Journal of Pre-Clinical and Clinical Research, Vol 1, No 1, 068-073
www.jpccr.eu

ORIGINAL ARTICLE

Helicobacter pylori and rheumatoid arthritis

Lykke B. Graff[1], Leif P. Andersen[2], Anne Gernow[3], Annie Bremmelgaard[4], Olaf Bonnevie*, Stig Bondesen[5], Bente Danneskiold-Samsøe[1], Else M. Bartels[1,6]

[1] *The Parker Institute, Frederiksberg Hospital, Denmark*
[2] *Frederiksberg Hospital, Department of Clinical Microbiology, Rigshospitalet, Denmark*
[3] *Department of Pathology, Denmark*
[4] *Department of Clinical Microbiology , Denmark*
[5] *Department of Gastroenterology, Denmark*
[6] *Copenhagen University Library, Section for Science and Medicine, Denmark*
* *Deceased*

Abstract: The aim of the research was to evaluate a possible relationship between *Helicobacter pylori* (*H. pylori*) and rheumatoid arthritis. The presence of and sero-prevalence of IgG antibodies to *H. pylori* were determined in 59 Danish rheumatoid-arthritis patients. The sero-prevalence was compared to the prevalence of controls and the background population. *H. pylori* in infected and uninfected patients were compared with regards to clinical and demographic characteristics, and the extent of their inflammatory disease activity. *H. pylori* was found in 18 patients (31%). The sero-prevalence of *H. pylori* was 32%, non-significant to the prevalence of controls (36%), and of the background population (26%). *H. pylori*/HLO positive and *H. pylori*/HLO negative patients were similar with regards to clinical and demographic characteristics and inflammatory disease activity. Our results did not show an aetiologic relationship between *H. pylori* and rheumatoid arthritis, but further studies are needed to discard the hypothesis completely.

Key words: *Helicobacter pylori*, rheumatoid arthritis, disease activity

INTRODUCTION

The aetiology of rheumatoid arthritis is still unknown, and the pathogenesis only partially understood. A frequently suggested idea is that rheumatoid arthritis, being an inflammatory disease, might be infective in origin [1-13].

An involvement of the gut in the aetiologic pathogenesis of rheumatoid arthritis has also been proposed [14, 15] and reasons for and against this are manifold. The gut is a highly evolved lymphoid organ, and due to the immense surface area, a very high proportion of the organism's contact with micro-organisms takes place in the gut. Indeed, reactive arthritis, a self-limited oligo-arthritis, is often a consequence of intestinal infections such as *Salmonella, Yersinia, Shigella,* and *Campylobacter* [16-19]. Intestinal bacteria are able to act as potential aetio-pathic candidates for inflammatory diseases through a transfer of antigenes, toxins or other structural bacterial components, i.e. peptido-glycans from bacteria cell walls, over the intestinal membrane [15, 20].

Warren and Marshall were the first to describe the presence of *Helicobacter pylori* (*H. pylori*), a Gram-negative curved and motile bacteria, in biopsy specimens from patients with gastritis [21]. During recent decades, *H. pylori* has been shown to be the major cause of gastritis and gastro-duodenal ulcers [22, 23]. *H. pylori* can therefore be identified in 70-90% of all incidences of gastritis and gastro-duodenal ulcers [24]. As the incidence of upper gastro-intestinal tract lesions is significantly higher in patients with rheumatoid arthritis [25-28], and a microbiological aetiology to rheumatoid arthritis is broadly considered, a relationship between *H. pylori* and rheumatoid arthritis should be a strong possibility.

Only a few studies have researched a possible connection between *H. pylori* and rheumatoid arthritis [29, 30], and the results are inconclusive. In order to obtain a more convincing answer to the question about a possible relationship between *H. pylori* and rheumatoid arthritis, we set up a study among the Danish population. This is the first study describing the prevalence of *H. pylori* in Danish rheumatoid-arthritis patients. The demographic characteristics, characteristics of the disease, as well as the inflammatory disease activity are described in relation to the *H. pylori* status of the patients.

METHODS

Study population. Outpatients from the Department of Rheumatology at the Frederiksberg Hospital in Copenhagen, Denmark, who met the criteria for rheumatoid arthritis – as determined by the American College of Rheumatism 1988 (ACR) [31] – were eligible for the study, for which there were two restrictions on this: 1) they had to be aged 18 or over, 2) they did not have any disease apart from rheumatoid arthritis.

A total of 194 patients diagnosed in the department with rheumatoid arthritis were identified using a computer search. Of these, 49 patients were not eligible because of age, concurrent disease, or because they no longer fulfilled the ACR criteria; another 94 eligible patients declined to participate.

A total of 51 outpatients consented to participate in the study and a further 8 patients reported voluntarily or at the suggestion of their general practitioner. Consequently, 59 patients – 47 women and 12 men, median age 60 years (range 19-80 years), and median disease duration 10 years (range 1-42 years), were included in the study. Patient characteristics

Corresponding author: Dr. Bente Danneskiold-Samsøe, Parker Instituttet, Frederiksberg Hospital, Nordre Fasanvej 57, 2000 Frederiksberg, Denmark.
E-mail: Danneskiold@fh.hosp.dk

Received: 23 April 2007; accepted: 30 June 2007

Table 1 *H. pylori*-infection related to gender ($p = 0.35$, Chi square test)

	Men	Women	Total
H. pylori/HLO positive Median age 59 years (36-79 years)	5 (45%)	13 (28%)	18 (31%)
H. pylori/HLO negative Median age 61 years (19-80 years)	7 (55%)	34 (71%)	41 (69%)
Total	12	47	59

Table 2 *H. pylori*-serology related to gender ($p = 0.26$, Chi square test)

	Men	Women	Total
Seropositive Median age 61 years (36-80 years)	6 (50%)	12 (25.5%)	18 (30.5%)
Borderline seropositive Median age 63 years (39-79 years)	2 (17%)	12 (25.5.%)	14 (23.7%)
Seronegative Median age 58 years (19-74 years)	4 (33%)	23 (49%)	27 (45.8%)
Total	12	47	59

Table 3 *H. pylori* serology related to *H. pylori*/HLO-status ($p < 0.001$, Chi square test)

	Seropositive	Borderline seropositive	Seronegative
H. pylori/HLO positive (n=18)	13	5	0
H. pylori/HLO negative (n=41)	5	9	27

Table 4 Sero-prevalence of *H. pylori* in patients with rheumatoid arthritis compared to age- and sex-matched controls and the estimated sero-prevalence of the Danish population (χ^2-test)

H. pylori seropositive (LMW-IgG > 400 EU)					
Patients (n=47)	Controls (n=47)	*p*	Patients (n=59)	Estimated Prevalence	*p*
15 (32%)	17 (36%)	0.66	31%	26%	0.54

are shown in Tables 1-6. Serum samples from 47 age- and sex-matched healthy controls were obtained from a serum bank.

Biopsies and histology. All patients had an upper endoscopy performed using an Olympus GIF Type 100 gastroscope, were pre-medicated with topical Xylocaine spray and offered intravenously titrated doses of Diazepam during biopsy sampling. A total of 10 biopsy specimens were taken at random from the antrum (6 biopsy specimens) and the corpus (4 biopsy specimens) for histological and microbiological examinations. For a period of 4 weeks prior to the endoscopy, the patients were prohibited from using ulcer medication or antibiotics.

Four biopsy specimens for histological examination (2 from the antrum and 2 from the corpus) were immediately formalin-fixed and routinely processed. Sections of paraffin-embedded biopsy specimens were stained with haematoxylin-eosin, van Gieson/Alcian-Blue and periodic-acid Schiff for morphological examination and detection of *H. pylori*-like organisms (HLO). Inflammation, gland atrophy, intestinal metaplasia and the presence of HLO were evaluated according to the Sydney System [32]. In addition, the biopsy specimens were immunohistochemically stained using polyclonal antibodies to *H. pylori* (DAKO, Copenhagen, Denmark) for further HLO detection.

Table 5 Patients' demographic and disease characteristics in relation to *H. pylori*/H. pylori like organisms (HLO) status (Mann Whitney's Rank Sum Test, χ^2-test and Fisher's Exact Test)

	All patients N=59	*H. pylori*/ /HLO positive patients N=18	*H. pylori*/ /HLO negative patients N=41	p
Age, years Median (range)	60 (19-80)	59 (36-79)	61 (19-80)	0.64
Disease duration, years Median (range)	10 (1-42)	12.5 (1-35)	8 (1–42)	0.36
Women, number (%)	47 (80%)	13 (72%)	34 (83%)	
Men, number (%)	12 (20%)	5 (28%)	7 (17%)	0.35
Patients with erosive arthritis, number (%)	44 (75%)	12 (67%)	32 (78%)	0.36
Patients with seropositive arthritis, number (%)	49 (83 %)	15 (83 %)	34 (83 %)	0.97
Patients with upper gastro--intestinal complaints (dyspepsia and epigastric pain, number (%)	33 (56%)	22 (54%)	11 (61%)	0.60
Patients with dyspepsia, number (%)	14 (24%)	6 (14%)	8 (44%)	0.04
Patients in NSAID/ASA--treatment*, number (%)	33 (56 %)	7 (39 %)	26 (63 %)	0.08
Patients in prednisolone treatment, number (%)	18 (31 %)	4 (22 %)	14 (34 %)	0.36
Patients in DMARD-treatment, number (%)	34 (57 %)	8 (44 %)	26 (63%)	0.18
Patients in gold compound treatment, number (%)	4 (7%)	1 (6%)	3 (7%)	0.80
Patients smoking, number (%)	20 (34%)	8 (44%)	12 (29%)	0.26
Patients with daily alcohol consumption, number (%)	2 (4%)	1 (6%)	1 (3%)	

* One patient received both NSAID and ASA, # statistical analysis not possible.

Table 6 Clinical and laboratory parameters of inflammatory disease activity in relation to H. *pylori*/HLO status (Mann Whitney's Rank Sum Test).

	All patients N = 59	*H. pylori*/ /HLO positive patients N= 18	*H. pylori*/ /HLO negative patients N=41	p
Morning stiffness (min) median (range)	23 (0-266)	60 (0-249)	0.5 (0-266)	0.04
Health assessment questionnaire HAQ – score (0-3) median (range)	1.125 (0-2.50)	1.125 (0-2)	1.125 (0-2.50)	0.81
Visual analogue scale VAS (0-100) median (range)	35 (0-86)	33.5 (0-78)	37 (9-86)	0.44
Swollen joints (number) median (range)	3 (0-16)	4 (0-13)	2 (0-16)	0.18
Tender joints (number) median (range)	5 (0-30)	9.5 (0-23)	5 (0-30)	0.57
Erythrocyte sedimentation rate – ESR (AE) median (range)	14 (1-90)	12 (3-47)	16 (1-90)	0.33
C-reactive protein CRP (nmol/l) median (range)	158 (48-1875)	66 (48-748)	242 (48-1875)	0.06

After completion of the endoscopy, 3 biopsy specimens for microbiological examination (1 from the antrum and 2 from the corpus), were immediately transported in sterile saline to chocolate agar plates and plated within 3 hours. The plates were incubated under micro-aerobic condition at 37°C for up to 5 days. *H. pylori* was identified as urease, oxidase, and catalase positive Gram-negative, motile, curved rods from small translucent colonies.

Serum sample *H. pylori* test. Serum samples from the patients and from the healthy controls were examined for IgG-antibodies to *H. pylori* by an enzyme-linked immuno-sorbent assay (ELISA), using a low molecular weight (LMW) antigen prepared by filtration of sonicated bacteria.

A selected strain of *H. pylori* (CH 20429) was grown for 24-48 hours, harvested in phosphate-buffered saline (pH 7.4), and centrifugated at 7,000*g* for 10 minutes. A suspension of 0.5g wet weight *H. pylori* per ml of phosphate-buffered saline was ultra-sonicated and filtrated through filters with molecular weight cut-offs at 100 kD and 30 kD. The filtrated preparation was dialysed against phosphate-buffered saline for 48 hours with the cut-off at 12 kD. The final antigen preparation consisted mainly of antigens with molecular weights from 15 kD – 30 kD.

Micro-titer plates were coated overnight with the antigen preparation and washed 5 times. The sera, diluted 1:500, were added to the plates which were incubated for 1 hour, washed 5 times, and then incubated for 1 hour with rabbit antibody to human IgG (DAKO, Copenhagen, Denmark) combined with horseradish peroxidase in a 1:2.000 dilution. The plates were washed 5 times and enzyme activity detected using the ortho-phenyle-diamine dihydrochloric acid-hydrogen peroxidase system. After 30 minutes, the chromogenic reaction was stopped with sulphuric acid, and the absorbance read in a photometer at 492 nm. The amount of antibody was expressed in ELISA units (EU), which are the absorbance values corrected for plate-to-plate and day-to-day variation. From previous validation of this procedure, ELISA seropositivity was defined as LMW-IgG values ≥ 400EU, and seronegativity as LMW-IgG values ≤ 100 EU [33]. Intermediate values were reported as borderline seropositive.

Disease activity scores. The inflammatory disease activity referred to the extent of current over-all inflammation and was measured by assessing the number of swollen and tender joints, duration of morning stiffness, level of functional disability (health assessment questionnaire, HAQ) [34], level of pain (visual analogue scale, VAS), erythrocyte sedimentation rate (ESR) and the level of C-reactive protein (CRP). The joint count system included 28 joints [35]. The joints were primarily evaluated for tenderness and swelling where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The joint score was ultimately reported in a binary manner as recommended by ACR [36].

Ethics. The study was conducted in accordance with the Helsinki II Declaration. All patients received oral and written information before initiation of the study, and all gave written consent before participation. The study was approved by the local Ethical Committee for the municipalities of Copenhagen and Frederiksberg.

Statistical analyses. Data were not distributed normally and statistical analyses conducted by Chi square test, Mann Whitney's rank sum test, and Spearman's correlation coefficient, as appropriate. Multiple comparisons were adjusted by the Bonferroni method. The level of significance was set at $p < 0.05$.

RESULTS

H. pylori was identified in 15 patients by culture (*H. pylori*) and by histological detection of *H. pylori*-like organisms (HLO). Additionally, HLO was detected in 3 patients who were culture-negative. All 3 patients had endoscopy performed on the same day. One patient agreed to a new endoscopy, and *H. pylori* was then identified by culture as well as by the histological examination; the other 2 patients had high LMW IgG-levels to *H. pylori,* supporting the histological evidence of a current *H. pylori* infection. In 41 patients, *H. pylori* was neither identified by histology nor by culture. Overall, *H. pylori* was identified in 18 patients (31%) – 13 women and 5 men, median age 59 years (36-79 years), and median disease duration of 13 years (1-35 years) (Table 1).

H. pylori-seropositivity was found in 18 patients (31%) – 12 women and 6 men, while 14 patients (23.7%) – 12 women and 2 men, were borderline seropositive, and 27 patients (45.8%) – 23 women and 4 men, were seronegative (Table 2). *H. pylori* infection was found to be unrelated to gender, although insignificantly more men than women were found to be *H. pylori*/HLO positive ($p = 0.35$) and *H. pylori* seropositive ($p = 0.35$).

In 13 cases, the *H. pylori*/HLO positive patients were found to be *H. pylori* seropositive, and in 5 cases *H. pylori* borderline seropositive. In addition to the *H. pylori*/HLO positive patients, 5 *H. pylori*/HLO negative patients were found to be *H. pylori* seropositive, and 9 – *H. pylori* borderline seropositive. The *H. pylori* serological status was therefore significantly associated with the *H. pylori*/HLO status ($p < 0.001$) (Table 3).

When comparing the *H. pylori* seropositivity in patients (32%) with that of an age- and gender-matched control group (36%), no significant difference was found ($p = 0.66$) (Table 4); nor did the sero-prevalence in patients (31%) differ from the estimated sero-prevalence in the Danish population (26%) ($p = 0.54$), (Table 4). Only 4 patients were treated with gold compounds, and were evenly distributed in relation to the *H. pylori*/HLO status. We have therefore decided not to take gold-compound treatment into account in this study.

Table 5 shows the patients' demographic and disease characteristics in relation to the *H. pylori*/HLO status. No significant differences were found between *H. pylori*/HLO positive and *H. pylori*/HLO negative patients with regard to age, disease duration, gender distribution, number of patients with erosive or IgM-rheuma-factor seropositive arthritis, number of NSAID-users, or number of patients treated with gold compounds or other DMARDs, including prednisolone. There was no significant relationship between patients with upper gastro-intestinal complaints and the occurrence of *H. pylori*, or with the effect of alcohol and/or smoking habits on the occurrence of *H. pylori*.

Significantly more *H.pylori*/HLO-positive patients showed dyspeptic symptoms (p=0.04) than those who were *H.pylori*/HLO-negative.

Table 6 and Figure 1 show the data for the clinical and laboratory parameters of the inflammatory disease activity. The duration of morning stiffness was significantly longer for

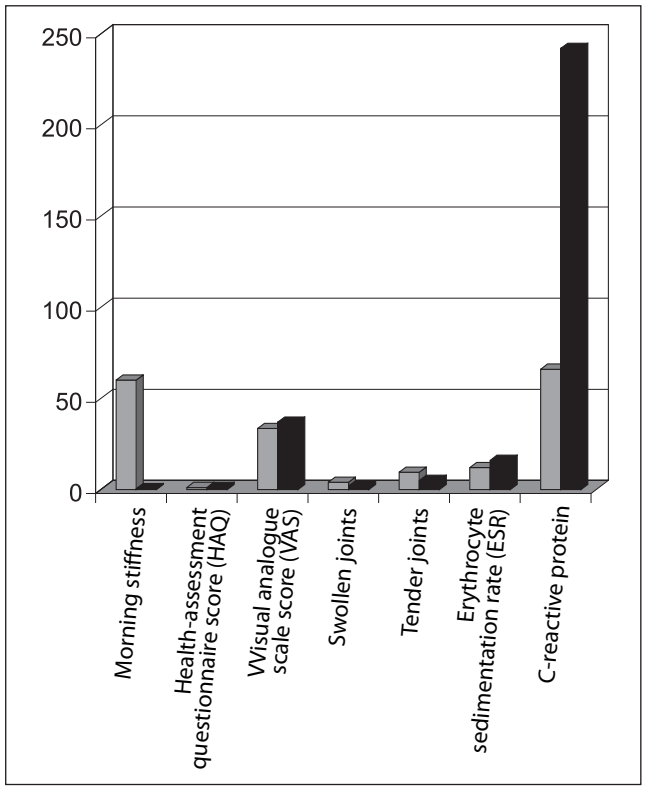

Figure 1 Clinical and laboratory parameters of inflammatory disease activity in *H. pylori* positive (grey) and *H. pylori*/HLO negative (black) patients with rheumatoid arthritis.

H. pylori/HLO-positive patients ($p = 0.04$) than for those who were *H.pylori*/HLO-negative, but no other significant differences were observed. This difference also proved to be insignificant after adjustment for multiple comparisons ($p = 0.28$).

The correlation coefficients between the LMW-IgG antibody level to *H. pylori* and the clinical and the laboratory parameters of the inflammatory disease activity are shown in Table 7 (all patients) and Table 8 (*H.pylori*/HLO positive patients). No significant correlations were found in the patient group taken as a whole, or specifically for the *H. pylori*/HLO positive patients.

DISCUSSION

Patients with rheumatoid arthritis have an increased risk of developing gastrointestinal tract lesions [25-27]. These conditions have proved to be predominantly caused by *H. pylori* [22] and therefore a higher prevalence of *H. pylori* in patients with rheumatoid arthritis could be expected. However, in previous studies the prevalence of *H. pylori* in NSAID-treated patients with rheumatoid arthritis has been reported to vary from 22%-68%, which is comparable to values in controls and the background population (Table 9). In concordance with these studies, we found comparable sero-prevalences of *H. pylori* in the patients and in age- and gender-matched controls (32% vs. 36%), and with the estimated sero-prevalence in the Danish population (31% vs. 26%) (Table 9). This might be interpreted as an indication of a lack of association between *H. pylori* and rheumatoid arthritis. On the other hand, the prevalence of gastritis and peptic-ulcer disease is markedly lower than the prevalence of *H. pylori,* while other intrinsic/extrinsic factors must be considerable. This may also be the case with rheumatoid arthritis. Since certain class II major histo-compatibility-complex (MHC II) epitopes are known to be strongly associated with rheumatoid arthritis [37], and since *H. pylori* probably acts through an activation of class II MHC molecules [38], a possible association might be restricted to patients with a particular class of II MHC type.

Gold salts are known to have anti-microbial activity, and *in vitro* studies of gold sodium thiomalate (GST) have indicated a 30-times stronger bactericidal effect against *H. pylori* cultures of GST, compared to bismuth [40]. Gold compounds are used as disease modifying anti-rheumatic drugs (DMARDs) in rheumatoid arthritis, and the prevalence of *H. pylori* in patients administered with gold compounds has also been evaluated (Table 10). A significantly lower prevalence of *H. pylori* in patients treated with gold compounds than those treated with with sulfazalasin has been reported [40], while others have failed to find a difference in prevalence [41, 42]. In our study, only 4 patients received gold compounds, and these patients were equally represented in the 2 groups of patients.

Rheumatoid arthritis predominates in women, with a female to male ratio of 2:1 to 4:1. The IgG and IgM antibody level to *H. pylori* has been described as increased in women compared to age-matched men [43], and a relationship between *H. pylori* and rheumatoid arthritis, according to this finding, could possibly be gender-related. However, our results do not confirm this hypothesis as we found more *H. pylori*/HLO positive and *H. pylori* seropositive men than women, although this was statistically insignificant (Table 1 and Table 2).

An association between *H. pylori* and rheumatoid arthritis could possibly be reflected in the patients' demographic and disease characteristics, but when comparing *H. pylori*/HLO positive with *H. pylori*/HLO negative patients, no significant

Table 7 Correlation between LMW-IgG antibody level to *H. pylori* and clinical and laboratory parameters of inflammatory disease activity in patients with rheumatoid arthritis (Spearman's correlation coefficient). N=59

	Morning-stiffness	HAQ -score	VAS -score	ESR	CRP	Swollen joints	Tender joints
LMW-IgG	rho=0,20 *p* = 0,17	rho =-0,17 *p* = 0,23	rho = -0,20 *p* = 0,21	rho = -0,10 *p* = 0,47	rho = -0,24 *p* = 0,08	rho = -0,224 *p* = 0,09	rho = -0,174 *p* = 0,19

Table 8 Correlation between LMW-IgG antibody level to *H. pylori* and clinical and laboratory parameters of inflammatory disease activity in *H. pylori*/HLO positive patients with rheumatoid arthritis (Spearman's correlation coefficient).

	Morning-stiffness	HAQ -score	VAS -score	ESR	CRP	Swollen joints	Tender joints
LMW-IgG	rho =-0,05 *p* =0,85	rho = -0,42 *p* = 0,11	rho = 0,35 *p* = 0,30	rho = -0,47 *p* = 0,06	rho = -0,39 *p* = 0,13	rho = 0,04 *p* = 0,88	Rho = 0,08 *p* = 0,77

Table 9 Previously reported *H. pylori* prevalence in NSAID-treated rheumatoid arthritis patients

Reference	Number of patients	Age (range)	Sex M/F	Biopsy specimens	H. pylori prevalence
Upadhyay R 1988	52	53 (26-72)	18/34	2 antrum	50 %2
Caselli M 1989	85	53 (27-82)	41/44	3 antrum	31 %2
Jones STM 1991	68	61 (19-83)	6/62	-	43 %4
Loeb DS 1992	50	65 (43-77)	12/38	6 antrum	22 % 1,2,3
Taha AS 1992a	174	59 (51-65)	29/145	3 antrum	32 % 1,2
Taha AS 1992b	31	55 *	5/26	* antrum	55 %1,2
Gubbins GP 1992	132	59 *	33/99	-	41%4
Goggin PM 1993	52	55 (24-75)	14/38	3 antrum	50%2,3
Henriksson K 1993	42	56 (50-66)	11/31	1 antrum	40 %1,2
Janssen M 1994	81	64 (20-85)	29/52	* antrum	68 %4

Prevalence determined by: 1) culture, 2) histology, 3) rapid urease test, 4) serology.
* not described – not relevant.

Table 10 Previous reported *H. pylori* prevalence in DMARD-treated rheumatoid arthritis patients

Reference	Number of patients	Age	Sex M/F	Biopsy specimens	*H. pylori* prevalence	Gold	Sulfasalazin	NSAID	Chloroquine
Gubbins GP [42]	132	59	33/92	-	41%[4]	yes (n=39)	*	Yes	*
					41%[4]	never (n=93)	*	Yes	*
Taha AS [41]	27	60	7/20	* antrum	33%[1,2]**	Yes	No	yes	no
	27	51	12/15		78%[1,2]	No	Yes	yes	no
Janssen M [43]	42	56	22/20	-	40%[4]	Yes	No	90%	no
	58	56	20/38		65%[4]	No	No	81%	yes

Prevalence determined by: 1) culture, 2) histology, 3) rapid urease test, 4) serology,
* not described, - not relevant,** significant

differences were found with regard to age, disease duration, gender distribution, erosive arthritis, IgM-rheuma-factor positive arthritis, NSAID and DMARD treatment, smoking and alcohol habits (Table 5).

It is interesting, however, that even though no specific symptoms have been reported as being related to the *H. pylori* infection, more *H. pylori*/HLO positive patients had dyspeptic symptoms.

When comparing the clinical and laboratory parameters for the inflammatory disease activity, the duration of morning stiffness was found to be significantly longer in *H. pylori*/HLO positive than in *H. pylori*/HLO negative patients (Table 6). Morning stiffness is thought to be caused by nocturnal accumulation of fluid in the joints. It cannot be ruled out that *H. pylori* has some influence on this accumulation, but it is more plausible that the observed difference is due to a statistical incident, since the difference was insignificant after adjustment for multiple comparisons.

An aetio-patho-genetical relationship between *H. pylori* and rheumatoid arthritis could possibly be reflected in the correlation between the serum antibody-level to *H. pylori* and the inflammatory disease activity. This is unlikely, however,since Showji et al. did not find a significant correlation between the serum IgG-antibody level to *H. pylori* and serum markers of inflammation (myeloid calcium-binding proteins) in patients with connective tissue diseases, including patients with rheumatoid arthritis [30]. Our results supported those of Showji in not revealing any significant correlation between the clinical and laboratory parameters of the inflammatory disease activity and the LMW-IgG antibody-level to *H. pylori*. However, both studies only included immunoglobulin class G, and it therefore remains to be shown whether there exists a correlation between other immunoglobulin classes or subclasses and the inflammatory disease activity of rheumatoid arthritis.

CONCLUSION

The sero-prevalence of *H. pylori* in patients with rheumatoid arthritis was found to be comparable to the estimated sero-prevalence in the Danish population (p=0.54). Furthermore, this sero-prevalence did not differ from the sero-prevalence in age- and gender-matched controls (p=0.66). *H. pylori* infection did not relate to any patient characteristic or inflammatory-disease activity parameter.

Our results show no direct relationship between rheumatoid arthritis and the presence of *H. pylori* in a group of Danish patients with rheumatoid arthritis.

The question remains: Does *H. pylori* have a secondary effect on the gastro-duodenal injuries seen frequently in patients with rheumatoid arthritis? This effect may either be in conjunction with the medical treatment received by these patients, or an independent effect.

It is possible that a wider ranging study might show a correlation between morning stiffness and other disease-related parameters and presence of *H. pylori*.

ACKNOWLEDGEMENTS

This study was supported by Grants from the OAK Foundation, Lykfeldt's Foundation, H.S. Research Foundation, William Nielsen's Foundation, the Danish Medic's Insurance, and Codan Insurance. The authors wish to thank laboratory technicians Hanne Kubert and Anne Skov, and Karen-Lisbeth Jensen, RN, for their competent assistance.

REFERENCES

1. Phillips PE: Infectious agents in the pathogenesis of rheumatoid arthritis. *Seminars in Arthr and Rheum* 1986, **16**, 1-10.
2. Bacon PA, Tunn EJ: Infection and arthritis. *Q J Med* 1986, **61(234)**, 897-899.
3. Espinoza LR: Rheumatoid arthritis: etiopathogenetic considerations. *Clin Lab Med* 1986, **6(1)**, 27-40.
4. Atkin S, Walker D, Mander M, Malcolm A, Dick WC: Observations on the causes of rheumatoid arthritis. *Br J Rheumatol* 1988, **27** (Suppl. 2), 173-175.
5. Phillips PE: Evidence implicating infectious agents in rheumatoid arthritis and juvenile rheumatoid arthritis. *Clin and Experim Rheum* 1988, **6**, 87-94.
6. Rodriguez MA, Williams RC: Infection and rheumatic diseases. *Clin Exp Rheumatol* 1989, **7(1)**, 91-97.
7. Wilder RL, Crofford LJ: Do infectious agents cause rheumatoid arthritis? *Clin Orthop* 1991, **265**, 36-41.
8. Inman RD: Infectious etiology of rheumatoid arthritis. *Rheum Dis Clin North Am* 1991, **17(4)**, 859-870.
9. Burmester GR: Hit and run or permanent hit? Is there evidence for a microbiological cause of rheumatoid arthritis? *J Rheumatol* 1991, **18(10)**, 1443-1447.
10. Ford DK: The microbiological causes of rheumatoid arthritis. *J Rheumatol* 1991, **18(10)**, 1441-1442.
11. Rook GA, Stanford JL: Slow bacterial infections or autoimmunity? *Immunol Today* 1992, **13(5)**, 160-164.
12. Rook GA, Lydyard PM, Stanford JL. A reappraisal of the evidence that rheumatoid arthritis and several other idiopathic diseases are slow bacterial infections. *Ann Rheum Dis* 1993, **52** (Suppl.1), 30-38.
13. Gaston JS: The role of infection in inflammatory arthritis. *QJM* 1994, **87(11)**, 647-651.
14. Greenstein AJ, Janowitz HD, Sachar DB: The extra-intestinal complications of Crohn's disease and ulcerative colitis: A study of 700 patients. *Medicine (Baltimore)* 1976, **55(5)**, 401-412.
15. Hazenberg MP, Klasen IS, Kool J, Ruseler-van Embden JG, Severijnen AJ: Are intestinal bacteria involved in the etiology of rheumatoid arthritis? Review article. *APMIS* 1992, **100(1)**, 1-9.
16. Granfors K, Jalkanen S, von Essen R, Lahesmaa-Rantala R, Isomaki O, Pekkola-Heino K et al.: Yersinia antigens in synovial-fluid cells from patients with reactive arthritis. *N Engl J Med* 1989, **320(4)**, 216-221.
17. Granfors K, Jalkanen S, Lindberg AA, Maki-Ikola O, von Essen R, Lahesmaa-Rantala R et al.: Salmonella lipopolysaccharide in synovial cells from patients with reactive arthritis. *Lancet* 1990, **335(8691)**, 685-688.
18. Granfors K, Jalkanen S, Toivanen P, Koski J, Lindberg AA.: Bacterial lipopolysaccharide in synovial fluid cells in Shigella triggered reactive arthritis. *J Rheumatol* 1992, **19(3)**, 500.
19. Toivanen A: Infection and arthritis. *Ann Med* 1994, **26(4)**, 245-248.
20. Midtvedt T: Intestinal bacteria and rheumatic disease. *Scand J Rheumatol* 1987, **64**, 49-54.
21. Warren JR, Marshall BJ: Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983, **1**, 1273.
22. Blaser MJ: Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. *J Infect Dis* 1990, **161(4)**, 626-633.
23. Axon AT: Treatment of Helicobacter pylori: An overview. *Aliment Pharmacol Ther* 2000, **14** (Suppl 3), 1-6.
24. Andersen LP, Nielsen H: Peptic ulcer: an infectious disease? *Ann Med* 1993, **25(6)**, 563-568.
25. Malone DE, McCormick PA, Daly L, Jones B, Long A, Bresnihan B et al.: Peptic ulcer in rheumatoid arthritis-intrinsic or related to drug therapy? *Br J Rheumatol* 1986, **25(4)**, 342-344.
26. Janssen M, Dijkmans BA, Lamers CB: Upper gastrointestinal manifestations in rheumatoid arthritis patients: Intrinsic or extrinsic pathogenesis *Scan J of Gastroenterology* 1990, **25** (Suppl.178), 79-84.
27. Janssen M, Dijkmans BA, van der Sluys FA, et al: Upper gastrointestinal complaints and complications in chronic rheumatic patients in comparison with other chronic diseases. *British J Rheumatol* 1992, **31**, 747-752.
28. Barr M, Buckley M, O'Morain C: Review article: Non-steroidal anti-inflammatory drugs and Helicobacter pylori. *Aliment Pharmacol Ther* 2000, **14** (Suppl 3), 43-47.
29. Showji Y, Nozawa R, Sato K, Suzuki H: Seroprevalence of Helicobacter pylori infection in patients with connective tissue diseases. *Microbiol Immunol* 1996, **40(7)**, 499-503.
30. Zentilin P, Savarino V, Garnero A, Accardo S, Seriolo B: Is Helicobacter pylori infection a risk factor for disease severity in rheumatoid arthritis? *Gastroenterology* 1999, **116(2)**, 503-504.
31. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988, **31(3)**, 315-324.
32. Price AB: The Sydney System: Histological division. *J Gastroenterol Hepatol* 1991, **6(3)**, 209-222.
33. Andersen LP, Espersen F, Souckova A, Sedlackova M, Soucek A: Isolation and preliminary evaluation of a low-molecular-mass antigen preparation for improved detection of Helicobacter pylori immunoglobulin G antibodies. *Clin Diagn Lab Immunol* 1995, **2(2)**, 156-159.
34. Fries JF, Spitz P, Kraines RG, Holman HR: Measurement of patient outcome in arthritis. *Arthritis Rheum* 1980, **23(2)**, 137-145.
35. Fuchs HA, Pincus T: Reduced joint counts in controlled clinical trials in rheumatoid arthritis. *Arthritis Rheum* 1994, **37(4)**, 470-475.
36. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C: American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* 1995, **38(6)**, 727-735.
37. Winchester R: The molecular basis of susceptibility to rheumatoid arthritis. *Adv Immunol* 1994, **56**, 389-466.
38. Engstrand L, Scheynius A, Pahlson C: An increased number of gamma/delta T-cells and gastric epithelial cell expression of the groEL stress-protein homologue in Helicobacter pylori-associated chronic gastritis of the antrum. *Am J Gastroenterol* 1991, **86(8)**, 976-980.
39. Braga LL, Sarosiek J, Marshal B, McCallum RW: Gold – a new potential drug for the treatment of *Helicobacter pylori* infection. *Gastroenterology* 1990, **98**, A25.
40. Taha AS, Sturrock RD, Russell RI: Helicobacter pylori and peptic ulcers in rheumatoid arthritis patients receiving gold, sulfasalazine, and nonsteroidal anti-inflammatory drugs. *Am J Gastroenterol* 1992, **87(12)**, 1732-1735.
41. Gubbins GP, Schubert TT, Attanasio F, Lubetsky M, Perez-Perez GI, Blaser MJ: Helicobacter pylori seroprevalence in patients with rheumatoid arthritis: Effect of nonsteroidal anti-inflammatory drugs and gold compounds. *Am J Med* 1992, **93(4)**, 412-418.
42. Janssen M, Dijkmans BA, Vandenbroucke JP, van Duijn W, Pena AS, Lamers CB: Decreased level of antibodies against Helicobacter pylori in patients with rheumatoid arthritis receiving intramuscular gold. Ann Rheum Dis 1992, 51(9), 1036-1038.
43. Andersen LP, Rosenstock SJ, Bonnevie O, Jorgensen T: Seroprevalence of immunoglobulin G, M, and A antibodies to Helicobacter pylori in an unselected Danish population. Am J Epidemiol 1996, 143(11), 1157-1164.