contribution.

Table 1

Research strategy for inclusion of relevant articles

<p>Medline search strategy ((((("scleroderma, systemic"[MeSH Terms] OR (((("scleroderma, systemic"[MeSH Terms] OR ("scleroderma"[All Fields] AND "systemic"[All Fields])) OR "systemic scleroderma"[All Fields]) OR ("systemic"[All Fields] AND "sclerosis"[All Fields])) OR "systemic sclerosis"[All Fields])) OR ("scleroderma, systemic"[MeSH Terms] OR "scleroderma, localized"[MeSH Terms])) OR (((((((("scleroderma, systemic"[MeSH Terms] OR ("scleroderma"[All Fields] AND "systemic"[All Fields])) OR "systemic scleroderma"[All Fields]) OR "scleroderma"[All Fields]) OR "scleroderma, localized"[MeSH Terms]) OR ("scleroderma"[All Fields] AND "localized"[All Fields])) OR "localized scleroderma"[All Fields]) OR "sclerodermas"[All Fields])) AND (((("mass spectrometry"[MeSH Terms] OR ("mass"[All Fields] AND "spectrometry"[All Fields])) OR "mass spectrometry"[All Fields]) OR ("2D"[All Fields] AND (((("electrophoresed"[All Fields] OR "electrophoresing"[All Fields]) OR "electrophoresis"[MeSH Terms]) OR "electrophoresis"[All Fields]) OR "electrophorese"[All Fields]) OR "electrophoreses"[All Fields])) OR (((("proteomics"[MeSH Terms] OR (((((((("proteom"[All Fields] OR "proteome"[MeSH Terms]) OR "proteome"[All Fields]) OR "proteomes"[All Fields]) OR "proteomical"[All Fields]) OR "proteomically"[All Fields]) OR "proteomics"[MeSH Terms]) OR "proteomics"[All Fields]) OR "proteomic"[All Fields])) OR "proteome"[MeSH Terms]) OR (((((((("proteom"[All Fields] OR "proteome"[MeSH Terms]) OR "proteome"[All Fields]) OR "proteomes"[All Fields]) OR "proteomical"[All Fields]) OR "proteomically"[All Fields]) OR "proteomics"[MeSH Terms]) OR "proteomics"[All Fields]) OR "proteomic"[All Fields])))) AND 2014/01/01:2020/12/31[Date - Publication]) NOT (((("amyotrophic lateral sclerosis"[MeSH Terms] OR ((("amyotrophic lateral sclerosis"[MeSH Terms] OR ((("amyotrophic"[All Fields] AND "lateral"[All Fields]) AND "sclerosis"[All Fields])) OR "amyotrophic lateral sclerosis"[All Fields])) OR ((("multiple sclerosis"[MeSH Terms] OR ("multiple"[All Fields] AND "sclerosis"[All Fields])) OR "multiple sclerosis"[All Fields])) OR "multiple sclerosis"[MeSH Terms]))</p>
<p>Scopus search strategy PUBYEAR > 2014 TITLE-ABS-KEY (systemic AND sclerosis OR scleroderma) AND mass AND spectrometry OR 2d AND electrophoresis OR proteomics OR proteome NOT mice OR animal.</p>

RESULTS

The search strategy retrieved 97 articles (70 indexed in Medline and 27 indexed in Scopus). After removal of 8 duplicates, a total number of 89 articles were included for the preliminary analysis. 21 articles were reviews articles and were excluded from the analysis. Also 4 editorials were excluded from the analysis. Two articles in Japanese and one article in Czech were also

excluded. 61 articles had their abstract and titles analyzed and among these 13 were included for full text analysis. After full text analysis, two articles were excluded due to the fact that they did not present exploratory proteomic studies, and two articles used SOMASCAN method. Therefore in the end 9 articles had their information retrieved in the database (Figure 1) [6–14].

Figure 1. Flowchart of the systematic review.

Patients and control groups

In total, 162 Ssc patients were recruited. In all studies, healthy controls were included. Most studies included healthy matched controls (for gender and age (8/9 studies) and one study clearly stated that healthy controls were not matched with Ssc

patients [12]. Matching is very important because some biomarkers are known to have gender and age variation and differences can rise only from the fact that controls were not matched with Ssc cases. In one study [7], healthy controls were further divided into smoker and non-smokers (as biological sample analyzed in this particular study was

BALF (bronchoalveolar lavage fluid), and thus smoking status is an important confounder due to the secondary inflammation caused by cigarette smoke).

Biological samples

Table 2 summarizes data extracted from the 9 articles included in the final analysis. As observed, most studies explored proteomics from biopsy of affected Ssc tissues (4/9 studies). 3 of these studies

evaluated modifications of the proteome from skin biopsies, and one study evaluated pulmonary biopsies from patients with ILD in Ssc. Two studies evaluated modifications from plasma samples, while only one study included BALF. Two studies evaluated “in vitro” changes of the fibroblast proteome (cultured from patient skin and pulmonary samples). These studies were also included in the analysis due to the fact that they could pinpoint relevant “in vivo” biomarkers that are related with fibroblasts, the key cell in Ssc pathogenesis.

Table 2

Summarization of included articles (biological sample, sample size and proteomic technique)

Reference	Biological sample	Sample size	Proteomic technique used
Ryu <i>et al.</i> , [6]	Plasma	14 Ssc with ILD /10 healthy controls	LC-MS/MS
Landi <i>et al.</i> , [7]	Bronchoalveolar lavage fluid	7 Ssc/ 10 healthy non-smoker controls/8 healthy smoker controls	2DE, MALDI-TOF/TOF
Corralo <i>et al.</i> , [8]	Skin biopsy	5 lcSsc/ 5 healthy controls	2 DE,MALDI-TOF/TOF
van Bon <i>et al.</i> , [9]	Plasma	67 lcSsc and 33 dcSSc/19 healthy controls	SELDI-TOF-MS
Chairta <i>et al.</i> , [10]	Skin biopsy	7 Ssc patients 2 biopsies/each patient - from affected and unaffected	LC-MS/MS
Mullenbrock <i>et al.</i> , [11]	Pulmonary myofibroblast cell culture supernatant	10 pulmonary fibrosis /10 Ssc patients /10 healthy controls	LC-MS/MS
Chaigne <i>et al.</i> , [12]	Cutaneous fibroblast cell culture supernatant	4 dSsc/3 unmatched healthy controls	2DE-DIGE/MALDI-TOF MS
Dumi <i>et al.</i> , [13]	Skin biopsy	3 Ssc patients/3 healthy controls	2DE, LC-MS/MS
Schiller <i>et al.</i> , [14]	Pulmonary biopsy	45 endstage Ssc with ILD/ 10 healthy controls	LC-MS/MS

ILD – interstitial lung disease, dSsc – diffuse systemic sclerosis, lSsc – localized systemic sclerosis, 2DE – two dimension electrophoresis, 2DE-DIGE – two-dimension electrophoresis fluorescence difference gel electrophoresis, MALDI – Matrix-assisted laser desorption/ionization, LC-MS/MS – Liquid chromatography-mass spectrometry, TOF – Time of flight, SELDI-TOF-MS – surface enhanced laser desorption/ionization-time of flight mass spectrometry.

Proteomic technique

Most studies used either 2 dimension electrophoresis or 2D DIGE as first step for protein separation. Spots and fractions were separated and compared using statistical measures with control group, thus identifying relevant candidate spots. For identification of spots, several mass spectrometry techniques were used (MALD-TOF, SELDI-TOF or LC-MS/MS). Most frequent used mass spectrometry technique was LC-MS/MS (5/9 studies).

Candidate proteomic biomarkers

Table 3 summarizes proteomic biomarkers found. In total, 539 candidate proteomic biomarkers from various samples of Ssc patients were identified being up- or downregulated compared to control group. Among these, 247 were downregulated, and 286 were upregulated. 2 proteins were either upregulated or downregulated (**alpha 1 antitrypsin** and **apoptosis**

regulator BAX). For the rest of four proteins, the expression was unknown [8]. Most biomarkers were identified from biopsy samples (320 from cutaneous samples, and 44 from pulmonary samples). 86 biomarkers were identified from fibroblast culture supernatant. 60 candidate biomarkers were found from plasma, and finally only 29 biomarkers were identified from BALF.

Quality assessment of reported data

All articles included in the final analysis presented good quality data, reporting most of criteria suggested by Mischak *et al.* (Table 4). However, some reporting criteria missed in a significant number of included articles. As in the previous systematic review, the most unfulfilled criteria were the last three (validation, limitation acknowledgement and clear specification of author contribution to the manuscript).

Table 3

Proteomic biomarkers identified using mass spectrometry studies in the literature. NSC-non smoking control, SC-smoking control

Article and biological sample	Biomarkers identified
<p>Ryu <i>et al.</i> [6] 38 biomarkers (6 downregulated, 32 upregulated) Plasma</p>	<p>Myelin basic protein (-4.05), Solute carrier family 35-member E2B (-2.17), Carboxypeptidase B2 (-1.83), Plasma serine protease inhibitor (-1.44), Protein BCAP (-1.27), Tallin-1 (-0.91), Complement C2 (0.67), Fibrinogen gamma chain (0.73), Fibrinogen beta chain (0.73), Fibrinogen alpha chain (0.81), Alpha-1-antichymotrypsin (0.83), Complement factor B (1.00), Immunoglobulin heavy variable 3-49 (1.03), Vitamin K-dependent protein S (1.04), Immunoglobulin lambda constant 2 (1.06), Serum albumin (1.06), Immunoglobulin heavy variable 4-28 (1.07), Alpha-1-antitrypsin (1.08), Inter-alpha-trypsin inhibitor heavy chain H3 (1.12), Immunoglobulin kappa light chain (1.12), Immunoglobulin kappa variable 1D-8 (1.13), Immunoglobulin lambda constant 7 (1.18), Immunoglobulin kappa variable 4-1 (1.22), Alpha-1-acid-glycoprotein 1 (1.25), serotransferrin (1.26), Immunoglobulin kappa variable 1-8 (1.27), Alpha-2-macroglobulin (1.31), Fibronectin (1.62), Alpha-1B-glycoprotein (1.63), Leucine-rich-alpha-2-glycoprotein (1.73), Immunoglobulin heavy variable 4-30-2 (1.75), Immunoglobulin lambda variable 3-19 (1.90), Immunoglobulin heavy constant alpha-1 (2.05), Immunoglobulin heavy variable 1-24 (2.47), Immunoglobulin kappa variable 1D-33 (2.53), Immunoglobulin heavy variable 3-64 (2.62), Sex hormone binding globulin (2.86), Pulmonary surfactant-associated protein B (4.89)</p>
<p>Landi <i>et al.</i>, [7] 29 biomarkers (21 upregulated, 7 down regulated, 1 protein - alpha 1 antitrypsin - upregulated in NSC vs SC and downregulated in SSc vs NSC group). Bronchoalveolar lavage fluid</p>	<p>Alpha-1-antichymotrypsin (-28.2 NSC vs SC), Alpha 1 antitrypsin (25.8 NSC vs SC; -22.41 SSc vs NSC), Ig alpha-2 chain C region (-34 SSc vs SC), Immunoglobulin J chain (26.43 SSc vs SC), Pulmonary surfactant-associated protein A2 (26.5 SSc vs NSC, SSc vs SC), Alpha-2-HS-glycoprotein (21.97 SSc vs NSC), Complement C3 alpha chain (-32.8 SSc vs SC), Complement Factor H (33.07 SSc vs NSC), Protein S100A6 (21.81 SSc vs NSC), Antithrombin-III (26.8 NSC vs SC), Angiotensinogen (22.9 SSc vs SC), Apolipoprotein AI (30.5 SSc vs NSC), Haptoglobin (36.36 SSc vs SC), Transferrin (32.29 SSc vs NSC), 32.66 SSc vs SC), Fatty acid-binding protein (23.71 SSc vs SC), Zinc-alpha-2-glycoprotein (24.39 SSc vs NSC, 22.16 SSc vs SC), Retinol-binding protein 4 (33.57 SSc vs NSC), 14-3-3 protein epsilon (18.93 SSc vs NSC), Selenium-binding protein (-31.55 SSc vs SC), Calcyphosin (27.93 SSc vs NSC, 24.18 SSc vs SC), Peroxiredoxin-1 (-30.5 NSC vs SC), Glutathione S-transferase P (29 NSC vs SC), Ceruloplasmin (28.71 SSc vs NSC, 25.89 SSc vs SC), Albumin (-29.8 SSc vs NSC, 23.86 SSc vs SC), Albumin C-term (18.14 SSc vs NSC, 21.9 NSC vs SC), Macrophage mannose receptor 1 (25.71 SSc vs NSC), Beta-2 microglobulin (24.76 SSc vs NSC), Lysozyme C (19.59 SSc vs NSC), Serpin B3 (19.64 SSc vs NSC)</p>
<p>Corallo <i>et al.</i>, [8] 37 biomarkers (26 upregulated, 7 downregulated, 4 unknown = present) Skin biopsy</p>	<p>ATP synthase subunit α, mitochondrial (-3.48), L-lactate dehydrogenase A chain (+2.13), Fructose-bisphosphate aldolase A (+8.44), Pyruvate kinase (-2.00), Phosphoglycerate kinase 1 (+4.50), Triosephosphate isomerase (+2.05), Transketolase (+2.24), Sodium/potassium-transporting ATPase subunit α-3 (+2.14), Ferritin light chain (+3.83), Rab GDP dissociation inhibitor β (present), Elongation factor 1-beta (+2.39), Elongation factor 2 (+2.20), Proteasome activator complex subunit 1 (+2.37), Calreticulin (present), Peptidyl-prolyl cis-trans isomerase A (+7.12), Heat shock cognate 71 kDa protein (+2.19), Peroxiredoxin-1 (present), Peroxiredoxin-6 (+2.40), Endoplasmic reticulum chaperone [Cu-Zn] (present), Superoxide dismutase [Mn] mitochondrial (-4.15), Glutathione S-transferase omega-1 (2.34), S-formylglutathione hydrolase (-3.03), Collagen α-1(I) chain (+2.84), Collagen α-2(I) chain (+47.72), Collagen α-1(VI) chain (-2.73), Prolyl 3-hydroxylase 3 (3.93), Calpain small subunit 1 (2.06), F-actin-capping protein subunit α-1 (-2.75), Gelsolin (+2.27), Tubulin β chain (+5.65), Cofilin-1 (+4.80), Vinculin (+4.87), Transgelin (+2.06), Vimentin (+2.45), Tropomyosin α-4 chain (+2.07), Galectin-1 (+2.00)</p>
<p>van Bon <i>et al.</i> [9] 22 biomarkers (8 downregulated, 13 upregulated, 1 upregulated and also downregulated = apoptosis regulator BAX) Plasma</p>	<p>IL-8 (-5.80), CXCL7 (-5.39), Cathepsin family (4.02), Platelet factor 4 variant, CCL4, CCL5, CXCL2 (2.11), beta-defensin 116 (-2.87), atrial natriuretic factor (-2.28), TNF alpha (2.24), Hepsidin 25 (-18.66), neutrophil defensin 4 (2.47), Apoptosis regulator BAX (1.17 and -1.64), Chymotrypsin family (2.17), Apolipoprotein CIII (2.86), Chymotrypsin family, minor HC antigen HB-1 (2.54), complement C1q (1.54), Neutrophil defensin 5 (3.13), Neutrophil defensin 3 (2.53), Pancreatic prothormone (3.69), PENK-A (-4.00), Platelet factor 4 variant, IGF1 (-2.20), S100A8 S100A9 and other members S100 family (4.06), Interferon-α-inducible protein 27-like protein 1 (-1.57), β-defensin 133, LL37 (1.62)</p>
<p>Mullenbrock <i>et al.</i> [11] 44 biomarkers (12 soluble upregulated, 14 insoluble upregulated, 14 soluble downregulated soluble, 4 insoluble downregulated) Skin biopsy</p>	<p>prolyl 4-hydroxylase subunit alpha3, cysteine-rich with EGF like domain protein 1, SERPING 1, ADMTS like protein 5, SLC4A4, hepatocyte growth factor, peroxidasin, elastin microfibril interfacer 2, SERP1-secreted frizzled related protein 1, Annexin A6, S100A10, TGF beta, tenascin C, heme binding protein 2, Host cell factor 1, neuropilin, intersectin 1, S100A6, lysyl oxidase, PLOD2, ADMATS like protein 4, microfibril associated protein 4, galectin 8, matrilin 2, plexin domain containing 2, tissue type plasminogen activator, dishevelled segment polarity protein 3, insulin like growth factor binding protein 7, lumican, perostin, TIMP3, matrix remodelling associated 5, collagen 5 alpha 1 chain, collagen 8 alpha 1 chain, ADAM 10, follistatin like protein 1, Inter-alpha trypsin inhibitor heavy chain 5, prolyl 4-hydroxylase subunit alpha 2, thrombospondin type 1 domain containing 4, osteonectin, leprecan, proline and arginine rich end leucine repeat protein, extracellular matrix protein 1, Decorin, Lysyl oxidase like 1, Wnt5A, EGF-like repeats and discoidin Domains 3, Growth arrest specific 6, Collagen 10 alpha 1 chain, Annexin A7, Fibrillin 2, Fibulin 5, fibulin 1, EGF containing fibrillin extracellular matrix protein, Latent Transforming growth factor beta binding protein 1, coagulation factor XIII A chain, Insulin like growth factor binding protein 5, Matrix remodelling associated 5, Gremlin 1</p>

<p>Chaigne <i>et al.</i> [12] 42 biomarkers (17 upregulated, 25 downregulated) Cutaneous fibroblast cell culture supernatant</p>	<p>Myosin-9 & Talin-1 (+1.64), Myosin-9 (+2.15), Talin-1 (+1.66), Myosin-9 (+1.68), Ras GTPase-activating-like protein IQGAP1 (-1.73), Hypoxia up-regulated protein 1 & Collagen alpha-1(VI) chain (+1.39), Hypoxia up-regulated protein 1 (+1.42), Protein phosphatase 1 regulatory subunit 12A & Integrin beta-1 (-1.51), Microtubule-associated protein 4 (-1.45), Leucine-rich PPR motif-containing protein (-1.32), Integrin alpha-V(-1.41), Neuroblast differentiation-associated protein AHNAK (-1.49), Ankyrin-1 heavy chain & Ubiquitin-like modifier-activating enzyme 1 & Major vault protein (-1.63), Neutral alpha-glucosidase AB & Hepatocyte growth factor-regulated tyrosine kinase substrate (+1.48), Alpha-actinin-4 & Endoplasmic (+1.75), LIM domain and actin-binding protein 1 (+1.48), Adseverin & LIM domain and actin-binding protein 1 (+1.94), X-ray repair cross-complementing protein 5 & LIM domain and actin-binding protein 1 (+1.52), Lysosomal alpha-glucosidase & Eukaryotic translation initiation factor 4B (-1.58), Zyxin & Threonine--tRNA ligase (+1.52), Glutamine--fructose-6-phosphate aminotransferase (+1.49), Threonine--tRNA ligase & Caldesmon (+1.64), Caldesmon (+1.81), Peptidyl-prolyl cis-trans isomerase FKBP9 (-1.61), Peptidyl-prolyl cis-trans isomerase FKBP9 (-1.43), Prelamin-A/C & Moesin (+1.35), TRIO and F-actin-binding protein (+2.86), Phosphoacetylglucosamine mutase (+1.49), Cytosol aminopeptidase (+1.38), Vimentin & Ribonuclease inhibitor (-1.46), Gamma-enolase & Vimentin (-1.57), Activating signal cointegrator 1 complex subunit 1 (+1.64), N(G),N(G)-dimethylarginine dimethylaminohydrolyase 1 (-1.3), 26S proteasome non-ATPase regulatory subunit 7 & Annexin A2 (-1.34), Aflatoxin B1 aldehyde reductase member 2 (+1.75), Beta-lactamase-like protein 2 & 40S ribosomal protein S3 (-1.58), Proteasome activator complex subunit 2 (-1.39)</p> <p>Aldehyde Dehydrogenase 1 Family Member A2 (0.42); ATP Binding Cassette Subfamily C Member 12 (2.64); Acyl-CoA Thioesterase 13 (0.52); Actin Alpha 2, Smooth Muscle (1.97); Actinin Alpha 1 (2.02); Angiotensinogen (1.53); Alpha 2-HS Glycoprotein (1.83); Aldo-Keto Reductase Family 1 Member C3 (0.60); AKT Serine/Threonine Kinase 3 (1.65); Aldehyde Dehydrogenase 3 Family Member A1 (1.62); Aldolase, Fructose-Bisphosphate C(1.83); Ankyrin Repeat Domain 18B (3.29); Adaptor Related Protein Complex 2 Subunit Alpha 1 (0.56); Amyloid P Component, Serum (2.58); Apolipoprotein D (2.55); ADP Ribosylation Factor Like GTPase 6 Interacting Protein 5 (0.66); Ankyrin Repeat And SOCS Box Containing 17 (1.80); ATPase Na+/K+ Transporting Subunit Alpha 3 (1.95); TPase H+ Transporting V0 Subunit D1 (0.59); Complement C4B (1.82); Complement Factor H Related 5 (1.76); Cofilin 2 (2.18); Clusterin (1.96); Cytochrome C Oxidase Subunit 6B1 (5.26); Carboxypeptidase N Subunit 2 (1.66); Cytochrome C1(0.58); Cytochrome P450 Family 4 Subfamily F Member 22 (0.59); Drebrin 1 (1.90); Dermcidin (1.66); DEAD-Box Helicase 55 (3.33); Deoxyribose-Phosphate Aldolase (0.59); Dehydrogenase/Reductase 7 (0.51); DEAH-Box Helicase 15 (0.61); Dermatopontin (1.88); Dynein Cytoplasmic 1 Light Intermediate Chain 2 (1.55); Eukaryotic Translation Initiation Factor 3 Subunit L (0.38); EYA Transcriptional Coactivator And Phosphatase 3 (4.18); Fibrillarin (0.62); Fibulin 1 (1.76); Fibromodulin (2.08); Formimidoyltransferase Cyclodeaminase (2.27); FTO Alpha-Ketoglutarate Dependent Dioxygenase (2.47); FUS RNA Binding Protein (0.63); G3BP Stress Granule Assembly Factor 1 (0.58); Glucosidase II Alpha Subunit (0.61); Glial Fibrillary Acidic Protein (1.68); G Protein Subunit Alpha 14 (1.86); Guanylate Kinase 1 (0.62); H1.0 Linker Histone (0.56); H2A.X Variant Histone (0.48); MacroH2A.1 Histone (0.58); H1.5 Linker Histone, Cluster Member (0.60); H1.4 Linker Histone, Cluster Member (0.56); H2B Clustered Histone 15 (0.50); H3 Clustered Histone 1 (0.46); Major Histocompatibility Complex, Class I, A (0.55); Major Histocompatibility Complex, Class I, G (2.08); Heterogeneous Nuclear Ribonucleoprotein A1 Like 2 (0.54); Heterogeneous Nuclear Ribonucleoprotein C (0.62); Haptoglobin (1.60); Hydroxysteroid 17-Beta Dehydrogenase 12 (0.37); Heat Shock Protein Family A (Hsp70) Member 4 (1.86); Intercellular Adhesion Molecule 1 (1.69); IQ Motif Containing GTPase Activating Protein 1 (2.04); Integrin Subunit Alpha 5 (2.10); Integrin Subunit Beta 1 (2.19); Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (1.62); Jumonji Domain Containing 7 (0.45); KN Motif And Ankyrin Repeat Domains 2 (1.64); Lysine Acetyltransferase 8 (2.37); Ladinin 1 (0.51); LanC Like 1 (0.59); Microtubule Associated Protein 4 (1.50); Methionyl-tRNA Synthetase 1 (0.59); Melanoma Cell Adhesion Molecule (2.15); MutL Homolog 3 (1.88); MOB Family Member 4, Phocin (0.58); MORC Family CW-Type Zinc Finger 2 (1.97); Myosin Light Chain Kinase (2.41); NCCRP1, F-Box Associated Domain Containing (0.53); NADH:Ubiquinone Oxidoreductase Subunit A13 (0.41); Nectin Cell Adhesion Molecule 1 (0.65); N-Myristoyltransferase 2 (0.44); Pericentriolar Material 1 (2.98); Progesterone Receptor Membrane Component 1 (2.45); Pleckstrin Homology Like Domain Family B Member 2 (0.42); Plastin 3 (1.51); Peptidylprolyl Isomerase D (2.50); Prostaglandin I2 Synthase (0.44); Purine Rich Element Binding Protein B (0.64); RAB3D, Member RAS Oncogene Family (1.64); Ribosomal Protein L13 (0.63); Ribosomal Protein L17 (0.57); Ribosomal Protein L23 (0.51); Ribosomal Protein L24 (0.53); Ribosomal Protein L30 (0.65); Ribosomal Protein L6 (0.54); Ribosomal Protein L7 (0.52); Ribosomal Protein L9 (0.50); Ribosomal Protein S13 (0.52); Ribosomal Protein S16 (0.53); Ribosomal Protein S23 (0.36); Ribosomal Protein S3A (0.58); Ribosomal Protein S5 (0.56); RNA Transcription, Translation And Transport Factor (2.39); SEC13 Homolog, Nuclear Pore And COPII Coat Complex Component (1.51); Sec23 Homolog A, COPII Coat Complex Component (0.56); Serpin Family B Member 1 (0.38); Serpin Family B Member 3 (0.63); Serpin Family C Member 1 (1.70); Sideroflexin 3 (0.67); Solute Carrier Family 25 Member 24 (0.57); Solute Carrier Family 25 Member 5 (0.56); Small Nuclear RNA Activating Complex Polypeptide 3(0.60); Small Nuclear Ribonucleoprotein Polypeptide G(0.60); Transcriptional Adaptor 2A (1.51); Transferrin (1.52); Triosephosphate Isomerase 1 (1.71); Tropomyosin 1 (2.30); Tropomyosin 2 (2.07); Tropomyosin 3 (2.06); Tropomyosin 4 (1.82); Ts Translation Elongation Factor, Mitochondrial (0.35); Tetraatricopectide Repeat Domain 9 (1.79); Twinkle MtDNA Helicase (2.00); Thioredoxin Domain Containing 17 (1.62); Ubiquitin C-Terminal Hydrolase L1 (2.52); Ubiquitin Specific Peptidase 31 (0.65); Ubiquitin Specific Peptidase 5 (1.83); Voltage Dependent Anion Channel 2 (1.54); Vitronectin (1.73);</p>
<p>Charta P <i>et al.</i>, [10] 132 biomarkers (69 upregulated; 63 downregulated) Skin biopsy</p>	

<p>Dumit <i>et al.</i> [13] 151 biomarkers (82 downregulated, 69 upregulated) Skin biopsy</p>	<p>Jun B proto-oncogene (-1.03); Protein FAM101B (-0.94); EPB41L2 protein variant (-0.93); Tropomyosin alpha-1 chain (-0.91); HEAT repeat-containing protein 5A; cDNA FLJ51408 (-0.77); 130 kDa GBBP-interacting protein; 90 kDa GBBP-interacting protein; Filamin A-interacting protein 1-like (-0.74); mitochondrial 3,2-trans-enoyl-CoA isomerase (-0.73); Signal transducer and activator of transcription 5B protein (-0.72); 40S ribosomal protein S19-binding protein 1 (-0.66); Barrier-to-autointegration factor; Breakpoint cluster region protein 1 (-0.66); Erythrocyte membrane protein band 4.1-like 2; 4.1G protein (-0.66); 7-dehydrocholesterol reductase (-0.63); CD95 antigen (-0.61); Uncharacterized protein KIAA0754 (-0.61); CF-1 64 kDa subunit tau variant (-0.59); Enapin (-0.59); Protein mago nashi homolog 2 (-0.54); Drebrin (-0.52); Inner nuclear membrane protein Man1 (-0.52); Pre-mRNA-splicing factor SRP75 (-0.51); Pleckstrin homology-like domain family B member 1 (-0.51); GMP kinase (-0.49); Glycine-rich RNA-binding protein CIRP; (-0.48); Nucleoporin Nup107 (-0.47); Aspartate aminotransferase, (-0.46); Nuclear fragile X mental retardation-interacting protein 2 (-0.45); 39S ribosomal protein L34, mitochondrial (-0.45); BRCA1-A complex subunit MERIT40 (-0.45); Coiled-coil-helix-coiled-coil-helix domain-containing protein 1; Nuclear protein C2360 (-0.44); Kindlin-2 (-0.43); Phosphatidylinositol-4-phosphate 5-kinase, type II, alpha (-0.43); Probable O-sialoglycoprotein endopeptidase (-0.41); Cadherin-2 (-0.41); DNA-directed RNA polymerase II subunit 11 (-0.40); Translocating chain-associated membrane protein 1 (-0.40); 11-zinc finger protein (-0.38); Seryl-tRNA synthetase (-0.37); Pumilio homolog 1 (Drosophila) (-0.37); Putative uncharacterized protein HCF1 (-0.37); Cation-chloride cotransporter 6 (-0.37); SH3 and PX domain-containing protein 1 (-0.36); Enhancer of rudimentary homolog (-0.36); KRAB-associated protein 1 (-0.34); 5-methylthioadenosine phosphorylase (-0.34); SAPK substrate protein 1 (-0.33); Exocyst complex component 1; Exocyst complex component Sec3; (-0.33); Catenin alpha-1 (-0.33); 300 kDa nuclear matrix antigen (-0.32); Wiskott-Aldrich syndrome protein family member 2 (-0.32); Oxysterol-binding protein homolog 1 (-0.32); Serpin B8 (-0.31); TAR RNA-interacting protein (-0.29); Extended synaptotagmin-2 ESYT2 (-0.29); Importin alpha Q1 (-0.28); A-kinase anchor protein 8 (-0.28); High mobility group nucleosome-binding domain-containing protein 1; Non-histone chromosomal protein HMG-14; High-mobility group nucleosome binding domain 1; Putative uncharacterized protein HMGN1 (-0.27); Protein FAM40A; cDNA FLJ7113; FAM40A protein (-0.27); Eukaryotic translation initiation factor 2C1 (-0.27); Coiled-coil and C2 domain-containing protein 1A (-0.27); cAMP-dependent protein kinase type II-alpha regulatory subunit (-0.26); TRM112-like protein (-0.26); Glucose-6-phosphate 1-dehydrogenase (-0.26) Basic transcription factor 3-like 4 (-0.25); Chloride intracellular channel protein 4 (-0.25); Pyridoxine kinase (-0.24); Active breakpoint cluster region-related protein (-0.23); Stromal antigen 2 (-0.22); 60S ribosomal protein L17 (-0.22); SAC2 suppressor of actin mutations 2-like protein (-0.22); DNA helicase V (-0.22); U6 snRNA-associated Sm-like protein Lsm3 (-0.20); Adapter-related protein complex 2 alpha-1 subunit (-0.20); Polyadenylate-binding protein 1 (-0.20); MAP kinase isoform p42 (-0.19); 60S ribosomal protein L26 (-0.18); Guanine nucleotide-binding protein G(y) subunit alpha (-0.16); Rab6-interacting protein 2 (-0.16); Adapter-related protein complex 2 beta subunit (-0.15); Serine/threonine-protein phosphatase (-0.14); Deubiquitinating enzyme FAF-X (-0.10); RNase L inhibitor (-0.08); GAP and centrosome-associated protein (0.13); ATP synthase subunit alpha (0.17); Adipocyte plasma membrane-associated protein (0.17); Methylmalonate semialdehyde dehydrogenase (0.17); ATP synthase subunit beta (0.18); Protein disulfide-isomerase A3 (0.18); Tissue-specific extingisher 1 (0.19); Fumarate hydratase (0.20); Lysyl hydroxylase 1 (0.20); Protein FAM176B (0.21); Protein canopy homolog 2 (0.23) CTP synthase 1 (0.23); Long-chain acyl-CoA synthetase 4 (0.24); Succinyl-CoA ligase [GDP-forming] subunit alpha (0.24); Putative uncharacterized protein MPST; (0.24); Alpha-2-macroglobulin receptor-associated protein (0.24); Interleukin-25 (0.25); 3-hydroxyisobutyrate dehydrogenase (0.26); 3-hydroxybutyrate dehydrogenase type 2 (0.26); Serpin B6 (0.28); Putative uncharacterized protein BCAP31 (0.29); Acyltransferase-like 2 (0.29); Acyl-Coenzyme A dehydrogenase, very long chain variant (0.30); Isocitric dehydrogenase subunit beta (0.30); Calpain-5 (0.31); Similar to cytoskeleton-associated protein 4 (0.31); Uncharacterized protein C22orf30 (0.32); Tomosyn-1 (0.32); Peroxiredoxin-4 (0.33); Nicotinamide nucleotide transhydrogenase (0.33); CTCL tumor antigen se20-7 (0.33); Putative uncharacterized protein SGCE; (0.36); Coiled-coil domain-containing protein 53 (0.38); Cell growth-inhibiting gene 4 protein (0.38); Nucleolar autoantigen No55 (0.43); Ornithine aminotransferase (0.45); Putative uncharacterized protein COX4NB (0.45); Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial (0.44); Calpain-5 (0.46); Similar to cytoskeleton-associated protein 4 (0.47); Uncharacterized protein C22orf30 (0.48); Tomosyn-1 (0.49); Peroxiredoxin-4 (0.50); Nicotinamide nucleotide transhydrogenase (0.51); 257 kDa golgin (0.52); Brain epsilon-sarcoglycan (0.54); Coiled-coil domain-containing protein 53; (0.55); Cell growth-inhibiting gene 4 protein (0.56); Nucleolar autoantigen No55 (0.57); Ornithine aminotransferase (0.58); Putative uncharacterized protein COX4NB (0.59); Isocitrate dehydrogenase [NAD] subunit beta (0.60); Calpain-5 (0.62); Cytoskeleton-associated protein 4 (0.63); Uncharacterized protein C22orf30 (0.64); Tomosyn-1; (Tomosyn) (0.65); Peroxiredoxin-4 (0.66); NAD(P) transhydrogenase, mitochondrial (0.67); 258 kDa golgin (0.69); Putative uncharacterized protein SGCE (0.70); Coiled-coil domain-containing protein 53 (0.71); Cell growth-inhibiting gene 4 protein (0.72); Nucleolar autoantigen No55 (0.73);</p> <p>Fe Fragment Of IgG Receptor IIb (-3); Structural Maintenance Of Chromosomes 4 (-5); Granulin Precursor (-4); Butyrophilin Like 9 (-3); CEA Cell Adhesion Molecule 8 (-3); Vanin 2 (-3); Peptidoglycan Recognition Protein 1 (-3); Cathelicidin Antimicrobial Peptide (-3); Proteinase 3 (-2); Azurocidin 1 (-4); Elastase, Neutrophil Expressed (-4); Cytidine Deaminase (-3); Resistin (-6); Lactotransferrin (-3); Matrix Metalloproteinase 9 (-3.5); Lipocalin 2 (-3); Matrix Metalloproteinase 8 (-5); Grancalcin (-2); Peptidyl Arginine Deiminase 4 (-2); Integrin Subunit Alpha M (-2); C-Reactive Protein (-4); GC Vitamin D Binding Protein (-4); Membrane Metalloendopeptidase (-3); Flavin Containing Dimethylamine Monooxygenase 5 (-3); Mast Cell Expressed Membrane Protein 1 (-3); Carbonic Anhydrase 4 (-4); Marginal Zone B And B1 Cell Specific Protein (+5); Keratin 17 (+5); Reticulocalbin 3 (+2.5); FKBP Prolyl Isomerase 10 Protein Coding 40 (+3); Glutathione Peroxidase 8 (Putative) (+3); ANTXR Cell Adhesion Molecule 1 (+2); Stromal Cell Derived Factor 4 (+2.5); Chromosome 1 Open Reading Frame 198 (+2.5); Periostin (+4); Myoglobin (+4); Matrix Metalloproteinase 23A (Pseudogene) (+3); CD99 Molecule (Xg Blood Group) (+3); Matrix Metalloproteinase 19 (+5); Alcohol Dehydrogenase 1C (Class I), Gamma Polypeptide (+3).</p>
<p>Schiller <i>et al.</i> [14] 44 biomarkers (18 upregulated, 26 downregulated) Pulmonary biopsy</p>	

Table 4

Recommended aspects to be reported in clinical proteomics applied to articles included in the analysis. WB: Western Blot, IHC-Immunohistochemistry, ELISA-Enzyme Linked Immunosorbent Assay MRM: Multiple Reaction Monitoring

Recommendation for reporting in scientific article	Included articles that fulfilled criteria
Identification and description of clinical question (9/9)	All articles included in the analysis
Assessment of subject description (8/9)	Ryu <i>et al.</i> , [6] Landi <i>et al.</i> , [7], van Bon <i>et al.</i> , [9], Chairta <i>et al.</i> , [10], Mullenbrock <i>et al.</i> , [11], Chaigne <i>et al.</i> , [12], Dumi <i>et al.</i> , [13], Schiller <i>et al.</i> , [14]
Sampling description (9/9)	All articles included in the analysis
Presentation of experimental methodology (9/9)	All articles included in the analysis
Statistical approach (9/9)	All articles included in the analysis
Validation of results (5/9)	Landi <i>et al.</i> , [7] (WB), Corallo <i>et al.</i> , [8] (IHC), van Bon <i>et al.</i> , [9] (ELISA), Chairta <i>et al.</i> , [10] (MRM), Schiller <i>et al.</i> , [14] (WB)
Limitation acknowledgement (2/9)	Ryu <i>et al.</i> , [6], Chaigne <i>et al.</i> , [12]
Clear presentation of each authors' contribution (5/9)	Ryu <i>et al.</i> , [6], Landi <i>et al.</i> , [7], Chairta <i>et al.</i> , [10], Mullenbrock <i>et al.</i> , [11], Schiller <i>et al.</i> , [14]

Validation of identified candidate biomarkers

Five articles out of 9 comprised validation of their findings. ELISA (Enzyme Linked Immunosorbent Assay), IHC (Immunohistochemistry), WB (Western Blot, 2 studies) and MRM (Multiple Reaction

Monitoring) were used for validation of some but not all identified candidate biomarkers. Only 13 biomarkers were validated (Table 5, **S100A8/A9, UCHL1, PPID, DDX55, COX6B1, APCS, 14-3-3-epsilon, S100A6, collagen 1, vimentin, KRT 17, SDF4 and MZB1**).

Table 5

Validation of biomarkers in included articles WB: Western Blot, IHC-Immunohistochemistry, ELISA-Enzyme Linked Immunosorbent Assay MRM: Multiple Reaction Monitoring

Article	Validated biomarkers	Validation technique
van Bon <i>et al.</i> , [9]	S100A8/A9	ELISA
Chairta <i>et al.</i> , [10]	UCHL1 (ubiquitin carboxy-terminal hydrolase L1), PPID (peptidylprolyl isomerase D), DDX55 (Dead-Box Helicase 55), COX6B1 (Cytochrome C oxidase 6B1) and APCS (Amyloid P)	MRM
Landi <i>et al.</i> , [7]	14-3-3epsilon and S100A6	WB
Corralo <i>et al.</i> , [8]	Collagen-1 and vimentin	IHC
Schiller <i>et al.</i> , [14]	KRT17 (keratin 17) and SDF4 (45kDa calcium-binding protein) MZB1 (Marginal Zone B And B1 Cell Specific Protein)	WB

DISCUSSION

This work is currently an update of a previous systematic review, aiming to summarize new biomarkers found in samples of Ssc patients to the already published data. This work focused on identifying biomarkers using mass spectrometry techniques and 2-dimensional electrophoresis. In the previous review, only 116 candidate biomarkers were identified, although many studies were included. In this review, another 539 candidate proteomic biomarkers are added, despite the fact that they were retrieved from a significantly smaller number of included articles. A possible explanation could be the fact that the current work included more articles that evaluated differential expression of biomarkers

in biopsies (mostly cutaneous). When analyzing candidate biomarkers in tissue samples, the chances to find an increased number of candidate biomarkers using mass spectrometry proteomics is high due to the fact that the concentration of these proteins would be higher in tissue samples, compared to other biological sample [15].

It is obvious that biomarkers easily quantified in the clinical laboratory setting in any patient are those that are found in serum/plasma/urine (as minimally invasive procedures are needed in order to obtain sufficient biological sample for analysis). Plasmatic proteome is complex, and among proteins that are normally found in abundance, there are also present so called "tissue leakage products", and also proteins that are aberrantly secreted by diseased

tissues (like tumors) [16]. As such, in addition to “in situ” validation and confirmation of a candidate biomarker, one could also be interested in its plasmatic profile (whether the protein is aberrantly present in circulation).

Therefore, studies that also analyze the distribution of candidate tissue biomarkers in the circulation are warranted. It is however expected that proteins that are secreted from the active site of inflammation/fibrosis to have low/very low abundance in plasma, and such characteristic would not make the biomarker easily detected using mass-spectrometry techniques. One could conclude that at least a part of the candidate biomarkers found from cutaneous and pulmonary specimens are candidate to be evaluated in plasma/serum of patients.

Interestingly, in this current update a higher number of candidate proteomic biomarkers were identified in plasma, compared to previous published data. There is a large majority of immunoglobulin chains that are candidate biomarkers, not a surprise having in mind the fact that Ssc is an autoimmune disease, and it is characterized by various autoantibodies. However, as they seem to be not specific, it is doubtful that they could be used for the prognosis and diagnosis of these patients. Specific autoantibodies carry higher predictive and prognostic values for the Ssc diagnosis and therapeutic response [17]. Compared to previous published data, some cytokines and chemokines also seem of interest for Ssc, being identified as candidate proteomic biomarkers using mass-spectrometry techniques. CXCL-2 and CXCL-7 seem promising candidate biomarkers, although CXCL-7 was found to be downregulated compared to controls. CXCL-7 is a platelet-derived chemokine, freed in the injury site, where it also transforms in its active form. This chemokine plays an important role in neutrophil recruitment, and neutrophil–platelet crosstalk [18]. Platelet derived chemokines have gained increased interest recently, as another chemokine CXCL-4 was associated with lung fibrosis and pulmonary arterial hypertension in Ssc patients [19]. Another interesting class of biomarkers identified is represented by defensins. Many defensins (human neutrophil peptides) were found to be candidate biomarkers for Ssc patients and independent validation studies have not yet been performed. It is possible there would be a connection between platelet chemokines (attracting and activating neutrophils in the active site of inflammation) and defensins. The exact role of this possible association alongside with implications

for prognosis of Ssc patients should be further explored.

As presented before, a large number of candidate biomarkers emerged from cutaneous biopsies. These include molecules involved in fibrogenesis – both intracellular and extracellular proteins (collagen subunits, various remodeling enzymes, extracellular matrix proteins, cytoskeleton molecules), all suggesting the high degree of extracellular matrix remodeling in cutaneous lesions of Ssc patients. These molecules could be “leaking” from the tissue damage site into circulation. As such, some of them could also be evaluated in the circulation of Ssc patients, as a hallmark of active remodeling, with implications upon patient’s prognosis and therapeutic responses. In addition to the biomarkers expressing inflammatory responses, another promising group of biomarkers are represented by molecules that are associated with oxidative stress. Enzymes like peroxiredoxin, glutathione peroxidase, glutathione reductase were found in multiple reports as potential biomarkers for Ssc. However, evaluation of circulatory levels of these enzymes could be difficult and it should be easier to identify their end-products, as it has been already proved that there is abnormal expression of these molecules [20]. An important aspect is the fact that markers of oxidative stress were found to be upregulated in many other pathologies, not only autoimmune, but also in various cancers or in elders [21, 22]. In addition, there are numerous reports in which oxidative stress molecules appear to have wide day to day fluctuations [23]. As such, evaluating enzymes that are upstream in the metabolic chain could be more useful, or at least as useful as evaluation of the end-products. Studies that tackle this approach should be performed in Ssc patients.

Only one study evaluated proteomic biomarkers from BALF [7]. There were only few additional candidate proteomic biomarkers identified, compared to the previous systematic review. Among these, protein 14-3-3-epsilon and S100A6 were validated. Protein 14-3-3-epsilon comes from a larger family of chaperons – 14-3-3 proteins. They are conserved proteins in all eukaryotic cells, and play chaperon role in protein correct folding. 14-3-3 proteins are considered to be at the crossroad between aging and cancer as they have shown consistent expression patterns in these two conditions [24]. Therefore, care should be taken when analyzing 14-3-3 epsilon as proteomic biomarker in Ssc patients, and adjustments for patient’s age should be taken under

consideration. However, studies are still needed in order to validate most of candidate proteomic BALF biomarkers.

Although there was a relatively high number of identified biomarkers, only 13 were also validated in the selected studies (Table 5). Most biomarkers were validated from biopsy samples, while only one in plasma samples, and two from BALF. Among them, keratin 17 is of interest. It could be a valuable biomarker, due to the fact that it has virtually no expression in normal skin, but it is induced under various stress conditions (like inflammation). Also, keratin 17 proved to play various functions, like regulating immune response in cancer development and psoriasis [25].

In conclusion, although many candidate biomarkers were additionally identified, independent validation studies are needed in order to evaluate the importance of these biomarkers. Many of them

could just be mirroring acute inflammation, autoimmune response and fibrosis, and they might have low specificity. However, some of them could not be good diagnostic biomarkers, but prognostic biomarkers (as it was the case of reticulocalbin (RCN) 1 and 3 validation study, which found similar expression in Ssc patients and healthy controls, but in the end a subgroup of patients with high RCN1 and 3 levels shared a common pattern of Ssc clinical characteristics [26]). This aspect should also be taken into account when performing validation studies. Trying to establish a threshold value in the healthy population is also very important, as few studies focused on the distribution of these candidate biomarkers in healthy population. The quest for promising biomarkers for Ssc patients is far from its ending, but alongside with development of high-throughput screening methods, the scientific community is getting close to achieving this goal.

Introducere. Scleroderma este o boală autoimună caracterizată prin fibroză cutanată și tisulară ce asociază modificări vasculare. Scopul acestei sinteze sistematice a fost de a aduce la zi lista de biomarkeri proteomici candidat indentificați prin tehnici de spectrometrie de masă din probele biologice ale pacienților cu sclerodermie.

Metode. Bazele de date Medline și Scopus au fost evaluate pe 1 septembrie 2020. Au fost identificate articolele relevante din perioada martie 2014–septembrie 2020. Doi evaluatori independenți au evaluat rezultatele obținute.

Rezultate. Au fost identificate 97 de articole din care au rămas doar 9 pentru analiză. Au fost identificați 539 de biomarkeri proteomici candidat dintr-o varietate de produse patologice (un număr mult mai ridicat de biomarkeri comparativ cu studiul anterior). Cei mai mulți biomarkeri au fost identificați din probe bioptice cutanate. Numai 5 articole au inclus și o etapă de validare având un număr de 13 biomarkeri validați.

Concluzii. Deși au fost identificați biomarkeri, studii independente de validare sunt necesare pentru a evalua importanța acestora la pacienții cu sclerodermie.

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