The evaluation of disease activity in Sjögren's syndrome based on the degree of MALT involvement: glandular swelling and cryoglobulinaemia compared to ESSDAI in a cohort study

S. De Vita, S. Gandolfo, S. Zandonella Callegher, A. Zabotti, L. Quartuccio

[1]*Department of Medical and Biological Sciences, Rheumatology Clinic, University of Udine, Italy.*

Salvatore De Vita,
Saviana Gandolfo,
Sara Zandonella Callegher,
Alen Zabotti,
Luca Quartuccio,

Please address correspondence to:
Prof. S. De Vita,
Rheumatology Clinic,
"Santa Maria della Misericordia" University Hospital,
Piazzale Santa Maria della Misericordia 15, 33100 Udine, Italy.
E-mail:
salvatore.devita@asuiud.sanita.fvg.it

Received on June 6, 2018; accepted in revised form on July 19, 2018.

Clin Exp Rheumatol 2018; 36 (Suppl. 112): S150-S156.

Key words: Sjögren's syndrome, lymphoma, disease activity

ABSTRACT

Objective. *To investigate if indicators of a heavier involvement of mucosa-associated lymphoid tissue (MALT) in primary Sjögren's syndrome (pSS), i.e. persistent salivary gland (SG) swelling and cryoglobulinaemia, might better evaluate the lymphoma risk compared to the ESSDAI. Therefore, the current concept of disease activity of pSS should be re-evaluated, based solely on ESSDAI.*

Methods. *A cohort of 255 pSS patients, including 30 pSS with B-cell lymphoma, was investigated. Three subgroups were distinguished, i.e. pSS developing lymphoma in the follow-up (n=12), pSS with lymphoma at cohort inclusion (n=18), and control pSS not developing lymphoma in the follow-up (n=225). SG swelling, cryoglobulinaemia and ESSDAI were evaluated at baseline, in the follow-up to one year before lymphoma diagnosis, and at lymphoma diagnosis.*

Results. *SG swelling and/or cryoglobulinaemia at baseline were significantly higher (p=0.0003) in pSS patients evolving into lymphoma if compared to pSS controls, while ESSDAI showed no significant difference. Both SG swelling and cryoglobulinaemia persisted and sometimes developed ex novo in the follow-up. SG swelling and cryoglobulinaemia were present in 24/30 (80%) cases the time of lymphoma diagnosis, and lymphoma itself was usually of MALT/marginal zone histotype (90%), leading to peculiar manifestation of lymphoma in pSS.*

Conclusion. *The autoimmune and lymphoproliferative involvement of MALT is the biological substrate of pSS. If this involvement is heavier, as reflected by SG swelling and cryoglobulinaemia, disease activity may be considered higher, and the risk of lymphoma is increased. The current concept and evaluation of activity of pSS, based solely on the ESSDAI, needs revision.*

Competing interests: none declared.

Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune and lymphoproliferative disease (1). The tissue mainly involved by pSS is the MALT (mucosa-associated lymphoid tissue) (2-4), and for this reason it was also called "autoimmune epithelitis" or "autoimmune exocrinopathy" (5). Connective tissue disease (CTD) and systemic vasculitis features, as well as an increased risk of lymphoma development, are also present (4, 6-16). However, while the clinical phenotype is different in disease subsets, the biologic substrate of pSS, *i.e.* the MALT autoimmune and lymphoproliferative involvement, is the common denominator. This MALT background is typical of pSS, and not of other CTDs or vasculitis. Many novel therapies are being evaluated in pSS, where effective treatments still represent an unmet need (17, 18). Currently, patient inclusion in clinical trials is mainly based on the degree of pSS activity as scored by the ESSDAI (19), which is a composite index for disease activity similar to other indexes for CTDs, such as SLEDAI for lupus (20). A significant contribution to the ESSDAI score is given by the different systemic features of pSS, some of which are not frequent. By contrast, the degree of glandular inflammation and non-malignant lymphoproliferation leading to mucosal dryness and to the increased risk of lymphoma evolution contributes in a more limited way (19). Of note, the majority of pSS patients observed in clinical practice have a low disease activity if based on the ESSDAI score <5 (12), but sicca manifestations, fatigue, constitutional symptoms and, possibly, lymphoma risk factors, may be present. The current exclusion of such patients from novel treatment approaches is hardly acceptable. These treatments might act positively on sicca syndrome and lymphoma risk factors,

and this could also represent an end point. Furthermore, as recently shown by two independent studies (12, 13), about one third of pSS patients who develop lymphoma in the subsequent follow-up show a very low ESSDAI at baseline. One additional issue is represented by the possible lack of a clear-cut distinction between pSS manifestations present at baseline, compared to those occurring during the subsequent disease course and, finally, shortly before the lymphoma diagnosis or at the lymphoma diagnosis (the latter being possibly linked to lymphoma itself, rather than being true predictors). Three steps may be usefully distinguished, including: 1) pSS at baseline, *i.e.* at the time of the first referral of the patient to the specialist for data collection; 2) pSS during the subsequent follow-up, at different times before the possible lymphoma evolution; and 3) pSS proximal or at the time of lymphoma diagnosis. In step 1, additional data at the time of pSS onset (*e.g.* first sicca manifestations) and at the time of pSS diagnosis or classification may antedate the baseline observation, and should be further distinguished. However, it may be difficult to obtain these data with precision. In step 2, the clinical features might change during the follow-up, and the risk of lymphoma might change accordingly. Overall, when dealing with steps 1 and 2, in our opinion it would be helpful to have all the prospective data collected very accurately within the same cohort. Step 3 may be characterised by delays in patient referral, in diagnostic tests, and in the definite diagnosis of indolent lymphoma. Symptoms might be also due to an already existing lymphoma, and in our Centre we consider one year before the diagnosis of lymphoma as the cut-off point to exclude an existing indolent lymphoma (although this is of course arbitrary). Since it was recently suggested that an increase in ESSDAI is a predictor of lymphoma in pSS (14), the clinical and laboratory manifestations of pSS patients developing lymphoma were evaluated in our cohort. In particular, we focused on the two well established lymphoma risk factors which are clinical surrogates for increased inflammation and lymphoproliferation of MALT in pSS (2-9, 21) *i.e.* persistent major salivary gland (SG) swelling and mixed cryoglobulinaemia. We aimed to understand whether and when either these indicators of a heavier MALT involvement, or ESSDAI, might better evaluate the lymphoma risk in pSS. Consequently, the observed findings, supporting the former, led to the conclusion that the current well-rooted concept of disease activity in pSS, based on the sole ESSDAI value, should be reconsidered. Disease activity should be evaluated based also on the degree of glandular inflammation and lymphoproliferation, *i.e.* the essence of pSS.

Materials and methods

The cohort included 255 consecutive unselected patients with pSS prospectively followed at the Clinic of Rheumatology, ASUIUD, University of Udine, Italy, a reference Italian Centre for pSS and for lymphomas occurring in rheumatic autoimmune diseases. The patients were all positive for the 2002 classification of pSS (22). Thirty of the 255 pSS patients either presented a B-cell non-Hodgkin's lymphoma (NHL) at baseline (*i.e.* at inclusion in the cohort; n=18) or developed a B-cell NHL in the follow-up (n=12). Therefore, data at B-cell NHL diagnosis were analysed in 30 cases, while prospective data were analysed in 12 patients, including data: a) at baseline (*i.e.* at the time of inclusion in the cohort; b) during the subsequent follow-up up to 1 year before the diagnosis of lymphoma; and c) at the time of the diagnosis of lymphoma. The 225 pSS patients who did not develop lymphoma in the follow-up were those, within our larger cohort, with a sufficiently long follow-up available (Table I), and represented the control group. Persistent salivary gland (SG) swelling and cryoglobulinaemia were investigated in detail, as definite indicators of inflammation and lymphoproliferation of MALT in pSS (2-9, 21). Persistent SG swelling was defined by either episodical (one or more episodes) of monolateral or bilateral parotid or submandibular swelling, or chronically persistent swelling, as evaluated by the clinician (2, 9, 19) and confirmed, whenever possible, by salivary gland ultrasonography. Cryoglobulinaemia was repeatedly searched, as recommended (15, 21).

Statistical analyses

Categorical variables are presented as frequencies and percentages. Quantitative variables are described with the mean ± standard deviation (SD), or with the median for the ESSDAI score (range). Chi square test was used to assess differences between subgroup of patients, and mean differences were expressed with 95% CI. In the case of distributions with extreme outliers a non-parametric Wilcoxon signed-rank test for paired data was used. All significance tests were two-tailed and values of $p<0.05$ were considered significant.

Results

The whole cohort included 255 patients with pSS (22), 236 women (92.5%) and 19 men (7.5%). Their mean age at baseline was 51.8±13.4 years. Anti-Ro/SSA and anti La/SSB antibodies were present in 195/255 (76.5%) and 126/255 (49.4%) of patients, respectively. The mean follow-up from the first evaluation (*i.e.* baseline data) to the last follow-up visit, also after NHL development, was 9.1±6.9 years. Data at baseline in the three subsets of pSS patients, *i.e.* pSS developing lymphoma in the follow-up (n=12), pSS with lymphoma at baseline (n=18), and pSS not developing lymphoma in the follow-up (n=225) are shown in Table I. The mean follow-up in these three groups was 14.8±7.8 years, 10.5±6.8 years, and 8.7±6.7 years, respectively. Additional data on the 12 pSS patients developing B-cell NHL are reported in Table II. They developed lymphoma after a mean follow-up of 3.75±2.8 years. Data on the whole 30 pSS patients with lymphoma are shown in Tables III and IV. A low grade B-cell NHL of MALT or of the marginal zone was present in 27/30 patients (90%) (Table IV).

At baseline, the evaluation of SG swelling and mixed cryoglobulinaemia, indicating a higher inflammation and lymphoproliferation of MALT, is better than ESSDAI to predict lymphoma evolution

At baseline, the prevalence of persistent

Table I. Data at baseline in the three subgoups of pSS patients of the cohort .

Patients features at baseline	pSS with NHL at inclusion (n=18)	pSS developing NHL in the follow-up (n=12)	pSS controls not developing NHL (n= 225)	*p*-value°
Age years, *mean ± SD*	51.8 ± 11.3	44.8 ± 8.9	52.2 ± 13.8	0.07
Females, *n (%)*	15/18 (83.3%)	10/12 (83.3%)	211/225 (93.8%)	0.19
Positive SG biopsy, *n (%)*	11/11 (100%)	7/7 (100%)	88/109 (80.7%)	0.35
Anti-Ro/SSA positive, *n (%)*	14/18 (77.8%)	10/12 (83.3%)	171/225 (76%)	0.74
Anti-La/SSB positive, *n (%)*	8/18 (44.4%)	8/12 (66.7%)	110/225 (48.9%)	0.23
ANA positive >1:160, *n (%)*	14/18 (77.8%)	12/12 (100%)	191/225 (84.9%)	0.22
Rheumatoid factor, *n (%)*	10/18 (55.6%)	6/12 (50%)	99/225 (44%)	0.68
Persistent SG swelling, *n (%)*	10/18 (55.6%)	8/12 (67%)	39/225 (17.3%)	0.0004
Cryoglobulinaemia, *n (%)* [§]	3/18 (16.7%)	3/12 (25%)	16/225 (7.1%)	0.06
Low C3, *n (%)**	5/18 (27.8%)	3/12 (25%)	48/225 (21.3%)	0.70
Low C4, *n (%)**	5/18 (27.8%)	4/12 (33.3%)	31/225 (13.8%)	0.06
Leukopenia, *n (%)**	2/18 (11.1%)	1/12 (8.3%)	18/225 (8%)	1.0
Lymphopenia, *n (%)**	4/18 (22.2%)	4/12 (33.3%)	33/225 (14.7%)	0.09

*Low C3 levels if <90 mg/dl; low C4 levels if <10 mg/dl; leukopenia if WBC <4000/mm^3; lymphopenia if lymphocytes <1000/mm^3.
°*p*-value between the baseline values in pSS patients developing NHL in the follow-up (n=12) *vs*. pSS controls non developing NHL (n=225).
[§]At baseline a cryoglobulinaemic vasculitis was present, concomitantly with cryoglobulinaemia, in 2/3 patients with NHL at inclusion, in 1/3 patients developing NHL in the follow-up, and in 11/16 pSS controls not developing NHL in the follow-up.

Table II. Glandular swelling, cryoglobulinaemia and ESSDAI in pSS patients developing lymphoma in the follow-up.

Indicators of heavy MALT involvement and ESSDAI score in pSS developing NHL (n=12)	Baseline	12 months to lymphoma	At lymphoma diagnosis	*p*-value at baseline: pSS NHL patients (n=12) *vs*. pSS controls (n=225)
Persistent glandular swelling, n (%)	8/12 (67%)	10/12 (83%)	11/12 (92%)	0.0004
Cryoglobulinaemia, n (%) [§]	3/12 (25%)	3/12 (25%)	4/12, (33%)	0.08
Persistent glandular swelling and/or cryoglobulinaemia, n (%)	9/12 (75%)	11/12 (92%)	11/12 (92%)	0.0003
Median ESSDAI (range)	5 (2-12)	10.5 (1-18)	19 (13-33)	0.128
Median ESSDAI (range) calculated by excluding lymphadenopathy domain	4.5 (0-8)	8 (1-14)	9 (1-21)	0.369
Median ESSDAI (range) calculated by excluding lymphadenopathy domain, SG swelling and cryoglobulinaemia	3 (0-3)	2 (1-10)	2 (1-10)	0.61

[§]One patient at baseline, and two patients at 12 months to lymphoma diagnosis and at lymphoma diagnosis presented cryoglobulinaemic vasculitis.

SG swelling, of cryoglobulinaemia and of low C4, was much higher in the pSS patients who developed NHL in the follow-up if compared to pSS controls (Table I). In detail (Table II), persistent SG swelling was present in 8 of the 12 pSS patients who developed lymphoma in the follow-up, while cryoglobulinaemia in 3/12 (1/3 also showing a cryoglobulinaemic vasculitis), and at least one of the two manifestations was present in 9/12 (75%; p=0.0003 *vs*. pSS controls). By contrast, the median ESSDAI at baseline in the 12 pSS patients developing NHL was 5 (2-12) and in pSS controls was 4 (0-26), *i.e*. not significantly different (p=0.128). In detail, among the pSS patients who developed NHL, the ESSDAI at baseline was <5 (low activity) in 4/12, and never reached at least 14 points (high activity) in all the 8 remaining patients. Among the 4 pSS patients with ESSAI <5, one patient showed a cryoglobulinaemic vasculitis with type II mixed cryoglobulinaemia, and another one parotid swelling with, *i.e*. half of the patients with an ESSDAI <5 presented risk factors for lymphoma evolution at baseline. The ESSDAI score in the pSS controls was <5 in 136/225 (60.4%), 5-13 in 76/225 (33.8%), and >13 in 13/225 (5.8%).

During the follow-up, SG swelling and cryoglobulinaemia persist and may further develop ex novo in some patients

During the follow-up, persistent SG swelling and/or cryoglobulinaemia, already present at baseline in 75% patients, persisted in the same patients, and two additional pSS patients developed them (persistent SG swelling in both, with one patient evolving from serum cryoglobulinaemia alone into an overt cryoglobulinaemic vasculitis (Table II). In total, 11/12 (92%) pSS patients of the Cohort who developed NHL in the follow-up had persistent salivary gland swelling and/or cryoglobulinaemia up to to one year before the diagnosis of lymphoma. The ESSDAI score concomitantly increased (Table II). By contrast, no significant increase in the prevalence of persistent salivary gland swelling and of cryoglobulinaemia or cryoglobulinaemic vasculitis, as well no significant increase in ESSDAI, was noticed in pSS controls who did not develop lymphoma (Table V).

At the time of lymphoma diagnosis, SG swelling, cryoglobulinaemia and heavy MALT involvement are usually present, leading to the peculiar manifestation of lymphoma in pSS

The data at the time of B-cell NHL diagnosis are reported in Tables III and IV. At the diagnosis of lymphoma, an indolent B-cell NHL (of MALT, or nodal of the marginal zone) was observed in 27/30 cases (90%) (Table III and IV). Persistent SG swelling was present in 21/30 (70%), cryoglobulinaemia in 7/30 (23.3%; also with cryoglobulinaemic vasculitis in 4/7) and at least one of the two in 24/30 (80%) (Table IV). However, among the 6 pSS patients lacking persistent SG swelling

Table III. Histological type and stage of B cell lymphoma in 30 pSS patients.

Age at NHL diagnosis (years), sex	Months of cohort follow-up before NHL diagnosis	Histological type	Stage	NHL localisation
61, F	48	DLBCL	III	Lymph nodes
50, F	12	MALT lymphoma	IV	Lung
39, F	46	MALT lymphoma	I E	Parotid gland
60, F	13	MALT lymphoma	III E	Parotid gland, lymph nodes
60, M	110	MALT lymphoma	I E	Parotid gland
66, F	96	MALT lymphoma	I E	Tongue
41, F	26	MALT lymphoma	I E	Parotid gland
52, F	29	MALT lymphoma	I E	Parotid gland
70, F	38	MALT lymphoma	IV	Parotid gland, lymph nodes, bone marrow
53, F	12	MALT lymphoma	I E	Parotid gland
56, F	77	Splenic marginal zone lymphoma	IV	Spleen, parotid gland, lymph nodes, bone marrow
67, F	72	DLBCL	IV	Spleen, stomach
55, M	-	MALT lymphoma	I E	Parotid and submandibular glands
49, F	-	MALT lymphoma	I E	Parotid gland
49, F	-	Nodal marginal zone lymphoma	IV	Lymph nodes, bone marrow
60, F	-	MALT lymphoma	I E	Parotid gland
50, F	-	MALT lymphoma	II E	Parotid gland, lymph nodes
52, M	-	Primary cutaneous marginal zone lymphoma	IE	Skin
50, F	-	MALT lymphoma	IV	Parotid gland, bone marrow
41, F	-	MALT lymphoma	IV	Parotid gland, bone marrow
60, F	-	MALT lymphoma	IV	Parotid gland, lung, liver
65, F	-	MALT lymphoma	I E	Lacrimal gland
39, F	-	MALT lymphoma	I E	Submandibular gland
65, F	-	MALT lymphoma	I E	Parotid gland
71, M	-	MALT lymphoma	III E	Parotid and minor salivary glands, lymph nodes
43, F	-	MALT lymphoma	I E	Parotid gland
71, F	-	MALT lymphoma	I E	Lacrimal gland
62, F	-	MALT lymphoma	I E	Parotid gland
63, F	-	Follicular lymphoma	III	Lymph nodes
67, F	-	MALT lymphoma	IV	Parotid gland, stomach, lymph nodes

Table IV. Glandular swelling, cryoglobulinaemia and MALT lymphoma are frequent at the diagnosis of B-cell NHL in pSS.

Indicators of heavy MALT involvement and ESSDAI score at NHL diagnosis (n=30)	Prevalence at the time of NHL diagnosis	*p*-value *vs.* pSS controls (n=225)
Cryoglobulinaemia, n (%) [§]	7/30 (23.3%)	
Persistent glandular swelling, n (%)	21/30 (70%)	
Persistent glandular swelling and/or cryoglobulinaemia, n (%)	24/30 (80%)	
MALT lymphoma, n (%)	27/30 (90%)	-
Persistent glandular swelling and/or cryoglobulinaemia and/or MALT lymphoma, n (%)	28/30 (93.3%)	-
Median ESSDAI (range)	19 (13-33)	<0.0001
Median ESSDAI (range) calculated by excluding lymphadenopathy domain	8 (1-19)	0.001
Median ESSDAI (range) calculated by excluding lymphadenopathy domain, SG swelling and cryoglobulinaemia	4 (1-12)	0.31

[§] Four patients at time of lymphoma diagnosis presented cryoglobulinaemic vasculitis.

and/or cryoglobulinaemia at the time of lymphoma diagnosis, a marginal zone B-cell NHL of MALT was in any case present in 4 patients, a marginal zone nodal B-cell NHL in one patient, and a diffuse large cell B-cell lymphoma in 1 patient. Overall, a MALT lymphoma and/or SG swelling and/or cryoglobulinaemia, *i.e.* a heavy MALT involvement, was noticed in 28/30 (93.3%) of pSS patients with lymphoma (Table IV). Their median ESSDAI at lymphoma onset in was 19 (13-33) *vs.* 5 (0-63) in the pSS controls who not develop lymphoma ($p<0.0001$). No statistically significant differences in the ESSDAI score and in clinical and laboratory features were noticed between the two Cohort NHL subgroups (pSS patients developing lymphoma in the follow-up, or with lymphoma at Cohort inclusion). In addition, their median ESSDAI was similar to that of pSS patients at lymphoma onset in an independent Italian Cohort of the Italian Study Group of SS (GRISS; data not shown). Importantly, when the ESSDAI score at lymphoma onset was calculated after excluding the lymphadenopathy domain, as done in another study to exclude overlap between lymphoma and pSS itself (14), the difference remained significant ($p=0.001$), the median ESSDAI was 8 (1-19) in the 30 NHL pSS cases and 4 (0-26) in the 225 pSS controls. However, when ESSDAI was calculated by also excluding SG swelling and cryoglobulinaemia, no significant difference was noticed between NHL and pSS without NHL ($p=0.31$), indicating that persistent SG swelling and cryoglobulinaemia themselves contributed significantly to the increase in ESSDAI at NHL diagnosis (Table IV).

Discussion

In the present prospective study, the two clinical manifestations of pSS representing widely recognised lymphoma predictors, *i.e.* persistent major SG (mainly parotid) swelling and mixed cryoglobulinaemia (6-13, 21) proved useful to identify pSS patients at higher risk of lymphoma evolution. These two manifestations were herein analysed in detail since they well reflect the degree of inflammation and lymphoproliferation of MALT in pSS (2-9, 21) and are very closely related biologically (21). They are therefore important to reveal the activity of pSS itself, when taking into account the disease pathophysiology and the concept of pSS as a disease

Table V. Non significant changes in glandular swelling, cryoglobulinaemia and ESSDAI in control pSS patients not developing lymphoma in the follow-up.

Indicators of heavy MALT involvement and ESSDAI score in pSS controls (n=225)	Baseline	During follow-up	*p*-value
Persistent glandular swelling, n (%)	37/225 (16.4%)	39/225 (17.3%)	0.75
Cryoglobulinaemia, n (%) [§]	16/225 (7.1%)	21/225 (9.3%)	0.12
Persistent glandular swelling and/or cryoglobulinaemia, n (%)	51/225 (22.6%)	53/225 (23.5%)	0.9

[§] Eleven patients (4.8%) presented cryoglobulinaemic vasculitis at baseline, and no patient developed it during the follow-up.

of MALT (1-4). Thus, starting from the lymphoma issue in pSS, an important conclusion emerged, *i.e.* that the degree of MALT involvement should represent a further criterion to evaluate pSS activity, even if the ESSDAI score is low. The majority of pSS patients who developed lymphoma in this study presented SG swelling and/or cryoglobulinaemia at Cohort inclusion. This cut-off point was investigated since it reflects the clinical practice where, if risk factors are present earlier, they quite often cannot be evaluated accurately. At baseline, SG swelling and/or cryoglobulinaemia were significantly increased in pSS patients who later developing lymphoma if compared to patients who did not. Overall, the usefulness of these two predictors of lymphoma in pSS is herein confirmed, and a longer follow-up in the present pSS control group might reveal an even stronger association. Other lymphoma predictors have been highlighted in pSS (6-14). They include lymphadenopathy and splenomegaly, anaemia, neutropenia and lymphopenia. It has been also suggested that the immunoglobulin monoclonal component in biologic fluids, free immunoglobulin light chains, increase in serum beta2-microglobulin and in rheumatoid factor titre (2, 6-13, 23). Other predictors are more difficult to be widely studied, such as the pattern of B-cell clonal expansion in metachronous salivary gland biopsies (22) and genetic alterations (23, 24). Novel tools and biomarkers are also being investigated (25). Overall, additional items would be helpful to predict lymphoma in pSS with greater sensitivity and specificity, and composite scores for lymphoma prediction have been proposed (7, 9, 10) and will be further evaluated (25).

However, SG swelling and cryoglobulinaemia show the best evidence, at present, of reflecting a heavier MALT involvement in pSS (2-9, 21, 24). Minor salivary gland pathologic data could also be useful to this end, but this needs to be further addressed (8, 10, 27), and additional tissue investigation and biomarkers are probably needed (25). From the accurate patient follow-up, we noticed that the prevalence of SG swelling and cryoglobulinaemia may further increase concomitantly with the reduction of the time to lymphoma diagnosis in pSS. This reinforces them as clinical manifestations, well reflecting a biologic background of pSS closely linked to lymphoma evolution. Of note, SG swelling and cryoglobulinaemia lead to a limited increase in the ESSDAI score, while cryoglobulinaemic vasculitis manifestations contribute more (19, 28). On the other hand, about one third of the pSS population who will develop lymphoma shows a low activity, as indicated by the ESSDAI, at baseline. This was shown in independent cohorts of pSS patients from Spain and from Greece (12, 13), and confirmed by the present results. In the study by Brito-Zeron, the increase in the ESSDAI at baseline was not selected in multivariate analysis indicating the variables which might better predict lymphoma, while salivary gland swelling and cryoglobulinaemia were selected (12). In the Greek study no significant difference in the ESSDAI score at baseline was noticed between pSS patients developing or not developing lymphoma in the follow up, as in the present study (13). Also, no patient who later developed lymphoma in our Cohort showed a very high ESSDAI at baseline. The fact that the ESSDAI is significantly increased at lymphoma diagnosis in this and other series (12-14) may be due not only to possible lymphoma manifestations (such as lymphadenopathy, splenomegaly, and constitutional symptoms) but also to cryoglobulinaemia, cryoglobulinaemic vasculitis features, and SG swelling themselves (28). This is supported by the analyses excluding these two manifestations for the calculation of the ESSDAI in the present study, where the ESSDAI itself resulted no longer significantly increased. Consistently, palpable purpura, vasculitis, peripheral neuropathy, renal involvement and SG swelling were frequent at lymphoma diagnosis in independent cohorts (12, 13). Furthermore, in a French multicentre study (14), SG enlargement, cryoglobulinaemia and low C4 were selected by multivariate analysis as significantly increased at 6 months before lymphoma diagnosis in pSS (when an existing, indolent lymphoma may be present). If compared to persistent SG swelling, cryoglobulinaemia is less frequent (5-10%), but implies a worse outcome in pSS (6, 7, 9-14, 28). Importantly, pSS-associated cryoglobulinaemia shows a pathobiology that is different from hepatitis C virus (HCV)-associated cryoglobulinaemia, since it is dependent on the involvement of MALT (21). Parotid swelling is detected about in 20-30% of pSS patients (10, 16), and only a part of them will develop lymphoma in the follow-up. Thus, it is relevant to better distinguish, among pSS patients with parotid swelling, those with a higher risk of lymphoma evolution. We pointed out that the persistent expansion of the same B-cell clone in metachronous parotid biopsies from the same patient is associated with a much higher risk of lymphoma evolution (29). Additional pathologic and molecular biopsy studies may further dissect the risk, and we and others (27) suggest that parotid biopsy should be done whenever possible in pSS patients with persistent parotid swelling. Furthermore, with the prospective evaluation of pSS patients with non-malignant parotid swelling (assessed by parotid biopsy) (9), those with a higher risk of lymphoma evolution could be identified in the presence of at

least 2 among: a) cryoglobulinaemia; b) low C4; c) leukopenia; and d) anti-SSB antibody positivity. Again, cryoglobulinaemia and the related reduction in C4 were identified on the background of SG swelling. Interestingly, peculiar heavy and light chain immunoglobulin genes are preferentially used in pSS-related B-cell lymphoproliferation of MALT, either with or without cryoglobulinaemia. These are the same genes implicated in the production of cryoglobulins in HCV-related (pSS-unrelated) cryoglobulinaemia (18, 30). The study of HCV-related autoimmunity and lymphoproliferation is therefore helpful for the study of pSS itself (31). The definite infectious trigger HCV can be effectively targeted, and growth factors which implicated for B-cell growth in pSS, such as BAFF and, recently, TSLP (32-34), are increased also in the course of HCV infection (35, 36). A final issue which needs to be highlighted, in our opinion, is the definition of the exact time frame when lymphoma predictors in pSS are investigated. As shown here, the risk of lymphoma at baseline might be different from that shown at different time points in the follow-up. A dynamic approach is recommended at present, requiring the re-evaluation of lymphoma risk factors at any follow-up visit, also with low ESSDAI values.

Overall, being prospective and linking SG swelling to cryoglobulinaemia, this study supports the notion that it is the heavier involvement of MALT that contributes to the lymphoma risk in pSS. Importantly, starting from the study of lymphoma in pSS, we conclude that the concept of disease activity of pSS itself, as built and currently evaluated (*i.e.* based on the sole ESSDAI composite score) should be revised. Also, the pSS patients showing a heavier glandular inflammatory and lymphoproliferative involvement (disclosed by SG swelling and cryoglobulinaemia in this study, and by improved methods in the future) may develop lymphoma and can be considered more active, even if the ESSDAI is low.

If well targeted, the MALT inflammation and the risk of lymphoma in pSS might be reduced by novel therapies available, and an adequate patient inclusion is required to reach this goal. We conclude that additional investigation is needed rather promptly needed.

References

1. ANDERSON LG, TALAL N: The spectrum of benign to malignant lymphoproliferation in Sjögren's syndrome. *Clin Exp Immunol* 1972; 10: 199-221.
2. DE VITA S, DE MARCHI G, SACCO S, GREMESE E, FABRIS M, FERRACCIOLI G: Preliminary classification of nonmalignant B cell proliferation in Sjögren's syndrome: perspectives on pathobiology and treatment based on an integrated clinico-pathologic and molecular study approach. *Blood Cells Mol Dis* 2001; 27: 757-66.
3. MITSIAS DI, KAPSOGEORGOU EK, MOUTSOPOULOS HM: The role of epithelial cells in the initiation and perpetuation of autoimmune lesions: lessons from Sjögren's syndrome (autoimmune epithelitis). *Lupus* 2006; 15: 255-61.
4. TZIOUFAS AG, KAPSOGEORGOU EK, MOUTSOPOULOS HM: Pathogenesis of Sjögren's syndrome: what we know and what we should learn. *J Autoimmun* 2012; 39: 4–8.
5. MOUTSOPOULOS HM: Sjögren's syndrome: autoimmune epithelitis. *Clin Immunol Immunopathol* 1994; 72: 162-5.
6. TZIOUFAS AG, BOUMBA DS, SKOPOULI FN, MOUTSOPOULOS HM: Mixed monoclonal cryoglobulinaemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjögren's syndrome. *Arthritis Rheum* 1996; 39: 767-72.
7. BAIMPA E, DAHABREH IJ, VOULGARELIS M, MOUTSOPOULOS HM: Hematologic manifestations and predictors of lymphoma development in primary Sjögren syndrome: clinical and pathophysiologic aspects. *Medicine* (Baltimore) 2009; 88: 284-93.
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 Sjögren's syndrome. *Ann Rheum Dis* 2011; 70: 1363-8.
9. QUARTUCCIO L, ISOLA M, BALDINI C *et al*.: Biomarkers of lymphoma in Sjögren's syndrome and evaluation of the lymphoma risk in prelymphomatous conditions: results of a multicenter study. *J Autoimmun* 2014; 51: 75-80.
10. FRAGKIOUDAKI S, MAVRAGANI CP, MOUTSOPOULOS HM: Predicting the risk for lymphoma development in Sjögren syndrome: An easy tool for clinical use. *Medicine* (Baltimore) 2016; 95: e3766.
11. RETAMOZO S, GHEITASI H, QUARTUCCIO L *et al*.: Cryoglobulinaemic vasculitis at diagnosis predicts mortality in primary Sjögren syndrome: analysis of 515 patients. *Rheumatology* (Oxford) 2016; 55: 1443-51.
12. BRITO-ZERÓN P, KOSTOV B, FRAILE G *et al*.: Characterization and risk estimate of cancer in patients with primary Sjögren syndrome. *J Hematol Oncol* 2017; 10: 90.
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: e0116189.
14. NOCTURNE G, VIRONE A, NG W-F *et al*.: Rheumatoid Factor and Disease Activity Are Independent Predictors of Lymphoma in Primary Sjögren's Syndrome. *Arthritis Rheumatol* 2016; 68: 977-85.
15. DE VITA S, GANDOLFO S, QUARTUCCIO L: Cryoglobulinemia. *In* HOCHBERG M, GRAVELLESE EM, SILMAN AJ, WEINBLATT ME, WEISMAN MH (Eds): Rheumatology, 2-Volume Set, 7th Ed., Elsevier 2017: 1421-7.
16. QUARTUCCIO L, BALDINI C, BARTOLONI E *et al*.: Anti-SSA/SSB-negative Sjögren's syndrome shows a lower prevalence of lymphoproliferative manifestations, and a lower risk of lymphoma evolution. *Autoimmun Rev* 2015; 14: 1019-22.
17. FOX RI, FOX CM: Sjögren's syndrome: why do clinical trials fail? *Rheum Dis Clin North Am* 2016; 42: 519-30.
18. DE VITA S, QUARTUCCIO L, SALVIN S *et al*.: Sequential therapy with belimumab followed by rituximab in Sjögren's syndrome associated with B-cell lymphoproliferation and overexpression of BAFF: evidence for long-term efficacy. *Clin Exp Rheumatol* 2014; 32: 490-4.
19. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69: 1103-9.
20. GLADMAN DD, IBAÑEZ D, UROWITZ MB: Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29: 288-91.
21. DE VITA S, QUARTUCCIO L, SALVIN S, CORAZZA L, ZABOTTI A, FABRIS M: Cryoglobulinaemia related to Sjögren's syndrome or HCV infection: differences based on the pattern of bone marrow involvement, lymphoma evolution and laboratory tests after parotidectomy. *Rheumatology* (Oxford) 2012; 51: 627-33.
22. DE VITA S, SACCO C, SANSONNO D *et al*.: Characterization of overt B-cell lymphomas in patients with hepatitis C virus infection. *Blood* 1997; 90: 776-82.
23. SANDHYA P, KURIEN BT, DANDA D, SCOFIELD RH: Update on pathogenesis of Sjögren's syndrome. *Curr Rheumatol Rev* 2017; 13 : 5-22.
24. TEOS LY, ALEVIZOS I: Genetics of Sjögren's syndrome. *Clin Immunol* 2017; 182: 41-7.
25. HARMONICSS – HARMONIzation and integrative analysis of regional, national and international Cohorts on primary Sjögren's Syndrome (pSS) towards improved stratification, treatment and health policy making. Website: harmonicss.eu.
26. 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; 30: S48-52.
27. HAACKE EA, VAN DER VEGT B, VISSINK A, SPIJKERVET FKL, BOOTSMA H, KROESE FGM: Germinal centres in diagnostic labial gland biopsies of patients with primary

Sjögren's syndrome are not predictive for parotid MALT lymphoma development. *Ann Rheum Dis* 2017; 76: 1781-4.
28. QUARTUCCIO L, BALDINI C, PRIORI R *et al*.: Cryoglobulinaemia in Sjögren syndrome: a disease subset that links higher systemic disease activity, autoimmunity, and local B cell proliferation in mucosa-associated lymphoid tissue. *J Rheumatol* 2017; 44: 1179-83.
29. DE VITA S, BOIOCCHI M, SORRENTINO D *et al*.: Characterization of prelymphomatous stages of B cell lymphoproliferation in Sjögren's syndrome. *Arthritis Rheum* 1997; 40: 318-31.
30. DE RE V, DE VITA S, GASPAROTTO D *et al*.: Salivary gland B cell lymphoproliferative disorders in Sjögren's syndrome present a restricted use of antigen receptor gene segments similar to those used by hepatitis C virus-associated non-Hodgkins's lymphomas. *Eur J Immunol* 2002; 32: 903-10.
31. DE VITA S, QUARTUCCIO L, FABRIS M: Hepatitis C virus infection, mixed cryoglobulinaemia and BLyS upregulation: targeting the infectious trigger, the autoimmune response, or both? *Autoimmun Rev* 2008; 8: 95-9.
32. PERS J-O, DEVAUCHELLE V, DARIDON C *et al*.: BAFF-modulated repopulation of B lymphocytes in the blood and salivary glands of rituximab-treated patients with Sjögren's syndrome. *Arthritis Rheum* 2007; 56: 1464-77.
33. QUARTUCCIO L, SALVIN S, FABRIS M *et al*.: BLyS upregulation in Sjögren's syndrome associated with lymphoproliferative disorders, higher ESSDAI score and B-cell clonal expansion in the salivary glands. *Rheumatology* (Oxford) 2013; 52: 276-81.
34. GANDOLFO S, FABRO C, BULFONI M *et al*.: Thymic stromal lymphopoietin (TSLP) expression is associated with lymphoproliferation and lymphoma in primary Sjögren's syndrome. *Arthritis Rheumatol* 2017; 69: Suppl. 10.
35. FABRIS M, QUARTUCCIO L, SACCO S *et al*.: B-Lymphocyte stimulator (BLyS) up-regulation in mixed cryoglobulinaemia syndrome and hepatitis-C virus infection. *Rheumatology* (Oxford) 2007; 46: 37-43.
36. SANSONNO D, RUSSI S, SANSONNO S, PAVONE F, DAMMACCO F: Thymic stromal lymphopoietin in hepatitis C virus-related cryoglobulinemic vasculitis: gene expression level and protein distribution. *Arthritis Res Ther* 2015; 17: 62.