LUND UNIVERSITY

Lymphoma and other malignancies in primary sjogren's syndrome A cohort study on cancer incidence and lymphoma predictors.

Theander, Elke; Henriksson, Gunnel; Ljungberg, Otto; Mandl, Thomas; Manthorpe, Rolf; Jacobsson, Lennart

Published in:
Annals of the Rheumatic Diseases

DOI:
10.1136/ard.2005.041186

2006

Lymphoma and other malignancies in primary sjögren's syndrome A cohort study on cancer incidence and lymphoma predictors

Elke Theander, Gunnel Henriksson, Otto Ljungberg, Thomas Mandl, Rolf Manthorpe and Lennart TH Jacobsson

Ann Rheum Dis published online 10 Nov 2005;
doi:10.1136/ard.2005.041186

ARD Online First, published on November 10, 2005 as 10.1136/ard.2005.041186

LYMPHOMA AND OTHER MALIGNANCIES IN PRIMARY SJÖGREN'S SYNDROME

A cohort study on cancer incidence and lymphoma predictors

Elke Theander MD, PhD, Gunnel Henriksson, MD, PhD, Otto Ljungberg MD, PhD, Thomas Mandl, MD, Rolf Manthorpe MD, PhD, Lennart T.H. Jacobsson MD, PhD

Department of Rheumatology, Department of Laboratory Medicine and Department of Pathology, Malmö University Hospital, Lund University, Lund, Sweden.

For correspondence or reprints contact:

Elke Theander
Department of Rheumatology,
Malmö University Hospital,
S - 20502 Malmö
Sweden
e-mail: elke.theander@medforsk.mas.lu.se

KEY WORDS:

Primary Sjögren's syndrome, lymphoma, cohort study, CD4+ T-lymphocytopenia, predictors

ABSTRACT

Objectives: Assessing the risk of lymphoproliferative disease or other malignancy (Standardized Incidence Ratios (SIR)), in patients with primary Sjögren's syndrome (pSS) according to the American-European Consensus Criteria (AECC), compared with patients with sicca-syndrome (non-AECC) and the background population. Identification of predictors of malignancy. Description of lymphoma types and survival probabilities .

Methods: Linked register study using information from the Malmö Primary SS Register combined with the Swedish Cancer Register and Cause-of-Death Register for calculation of SIRs. Re-classification of the detected lymphomas according to WHO classification. COX regression analysis to study the predictive value of clinical, laboratory and histological findings at the time of diagnosis.

Results: 507 patients (286 AECC-SS) with a median follow-up of 8 years (range 1 month up to 19 years) were included. SIRs (95% Confidence Interval (CI)) for malignancies in total and for non-Hodgkin's lymphomas (NHL) were 1.42 (0.98-2.00) and 15.57 (7.77-27.85) respectively in those fulfilling the AECC (n=286). In non-AECC sicca patients (n=221) SIR for malignancy of any kind was 0.77 (0.41-1.32), no lymphoproliferative neoplasms were detected. Significant predictors of lymphoproliferative disease were purpura/skin vasculitis (Hazard Ratio (HR): 4.64, 95%CI: 1.13-16.45), low complement factor C3 (HR: 6.18, 95%CI: 1.57-24.22), low C4 (HR: 9.49, 95%CI: 1.94-46.54), CD4+ T-lymphocytopenia (HR: 8.14, 95%CI: 2.10-31.53) and a low CD4+/CD8+ T-cell ratio ≤0.8 (HR: 10.92, 95%CI: 2.80-41.83). 58 % of the NHLs were diffuse large B-cell lymphomas.

Conclusion: The present study reveals 16 fold increased risk for development of NHL. In addition to previously recognized predictors CD4+ T-lymphocytopenia is a strong risk factor for developing lymphoma.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is an autoimmune connective tissue disease with an estimated prevalence of 0.5% among adults, when classified according to the American-European Consensus Criteria (AECC)[1-3]. The aetiology is unclear. Genetic, hormonal, environmental (mainly infectious) and other factors (such as birth weight [4]) interact in its pathogenesis [5, 6].

Patients with pSS experience mouth and/or eye dryness as the main consequence of an autoimmune destruction or functional blockade of the exocrine gland tissue. The most frightening complication of pSS is lymphoproliferative malignancy. In 142 SS patients admitted to the National Institute of Health (NIH) between 1954 and 1975 seven lymphomas were observed and resulted in a relative risk of lymphoma of 44.4 for pSS [7]. Several studies have confirmed this association and a lifetime risk of around 5%-10% [8-11]. Malignant lymphoma is the only cause of death, for which pSS patients are at increased risk [11, 12]. Several predictors of lymphoma development have been identified. Clinical signs such as lymphadenopathy [10, 11, 13], swollen salivary glands [7, 10, 11, 13], palpable purpura or skin vasculitis [11, 14], peripheral nerve involvement [14], leg ulcers [13], low-grade fever [14], use of cytotoxic drugs [7], younger onset pSS [7, 9] and laboratory predictors such as anaemia [14], lymphopenia [14], low levels of C3 [12, 15] and C4 [11, 12, 15], and

cryoglobulinaemia [15, 16] have been described. Reports from our group [17, 18] and others [19-21] have drawn attention to the high frequency of CD4+ T-lymphocytopenia and its possible connection to lymphoma development.
After 1976 [7] no studies have contributed with prospectively followed cohorts, high precision of assessment of the associated malignancies and comparison with solid, reliable background population data.
Following Kassan's [7] original description, risks of non-haematological malignancies have only been analysed in patient populations identified by hospital discharge registries [9, 22], which are likely to be biased towards more severe cases of pSS.
Lymphoma (and malignancy) incidence and prevalence in the background population is subject to continuous change [23, 24], attributable to population dynamics, true changes in SIRs, improved diagnosis and survival rates. Risks in the target cohort must be carefully calculated and compared to age, sex and calendar period adjusted expected risks. Swedish Health Registers, including the Cancer Register [25, 26] and Cause-of-Death Register [27], allow to implement a reliable comparison by linking patient registers to official registers using the Swedish personal identification number [28].

The aim of the present study was to analyse the degree of risk of lymphoproliferative malignancy in a prospectively collected mono-centre pSS cohort, identified in an out-patient clinic setting, taking advantage of the Swedish health system registers allowing exact detection of incident cases of malignancies [25]. Primary objectives were calculation of SIRs for lymphomas and other malignancies and detection of predictors of lymphoma.

PATIENTS AND METHODS

Malmö Sjögren´s Syndrome Register:

Since 1984 patients with pSS were consecutively registered and followed prospectively with intervals of 6 months to 2 years collecting clinical, laboratory and histological data.

Swedish health care registers:

Nationwide population-based mandatory registers for census data, death and cancer incidence, identifying individuals according to the unique national identification number [28], allow for linkage of these registers with each other and local registers such as the Malmö SS Register. The coverage (99%) and quality of information has been found to be excellent [25, 29].

***Study cohort and observation time*:**

The study cohort consisted of all 507 patients included in the pSS register up to December 2002, who lived in Sweden and fulfilled either the Copenhagen [30], the 1993 European [31] or the American-European Consensus Criteria (AECC) [1] (n=286) for pSS. None of the patients had known Hepatitis C, HIV, Sarcoidosis or preexisting lymphoma. The observation period covered the time from 1984 until December 31, 2002, up to which information from the National Death and Cancer Register were available. Thus the individual observation time was from the time-point of diagnosing pSS until the first malignancy, death or the closure date of December 31, 2002, whichever appeared first. Four AECC and 6 non-AECC patients could not be matched with the national registries, thus being lost to follow-up.The individual

observation time was in median 8 years (1 month to 19 years). The total observation time was 2464 years in AECC SS and 1840 in the non-AECC sicca patients.

Representativeness of the study cohort:

The majority of the patients lived in Malmö and surroundings. Our cohort covers about 20% to 30% of those expected to fulfil the AECC in our region including those with subclinical disease [12].

Verification of lymphoma types:

The type of the lymphoproliferative malignancies was re-classified according to the 2001 WHO classification for Tumours of Haematopoietic and Lymphoid Tissues [32] by one of the authors (OL).

Variables in the predictor analysis (only AECC-group, n=286):

As possible predictor variables for lymphoma development we included salivary gland swelling, purpura or skin vasculitis, autoantibodies (ANA, anti-SSA/Ro, anti-SSB/La, IgM-RF), salivary gland biopsy, serum immunoglobulins, levels of complement factors C3 and C4 and lymphocyte subtype abnormalities, especially absolute or relative CD4+ T-lymphocytopenia or a low ratio of CD4+/CD8+ T-cells. Normal ranges: IgG: 6.19-14.9 g/l, IgA: 0.7-3.65 g/l, IgM: 0.39-2.08 g/l, C3: 0.77-1.38g/l, C4: 0.12-0.33g/l, CD4+%: 30-50% of lymphocytes, CD4+ absolute count: 300-2000 cells/μl, CD4+/CD8+ ratio: 0.8-3.0. Determination of peripheral blood lymphocyte subtype distribution had been performed by flow cytometry, beginning 1988, as described [17, 33], in 165 (57%) AECC patients. CD4+ T-lymphocytopenia was defined according to the reference limits of our laboratory. Patients with severe CD4+ T-lymphocytopenia were tested repeatedly to ensure the reliability of the results and investigated for predisposing conditions. Only CD4+ T-lymphocytopenia without evidence for drug induction or virus infection was included in the statistical calculations. Cryoglobulins were not analysed routinely, since cryoglobulinaemia has been shown to be unusual in Nordic populations [34].

Linkage procedure:

Using the national personal identification number, issued to all permanent residents in Sweden, the SS register was linked to the National Cancer and Cause-of-Death Registers by the National Board of Health and Welfare, retrieving all cancer diagnoses, deaths and causes of death in the study population until December 31, 2002.

Statistics:

Calculation of SIRs:

Expected risks for malignancies were calculated by comparison with the background population (region of Southern Sweden) matched for age, sex and calendar period. A SIR was calculated by dividing observed by expected risk. Exact 95% confidence intervals calculated from binomial distributions were created for the SIRs.

Cancer diagnoses were grouped (for detail see table 2) according to *International Classification of Diseases*, 7th Revision (ICD 7). Risk estimates were calculated for the first malignancy after the pSS diagnosis without latency period.

Predictor analysis:

COX regression analysis with proportional hazards assumption adjusted for age was applied in order to study the influence of laboratory, clinical and histological findings at the time of diagnosis on lymphoma incidence. For CD4+ T-lymphocytopenia observation time from the first available flow cytometry (in all cases before lymphoma development) until lymphoma or cancelling date was used. The low number of events of lymphoproliferative malignancies (12 patients) did not allow for the use of multivariate analysis. The high prevalence of positive salivary gland biopsy both in patients without (90%) and those with (100%) lymphoma makes this variable unsuitable as a predictor. Predictors were used as continuous variables

whenever possible, expressing HRs as risks per 1 standard-deviation change. For complement and immunoglobulins due to partly u-shaped distributions of the risk estimates the results were divided into quartiles, comparing highest and lowest quartiles with the two in between.

Power analysis:

With the available patient number and events of NHLs compared to expected events our study had an 80% power of detecting a statistically significant increase of the risk of lymphoma by 500% to a SIR of 5.1, within the patient group fulfilling the AECC. The detected SIR and its 95% confidence interval are above this level.

RESULTS

Demographic and basic clinical variables*:*

Ninety-two % of the patients were Scandinavian, 90% were women. The disease duration from appearance of the first symptom until diagnosis (estimated by the patient at the first contact) was in median 7 years. Table 1 gives baseline characteristics by patient group.

Table 1. Baseline patient and disease characteristics

	AECC SS without malignancy n=253	**available data n**	**AECC SS + lymphoma/ myeloma n=12**	**available data n**	**AECC SS + other malignancy n=21**	**available data n**
Female/male	232/21	253	10/2	12	19/2	21
Age at diagnosis	56 (16-82)	254	58 (25-75)	12	66 (40-80)	21
Biopsy positive°	213 (90%)	240	10 (100%)	10	18 (86%)	21
SSA/B positive°	143 (57%)	250	10 (83%)	12	12 (48%)	21
RF positive°	139 (58%)	241	9 (75%)	12	9 (40%)	20
ANA positive°	212 (85%)	251	12 (100%)	12	16 (76%)	21
Salivary gland swelling°	74 (31%)	242	5 (42%)	12	2 (10%)	21
Purpura or skin vaculitis°	25 (10%)	240	4 (33%)*	12	0 (0%)	21
IgG (g/l)	16.2(1.0-92.0)	240	15.2(8.2-36.0)	12	15.5(5.4-33.9)	21
C3 (g/l)	0.97(0.17-1.73)	207	0.78(0.50-1.01)**	11	1.1(0.70-1.46)	20
C4 (g/l)	0.23(0.02-1.60)	207	0.16 (0.01-0.37)	11	0.29(0.1-0.75)	20
CD4+ (%)§	45 (4-75)	142	35 (12-54.)**	11	40 (12-47)	12
CD8+ (%)§	25 (3-65)	142	44 (22-68)***	11	28 (14-79)	12
CD4/CD8	1.7 (0.3-8.1)	142	0.7 (0.2-2.3)**	11	1.4 (0.2-3.3)	12
CD4-penia§§ °	24 (17%)	142	8 (73%)***	11	3 (25%)	12

Age: years (median/range). Biopsy positive: lower lip salivary gland biopsy with a focus score >1. IgG, C3, C4, CD4+, CD8+, CD4/CD8: median (range). ° n-observed (% of available). § % of total lymphocyte count. §§CD4penia defined as either CD4+ T-cells<300 cells/µl or CD4+ T-cells <30% of total lymphocyte count or CD4/CD8 ratio <0.8. * significantly different from AECC SS without malignancy with $p < 0.05$, ** significantly different from AECC SS without malignancy with $p < 0.01$, *** significantly different from AECC SS without malignancy with $p < 0.001$

Standardized incidence ratios (SIRs) for lymphomas and solid tumours (Table 2)

In the AECC group 33 tumours were detected during the 2464 years of observation, while 23.21 were expected, resulting in an SIR of 1.42 (95% CI:0.98-2.00). Two of these patients had suffered other malignancies before the diagnosis of pSS. Furthermore prior to their pSS diagnosis 11 patients had diagnosed malignancies, including a chronic myeloic leukaemia, but did not develop malignancies later on. After the pSS diagnosis 2 patients developed more than 1 malignancy: one patient developed a myeloma after a lymphoma, one patient renal cancer after a breast cancer. At least 4 patients later developing lymphoproliferative neoplasms had skin cancers previously, (two cases basal-cell cancers, one squamous-cell cancer and in the fourth a combination of both types), detected by re-reading the lymphoma patients' case records (reporting of basal-cell carcinomas was not mandatory before 2003). For non-AECC patients the SIR for all cancers was 0.77 (0.41-1.32), no lymphoproliferative diseases were observed after pSS diagnosis.

In 286 AECC patients with a median observation time of 7 years, 11 NHLs, and 1 myeloma occurred as first malignancy after pSS diagnosis. The expected number for NHL was 0.79, resulting in a SIR of 15.57 (95% CI 7.77–27.85, $p < 0.0001$). The patient registered as myeloma in the cancer register had simultaneously a diffuse large B-cell lymphoma (DLBC) according to several re-evaluations.

Table 2 gives the SIRs for selected malignancies. To summarize, patients with pSS according to AECC have a non-significant increase in total risk of malignancy (point estimate 1.42). There was an excess of 10.2 malignancies, completely attributable to the excess in lymphoma/myeloma. This results in an excess malignancy of 4.2 per 1000 patient years at risk. The risk of lymphoma increased with time after the diagnosis of pSS: During the first 5 years the SIR for NHL was 6.4 (95% CI 1.3-18.7), during year 6 to 10 it was 11.1 (3.0-28.5) and during year 10-15 20.8 (6.8-48.6). The shortest duration between diagnosing pSS and lymphoma was 10 months in a patient with a 10-year history of sicca symptoms prior to pSS diagnosis. The point estimate for risk of pulmonary carcinomas was increased, although the small number makes precision poor: SIR 2.47 (0.67-6.32). The SIR for all non-haematological malignancies in the AECC SS patients was 0.93 (0.59-1.40).

Table 2. SIRs and 95% CI for selected types of malignancies, detected after the diagnosis of SS

AECC Sjögren's Syndrome
n=286, years at risk: 2464

	ICD7	n observed	n expected	SIR	95% CI
All malignancies		**33**	**23.21**	**1.42**	**0.98-2.00**
NHL	**200,202**	**11**	**0.71**	**15.57**	**7.77-27.85**
Myeloma	203	1	0.31	3.27	0.08-18.23
Mb Hodgkin	201	0	0.05		
Leukemia	201,2041-208	0	0.45		
Mouth and throat	140-148	1	0.33	3.03	0.08-16.88
Gastrointestinal	150-157	8	4.56	1.75	0.76-3.46
Lung	1620-1622	4	1.48	2.71	0.74-6.94
Breast	170	3	5.93	0.51	0.10-1.48
Female reproductive system	171-176	0	2.90		
Prostate/testis	177-178	0	0.72		
Kidneys/urinary tract	180-181	1	1.30	0.77	0.02-4.29
Skin/non melanoma	190	2	1.03	1.93	0.23-6.98
Skin/melanoma	191	0	0.87		
Brain	193	0	0.64		
Thyroid gland	194	1	0.15	6.86	0.17-38.21
Connective tissue	196-197	1	0.14	7.14	0.18-39.80

Non-AECC Sicca Syndrome
n=221, years at risk: 1840

	ICD7	n observed	n expected	SIR	95% CI
All malignancies		**13**	**16.89**	**0.77**	**0.41-1.32**
NHL	**200,202**	0	0.56		
Myeloma	203	0	0.24		
Mb Hodgkin	201	0	0.03		

Lymphoma types after re-evaluation:

Details of the 12 cases with re-classification of the WHO histopathology of the lymphoma biopsies are shown in table 3. Eleven of 12 patients had B-cell NHLs, (two of them appearing simultaneously with or followed by a myeloma). One patient had a T-cell lymphoma. Seven of the 11 B-cell lymphomas were DLBC lymphomas. This type comprised 58% of all NHLs in our cohort. Only 2 lymphomas (a follicular and a DLBC) were localized to the salivary gland region, but both were interpreted as originating from lymph nodes, in one case within and in the other adjacent to the parotid gland. In one patient with high grade DLBC lymphoma transformation from a MALT lymphoma could not be excluded. One patient had had a submandibular pseudolymphoma 18 years before the DLBC lymphoma in a neck lymph gland. Re-evaluation of the tissue from this submandibular gland only revealed a lymphoepithelial lesion.

Table 3. Clinical, laboratory and histological features of patients developing lymphoproliferative neoplasms

Pat	1	2	3	4	5	6	7	8	9	10	11	12
Age at pSS diagnosis	75	52	69	54	67	66	59	50	52	53	25	57
Age at lymphoma/ myeloma onset	76	58 61	72	61	78	73	71	57	65	59	33	67
Death /Age at death	+ 82	+ 62	-	-	+ 79	+ 74	-	+ 57	-	+ 61	+ 39	-
Symptom duration before pSS diagnosis	10	16	21	12	18	0	6	18	25	11	2	4
Sex	female	male	male	female	female	female	female	female	female	female	female	female
Lymphoma type (WHO)	Small lympho-cytic B-cell/chronic lymphatic leucemia	Small lympho-cytic B-cell+ Myeloma	Follicular B-cell	Small lympho-cytic B-cell = Walden-ström MG	Diffuse large B-cell	Diffuse large B-cell	Diffuse large B-cell °	Anaplastic large T-cell	Diffuse large B-cell	Diffuse large B-cell	Diffuse large B-cell	Myeloma + diffuse large B-cell
Grade	low	low	low	low	high	high	high	high	high	high	high	(high)
Primary localisation	BM, LNs	BM, LNs	Salivary glands	BM	LN groin	Right knee hollow	Saliv.gl.? LNs neck, intraabdo-minal,BM	LNs, liver spleen, BM	Lung-paren-chyma	BM, LNs	Salivary glands, LNs	LNs
Salivary gland swelling	-*	+*	-*	+°	+*	+*	+*	-*	+°	+°	+*	-*
Palpable purpura / skin vasculitis	-*	+*	-*	-*	-*	-*	+*	+*	+°	+°	+*	+°
Peripheral Neuropathy	-*	+*	-*	-*	+°	+°	-*	+°	+°	+°	+°	-*

Enlarged LN or spleen	-*	-*	-*	-*	-* +°	-*	-*	+°	+°	+	-*	+*
Concomitant lymphoma predisposing condition	Squa-mous cell cancer *	Psoriasis°	Psoriasis* *H pylori* ° Chronic gastritis	-	Basal cell cancer°	Hashi-moto*	H pylorSqua-mous + basal cell cancer°	-	Coeliak disease* *H pylori*° Hashi-moto*	-	Sibling with psoriasis	Basal cell cancer°
RF	+*	+*	-*	+*	+°	+*	+*	-*	+*	-	+*	+*
SSA/SSB	-°	+*	+°	+*	+*	+°	-*	+°	+°	+*	+*	+*
ANA	+*	+*	+*	+*	+*	+*	+*	+°	+*	+*	+*	+*
Cryoglob	ND	+°	ND	ND	ND	ND	-*	-*	+*	ND	+*	ND
Lymphopenia	-*	-*	-*	-*	-*	+*	+*	+°	+*	-*	+*	-*
Anemia	-*	-*	-*	-*	-*	+*	-*	-°	+*	-*	+*	-*
C3 g/l	0.70*	0.55*	0.98*	0.83*	0.84*	0.75*	0.50*	0.83*	0.78*	1.01*0.81°	0.73*	1.06°
C4 g/l	0.36*	0.13*	0.27*	0.29*	0.14*	0.16*	0.01*	0.16*	0.18*	0.37*0.12°	0.06*	0.23°
CD4penia§	ND	+*	+*	-*	+*	+*	-°	+*	+*	-*	+*	+*
IgG g/l	8.2*	14.0*	11.9*	13.8*	16.0*	27.0*	11.3*	23.0*	34.1*	14.4*	36.0*	24.6*
IgA g/l	1.72*	2.20*	2.20*	2.14*	2.82*	1.38*	1.29*	1.00*	0.13*	2.30*	3.31*	2.98*
IgM g/l	0.63*	2.30*	1.76*	1.54*	0.86*	2.03*	1.11*	1.03*	1.33*	0.40*	1.98*	1.51*
Monoclonality	-*	+* IgG	-*	+° IgM	+° IgM	-*, oligo°	-*	-*	-*	-*	+* IgM	Oligo*
Saliv.biopsy#	+*	+*	##	+*	+*	+*	+*	+*	+*	+*	+*	ND

* evaluated at diagnosing pSS, °developing during the course of disease before lymphoma development, but not present or not analysed at first visit. ND not done, BM = bone marrow. LN = lymph node, ## biopsy in this case contained insufficient glandular tissue for evaluation. §CD4-penia is defined as number of CD4 below 300 cells/µl, % of CD4 below 30, or ratio of CD4/CD8 below 0.8. # + = focus score >1

°This patient had had a pseudolymphoma in a submandibular gland before her pSS was diagnosed (18 years before the large B-cell lymphoma). At reevalution of the tissue the lymphoepithelial lesion without full-blown malignancy was confirmed.

Survival after lymphoma diagnosis:
Seven of 12 patients with lymphoma/myeloma had died (Table 3). The median survival time for all lymphoma patients was 43 months (low-grade 76, high-grade 31, log rank test: 0.08).
Predictors of lymphoma development: Cox regression analysis within the AECC group (n=286) Table 4:
The strongest predictor of lymphoma was a lowered CD4+/CD8+ T-cell ratio. Eight of 11 patients with available lymphocyte subtyping had a CD4+/CD8+ T-cell ratio of ≤0.8, resulting in a HR of 10.92 (95% CI: 2.80-41.83). The low number of events made multivariate regression analysis impossible, only adjustment for age was performed. Levels of immunoglobulins were not significantly associated with increased risks. Patients later developing lymphoma/myeloma had significantly lower relative numbers of CD4+ T-lymphocytes, increased CD8+ T-lymphocytes and lowered ratio of CD4+/CD8+ ($p < 0.01$, < 0.001 and < 0.01 respectively) (Table 1). Figure 1 shows Kaplan-Meier plots for the risk of developing NHL/myeloma in patients presenting with or without CD4+ T-lymphocytopenia. The time between the lymphocyte count and the lymphoma was in median 88 months (4-156). Low levels of complement factors C3 and C4 predicted haematological malignancy. Nine of the 12 patients had disorders associated with lymphoma development themselves, such as celiac disease [35], *Helicobacter pylori* [36], psoriasis [37], autoimmune thyreoiditis [38], skin cancers [39, 40]. Methotrexate, auranofin, ocular (topical) cyclosporine and chloroquine were the only anti-rheumatic drugs used before lymphoma appearance (Table 3).

Table 4. Predictors of lymphoproliferative disease (n=12) within the AECC group (n=286).

Predictor variable	Total AECC cohort n available	Lymphoma patients n available	HR (95% CI)	p
Age at diagnosis	286	12	1.02/year (0.97-1.06)	0.516
Salivary gland swelling yes/no	81/194	5/7	2.02 (0.62-6.61)	0.247
Purpura/skin vasculitis yes/no	29/244	4/4	4.64 (1.13-16.45)	**0.017**
ANA positivity yes/no	240/44	12/0	-	
RF positivity yes/no	156/117	9/3	3.03 (0.80-11.24)	0.102
SSA/SSB yes/no	163/120	10/2	2.58 (0.69-9.63)	0.159
CD4-penia yes/no	35/130	8/3	8.14 (2.10-31.53)	**0.002**
CD4+ (%)*	165	11	0.57 (0.34-0.93)	**0.026**
CD8+ (%)*	165	11	1.76 (1.14-2.73)	**0.011**
CD4+/CD8+ratio*	165	11	0.23 (0.07-0.73)	**0.013**
CD4+/CD8+ratio ≤ 0.8 yes/no	29/136	8/3	10.92 (2.8-41.83)	**0.000**
C3 ≤ 0.83 g/l °	60	8	6.18 (1.57-24.22)	**0.009**
C3 0.84-1.12 g/l °	118	3	1.0 (referent)	
C3 ≥1.13 g/l °	60	0	-	-
C4 ≤ 0.18 g/l °	62	7	9.49 (1.94-46.54)	**0.006**

C4 = 0.19-0.30 g/l °	116	2	1 (referent)	
C4 ≥ 0.31 g/l °	60	2	1.60(0.22-11.42)	0.641
IgG ≤ 12.0 g/l °	71	3	1.41 (0.31–6.32)	0.65
IgG = 12.1-21.4 g/l °	133	5	1 (referent)	
IgG ≥ 21.5 g/l °	68	5	2.54 (0.67-9.65)	0.17

The analysis was performed using COX regression with adjustment for age. * HR per 1 standard deviation (SD) increase. CD4+ (%) mean: 43.32, SD: 12.20, CD8+ (%) mean: 28.59, SD:13.01, CD4+/CD8+ratio: 1.90, SD: 1.18. ° Due to a u-shaped distribution for the risk of lymphoma, quartiles for C3, C4 and IgG were used, testing the risks for the highest and lowest quartiles versus the two middle ones. For all predictors first ever assessment is used.

DISCUSSION

We have performed an analysis of the risk of malignancy in general and lymphoproliferative neoplasms in particular in our prospectively followed cohort of pSS patients. The focus of this study was on patients fulfilling the AECC for pSS [1].

There are mainly 2 new messages:

1) The often cited risk estimate for NHL of 44 fold increase compared with the background population is probably valid only for highly selected patient populations with severe disease, while a lower risk estimate as found in this study (16 fold increase) is probably more representative for an average pSS population. No other malignancies were overrepresented with statistical significance, although the power to detect such deviations was low.

2) Our results show a substantial risk increase for developing lymphoproliferative malignancy in patients with a decreased CD4+/CD8+ T-lymphocyte ratio. No previous longitudinal cohort study has evaluated the significance of T-cell disturbances with respect to outcome in pSS despite the fact that the presence of CD4+ T-lymphocytopenia was described years ago and suggested to be associated with cases of NHL [17]. Earlier proposed risk factors such as hypocomplementaemia and skin vasculitis are confirmed.

Additional interesting observations include the presence of further predisposing factors such as autoimmune thyroiditis, celiac disease, *H pylori* infection, skin cancers or psoriasis, which may deserve increased awareness and intensified search for lymphoma when combined with suspicious clinical or laboratory signs. The significance of these coincidences requires confirmation. However, the high frequency of earlier non-melanoma skin cancers in our

lymphoma patients is in concordance with several recent reports on increased lymphoma risks in skin cancer patients [39, 40]. Surprisingly, the NHLs in our pSS cohort are in 58% high-grade, diffuse large B-cell (DLBC) lymphomas.

In concordance with our previous study on mortality also the present investigation underlines the importance of strict and universally accepted classification criteria, as patients not fulfilling the AECC criteria do not show any increased lymphoma risk in contrast to those who do so.

Strengths of the present study are the strict mono-centre prospective design of the data collection in combination with the highly reliable Swedish general health registers [25]. In addition, the follow-up time of up to 19 years (median 8 years) is relatively long. This seems to be a prerequisite to allow evaluation of long-term severe outcomes such as death or cancer development. When Kirtava 1995 [17] described 6 patients with CD4+ T-lymphocytopenia from our department (follow-up up to 7 years), only 1 had developed lymphoma. In the present study we found that another 2 of these patients had developed a lymphoma. The mean time between diagnosing pSS and the appearance of the lymphoma was in the present study 8 years (1-13years). The risk of lymphoma increases with follow-up time, exemplified by the highest SIR of >20 being observed in those followed more than 10 years.

CD4+ T-lymphocytopenia has been described in association with and preexisting before NHLs [41-44]. In our study all the risk calculations were performed using the first available CD4+ T-cell analysis, most often performed at or shortly after the time of diagnosing pSS, and always before the lymphoma detection (Table 3), which excludes the possibility of a lymphoma induced CD4+ T-lymphocytopenia. We have to acknowledge the lack of systematic cryoglobulin analysis in our cohort as an important drawback. The assumption that cryoglobulinaemia is rare in Swedish pSS patients [34] needs to be revised in the light of the new classification criteria.

Our study differs from previous studies in several important aspects. The lymphoma incidence was lower than in Kassan's original description, which however, as the authors themselves point out, may not be generalized to other populations less prone to selection bias [7]. Furthermore their study had slightly less precision, being based on only 7 (4 primary SS lymphoma) cases. The described histiocytic diffuse and Lennert's lymphomas in 6 of 7 cases would correspond to high-grade DLBC and T-cell lymphomas in the present WHO classification. This is in accordance with our cases with predominantly high-grade DLBC. In contrast, two other case series have documented a predominance of low-grade lymphomas, quite often in salivary glands and of MALT type [10, 45], while another study did not find any MALT lymphomas among 4 SS associated NHLs [8]. Only one of our cases could possibly be classified as MALT lymphoma. Our approach with linkage to the validated national cancer register excludes any major detection bias, which may operate when cases are identified in routine clinical settings. Transformation from earlier MALT lymphoma into DLBC lymphoma can however not be excluded. Survival after lymphoma was comparable with previous reports when comparing groups of high- and low-grade lymphomas separately [10].

The lymphoma types found in our study are similar to those found in Swedish RA cohorts [46]. Also in SLE, the associated lymphomas are predominantly of the DLBC type [47]. The reported increase in risk with disease duration is similar to studies in RA, but in contrast to studies in SLE, where the highest risk is observed within the first 5 years after diagnosis [47]. In RA high disease activity is the most important predictive factor for lymphoma [48]. Disease activity is difficult to assess in pSS. Correlation between extraglandular disease and

CD4+ T-lymphocytopenia due to apoptosis was described in pSS [19]. It seems conceivable that a longstanding deficiency in immune surveillance finally allows malignant transformation in antigen-stimulated proliferating B-cells.
The causes of CD4+ T-lymphocyte depletion or disturbed balance between CD4+ and CD8+ T-cells are unknown. Anti-CD4+ antibodies have been documented in pSS patients without correlation to the level of CD4+ T-cells [49]. Virus infections are typical causes of lymphopenia, and HIV infection is the prototype of virus-induced CD4+ T-lymphocytopenia, associated with lymphoma development [50]. Hepatitis C [51] and EBV [52] are viruses associated with lymphoma development and autoimmunity. Coxsackie B virus was recently proposed as an etiologic factor in pSS [53, 54], but its potential to induce cytopenia has not been studied. CD4+ T cells and subsets of the CD4+ T-lymphocytes are important in tumour immunity [55]. To what extent the different predictors for lymphoma development, such as cryoglobulinemia, hypocomplementemia, B-cell activation and CD4+ T-cell depletion have a shared etiology or represent different aspects of risk needs to be elucidated.
In summary our results suggest that CD4+ T-lymphocytopenia is a useful clinical predictor, which possibly is of crucial importance in the sequence of events leading to lymphoma development in pSS patients. Previously proposed risk factors, such as hypocomplementemia and skin vasculitis could be confirmed, while aggressive types of lymphomas were frequent in our cohort. The overall lymphoma risk was lower than earlier proposed, but increased with longer disease duration.

ACKNOWLEDGMENTS
We thank biostatistician Jan Åke Nilsson (Malmö University Hospital) for the professional support in performing the statistical analyses and Dr Anna Bladström (Southern Swedish Cancer Registry) for calculating cancer specific SIRs. This study was supported by the Swedish Rheumatism Association and the Skane County Council's Research and Development Foundation and the European BIOMED concerted action BMH4-96-0595.

COMPETING INTERESTS
None of the authors have to declare any competing interests

ETHIC CONSIDERATIONS
The study was approved by the Ethics Committee at Lund University.

REFERENCES

1. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002; 61(6): 554-8.
2. Bowman SJ, Ibrahim GH, Holmes G, Hamburger J, Ainsworth JR. Estimating the prevalence among Caucasian women of primary Sjögren's syndrome in two general practices in Birmingham, UK. Scand J Rheumatol 2004; 33: 39-43.
3. Theander E, Jacobsson L. Reply: Prevalence of primary SS. Arthritis Rheum 2005; 52(1): 369-70.
4. Mostafavi B, Akyuz S, Jacobsson ME, Nilsen LV, Theander E, Jacobsson LH. Perinatal characteristics and risk of developing primary Sjögren's syndrome: a case-control study. J Rheumatol 2005; 32(4): 665-8.
5. Delaleu N, Jonsson R, Koller MM. Sjögren's syndrome. Eur J Oral Sci 2005; 113(2): 101-13.
6. Jonsson R, Bowman S, Gordon T. Sjögren's Syndrome. In: Koopman W and Moreland L, editors. Arthritis and Allied Conditions - A Textbook of Rheumatology. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 1681-705.
7. Kassan SS, Thomas TL, Moutsopoulos HM, Hoover R, Kimberly RP, Budman DR, et al. Increased risk of lymphoma in sicca syndrome. Ann Intern Med 1978; 89: 888-92.
8. Pariente D, Anaya JM, Combe B, Jorgensen C, Emberger JM, Rossi JF, et al. Non-Hodgkin's lymphoma associated with primary Sjögren's syndrome. Eur J Med 1992; 1: 337-42.
9. Kauppi M, Pukkala E, Isomäki H. Elevated incidence of hematologic malignancies in patients with Sjögren's syndrome compared with patients with rheumatoid arthritis (Finland). Cancer Causes and Control 1997; 8: 201-4.
10. Voulgarelis M, Dafni U, Isenberg DI, Moutsopoulos HM, Jonsson R, Haga H-J, et al. Malignant lymphoma in primary Sjögren's syndrome. Arthritis Rheum 1999; 42: 1765-72.
11. Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjögren's syndrome. Arthritis Rheum 2002; 46(3): 741-7.
12. Theander E, Manthorpe R, Jacobsson LTH. Mortality and causes of death in primary Sjögren's syndrome. Arthritis Rheum 2004; 50(4): 1262-9.
13. Sutcliffe N, Inanc M, Speight P, Isenberg D. Predictors of lymphoma development in primary Sjögren's syndrome. Semin Arthritis Rheum 1998; 28(2): 80-7.
14. Voulgarelis M, Moutsopoulos HM. Malignant lymphoma in primary Sjögren's syndrome. IMAJ 2001; 3: 761-6.
15. Ramos-Casals M, Brito-Zeron P, Yague J, Akasbi M, Bautista R, Ruano M, et al. Hypocomplementaemia as an immunological marker of morbidity and mortality in patients with primary Sjögren's syndrome. Rheumatology (Oxford) 2005; 44(1): 89-94.

16. Tzioufas AG, Boumba DS, Skopouli FN, Moutsopoulos HM. Mixed monoclonal cryoglobulinemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the devolopment of lymphoma in primary Sjögren's syndrome. Arthritis Rheum 1996; 39(5): 767-72.
17. Kirtava Z, Blomberg J, Bredberg A, Henriksson G, Jacobsson L, Manthorpe R. CD4+ T-lymphocytopenia without HIV infection: increased prevalence among patients with primary Sjögren's syndrome. Clin Exp Rheumatol 1995; 13(5): 609-16.
18. Mandl T, Bredberg A, Jacobsson LT, Manthorpe R, Henriksson G. CD4+ T-lymphocytopenia-a frequent finding in anti-SSA antibody seropositive patients with primary Sjogren's syndrome. J Rheumatol 2004; 31(4): 726-8.
19. Zeher M, Szodoray P, Gyimesi E, Szondy Z. Correlation of increased susceptibility to apoptosis of CD4+ T cells with lymphocyte activation and activity of disease in patients with primary Sjogren's syndrome. Arthritis Rheum 1999; 42(8): 1673-81.
20. Ferraccioli GF, Tonutti E, Casatta L, Pegoraro I, De Vita S, Sala P, et al. CD4 cytopenia and occasional expansion of CD4+CD8+ lymphocytes in Sjögren's syndrome. Clin Exp Rheumatol 1996; 14: 125-30.
21. Kroneld U, Halse AK, Jonsson R, Bremell T, Tarkowski A, Carlsten H. Differential immunological aberrations in patients with primary and secondary Sjögren's syndrome. Scand J Immunol 1997; 45(6): 698-705.
22. Thomas E, Brewster DH, Black RJ, Macfarlane GJ. Risk of malignancy among patients with rheumatic conditions. Int J Canc 2000; 88: 497-502.
23. Stenbeck M, Rosén M, Sparén P. Causes of increasing cancer prevalence in Sweden. Lancet 1999; 354: 1093-4.
24. Clarke CA, Glaser SL. Changing incidence of non-Hodgkin lymphomas in the United States. Cancer 2002; 94: 2015-23.
25. Mattsson B, Wallgren A. Completeness of the Swedish Cancer Register. Acta Radiologica 1984; 23(5): 305-13.
26. Cancer incidence in Sweden 2002. In Statistics, Health and Diseases. The National Board of Health and Welfare CfE, Editor. 2003: Stockholm.
27. Dödsorsaker 2002. In Official Statistics of Sweden, Health and Diseaes. 2004; National Board of Health and Welfare: Stockholm.
28. Lunde AS. The Person-Number System of Sweden, Norway, Denmark, and Israel. In Vital and Health Statistics. Services USDoHaH, Editor. 1990; Office of Health Research, Statistics, and Technology: Hyattsville. 1-40.
29. Sundman L, Jakobsson S, Nystrom L, Rosen M. A validation of cause of death certification for ischaemic heart disease in two Swedish municipalities. Scand J Prim Health Care 1988; 6(4): 205-11.
30. Manthorpe R, Oxholm P, Prause JU, Schiödt M. The Copenhagen criteria for Sjögren's syndrome. Scand J Rheumatol 1986; Suppl 61: 19-21.
31. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, M BR, et al. Preliminary criteria for the classification of Sjögren's syndrome. Arthritis Rheum 1993; 36: 340-7.
32. Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Pathology and genetics: Tumours of the haematopoietic and lymphoid tissue. World Health Organization classification of tumours. Kleihaus P and Sobin L. Lyon: IARC Press; 2001.

33. Henriksson G, Brant M, Sallmyr A, Fukushima S, Manthorpe R, Bredberg A. Enhanced DNA damage-induced p53 peptide phosphorylation and cell-cycle arrest in Sjögren's syndrome cells. Eur J Clin Invest 2002; 32: 458-65.
34. Verbaan H, Carlson J, Eriksson S, Larsson Å, Liedholm R, Manthorpe R, et al. Extrahepatic manifestations of chronic hepatitis C infection and the interrelationship between Sjögren´s syndrome and hepatitis C in Swedish patients. J Intern Med 1999; 245: 127-32.
35. Askling J, Linet M, Gridley G, Halstensen TS, Ekstrom K, Ekbom A. Cancer incidence in a population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis. Gastroenterology 2002; 123(5): 1428-35.
36. Carroll IM, Khan AA, Ahmed N. Revisiting the pestilence of Helicobacter pylori: insights into geographical genomics and pathogen evolution. Infect Genet Evol 2004; 4(2): 81-90.
37. Hannuksela-Svahn A, Pukkala E, Laara E, Poikolainen K, Karvonen J. Psoriasis, its treatment, and cancer in a cohort of Finnish patients. J Invest Dermatol 2000; 114(3): 587-90.
38. Kossev P, Livolsi V. Lymphoid lesions of the thyroid: review in light of the revised European-American lymphoma classification and upcoming World Health Organization classification. Thyroid 1999; 9(12): 1273-80.
39. Frisch M, Hjalgrim H, Olsen JH, Melbye M. Risk for subsequent cancer after diagnosis of basal-cell carcinoma. A population-based, epidemiologic study. Ann Intern Med 1996; 125(10): 815-21.
40. Wassberg C, Thorn M, Yuen J, Ringborg U, Hakulinen T. Second primary cancers in patients with squamous cell carcinoma of the skin: a population-based study in Sweden. Int J Cancer 1999; 80(4): 511-5.
41. Sewell HF, MacKenzie RH, Dawson AA, Ratcliffe MA, King DJ, Bennett NB. Phenotypic abnormality of T cells in B cell non-Hodgkin's lymphoma. Dis Markers 1990; 8(3): 145-9.
42. Cook M, Bareford D, Kumararatne D. Non-Hodgkin's lymphoma: an unusual complication of idiopathic CD4+-lymphocytopenia. Hosp Med 1998; 59: 582.
43. Campbell J, Prince H, Juneja S, Seymour J, Slavin M. Diffuse large cell lymphoma and t(8;22)(q24;q11) in a patient with idiopathic CD4+T-lymphopenia. Leuk Lymphoma 2001; 41: 421-3.
44. Guilloton L, Drouet A, Bernard P, Berbineau A, Berger F, Kopp N. Cerebral intravascular lymphoma during T-CD4+idiopathic lymphopenia syndrome. Presse Med 1999; 28(25): 1513-5.
45. Masaki Y, Sugai S. Lymphoproliferative disorder in Sjögren's syndrome. Autoimmunity Reviews 2004; 3: 175-82.
46. Baecklund E, Sundström C, Ekbom A, Catrina AI, Biberfeld P, Feltelius N, et al. Lymphoma sybtypes in patients with rheumatoid arthritis. Arthritis Rheum 2003; 48(6): 1543-50.
47. Bernatsky S, Boivin JF, Joseph L, Rajan R, Zoma A, Manzi S, et al. An international cohort study of cancer in systemic lupus erythematosus. Arthritis Rheum 2005; 52(5): 1481-90.
48. Baecklund E, Askling J, Rosenquist R, Ekbom A, Klareskog L. Rheumatoid arthritis and malignant lymphomas. Curr Opin Rheumatol 2004; 16(3): 254-61.

49. Henriksson G, Manthorpe R, Bredberg A. Antibodies to CD4 in primary Sjögren´s syndrome. Rheumatology 2000; 39: 142-7.
50. Ihrler S, Zietz C, Sendelhofert A, Menauer F, Blasenbreu-Vogt S, Löhrs U. Zur Differenzialdiagnose lymphoepithelialer Speicheldrüsenläsionen. Pathologe 2000; 6: 424-32.
51. Ramos-Casals M, Trejo O, García-Carrasco M, Cervera R, de la Red G, Gil V, et al. Triple association between hepatitis C virus infection, systemic autoimmune diseases, and B cell lymphoma. J Rheumatol 2004; 31: 495-9.
52. Young LS, Rickinson AB. Epstein-Barr Virus: 40 years on. Nature Reviews Cancer 2004; 4: 757-68.
53. Triantafyllopoulou A, Tapinos N, Moutsopoulos HM. Evidence for coxsackievirus infection in primary Sjögren's syndrome. Arthritis Rheum 2004; 50(9): 2897-902.
54. Liakos DA, Triantafyllopoulou A, Kapsogeorgou EK, Moutsopoulos HM. Autoimmune diseases: role of coxsackieviruses in their pathogenesis. Autoimmun Rev 2004; 3 Suppl 1: S71-3.
55. Rajnavölgyi É, Lány À. Role of CD4+ T lymphocytes in antitumor immunity. Adv Cancer Res 2003; 87: 195-249.

FIGURE LEGEND

Figure 1. Kaplan-Meier plots for the risk of lymphoproliferative disease in patients with or without CD4+ T-lymphocytopenia