Validation of thymic stromal lymphopoietin as biomarker of primary Sjögren's syndrome and related lymphoproliferation: results in independent cohorts

S. Gandolfo[1], C. Fabro[1], E. Kapsogeorgou[2], S. Colafrancesco[3], F. Ferro[4], E. Bartoloni[5], L. Quartuccio[1], A. Goules[2], R. Priori[3], A. Alunno[5], F. Conti[3], G. Valesini[3], R. Giacomelli[6], C. Baldini[4], R. Gerli[5], A.G. Tzioufas[2], S. De Vita[1]

[1]*Rheumatology Clinic, Udine University Hospital, Department of Medical Area, University of Udine, Italy;*
[2]*Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, Greece;*
[3]*Rheumatology Unit, Sapienza University of Rome, Italy;*
[4]*Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Italy;*
[5]*Rheumatology Unit, Department of Medicine, University of Perugia, Italy;*
[6]*Rheumatology Unit, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy.*

Saviana Gandolfo, MD
Cinzia Fabro, MSc
Efstathia Kapsogeorgou, PhD
Serena Colafrancesco, MD, PhD
Francesco Ferro, MD
Elena Bartoloni, MD
Luca Quartuccio, MD, PhD
Andreas Goules, MD
Roberta Priori, MD, PhD
Alessia Alunno, MD, PhD
Fabrizio Conti, MD, PhD
Guido Valesini, MD
Roberto Giacomelli, MD, PhD
Chiara Baldini, MD, PhD
Roberto Gerli, MD
Athanasios G. Tzioufas, MD
Salvatore De Vita, MD

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

Received on August 21, 2020; accepted in revised form on September 14, 2020.

Clin Exp Rheumatol 2020; 38 (Suppl. 126): S189-S194.

Key words: Sjögren's syndrome, thymic stromal lymphopoietin, biomarkers, lymphoma, mucosa-associated lymphoid tissue

Competing interests: none declared.

ABSTRACT

Objective. *Thymic stromal lymphopoietin (TSLP) has been implicated in primary Sjögren's syndrome (pSS) and related B-cell lymphoproliferation and lymphoma (NHL) by studies on salivary pathologic tissues and serum. The purpose of this work was to validate serum TSLP as biomarker of pSS and related lymphoproliferation by the study of two additional independent cohorts.*

Methods. *Serum TSLP was measured by ELISA in the original published Cohort-1 from Udine, Italy, including 91 patients. Two additional cohorts were then studied for validation: Cohort-2, including 4 sub-cohorts comprising 125 patients from the Universities of Roma, L'Aquila, Pisa and Perugia, belonging to the Italian SS Study Group (GRISS), and Cohort-3, including 59 patients from the University of Athens, Greece. Overall, 159 control subjects were enrolled. Active pSS-NHL, as well as pre-lymphomatous conditions, i.e. persistent salivary gland swelling and mixed cryoglobulinaemia, were investigated in detail. In addition, serum samples from pSS-NHL in complete remission were analysed (n=27).*

Results. *TSLP serum levels were confirmed to be significantly higher in pSS compared to controls in both Cohort-2 and Cohort-3, in particular in patients with lymphoproliferation. Serum TSLP was much higher in pSS pre-lymphomatous conditions. Finally, active NHL showed the highest TSLP serum levels, while in NHL in remission TSLP resulted undetectable or significantly lower than in benign pSS.*

Conclusion. *By the study of independent cohorts, it was again demonstrated that serum TSLP levels are increased in pSS, above all in more advanced B-cell lymphoproliferation and NHL. Serum TSLP can therefore represent a novel biomarker for pSS-related lymphoproliferation.*

Introduction

Primary Sjögren's syndrome (pSS) is a highly polymorphic autoimmune and lymphoproliferative systemic disease characterised by an increased risk of malignant B cell lymphoma development, significantly impacting on survival and mortality of pSS patients (1-3).

In pSS, B cell lymphoma originates from key pathogenetic events which take place in the context of the mucosa-associated lymphoid tissue (MALT), an acquired lymphocytic tissue mainly involving salivary gland (SG), where inflammation self-maintains and B cell lymphoproliferation occurs at different degrees (4-7).

In recent decades, research efforts have been focused on the development of algorithms aiming to stratify pSS patients for the risk of lymphoma (8-10), by combining pSS clinical, pathological and laboratory features, which may serve as adverse predictors. Pre-lymphomatous conditions have been also identified, *i.e.* persistent SG enlargement and mixed cryoglobulinaemia, both representing well-established red flags to be carefully considered in order to exclude a lymphoma already in place or to unveil a major risk stage of disease to be strictly followed-up for potential lymphoma development (9, 11-13).

Therapies specifically approved for pSS or to decrease lymphoma risk in this disease are still lacking, and current available instruments are not yet sufficient to comprehensively capture the complexity of pSS (8, 14-16). For these purposes, very recently, cutting-edge devel-

opments in already known techniques, such as SG ultrasound (17, 18), tools derived from other fields of medicine and also from different sciences, such as engineering technology (14, 19, 20), and novel laboratory biomarkers are being successful developed (21).

Among the latter, thymic stromal lymphopoietin (TSLP) has been recently proposed as a promising novel biomarker for pSS and related lymphoproliferation (22). TSLP expression, as epithelial-derived and lymphopoietic cytokine, was studied by histopathological and molecular analysis directly in the SG biopsies showing a different degree of severity of B-cell lymphoproliferation, and a significant increase in the percentage of TSLP-positive infiltrating SG B-cells was found along with the progression of pSS B-cell lymphoproliferation itself. This percentage was maximal in malignant lymphoma, where almost all the B-cells were TSLP-positive, and settled at intermediate levels in more advanced but still benign lymphoproliferation of myoepithelial sialadenitis (MESA) (22).

An analogous increasing trend of TSLP expression was mirrored by the results obtained by the analysis of TSLP levels in the serum of the corresponding pSS patients (22). In this study, serum TSLP resulted significantly higher in pSS compared to healthy and non-autoimmune sicca (nSS) control subjects, and showed a progressively increasing value in sera of pSS patients stratified according to their degree of B cell lymphoproliferation, with the highest levels in NHL (22). Patients with the two better recognised pre-lymphomatous pSS conditions abovementioned, *i.e.* persistent SG swelling and mixed cryoglobulinaemia, showed also significantly higher TSLP serum levels compared to pSS without these features (22). Taken together, these data strongly suggested a promising role of TSLP as a biomarker of pSS and related lymphoproliferation, encouraging further studies.

The aim of the present study was therefore to confirm these results by the study of TSLP serum levels in additional independent cohorts of pSS patients with a different degree of lymphoproliferation.

Methods

Patients

Firstly, a comparative analysis was performed between TSLP serum levels measured in pSS patients and controls from three independent cohorts.

The original cohort of pSS patients from Udine (UD), including 91 pSS patients previously studied (22) was Cohort 1. Two additional cohorts were studied for comparison: Cohort 2 came from the Italian SS Study Group (GRISS) and included 4 sub-cohorts comprising 125 pSS patients from the Universities of Roma (RO), L'Aquila (AQ), Pisa (PI) and Perugia (PG). Cohort 3 came from the National and Kapodistrian University of Athens, Greece, and included 59 pSS patients. All pSS patients from the three cohorts fulfilled the pSS 2016 ACR-EULAR classification criteria (23).

Since TSLP levels have been demonstrated significantly higher in the serum of pSS patients with active NHL than benign pSS (22), comparative analyses between the cohorts have been performed after excluding NHL patients (n=12 in Cohort 1; n=1 in Cohort 2; n=8 in Cohort 3), which were then analysed and compared separately.

Of note, pSS patients with uncontrolled active asthma were also excluded *a priori* from this study to avoid possible bias (24).

Relevant pSS features, especially those linked to lymphoproliferation and heavier MALT involvement (*i.e.* persistent SG swelling and mixed cryoglobulinaemia) (9, 12), were carefully collected and investigated in detail.

Overall, 159 control subjects were enrolled in this study: 101 from Italy, including 80 sex and age-matched healthy blood donors (HBDs) and 21 patients with nSS (*i.e.* subjective oral or ocular dryness unrelated to an autoimmune disease), and 58 from Greece, including 31 sex and age-matched HBDs and 27 nSS. Comparative analyses have been performed between patients and controls belonging to the same geographical region.

Finally, a separate analysis was performed in order to assess TSLP levels in serum samples from additional patients with a pSS-related MALT NHL in stable and complete remission (n=27: n=13 from Italy and n=14 from Greece).

TSLP serum level assessment

Serum samples were collected and stored at -80°C until their use, according to a common protocol shared between the three cohorts. Samples were then centralised and serum levels of TSLP, expressed as pg/mL, were measured by ELISA according to manufacturer's (R&D Systems) protocol. All determinations were performed in duplicate.

Statistical analysis

Analyses were performed using GraphPad Prism software (v. 7.02; GraphPad Software). Student t-test or the non-parametric Mann-Whitney test were used to calculate the statistical significance between groups. Paired samples were analysed with the t-test or Wilcoxon signed-rank test, as appropriate. A p-value <0.05 was considered significant.

Results

TSLP is elevated in the serum of pSS patients from three independent cohorts

The main features of pSS patients from the three cohorts are shown in Table I, although clinical data of Cohort 1 are described in detail elsewhere (22).

Cohort 2 included 125 pSS patients (female n=114, 91.2%; mean age 58.1 years, range 23–84): 124 with a benign pSS and 1 with active pSS-related MALT NHL, not evaluated for comparative analysis, as previously explained. As in Cohort 1 (22) (Fig. 1-A), also in Cohort 2 serum levels of TSLP were confirmed significantly higher than Italian controls (mean 30.26 pg/mL, 0.41–95.21; $p<0.0001$) (Fig. 1-B) and resulted comparable to those previously measured in Cohort 1 (p=not significant) (Fig. 1-E).

By separately analysing and comparing each single sub-cohort belonging to Cohort 2, no difference was found in serum TSLP levels (RO n=49, mean 33.21 pg/mL, range 1.4-95.21; AQ n=34, mean 38.6 pg/mL, range 16.31–85.11; PI n=28, mean 20.23 pg/mL, range 0.41–56.67; PG n=13, mean 19.39 pg/mL, 1.03–68.38; p=not significant) (Fig. 1-C). Individually taken, TSLP levels

Table I. Main features of pSS patients from the three cohorts.

	Cohort 1 n=91	Cohort 2 n=125	Cohort 3 n=59
Age, years: mean (range)	57.2 (25-80)	58.1 (23-84)	57.9 (27-82)
Female: n° (%)	86 (94.5%)	114 (91.2%)	57 (96.6%)
Duration of disease, months: mean (range)	54.1 (9-185)	60.6 (1-564)	141.1 (6-408)
Persistent salivary gland swelling: n° (%)	35 (38.5%)	29 (23.2%)	15 (25.4%)
Anti-SSA and/or anti-SSB antibodies: n° (%)	91 (100%)	85 (68%)	47 (79.7%)
Rheumatoid Factor: n° (%)	36 (39.6%)	40 (32%)	34/54 (63%)
Cryoglobulinaemia: n° (%)	18 (19.8%)	1 (0.8%)	5 (8.47%)
Salivary gland biopsy positive for GCs: n° (%)	28 (30.8%)	34 (27.2%)	9/49 (18.37%)
Active NHL: n° (%)	12 (13.2%)	1 (0.8%)	8 (13.6%)

in each single sub-cohort were also comparable to those measured in Cohort 1 (*p*=not significant) (Fig. 1-C).

Cohort 3 included 59 pSS patients (female n=57, 96.6%; mean age 57.9 years, range 27–82): 51 with a benign pSS and 8 with active pSS-related NHL, not included in comparison between cohorts. Serum levels of TSLP in Cohort 3 resulted significantly higher compared to the Greek controls (mean 43.25 pg/mL; range 0–128.1; $p<0.0001$) (Fig. 1-D), comparable to those measured in Cohort 1 (*p*=not significant), but higher compared to Cohort 2 (*p*=0.0006) (Fig. 1-E).

TSLP serum levels are independent of anti-SSA/anti-SSB status and of the presence of rheumatoid factor

Serum levels of TSLP were not influenced by anti-SSA/anti-SSB status, with no difference observed between seropositive and seronegative pSS patients both in Italy (seropositive: mean 32.07 pg/mL, range 0–140.8; seronegative 28.48 pg/mL, range 1.4–67.04; *p*=not significant) (Fig. 2-A) and in Greece (seropositive: mean 44.56 pg/mL, range 0–128.1; seronegative mean 38.5 pg/mL, range 0–78.95; *p*=not significant) (Fig. 2-B).

No difference was found also in serum levels of TSLP between rheumatoid factor (RF)-positive and -negative pSS patients, both in the Italian Cohorts (seropositive: mean 27.04 pg/mL, range 0–140.8; seronegative 30.29 pg/mL, range 0–85.11; *p*=not significant) (Fig. 2-C) and in the Greek Cohort (seropositive: mean 44.05 pg/mL, range 0–128.1; seronegative mean 29.07 pg/mL, range 0–67.71; *p*=not significant) (Fig. 2-D).

TSLP levels are higher in the serum of pSS patients with pre-lymphomatous conditions

As previously reported (22), pSS patients with the two better recognised pre-lymphomatous pSS conditions, *i.e.* persistent SG swelling and mixed

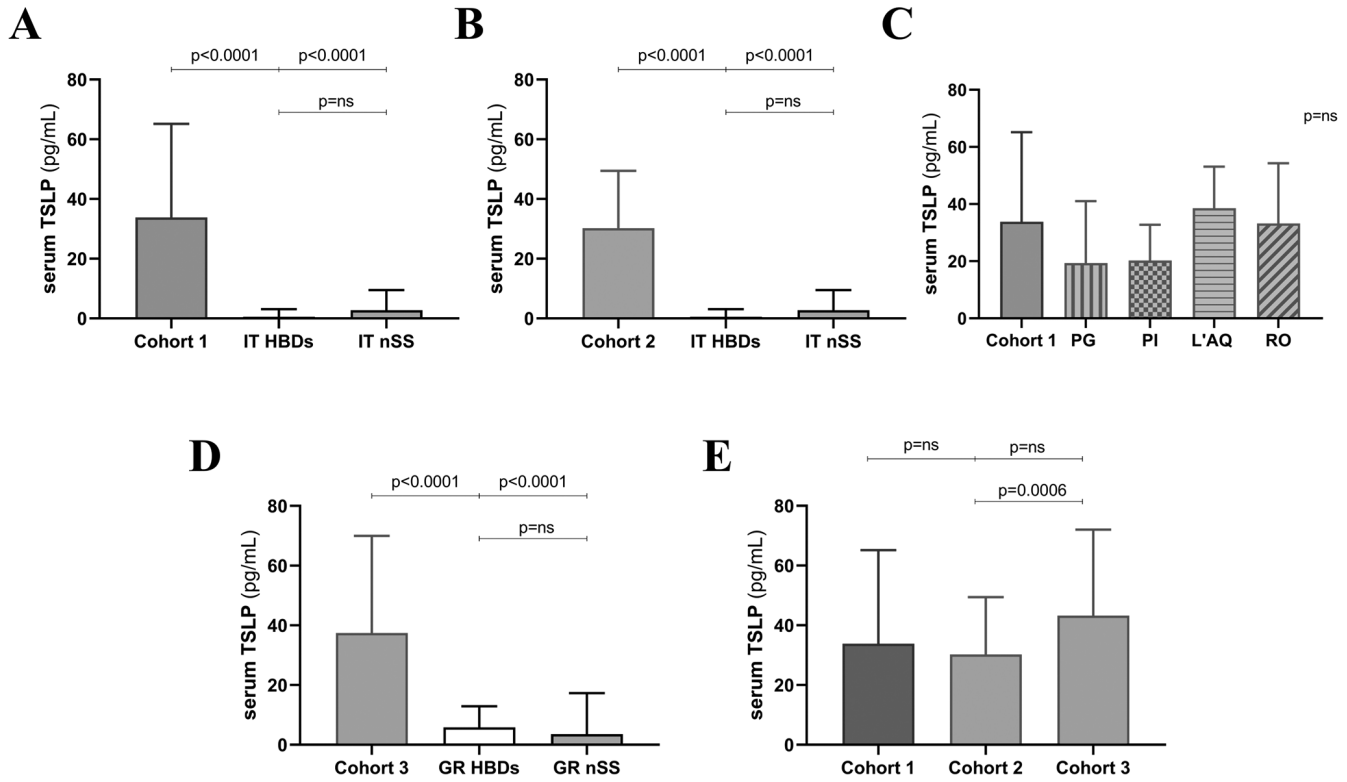

Fig. 1. Serum levels of TSLP in three independent cohorts.
A. Serum levels of TSLP are significantly higher in pSS patients belonging to Cohort 1 compared to Italian HBDs and nSS controls, **B.** this was confirmed also in Cohort 2. **C.** By separately analysing and comparing each single sub-cohort belonging to Cohort 2, no difference was found in serum TSLP levels. Individually taken, TSLP levels in each single sub-cohort were also comparable to those measured in Cohort 1. **D.** Serum levels of TSLP are significantly higher in pSS patients belonging to Cohort 3 compared to Greek HBDs and nSS controls. **E.** Serum levels of TSLP in Cohort 3 resulted significantly higher compared to Greek controls, and comparable to those measured in Cohort 1, but higher compared to Cohort 2.

cryoglobulinaemia (9, 12), showed significantly higher TSLP serum levels compared to pSS without glandular enlargement and to pSS without cryoglobulinaemia, respectively. With regard to persistent SG enlargement, this finding was also confirmed in Cohort 2 (pSS patients with SG swelling: mean 39.66 pg/mL, range 5.12–95.21; pSS patients without SG swelling: mean 28.7 pg/mL, range 0.41–84.7; p=0.024) (Fig. 3-A), and by analysing altogether the Italian pSS (pSS patients with SG swelling: mean 43.10 pg/mL, range 5.12–140.8; pSS patients without SG swelling: mean 28.14 pg/mL, range 0–85.11; p=0.0009) (Fig. 3-B). Analogous result was noticed in Cohort 3 (pSS patients with SG swelling: mean 64.83 pg/mL, range 25.63–128.1; pSS patients without SG swelling: mean 35.87 pg/mL, range 0–102.6; p=0.0011) (Fig. 3-C).
Comparative analysis regarding cryoglobulinaemia in Cohort 2 was not performed, given the very low representation of this specific feature (n=1). In Cohort 3, not-malignant pSS patients with mixed cryoglobulinaemia, as previously observed in Cohort 1 (22), showed higher serum levels of TSLP (mean 63.78 pg/mL; range 28.48–117.9) compared to pSS patients with no cryoglobulinaemia (mean 41.5 pg/mL; range 0–128.1; p=not significant) (Fig. 3-D), although the significance was not reached.

TSLP is detected at high levels in the serum of pSS-related active NHL

The only pSS patient in Cohort 2 with active NHL showed serum TSLP of 160.91 pg/mL, comparable to the mean TSLP in the 12 pSS with NHL (151.96 pg/mL) belonging to Cohort 1, previously analysed (22).
In Cohort 3, pSS patients with NHL of MALT histotype showed higher levels of TSLP (mean 72.65 pg/mL, range 22.76–244) compared to non-malignant pSS patients (p=0.045) (Fig. 4-A).

TSLP serum levels are undetectable or detectable at very low levels in pSS-related NHL in remission

In additional 13 serum samples from Italian pSS patients with a NHL in complete remission, serum levels of TSLP resulted undetectable (7/13) or

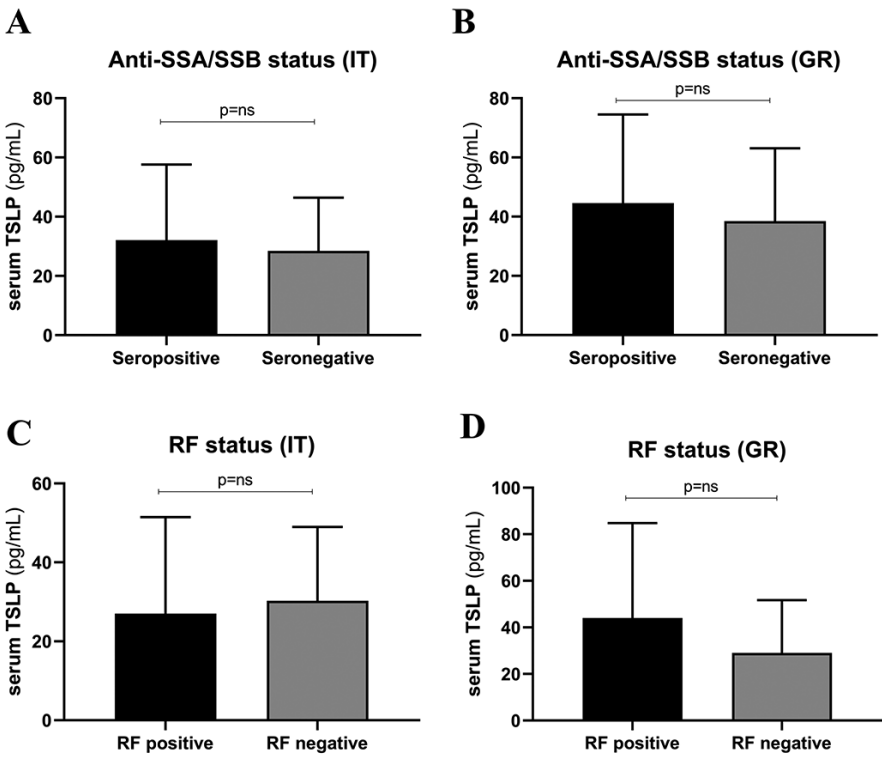

Fig. 2. Serum levels of TSLP serum levels in pSS patients stratified for anti-SSA/anti-SSB status and for the presence of rheumatoid factor. **A.** Serum levels of TSLP resulted independent from anti-SSA/anti-SSB status, with no difference observed between seropositive and seronegative pSS patients both in Italian and **B.** in Greek Cohort. **C.** No difference was found also in serum levels of TSLP between rheumatoid factor (RF) positive and negative pSS patients, both in Italian and **D**. in Greek Cohort.

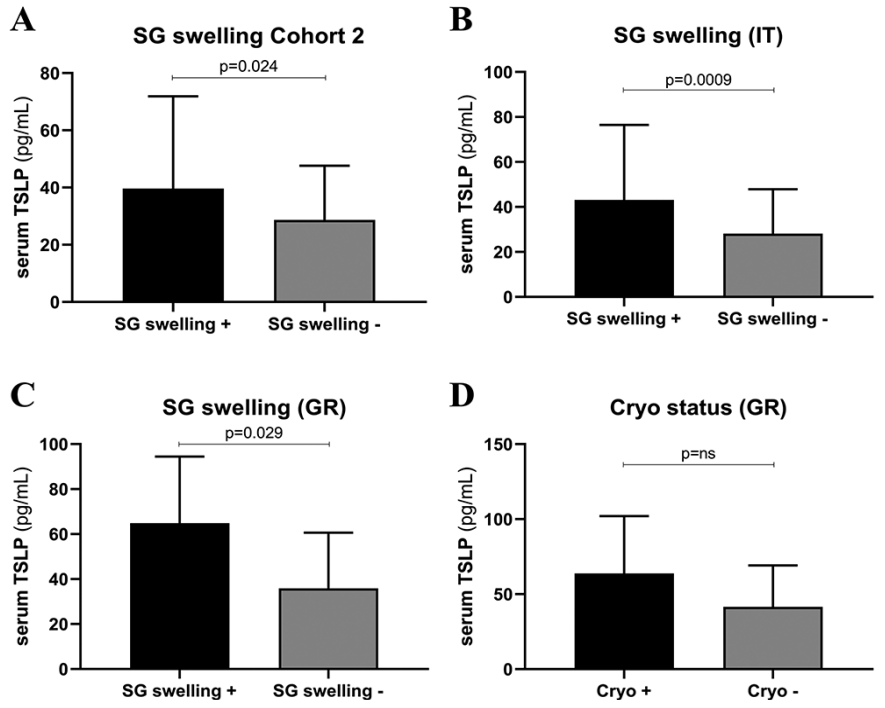

Fig. 3. Serum levels of TSLP levels in pSS patients stratified for the presence of pre-lymphomatous conditions. **A.** In Cohort 2, pSS patients with SG swelling showed higher serum levels of TSLP pSS patients without SG swelling, as previously observed in Cohort 1 (22). **B.** This observation was confirmed in the whole Italian cohort, and **C**. in Cohort 3. **D.** In Cohort 3, pSS patients with mixed cryoglobulinaemia, as previously observed in Cohort 1 (22), showed higher serum levels of TSLP compared to pSS patients negative, even if significance was not reached.

detectable at very low levels (6/13) (mean 10.46 pg/mL, 0–38.5), and significantly lower than in benign Italian pSS patients (mean 31.48 pg/mL, 0–140.8; *p*=0.0022) (Fig. 4-B).

These results were confirmed also in additional 14 serum samples from Greek pSS patients with a NHL in remission, serum levels of TSLP being undetectable (3/14) or detectable at very low levels (11/14) (mean 21.81 pg/mL, 0–67.65), and significantly lower than in benign Greek pSS patients (mean 43.25 pg/mL; range 0–128.1; *p*=0.011) (Fig. 4-C).

Finally, serum levels of TSLP in pSS patients with NHL in remission were comparable between the Italian and Greek cohort (*p*=not significant) (Fig. 4-D).

Interestingly, metachronous samples from one Italian patient, collected firstly at the stage of NHL activity and then at NHL remission, showed a dramatic decrease of TSLP from 128.04 pg/mL to undetectable levels.

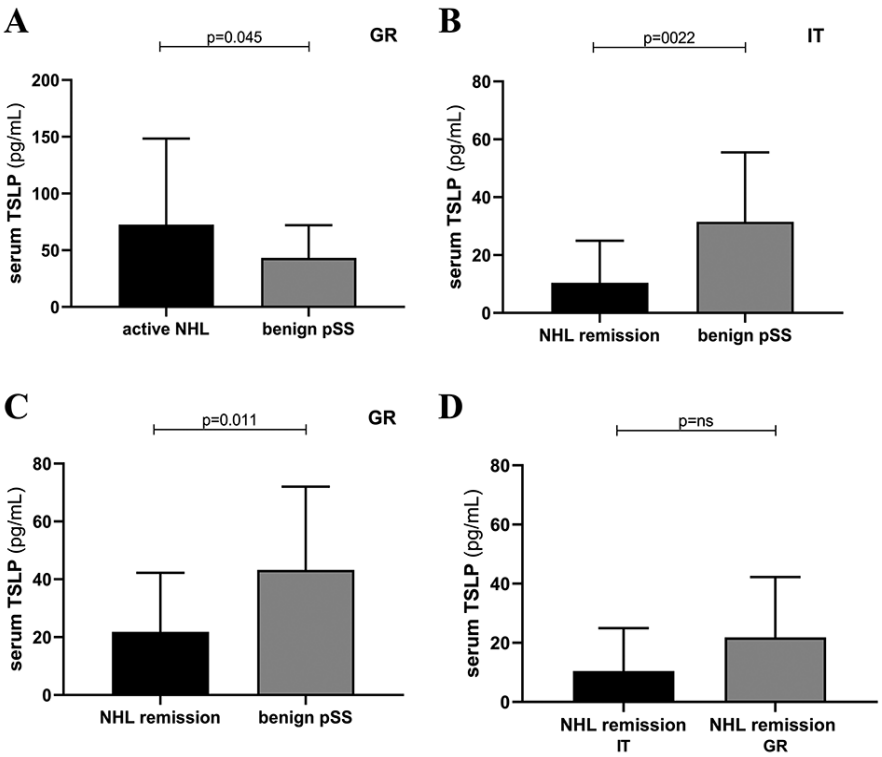

Fig. 4. TSLP serum levels in pSS-related NHL. **A.** TSLP is detected at higher levels in serum of Greek patients with a pSS-related active NHL compared to benign pSS Greek patients. **B.** TSLP serum levels are undetectable or detectable at very low levels in pSS-related NHL in remission in additional 13 serum samples from Italy, and **C.** in additional 14 serum samples from Greece, in both cases at a significantly lower level than in correspondent benign pSS patients. **D.** Serum levels of TSLP in pSS patients with a NHL in remission were comparable between Italy and Greece.

Discussion

This study, by means of data replication in independent cohorts of pSS patients with different degrees of lymphoproliferation, confirms serum TSLP as a biomarker of pSS and related lymphoproliferation.

The important role of TSLP, a cytokine originally identified by its ability to promote the proliferation and development of B cells, in regulating B-cell autoimmune response e in B-cell malignancy development, consolidated in the last very few years (22, 25-29).

Besides the potential role of serum or plasma TSLP as a biomarker of some non-malignant diseases (22, 30-32), a role of TSLP as biomarker emerged also in malignant disorders, such as gastric cancer and Hodgkin's lymphoma (HL) (29, 33), providing insights also for its potential impact in clinical care. In this regard, in HL basal plasmatic TSLP levels have been suggested as indicator of high risk disease and as predictor of response to chemotherapy (29, 34). Furthermore, the direct tissue demonstration of TSLP and TSLP-R expression by both malignant and immune cells populating the HL lymph node microenvironment (29) suggested that TSLP may play a role in numerous paracrine and/or autocrine pathogenetic interactions in the affected tissue.

An analogous observation has been highlighted also in pSS and related lymphoproliferation and NHL, by SG tissue studies (22). In SG of pSS patients, TSLP was expressed by SG-infiltrating B cells, whose percentage increased along with the progression of pSS B-cell lymphoproliferation. This increase was maximal in malignant lymphoma, where almost all the B-cells were TSLP-positive, and settled at an intermediate level in the pre-lymphomatous stage of MESA, compared to SG with a fully benign acquisition of MALT (22). TSLP serum levels behaved in a consensual specular way of what observed in pSS SG stratified tissues (22).

This work validates, in two additional independent cohorts of pSS patients, the previous results (22), supporting the role of TSLP as serum biomarker for pSS and related lymphoproliferation.

Serum TSLP was indeed found higher in pSS patients compared to controls in both Italian and Greek cohorts. Neither anti-SSA/SSB nor RF status seem to influence TSLP serum levels.

Among benign pSS patients, those with the well-recognised pre-lymphomatous condition of persistent SG enlargement showed much higher TSLP serum levels in all the three cohorts compared to pSS patients without this feature. A similar trend was observed also in cryoglobulinaemic Greek patients while analysis could not be performed in Cohort 2 due to very low prevalence of cryoglobulin positivity. The much lower prevalence of cryoglobulinaemia in Cohort 2 than in Cohort 3 might also explain the lower serum levels of TSLP measured in Cohort 2 than in Cohort 3. Further analyses are ongoing in order to more extensively study the possible correlations between serum levels of TSLP with specific histopathologic features linked to a heavier local acquisition of salivary MALT in pSS, *e.g.* the presence of GCs-like ectopic structures, yet correlated with higher serum TSLP (22), of lymphoepithelial lesions, and other laboratory features.

Finally, the separate analysis of the serum of pSS patients with active MALT NHL confirmed the maximal increase of TSLP in both the Italian and Greek cohorts.

The increase in serum TSLP levels from MESA to NHL may suggest that circulating TSLP could mirror tissue lymphoproliferative events occurring in the context of the key pathologic tissue of pSS, *i.e.* the SG with acquisition of MALT. In addition, integrating TSLP serum measurement in a stratification model of pSS for the risk of NHL development could be worthwhile, as suggested by the significant down-regulation of serum TSLP in pSS patients with NHL in full remission.

A limitation of this study could be the different prevalence of some features (*e.g.* cryoglobulins positivity) among the three cohorts, due to geographic and/or referral issues. Then, larger validation studies are definitely worthwhile on higher numbers of patients, as planned in the HarmonicSS project (European Union Grant 731944; https://harmonicss.eu/) (14, 15).

In conclusion, TSLP serum levels are increased in pSS, mainly in more advanced pSS-related lymphoproliferation and NHL, as herein confirmed in three independent cohorts. TSLP likely represents a novel biomarker to monitor lymphoproliferation in pSS.

References

1. BRITO-ZERÓN P, BALDINI C, BOOTSMA H *et al*.: Sjögren syndrome. *Nat Rev Dis Primers* 2016; 2: 16047.
2. VIVINO FB, BUNYA VY, MASSARO-GIORDANO G *et al*.: Sjögren's syndrome: An update on disease pathogenesis, clinical manifestations and treatment. *Clin Immunol* 2019; 203: 81-121.
3. VOULGARELIS M, ZIAKAS PD, PAPAGEORGIOU A, BAIMPA E, TZIOUFAS AG, MOUTSOPOULOS HM: Prognosis and outcome of non-Hodgkin lymphoma in primary Sjögren syndrome. *Medicine* 2012; 91: 1-9.
4. ANDERSON LG, TALAL N: The spectrum of benign to malignant lymphoproliferation in Sjögren's syndrome. *Clin Exp Immunol* 1972; 10: 199-221.
5. MOUTSOPOULOS HM: Sjögren's syndrome: autoimmune epithelitis. *Clin Immunol Immunopathol* 1994; 72: 162-5.
6. 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.
7. DE VITA S, GANDOLFO S, ZANDONELLA CALLEGHER S, ZABOTTI A, QUARTUCCIO L: 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. *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S150-6.
8. DE VITA S, GANDOLFO S: Predicting lymphoma development in patients with Sjögren's syndrome. *Expert Rev Clin Immunol* 2019; 15: 929-38.
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* 2016; 95: e3766.
11. 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.
12. TZIOUFAS AG, BOUMBA DS, SKOPOULI FN, MOUTSOPOULOS HM: Mixed monoclonal cryoglobulinemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjögren's syndrome. *Arthritis Rheum* 1996; 39: 767-72.
13. QUARTUCCIO L, BALDINI C, PRIORI R *et al*.: Cryoglobulinemia 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.
14. DE VITA S, ZABOTTI A, GANDOLFO S: Current limitations and ongoing initiatives to evaluate the activity, damage and outcome of primary Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S24-6.
15. GOULES AV, EXARCHOS TP, PEZOULAS VC *et al*.: Sjögren's syndrome towards precision medicine: the challenge of harmonisation and integration of cohorts. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S175-84.
16. GANDOLFO S, DE VITA S: Emerging drugs for primary Sjögren's syndrome. *Expert Opin Emerg Drugs* 2019; 24: 121-32.
17. VUKICEVIC AM, MILIC V, ZABOTTI A *et al*.: Radiomics-based assessment of primary Sjögren's syndrome from salivary gland ultrasonography images. *IEEE J Biomed Health Inform* 2020; 24: 835-43.
18. ZABOTTI A, ZANDONELLA CALLEGHER S, GANDOLFO S *et al*.: Hyperechoic bands detected by salivary gland ultrasonography are related to salivary impairment in established Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S146-52.
19. PEZOULAS VC, KOUROU KD, KALATZIS F *et al*.: Medical data quality assessment: On the development of an automated framework for medical data curation. *Comput Biol Med* 2019; 107: 270-83.
20. PEZOULAS VC, KOUROU KD, KALATZIS F *et al*.: Enhancing medical data quality through data curation: a case study in primary Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S90-6.
21. GOULES AV, TZIOUFAS AG: Lymphomagenesis in Sjögren's syndrome: Predictive biomarkers towards precision medicine. *Autoimmun Rev* 2019; 18: 137-43.
22. GANDOLFO S, BULFONI M, FABRO C *et al*.: Thymic stromal lymphopoietin expression from benign lymphoproliferation to malignant B-cell lymphoma in primary Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S55-64.
23. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* 2017; 76: 9-16.
24. CORREN J, PARNES JR, WANG L *et al*.: Tezepelumab in adults with uncontrolled asthma. *N Engl J Med* 2017; 377: 936-46.
25. VETTER T, BOROWSKI A, WOHLMANN A *et al*.: Blockade of thymic stromal lymphopoietin (TSLP) receptor inhibits TSLP-driven proliferation and signalling in lymphoblasts from a subset of B-precursor ALL patients. *Leuk Res* 2016; 40: 38-43.
26. BROWN VI, HULITT J, FISH J *et al*.: Thymic stromal-derived lymphopoietin induces proliferation of pre-B leukemia and antagonizes mTOR inhibitors, suggesting a role for interleukin-7Ralpha signaling. *Cancer Res* 2007; 67: 9963-70.
27. NAKAJIMA S, FUJIWARA T, OHGUCHI H *et al*.: Induction of thymic stromal lymphopoietin in mesenchymal stem cells by interaction with myeloma cells. *Leuk Lymphoma* 2014; 55: 2605-13.
28. 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.
29. FERRETTI E, HOHAUS S, DI NAPOLI A *et al*.: Interleukin-31 and thymic stromal lymphopoietin expression in plasma and lymph node from Hodgkin lymphoma patients. *Oncotarget* 2017; 8: 85263-75.
30. KOYAMA K, OHBA T, HARO H, NAKAO A: Positive association between serum thymic stromal lymphopoietin and anti-citrullinated peptide antibodies in patients with rheumatoid arthritis. *Clin Exp Immunol* 2015; 181: 239-43.
31. LEE EB, KIM KW, HONG JY, JEE HM, SOHN MH, KIM KE: Increased serum thymic stromal lymphopoietin in children with atopic dermatitis. *Pediatr Allergy Immunol* 2010; 21: e457-60.
32. CORREN J, ZIEGLER SF: TSLP: from allergy to cancer. *Nat Immunol* 2019; 20: 1603-9.
33. WATANABE J, SAITO H, MIYATANI K, IKEGUCHI M, UMEKITA Y: TSLP expression and high serum TSLP level indicate a poor prognosis in gastric cancer patients. *Yonago Acta Med* 2015; 58: 137-43.
34. ZINZANI PL, BROCCOLI A, GIOIA DM *et al*.: Interim positron emission tomography response-adapted therapy in advanced-stage hodgkin lymphoma: final results of the phase II part of the HD0801 study. *J Clin Oncol* 2016; 34: 1376-85.