

Risk, Predictors, and Clinical Characteristics of Lymphoma Development in Primary Sjögren's Syndrome

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Objective: To assess the risk and predictors of lymphoma development in a large cohort of patients with primary Sjögren's syndrome (pSS).

Methods: Cox-regression analyses were used to study the predictive value of clinical and laboratory findings at pSS diagnosis, and Kaplan-Meier survival curves to compare survival probability between patients who developed lymphoma and the total cohort. Expected risk for lymphoma was calculated by comparison with the background population.

Results: Eleven (4.5%) from 244 patients developed a non-Hodgkin lymphoma (NHL). Diffuse large B-cell and mucosa-associated lymphoid tissue lymphomas occurred at a similar frequency. Three (27.3%) patients died: 2 due to transformation from mucosa-associated lymphoid tissue to diffuse large B-cell. Purpura (HR 8.04, 95% confidence interval [CI] 2.33-27.67), parotidomegaly (HR 6.75, 95%CI 1.89-23.99), anemia (HR 3.43, 95%CI 1.04-11.35), leukopenia (HR 8.70, 95%CI 2.38-31.82), lymphocytopenia (HR 16.47, 95%CI 3.45-78.67), hypergammaglobulinemia (HR 4.06, 95%CI 1.06-15.58), low C3 (HR 36.65, 95%CI 10.65-126.18), and low C4 (HR 39.70, 95%CI 8.85-126.18) levels at pSS diagnosis were significant predictors of NHL development, but only hypocomplementemia and lymphocytopenia were independent risk factors. Hypocomplementemia was related to earlier development of NHL and higher mortality. The cumulative risk of developing lymphoma ranged from 3.4% in the first 5 years to 9.8% at 15 years. Standardized incidence ratio (95%CI) for NHL development was 15.6 (95%CI 8.7-28.2).

Conclusions: Patients with pSS have a 16-fold increased risk of developing lymphoma. This risk increases with time. Hypocomplementemia and lymphocytopenia at pSS diagnosis are the strongest predictors. Survival is clearly reduced in patients with hypocomplementemia. Indolent lymphomas tend to evolve over time toward a more aggressive histologic type.

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Primary Sjögren syndrome (pSS) is a systemic autoimmune rheumatic disease characterized by lymphocytic infiltration of the exocrine glands mainly involving the salivary and lachrymal glands, but fre-

quently affecting other sites too (1-3). Patients with pSS also present broad spectrum analytical features (cytopenias, hypergammaglobulinemia, hypocomplementemia, cryoglobulins), and autoantibodies (antinuclear antibodies, anti-SS-A/Ro and anti-SS-B/La antibodies) (1-5).

pSS is considered to be a benign autoimmune disease with a relatively chronic and indolent course. However, several reports have noted an increased incidence of malignant non-Hodgkin lymphomas (NHL) in pSS patients (5-9), with an estimated risk of up to 44 times greater than that observed in a comparable normal population (6). Various histological subtypes of lymphoma have been de-

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scribed, including follicular lymphoma, diffuse large B cell lymphoma (DLBCL), and especially mucosa-associated lymphoid tissue (MALT) lymphoma (6-9), preferentially occurring in extranodal sites, mainly in the major salivary glands (5-9). Several predictors of lymphoma development have been described such as lymphadenopathy (5,7,8), parotid gland enlargement (5-8), skin vasculitis (7-10), peripheral neuropathy (7,11), anemia (5,7), lymphocytopenia (5,7), hypocomplementemia (5,8-10), and serum cryoglobulins (5,10,12), but factors implied in the pathogenesis of lymphoma are poorly understood.

The objectives of this study were to evaluate the risk of developing lymphoma in a large prospectively collected cohort of patients with pSS from 1 center, and to identify laboratory and clinical findings present at the time of pSS diagnosis that may be significantly related with the development of lymphoma (prognostic factors). The clinical presentation, response to treatment, and outcome of patients who developed lymphoma are also described.

PATIENTS AND METHODS

All patients consecutively diagnosed with pSS and followed up consecutively at our department between January 1988 and December 2008 were eligible for the study. Patients were followed at regular intervals of 6 to 12 months or more frequently if required. All patients fulfilled 4 or more of the preliminary diagnostic criteria for pSS proposed in 1993 by the European Community Study Group (13). A complete history and physical examination was obtained in all cases. Diagnostic tests for xerophthalmia (Schirmer's test, Rose Bengal staining, and tear break-up time), xerostomia (parotid scintigraphy), and lip minor salivary gland biopsy were applied according to the recommendations of the European Community Study Group (13). Immunologic tests included antinuclear antibodies determined by indirect immunofluorescence using triple tissue cryostat sections (liver-stomach-kidney); precipitating antibodies to the extractable nuclear antigens Ro/SS-A and La/SSB detected by ELISA, and rheumatoid factor, serum cryoglobulins, and complement fractions C3 and C4 detected by nephelometry. Clinical and laboratory data were prospectively collected and computerized according to a standard protocol. Exclusion criteria were chronic viral infections, sarcoidosis, previous lymphoproliferative processes, and associated systemic autoimmune diseases. Data recorded on each subject included gender, date of birth, date of first study visit, date of last follow-up, survival status, date of diagnosis and type of lymphoma, clinical staging, and final outcome (remission, relapse(s), progressive disease, and death). We systematically evaluated the presence of ocular and oral symptoms, ocular signs, bilateral or unilateral parotid gland enlargement, Raynaud's phenomenon, palpable purpura, arthritis/arthralgia, fatigue, lymphadenopathy, splenomegaly, lung involvement, renal involvement, and peripheral neuropathy, as well as results of laboratory

tests, at diagnosis and during the follow-up. Hepatitis C was actively excluded in all patients. Serology to Epstein-Barr Virus (EBV), cytomegalovirus, and human immunodeficiency virus was investigated in all patients who developed lymphoma. Additionally, the presence of EBV genome was detected in these patients using the fluorescein-conjugated EBV nucleotides Eber 1 and Eber 2, complementary to the nuclear RNAs portions of the Eber gens, actively transcribed in the latently infected cells. *Helicobacter pylori* infection was discarded in all patients with MALT lymphoma.

All patients were regularly followed-up from diagnosis of pSS to either death, loss of follow-up, or December 2008 (the censoring date).

Clinical lymphoma staging included physical examination, computed tomographic scans of the thorax and abdomen, bone marrow biopsy, and biopsy of the involved tissues. The type of the lymphoproliferative malignancies was classified according to the 2001 World Health Organization Classification for tumors of hematopoietic and lymphoid tissues (14). Tumor response was recorded as complete or partial remission, no response, or progressive disease according to the Cheson criteria (15). Performance status was graded at the time of lymphoma diagnosis using the Eastern Cooperative Oncology Group (ECOG) score (16). The study was approved by the institutional review board and all patients gave informed consent.

Statistical Analysis

The statistical processing of the study was performed using the SPSS program version 15.0 for Windows (SPSS Inc, Chicago, IL). Comparison of categorical variables was performed by means of the Fisher exact test. Subgroup differences of continuous variables were compared with the nonparametric Mann-Whitney *U* test. Demographic, clinical, and laboratory characteristics recorded at the initial evaluation of patients were assessed as predictors of lymphoma development and death by using Cox regression analysis with age-adjusted proportional hazard models. Survival was estimated through the Kaplan-Meier curves, and comparisons were made by the log-rank test. Statistical significance was defined as $P < 0.05$. Expected risk for malignancies was calculated by comparison with the background population. The incidence of cancer in the general population of Spain, by gender and age strata, was obtained from the GLOBOCAN database (17). This database contains data reported to the Descriptive Epidemiology Group of the International Agency for Research on Cancer, a section of the World Health Organization. The standardized incidence ratio of NHL was estimated by indirect age and sex standardization and represented the ratio between observed and expected cases. The expected cases were obtained from the incident cases of NHL (or deaths due to NHL) in the general population of Spain reported to the

GLOBOCAN database. Confidence intervals (95%CI) were calculated assuming a Poisson distribution.

RESULTS

The study cohort consisted of 244 patients (235 females and 9 males), with a mean age of 57.8 ± 13.6 years (median 59 year; range 17-88 years) at pSS diagnosis. All patients fulfilled the diagnostic criteria for pSS proposed by the European Community Study Group (13), and 201 (82.38%) fulfilled the American-European Consensus criteria (18). The median follow-up was 8.6 years (1 to 20 year).

Eleven patients developed a NHL: 4 of MALT type, 3 DLBCL, 3 follicular lymphoma, and 1 both MALT and DLBCL. Thus the estimated prevalence of NHL in our cohort was 4.5% (95%CI 2.4-8.2%). The mean age of patients at NHL diagnosis was 63.2 ± 12.5 years (median 60 years; range 32-77 years). The mean time from pSS diagnosis to lymphoma diagnosis was 5.5 ± 4.5 years (median 4.23 years; range 1-14 years), and the mean time from the onset of sicca symptoms (pSS initial symptoms) to lymphoma diagnosis was 10.5 ± 7.75 years (median

7.3 years; range 3-24 years). No patient was simultaneously diagnosed as having pSS and lymphoma. Ocular and oral symptoms were reported by all patients who developed lymphoma. Parotid gland enlargement was present in 7 (63.64%) cases. Arthralgia (81.8%), fatigue (81.8%), and purpura (36.4%) were the most frequent systemic manifestations in these patients at the time of pSS diagnosis. The hemogram revealed anemia (hemoglobin <12 g/dL) in 6 (54.5%) patients at pSS diagnosis, leukopenia (<4000 cells/ μ L) in 7 (63.6%) patients, and lymphocytopenia (≤ 1000 cells/ μ L) in 9 (81.8%) patients. Serum protein electrophoresis and serum immunoglobulin quantification showed the presence of hypergammaglobulinemia (gammaglobulin fraction $>22\%$ of total serum proteins) in 8 (72.7%) patients and monoclonal immunoglobulins in 2 (cases 4 and 10). Six (54.5%) patients had low C3 levels and 4 had (36.4%) low C4 levels. Cryoglobulins were detected in 4 (36.4%) patients and all of them were type II. Primary SS was confirmed by minor salivary gland biopsy in 7 patients. Clinical, epidemiological, and histological features of patients with pSS and lymphoma are summarized in Table 1.

Case/ Sex	Age (yr) at NHL Diagnosis	Lymphoma Type	Lymphoma Location	Clinical Staging	Time (mo) from SS Diagnosis	Glandular pSS Manifestations	Extraglandular pSS Manifestations	Lip Salivary Gland Biopsy
1/F	60	DLBCL	Mediastinum liver	IV-A	75	XF, XS	RP, VL, arthritis, AH lung fibrosis, fatigue	ND
2/F	63	DLBCL leg type	Legs	I _E -A	40	XF, XS	Polineuropathy, fatigue arthralgia	Positive*
3/F	68	MALT	Parotid gland	I-A	62	XF, XS, PGE parotitis	Polyarthralgia	ND
4/F	68	Follicular grade 2	Mesenterium	I-A	25	XF, XS	VL, arthralgia	Positive*
5/F	50	Follicular grade 3	Lachrymal gland Mesenterium	IV _E -A	25	XF, XS, PGE	Fatigue, arthralgia, hypothyroidism	Positive*
6/F	32	MALT	Parotid gland	I _E -A	81	XF, XS, PGE parotitis	RP, VL, arthritis fatigue	Positive
7/F	70	MALT	Parotid gland	I _E -A	51	XF, XS, PGE	Fatigue, arthralgia	Positive
8/F	73	DLBCL	Parotid gland Splenomegaly	III _S -A	195	XF, XS, PGE	RP, VL, polyneuropathy PBC, arthralgia, fatigue	Positive*
9/F	68	Follicular grade 3b	Abdominal splenomegaly	II _S -B	162	XF, XS, PGE	Hypothyroidism, arthralgia fatigue	ND
10/F	67	MALT	Bronchial	II-A	27	XF, XS	Fatigue, arthralgia	Positive*
11/F	76	DLBCL	Bilateral parotid gland	IV-A	68	XF, XS, PGE parotitis	Fatigue, arthralgia	ND

AH, autoimmune hepatitis; DLBCL, diffuse large B cell lymphoma; MALT, mucosa-associated lymphoid tissue lymphoma; ND, not done; PBC, primary biliar cirrhosis; PGE, parotid gland enlargement; pSS, primary Sjögren disease; RP, Raynaud's phenomenon; VL, biopsy-proven cutaneous vasculitis; XS, xerostomia; XF, xerophthalmia.
*Positive lip salivary gland biopsy: grade III/IV according to the Chilsholm and Mason focus score classification.

During the follow-up, clinical findings suggesting NHL development included asymmetrical enlargement of parotid or lachrymal glands (4 cases), intraglandular palpable mass (2 cases), peripheral lymph node enlargement (1 case), splenomegaly (2 cases), and recurrent fever (2 cases). New onset of Raynaud's phenomenon preceded lymphoma development in 1 case, and palpable purpura in 2 cases. At the time of lymphoma diagnosis, 7 (63.6%) of the 11 patients had low C3 levels, 5 (45.5%) had low C4 levels, and 5 (45.5%) had detectable cryoglobulins. A monoclonal immunoglobulin was identified in the serum of 5 (45.5%) patients (in 3 cases associated with mixed type II cryoglobulins), and low serum levels of immunoglobulin were detected in 3 patients. Thymidine kinase and beta-2 microglobulin serum levels were above the normal values in 7 and 6 patients, respectively. LDH was found elevated in 7 (63.6%) patients. EBV-encoded small RNAs of the EBV were not expressed by the lymphoma cells in any case. In addition, antibodies to EBV, cytomegalovirus, and human immunodeficiency virus were not detected in the patient's sera. *H. pylori* serology was negative in all patients with MALT lymphoma and the bacterium was not detected in the biopsy of the patient who developed a gastric MALT lymphoma. Three pa-

tients (cases 1, 2, and 8) received an immunosuppressive therapy before the onset of lymphoma: prednisone for all 3 and 1 (case 2) intravenous cyclophosphamide for severe polyneuropathy.

Lymphoma was indolent in 7 cases. Clinical staging at diagnosis disclosed a limited disease (stage I and II) in 6 cases. Extranodal involvement was present in 8 cases (Table 1). The extranodal sites were salivary gland (5 patients), lachrymal gland (1 patient), legs (1 patient), and lung (1 patient). Three patients had bone marrow infiltration. The patient's performance status (ECOG) was mostly grade 0 (7 patients). Only 2 patients reported B symptoms. All patients were treated with chemotherapy, sometimes in conjunction with surgery or complementary radiotherapy (Table 2). Complete remission was achieved in 10 cases. However, 7 patients suffered a relapse and required new lines of chemotherapy and/or radiotherapy. Rituximab was given to 6 patients initially or during the follow-up. The relapse-free period after initial therapy ranged between 11 and 28 months (median 17.9 months). Relapses occurred at initial site in 4 patients. Two patients (cases 3 and 10) experienced a high-grade transformation to DLBCL and died, and 1 patient (case 8)

Table 2 Therapy and Disease Evolution in Patients with pSS and Lymphoproliferative Disease

Case	Chemotherapy	Number of Cycles	Radiotherapy	Response to Therapy	Relapses Number/ Location	Treatment at Relapse	Follow-Up Period ^a (mo)	Disease Evolution
1	CHOP	6	36 Gy	CR	None	—	120	Free of disease
2	R-CHOP	4	36 Gy	CR	None	—	87	Free of disease
3	CVP	3	30 Gy	CR	1/DLBCL mediastinal mass	CHOP	24	Death by disease progression
4	CHOP/NOPP/CVP	2/2/2	No	CR	1/mesenterium	Rituximab	70	Free of disease
5	CVP/NOPP/CHOP	2/2/2	No	CR	2/lacrima and intrabdominal	Rituximab CVP-R R maintenance	65	On maintenance treatment with R
6	FC	4	No	PR	4/parotid gland adenopathies	Rituximab + CBZ Radiotherapy R-CVP	73	On maintenance treatment with R
7	CBZ	1	No	CR	None	—	31	Free of disease
8	R-CHOP	4	36 Gy	CR	1/gastric (MALT)	Rituximab + CBZ	86	Free of disease
9	R-CHOP + R maintenance	6	No	CR	None	—	12	Free of disease
10	CHOP	6	No	CR	2/mediastinal and thoracic (DLBCL)	R-ESHAP Radiotherapy	85	Death by disease progression
11	R-CHOP	6	30 Gy	CR	1/parotid gland	Rituximab + CVP	12	Death by disease progression

CBZ, Chorambucyl; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; CR, complete remission; CVP, cyclophosphamide, vincristine, prednisolone; PR, partial remission; R: Rituximab; R-CHOP, Rituximab + CHOP.

^aFollow-up period (mo) since lymphoma diagnosis.

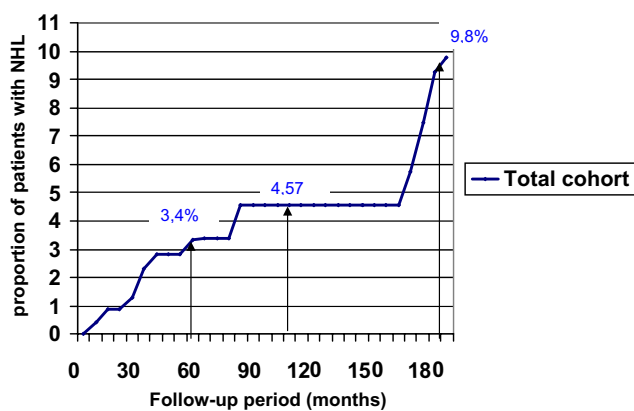


Figure 1 Cumulative risk of lymphoma development in patients with primary SS. (Color version of figure is available online.)

developed a second lymphoma (gastric MALT lymphoma) that was successfully treated.

Univariate Cox regression analysis identified parotid enlargement (HR 6.75, 95% CI 1.89-23.99), palpable purpura (HR 8.04, 95% CI 2.33-27.67), anemia (HR 3.43, 95% CI 1.04-11.35), leukopenia (HR 8.70, 95% CI 2.38-31.82), lymphocytopenia (HR 16.47, 95% CI 3.45-78.76), hypergammaglobulinemia (HR 4.06, 95% CI 1.06-15.58), low C4 levels (HR 39.70, 95% CI 8.85-126.18), and low C3 levels (HR 36.65, 95% CI 10.65-116.12) at the time of pSS diagnosis, as significant predictors of lymphoproliferative disease. Cryoglobulins were more frequently detected at pSS diagnosis in patients who developed lymphoma but no statistical significance was reached ($P = 0.54$).

Stepwise multivariate Cox regression was adjusted for age-identified lymphocytopenia and low C3/C4 levels at pSS diagnosis as independent predictors of lymphoma development.

The cumulative risk for developing lymphoma ranged from 3.4% in the first 5 years after pSS diagnosis to 9.8% at 15 years (Fig. 1) and was significantly higher in patients

with at least 1 risk factor at pSS diagnosis (Fig. 2). Low C3/C4 levels were associated with early development of NHL (in the first 5 years of the disease), and lymphocytopenia with later development of NHL (5 to 10 years after pSS diagnosis) (Fig. 2). The risk of lymphoma development was minimal among patients without adverse predictors (Fig. 2).

The relative risk of developing lymphoma was calculated to be 15.6 times that in the general population (standardized incidence ratio 15.6; 95%CI, 8.7- 28.2).

By the closing date for this study, 3 patients died and 8 were alive; 6 were in complete remission, and 3 were receiving maintenance therapy with Rituximab. Deaths were due to transformation to a high-grade lymphoma (2 cases) and disease progression despite therapy (1 case). Two of the 3 patients who died had low C3 and/or C4 levels at the first study visit. All deaths occurred within 28 and 84 months after diagnosis. Median follow-up from the time of lymphoma diagnosis to the last visit or the patient's death was 64.54 months (range 14 to 126 months). The overall survival at 10 years was 91% for the whole pSS patients group and 69% for patients developing lymphoma. Survival curves of the total cohort and of patients with pSS-associated lymphoma are shown in Figure 3.

DISCUSSION

The present series is 1 of the largest single-center experiences on patients with pSS-associated lymphoproliferative disease. In accordance with previous reports (5,7-9) we confirmed the preponderance of B-cell malignancies in pSS patients. Eleven of 244 (4.5%) patients developed a NHL in our series, similar to others that estimated this probability about 5 to 8% (5,7-9,11). The relative risk of developing lymphoma was estimated to be 16 times higher than the general population, identical to that recently reported by Theander and coworkers (9) and similar to the pooled risk of 18.8, estimated in a recent meta-analysis (19). This risk increased with time and was maintained high even 15 years after pSS diagnosis. The

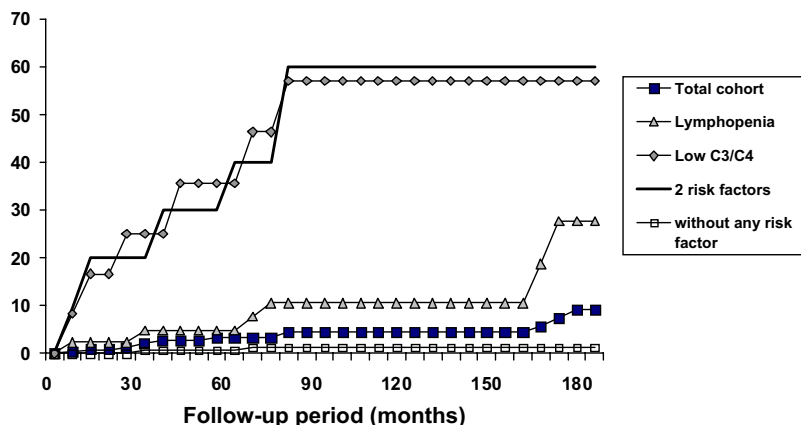


Figure 2 Cumulative risk of lymphoma development according to the presence of lymphocytopenia, hypocomplementemia, or both risk factors at pSS diagnosis. (Color version of figure is available online.)

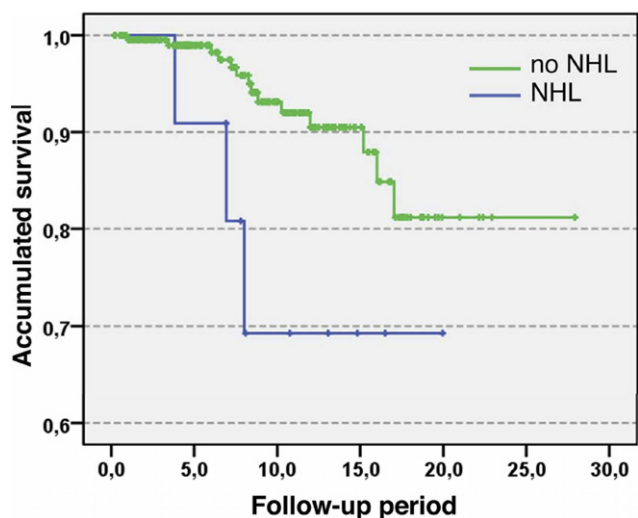


Figure 3 Kaplan-Meier plots for risk of death in the total pSS patients cohort and in patients who developed NHL. (Color version of figure is available online.)

risk was not related to the patient age at time of pSS diagnosis and was minimal among patients without any adverse predictor factor.

Lymphomas usually appeared at sites previously involved by lymphoproliferative lesions (ie, the parotid and the lachrymal glands), but in contrast to other series (5,7,20,21). MALT and DLBCL lymphomas occurred at a similar frequency in our patients, suggesting that the predominance of MALT lymphomas may not be as large as formerly believed and that DLBCLs are also frequent in patients with pSS. DLBCLs usually appeared later than MALT lymphomas during the follow-up and sometimes emerged as a transformation of an indolent lymphoma. This fact would indicate that this type of lymphoma with slower evolution may occur in patients with longstanding disease.

Specific clinical findings most frequently associated with NHL development were asymmetrical enlargement of parotid or lachrymal glands, intraglandular palpable mass, persistent lymphadenopathy, and splenomegaly. B symptoms were unusual in our patients, as reported in other series (5,7). New onset of Raynaud's phenomenon and palpable purpura heralded lymphoma development in 3 cases. Mixed type II cryoglobulin was found in both patients with purpura suggesting that these findings were correlated. Nonexocrine manifestations occurring more often in patients with lymphoma than in the general pSS cohort were arthralgia, fatigue, and palpable purpura.

Parotid gland enlargement, palpable purpura, anemia, leukopenia, lymphocytopenia, hypergammaglobulinemia, and low C3/C4 levels at initial pSS diagnosis were identified as risk factors for NHL development in the course of the disease, but only low C3/C4 levels and lymphocytopenia were independent predictors of lymphoma. Interestingly, low C3/C4 levels at the time of pSS diagnosis were associated with early development of NHL in

the first 5 years of the disease, while lymphocytopenia was associated with NHL development later, 5 to 10 years after disease evolution. Most of these disease characteristics have been identified in the past as risk factors for lymphoma development in pSS (5,7-10,21). However, to our knowledge, no study has evaluated the relationship between the presence of hypocomplementemia and/or lymphocytopenia at the time of pSS diagnosis and the time interval to appearance of lymphoma, although recently Baimpa and coworkers (5) have linked the presence of hypocomplementemia with the development of MALT lymphomas, which usually appear earlier in disease evolution, and the presence of lymphocytopenia with the development of DLBCL, which usually appear later in disease evolution.

Hypocomplementemia has previously been related to lymphoma development and to a poor prognosis in patients with pSS (7-9,22,23). In healthy subjects it seems that the complement facilitates the negative selection of autoreactive B cells and induces their apoptosis in the bone marrow and peripheral blood (24,25). Low C3/C4 levels may favor a greater survival of self-reactive B cells in pSS patients and increase the potential risk of unfavorable mutations occurring and leading to a lymphoid malignancy. Noteworthy, we found low C3/C4 levels at pSS diagnosis in 2 of the 3 patients who died from lymphoma, suggesting that complement levels at the time of pSS diagnosis are important prognostic factors not only for lymphoma development but also for overall survival.

Lymphocytopenia, and especially CD4+ T-cells lymphocytopenia, has also been associated with lymphoma development in pSS patients, mainly with DLBCL subtype (5,9). CD4+ T-cells lymphocytopenia may favor the development of autoantigen-specific proinflammatory T cells (26) and impair the immune control of B-cell proliferation due to the lack of regulatory cytokines from CD4+ T-lymphocytes (27). This may enhance malignant transformation. The causes of lymphocytopenia and disturbed balance between CD4+ and CD8+ T cells in pSS patients are unknown, although it has been suggested that low CD4+ counts reflect the elimination of CD4+ T populations by sustained antigenic activation-induced CD4+ apoptosis (26). Due to the lack of routinely performed flow cytometry in the samples of our patients, we were unable to investigate the distribution of peripheral lymphocytes subtypes. Nevertheless, our results reflect that lymphocytopenia is 1 of the stronger predictors of lymphoma development in these patients.

In keeping with previous studies (5,10,12), cryoglobulins were more frequently detected at the time of pSS diagnosis in patients who developed lymphoma, but this finding did not reach statistical significance. Mixed monoclonal cryoglobulinemia has typically been linked to the transition from polyclonal B-cell hyperactivity to monoclonal expansion of B cells, preceding NHL development by months to years (28).

In contrast to other reported data (29), raised levels of serum Beta-2 microglobulin were not predictive of lymphoma development in our patients (data not shown). Instead, this B-cell activation marker seemed to be related to the presence of extraglandular involvement in pSS, as previously suggested (30). However, since Beta-2 microglobulin has been shown to be an independent prognostic marker in follicular and DLBCL lymphomas in addition to the classical prognostic scores (31), we recommend its routine examination in patients with lymphoma. We would like to emphasize that some hematologic abnormalities related to lymphoma development may not be present at pSS diagnosis and may evolve during the follow-up. So, in our series, 1 patient showed low C3 and C4 levels when lymphoma was diagnosed, while their complement values were within the normal range at the time of pSS diagnosis. Similarly, cryoglobulins were detected at the time of lymphoma diagnosis in 2 patients with no detectable cryoglobulins at pSS diagnosis. Finally, 3 patients showed a serum monoclonal immunoglobulin (IgM kappa) when lymphoma was diagnosed, and 3 others had a decrease in serum polyclonal immunoglobulins. The demonstration of a dynamic association between oscillations of these blood parameters and progression to overt lymphoma would be of great interest. We therefore recommend monitoring complement levels, cryoglobulins, and serum protein electrophoresis and immunoglobulin quantification, in patients with risk factors for lymphoma development, especially in those with cytopenias at pSS diagnosis.

The mechanisms underlying the pathogenesis of lymphoma in patients with pSS have not yet been identified. Progression from benign polyclonal lymphocytic infiltration characteristic of pSS, to monoclonal B cell expansion, seems to play a pivotal role in the transition from the autoimmune state to NHL. However, the simple detection of B-cell clonality cannot be used as a criterion for diagnosis of B-cell lymphoma (28,32-40). Different mechanisms such as disturbed B-cell biology, defects in apoptosis, T-cell modulation, persistent antigenic stimulation, inactivation of tumor suppression genes, and sequential activation of proto-oncogenes by amplifications or mutations and/or viral infections may contribute to the complex process of lymphomagenesis in pSS (11,24-26,28,35-38). Some viruses (EBV, human herpes virus) (37,41) and bacteria (*H. pylori*, *Chlamydia psittaci*) (42,43) have been proposed as possible triggers for NHL development, especially of MALT-subtype. Antigens derived from these infectious agents may act as "molecular mimics," leading to chronic stimulation and perpetual proliferation of B cells to eliminate the antigen (44), ultimately promoting the onset of lymphoma (36). In this sense, it has been demonstrated that *H. pylori* infection triggers a chronic stimulus that would drive the development of gastric MALT lymphoma (45), and other non-gastric MALTs (42). A similar pathogenic model has been hypothesized for *C. psittaci* infection and ocular adnexal lymphoma development (43), although data are contro-

versial (46). We did not find in our series any relationship between EBV infection and lymphoma development, as previously reported by Royer and coworkers (37). Similarly, no relationship between *H. pylori* infection and subsequent MALT lymphoma development was found, our results suggesting that these lymphomas are not associated with infectious agents known to be present in other type of lymphomas. Finally, we did not find any relationship between prior administration of immunosuppressive therapy and lymphoma occurrence.

The sustained risk of lymphoma over time observed in the present study supports the hypothesis that chronic antigen-driven B-cell stimulation plays a pivotal role in lymphoma development in pSS. Our results also suggest that at least a proportion of DLBCLs arise from MALT transformation, as previously proposed (7,37,39,40). The linkage between these 2 NHL subtypes has been confirmed by molecular analysis (39,40). The prevalence and the time interval of histologic transformation for MALT lymphomas are unknown. Therefore, lifelong follow-up of patients with pSS and MALT lymphoma is recommended.

In conclusion, patients with pSS have a disproportionate tendency to develop lymphoproliferative diseases (a 16-fold increased risk compared with the general population). This risk increases with disease duration. DLBCLs appear in a similar frequency as MALT lymphomas, but tend to appear in later stages of the disease and at least a proportion of them arise from transformation of indolent lymphomas. Parotidomegaly, palpable purpura, hypergammaglobulinemia, anemia, leukopenia, and, especially, lymphocytopenia and low C3/C4 levels at time of pSS diagnosis, are predictors of lymphoma development during the disease course. Patients with these findings should be considered high-risk patients and followed up closely throughout life. It must be considered that some hematological abnormalities related to lymphoma development may not be present at pSS diagnosis and may appear as heralding lymphoma occurrence. Consequently, monitoring complement levels, cryoglobulins, serum protein electrophoresis, and immunoglobulin quantification should be recommended in high-risk patients.

The prospective design of our study in combination with the long-term follow-up time of up to 20 years (median 8.6 years) reinforces our results. More studies are needed to define algorithms for the identification of patients with high risk of lymphoma and to evaluate their pretreatment lymphoma risk profile.

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