Taylor & Francis
Taylor & Francis Group

Expert Review of Clinical Immunology

ISSN: 1744-666X (Print) 1744-8409 (Online) Journal homepage: https://www.tandfonline.com/loi/ierm20

Predicting lymphoma development in patients with Sjögren's syndrome

Salvatore De Vita & Saviana Gandolfo

To cite this article: Salvatore De Vita & Saviana Gandolfo (2019): Predicting lymphoma development in patients with Sjögren's syndrome, Expert Review of Clinical Immunology, DOI: 10.1080/1744666X.2019.1649596

To link to this article: https://doi.org/10.1080/1744666X.2019.1649596

Accepted author version posted online: 26 Jul 2019.

Publisher: Taylor & Francis

Journal: *Expert Review of Clinical Immunology*

DOI: 10.1080/1744666X.2019.1649596

Review

Predicting lymphoma development in patients with Sjögren's syndrome

Salvatore De Vita, Saviana Gandolfo

Rheumatology Clinic, Udine University Hospital, Department of Medical Area, University of Udine, Udine, Italy

Correspondence:
Salvatore De Vita
Rheumatology Clinic, University Hospital "S. Maria della Misericordia", piazzale S. Maria della Misericordia 15, 33100, Udine, Italy
Email: salvatore.devita@asuiud.sanita.fvg.it
Telephone: + 39 0432 559808
Fax: +39 0432 559472

ABSTRACT

Introduction: The issue of predicting lymphoma in primary Sjögren's syndrome (pSS) starts from its clinical and biologic essence, i.e., an autoimmune exocrinopathy with sicca syndrome, inflammation and lymphoproliferation of MALT (mucosa-associated lymphoid tissue) in exocrine glands.

Areas covered: The two major predictors to be firstly focused are persistent salivary gland swelling and cryoglobulinemic vasculitis with related features as purpura and low C4, or the sole serum cryoglobulinemia repeatedly detected. They are pathogenetically linked and reflect a heavier MALT involvement by histopathology, with the expansion of peculiar rheumatoid factor (RF)-positive clones/idiotypes. Other predictors include lymphadenopathy, splenomegaly, neutropenia, lymphopenia, serum beta2-microglobulin, monoclonal immunoglobulins, light chains, and RF. Composite indexes/scores may also predict lymphoma.

Expert opinion: Prediction at baseline needs amelioration, and must be repeated in the follow-up. Careful clinical characterization, with harmonization and stratification of large cohorts, is a relevant preliminary step. Validated and new biomarkers are needed in biologic fluids and tissues. SG echography with automatic scoring could represent a future imaging biomarker, still lacking. Scoring MALT involvement in pSS, as an additional tool to evaluate disease activity and possibly to predict lymphoma, is welcomed. All these efforts are now ongoing within the HarmonicSS project and in other research initiatives in pSS.

Keywords: Sjögren's, lymphoma, cryoglobulinemia, rheumatoid factor, HCV, MALT, ESSDAI, activity, swelling, autoimmunity

ARTICLE HIGHLIGHTS

1. The clinical hallmark of pSS is sicca syndrome, and the pathobiological hallmark is the acquisition of MALT (mucosa-associated lymphoid tissue) in exocrine glands, predisposing to lymphoma.
2. Although many lymphoma predictors (clinical, laboratory and pathologic) have been suggested in pSS, only two of them may be currently considered as major predictors: persisting salivary gland swelling and mixed cryoglobulinemia, which are pathogenetically linked.
3. B-cell clones linked to rheumatoid factor specificity are implicated in pSS-related lymphomagenesis, and they have a strong selection advantage within a polyclonal, benign B-cell expansion. They may either show or lack a rheumatoid factor-related cryoglobulinemic activity.
4. An easy-to-perform composite index of MALT involvement in pSS is needed, as an additional tool to evaluate activity and possibly to better predict lymphoma in pSS. Clinical, laboratory and imaging (salivary gland ultrasonography) tools relate to salivary gland histopathology, and might represent surrogates of it in pSS. By contrast, ESSDAI does not predict lymphoma.
5. The preliminary harmonization and stratification of large pSS cohorts, now in course within the HarmonicSS project (European Union Grant 731944; https://harmonicss.eu), is a crucial preliminary step for further clinical and applied research.

1. INTRODUCTION

Primary Sjögren's syndrome (pSS) is an autoimmune and lymphoproliferative connective tissue disease (CTD) [1], and its pathobiological hallmark is the acquisition of MALT (mucosa-associated lymphoid tissue) in exocrine glands [2, 3, 4]. Also for this reason pSS was called "autoimmune epithelitis" or "autoimmune exocrinopathy" [5]. Severe manifestations of CTDs are much less frequent [4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16] than sicca syndrome in pSS. By contrast, patients with pSS have a greatly increased risk to develop a B cell non-Hodgkin's lymphoma (NHL), which has a prevalence of about 5% [17, 18, 19, 20, 21, 22, 23, 24, 25], and which represents the major cause of patient decreased survival [26].

Although the clinical phenotype may be different in subsets of patients with pSS, the autoimmune and lymphoproliferative involvement of MALT sites, and in particular of the salivary and the lachrymal glands, is a common denominator in disease pathobiology, and leads to the key symptoms of dryness [2, 3, 4, 27]. This MALT background is typical of pSS, and not of other CTDs. Thus, it is not surprising that the MALT histotype or, in general, the marginal zone histotype, is detected in the vast majority of B cell NHLs arising in pSS. The second, but rarer lymphoma histotype is diffuse large B cell lymphoma, which may also derive from the transformation of a MALT lymphoma [24, 28, 29]. The very high prevalence of marginal zone lymphoma (84.2%; De Vita, personal observation) was recently confirmed in a large tri-centric Italian and Greek cohort from Udine, Pisa and Athens (UPA), involving 140 pSS-NHLs [30]. Strikingly, lymphoma localization is often salivary, i.e., a crucial localization of pSS itself.

In pSS MALT lesions, B lymphocytes are continuously stimulated, and epithelial cells play an active pathogenetic role [3, 4, 5]. Chronic triggering, immune cells mediating both the innate and adaptive response, cytokines and growth factors, are all present in the local microenvironment [4], and support the proliferation by B cells with different antibody specificity [31, 32]. The emergence of rheumatoid factor (RF)-positive clonal B cell expansions is later facilitated, and these cells in turn become more prone to additional events favouring their expansion, with the final possibility of overtly malignant transformation, antigen-selected [2, 33, 34, 35, 36, 37, 38, 39, 40, 41].

The issue of lymphoma prediction is of extreme importance in pSS, and not only due to the severity of this possible complication, but also to understand the pathophysiology of pSS itself with the available history (and pathologic tissues) from the fully benign to the overtly malignant stages of B cell expansion. Despite several years of efforts, however, the possibility to predict the outcome of pSS at the beginning of the disease, and also during the subsequent follow-up, is still limited. Lymphoma, as a possible outcome of pSS, makes no exception, although important steps to highlight some clinical and laboratory lymphoma predictors have been done over the years.

We will discuss the possible prediction of lymphoma development in pSS in clinical practice, at present. The currently used and easy-to-perform methods to evaluate the risk of lymphoma progression at the onset and during the course of pSS will be presented (Table 1). Secondly, we

will focus to some recent observations which could be useful, in our mind, to improve the study of lymphoma predictors. Finally, the most recent proposals and collaborative ongoing research approaches to this topic will be briefly updated, mainly in the HarmonicSS project (HarmonicSS, European Union Grant 731944; https://harmonicss.eu) [42].

2. PREDICTORS OF LYMPHOMA RISK IN pSS

Many lymphoma predictors (clinical, laboratory, and pathologic) have been searched in pSS since the 1970s. The more clinically relevant, as supported by the literature, are persistent salivary gland (SG) swelling and mixed cryoglobulinemia repeatedly detected and/or cryoglobulinemic vasculitis (CV; i.e. the presence of clinical vasculitis in addition to positive serum cryoglobulins) [2, 6, 7, 8, 9, 10, 11, 12, 13, 15, 27, 43], as well as low C4 and purpura, which may be cryoglobulinemia-related (Table 1). These two main clinical predictors are, in any case, strictly linked to additional clinical, pathologic and laboratory proposed predictors of lymphoma in pSS [6, 7, 8, 9, 10, 11, 12, 13, 14]. These include other CV-associated features (glomerulonephritis, peripheral neuropathy) [15], a heavier involvement of MALT by SG biopsy [2, 3, 4, 5, 6, 7, 8, 9, 43, 44], and laboratory features as monoclonal gammopathy and rheumatoid factor and its peculiar idiotypes, which also determine the pathophysiology of cryoglobulinemia [15] (Table 1).
Many other predictors have been also suggested, such as lymphadenopathy, splenomegaly, neutropenia, lymphopenia, free immunoglobulin light chains, increased serum beta2 microglobulin, positive rheumatoid factor in general, genetic abnormalities, oncogenetic events, cytokines and growth factors, chemokines, ongoing monoclonal B-cell expansion in metachronous tissue biopsies, and recently the European League Against Rheumatism (EULAR) Sjögren's syndrome disease activity index (ESSDAI) [2, 6, 7, 8, 9, 10, 11, 12, 13, 14, 26, 44, 45, 46] (Table 1).
Of note, it is crucial to know exactly whether a so called “predictor” of lymphoma in pSS may rather relate to the progression to B-cell lymphoma ad/or to fully deregulating oncogenetic events. If so, it is not a true predictor, even if very useful to monitor the disease and to catch the progression to a malignant lymphoproliferation, True predictors, by contrast, may be present many years before the lymphoma diagnosis. In addition, predictors may be different in different subgroups of pSS patients: the stratification of pSS is, thus, of crucial importance.

2.1 Persistent salivary gland swelling and cryoglobulinemia: the two major lymphoma predictors, related to tissue inflammation and lymphoproliferation in pSS

The major risk factor for lymphoma evolution in pSS is represented by persistent SG, which has been defined as swelling lasting at least two months [2, 27, 33]. A number of studies supports this statement [2, 6, 7, 8, 9, 10, 11, 12, 13, 27, 43], although how glandular swelling may be better detected still remains elusive. Importantly, SG enlargement is accompanied by gland inflammation, a higher focus score [10, 47, 48], and a higher degree of lymphoid organization. The organization

of the infiltrating lymphoid cells in SG biopsies in pSS may range from unorganized foci to organized foci, with or without germinal centres. The presence of germinal centres may imply a higher risk of lymphoma in pSS, although the issue is still controversial [8, 44, 49, 50]. Furthermore, SG localization is largely overrepresented in pSS-associated lymphomas, consistent with the earliest clinical observations by Talal [1]. In turn, SG swelling is phenotypically and biologically strictly linked to mixed cryoglobulinemia in pSS [15, 43].

In pSS, fully benign SG MALT contains the expansion of B cells with different specificity [31], but the B-cell clones heavily overexpanded in pSS-related myoepithelial sialadenitis and MALT lymphoma (i.e., those with a strong selection advantage) often express the same immunoglobulin genes and idiotypes of cryoglobulins, e.g. IGHV1-69, IGKV3-20 and the 17.109 idiotoype, and may originate cryoglobulins [6, 33, 38, 39, 40, 51]. In addition, a heavier SG inflammation and lymphoproliferation is significantly related to cryoglobulinemia in pSS [52]. Furthermore, the sole physical excision of a MALT lymphoma expressing these genes (by parotidectomy) was followed by a marked decrease in serum cryoglobulins, and then by their increase concomitantly to lymphoma relapse [43]. Thus, the salivary and, in general, the MALT involvement, i.e., the biologic hallmark of pSS, appears as the substrate of cryoglobulinemia in pSS, and these two main predictors of lymphoma in pSS are definitely linked each other. The MALT involvement appears to be, in any case, the original substrate: consistently, SG swelling, which reflects MALT expansion, is much more frequent than cryoglobulinemia [9, 10, 27].

One major clinical issue is that while SG swelling is present in about one third of patients with pSS, only a minor part of them will develop lymphoma. The risk of lymphoma was specifically investigated in pSS patients with SG swelling in only one study [9], providing a useful instrument for a further stratification, in clinical practice, of the whole SG swelling subgroup. In this study, analysing the lymphoma risk in a large multicentre Italian cohort in general, patients with the two major lymphoma predictors (CV and SG/parotid enlargement) were investigated in separate groups. All the selected 661 pSS patients, fulfilling the American European Criteria for the classification of SS, were hepatitis C virus (HCV)-negative and repeatedly tested for a careful evaluation of cryoglobulinemia. Patients were categorized into four groups, including pSS with lymphoma, with CV and without lymphoma, with persistent SG swelling without lymphoma (with or without CV), and pSS controls without lymphoma and without CV or SG swelling. In addition, pSS patients with SG swelling were further differentiated, either evolving or not into lymphoma in the follow-up. Within pSS patients with SG swelling, those with at least two additional risk factors among four (cryoglobulinemia, low C4, leukopenia and anti-La/SSB positivity) were at high risk of lymphoma, whereas the positivity of only one or none of the four risk factors provided a negative predictive value for lymphoma of about 90%. Strikingly, cryoglobulinemia and low C4 were selected as additional predictors together with SG swelling [9].

The second major predictor of lymphoma in pSS, besides persistent SG swelling, is the presence

of serum cryoglobulins (cryoglobulinemia) and, more consistently, a frank cryoglobulinemic vasculitis - CV, both predictors of lymphoma in pSS [2, 7, 26, 53, 54]. The association between mixed monoclonal (type II) cryoglobulinemia and the risk of lymphoma in pSS was first documented by Tzioufas et al. [6], who also reported an increased risk related to the presence of specific monoclonal RF-associated cross-reactive idiotypes (CRI), such as 17109 and G-6. Serum mixed cryoglobulinemia, occurs in about 7- 15 % patients with pSS, while CV is less frequent (3-7%), though it greatly affects the pSS-related morbidity [7, 52, 55]. Of note, the current international classification criteria for CV [56] have been further validated both in HCV-positive patients (i.e., the large majority of CV, i.e. about 70-90%, lacking pSS) [57] and in patients with pSS [58], which resulted the most common condition of HCV-unrelated CV [56]. This is consistent with the clinical phenotype showing many similarities [15]. Anyway, clinical differences also exist and, overall, two distinct entities (i.e., HCV-related and pSS-related/HCV-unrelated CV) are associated with mixed cryoglobulinemia and both predispose to B-cell NHL [15, 43, 59, 60]. Of note, phenotypic similarities and differences are also observed between lymphomas complicating the course of HCV-related and pSS-related CV in several studies [51, 61]. Furthermore, the similarities and differences are also biologic, since HCV-related cryoglobulinemia is mainly a bone marrow and hepatic lymphoproliferative disorder, while pSS-related cryoglobulinemia depends on lymphoproliferation of MALT [43]. Both in HCV-related and in pSS-related CV, however, there is an expansion of RF-positive B cell clones which employ the same immunoglobulin heavy and light genes [51], indicating common pathogenetic pathways. As a conclusion, HCV infection therefore represents a very good model to study the link between infection, autoimmunity and lymphoproliferation in pSS, where the triggering exogenous antigen(s) and/or autoantigen(s) remain unknown [59, 60, 62]. Finally, while cryoglobulinemia is a predictor of lymphoma in pSS, there are very limited data about additional predictors in patients who already have cryoglobulinemia [9, 63]. Further oncogenetic events definitely occur after the development of cryoglobulinemia itself in the course of HCV infection, since only a fraction of these patients evolves into lymphoma: in turn, this also may occur in pSS.

After the first report on cryoglobulinemia as lymphoma predictor in pSS [6] many other studies focused on this issue. Skoupouli and co-workers [53] reported that the development of pSS lymphoma was associated with the presence of mixed monoclonal cryoglobulins (relative risk, 7.9; p= 0.0012), low levels of C4 complement (relative risk, 7.5; p=0.0016), and purpura (relative risk, 3.9; p=0.037), the two latter manifestations being related to cryoglobulinemia. Low levels of C4 were indicated as the strongest predictor for mortality in pSS (relative risk, 6.5; p=0.0041). Of interest, the Authors observed that the initial presentation of pSS with these manifestations could determine the outcome and mortality of pSS itself [53].

Hypocomplementemia as a risk factor of lymphoma in pSS is herein discussed together with cryoglobulinemia and CV, since these are pathogenetically related to complement consumption

[15]. Hypocomplementemia is closely associated with the two main adverse outcome scenarios in pSS, i.e., lymphoma development and death [53]. In addition, low complement, as a lymphoma predictor, may be caused by an immune complex-mediated complement consumption also without detectable cryoglobulins. In fact, rheumatoid factor positivity itself, not necessarily linked to cryoprecipitability, may be as well related to the formation of immune complex and to complement consumption, and pSS-related lymphomas express B-cell receptor sequences with RF reactivity [33, 36, 40]. Thus, both peculiar rheumatoid factor specificities / idiotypes and low C4 deserve additional evaluation as relevant predictors of lymphoma in pSS, even without cryoglobulinemia.

2.2 Other risk factors

There are less data in the literature as concerns additional proposed risk factors for lymphoma in pSS.

Lymphopenia was reported as predictor of lymphoma in a cohort study by Theander et al. [64] who identified $CD4^+/CD8^+$ T cell ratio ≤0.8 (HR = 10.92, 95% CI 2.80 to 41.83) as the strongest risk factor for developing lymphoma in pSS. Other predictors in this study were $CD4^+$ T lymphocytopenia (HR = 8.14, 95% CI 2.10 to 31.53), low C4 (HR = 9.49, 95% CI 1.94 to 46.54), purpura/skin vasculitis (HR = 4.64, 95% CI 1.13 to 16.45) and decreased C3 (HR = 6.18, 95% CI 1.57 to 24.22). Of note, a diffuse large cell lymphoma histotype was more common than a marginal zone histotype in this cohort [64]. Consistently, Baimpa et al. reported that $CD4^+$ lymphocytopenia was the only independent variable predicting a lymphoma histotype different from marginal zone lymphoma in pSS, the major histotype being again diffuse large B cell NHL [7].

Among the other possible hematologic manifestations of pSS, Baimpa et al. reported neutropenia as a lymphoma risk factor in pSS (HR 8.9), correlating with splenomegaly [7]. In general, neutropenia appears as one laboratory factor able to predict the transformation of IgM disorders into lymphoid malignancy [65]. The role of anaemia has been sporadically suggested [10, 12].

Data regarding lymphadenopathy and splenomegaly as risk factors of lymphoma in pSS are scanty [7], may be difficult to be assessed if mild and inconstant, and finally a lymphoma evolution, rather than a true lymphoma prediction, may be also hypothesized.

High levels of serum beta2 microglobulin, of serum and urine immunoglobulin light chains ad their kappa/lambda ratio may also correlate with the burden of proliferating B cells [66, 67, 68].

More recently, by comparing the clinical and laboratory features in pSS patients positive or negative for the anti-Ro/SSA and/or anti-La/SSB antibodies, it was found that anti-SSA/SSB-negative pSS showed a lower risk of lymphoma evolution [16].

Moreover, a younger age at the pSS onset seems associated with an increased risk for lymphoproliferation [69, 70].

Genetic abnormalities, oncogenetic events, the involvement of NF-kB pathway, of cytokines, growth factors and chemokines [8, 44, 47, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85]

and the ongoing expansion in tissue lesions of the same B-cell clone [33], are also linked to the increased risk of lymphoma development or to multistep lymphomagenesis in pSS (reviewed in [86] and in [26]). They include B cell activating factor (BAFF) and Tumor necrosis factor alpha induced protein 3 (TNFAIP3) variants, BAFF receptor His159Tyr mutation, chromosomal translocations and oncogene deregulation, increased serum levels of Fms-related tyrosine kinase 3 ligand (Flt3-L), of C-X-C motif ligand 13 (CXCL13), and high Interferon (IFN) γ/IFNα ratio in minor SG biopsy. They are not however, at present, easy-to-access tests in current clinical practice, and are not herein discussed.

2.3 Composite indexes and scores

Composite indexes and scores might improve the prediction of lymphoma development in pSS [7, 9, 10, 87], although focusing on single predictors in the single individual patient remains a useful preliminary step in the clinical approach to the patient. Composite indexes might overweight and/or mainly evaluate the most important predictors. Whether/which additional predictors might be added, and how much they should be weighted, is also a question. Notably, SG swelling and cryoglobulinemia (or monoclonal gammopathy related to it) are included in all the currently available composite indexes for lymphoma prediction in pSS.

The attempt to stratify pSS patients on the basis of their lymphoproliferative risk was assessed for the first time by Ioannidis et al. [87], and confirmed the crucial role of cryoglobulinemia. The presence of parotid enlargement (hazard ratio - HR 5.21, 95% CI 1.76–15.4), palpable purpura (HR 4.16, 95% CI 1.65–10.5) and low C4 levels (HR 2.40, 95% CI 0.99–5.83) at the first visit resulted as independent predictors of lymphoproliferation in pSS. The Authors then proposed a stratification of pSS in two distinct risk categories, according to the presence or absence of low C4 levels and/or palpable purpura (both related to cryoglobulin positivity): the type I high-risk pSS, that accounts of about 20% of pSS at diagnosis, and the more frequent (80%) type II low-risk pSS phenotype, without a significant increase in risk of lymphoma evolution [87]. Brito-Zeròn and co-workers [55], in their cohort analysis, confirmed these data and added to the model the additional major risk factors of lymphoma development, i.e., severe parotid involvement, demonstrated by scintigraphy, and cryoglobulinemia. A survival analysis performed in this cohort found that pSS patients with at least two adverse factors at diagnosis among four, i.e. parotid involvement by scintigraphy, cryoglobulinemia, purpura and low C4, had a significantly lower survival than patients without risk factors.

A subsequent retrospective study investigated hematologic manifestations and predictors of lymphoma in 536 pSS patients (40 with lymphoma) [7]. Among 5 risk factors identified by multivariate analysis as predictors (cryoglobulinemia, low C4 levels, neutropenia, splenomegaly and lymphadenopathy) the proportion of pSS patients developing lymphoma by number of risk

factors was 3.62%, 11.96%, 34.78%, 80% and 100% in patients with 0, 1, 2, 3, and 4 risk factors, respectively.

Finally, a more recent case control study by Fragkioudaki et al. [10] compared 92 pSS patients developing lymphoma in the follow-up to 381 pSS controls without this evolution. Distinct multivariate analyses disclosed independent clinical, laboratory and histopathological risk factors for lymphoma in pSS, and a final multivariate analysis of all variables found seven independent lymphoma risk factors, the highest significance being for SG enlargement. Other risk factors included not only items which may be cryoglobulinemia-related, i.e., low C4, monoclonal gammopathy and positive rheumatoid factor, but also lymphadenopathy, Raynaud's phenomenon, anti-Ro/SSA and/or anti-La/SSB positivity. Development of lymphoma was 3.8% for patient with ≤ 2 risk factors, 39.9% for patients with 3 to 6 risk factors, and 100% in the presence of all the 7 risk factors [10].

By contrast, there is only one study where the risk of lymphoma was evaluated in pSS patients who already have a major risk factor, represented by SG enlargement [9]. When comparing 22 pSS patients with persistent SG swelling who developed lymphoma in the follow-up with 19 pSS patients with SG swelling who did not develop it after ≥ 5 years of follow-up, at least 2/4 among serum cryoglobulins, low C4, leukopenia and anti-La/SSB positivity provided a 10.2 odds ratio for lymphoma development (12/22 vs. 2/19 patients correctly classified) [9].

3. RECENT OBSERVATIONS TO IMPROVE LYMPHOMA PREDICTION STUDIES

3.1 An improved evaluation of disease activity of pSS is needed

Persistent SG swelling and mixed cryoglobulinemia [6, 7, 8, 9, 10, 11, 12, 13, 43] well reflect the degree of inflammation and lymphoproliferation of MALT in pSS [2, 3, 4, 5, 6, 7, 8, 9, 43]. Thus, they reveal the activity of pSS itself [1, 2, 3, 4]. In turn, increased activity of pSS is conceivably linked to a higher lymphoma risk. SG swelling and cryoglobulinemia were then analysed in detail [27], in the follow-up pSS patients developing lymphoma (30 cases) or not (225 pSS controls), to assess whether it is better to investigate definite lymphoma predictors rather than the ESSDAI, i.e., a putative lymphoma predictor [14] including a number of lymphoma-unrelated or poorly-related items. Of note, SG swelling and/or cryoglobulinemia at baseline were significantly higher ($p=0.0003$) in pSS patients evolving into lymphoma if compared to pSS controls, while the ESSDAI score showed no significant difference [27].

That ESSDAI is not a good lymphoma predictor was also shown in two different studies in independent large cohorts [10, 11], besides the aforementioned one [27]. Of note, about one third of pSS patients who will develop lymphoma shows a low activity, as calculated by ESSDAI, at baseline [10, 11, 27].

Therefore, the concept of the priority to consider the key disease manifestations and biomarkers predicting lymphoma in pSS, rather than a generical index of disease activity including lymphoma-

unrelated items, clearly emerges. Consistently, the possible increase in ESSDAI in pSS patients developing lymphoma appears to be mainly linked to the concomitant positivity of true lymphoma predictors (i.e., SG enlargement, cryoglobulinemia, purpura and low C4) and/or to possible lymphoma manifestations (lymphadenopathy) [27].

If pSS activity is evaluated with a composite index scoring the extent of MALT involvement, such pSS activity index might possibly prove useful for lymphoma prediction in pSS, where lymphoma usually arises from MALT.

3.2 The risk of lymphoma may change over time

A second important concept recently underlined [27] is that the risk of lymphoma evolution may change over time in pSS, i.e., the risk at different time points in the follow-up at may differ with that at baseline. As an example, SG swelling or cryoglobulinemia sometimes are lacking at baseline and develop ex novo in the follow-up of pSS. This is now under more extensive investigation in larger joined cohorts.

A dynamic approach is therefore recommended at present, requiring the re-evaluation of lymphoma risk factors by the general practitioner and the medical specialist at any follow-up visit of the pSS patient, also with low ESSDAI values (see Section 3).

3.3 Laboratory biomarkers will be crucial to improve lymphoma prediction

Laboratory biomarkers may be investigated either in biologic fluids (blood, saliva, urine) or directly in pathologic tissue biopsies in pSS [88]. Within the HarmonicSS project (European Union Grant 731944; https://harmonicss.eu), the investigation of biomarkers is one major task to improve patient stratification. A shorter list of laboratory biomarkers to be mainly focused within HarmonicSS was agreed by Experts during the last meeting of the EULAR Sjögren's syndrome experimental and translational investigative alliance study group (ESSENTIAL) held at the 2018 annual EULAR congress, Amsterdam. Most of the enclosed biomarkers may relate to disease activity and to the risk of lymphoma evolution.

These include the so called “old biomarkers”, i.e., those proposed by several studies in the past (beta2 microglobulin, free light chains, RF), the so called “old-new” biomarkers, i.e. those more recently proposed by different groups by preliminary studies (BAFF, CXCL13, Flt3-L, TNFAIP3 polymorphisms, IFN signature), and two definitely novel biomarkers proposed by single groups within HarmonicSS, i.e. miRNA200b-5p and thymic stromal lymphopoietin (TSLP) [89, 90]. In a very recent paper [91], low miRNA200b-5p was reduced in prelymphoma and lymphoma pSS patients, proving to be a useful predictor. TSLP increases in the serum of pSS patients, with higher levels in the myoepithelial sialadenitis and mixed cryoglobulinemia, and with the highest level in patients with B-cell lymphoma [92]. Importantly, a concomitant increased expression in tissue B-lymphocytes, maximal in lymphomatous ones, is noticed [93].

These biomarkers will be validated in the large harmonized cohort of pSS patients within the HarmonicSS study. Of course, the simultaneous investigation of a much wider panel of possible laboratory biomarkers is equally needed, and is also planned within HarmonicSS itself and other cooperative research projects in pSS (see Section 3).

4. CURRENT NOVEL INITIATIVES

Different ongoing cooperative initiatives in Europe are expected to contribute to the issue of lymphoma prediction, including, besides HarmonicSS, the BIG DATA Sjögren project [94, 95], the PRECISESADS study (European Union Grant 115565; https://www.precisesads.eu), and the NECESSITY project (European Union Grant 806975).

Evaluation of lymphoma risk is a major goal of the HarmonicSS project, and the following current initiatives have been taken in it:

a. The development of a novel composite index for lymphoma prediction in pSS, based on harmonized data in the largest expected cohort of cases and controls, is one of the key clinical unmet needs in HarmonicSS. Both traditional statistical methods and novel engineer-based methodologies [42, 96] will be used to achieve the best result. Two previous scores for lymphoma prediction, i.e. the more recent one in the largest series [10] and the only one available in patients who already have persistent SG swelling [9], will be also validated.
b. The investigation of very large array of serum and plasma biomarkers for pSS has been planned, including possible novel ones and those which could be more implicated for lymphoma prediction. The use of a unique, customized kit, and also saliva biomarkers, are under investigation.
c. The development of a possible imaging biomarker will be investigated in pSS, by means of SG ultrasound [97]. This will then occur not only for diagnostic purposes, but also to improve pSS follow-up, including lymphoma prediction. To this end, very novel techniques, including image segmentation and artificial intelligence, are being applied to this powerful, imaging tool [97, 98].
d. A final task of HarmonicSS is the development of a composite index of MALT involvement as an additional tool to evaluate disease activity in pSS. As said, MALT inflammation and lymphoproliferation represent the biologic essence of pSS. Many pSS patients cannot be recruited in clinical trials, despite having sicca symptoms, since the ESSDAI is low. This does not exclude, however, biologic and tissue disease activity in pSS [27]. The MALT composite index for pSS will be developed based on histopathology as the golden standard for the definition of the extent and the quality of tissue inflammation and lymphoproliferation. One novel concept is to simultaneously investigate, within the large harmonized pSS cohort, additional “surrogates” of MALT histopathology, i.e., the different

possible clinical, laboratory and imaging abnormalities significantly correlated. These may include SG swelling and CV (clinical), cryoglobulinemia, rheumatoid factor positivity and titre, low C4, and other tests in biologic fluids (laboratory), and SG abnormalities by ultrasound (imaging). Finally, the composite index, based both on bioptic and "surrogate" items, will be developed and then validated.

5. EXPERT OPINION

Primary Sjögren's syndrome is an autoimmune and lymphoproliferative connective tissue disease. Its clinical hallmark is the sicca syndrome, and its pathobiological hallmark is the acquisition of MALT (mucosa-associated lymphoid tissue) in exocrine glands. The issue of predicting lymphoma in pSS should start from these clinical and biological cornerstones.

Lymphoma prediction is relevant, both at baseline and in the follow-up assessment of pSS, for two main reasons. A clinical reason, since lymphoma represents the main cause of increased mortality, and a biologic reason, since it gives the rare possibility to investigate prospectively the different multistep pathogenetic events implicated in lymphoproliferation, and to pSS itself.

Many lymphoma predictors (clinical, laboratory and pathologic) have been highlighted in pSS. Only two of them, however, may be currently considered as major predictors, since they are the ones more consistently reported in the literature and also well linked to MALT autoimmunity and lymphoproliferation in pSS: they are persistent salivary gland (SG) swelling, and cryoglobulinemic vasculitis (CV; with related clinical and laboratory features such as purpura and low C4). Repeatedly detected cyoglobulinemia, even without overt clinical features of vasculitis, should be also considered in pSS.

Of note, persistent SG swelling and cryoglobulinemia reflect a heavier MALT acquisition in pSS by pathologic studies, which has been also related to an increased lymphoma risk.

Although pSS-related fully benign SG MALT contains B cells with different antigen specificity, the B-cell clones using peculiar immunoglobulin genes linked to rheumatoid factor (RF) specificity have a strong selection advantage, and are those overexpanded in pSS-related myoepithelial sialadenitis and MALT lymphoma. Then, RF-related peculiar clones/idiotypes are implicated in pSS-related lymphomagenesis, possibly also in the lack of RF-related cryoglobulinemic activity.

Overall, and consistent with the clinical finding of SG swelling being more frequent than cryoglobulinemia both in pSS and in pSS-related lymphomas, the MALT acquisition, with possible SG swelling, appears as the earliest pathogenetic event. Consequently, the evaluation of MALT in pSS is a starting point to predict lymphoma and to ameliorate this prediction in the future.

At present, all the pSS patients should be carefully evaluated with clinical, laboratory and imaging tools for present and past SG swelling, and CV features or cryoglobulinemia, also by careful laboratory testing. Other reported lymphoma predictors should be then studied, including clinical ones (lymphadenopathy, splenomegaly) and laboratory ones (neutropenia, lymphopenia,

increased serum beta2 microglobulin, immunoglobulin monoclonal components and light chains in biologic fluids, and RF, in particular its higher titres).

Two composite indexes/scores currently available to predict lymphoma in the general pSS population or in the pSS patients who already suffer of SG swelling may be finally used. Conversely, the ESSDAI score does not appear useful to predict lymphoma. The evaluation in a reference Centre for pSS would be required if an increased lymphoma risk is suspected, with patients also undergoing thorax computed tomography, abdomen and pelvic ultrasound, the study of gastric MALT, and bone marrow biopsy/aspiration if cryoglobulinemia is present. HCV infection should be always searched.

Recent observations and cooperative initiatives to improve lymphoma prediction in pSS in the future are crucial, and include:

- a dynamic approach requiring the re-evaluation of lymphoma risk factors by the general practitioner and the medical specialist at any follow-up visit, also in pSS with low ESSDAI values;
- the importance of validated and novel laboratory biomarkers in biologic fluids (blood, saliva, urine) and directly in pathologic tissue biopsies in pSS. Their more widespread use will be needed in the future;
- the importance of large multicentric collaborations, preceded by a very careful patient characterization. To this end, the preliminary harmonization and stratification of large pSS cohorts is crucial, and is now in course within the HarmonicSS (European Union Grant 731944; https://harmonicss.eu);
- the need also of a powerful imaging biomarker, which could be easily repeated during the follow-up, and showing a good reproducibility. SG ultrasound with the use of automatic scoring and artificial intelligence is being investigated to this end in HarmonicSS;
- finally, the need of an easy-to-perform composite index of MALT involvement in pSS, as an additional tool to evaluate disease activity and possibly to predict lymphoma in pSS.

Funding

This paper was not funded.

Declaration of interest

S. De Vita has acted as Consultant Expert for Roche, Human Genome Science, Glaxo Smith Kline and Novartis. Doctor S. Gandolfo has no conflicts of interest to declare. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

REFERENCES

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Anderson LG, Talal N. The spectrum of benign to malignant lymphoproliferation in Sjogren's syndrome. Clinical and experimental immunology. 1972 Feb;10(2):199-221. PubMed PMID: 4625796; PubMed Central PMCID: PMCPMC1713180. eng.
2. De Vita S, De Marchi G, Sacco S, et al. Preliminary classification of nonmalignant B cell proliferation in Sjogren's syndrome: perspectives on pathobiology and treatment based on an integrated clinico-pathologic and molecular study approach. Blood cells, molecules & diseases. 2001 Jul-Aug;27(4):757-66. doi: 10.1006/bcmd.2001.0446. PubMed PMID: 11778660; eng.
3. Mitsias DI, Kapsogeorgou EK, Moutsopoulos HM. The role of epithelial cells in the initiation and perpetuation of autoimmune lesions: lessons from Sjogren's syndrome (autoimmune epithelitis). Lupus. 2006;15(5):255-61. doi: 10.1191/0961203306lu2290rr. PubMed PMID: 16761498; eng.
4. Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjogren's syndrome: what we know and what we should learn. Journal of autoimmunity. 2012 Aug;39(1-2):4-8. doi: 10.1016/j.jaut.2012.01.002. PubMed PMID: 22326205; eng.
5. Moutsopoulos HM. Sjogren's syndrome: autoimmune epithelitis. Clinical immunology and immunopathology. 1994 Aug;72(2):162-5. PubMed PMID: 8050187; eng.
6. Tzioufas AG, Boumba DS, Skopouli FN, et al. Mixed monoclonal cryoglobulinemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjogren's syndrome. Arthritis and rheumatism. 1996 May;39(5):767-72. PubMed PMID: 8639173; eng.
7. Baimpa E, Dahabreh IJ, Voulgarelis M, et al. Hematologic manifestations and predictors of lymphoma development in primary Sjogren syndrome: clinical and pathophysiologic aspects. Medicine. 2009 Sep;88(5):284-93. doi: 10.1097/MD.0b013e3181b76ab5. PubMed PMID: 19745687; eng.
8. Theander E, Vasaitis L, Baecklund E, 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 Aug;70(8):1363-8. doi: 10.1136/ard.2010.144782. PubMed PMID: 21715359; PubMed Central PMCID: PMCPMC3128323. eng.
9. Quartuccio L, Isola M, Baldini C, et al. Biomarkers of lymphoma in Sjogren's syndrome and evaluation of the lymphoma risk in prelymphomatous conditions: results of a multicenter

study. Journal of autoimmunity. 2014 Jun;51:75-80. doi: 10.1016/j.jaut.2013.10.002. PubMed PMID: 24231556; eng.

10. Fragkioudaki S, Mavragani CP, Moutsopoulos HM. Predicting the risk for lymphoma development in Sjogren syndrome: An easy tool for clinical use. Medicine. 2016 Jun;95(25):e3766. doi: 10.1097/md.0000000000003766. PubMed PMID: 27336863; PubMed Central PMCID: PMCPMC4998301. eng.
11. Retamozo S, Gheitasi H, Quartuccio L, et al. Cryoglobulinaemic vasculitis at diagnosis predicts mortality in primary Sjogren syndrome: analysis of 515 patients. Rheumatology (Oxford). 2016 Aug;55(8):1443-51. doi: 10.1093/rheumatology/kew194. PubMed PMID: 27107430; eng.
12. Brito-Zeron P, Kostov B, Fraile G, et al. Characterization and risk estimate of cancer in patients with primary Sjogren syndrome. Journal of hematology & oncology. 2017 Apr 17;10(1):90. doi: 10.1186/s13045-017-0464-5. PubMed PMID: 28416003; PubMed Central PMCID: PMCPMC5392920. eng.
13. Papageorgiou A, Ziogas DC, Mavragani CP, et al. Predicting the outcome of Sjogren's syndrome-associated non-hodgkin's lymphoma patients. PLoS One. 2015;10(2):e0116189. doi: 10.1371/journal.pone.0116189. PubMed PMID: 25723713; PubMed Central PMCID: PMCPMC4344229. eng.
14. Nocturne G, Virone A, Ng WF, et al. Rheumatoid Factor and Disease Activity Are Independent Predictors of Lymphoma in Primary Sjogren's Syndrome. Arthritis & rheumatology (Hoboken, NJ). 2016 Apr;68(4):977-85. doi: 10.1002/art.39518. PubMed PMID: 26606524; eng.
15. De Vita S, Gandolfo S, Quartuccio L. Cryoglobulinemia. 7th ed. Hochberg M, Gravellese E, Silman A, et al., editors.: Elsevier; 2017. (Rheumatology, 2-Volume Set).
16. Quartuccio L, Baldini C, Bartoloni E, et al. Anti-SSA/SSB-negative Sjogren's syndrome shows a lower prevalence of lymphoproliferative manifestations, and a lower risk of lymphoma evolution. Autoimmunity reviews. 2015 Nov;14(11):1019-22. doi: 10.1016/j.autrev.2015.07.002. PubMed PMID: 26162302; eng.
17. Hernandez JA, Olive A, Ribera JM, et al. Probability of the development of non-Hodgkin's lymphoma in primary Sjogren's syndrome. Scandinavian journal of rheumatology. 1996;25(6):396-7. PubMed PMID: 8996477; eng.
18. Kruize AA, Hene RJ, van der Heide A, et al. Long-term followup of patients with Sjogren's syndrome. Arthritis and rheumatism. 1996 Feb;39(2):297-303. PubMed PMID: 8849383; eng.
19. Lazarus MN, Robinson D, Mak V, et al. Incidence of cancer in a cohort of patients with primary Sjogren's syndrome. Rheumatology (Oxford). 2006 Aug;45(8):1012-5. doi: 10.1093/rheumatology/kei281. PubMed PMID: 16490754; eng.

20. Martens PB, Pillemer SR, Jacobsson LT, et al. Survivorship in a population based cohort of patients with Sjogren's syndrome, 1976-1992. The Journal of rheumatology. 1999 Jun;26(6):1296-300. PubMed PMID: 10381046; eng.
21. Pavlidis NA, Drosos AA, Papadimitriou C, et al. Lymphoma in Sjogren's syndrome. Medical and pediatric oncology. 1992;20(4):279-83. PubMed PMID: 1608349; eng.
22. Sutcliffe N, Inanc M, Speight P, et al. Predictors of lymphoma development in primary Sjogren's syndrome. Semin Arthritis Rheum. 1998 Oct;28(2):80-7. PubMed PMID: 9806368; eng.
23. Valesini G, Priori R, Bavoillot D, et al. Differential risk of non-Hodgkin's lymphoma in Italian patients with primary Sjogren's syndrome. The Journal of rheumatology. 1997 Dec;24(12):2376-80. PubMed PMID: 9415645; eng.
24. Voulgarelis M, Dafni UG, Isenberg DA, et al. Malignant lymphoma in primary Sjogren's syndrome: a multicenter, retrospective, clinical study by the European Concerted Action on Sjogren's Syndrome. Arthritis and rheumatism. 1999 Aug;42(8):1765-72. doi: 10.1002/1529-0131(199908)42:8<1765::Aid-anr28>3.0.Co;2-v. PubMed PMID: 10446879; eng.
25. Zufferey P, Meyer OC, Grossin M, et al. Primary Sjogren's syndrome (SS) and malignant lymphoma. A retrospective cohort study of 55 patients with SS. Scandinavian journal of rheumatology. 1995;24(6):342-5. PubMed PMID: 8610217; eng.
26. Goules AV, Tzioufas AG. Lymphomagenesis in Sjogren's syndrome: Predictive biomarkers towards precision medicine. Autoimmunity reviews. 2019 Feb;18(2):137-143. doi: 10.1016/j.autrev.2018.08.007. PubMed PMID: 30572133; eng.
27. De Vita S, Gandolfo S, Zandonella Callegher S, et al. The evaluation of disease activity in Sjogren's syndrome based on the degree of MALT involvement: glandular swelling and cryoglobulinaemia compared to ESSDAI in a cohort study. Clin Exp Rheumatol. 2018 May-Jun;36 Suppl 112(3):150-156. PubMed PMID: 30156548; eng.
28. Voulgarelis M, Ziakas PD, Papageorgiou A, et al. Prognosis and outcome of non-Hodgkin lymphoma in primary Sjogren syndrome. Medicine. 2012 Jan;91(1):1-9. doi: 10.1097/MD.0b013e31824125e4. PubMed PMID: 22198497; eng.
29. Royer B, Cazals-Hatem D, Sibilia J, et al. Lymphomas in patients with Sjogren's syndrome are marginal zone B-cell neoplasms, arise in diverse extranodal and nodal sites, and are not associated with viruses. Blood. 1997 Jul 15;90(2):766-75. PubMed PMID: 9226177; eng.
30. Goules A, Baldini C, Gandolfo S, et al. Lymphoma in primary Sjögren's syndrome: a retrospective clinical study with patients from the UPA (Udine, Pisa, Athens) group. Annals of the Rheumatic Diseases. 2019;78(Suppl 2):1169.2-1170.

31. Bende RJ, Slot LM, Hoogeboom R, et al. Stereotypic rheumatoid factors that are frequently expressed in mucosa-associated lymphoid tissue-type lymphomas are rare in the labial salivary glands of patients with Sjogren's syndrome. Arthritis & rheumatology (Hoboken, NJ). 2015 Apr;67(4):1074-83. doi: 10.1002/art.39002. PubMed PMID: 25546553; eng.
32. Visser A, Doorenspleet ME, de Vries N, et al. Acquisition of N-Glycosylation Sites in Immunoglobulin Heavy Chain Genes During Local Expansion in Parotid Salivary Glands of Primary Sjogren Patients. Frontiers in immunology. 2018;9:491. doi: 10.3389/fimmu.2018.00491. PubMed PMID: 29662487; PubMed Central PMCID: PMCPMC5890187. eng.
33. De Vita S, Boiocchi M, Sorrentino D, et al. Characterization of prelymphomatous stages of B cell lymphoproliferation in Sjogren's syndrome. Arthritis and rheumatism. 1997 Feb;40(2):318-31. PubMed PMID: 9041944; eng.
34. Bahler DW, Swerdlow SH. Clonal salivary gland infiltrates associated with myoepithelial sialadenitis (Sjogren's syndrome) begin as nonmalignant antigen-selected expansions. Blood. 1998 Mar 15;91(6):1864-72. PubMed PMID: 9490668.
35. 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 Jun 15;95(12):3878-84. PubMed PMID: 10845923.
36. Martin T, Weber JC, Levallois H, et al. Salivary gland lymphomas in patients with Sjogren's syndrome may frequently develop from rheumatoid factor B cells. Arthritis and rheumatism. 2000 Apr;43(4):908-16. doi: 10.1002/1529-0131(200004)43:4<908::AID-ANR24>3.0.CO;2-K. PubMed PMID: 10765938.
37. Anderson LG, Cummings NA, Asofsky R, et al. Salivary gland immunoglobulin and rheumatoid factor synthesis in Sjogren's syndrome. Natural history and response to treatment. Am J Med. 1972 Oct;53(4):456-63. PubMed PMID: 4627721.
38. Kipps TJ, Tomhave E, Chen PP, et al. Molecular characterization of a major autoantibody-associated cross-reactive idiotype in Sjogren's syndrome. Journal of immunology (Baltimore, Md : 1950). 1989 Jun 15;142(12):4261-8. PubMed PMID: 2542406.
39. Fox RI, Chen P, Carson DA, et al. Expression of a cross-reactive idiotype on rheumatoid factor in patients with Sjogren's syndrome. Journal of immunology (Baltimore, Md : 1950). 1986 Jan;136(2):477-83. PubMed PMID: 3079786.
40. Bende RJ, Aarts WM, Riedl RG, et al. Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. The Journal of experimental medicine. 2005 Apr 18;201(8):1229-41. doi: 10.1084/jem.20050068. PubMed PMID: 15837810; PubMed Central PMCID: PMCPMC2213160.

41. De Vita S, Ferraccioli G, Avellini C, et al. Widespread clonal B-cell disorder in Sjogren's syndrome predisposing to Helicobacter pylori-related gastric lymphoma. Gastroenterology. 1996 Jun;110(6):1969-74. PubMed PMID: 8964425; eng.
42. Kourou KD, Pezoulas VC, Georga EI, et al. Cohort Harmonization and Integrative Analysis From a Biomedical Engineering Perspective. IEEE reviews in biomedical engineering. 2019;12:303-318. doi: 10.1109/rbme.2018.2855055. PubMed PMID: 30004887; eng.
43. De Vita S, Quartuccio L, Salvin S, et al. Cryoglobulinaemia related to Sjogren's syndrome or HCV infection: differences based on the pattern of bone marrow involvement, lymphoma evolution and laboratory tests after parotidectomy. Rheumatology (Oxford). 2012 Apr;51(4):627-33. doi: 10.1093/rheumatology/ker407. PubMed PMID: 22210656; eng.
44. Teos LY, Alevizos I. Genetics of Sjogren's syndrome. Clinical immunology (Orlando, Fla). 2017 Sep;182:41-47. doi: 10.1016/j.clim.2017.04.018. PubMed PMID: 28476436; PubMed Central PMCID: PMCPMC5660941. eng.
45. De Vita S, Sacco C, Sansonno D, et al. Characterization of overt B-cell lymphomas in patients with hepatitis C virus infection. Blood. 1997 Jul 15;90(2):776-82. PubMed PMID: 9226178; eng.
46. Sandhya P, Kurien BT, Danda D, et al. Update on Pathogenesis of Sjogren's Syndrome. Current rheumatology reviews. 2017;13(1):5-22. doi: 10.2174/1573397112666160714164149. PubMed PMID: 27412602; PubMed Central PMCID: PMCPMC5280579. eng.
47. Risselada AP, Kruize AA, Goldschmeding R, et al. The prognostic value of routinely performed minor salivary gland assessments in primary Sjogren's syndrome. Ann Rheum Dis. 2014 Aug;73(8):1537-40. doi: 10.1136/annrheumdis-2013-204634. PubMed PMID: 24525912.
48. Carubbi F, Alunno A, Cipriani P, 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 Mar;24(3):315-20. doi: 10.1177/0961203314554251. PubMed PMID: 25297554; eng.
49. Haacke EA, van der Vegt B, Vissink A, et al. Germinal Centers in Diagnostic Biopsies of Patients With Primary Sjogren's Syndrome Are Not a Risk Factor for Non-Hodgkin's Lymphoma but a Reflection of High Disease Activity: Comment on the Article by Sene et al. Arthritis & rheumatology (Hoboken, NJ). 2019 Jan;71(1):170-171. doi: 10.1002/art.40715. PubMed PMID: 30178631; eng.
50. Haacke EA, van der Vegt B, Vissink A, et al. Germinal centres in diagnostic labial gland biopsies of patients with primary Sjogren's syndrome are not predictive for parotid MALT lymphoma development. Ann Rheum Dis. 2017 Oct;76(10):1781-1784. doi: 10.1136/annrheumdis-2017-211290. PubMed PMID: 28710097; eng.

51. De Re V, De Vita S, Gasparotto D, et al. Salivary gland B cell lymphoproliferative disorders in Sjogren's syndrome present a restricted use of antigen receptor gene segments similar to those used by hepatitis C virus-associated non-Hodgkins's lymphomas. European journal of immunology. 2002 Mar;32(3):903-10. doi: 10.1002/1521-4141(200203)32:3<903::Aid-immu903>3.0.Co;2-d. PubMed PMID: 11870635; eng.
52. Quartuccio L, Baldini C, Priori R, et al. Cryoglobulinemia in Sjogren Syndrome: A Disease Subset that Links Higher Systemic Disease Activity, Autoimmunity, and Local B Cell Proliferation in Mucosa-associated Lymphoid Tissue. The Journal of rheumatology. 2017 Aug;44(8):1179-1183. doi: 10.3899/jrheum.161465. PubMed PMID: 28507188; eng.
53. Skopouli FN, Dafni U, Ioannidis JP, et al. Clinical evolution, and morbidity and mortality of primary Sjogren's syndrome. Semin Arthritis Rheum. 2000 Apr;29(5):296-304. PubMed PMID: 10805354.
54. Quartuccio L, Corazza L, Gandolfo S, et al. Persistent Rather Than Fluctuating Serum Cryoglobulins are Associated with Cryoglobulinemic Vasculitis in Primary Sjögren's Syndrome, and Related to the Risk of Lymphoma [abstract]. Ann Rheum Dis. 2015;74 (suppl. 2).
55. Brito-Zeron P, Ramos-Casals M, Bove A, et al. Predicting adverse outcomes in primary Sjogren's syndrome: identification of prognostic factors. Rheumatology (Oxford). 2007 Aug;46(8):1359-62. doi: 10.1093/rheumatology/kem079. PubMed PMID: 17569749; eng.
56. De Vita S, Soldano F, Isola M, et al. Preliminary classification criteria for the cryoglobulinaemic vasculitis. Ann Rheum Dis. 2011 Jul;70(7):1183-90. doi: 10.1136/ard.2011.150755. PubMed PMID: 21571735; PubMed Central PMCID: PMCPMC3103668.
57. Quartuccio L, Isola M, Corazza L, et al. Validation of the classification criteria for cryoglobulinaemic vasculitis. Rheumatology (Oxford). 2014 Dec;53(12):2209-13. doi: 10.1093/rheumatology/keu271. PubMed PMID: 24994905.
58. Quartuccio L, Isola M, Corazza L, et al. Performance of the preliminary classification criteria for cryoglobulinaemic vasculitis and clinical manifestations in hepatitis C virus-unrelated cryoglobulinaemic vasculitis. Clin Exp Rheumatol. 2012 Jan-Feb;30(1 Suppl 70):S48-52. PubMed PMID: 22410397; eng.
59. De Vita S. Treatment of mixed cryoglobulinemia: a rheumatology perspective. Clin Exp Rheumatol. 2011 Jan-Feb;29(1 Suppl 64):S99-103. PubMed PMID: 21586203; eng.
60. De Vita S, Quartuccio L, Fabris M. Hepatitis C virus infection, mixed cryoglobulinemia and BLyS upregulation: targeting the infectious trigger, the autoimmune response, or both? Autoimmunity reviews. 2008 Dec;8(2):95-9. doi: 10.1016/j.autrev.2008.05.005. PubMed PMID: 18589005; eng.

61. De Re V, De Vita S, Marzotto A, et al. Sequence analysis of the immunoglobulin antigen receptor of hepatitis C virus-associated non-Hodgkin lymphomas suggests that the malignant cells are derived from the rheumatoid factor-producing cells that occur mainly in type II cryoglobulinemia. Blood. 2000 Nov 15;96(10):3578-84. PubMed PMID: 11071657.
62. Fabris M, Dolcetti R, Pasini E, et al. High prevalence of Chlamydophila psittaci subclinical infection in Italian patients with Sjogren's syndrome and parotid gland marginal zone B-cell lymphoma of MALT-type. Clin Exp Rheumatol. 2014 Jan-Feb;32(1):61-5. PubMed PMID: 24447326.
63. Fabris M, Quartuccio L, Salvin S, et al. Fibronectin gene polymorphisms are associated with the development of B-cell lymphoma in type II mixed cryoglobulinemia. Ann Rheum Dis. 2008 Jan;67(1):80-3. doi: 10.1136/ard.2006.067637. PubMed PMID: 17526550; eng.
64. Theander E, Henriksson G, Ljungberg O, et al. Lymphoma and other malignancies in primary Sjogren's syndrome: a cohort study on cancer incidence and lymphoma predictors. Ann Rheum Dis. 2006 Jun;65(6):796-803. doi: 10.1136/ard.2005.041186. PubMed PMID: 16284097; PubMed Central PMCID: PMCPMC1798187.
65. Morra E, Cesana C, Klersy C, et al. Clinical characteristics and factors predicting evolution of asymptomatic IgM monoclonal gammopathies and IgM-related disorders. Leukemia. 2004 Sep;18(9):1512-7. doi: 10.1038/sj.leu.2403442. PubMed PMID: 15322559; eng.
66. Anaya JM, McGuff HS, Banks PM, et al. Clinicopathological factors relating malignant lymphoma with Sjogren's syndrome. Semin Arthritis Rheum. 1996 Apr;25(5):337-46. PubMed PMID: 8778989; eng.
67. Walters MT, Stevenson FK, Herbert A, et al. Urinary monoclonal free light chains in primary Sjogren's syndrome: an aid to the diagnosis of malignant lymphoma. Ann Rheum Dis. 1986 Mar;45(3):210-9. PubMed PMID: 3082300; PubMed Central PMCID: PMCPMC1001853. eng.
68. Pertovaara M, Pukkala E, Laippala P, et al. A longitudinal cohort study of Finnish patients with primary Sjogren's syndrome: clinical, immunological, and epidemiological aspects. Ann Rheum Dis. 2001 May;60(5):467-72. PubMed PMID: 11302868; PubMed Central PMCID: PMCPMC1753646. eng.
69. Ramos-Casals M, Cervera R, Font J, et al. Young onset of primary Sjogren's syndrome: clinical and immunological characteristics. Lupus. 1998;7(3):202-6. doi: 10.1191/096120398678920019. PubMed PMID: 9607645; eng.
70. Haga HJ, Jonsson R. The influence of age on disease manifestations and serological characteristics in primary Sjogren's syndrome. Scandinavian journal of rheumatology. 1999;28(4):227-32. PubMed PMID: 10503559; eng.
71. Joao C, Farinha P, da Silva MG, et al. Cytogenetic abnormalities in MALT lymphomas and their precursor lesions from different organs. A fluorescence in situ hybridization (FISH)

study. Histopathology. 2007 Jan;50(2):217-24. doi: 10.1111/j.1365-2559.2007.02580.x. PubMed PMID: 17222250; eng.

72. Nezos A, Papageorgiou A, Fragoulis G, et al. B-cell activating factor genetic variants in lymphomagenesis associated with primary Sjogren's syndrome. Journal of autoimmunity. 2014 Jun;51:89-98. doi: 10.1016/j.jaut.2013.04.005. PubMed PMID: 23845207; eng.
73. Papageorgiou A, Mavragani CP, Nezos A, et al. A BAFF Receptor His159Tyr Mutation in Sjogren's Syndrome-Related Lymphoproliferation. Arthritis & rheumatology (Hoboken, NJ). 2015 Oct;67(10):2732-41. doi: 10.1002/art.39231. PubMed PMID: 26097183.
74. Nocturne G, Boudaoud S, Miceli-Richard C, et al. Germline and somatic genetic variations of TNFAIP3 in lymphoma complicating primary Sjogren's syndrome. Blood. 2013 Dec 12;122(25):4068-76. doi: 10.1182/blood-2013-05-503383. PubMed PMID: 24159176; PubMed Central PMCID: PMCPMC3862283. eng.
75. Bombardieri M, Barone F, Humby F, 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. Journal of immunology (Baltimore, Md : 1950). 2007 Oct 1;179(7):4929-38. PubMed PMID: 17878393; eng.
76. Le Pottier L, Devauchelle V, Fautrel A, et al. Ectopic germinal centers are rare in Sjogren's syndrome salivary glands and do not exclude autoreactive B cells. Journal of immunology (Baltimore, Md : 1950). 2009 Mar 15;182(6):3540-7. doi: 10.4049/jimmunol.0803588. PubMed PMID: 19265132; eng.
77. Amft N, Curnow SJ, Scheel-Toellner D, 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 and rheumatism. 2001 Nov;44(11):2633-41. PubMed PMID: 11710719; eng.
78. Barone F, Bombardieri M, Rosado MM, 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. Journal of immunology (Baltimore, Md : 1950). 2008 Apr 1;180(7):5130-40. PubMed PMID: 18354239; eng.
79. Nocturne G, Seror R, Fogel O, et al. CXCL13 and CCL11 Serum Levels and Lymphoma and Disease Activity in Primary Sjogren's Syndrome. Arthritis & rheumatology (Hoboken, NJ). 2015 Dec;67(12):3226-33. doi: 10.1002/art.39315. PubMed PMID: 26359802; eng.
80. Jonsson MV, Szodoray P, Jellestad S, et al. Association between circulating levels of the novel TNF family members APRIL and BAFF and lymphoid organization in primary Sjogren's syndrome. Journal of clinical immunology. 2005 May;25(3):189-201. doi: 10.1007/s10875-005-4091-5. PubMed PMID: 15981083; eng.

81. Quartuccio L, Salvin S, Fabris M, et al. BLyS upregulation in Sjogren's syndrome associated with lymphoproliferative disorders, higher ESSDAI score and B-cell clonal expansion in the salivary glands. Rheumatology (Oxford). 2013 Feb;52(2):276-81. doi: 10.1093/rheumatology/kes180. PubMed PMID: 22879463; eng.
82. Mariette X, Roux S, Zhang J, et al. The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjogren's syndrome. Ann Rheum Dis. 2003 Feb;62(2):168-71. PubMed PMID: 12525388; PubMed Central PMCID: PMCPMC1754442. eng.
83. Tobon GJ, Renaudineau Y, Hillion S, et al. The Fms-like tyrosine kinase 3 ligand, a mediator of B cell survival, is also a marker of lymphoma in primary Sjogren's syndrome. Arthritis and rheumatism. 2010 Nov;62(11):3447-56. doi: 10.1002/art.27611. PubMed PMID: 20556820.
84. Hall JC, Baer AN, Shah AA, et al. Molecular Subsetting of Interferon Pathways in Sjogren's Syndrome. Arthritis & rheumatology (Hoboken, NJ). 2015 Sep;67(9):2437-46. doi: 10.1002/art.39204. PubMed PMID: 25988820; PubMed Central PMCID: PMCPMC4551661. eng.
85. Nezos A, Gravani F, Tassidou A, et al. Type I and II interferon signatures in Sjogren's syndrome pathogenesis: Contributions in distinct clinical phenotypes and Sjogren's related lymphomagenesis. Journal of autoimmunity. 2015 Sep;63:47-58. doi: 10.1016/j.jaut.2015.07.002. PubMed PMID: 26183766; PubMed Central PMCID: PMCPMC4564326. eng.
86. Voulgarelis M, Skopouli FN. Clinical, immunologic, and molecular factors predicting lymphoma development in Sjogren's syndrome patients. Clinical reviews in allergy & immunology. 2007 Jun;32(3):265-74. doi: 10.1007/s12016-007-8001-x. PubMed PMID: 17992593; eng.
87. Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjogren's syndrome. Arthritis and rheumatism. 2002 Mar;46(3):741-7. doi: 10.1002/art.10221. PubMed PMID: 11920410.
88. Giacomelli R, Afeltra A, Alunno A, et al. Guidelines for biomarkers in autoimmune rheumatic diseases - evidence based analysis. Autoimmunity reviews. 2019 Jan;18(1):93-106. doi: 10.1016/j.autrev.2018.08.003. PubMed PMID: 30408582; eng.
89. Astrakhan A, Omori M, Nguyen T, et al. Local increase in thymic stromal lymphopoietin induces systemic alterations in B cell development. Nature immunology. 2007 May;8(5):522-31. doi: 10.1038/ni1452. PubMed PMID: 17401368; eng.
90. Tsilingiri K, Fornasa G, Rescigno M. Thymic Stromal Lymphopoietin: To Cut a Long Story Short. Cellular and molecular gastroenterology and hepatology. 2017 Mar;3(2):174-182.

doi: 10.1016/j.jcmgh.2017.01.005. PubMed PMID: 28275684; PubMed Central PMCID: PMCPMC5331833. eng.

91. Kapsogeorgou EK, Papageorgiou A, Protogerou AD, et al. 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 Aug;77(8):1200-1207. doi: 10.1136/annrheumdis-2017-212639. PubMed PMID: 29779010; eng.
92. Gandolfo S, Fabro C, Bulfoni M, et al. Serum Levels of Thymic Stromal Lymphopoietin: A Possible Novel Biomarker in Primary Sjögren's Syndrome and Related Lymphoproliferation [abstract]. Arthritis & rheumatology (Hoboken, NJ). 2018;70 (suppl 10).
93. Gandolfo S, Fabro C, Bulfoni M, et al. Thymic stromal lymphopoietin expression from benign lymphoproliferation to malignant B-cell lymphoma in primary Sjögren's syndrome Clin Exp Rheumatol. 2019 (In Press.).
94. Brito-Zeron P, Acar-Denizli N, Zeher M, et al. Influence of geolocation and ethnicity on the phenotypic expression of primary Sjogren's syndrome at diagnosis in 8310 patients: a cross-sectional study from the Big Data Sjogren Project Consortium. Ann Rheum Dis. 2017 Jun;76(6):1042-1050. doi: 10.1136/annrheumdis-2016-209952. PubMed PMID: 27899373; eng.
95. Brito-Zeron P, Acar-Denizli N, Ng WF, et al. How immunological profile drives clinical phenotype of primary Sjogren's syndrome at diagnosis: analysis of 10,500 patients (Sjogren Big Data Project). Clin Exp Rheumatol. 2018 May-Jun;36 Suppl 112(3):102-112. PubMed PMID: 30156539; eng.
96. Pezoulas VC, Kourou KD, Kalatzis F, et al. Medical data quality assessment: On the development of an automated framework for medical data curation. Computers in biology and medicine. 2019 Apr;107:270-283. doi: 10.1016/j.compbiomed.2019.03.001. PubMed PMID: 30878889; eng.
97. Baldini C, Zabotti A, Filipovic N, et al. Imaging in primary Sjogren's syndrome: the 'obsolete and the new'. Clin Exp Rheumatol. 2018 May-Jun;36 Suppl 112(3):215-221. PubMed PMID: 30156542; eng.
98. De Vita S, Lorenzon G, Rossi G, et al. Salivary gland echography in primary and secondary Sjogren's syndrome. Clin Exp Rheumatol. 1992 Jul-Aug;10(4):351-6. PubMed PMID: 1395220.

TABLE 1. Lymphoma predictors in pSS

LYMPHOMA PREDICTORS in pSS

1. Major predictors

- persistent salivary gland swelling
- cryoglobulinemic vasculitis and serum cryoglobulinemia

Strictly related predictors

- Manifestations of cryoglobulinemic vasculitis: purpura, low C4, others (glomerulonephritis, peripheral neuropathy)
- Heavier MALT involvement by tissue salivary histopathology
- B-cell clonal expansion with peculiar rheumatoid factor-related genes / idiotypes, and ongoing expansion of the same B-cell clone in metachronous tissue biopsies

2. Other possible predictors

Clinical

- Younger age at disease onset
- Lymphadenopathy
- Splenomegaly
- Raynaud's phenomenon

Blood

- Neutropenia
- Lymphopenia / CD4+ T lymphocytopenia
- Anti-SSA/SSB positivity
- Rheumatoid factor and its titre
- Increased serum beta2 microglobulin
- High levels of immunoglobulin light chains
- Increased serum levels of Flt3-L
- Increased serum levels CXCL13
- Increased serum levels of TSLP

Genetic and tissue

- Chromosomal translocations and oncogene deregulation
- TNFAIP3 polymorphisms
- BAFF polymorphisms

- BAFF receptor His159Tyr mutation
- High Interferon IFNγ/IFNα ratio in minor SG biopsy
- Decreased miR200b-5p in minor SG biopsy
- Tissue TSLP

3. Composite indexes

- lymphoma prediction in all pSS patients [10]
- lymphoma prediction in pSS patients with persistent SG swelling [9]

4. Investigational

- **HarmonicSS**
  - Novel score for lymphoma prediction
  - Blood, saliva and tissue biomarkers
  - Imaging biomarkers: salivary gland echography
  - Composite index of MALT involvement
- **Other research projects**

  BIG DATA SjÖGREN PROJECT

  PRECISESADS

  NECESSITY