Short Communication

Novel Autoantibodies in Sjogren's Syndrome

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Abstract

Sjogren's syndrome (SS) is a chronic autoimmune disease of exocrine glands. It affects lacrimal and salivary glands resulting in dryness of the eyes and mouth. It can also cause systemic disease manifestations. It is usually classified as primary or secondary Sjogren's syndrome. Secondary SS can occur in the presence of Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Systemic Sclerosis (SSc) or Mixed Connective Tissue Disease (MCTD).

INTRODUCTION

Sjogren's syndrome has a substantial burden on patients' quality of life and the healthcare system. Epidemiological review and meta-analysis of Sjogren's syndrome published in 2014 depicted the incidence of SS as: 6.0-11.8 per 100 000 person-years in Asia, 3.9-5.3 per 100 000 person-years in Europe and 3.9 per 100 000 person-years in North America. It primarily occurs in women with an average age of 56.2 years. Men are diagnosed at the age of 65 years and older [1].

Sjogren's syndrome is difficult to diagnose as there is no screening test available with high sensitivity. Instead, the diagnosis is made based on the criteria developed by American-European Consensus Group (ACEG) and American College of Rheumatology (ACR)that use combinations of clinical symptoms, specific tests for dry eye and dry mouth, salivary gland histology and autoantibodies. Some of the important antibodies that currently help make the diagnosis include anti-nuclear antibody (ANA), rheumatoid factor (RF), anti-Ro/SSA and anti La/SSB [2]. However, the criteria are imperfect as the average time from disease onset to diagnosis is 5 years [3]. Recently novel biomarkers anti-salivary gland protein 1 (SP1), anti-parotid secretory protein (PSP) and anti-carbonic anhydrase VI (CAVI) were studied. These antibodies were found to occur earlier in the disease course [4].

Anti-La/Ssb and Anti-Ro/Ssa

The Ro/SSA antigen consists of two cellular proteins, "Ro52" and "Ro60". The protein Ro60 is localized in the nucleus and nucleolus while Ro52 is localized in the cytoplasm. Ro60 recognizes and binds miss-folded non coding RNA [5], while Ro52 is an E3 ubiquitin ligase, an enzyme that adds ubiquitin molecules to the target proteins [6]. The La antigen is a 47-Kd protein that travels between the nucleus and cytoplasm; it is primarily located in the nucleus and is involved in processing of small, non-coding RNA [7].

The anti-Ro and anti-La antibodies are very important as they are part of ACEG and ACR Sjogren's syndrome classification criteria. As per ACEG criteria, for patients to be classified as SS they must have clinical symptoms along with a positive anti-Ro, anti-La or a positive salivary gland biopsy. Alternatively, as per the ACR criteria, a patient can be classified by having either a positive anti-Ro, anti-La, both RF and ANA or a positive salivary gland biopsy [8,9].

In 1989, Venables et al., examined the sensitivity and specificity of anti-Ro and anti-La for SS and they found that 56% of the patient with SS had anti-Ro while 42% had anti-La. However, they also found 38% and 6% of the patients with SLE had anti-Ro and anti-La, respectively. The positive predictive value for anti-Ro was calculated to be only 42%, while for anti-La was 83% deeming anti-La antibodies to be more specific for SS [10].

A study conducted by Harley et al., in 2005 showed anti-La to be less sensitive in patients with RA and secondary SS versus primary SS. In patients with primary SS, 100% of the patients were anti-Ro positive and 98% of them were anti-La positive. On the other hand, in the patients with secondary SS, 92% were anti-Ro positive and only 70% were anti-La positive. Furthermore, in the patients with secondary SS that were anti-Ro and anti-La negative, 67% and 55% of them, respectively, had RA. These antibodies seem to have decreased sensitivity in patients with secondary SS and RA [11].

Anti-nuclear antibody and rheumatoid factor

Anti-Nuclear Antibody (ANA) is an autoantigen that is present in the nucleus. ANA is present in patients with and without rheumatologic illnesses. In 1999, Tan et al., reported an average of 31.7% healthy individuals have titers of ≥ 1:40 while 13.0%, 5.0% and 3% of healthy individuals had titers of ≥ 1:80, 1:160 and 1:320, respectively [12]. The average sensitivity for ANA in SS is 48% and the specificity is 52% [13].

Rheumatoid Factor (RF) is involved in clearing immune complexes and B cell antibody production. Its production increases in chronic immune responses and infectious diseases [14]. RF is one of the antibodies used in diagnosis of rheumatoid arthritis (RA). However, it has a very low specificity and sensitivity for SS. In 2009, Filipe et al., showed that there was a significant increase in RF IgA and IgM in patients with SS. However, there was no significant difference in RF between patients with RA versus RA with SS [15].

Hence ANA and RF alone are not useful in ruling out SS. However, an article on ACR classification by Shiboski et al., showed that combined positive RF and ANA titer of ≥ 1:320 had good specificity but low sensitivity for diagnosis of SS. This was found to be a good substitute for anti-SSA and anti-SSB antibodies [9]. As a result, the presence of both ANA and RF antibodies was added to the ACR classification criteria. .

Novel autoantibodies

Anti-carbonic anhydrase isoenzyme VI, anti-salivary gland protein 1 and anti-parotid secretory protein: Some of the new antibodies being studied include anti-Carbonic Anhydrase isoenzyme VI (anti-CAVI), anti-Salivary gland protein 1 (anti-SP1) and anti-Parotid Secretory Protein (anti-PSP). Carbonic anhydrases are enzymes that are involved in pH homeostasis in tissues [16]. One of these includes carbonic anhydrase iso-enzyme VI (CAVI). It is the only secretory iso-enzyme that is exclusively expressed in the serous acinar cells of the parotid and submandibular glands and is secreted in saliva [17]. These autoantibodies were first discovered in Interleukin14 alpha transgenic (IL14aTG) mice and were found to occur early in the course of the disease, when there is minimal salivary gland injury. They have also been found in patients that have not met the criteria for SS but have had unexplained dry eyes [18]. Parotid secretory protein (PSP) was initially discovered in murine parotid saliva; however, it is also found in lung, skin and gonads. It is postulated that this protein plays an important part in innate immunity [19]. In SS, anti-CAVI and anti-PSP usually occur in the same patient population [20].

On the other hand, expression of anti-SP1 is not related to anti-CAVI or anti PSP. Salivary gland protein (SP1) was first demonstrated in murine models. In 2014, Shen et al., showed that similar proteins also existed in human parotid gland and more importantly antibodies to this protein were found in patients with SS [20]. This antibody was found to rarely occur in normal controls or patients with RA [18]. However, itdid occur in 50% of patients with secondary SS and RA, and together with anti-Ro and anti-La these antibodies were found in 80% of patients with secondary SS and RA [20].

The novel antibodies, anti-CAVI, anti-PSP and anti-SP1, occur in increased number of patients with unexplained dry eyes and dry mouth, as opposed to anti-Ro and anti-La [18,20]. In a study where dry eyes were evaluated using Schirmer's test, it was found that the patients whose Schirmer's test was > 3mm but < 6 mm, the expression of anti-SP1, anti-CAVI and anti-PSP was significantly higher than that of anti-Ro and anti-La. However, when the patients whose Schirmer's test was < 3 mm, the expression of anti SP1, anti-CAVI and anti-PSP was not significantly greater than the expression of anti-Ro/La. However, it was still significantly greater than in normal controls. Another subset of the patients was evaluated in the same study which included patients with Schirmer's test > 3mm and < 6 mm who had symptomatic dry eyes for < 2 years and did not have dry mouth. This subset did not express anti-Ro/La but did express anti-SP1, anti-PSP and anti-CAVAI with a statistically significant difference. This shows that the expression of anti-SP1, anti-CAVI and anti-PSP occurs earlier in the disease while expression of anti Ro/La lags behind [4].

It has been previously noted that in long standing SS these novel antibodies may disappear. However, the ELISA assays were previously only done for IgM and IgG. In a recent study IgA was also evaluated in patients with long standing SS. This is important as IgA predominates in the mucosal areas. It was found that 38%, 7% and 9% of patients had IgA anti-CAVI, anti-SP1 and anti-PSP, respectively which was highly significant when compared with normal controls [21].

In the same study, the novel autoantibodies were evaluated in SLE, SSc or MCTD with or without secondary SS. In the patients with SLE and SS, 44% of the patients were positive for IgA anti-PSP, which was statistically significant. In patients with SSc and SS, 71% of the patients had positive anti-SP1, anti-CAVI and anti-PSP antibodies, the majority of them being IgA. It was statistically significant when compared with anti-Ro in patients with SSc and SS; however, when compared with patients with SSc without secondary SS it was not statistically significant. There was a small sample size evaluated for MCTD which showed these novel antibodies were found in 58% of patients with SS and MCTD and none in patients with MCTD without secondary SS, again majority of these being IgA antibodies. Unfortunately, this was not statistically significant due to small sample size [21].

In a recent analysis of 488 patients diagnosed with Sjogren's syndrome by Ophthalmologists and Rheumatologists, the presence of only the classical autoantibodies (anti-Ro/anti-La) was found in 62 patients. The novel autoantibodies (anti-SP1, anti-CA6 and anti-PSP) were found exclusively in 293 patients and both classical and novel autoantibodies were found in 133 patients (Figure 1). Thus, the addition of the novel autoantibody testing adds significantly to the sensitivity of testing for Sjogren's syndrome.
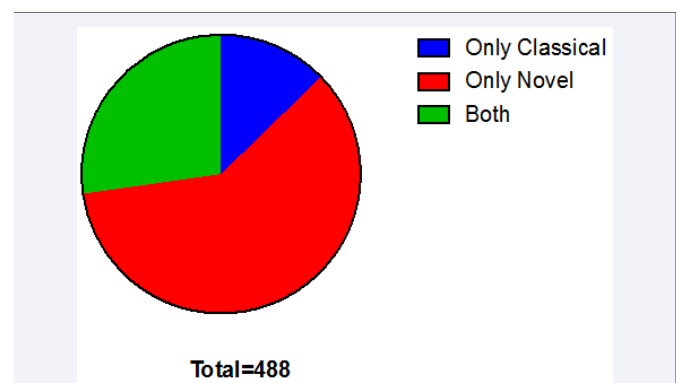

Figure 1 Anti-Muscarinic acetylcholine receptor 3.

Muscarinic acetylcholine receptor subtype 3 (m3AChR) is a membrane protein that is present in multiple locations in our body including salivary glands, lacrimal glands, brain and pancreas. In salivary and lacrimal glands these receptors aid in transmitting parasympathetic signal to secretory acinar epithelial cells [22]. The protein m3AChR consists of 4 extracellular domains: N terminal and three extracellular loops [23]. Antibodies against the second extracellular loop has been of particular interest. In 2008, a preclinical study by Kovacs et al., found that antibody to a peptide on second extracellular loop of human m3AChR purified from patients with primary SS bound salivary gland acinar cells from a healthy human. They were seen to bind in a dot-like or short linear pattern at the basolateral membrane of epithelial cells that correlates with the location of the nerve endings innervating the epithelial cells [22]. In 2012, He et al., showed that antibodies to m3AChR polypeptide was present in 69% of the patients with primary SS. It was also present in patients with SLE, RA and healthy controls; however, the expression in primary SS was significantly higher [24].

Anti-Carbonic anhydrase II: Carbonic anhydrase II (CAII) is involved in acid base equilibrium. It acts on the proximal and distal tubule and is involved in bicarbonate reabsorption and regeneration. Anti-CAII has been found in numerous rheumatologic illnesses including: SLE, SS, SSc, dermatomyositis, RA, primary biliary cholangitis, autoimmune cholangitis and graves thyroiditis [25]. Renal tubular acidosis (RTA) is one of systemic manifestations of SS. Anti-CAII antibodies have been hypothesised to be associated with RTA in patients with SS. In 2005, Takemoto et al., showed that patients with SS with renal tubular acidosis had higher level of anti-CAII than patients without acidosis. Their study also showed that the level of the antibody titers was proportional to the amount of acidemia [26].

Involvement of antibodies in pathogenesis

Sjogren's Syndrome is a disease of chronic inflammation with lymphocytic infiltration in the salivary and lacrimal glands [27]. As mentioned previously, SS is associated with many autoantigens which likely play a role in the disease process. One of the theories regarding pathophysiology of SS consists of viral infection inducing type I IFN, predominantly IFN-α, production by plasmacytoid dendritic cells (PDCs). IFN-α has various effects on the adaptive immune system which leads to local and systemic manifestations of SS. However viral infection is not the only inducer of IFN-α. There are endogenous autoantigens such as RNA molecules present that can lead to increased production of IFN-α [27]. In 2005, Bave et al., speculated that the autoantibodies to RNA-binding proteins, such as SSA and SSB, may also lead to induction of IFN-α. Their research showed that when sera contained anti-SSA antibodies significantly greater production of IFN-α was observed in peripheral blood mononuclear cells when combined with apoptotic and necrotic material. However, they were not able to show statistically significant increase in IFN-α with sera containing anti-SSB antibodies [28].

In 2011, Tsuboi et al., exhibited that antibodies to 2nd extracellular loop of M3AChR stopped the calcium influx in Cevimeline stimulated human salivary gland cells [29]. This provided some functional evidence for involvement of anti-m3AChR in pathogenesis of SS. Moreover, He et al., showed that the salivary flow rate was significantly lower in patients with anti-M3AChR antibodies versus the control group [24]. On the other hand, in 2016 a study by Chen et al. contradicted these claims. In their study, mice were immunized to produce antibodies against six peptides found on m3AChR protein. The binding of these antibodies was seen on the salivary gland, however no change in tear and saliva production was observed. This does not completely rule out the role of anti-m3AChR antibodies in salivary gland dysfunction as the peptide antibodies were either in linear or circular form and anti-M3AChR antibody may need its protein structure to function [23].

The involvement of anti-CAII in pathogenesis was presented by Nishimori et al., in 1995. Immunization of mice with anti-CAII produced large foci of mononuclear cells in salivary gland which was significant when compared with control mice. They also showed that SP-1 binds human and bovine CAII [30]. Both anti-SP1 and anti-CAII are found in patients with SS [20,25]. However, the significance of interaction between SP-1 and CAII is unknown and it may have been an artifact that was observed [30]. Furthermore, as mentioned earlier anti-CAII has been associated with patients with SS and RTA. In 2007, a study showed that mice immunized to produce anti-CAII antibodies developed incomplete distal RTA; they had urine acidification defect however they did not develop metabolic acidosis [31].

There are numerous gaps in knowledge regarding the involvement of autoantibodies in the pathogenesis of SS. As in other autoimmune diseases, the question has been raised as to whether initial IgM autoantibodies that are seen early in the disease are an attempt to cover up damaged tissue and help in the healing process. Later IgG and IgA antibodies could potentially have roles both in tissue injury and or tissue healing. Further work is needed to understand these issues further. The data supporting pathophysiological roles for anti-SSA/Ro, anti-M3AChR and anti-CAII is scarce. No studies have yet determined roles for anti-CA IV, anti-SP1 and anti-PSP.

CONCLUSION

There are no curative agents available for SS, all of the treatments are for symptomatic relief or to stop the progression of the disease. Hence, early detection of the SS is essential. Unfortunately, the diagnosis of SS is often delayed as most diagnostic markers available occur later in the disease course. However, now with the novel antibodies, early detection seems possible. Anti-SP1, anti-CAVI and anti-PSP expression was significantly higher in patients with early eye dryness as opposed to anti Ro/La. Now an advanced antibody panel is available which includes anti-CAVI, anti-PSP and anti-SP1 along with anti-Ro/anti-La, this should facilitate earlier detection of SS [4].

Further studies with bigger sample sizes need to be conducted to further evaluate these antibodies in secondary SS as the results in previous studies in patients with SS and SSc or SS and MCTD was not statistically significant due to small sample sizes. Moreover, research on antibodies against carbonic anhydrase isozyme II and muscarinic receptor antibodies is still in early stages. These antibodies are very important as they may be involved in pathogenesis of SS and likely occur earlier in the disease course, however more research is needed to make any further conclusions.

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Another interesting area of future research is the role of these autoantibodies in disease pathogenesis. Studies in various autoimmune diseases have suggested that early IgM autoantibodies may be part of a repair process, rather than contributors to tissue injury. Similar questions have not yet been raised with regards to the IgG and IgA antibodies that are seen later in the course of the disease. Furthermore, the prevalence of IgA autoantibodies in secondary SS deserves further investigation.

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