Candidate autoantibodies for primary Sjögren's syndrome: where are they now?

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ABSTRACT

Although ANA, SSA and SSB antibody testing are universally accepted biomarkers for Sjögren's syndrome (SS) diagnosis, they do not occur in all patients. Up to 18% of SS patients are seronegative, with potential for delayed or missed diagnosis. There are no clinically available autoantibodies with predictive value for SS end-organ complications. Over the last three decades, novel autoantibodies for SS diagnosis and monitoring have been identified but few have transitioned from research studies to clinical use. We performed a literature review of candidate serum autoantibodies to examine their persistence in the literature and potential clinical utility. Of the nineteen autoantibodies we identified, AQP5, SP-1, CA6, and PSP Abs have the most promise. Larger cohort studies are needed to determine their potential contribution in SS management.

Introduction

Sjögren's syndrome (SS) is a systemic autoimmune condition characterised by immune-mediated injury primarily affecting exocrine gland in addition to systemic complications including autoimmune cytopenias, increased lymphoma risk, autonomic and peripheral neuropathy. It is the most common autoimmune connective tissue disorder with a prevalence between 0.01% to 3% and an overall female/male ratio of 10.72 (95% confidence interval [95% CI] 7.35 to 15.62) (1).

The four classical autoantibodies used for primary SS management include anti-nuclear antibodies (ANA), Ro/SSA Abs (SSA), La/SSB Abs (SSB), and rheumatoid factor (RF). ANA are found in 59-85% of primary SS patients. SSA and SSB occur in 50–70% of SS patients and are associated with longer disease duration, extra-glandular involvement, and increased lymphocyte infiltrates in glandular biopsies (2, 3). Isolated detection of SSB is rare and not necessarily associated with primary SS, but co-positivity of SSA and SSB is associated with increased prevalence of lymphoproliferative disorder and lymphoma (4). RF levels are elevated in 36-74% of patients and may be associated with articular disease, cutaneous vasculitis, polyclonal hypergammaglobulinaemia, renal and central nervous system involvement (2).

Inclusion of autoantibodies as a SS diagnostic criterion has varied across several revisions of the American College of Rheumatology (ACR) SS diagnostic criteria. All four autoantibodies were part of the 2012 ACR SS diagnostic criteria (5). ANA and RF, however, were removed from the 2002 and 2016 ACR criteria, since they did not improve collective sensitivity and specificity (6). The diagnostic criteria can identify seronegative patients with histopathological evidence of primary SS. In fact, seronegative SS is well-documented in the literature with prevalence ranging from 4.5% to 18% (7, 8), highlighting the risk of missed diagnosis and the potential value of additional non-invasive biomarkers (9).

Despite published guidelines, diagnosis of SS is not always straight forward since it has a slow, chronic nature; the most common presentation of sicca symptoms is often vague and non-specific, and its clinical phenotype is protean. Novel serum autoantibodies that are complication-specific, have prognostic significance, and assist diagnosis of seronegative SS patients could greatly improve management. Many potential serum biomarkers had been identified over the last few decades. This review examines novel candidate autoantibod-

ies for sero-diagnosis of SS and their potential clinical utility. We review the chronology of their description, detection methodology and persistence in the literature.

Methods

We performed a literature review of SS candidate serum autoantibodies using the electronic database "PubMed". Search terms used included "Sjögren's syndrome", "Sjögren's disease", "novel autoantibodies", "serum autoantibodies", "serum biomarkers" and reviewed studies that were published prior to June 2021. We excluded studies of autoantibodies that were confined to murine models that had not been tested in human sera. Furthermore, antibodies described in SS with a more dominant primary autoimmune disease association were excluded, such as aquaporin 4 Abs, thyroid autoantibodies. We also excluded routinely available antibodies used in current evaluation of potential SS, *e.g.* RF.

Results

We identified nineteen candidate serum autoantibodies described before June 2021. Table I summarises their prevalence, detection methods and clinical associations. Thirteen of these autoantibodies were first published in the last decade. Figure 1 describes the discovery and durability of these autoantibodies in the literature. We describe the key attributes of these autoantibodies in descending order of clinical promise.

Table I. Candidate autoantibodies for primary Sjögren's syndrome.

Autoantibodies	Prevalence	Detection method*	Clinical association
MR3	63–81%	ELISA RIA Fluorimetry IIF Flow cytometry	Higher focus score Lower salivary flow
Alpha-fodrin	38–42%	Immunoblot ELISA RIA	Hypergammaglobulinaemia
AQP5	76.8%	RIPA	Low resting salivary flow Sicca symptoms
SP1, CA6, PSP	40–67%	ELISA	Sicca symptoms Precedes SSA/SSB detection
ENO1	61.5% **	ELISA	Higher rate of RF positivity Reduced salivary flow rate
DNase I	43.5%	ELISA	Neutropenia
PUF60	30%	ELISA	More common in Asians, African American Also detected in dermatomyositis
IFI16	29–34%	ELISA	Hypergammaglobulinaemia Severe oral sicca symptoms
gAChR	23.1%	LIPS	Autonomic dysfunction
P selectin	22.8%	ELISA	Thrombocytopenia
MDM2	21%	ELISA	Positive correlation with ESSDAI Anaemia, thrombocytopenia
NR2	19.7%	ELISA	Depression, memory loss Lower hippocampal grey matter volume
STMN 4	15.3%	ELISA	Polyneuropathy, vasculitis
NA14	13.6%	ELISA	Higher IgA level
Cal 3	11%	ELISA	Peripheral neuropathy
TRIM38	10.2%	RIPA	High focus score

*Detection methods: ELISA: enzyme-linked immunosorbent assay; RIA: radioimmunoassay; LIPS: luciferase immunoprecipitation; RIPA: radio-immunoprecipitation; IIF: indirect immunofluorescence. **Higher serum level in SS *vs.* healthy control (19.33 ng/ml *vs.* 8.7 ng/ml, $p<0.01$), AUC of ROC 0.81.

Candidate autoantibodies with high prevalence in SS

Aquaporin (AQP)

Of the reported autoantibodies, aquaporin 5 (AQP5) Ab had the highest prevalence in SS. AQPs are a family of membrane proteins responsible for transportation of water and solutes across cell membranes. Antibodies to several AQPs, including AQP1, APQ3, AQP8, and AQP9, had been detected in SS patients (10) with AQP5 Ab the most studied. AQP5 normally occurs in the apical membrane of acinar cells. Transgenic mice lacking AQP5 showed decreased saliva secretion, suggesting AQP5 plays a role in salivary function (10). Abnormal distribution of AQP5 was described in the minor salivary glands of 10 SS patients, differing from biopsies of healthy controls or those with non-SS dry mouth disorders such as sarcoidosis (11).

AQP5 Ab was studied in the sera of 112 SS patients and 53 healthy controls in a Korean study (12). Of these, 73.2% of SS patients and 32.1% of healthy controls were positive for AQP5 IgG by indirect immunofluorescence with a significant difference in intensity ($p<0.0001$). The sensitivity was 73% and specificity was 68% (12). This was replicated in a non-Korean cohort, using 111 primary SS sera from the Sjögren's International Collaborative Clinical Alliance (SICCA) registry, 43 non-SS controls, as well as 35 systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) patients (13). AQP5 Abs were detected using ELISA and cell-based immunofluorescence cytochemistry. Based on ROC curve analysis, AQP5 IgG directed to epitope E1 were most well-powered to differentiate between SS and non-SS ($p<0.0001$), with sensitivity of 61% and specificity of 77% (13). ELISA and immunofluorescence cytochemistry assays exhibited a high degree of agreement. Wider clinical use of AQP5 Ab may be limited by its moderate sensitivity. Further prospective studies in a larger cohort are needed to validate the diagnostic advantage of AQP5 Abs for SS diagnosis and management.

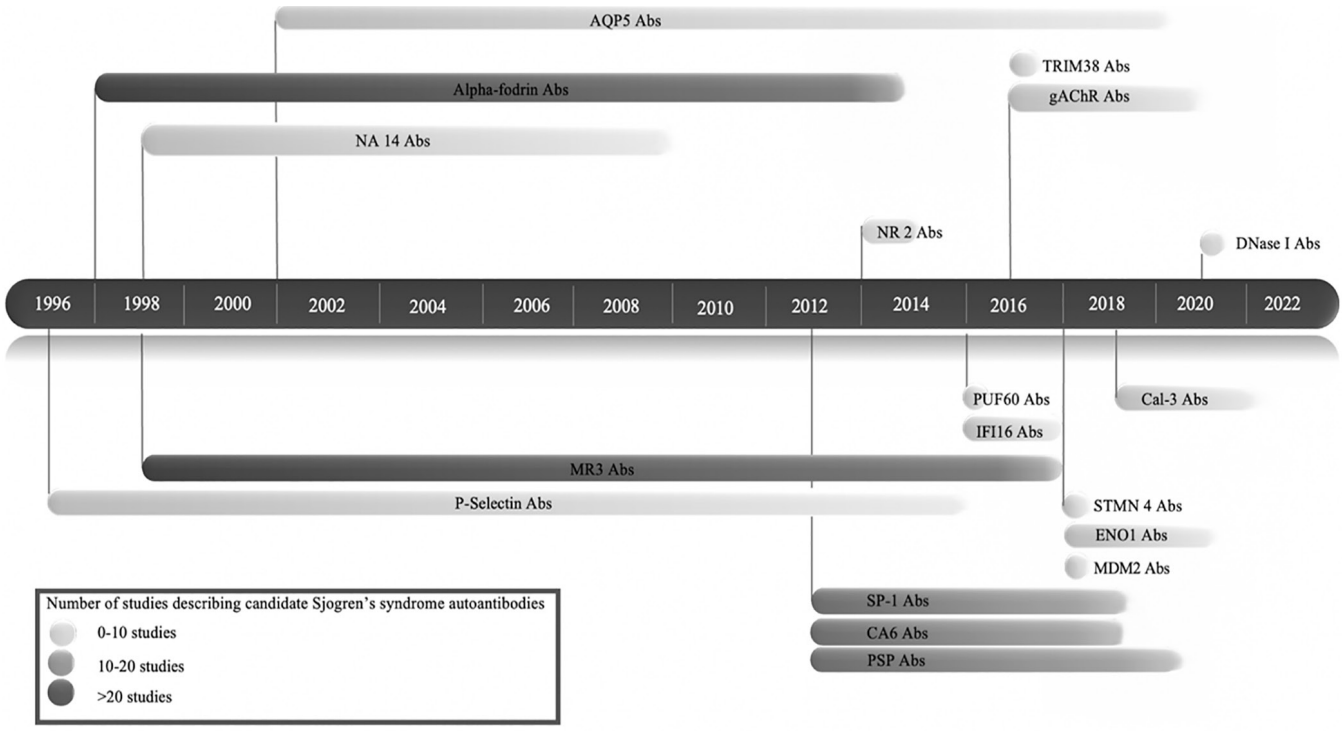

Fig. 1. Chronology of candidate serum autoantibodies. The discovery and durability of candidate Sjögren's syndrome autoantibodies over the last 25 years. Autoantibodies are further divided into three groups based on the number of published studies.

Salivary gland protein 1 (SP-1), carbonic anhydrase 6 (CA6), parotid secretory protein (PSP)

Autoantibodies to salivary gland protein 1 (SP-1), carbonic anhydrase 6 (CA6) and parotid secretory protein (PSP) have been reported in patients with immune-mediated and non-immune-mediated sicca symptoms. They were first identified in murine models as potential SS biomarkers in 2012 (14). These autoantibodies were then evaluated in 13 patients who met SS ACR diagnostic criteria. Each patient had at least one novel autoantibody. Serological studies were performed on a further 29 patients with less than 2 years of sicca symptoms. In this group, 76% had SP-1 Abs or CA6 Abs while only 31% had either SSA or SSB. A further 20 SS patients with positive salivary gland biopsies without SSA or SSB were found to have SP-1 Ab in 45% and CA6 Ab in 5% (14).

These promising findings were extended in a larger study using sera from the SICCA registry. Using lymphocyte focus scores from lip biopsies as a marker for disease severity, over 60% of SS patients with mild disease activity were found to have at least one of SP-1, CA6, or PSP Abs. By comparison only 30% of these patients had SSA and SSB (15).

CA6 Abs had the strongest association with ocular sicca symptoms based on a prospective study of 60 patients (46 confirmed SS, 14 without SS) (16). The prevalence of SP1 Abs was studied in two different ethnic cohorts: 52% of 123 Greek patients with primary SS had SP-1 Abs, whilst 40% of 124 Chinese primary SS patients were positive (17, 18). Both studies described a positive association between SP-1 Abs and salivary gland dysfunction.

Deoxyribonuclease I (DNase I)

Deoxyribonuclease I (DNase I) is one of the latest autoantigens studied in SS patients. It is an extracellular enzyme assisting apoptotic debris clearance. Reduced DNase enzymatic activity has been demonstrated in dry eye disease patients (19). A study of 85 primary SS patients, 50 RA patients and 88 healthy controls, found DNase I Ab in 43.5% of SS patients but not in RA and healthy controls (20). DNase I Abs were positively associated with neutropenia and negatively with peripheral neuropathy (20).

Autoantibodies with organ-specific associations

Neurological complications

- Ganglionic acetylcholine receptor (gAChR)

Ganglionic acetylcholine receptor (gAChR) antibodies were first reported in one patient with primary SS and progressive dysautonomia who responded to oral corticosteroids, while a phenotypically similar patient without gAChR Ab did not response to immunosuppression (21).

The potential clinical utility of gAChR Abs for assessment of SS patients with autonomic neuropathy was further examined in a subsequent observational study. Thirty-nine patients with primary SS and 39 healthy controls were assessed for gAChR Abs using a luciferase immunoprecipitation system. gAChR-alpha3 Abs were absent in healthy controls but present in 9 of 39 SS patients, of whom 5 had autonomic neuropathy (22). In another clinical cohort, gAChR Abs were detected in 8 of 10 SS patients with autonomic dysfunction, 1 of 34 non-SS autonomic neuropathy patients and not in healthy controls (22). The most common clinical complications in gAChR positive SS patients were ortho-

static hypotension and gastrointestinal disturbance but titres did not correlate with symptom severity (22). Although gAChR is a well-described autoantigen in autoimmune autonomic gangliopathy, further research is needed to characterise its significance in SS autonomic neuropathy.

- N-methyl-d-aspartate receptor subtype 2 (NR2)

NR2 is a N-methyl-d-aspartate (NMDA) receptor abundant in the hippocampus. Initially NR2 Abs were investigated as a biomarker for cognitive dysfunction in SLE patients with inconclusive results (23, 24). Subsequently, stronger associations were found in primary SS patients with neuropsychiatric complications (25). NR2 Abs were present in 13 of 66 primary SS with neuropsychiatric complications but also 7 of 66 healthy controls (25). NR2 Abs were most closely associated with depression. Worsening memory and learning performance was associated with presence of NR2 Abs in cerebral spinal fluid, but not serum (25).

- Stathmin 4 (STMN-4)

Stathmins are members of a phosphoprotein family important in neuronal development. Stathmin-deficient mice develop peripheral vascular axonopathy (26). Stathmin 4 (STMN-4) occurs in the golgi apparatus. The highest seroprevalence of STMN-4 Abs in a large cohort of 228 systemic autoimmune disorder patients was found in RA (18%), primary SS (15.2%), with lower rates in SLE patients (8%) and blood donor controls (5% of 113) (26). SS patients with STMN-4 Abs had higher rates of peripheral neuropathy (33% *vs*. 7.8%, p=0.01) (26). The study did not comment on the rate of peripheral neuropathy in SLE and RA patients.

- Calponin 3 (Cal-3)

Calponin 3 (Cal-3) is a 40kd protein widely expressed in central nervous system neuronal and glial cells and peripheral nervous system perineuronal satellite cells. Cal-3 Abs were evaluated in 209 patients with primary SS, 138 patients with inflammatory myopathy, 138 SLE patients, 44 multiple sclerosis and 46 healthy controls (27). Cal-3 Abs were detected in 11% of 209 SS patients, 8.7% of 138 SLE, 5.1% of 138 myositis patients, and 6.8% of 44 multiple sclerosis patients (27). Subgroup analysis revealed a strong association between Cal-3 Ab and electrophysiologically-confirmed peripheral neuropathy in primary SS patients (p=0.02) (27).

Haematological complications

- P-selectin

P-selectin is an adhesion molecule that modulates interactions between leukocytes, platelets, and endothelium (28). P-selectin Abs may be a marker for thrombocytopenia in primary SS. A 2015 study examined P-selectin Abs in 70 SS patients, 32 idiopathic thrombocytopenic purpura (ITP), and 35 healthy controls and found P-selectin Abs in 22.8% of SS compared with 15.6% of ITP patients (29). The plasma P-selectin Ab level in pSS and ITP were significantly higher than that of health controls (p=0.024). Within the SS cohort, 32 patients had thrombocytopenia and 38 did not. P-selectin Abs were found in 40.6% of in primary SS with thrombocytopenia and only 7.9% primary SS patients without thrombocytopenia (p=0.05), suggesting P-selectin may be a marker for SS-mediated thrombocytopenia (29).

- Mouse double minute 2 (MDM-2)

Mouse double minute 2 (MDM-2), also known as E3 ubiquitin-protein ligase, is an oncoprotein that mediates cell cycle regulators including p53. Autoantibodies to MDM-2 have been identified in patients with SLE and primary SS (30). Higher levels of MDM-2 Abs were detected in labial gland tissues in 73.3% of 100 SS patients, compared to 22.2% of 74 healthy controls. Serum MDM-2 Abs were detected in 21% of 100 SS patients compared to 5.4% of 74 healthy controls (p<0.05) (31). The titre of MDM-2 Abs had a positive correlation with ESSDAI (31). Although SSA and SSB were more abundant in this SS cohort at 47%, MDM2 Abs were associated with longer disease duration, anaemia and thrombocytopenia, indicating potential predictive value for severity of haematological complications (31).

Sicca symptoms

Interferon-inducible protein 16 (IFI16) is an intracellular pathogenic DNA receptor targeted in autoimmune pathogenesis. IFI16 Abs have been detected in SLE, scleroderma and RA (32). A study of 67 SS patients and 100 healthy controls found IFI16 Abs in 34% of SS patients and 5.2% of healthy controls (p<0.0001). No correlation was observed between antibody titre and disease severity (33). A subsequent study using sera from 133 SS patients from the SICCA registry found IFI16 Abs in 28.6% SS patients, compared to 23.5% of SLE patients and 2.1% healthy controls. IFI16 Ab detection was associated with abnormal Schirmer's test, polyclonal hypergammaglobulinaemia, and high focus score on labial salivary gland biopsies (p<0.02) (32).

Important autoantibodies not amenable to clinical laboratory use

Muscarinic receptor type 3 (MR3)

Autoantibodies against lacrimal gland MR3 muscarinic receptors were first described in SS patients in 1998 (34). Muscarinic receptors are G-protein coupled acetylcholine receptors present in neuron membranes. MR3 Abs have been implicated in impaired saliva secretion (34). Many subsequent studies examined the value of MR3 Abs for SS diagnosis. A meta-analysis of 11 studies with 965 SS patients and 1289 controls found high specificity but low sensitivity (pooled sensitivity 0.43 [95% CI, 0.28–0.58]; pooled specificity 0.95 [95% CI, 0.91–0.97]) (35). MR3 Ab levels were associated with higher labial gland focus score and reduced saliva flow rate (36). The area under the summary receiver operating characteristic curve (SROC) was 0.89 (95% CI 0.86–0.92), indicating modest overall diagnostic accuracy for SS (35).

Alpha fodrin (AF)

Antibodies against alpha-fodrin (AF) were first described in the salivary gland tissue of 14 Japanese SS patients in 1997 and proposed as an early marker for SS (37). AF occurs in many human tissues including salivary glands, muscle cells and postsynaptic mem-

branes. It plays a role in glandular secretion, is associated with membrane ion channels, and had been considered a surrogate marker of synaptic activity (38). Studies on AF Abs in SS demonstrated conflicting sensitivity and specificity, due to variable study designs and criteria for detection (39).

A meta-analysis of 23 studies of AF Abs found high pooled specificity of 83% but low pooled sensitivity 39.3% (40), in common with the MR3 Ab meta-analysis. AF Ab detection methods in the studies included radioimmunoassay, immunoblot and ELISA. One study examined IgA AF Ab alone, 12 examined IgG AF Ab alone, whilst 10 examined IgA and IgG (40). IgA AF Ab had higher sensitivity at 41.9% compared to 38% for IgG AF Ab (40). Overall diagnostic accuracy, based on positive and negative likelihood ratios, was moderate at best.

In a study of SSA, SSB and AF Abs in primary SS, SLE, RA and healthy controls, AF Abs were not detected in RA or SLE. Despite specificity, AF Ab added little to SSA and SSB for SS diagnosis (41).

Other candidate autoantibodies

Poly(U)-binding-splicing factor 60kDa (PUF60)

Antibodies to poly(U)-binding-splicing factor 60kDa (PUF60), a protein of the large U2 snRNP complex, were first described in otherwise sero-negative dermatomyositis patients (42). Subsequently, the prevalence of PUF60 Ab was determined in 84 patients with primary SS, 71 with SLE, 45 with polymyositis, 45 with inclusion body myositis and 38 healthy controls. A significantly higher portion of primary SS patients (30%) and dermatomyositis patients (18%) had PUF60 Abs compared to other systemic rheumatic conditions and healthy controls (42). Polyclonal hypergammaglobulinaemia and Ro52 co-positivity was associated with the presence of PUF60 Abs.

Alpha-enolase (ENO1)

Alpha-enolase (ENO1) is a salivary autoantigen up-regulated in primary SS patients who later develop MALT lymphoma (43). Compared to healthy controls, ENO1 is overexpressed in saliva secretions and labial gland biopsies of primary SS patients ($p<0.01$) (43). Higher serum ENO1 Ab levels were found in 26 primary SS patients compared to 20 healthy controls. ENO1 Abs were associated with reduced salivary flow rate (44). Whether serum ENO1 Ab have predictive value for MALT lymphoma in SS has yet to be investigated.

Nuclear autoantigen of 14kDa (NA14)

Nuclear autoantigen of 14 kDa (NA14) were first identified from a SS patient in 1998. The first formal evaluation of NA14 Abs was undertaken in 2009, including 132 primary SS patients, 50 secondary SS patients, 100 SLE patients, 43 scleroderma patients, 54 RA patients, 29 inflammatory myositis patients, and 58 healthy controls (45). NA14 Abs were detected in 13.6% primary SS, a significantly greater prevalence compared to all other patient groups. Incubation of human salivary gland cell lines with interferon γ induced expression of NA14 but not SSA antigen, suggesting a distinct pathway for NA14 production in primary SS (46). Presence of NA14 Ab did not correlate with any specific SS clinical features (45).

Tripartite motif-containing protein 38 (TRIM-38)

The tripartite motif-containing protein family includes TRIM-21, usually referred to as Ro52, and TRIM-38 (47). TRIM-38 Ab was first described in a cohort of 8 primary SS patients. Further study of a larger cohort with 235 primary SS patients (90% co-positive for Ro52 and SSA) and 50 controls described a prevalence of 10.2% in SS patients compared to 4% of healthy controls (48). The presence of TRIM-38 Ab was strongly associated sicca severity, low Schirmer score and higher focus score (48).

Discussion

Autoantibody production in SS is complex with environmental factors, infective triggers and dysregulation of cellular apoptosis all incriminated. Molecular mimicry between autoantigens and viral molecules such as Epstein-Barr virus, hepatitis C, and Coxsackie virus may elicit autoantibody production in SS (49). Infiltrating lymphocytes in SS salivary glands increase expression of CD40/CD40 ligand, Bcl-2 family proteins, as well as increased B-cell activating factor signal (49), providing aberrant resistance to normal apoptosis. Consequent fragmentation and redistribution of autoantigen may result in autoantibody production (49).

The classical SS autoantibodies, SSA and SSB, were first called SjT and SjD Abs by Anderson *et al*. in 1961 (50). Over the next decade, Reichlin *et al*. described anti-Ro and anti-La in SLE and SS patients (50). Subsequent immunochemical equivalence was demonstrated for Ro with SjT and La with SjD, allowing a convergent nomenclature as SSA and SSB, respectively (50). Such complex evolution of autoantibody names is not unusual by the time they reach clinical fruition. The durable clinical value of these autoantibodies lies in their sero-diagnosis of primary and secondary SS. Apart from SSA positivity indicating a risk of neonatal lupus, congenital heart block or subacute cutaneous lupus, these autoantibodies do not have predictive value for activity or severity of SS and its complications.

For new autoantibodies to achieve widespread clinical use in SS, they would need to identify either seronegative patients, indicate disease activity, or predict complications. Furthermore, they must be amenable to reliable, reproducible estimation in clinical laboratories. Our review identified 19 candidate serum autoantibodies for primary SS. AQP5 Ab may have the greatest potential clinical utility. For sero-diagnosis, AQP5 Abs may have greater seroprevalence than SSA and SSB while retaining high specificity. The presence of AQP5 Ab is associated with sicca severity. However, studies on AQP5 Ab have been limited to cohorts of 150 patients or less. Since SS is one of the most common autoimmune disorders, these studies should be extended to larger, ethnically diverse populations. Comparative analysis should be performed on the potential clinical utility of AQP5 Ab against SSA and SSB.

For early SS diagnosis, SP-1, CA6 and PSP Ab have the greatest potential as they may precede SSA and SSB in primary SS (15-18). At present, the clinical benefit of early SS sero-diagnosis is unclear since most patients have minimal clinical symptoms with low disease activity, for whom current therapeutic interventions are limited. Examination of the clinical trajectory of SP-1, CA6, and PSP Ab positive patients in longitudinal studies could clarify the clinical utility of these autoantibodies.

ANA, SSA and SSB alone do not identify all SS patients. The diagnosis of primary SS can be frustrating for patients and challenging to clinicians due to its slow and chronic nature, variable phenotype, and non-specific clinical presentations. Two of the most promising earlier neo-autoantibodies for seronegative SS were MR3 and AF Abs. However, sensitivity and specificity of AF Ab alone were inferior to SSA and SSB (36, 41). Similarly, studies of MR3 Ab were inconsistent mainly due to diverse laboratory detection methods (51). Cumbersome functional assays for MR3 Ab detection required complex, laborious primary cell preparation and rendered the test impractical for large scale, clinical laboratory use (52).

Whether new autoantibody detection technologies could identify novel autoantibodies in SS remains to be seen. For example, the luciferase immunoprecipitation system (LIPS) can identify efficiently linear and conformational epitopes of autoantigens often missed in solid-phase immunoassays (53). This was used to study gAChR in SS and salivary protein autoantibody profiling in sicca conditions (22, 54). Antigen microarrays can allow simultaneous detection of autoantibodies to a large number of DNA, peptide, and recombinant proteins targets but are frustrated by nonspecific background and high false-positive rates (53).

Autoantibodies with predictive value for serious SS complications, such as neurological disorders including peripheral and autonomic neuropathy, ITP, cytopenia, and lymphoma, are clinically desirable. RF, SSA and SSB positivity have been identified as independent risk factors for lymphoma development in SS patients (4). Potential candidate autoantibodies identified in this review were gAChR, STMN-4, Cal-3, ENO1, P-selectin, MDM-2, and IFI16 Abs. Unfortunately, most studies of these autoantibodies were too underpowered to support significant conclusions, in part due to the low prevalence of these complications. Some of these autoantibodies are encountered in other diseases, such as gAChR Abs in autoimmune autonomic ganglionopathy and PUF60 Abs in dermatomyositis. Before adoption for clinical use, extensive studies of the value of these autoantibodies for the diagnosis SS-specific complications are needed.

Our review has focused on human autoantibodies. Over 20 murine models of SS have provided functional insights of autoantibody and cellular immune dysfunction and exocrine dysregulation characteristic of SS (55). No one murine model encompasses all the clinical features of SS. Studies of functional autoantibodies from germinal centres of human SS salivary glands have the potential for greater pathological relevance. However, clinical laboratory application of functional autoantibody assays has disappointed, such as the MR3 Ab assay (52).

Further studies of SS pathogenesis may allow identification of diagnostic, prognostic and therapeutic markers which may not be autoantibodies. Limited progress, despite extensive studies over three decades on autoantibodies to add to or replace SSA/SSB, suggest that non-antibody markers could be a more fruitful area of research. For example, serum lymphoneogenic chemokine CXCL12 and CXCL13 levels have been implicated in lymphoma development, whilst serum Krebs von den Lungen-6 levels may have a role interstitial lung disease assessment (56, 57). Future studies combining serum autoantibodies with biomarkers may further improve SS diagnosis, classification, and complication management. Prospective, longitudinal studies that examine long-term clinical evolution of biomarkers and autoantibodies with prediction of clinically useful endpoints and stratification of treatment options are needed.

Conclusion

Additional autoantibodies for SS could assist with sero-diagnosis in SSA and SSB negative patients, identification of early SS, and have predictive value for complications. Only SSA and SSB are consistently included in SS diagnostic criteria and are available widely in clinical laboratories. Nonetheless, 4.5 to 18% of SS patients are seronegative (7, 8). Many autoantibodies have been identified with the potential to improve SS diagnosis. At present, only AQP5, SP-1, CA6, PSP Abs showed durable clinical promise but need further development before they can be considered in clinical laboratory practice.

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