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Lymphomagenesis in Sjögren's syndrome: Predictive biomarkers towards precision medicine

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ABSTRACT

Sjögren's syndrome (SS) is characterized by B cell hyperactivity documented by the production of plethora of autoantibodies and a strong tendency for NHL of B cell origin. Classical predictors of lymphoma have been already proposed and proved their validity, including clinical, serological and histopathologic biomarkers. The process of lymphomagenesis is multistep and encompasses mechanisms of antigen driven selection of the BCR with RF activity and various genetic contributors implicated in B cell proliferation, cell growth and cell cycle control, enhanced by a complex milieu of cytokines and trophic agents that are abundant within the inflammatory lesion of minor salivary glands of SS patients. Extensive efforts in the basic research field have revealed several novel biomarkers for lymphoma prediction while the major cellular and molecular mechanisms of evolutionary transition of B cells towards malignancy are under investigation. In this review, we present the current data regarding the newly proposed biomarkers for SS associated lymphoma prediction and a hypothetical model of lymphomagenesis based on the emerging data.

1. Introduction

Among systemic autoimmune diseases, Sjögren's syndrome (SS) carries the higher risk for the occurrence of B cell lymphoproliferative disorders [1]. Although SS is mainly confined to the salivary and lacrimal glands resulting in mucosal dryness, the chronic and low-grade inflammation, appears to be an important contributor for the development of lymphoma, arising mainly from the diseased tissues. However, not all patients with SS are at high risk for lymphoproliferation but only those with distinct phenotypic characteristics that reflect an underlying multistep transition from a premalignant oligoclonal/monoclonal B cell state to mature B cell malignancy [2,3]. Patients with B cell mediated clinical manifestations constitute the systemic form of the disease and draw clinical attention as they display increased morbidity and a strong propensity for lymphoma development. Cryoglobulinemic mediated mechanisms are involved in the production of the clinical picture among patients with the systemic form of the disease, revealing the dynamic pathogenetic role of the B cell component. At some point during the disease course, the underlying biological process of B cell evolution towards malignancy is completed and is clinically manifested as lymphoma. The mechanistic link between the evolving B cell biology and the specific clinical spectrum of immune complex mediated extraepithelial manifestations, allowed us to focus on this specific subgroup of SS patients to identify predictors of lymphomas for research and clinical purposes [2,4].

Over the past decades, well established and validated risk factors for lymphomas have been reported to the literature and the clinical picture and outcome of patients with SS associated lymphomas have been extensively explored [5,6]. Lymphomas confer slightly increased mortality rates among SS patients compared to general population, but the majority of SS patients with lymphomas display a favorable prognosis with high overall survival. Classical predictors of lymphomas define those SS patients who require closer follow up in order to early diagnose and manage the complication of lymphoproliferative disorders. The era of biological agents and sophisticated biotechnologies led to the necessity for novel biomarkers linked to the major biological and molecular pathogenetic mechanisms of the disease, including lymphomagenesis. [2]. Although, lymphomagenesis in the context of SS is a multistep and complex process, it remains an attractive field of extensive basic and clinical research. As a result, novel genetic and molecular predictors of lymphoma have been proposed in addition to the already established clinical and serological biomarkers. In this review, we summarize the current knowledge about lymphoproliferation in SS and we focus on the clinical utility of previous and promising predictors of lymphoma.

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2. The clinical phenotypes of Sjögren's syndrome

The presence of lymphocytic infiltration around the epithelial structures of affected tissues in patients with SS, is the histopathologic hallmark of the disease, observed mainly at the salivary and lacrimal glands [7]. The composition of the infiltrate is diverse but it consists of T and B cell populations while plasma cells and macrophages do not exceed 5% of the total infiltrating cells [8]. Of note, It has been shown that the composition of the infiltrate is associated with the clinical phenotypes of the disease. Considering the immunopathogenesis of SS, symptomatology is thought to result either from lymphocytic infiltration of the epithelium that leads progressively to functional impairment of the affected tissue or as a consequence of cryoglobulin immune complex mediated inflammation of the involved organs, following deposition and complement activation [9]. Interestingly, the diseased epithelium is not just an innocent bystander that is slowly damaged but an active player, participating in maintenance and perpetuation of the autoimmune vicious cycle within the pathologic lesion. Thus, epithelial cells acquire antigen presenting properties by expressing MHC I and II molecules, secrete cytokines and chemokines necessary to attract and expand lymphocytic clones and are considered a continuous source of autoantigens release either by apoptotic blebs or through exosomes, fueling the local autoimmune response with antigenic load [9]. On the other hand, the B cell compartment within the lesion is of great importance and is implicated in the production of autoantibodies, hypergammaglobulinemia and cryoglobulins. However, B cell biology defines the plasticity of the inflammatory lesion, with non-specific benign B cell hyperactivity being responsible for the diffuse hypergammaglobulinemia and anti-Ro/SSA and anti-La/SSB antibodies in SS while B cell monoclonality for cryoglobulin production [9]. As mentioned previously, the histopathologic intralesional inflammatory features were correlated with the clinical phenotypes of the disease. The predominance of T cells within mild or intermediate lesions was linked to glandular and extraglandular manifestations of SS while B cell expansion, observed in advanced lesions was associated with the immune complex mediated complications [8].

The clinical manifestation of SS can be categorized based on anatomical and immunopathologic grounds, into periepithelial glandular or extraglandular and extraepithelial [6,10]. The lymphocytic infiltrate around the epithelial cells of the involved tissues and organs, is considered the common immunopathologic feature observed between the affected glandular and extraglandular sites. Mucosal dryness dominates the clinical picture of almost all SS patients with oral and eye dryness to afflict > 95% of SS population while other forms of dryness include xerotrachea resulting in dry cough, vaginal dryness accompanied by dyspareunia as well as skin and nose dryness [6,10]. In addition, 40% of pSS patients are expected to present with bilateral or unilateral parotid swelling that is usually painless and subsides spontaneously within weeks. Dryness symptoms vary in severity, depending also on the subjective perception of each patient but definitely have an impact on the quality of life [6,10]. Similarly, extraglandular periepithelial manifestations are attributed to the same immunopathologic mechanism of lymphocytic infiltration involving the tubular epithelium (interstitial nephritis/tubulitis), biliary epithelium (primary biliary cholangitis) or bronchus/bronchiolar epithelium (xerotrachea/small airways disease). However, there are systemic non-specific extraglandular manifestations such as Raynaud's phenomenon, fatigue, non-erosive arthritis of small hands and interstitial lung disease (ILD) with less defined pathogenesis [6,10]. For most SS patients, the clinical spectrum of the disease has been already completed at the time of diagnosis, considering that patients seek medical evaluation many years after the occurrence of sicca symptoms and mucosal dryness [6]. The clinical picture of SS patients remains quite stable for many years as a consequence of the benign and slowly progressive nature of the periepithelial lymphocytic infiltration, although in some cases the cumulative effect of tissue damage may result in functional deregulation with significant organ impairment [6,11].

The intralesional expansion of B cell component is reflected by the occurrence of B cell related symptoms, pointing out the very dynamic potential of B cells in SS pathogenesis. Almost 15% of SS patients have serum monoclonal component while the identification of type II circulating cryoglobulins is an early laboratory finding of the syndrome, both suggesting that B cell monoclonality is already present and detectable during the early stages of the disease [6,12]. The B cell associated extraepithelial manifestations characterize the systemic form of the disease and affect approximately 10–15% of SS population. These manifestations are driven by the underlying B cell monoclonality and are mediated mainly by type II cryoglobulins with rheumatoid factor activity. The pathogenetic mechanism of B cell mediated clinical manifestations is attributed to immune complex formation and tissue deposition leading to complement activation with subsequent inflammation, explaining also the low C4 complement levels that are frequently observed in these patients [6]. Palpable purpura of the lower extremities due to small vessel vasculitis may occur in 10% of SS patients and is considered the most common extraepithelial manifestation that usually follows the appearance of sicca symptoms, although in some rare cases purpura precedes the development of mucosal dryness [6,10]. Membranoproliferative glomerulonephritis is the typical histologic type manifested as nephritic syndrome with or without renal impaired function and is produced by immune complex deposition at the glomeruli [13,14]. Overall, glomerulopathies affect 2–3% of SS patients and include also other histologic types such as membranous and mesangial with similar clinical features. Finally, peripheral neuropathy may be observed in 1–2% of SS patients resulting from immune complex deposition in the vasa nervosum, producing either mononeuritis multiplex or sensorimotor axonal polyneuropathy [6]. Extraepithelial manifestations as opposed to glandular and extraglandular periepithelial manifestations, have the tendency to occur as a late sequel of SS and are characterized by worse prognosis if left untreated. The fact that extraepithelial manifestations of SS respond very well to anti-B cell depletion therapies, further support the pathogenetic association with the underlying intralesional B cell component [15]. Interestingly, SS patients with the systemic form display much higher risk to develop also lymphoproliferative disorders compared to those SS patients without, underlining the evolutionary continuum of B cells towards lymphomagenesis in this subgroup.

3. The clinical features of Sjögren's syndrome non Hodgkin's associated lymphomas

In the past decades, many different groups have shown that SS predisposes to the development of lymphoproliferative disorders of B cell origin, affecting survival and morbidity of SS patients. In previous studies, the prevalence of lymphoproliferative disorders varies from 2.7% to 9.8% [16–24] while SS displays the higher standardized incidence ratio (SIR) (18.9, 95% CI = 9.4–37.9) for lymphomas among other systemic autoimmune diseases including SLE and RA [1]. Interestingly, the estimated 10 year risk of lymphoma is approximately 4% while the life time risk is expected to be > 5% in SS population [3]. According to the estimated standardized mortality ratio (SMR), ranging from 1.02 (95% CI = 1.03–3.71) to 4.66 (95% CI = 3.85–5.60), SS patients exhibit slightly increased mortality rates compared to the general population, mainly due to non Hodgkin's lymphomas (NHL) [3,6,25–29]. In this line, Theander et al. found a SMR = 1.17 (95% CI = 0.81–1.53) that was exclusively attributed to lymphomas while the specific SMR was 7.89 (95% CI = 2.89–17.18), strongly suggesting a close relation between lymphomas and reduced survival in SS [28]. Finally, in a mortality study that included 53 SS patients with lymphoma and 531 SS patients without, the SMR was found 3.25 (95% CI = 1.32–6.76) and 1.08 (95% CI = 0.79–1.45) respectively, with 1.58/1000 person years excess death due to lymphoproliferative malignancies [29].

Approximately 65% of SS patients with associated lymphomas suffer from indolent extranodal marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) [30]. The other 2 histologic types include diffuse large B cell lymphomas (15%) and nodal marginal zone B cell lymphomas (10%). The most common lymphoma associated clinical manifestation was parotidomegaly (67%) and lymphadenopathy (61%) while 70% of patients present with hematologic manifestations including anemia, neutropenia or thrombocytopenia [30]. Interestingly, almost all patients had at least one laboratory finding suggestive of B cell deregulation such as serum monoclonal component, cryoglobulinemia, hypocomplementemia or hypogammaglobulinemia. Of note, more than half of patients had already at the time of lymphoma diagnosis, skin vasculitis/palpable purpura, 25% glomerulonephritis and 20% evidence of peripheral neuropathy [30]. The fact that SS associated lymphomas are of B cell origin and patients have prominent and detectable B cell abnormalities combined with high prevalence of extraepithelial manifestations, clearly point out the underlying evolutionary transition from a benign polyclonal B cell hyperactivity to premalignant B cell monoclonality and finally to malignant B cell lymphoma within the clinical spectrum of SS.

The outcome and prognosis of SS patients with associated lymphomas is favorable [30]. In a series of 77 patients, the 5-year overall survival (OS) rate was found 91% (95%CI: 82.14–95.80%) for the entire population. However, after analyzing according to histologic subtype, the 5 year (OS) was 94% (95%CI: 83.46–98.59%) for the subgroup of SS patients with MALT type, 87% (95%CI: 50.78–99.89%) for SS patients with nodal marginal zone and 75% (95%CI: 46.15–91.73%) for those with DLBC, suggesting that SS patients with DLBC display a worse prognosis due to higher relapse and death rates. In the multivariate analysis, an International Prognostic Index (IPI) score ≥ 3 was identified as an independent adverse predictor associated with reduced 5-year (OS) (adjusted HR = 8.529, 95% CI:1.325–54,904, $p = .024$) [30]. Regarding the management strategy of SS associated lymphomas, it depends primarily on the Ann Arbor stage, the histologic type and patient's comorbidities and performance status. For SS patients with MALT type and without dissemination of other hematological sites, a wait and see policy is recommended while in more advanced stages rituximab plus chemotherapy is a reasonable option. For DLBC cases, R-CHOP is by far the first line treatment while other regimens are administered after relapses [30].

4. Lymphomagenesis in SS

As mentioned previously, SS patients have the propensity to develop extranodal marginal zone B (MZB) cell MALT lymphomas originated from the salivary glands as a result of clonal expansion and malignant transformation of the B cell component within the inflammatory lesion. The histopathologic diagnosis of MALT lymphomas is based on morphological, immunophenotypic and genetic characteristics including documentation of monoclonality of the neoplastic population. Typically, neoplastic cells consist of small lymphocytes with morphologic features of centrocytes and less commonly centroblasts that invade the affected epithelium producing the characteristic picture of lymphoepithelial lesion [31,32]. The neoplastic lymphocytes also express specific surface markers such as IgM+, CD19+, CD20+, CD21+ and quite often CD43+ but they lack CD5 and CD10 [31,32]. Almost 50% of them show monotypic cytoplasmic Ig expression [32] while genetic studies have revealed various chromosomal abnormalities and high degree of somatic mutations, implying a post germinal center stage [33,34]. Normally, marginal zone B cells, are derived from TII transitional B cells (B-2 cells) after strong TLR engagement and reside outside the mantle area of GC structures in the lymph nodes and in the interphase zone of red and white splenic pulp [35]. These cells are designed to respond to thymus independent antigens after BCR and TLR ligation, producing low affinity antibodies. Apart from the high expression levels of BCR and TLRs, they also express the BAFF receptor TACI and it seems that the activating intracellular pathway results from the enhanced signal of all three surface receptors. Interestingly, MZ B cells have the capacity to undergo class switch to IgG or IgA and to differentiate into plasma cells [36]. In the minor salivary glands (MSG) of SS patients, infiltrating cells with immunophenotypic features resembling TII transitional and MZ B cells have been described around the ectopic GC like structures, along with autoreactive B cell clones producing autoantibodies [36]. Although, genuine GC as documented by the expression of AID are rare in MSG [37], salivary ectopic GC like structures are observed in 25% of SS patients at the time of diagnosis and have been proposed as a predictor of lymphoma [38]. The formation of ectopic GC like structures within the inflamed salivary glands, is complex and requires a milieu of chemokines such as CXCL12, CXCL13 and CCL11, cytokines and adhesions molecules. Given that ectopic GC like structures reflect disease severity, are considered also a potential active site of lymphomagenesis in SS [38–43]. However, in a recent study ectopic GC like structures were not identified as risk factor for lymphomas [44]. Taken together, MZ B cells and ectopic GC like structures are potential active contributors of lymphomagenesis in SS but further studies are needed to document their exact role in this process.

Gene sequencing analysis of the CDR3 region of the variable segment of heavy chain (IgV_H) in the salivary glands of SS patients with MALT lymphomas, revealed homology to V1–69, V3–7 encoded RFs and WOL – RFs in 40% of patients, with IgG binding capacity in vitro, while neoplastic B cells have been found to express BCR with RF activity, implying that MALT lymphomas may arise from RF (+) B cells clones [45,46]. Indeed, similar IgV_H repertoire has been previously described by other groups within the inflammatory lesion of MSG [46] while autoreactive B cell clones with RF activity have been reported in the salivary glands of SS patients along with IgG or IgM plasma cells, replacing the normally observed IgA plasma cells of mucosae [47–51]. Additionally, circulating type II cryoglobulins with RF activity probably produced within the diseased MSGs, have been identified as predictors of lymphoma in SS patients, underlining the important role of RF(+) B cell clones in lymphomagenesis [12,52]. Furthermore, immune complexes containing chromatin or CpG DNA have the capacity to induce proliferation of RF(+) B cells in vitro, most likely through engagement of BCR and TLR9 [53,54]. The fact that IgG autoreactive B cell clone specificity within the salivary glands extends beyond the spectrum of Ro/SSA and La/SSB supports the notion that immune complex formation may take place in situ leading to RF+ B cell proliferation. In addition, the microenvironment of the inflammatory lesion is rich in cytokines, growth factors and trophic agents capable of supporting the expansion and survival of malignant B cells such as BAFF and IL-6.

At the cellular and molecular level, the NF-kB pathway, BAFF and TNFAIP3 have drawn much of attention in the past years. More specifically, the NF-kB pathway seems to be activated through BCR ligation by autoantigens leading to enhanced proliferative and survival signals upon neoplastic B cells [55]. The SNP polymorphism rs2230926 of TNFAIP3 has been associated with increased risk of lymphoma in SS while 77% of SS patients with lymphoma show either germline or tumoral mutations of TNFAIP3 resulting in loss of function and impaired negative control on the NF-kB pathway [56]. B cell activating factor (BAFF) is a trophic agent implicated in B cell proliferation and differentiation by activating the NF-kB pathway and delivering anti-apoptotic and survival signals [57,58]. More specifically, BAFF-R ligation is involved in the non-canonical NF-kB2 pathway, leading to the recruitment of TRAF3 into the cytoplasmic tail of the receptor, thus inhibiting the NF-kB inducing kinase degradation and promoting the NF-kB2 transcriptional program [55]. The levels of BAFF have been found elevated in the serum of SS patients and specific BAFF polymorphisms have been associated with lymphoma development and increased BAFF serum levels [59–62]. BAFF is produced by a variety of cell types including salivary epithelial cells, under the induction of both IFN type I and II which are abundant within the salivary histopathologic lesion of SS. Interestingly, BAFF has been proposed to be involved in both

autoimmunity and NHLs [58]. A mutation of the BAFF receptor in NHL patients, encoding a His159Tyr substitution in the cytoplasmic tail, was found to facilitate TRAF2,3,6 recruitment leading to increased immunoglobulin production after CD40 stimulation and activation of both NF-kB1 and 2 pathways [63]. Similarly, almost 70% of SS patients with MALT lymphomas at the 3rd decade of life, were carriers of this specific mutation and isolated B cells were found with increased NF-kB2 levels at the mRNA and protein level but not NF-kB1 [64]. Overall, the NF-kB pathway and its normal inducers and regulators such as the proliferative BAFF/BAFF-R pathway and the tumor suppressor TNFAIP3 respectively, seem to be implicated in lymphomagenesis of SS.

The hypothetic model of SS associated lymphoproliferation, is considered a multistep and chronic process during which 2 distinct stages are taking place within the inflammatory lesion of the MSG, driving the transition from a benign polyreactive B cell population to a malignant monoclonal B cell component [46,65,66]. In the first stage, the persistent and chronic antigenic stimulation defines and shapes the BCR repertoire from a reactive and polyclonal state to a restricted oligoclonal RF+ spectrum as a result of an antigen driven, yet unknown, selection. In the second stage, the network of cytokines, growth factors and trophic agents within the lesion in combination with stimulating signals through the BCR and TLR receptors on B cells, maintain the proliferative and survival potential of the expanding B cell oligoclonal population. Further accumulation of germline mutations leading to activation of oncogenes or inactivation of tumor suppression genes are expected to provide acquired traits involved in cell cycle control, growth and proliferation, necessary to drive and complete the malignant transformation into phenotypically mature lymphomas (Fig. 1). However, the key molecules and intracellular signaling pathways that govern this process remain unclear. Better understanding of the mechanisms of lymphomagenesis is expected not only to facilitate the development of new and reliable biomarkers for lymphoma but also to reveal novel therapeutic targets.

5. Biomarkers for lymphoma prediction in SS

5.1. Classical biomarkers

Several, clinical, serological and histological biomarkers have been identified and proposed as potential predictors of SS associated NHLs, given that lymphomas are considered the most serious complication of SS with an impact on morbidity and mortality [2,4,5]. Recurrent episodes of unilateral or bilateral parotid swelling have been proven as the most reliable clinical predictor of lymphoma by many different groups [3,21,67–69]. Similarly, the occurrence of extraepithelial B cell mediated manifestations have been correlated with future development of lymphomas among SS population. Skin small vessel vasculitis of the lower extremities manifested as palpable purpura or leg ulcers, is also an independent risk factor of lymphoma while the appearance of peripheral neuropathy has been found to herald the upcoming development of lymphoproliferative disorders [3,6]. Glomerulonephritis, another immune complex mediated manifestation, although following a close temporal relationship with lymphomas in some SS patients, carries an increased risk for SS associated NHLs [6,14]. Finally, lymphadenopathy reflecting the hyperactive state of lymphocytes is another useful and well documented clinical biomarker to predict lymphoma complication in SS patients [3,21,70]. In the field of serologic biomarkers, serum mixed monoclonal cryoglobulins (MMC) of type II with rheumatoid factor (RF) activity and hypocomplementemia are the strongest and most validated predictors of lymphoma in SS [3,6,52,67,68,70]. It has been shown that the detection of type II cryoglobulins may precede the development of lymphoma in SS patients while low serum levels of C4 have been associated with increased risk of lymphoma and death. Interestingly, after combining palpable purpura and C4 serum hypocomplemetemia at the first evaluation visit, Iaonnidis et al. proposed a simple predictive model according to which SS patients with palpable purpura and low C4 serum complement levels with or without detectable type II cryoglobulins, can be classified as high risk with a strong tendency to present with NHL in the future [3]. Among hematologic numerical abnormalities, absolute CD4 lymphopenia and low CD4/CD8 ratio have been correlated with DLBC lymphomas while neutropenia at the diagnosis was related to increased risk of MALT lymphomas [70]. The high degree of inflammation within the MSG and especially a focus score ≥ 3 have been identified as independent risk factors for lymphoproliferation [71,72]. In addition, approximately 25% of SS patients have ectopic GC like structures at the salivary gland biopsy during initial evaluation and although debated, the presence of these structures is considered a risk factor for lymphoma and potential active site of lymphomagenesis by most research groups and clinicians [38]. As mentioned previously, the intralesional predominance of B cells constituting > 50% of total infiltrating cells in advanced lesions, characterizes a subset of SS patients with extraepithelial immune complex manifestations more prone to develop also lymphoproliferative disorders [8]. Finally, the presence of IL-18 expressing cells with macrophage features within the inflammatory infiltrates of MSG, was found to occur before the development of mature

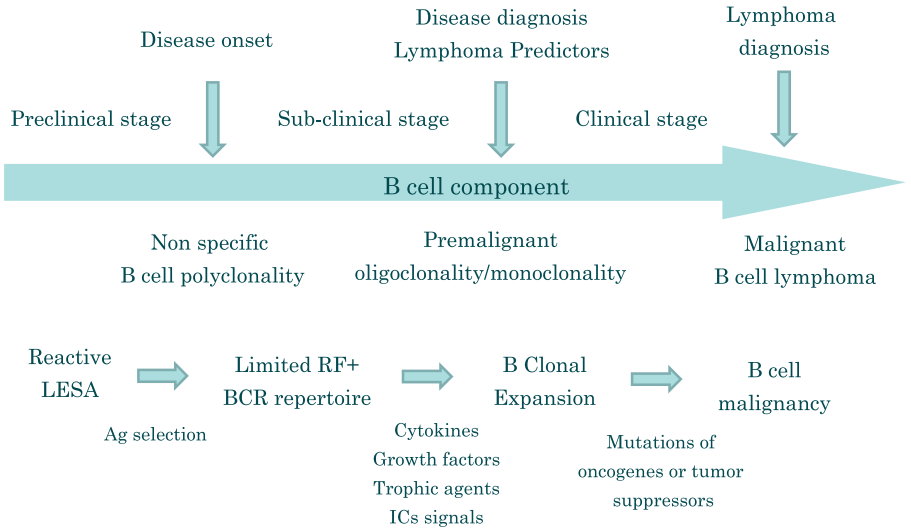

Fig. 1. Clinical and biological evolution of lymphomagenesis in Sjögren's syndrome. LESA: lymphoepithelial sialadenitis, RF: rheumatoid factor, ICs: immune complexes.

Table 1
Biomarkers predicting lymphoma development in Sjögren's syndrome.

Classical	Newly proposed
Clinical	Molecular
• Parotidomegaly • Palpable purpura/leg ulcers • Peripheral neuropathy • Lymphadenopathy	• Increased serum levels of CCL11 and CXCL13 • Increased serum levels of Flt3L • Upregulation of ductal CXCL12 • Decreased miR200b levels in MSG • High IFNγ/IFNα ratio in MSG • AID distribution in ectopic GC
Serological	Genetic
• Mixed monoclonal cryoglobulins • Hypocomplementemia • Neutropenia/Lymphopenia	• BAFF polymorphisms • TNFAIP3 polymorphism • His159Tyr
Histological	
• FS ≥ 3 • Ectopic GC	

Abbreviations: GC = germinal centers, FS = focus score, AID = activation induced cytidine deaminase.

lymphoma while higher macrophage incidence was prominent in patients with SS associated NHL compared to those without [8,11,73]. Overall, various clinical signs, serological parameters and histopathological findings have been analyzed and identified with significant predictive value for the development of lymphomas in SS population, most of which point out the dynamic and evolutionary transition of intralesional polyclonal B cells to malignant lymphomatous neoplastic lymphocytes of B cell origin. These simple and feasible biomarkers can be potentially used to define the high risk patient in order to study and provide new insights regarding the cellular and molecular events that drive lymphomagenesis in SS.

5.2. Novel biomarkers

Apart from the classical predictors of lymphoma mentioned above, novel biomarkers have been proposed as a result of extensive and continuous research effort motivated by the new biotechnologies that were applied in different biological specimens including saliva, serum and MSG tissue [2] (Table 1). Exploring the possible role of lymphotoxins and chemokines involved in the formation of GC, increased serum levels of CCL11 and CXCL13 were found in SS patients with lymphomas while CXCL12 expression was upregulated mainly within the infiltrated salivary ducts and areas of neoplastic B cells as opposed to CXCL13 and CCL21 that were elevated in reactive lymphoid aggregates only, suggesting a regulatory role for CCL12 in survival of B cells [40,74]. In this line and analyzing the topology and cellular distribution of activation induced cytidine deaminase (AID) within ECG in order to better understand the stages of lymphomagenesis, AID was identified in residual ECG strictures and not in the marginal like B cell population in SS patients with MALTs compared to SS patients without MALTs whereas (AID) was expressed by follicular dendritic cells within ECG and large B cells residing in a T rich zone outside the ECG [41]. Furthermore, studying the role of Flt3/Flt3L pathway of lymphoid bone marrow progenitors that delivers survival signals, it was found that Flt3L serum levels were elevated in lymphoma SS patients with significant predictive value for lymphoma development [75]. Regarding the newly genetic proposed markers, specific BAFF variants (rs1224141, rs12583006, rs9514828, rs1041569, rs9514827), the BAFF receptor His159Tyr mutation and the TNFAIP3 rs2230926 polymorphism have been implicated in SS associated lymphoproliferation and have been proposed as potential biomarkers for lymphoma prediction [56,62,64]. Similarly, considering the pathogenetic role of type I and II interferons, the high mRNA ratio of IFNγ/IFNα in the MSG tissues of SS patients, was proposed as an additional histopathologic biomarker for predicting in situ lymphoma in the context of SS [76]. Finally, in a recently published study, the mRNA levels of the miR200b family exerting properties as negative regulator of epithelial to mesenchymal transition and as tumor suppressor element, were measured with quantitative real time PCR in the MSG tissues of SS patients with sequential prelymphoma/lymphoma and without lymphoma [77]. Expression of miR200b-5p was reduced in prelymphoma/lymphoma patients compared to those without lymphoma, with no significant change upon transition to lymphoma. Interestingly, low miR200b-5p levels were proven to distinguish patient who will develop lymphomas from those who already have or will not develop lymphomas and were identified as an independent risk factor for lymphoproliferative disorders. Although research groups systematically attempt to utilize the new biotechnologies for measuring and evaluating numerous biological parameters that could be potentially used as biomarkers, confirmation and validation studies are required by many different groups for every newly proposed biomarker before the introduction in the clinical practice.

6. Conclusions

Lymphoproliferation is the most serious complication of SS and in combination with the immune complex mediated systemic form of the disease afflicting almost 10–15% of patients, define phenotypically unique subsets of SS characterized by the intralesional expansion of B cells within the MSG as opposed to glandular or extraglandular periepithelial manifestations in which T cells predominate within the inflammatory lesion. The spectrum of B cells extends from a benign polyclonal state producing hypergammaglobulinemia and various autoantibodies to premalignant oligoclonality/monoclonality accounting for cryoglobulinemic mediated symptoms and ends to malignant neoplastic MZ like B cells. In this transitional continuum, B cells evolve towards malignancy by an antigen driven process favoring RF positivity and subsequently by accumulating critical, yet unknown mutations involved in cell growth and proliferation until the mature phenotype of malignant lymphomas is complete. Although various established and validated predictors of lymphoma have been reported in the literature, the primary cellular and molecular events that drive the stages of lymphomagenesis remain to be elucidated. Apart from the classical clinical, serological and histopathological biomarkers for SS associated lymphomas, additional novel molecular and genetic biomarkers have been proposed, although validation is needed also from other groups before incorporation in the clinical management of SS patients. It is generally accepted for a specific biologic parameter in order to become a useful biomarker that some characteristic should be met, including simplicity, feasibility, reproducibility and cost-benefit adequacy. Regarding the biological significance of biomarkers related to SS associated lymphomas, ideally is expected to reflect the different stages of lymphomagenesis facilitating evaluation of response to treatment and more sophisticated patients' stratification.

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Conflicts of interest

The authors have no conflicts of interest to declare.

References

[1] Zintzaras E, Voulgarelis M, Moutsopoulos HM. The risk of lymphoma development in autoimmune diseases: a meta-analysis. Arch Intern Med 2005;165:2337–44.
[2] Goules AV, Tzioufas AG. Primary Sjgren's syndrome: clinical phenotypes, outcome and the development of biomarkers. Autoimmun Rev 2016;15:695–703.
[3] Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjogren's syndrome. Arthritis Rheum 2002;46:741–7.
[4] Papageorgiou A, Voulgarelis M, Tzioufas AG. Clinical picture, outcome and predictive factors of lymphoma in Sjgren syndrome. Autoimmun Rev 2015;14:641–9.
[5] Nishishinya MB, Pereda CA, Munoz-Fernandez S, Pego-Reigosa JM, Rua-Figueroa I, Andreu JL, et al. Identification of lymphoma predictors in patients with primary Sjogren's syndrome: a systematic literature review and meta-analysis. Rheumatol Int 2015;35:17–26.
[6] Skopouli FN, Dafni U, Ioannidis JP, Moutsopoulos HM. Clinical evolution, and morbidity and mortality of primary Sjogren's syndrome. Semin Arthritis Rheum 2000;29:296–304.
[7] Moutsopoulos HM. Sjogren's syndrome: autoimmune epithelitis. Clin Immunol Immunopathol 1994;72:162–5.
[8] Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjogren's syndrome. J Autoimmun 2010;34:400–7.
[9] Goules AV, Kapsogeorgou EK, Tzioufas AG. Insight into pathogenesis of Sjogren's syndrome: dissection on autoimmune infiltrates and epithelial cells. Clin Immunol 2017;182:30–40.
[10] Mavragani CP, Moutsopoulos HM. Sjogren syndrome. CMAJ 2014;186:E579–86.
[11] Kapsogeorgou EK, Christodoulou MI, Panagiotakos DB, Paikos S, Tassidou A, Tzioufas AG, et al. Minor salivary gland inflammatory lesions in Sjogren syndrome: do they evolve? J Rheumatol 2013;40:1566–71.
[12] Tzioufas AG, Manoussakis MN, Costello R, Silis M, Papadopoulos NM, Moutsopoulos HM. Cryoglobulinemia in autoimmune rheumatic diseases. Evidence of circulating monoclonal cryoglobulins in patients with primary Sjogren's syndrome. Arthritis Rheum 1986;29:1098–104.
[13] Goules A, Masouridi S, Tzioufas AG, Ioannidis JP, Skopouli FN, Moutsopoulos HM. Clinically significant and biopsy-documented renal involvement in primary Sjogren syndrome. Medicine (Baltimore) 2000;79:241–9.
[14] Goules AV, Tatouli IP, Moutsopoulos HM, Tzioufas AG. Clinically significant renal involvement in primary Sjogren's syndrome: clinical presentation and outcome. Arthritis Rheum 2013;65:2945–53.
[15] Mavragani CP, Nezos A, Moutsopoulos HM. New advances in the classification, pathogenesis and treatment of Sjogren's syndrome. Curr Opin Rheumatol 2013;25:623–9.
[16] Hernandez JA, Olive A, Ribera JM, Tena X, Cuxart A, Feliu E. Probability of the development of non-Hodgkin's lymphoma in primary Sjogren's syndrome. Scand J Rheumatol 1996;25:396–7.
[17] Kruize AA, Hene RJ, van der Heide A, Bodeutsch C, de Wilde PC, van Bijsterveld OP, et al. Long-term followup of patients with Sjogren's syndrome. Arthritis Rheum 1996;39:297–303.
[18] Lazarus MN, Robinson D, Mak V, Moller H, Isenberg DA. Incidence of cancer in a cohort of patients with primary Sjogren's syndrome. Rheumatology (Oxford) 2006;45:1012–5.
[19] Martens PB, Pillemer SR, Jacobsson LT, O'Fallon WM, Matteson EL. Survivorship in a population based cohort of patients with Sjogren's syndrome, 1976–1992. J Rheumatol 1999;26:1296–300.
[20] Pavlidis NA, Drosos AA, Papadimitriou C, Talal N, Moutsopoulos HM. Lymphoma in Sjogren's syndrome. Med Pediatr Oncol 1992;20:279–83.
[21] Sutcliffe N, Inanc M, Speight P, Isenberg D. Predictors of lymphoma development in primary Sjogren's syndrome. Semin Arthritis Rheum 1998;28:80–7.
[22] Valesini G, Priori R, Bavoillot D, Osborn J, Danieli MG, Del Papa N, et al. Differential risk of non-Hodgkin's lymphoma in Italian patients with primary Sjogren's syndrome. J Rheumatol 1997;24:2376–80.
[23] Voulgarelis M, Dafni UG, Isenberg DA, Moutsopoulos HM. Malignant lymphoma in primary Sjogren's syndrome: a multicenter, retrospective, clinical study by the European Concerted Action on Sjogren's Syndrome. Arthritis Rheum 1999;42:1765–72.
[24] Zufferey P, Meyer OC, Grossin M, Kahn MF. Primary Sjogren's syndrome (SS) and malignant lymphoma. A retrospective cohort study of 55 patients with SS. Scand J Rheumatol 1995;24:342–5.
[25] Alamanos Y, Tsifetaki N, Voulgari PV, Venetsanopoulou AI, Siozos C, Drosos AA. Epidemiology of primary Sjogren's syndrome in north-west Greece, 1982–2003. Rheumatology (Oxford) 2006;45:187–91.
[26] Brito-Zeron P, Kostov B, Solans R, Fraile G, Suarez-Cuervo C, Casanovas A, et al. Systemic activity and mortality in primary Sjogren syndrome: predicting survival using the EULAR-SS Disease Activity Index (ESSDAI) in 1045 patients. Ann Rheum Dis 2014;75:348–55.
[27] Pertovaara M, Pukkala E, Laippala P, Miettinen A, Pasternack A. A longitudinal cohort study of Finnish patients with primary Sjogren's syndrome: clinical, immunological, and epidemiological aspects. Ann Rheum Dis 2001;60:467–72.
[28] Theander E, Manthorpe R, Jacobsson LT. Mortality and causes of death in primary Sjogren's syndrome: a prospective cohort study. Arthritis Rheum 2004;50:1262–9.
[29] Voulgarelis M, Ziakas PD, Papageorgiou A, Baimpa E, Tzioufas AG, Moutsopoulos HM. Prognosis and outcome of non-Hodgkin lymphoma in primary Sjogren syndrome. Medicine (Baltimore) 2012;91:1–9.
[30] Papageorgiou A, Ziogas DC, Mavragani CP, Zintzaras E, Tzioufas AG, Moutsopoulos HM, et al. Predicting the outcome of Sjogren's syndrome-associated non-hodgkin's lymphoma patients. PLoS One 2015;10:e0116189.
[31] Schmid C, Kirkham N, Diss T, Isaacson PG. Splenic marginal zone cell lymphoma. Am J Surg Pathol 1992;16:455–66.
[32] Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016;127:2375–90.
[33] Bahler DW, Miklos JA, Swerdlow SH. Ongoing Ig gene hypermutation in salivary gland mucosa-associated lymphoid tissue-type lymphomas. Blood 1997;89:3335–44.
[34] Qin Y, Greiner A, Trunk MJ, Schmausser B, Ott MM, Muller-Hermelink HK. Somatic hypermutation in low-grade mucosa-associated lymphoid tissue-type B-cell lymphoma. Blood 1995;86:3528–34.
[35] Daridon C, Pers JO, Devauchelle V, Martins-Carvalho C, Hutin P, Pennec YL, et al. Identification of transitional type II B cells in the salivary glands of patients with Sjogren's syndrome. Arthritis Rheum 2006;54:2280–8.
[36] Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nat Rev Immunol 2013;13:118–32.
[37] Le Pottier L, Devauchelle V, Fautrel A, Daridon C, Saraux A, Youinou P, et al. Ectopic germinal centers are rare in Sjogren's syndrome salivary glands and do not exclude autoreactive B cells. J Immunol 2009;182:3540–7.
[38] Theander E, Vasaitis L, Baecklund E, Nordmark G, Warfvinge G, Liedholm R, et al. Lymphoid organisation in labial salivary gland biopsies is a possible predictor for the development of malignant lymphoma in primary Sjogren's syndrome. Ann Rheum Dis 2011;70:1363–8.
[39] Amft N, Curnow SJ, Scheel-Toellner D, Devadas A, Oates J, Crocker J, et al. Ectopic expression of the B cell-attracting chemokine BCA-1 (CXCL13) on endothelial cells and within lymphoid follicles contributes to the establishment of germinal center-like structures in Sjogren's syndrome. Arthritis Rheum 2001;44:2633–41.
[40] Barone F, Bombardieri M, Rosado MM, Morgan PR, Challacombe SJ, De Vita S, et al. CXCL13, CCL21, and CXCL12 expression in salivary glands of patients with Sjogren's syndrome and MALT lymphoma: association with reactive and malignant areas of lymphoid organization. J Immunol 2008;180:5130–40.
[41] Bombardieri M, Barone F, Humby F, Kelly S, McGurk M, Morgan P, et al. Activation-induced cytidine deaminase expression in follicular dendritic cell networks and interfollicular large B cells supports functionality of ectopic lymphoid neogenesis in autoimmune sialoadenitis and MALT lymphoma in Sjogren's syndrome. J Immunol 2007;179:4929–38.
[42] Nocturne G, Mariette X. Advances in understanding the pathogenesis of primary Sjogren's syndrome. Nat Rev Rheumatol 2013;9:544–56.
[43] Salomonsson S, Jonsson MV, Skarstein K, Brokstad KA, Hjelmstrom P, Wahren-Herlenius M, et al. Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjogren's syndrome. Arthritis Rheum 2003;48:3187–201.
[44] Johnsen SJ, Berget E, Jonsson MV, Helgeland L, Omdal R, Jonsson R. Evaluation of germinal center-like structures and B cell clonality in patients with primary Sjogren syndrome with and without lymphoma. J Rheumatol 2014;41:2214–22.
[45] Bende RJ, Aarts WM, Riedl RG, de Jong D, Pals ST, van Noesel CJ. Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. J Exp Med 2005;201:1229–41.
[46] Miklos JA, Swerdlow SH, Bahler DW. Salivary gland mucosa-associated lymphoid tissue lymphoma immunoglobulin V(H) genes show frequent use of V1-69 with distinctive CDR3 features. Blood 2000;95:3878–84.
[47] Deacon EM, Matthews JB, Potts AJ, Hamburger J, Bevan IS, Young LS. Detection of Epstein-Barr virus antigens and DNA in major and minor salivary glands using immunocytochemistry and polymerase chain reaction: possible relationship with Sjogren's syndrome. J Pathol 1991;163:351–60.
[48] Fox RI, Chen P, Carson DA, Fong S. Expression of a cross-reactive idiotype on rheumatoid factor in patients with Sjogren's syndrome. J Immunol 1986;136:477–83.
[49] Halse A, Harley JB, Kroneld U, Jonsson R. Ro/SS-A-reactive B lymphocytes in salivary glands and peripheral blood of patients with Sjogren's syndrome. Clin Exp Immunol 1999;115:203–7.
[50] Matthews JB, Deacon EM, Wilson C, Potts AJ, Hamburger J. Plasma cell populations in labial salivary glands from patients with and without Sjogren's syndrome. Histopathology 1993;23:399–407.
[51] Speight PM, Cruchley A, Williams DM. Quantification of plasma cells in labial salivary glands: increased expression of IgM in Sjogren's syndrome. J Oral Pathol Med 1990;19:126–30.
[52] Tzioufas AG, Boumba DS, Skopouli FN, Moutsopoulos HM. Mixed monoclonal cryoglobulinemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjogren's syndrome. Arthritis Rheum 1996;39:767–72.
[53] Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 2002;416:603–7.
[54] Viglianti GA, Lau CM, Hanley TM, Miko BA, Shlomchik MJ, Marshak-Rothstein A. Activation of autoreactive B cells by CpG dsDNA. Immunity 2003;19:837–47.
[55] Gardam S, Brink R. Non-Canonical NF-kappaB Signaling Initiated by BAFF Influences B Cell Biology at Multiple Junctures. Front Immunol 2014;4:509.
[56] Nocturne G, Boudaoud S, Miceli-Richard C, Viengchareun S, Lazure T, Nititham J, et al. Germline and somatic genetic variations of TNFAIP3 in lymphoma complicating primary Sjogren's syndrome. Blood 2013;122:4068–76.
[57] Bossen C, Schneider P. BAFF, APRIL and their receptors: structure, function and signaling. Semin Immunol 2006;18:263–75.
[58] Ferrer G, Hodgson K, Montserrat E, Moreno C. B cell activator factor and a

proliferation-inducing ligand at the cross-road of chronic lymphocytic leukemia and autoimmunity. Leuk Lymphoma 2009;50:1075–82.
[59] Jonsson MV, Szodoray P, Jellestad S, Jonsson R, Skarstein K. Association between circulating levels of the novel TNF family members APRIL and BAFF and lymphoid organization in primary Sjogren's syndrome. J Clin Immunol 2005;25:189–201.
[60] Mariette X, Roux S, Zhang J, Bengoufa D, Lavie F, Zhou T, et al. The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjogren's syndrome. Ann Rheum Dis 2003;62:168–71.
[61] Novak AJ, Slager SL, Fredericksen ZS, Wang AH, Manske MM, Ziesmer S, et al. Genetic variation in B-cell-activating factor is associated with an increased risk of developing B-cell non-Hodgkin lymphoma. Cancer Res 2009;69:4217–24.
[62] Nezos A, Papageorgiou A, Fragoulis G, Ioakeimidis D, Koutsilieris M, Tzioufas AG, et al. B-cell activating factor genetic variants in lymphomagenesis associated with primary Sjogren's syndrome. J Autoimmun 2014;51:89–98.
[63] Hildebrand JM, Luo Z, Manske MK, Price-Troska T, Ziesmer SC, Lin W, et al. A BAFF-R mutation associated with non-Hodgkin lymphoma alters TRAF recruitment and reveals new insights into BAFF-R signaling. J Exp Med 2010;207:2569–79.
[64] Papageorgiou A, Mavragani CP, Nezos A, Zintzaras E, Quartuccio L, De Vita S, et al. A BAFF receptor His159Tyr mutation in Sjogren's syndrome-related lymphoproliferation. Arthritis Rheumatol 2016;67:2732–41.
[65] Jordan RC, Speight PM. Lymphoma in Sjogren's syndrome. From histopathology to molecular pathology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;81:308–20.
[66] Moisini I, Davidson A. BAFF: a local and systemic target in autoimmune diseases. Clin Exp Immunol 2009;158:155–63.
[67] Brito-Zeron P, Ramos-Casals M, Bove A, Sentis J, Font J. Predicting adverse outcomes in primary Sjogren's syndrome: identification of prognostic factors. Rheumatology (Oxford) 2007;46:1359–62.
[68] Quartuccio L, Isola M, Baldini C, Priori R, Bartoloni Bocci E, Carubbi F, et al. Biomarkers of lymphoma in Sjogren's syndrome and evaluation of the lymphoma risk in prelymphomatous conditions: results of a multicenter study. J Autoimmun 2013;51:75–80.
[69] Risselada AP, Kruize AA, Bijlsma JW. Clinical features distinguishing lymphoma development in primary Sjogren's Syndrome–a retrospective cohort study. Semin Arthritis Rheum 2013;43:171–7.
[70] Baimpa E, Dahabreh IJ, Voulgarelis M, Moutsopoulos HM. Hematologic manifestations and predictors of lymphoma development in primary Sjogren syndrome: clinical and pathophysiologic aspects. Medicine (Baltimore) 2009;88:284–93.
[71] Risselada AP, Kruize AA, Goldschmeding R, Lafeber FP, Bijlsma JW, van Roon JA. The prognostic value of routinely performed minor salivary gland assessments in primary Sjogren's syndrome. Ann Rheum Dis 2014;73:1537–40.
[72] Carubbi F, Alunno A, Cipriani P, Bartoloni E, Baldini C, Quartuccio L, et al. A retrospective, multicenter study evaluating the prognostic value of minor salivary gland histology in a large cohort of patients with primary Sjogren's syndrome. Lupus 2015;24:315–20.
[73] Manoussakis MN, Boiu S, Korkolopoulou P, Kapsogeorgou EK, Kavantzas N, Ziakas P, et al. Rates of infiltration by macrophages and dendritic cells and expression of interleukin-18 and interleukin-12 in the chronic inflammatory lesions of Sjogren's syndrome: correlation with certain features of immune hyperactivity and factors associated with high risk of lymphoma development. Arthritis Rheum 2007;56:3977–88.
[74] Nocturne G, Seror R, Fogel O, Belkhir R, Boudaoud S, Saraux A, et al. CXCL13 and CCL11 serum levels and lymphoma and disease activity in primary Sjogren's syndrome. Arthritis Rheumatol 2015;67:3226–33.
[75] Tobon GJ, Saraux A, Gottenberg JE, Quartuccio L, Fabris M, Seror R, et al. Role of Fms-like tyrosine kinase 3 ligand as a potential biologic marker of lymphoma in primary Sjogren's syndrome. Arthritis Rheum 2013;65:3218–27.
[76] Nezos A, Gravani F, Tassidou A, Kapsogeorgou EK, Voulgarelis M, Koutsilieris M, et al. Type I and II interferon signatures in Sjogren's syndrome pathogenesis: contributions in distinct clinical phenotypes and Sjogren's related lymphomagenesis. J Autoimmun 2015;63:47–58.
[77] Kapsogeorgou EK, Papageorgiou A, Protogerou AD, Voulgarelis M, Tzioufas AG. Low miR200b-5p levels in minor salivary glands: a novel molecular marker predicting lymphoma development in patients with Sjogren's syndrome. Ann Rheum Dis 2018;77:1200–7.