Seroprevalence of *Helicobacter pylori* in primary Sjögren's syndrome

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Abstract

Objective

To study the seroprevalence of Helicobacter pylori *(*H. pylori*) infection in patients with primary Sjögren's syndrome (SS), fulfilling the 1993 European classification criteria compared with three different control groups.*

Methods

Serological tests investigating the presence of antibodies against H. pylori *were performed by Enzyme Immuno Assay (EIA) and confirmed by immunoblot (IB). The samples were tested for antibodies against cytotoxin-associated-protein A (CagA). The three control groups included were: one simultaneously collected age-matched group of orthopaedic outpatients without rheumatological disease, a random primary care patient sample from the same geographic region and a group of age-matched blood donors.*

Results

45% of the SS patients (n = 164) were EIA-positive for H. pylori *and 30% were positive in the confirming IB assay. 23% had antibodies to the CagA protein. We found a clear and statistically significant increase in seroprevalence with increasing age. These estimates were lower compared to the control group of orthopaedic patients but similar to those in the other two control groups, thus showing the importance of multiple control groups in case control studies. In the group of SS patients there were no significant associations between a positive EIA, IB or CagA for* H. pylori *and the presence of abnormal serum levels of autoantibodies (ANA, anti-SSA, anti-SSB, rheumatoid factor (RF)) or an abnormal lip biopsy.*

Conclusion

Swedish patients with primary SS do not have higher H. pylori *seroprevalence rates than controls. Neither was* H. pylori *seropositivity associated with the presence of immunological markers of SS such as circulating autoantibodies or a lip biopsy with abnormal focus score.*

Key words

Primary Sjögren's syndrome, *Helicobacter pylori*, autoimmune disease.

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Introduction

Primary Sjögren's syndrome (SS) is one of the most common systemic inflammatory autoimmune connective tissue diseases. Its aetiology is unknown. The prevalence varies between 0.04% and 4.8%, depending on the type of diagnostic criteria used and the population studied (1). Besides the obligatory exocrine organ involvement some patients show non-exocrine disease manifestations. One of the most serious complications of SS is the about 40-fold increased risk of developing malignant non-Hodgkin lymphoma (MNHL) that may develop in about 5% of the SS patients (2). Patients with salivary gland enlargement, lymphadenopathy, skin vasculitis, peripheral neuropathy, lymphopenia and anaemia seem to run a higher risk of developing MNHL than other SS patients (3). No published data exist regarding the prevalence of mucosa-associated-lymphoid-tissue (MALT-) lymphoma in SS.

Helicobacter pylori (*H. pylori*) is probably the most prevalent bacterial infection in man world-wide, most often acquired early in life. Prevalence rates differ greatly depending on age, geographic region and socio-economic status (4-6). *H. pylori* colonises the gastric epithelium and causes chronic type B gastritis and predicts the development of distal gastric adenocarcinoma (7) as well as MALT lymphoma (8, 9). A causal link between *H. pylori* infection and MALT lymphoma is further supported by the fact that eradication of *H. pylori* with antibiotics has resulted in regression of lymphoma (10, 11).

SS is a disease of unknown aetiology and associated with gastritis, though not apparently due to *H. pylori* (12, 13) as well as with an increased risk for development of MNHL (3). Hypothetically, it is thus possible that *H. pylori* infection may be of importance in SS either as an etiological agent or by interacting with the clinical course. Studies of patients with SS have shown that some patients may improve with respect to their SS symptoms in connection with treatment of *H. pylori* infection (14).

Studies on the frequency of *H. pylori* infection in patients with SS have so far been relatively small and yielded conflicting results (12, 13, 15, 16) (Table I).

The aims of the current study were:

1) To determine the seroprevalence of *H. pylori* infection, the bacteria's CagA status as a virulence factor and the antibody titres in SS patients compared with three different control groups.

2) To assess the associations between *H. pylori* seropositivity and signs of autoimmune dysregulation (autoantibody status, salivary gland biopsy focus score).

Materials and methods

Patients

The patient group consisted initially of 184 consecutive patients with primary SS fulfilling the Copenhagen criteria (17) from the Sjögren's syndrome research centre at the rheumatological outpatient clinic, Malmö University Hospital, Sweden. On the suggestion of the referees the group was reduced to 164 patients who all fulfilled not only the Copenhagen criteria but also the 1993 European classification criteria for primary SS (18). The median age of the patients was 63 years (16-88); 92% were women. The disease duration was in median 11.5 years (1-44). 51% were ANA positive, 33% were SSA-, 26% SSB- and 30% RF positive. In 51% of the patients the salivary gland biopsy had shown a focus score >1. 25% used NSAIDs and 17% were smokers while another 31% were former smokers. All patients were currently living in Sweden, most of them in Malmö County and the nearby surroundings. Almost all patients were Caucasians.

Patient group data on autoantibody status, dyspeptic symptoms, smoking habits and NSAID treatment were collected at the same time as blood samples for Helicobacter serology were drawn. A lower lip salivary gland biopsy was usually done at the time of diagnosis and the focus score from that biopsy was used.

Controls

Control group I (orthopaedic): An age-matched control group consisting of consecutive orthopaedic outpatients without rheumatological autoimmune

Table I. Studies on *Helicobacter pylori* seroprevalence in patients with primary Sjögren's syndrome.

Study (ref.)	Sjögren's syndrome Number Classification criteria Age: mean or median (range)	Controls Number Type Age: mean or median (range)	Methods Tests	Results % positive Patients vs controls (if applicable)
Ferraccioli *et al.* 1996 (17)	21 1993 European criteria 52 (30 - 70) years	80 Dyspepsia Age not given	Endoscopy Histopathology	71% vs 63%
Showji *et al.* 1996 (22)	7 Age not given	24 Pulmonary diseases Median not given (range 40-69 years)	ELISA Western blotting Age-matched	No seroprevalence given Higher titers in cases than controls
Collin *et al.* 1997 (20)	32 San Diego criteria 55 (27-71) years	64 Dyspepsia 55 (26-70) years	Endoscopy Histopathology	31.2% vs 39.1% n.s.
Aragona *et al.* 1999 (21)	34 primary SS 1993 European criteria 46 (13-72) years 19 Secondary SS 50.2 (23-70) years	22 Autoimmune diseases 44.3 (25-67) years 43 Eye surgery patients 44.7 (22-77) years	*H. pylori* ELISA Anti-Hsp60 ELISA	Results from *H. pylori* ELISA : prim SS: 79.4% sec SS: 57.9% autoimm: 18.2% eye surgery: 48.5%

disease from the Malmö University Hospital was used for comparison of the seroprevalence rate. 142 SS patients were paired with 142 orthopaedic patients so that each SS patient received a control that was at the most 3 years older or younger. The median age was 62 years (16-88). No clinical data were collected from the control group.

Control group II (blood donors): We had earlier tested 41 healthy blood donors from Malmö County for *H. pylori* by EIA. This group was compared with an age-matched subgroup of 41 primary SS patients.

Control group III (historical/primary care): In 1996 Bergenzaun *et al.* studied 393 persons from the region of Lund (20 km north-east of Malmö) (6). In both that and the present study anti-*H. pylori* antibodies were tested with EIA at the same laboratory and by the same method. This control group consisted of randomly chosen primary care patients from urban, suburban and rural areas without gastrointestinal complaints (6).

Analysis of H. pylori *serology*

Serum samples of the patients and controls were analysed for antibodies to *H. pylori* and antibodies to the CagA protein using EIA and immunoblot. In control groups II and III only EIA analysis was carried out.

EIA: The methods and reagents for the indirect IgG-EIA have earlier been described in detail (19, 20). The cut-off value for seropositivity was set to a relative antibody activity (RAA) of > 35 and values between 25 and 35 RAA were regarded as borderline results (19). The cut-off values are based on EIA and immunoblot analyses of serum samples from patients with positive and negative gastric culture for *H. pylori*, blood donors and children. The sensitivity of the EIA test is 90%, its specificity 97% (20).

Immunoblot assay (IB): IB analysis was carried out as recently described (21). Test sera staining proteins with a high molecular mass protein (110/120 kDa) or at least 2 of 5 proteins with low molecular masses (26, 29, 30, 31 and 33 kDa), or combinations of both, were defined as IB positive. Positive IB results correlate to *H. pylori* culture positivity in 97.5% (21).

Immunological data

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence using HEp2-cells with the following cut-off values: For women titres ≥ 64, ≥ 128 or ≥ 256 were considered positive in the age groups ≤ 45 years, 46-60 years or ≥ 61 years, respectively. For men one titre lower in the corresponding age group was considered positive.

Anti-SSA (Ro 52 and/or Ro 60) and anti-SSB (La 48) antibodies were analysed by immunodiffusion and results were registered as either positive or negative.

IgM RF measurement was done by ELISA,with positive results of >20 IU/ml, based on standardisation with a WHO RF reference preparation.

Statistical analysis

The numbers of controls in the control group I were calculated in order to allow the detection of a 10% difference of prevalence between patients and controls with an expected prevalence of 35% in the general adult population. The seroprevalence rates in the patient group and the group of the age-matched controls were compared using ^{2}-tests. For matched pairs with dichotomous outcome McNemar's-test was used, for variables with three different outcomes the Stewart-Maxwell-test was applied. The antibody levels in patients and controls were compared using the Mann-Whitney-U-test.

In order to evaluate the association between SS related clinical and serological data and Helicobacter-serology logistic regression with adjustment for age

was applied.
All tests were performed two-sided at a significance level of 5%.

Results

Primary Sjögren's syndrome patients
In total 73 (45%) of the 164 patients were EIA-positive, and 15 (9%) showed borderline results. IB was performed with EIA-positive/borderline sera only, and antibody reactivity to the CagA protein was evaluated. In the following presentation those negative in the EIA test are considered as negative by the IB and for CagA. Forty-nine (30%) patients had IB-positive results (67% of the EIA positives) and 37 (23%) had CagA antibodies (50% of the EIA positives). We found significantly higher prevalence rates with increasing patient age. (p-values for test for linear trend in the patient group for EIA,IB and CagA results respectively: 0.042, 0.001 and 0.008). The median (range) level of antibody units in the EIA analysis was 25.5 (0-127), if all patients, and 74 (36-127), if only those with values above the cut-off level were included (Figs. 1 and 2). Sixty patients (37%) complained about upper gastrointestinal problems, of those 40 (67%) were negative and 20 (33%) were positive in the *H. pylori* immunoblot test (p = 0.71). One patient in this study developed MNHL in a parotic gland, diagnosed some months after eradication therapy for *H. pylori*. Three more patients had a history of lymphoma or pseudolymphoma; all these were IB and CagA negative.

Control group I (142 orthopaedic out-patients)
In the group of orthopaedic patients 75 (53%) were EIA positive (plus 10% borderline results), 63 (44%) were positive by IB and 53 (37%) showed CagA antibodies. In contrast to the results obtained in the patient group the tests for linear correlation between age and seropositivity did not show significant results (p-values for EIA, IB and CagA: 0.720, 0.378 and 0.550 respectively). The median (range) level of antibody units in the EIA analysis was 36.5 (0-118) for all the controls and 76 (36-118) for those with results above the cut-off level (Table II, Fig. 1, Fig. 2)
The difference in seroprevalence between the SS patient group and the age-matched control group was statistically significant with a lower prevalence in the SS patients than in the controls in IB and CagA, but not in the EIA-test. (p = 0.01 for IB, p = 0.007 for CagA-(Table II, Fig. 1).

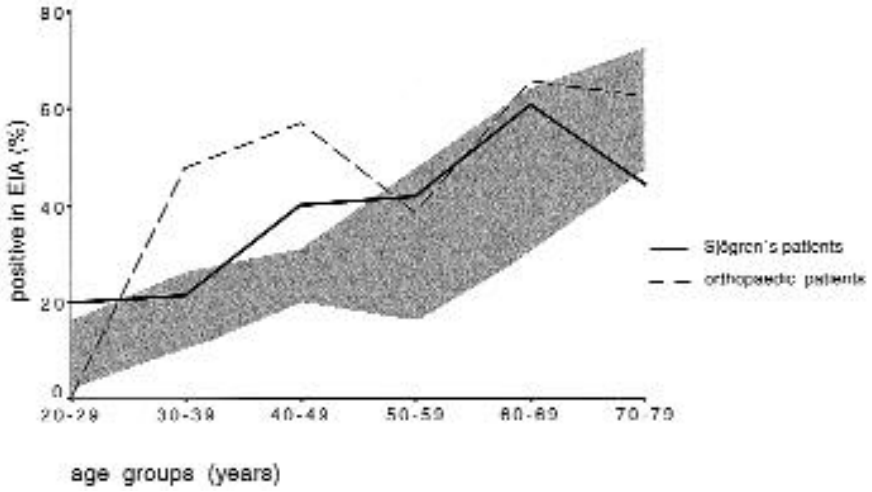

Fig. 1. Prevalence of *Helicobacter pylori* seropositivity in age groups analysed by EIA. The lines show the group of Sjögren's patients and the control group I (orthopaedic patients) (p > 0.05). The shadowed area represents the 95% confidence interval in the population study by Bergenzaun *et al.* (control group III) (ref. 8).

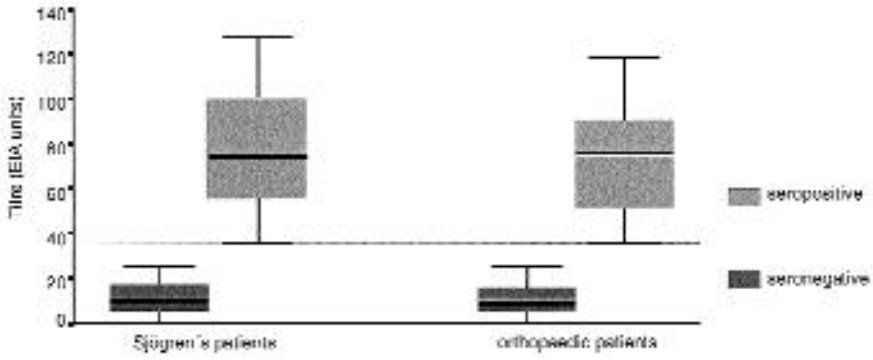

Fig. 2. Comparison of the levels of EIA titres in SS patients and control group I (orthopaedic patients). The graph shows the median titre, interquartile range and non-outliers range for both groups, devided into 'seropositives' and 'seronegatives' according to the defined cut-off value of a relative antibody activity of 35. The borderline cases are excluded. p > 0.05.

Control group II (41 healthy blood donors)
In this control group 15 (37%) had positive EIA results. Among the 41 age-matched SS patients 15 (37%) were seropositive and thus there was no statistically significant difference between these two groups.

Control group III (historic population study)
The results of the control group III are published in detail elsewhere (6). In that study only EIA-tests were performed. A graphic illustration shows that the seroprevalence in the group with primary SS is within the 95% confidence limits (shadowed area) of the control group for most age groups (Fig. 1).

Subgroup analysis, (Table II)
Fulfilling increasing number of items of the European criteria was not associated with higher seroprevalence for *H. pylori*. Separate parameters as abnormal focus score in salivary gland biopsy, presence of autoantibodies in peri-

Table II. Seroprevalence of antibodies to *Helicobacter pylori* in patients with Sjögren's syndrome and in orthopaedic patients (control group I) in age groups.

	Sjögren's syndrome patients results in %				Orthopaedic patients results in %			
Age years	n	EIA pos (borderline)	Immunoblot pos	CagA pos	n	EIA pos (borderline)	Immunoblot pos	CagA pos
≤ 19	1	100 (0)	0	0	1	0 (100)	0	0
20-29	5	20 (0)	0	0	3	0 (67)	33	33
30-39	9	22 (0)	22	11	13	48 (8)	46	31
40-49	15	40 (0)	20	13	14	57 (7)	50	50
50-59	31	42 (19)	13	13	26	39 (19)	31	31
60-69	43	61 (12)	49	37	41	66 (2)	46	39
70-79	30	43 (7)	43	37	34	62 (6)	53	44
≥ 80	8	38 (13)	25	13	10	30 (10)	40	20
all	142	46 (10)	32	24	142	53 (10)	44	37

Table III. Associations (age adjusted) between positive immunoblot test for *H. pylori*, disease characteristics of primary SS and dyspeptic symptoms.

Groups	n	median age	%pos by IB	OR(95%CI)
All patients	164	63	30	not applicable
1993 European criteria				
5 items / 4 items	51 / 67	63 / 61	39 / 25	1.97 (0.88-4.39)
6 items / 4 items	46 / 67	63 / 61	26 / 25	0.97 (0.40-2.36)
ANA (pos / neg)	83 / 80	63 / 61	29 / 30	0.86 (0.43-1.74)
Ro and/or La (pos / both neg)	53 / 110	63 / 62	26 / 31	0.75 (0.35-1.62)
RF (pos / neg)	50 / 113	63 / 61	28 / 30	0.84 (0.39-1.80)
Lip biopsy (pos / neg)	83 / 60	63 / 60.5	31 / 25	1.28 (0.59-2.79)
Dyspepsia (yes / no)	60 / 97	64 / 60	33 / 29	1.14 (0.56-2.34)

pheral blood (ANA, anti-SSA, anti-SSB, RF), disease duration, age at onset, van Bijsterveld score, unstimulated whole sialometry or SS unrelated characteristics as dyspeptic symptoms and NSAID use did not show any statistically significant associations with *H. pylori* seropositivity. Subjects who had never smoked had a tendency to lower seroprevalence than former and current smokers (37% vs. 52% and 40% in EIA) without reaching statistical significance. (Data partly shown in Table III). When analysing the results from the initial 184 patients no further statistical significances appeared.

Discussion

Our study does not support a major role of *H. pylori* in the development of primary SS in Swedish patients as the patient group on the whole had neither higher prevalence nor higher antibody levels compared with three different control groups. No association between seropositivity for *H. pylori* and SS related disease characteristics could be detected. During recent years conflicting results regarding the prevalence of *H. pylori* infection in SS patients have been published (12,13,16). Our study of 164 primary SS patients is the largest one by now and includes a sufficient number of patients to allow the analysis of different subgroups. Multiple control groups have been used to enhance the validity of the study. The results are in accordance with another northern European study (12) but in contrast to the conclusions of two smaller Italian and Japanese studies (13, 16).

How should we explain these discrepancies? The *H. pylori* prevalence in southern European populations including Italy is higher than in Sweden (5, 6) and different antigenic properties have been shown in *H. pylori* strains from different European countries (22). The age distribution in the studied groups is very important, as higher age is clearly associated with higher prevalence in epidemiological studies. The other studies, especially the one from Japan, may be too small in size to draw reliable conclusions about prevalence differences. Other factors that may contribute to the conflicting results include different genetic backgrounds in different SS populations. Other studies have found different HLA class II associations in Caucasoid, Chinese and Japanese patients with SS (23), but significant differences between European populations have not been described.

A methodological concern in our study is the lack of association between age and *H. pylori* seropositivity in our prospectively collected control group of orthopaedic patients which is in contrast to all other epidemiological studies including our own patient group. One possible explanation for this might be the higher rate of immigrants in the younger age strata of this control group. The comparison with two additional control groups, one contemporary and one historic (6) gives strong support to our conclusion that the frequency of *H. pylori* infection in patients with primary SS is the same as in

controls. It also illustrates the importance of having multiple control groups in case control studies.

Despite the absence of difference in seroprevalence between cases and controls it is still conceivable that *H. pylori* infection may be of importance in smaller subgroups of patients with SS. Apart from an increased frequency of gastritis (12), these patients are also characterised by an association with MNHL (2) and primary biliary cirrhosis (PBC) (24, 25), a disease which by some but not all authors has been suggested to be associated with *H. pylori* infection (26, 27). Another intriguing observation in patients with SS is that of abnormal distribution of Lewis blood types (28). These blood group determinants are found on human gastric epithelium, on glandular tissue and in saliva. There is molecular mimicry between these epitopes and lipopolysaccharides of *H. pylori* giving rise to the production of cross-reacting (auto)-antibodies in patients developing chronic gastritis (29).

To evaluate whether antibodies towards *H. pylori* in our patient group were associated with gastritis or possibly development of MALT lymphoma, would have required gastroscopy with histological examination, which was beyond the scope of the present study. Nevertheless our study strongly supports that *H. pylori* infection is not a major etiological factor in SS.

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