ORIGINAL ARTICLE

Does infection with *Chlamydia pneumoniae* and/or *Helicobacter pylori* increase the expression of endothelial cell adhesion molecules in humans?

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Objective To investigate if *Chlamydia pneumoniae* and/or *Helicobacter pylori* seropositivity is associated with elevated levels of soluble endothelial cell adhesion molecules (sCAMs) as markers of atherosclerotic activity.

Methods Immunoglobulin A (IgA) and IgG antibodies to the two bacteria, soluble intercellular cell adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and E-selectin were measured in coronary heart disease (CHD) patients ($n = 193$) and age- and sex-matched controls ($n = 193$). Two different serological methods were used for the detection of *Chlamydia* antibodies: Labsystems microimmunofluorescence to detect species-specific *C. pneumoniae* antibodies and Medac's recombinant enzyme-linked immunosorbent assay to detect genus-specific lipopolysaccharide antibodies.

Results The concentrations of sICAM-1 and E-selectin were higher in CHD patients with positive vs. negative *Chlamydia* lipopolysaccharide IgA ($P = 0.044$ for both). *H. pylori* antibodies alone did not predict raised levels of sCAMs, but in CHD patients sICAM-1 was increased with IgA seropositivity to both bacteria compared to double seronegativity ($P = 0.034$). Concentrations of sVCAM-1 were elevated in CHD patients with double IgA seropositivity compared to those with *Chlamydia* lipopolysaccharide IgA seropositivity alone ($P = 0.018$).

Conclusion Our results may indicate that *C. pneumoniae* contributes to increased inflammation in CHD, and that this contribution is even more pronounced when present in combination with *H. pylori* IgA antibodies.

Keywords *Chlamydia pneumoniae*, *Helicobacter pylori*, vascular cell adhesion molecule, intercellular cell adhesion molecule, E-selectin, coronary heart disease, inflammation

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INTRODUCTION

Atherosclerosis is now generally accepted as an inflammatory disorder in the arterial wall [1]. Early stages in the development of atherosclerosis are characterized by subendothelial lipid accumulation and leucocyte adhesion to the endothelium resulting in infiltration of macrophages and T lymphocytes into the arterial intima [1]. Endothelial cell adhesion molecules play a central role in the adherence of leucocytes, and the activated endothelium has been shown to express various adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin [2,3]. Elevated levels of soluble adhesion molecules (sCAMs) have also been found in patients with coronary

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heart disease (CHD) and peripheral atherosclerosis [4–7] compared to healthy individuals.

The possible relationship between microbial agents and atherosclerosis remains controversial. Most seroepidemiological studies published have shown a higher prevalence of antibodies to *Chlamydia pneumoniae* and/or *Helicobacter pylori* among patients with CHD compared to healthy individuals [8–10], but there are also studies which do not show this [11,12]. There is a substantial amount of evidence for the presence of *C. pneumoniae* within atherosclerotic plaques [13–15], but its pathogenetic role is still uncertain.

Presuming that micro-organisms contribute to atherosclerosis, local as well as systemic pathways may be operative. Experimental studies have shown that *C. pneumoniae* can maintain infection in endothelial cells, macrophages and smooth muscle cells and induce foam cell formation [16–18]. Infected human aortic endothelial cells have been shown in vitro to express increased amounts of ICAM-1, VCAM-1 and E-selectin on the surface [19,20]. It is not settled, however, settled whether in vivo infection with *C. pneumoniae* leads to an increase in sCAMs in humans.

Although there have been a few positive reports [21], most investigators have failed to demonstrate the presence of *H. pylori* in the arterial wall [22,23]. However, this micro-organism's tendency to produce chronic infection and inflammation is well known from gastroenterology, and there is still a possibility of systemic influence causing vascular inflammation.

The aim of the present study was to investigate if seropositivity to *C. pneumoniae* and/or *H. pylori* is associated with increased levels of markers of vascular inflammation, possibly indicating that infection with these microbes contributes to the process of atherosclerosis. Levels of sVCAM-1, sICAM-1 and E-selectin were measured as markers of endothelial inflammation and were compared between seropositive and seronegative individuals. Both CHD patients and matched healthy controls were included and investigated separately. In the absence of a reference standard for measurements of *C. pneumoniae* antibodies, and knowing that the choice of method might influence the serological results [24], we included two fundamentally different methods for *C. pneumoniae* serology, one detecting species-specific antibodies [Labsystems microimmunofluorescence (MIF)] and another method detecting genus-specific lipopolysaccharide (LPS) antibodies [Medac's recombinant enzyme-linked immunosorbent assay (rELISA)].

METHODS

Study population

The study population comprised 193 patients with documented CHD and 193 age- and sex-matched healthy controls from the county of Vestfold, Norway. The CHD patients were included at a median of 16 days after an acute myocardial infarction (AMI) ($n = 74$), percutaneous transluminal coronary angioplasty ($n = 38$), or coronary artery bypass surgery ($n = 50$). AMI was defined as typical clinical symptoms (chest pain and/or dyspnoea) accompanied by typical electrocardiographic findings [development of pathological Q waves or ST deviations (non-Q)] and significant increase of cardiac enzymes [creatine kinase (CK) >250 U/L, and/or creatine kinase myocardial-specific isozyme CKMB >10 μg/L; World Health Organization criteria]. In addition, 31 patients in a chronic stage of CHD, with more than a 3 month lapse of time since their last acute event, were included. Of the CHD patients, 135 (70%) had a previous myocardial infarction in their history. None of the patients had severe heart failure.

Four hundred healthy controls were recruited from four working sites in Vestfold county, and for each CHD patient included in the study, one age- and sex-matched control subject was drawn from this control pool. This apparently healthy individual was then included after an interview and a clinical examination with a near-maximal bicycle electrocardiogram, if no symptoms or clinical evidence of atherosclerotic disease were present. We also aimed to match educational level, but there was a somewhat higher proportion of healthy individuals with academic education in the control group (43%) than in the CHD group (34%).

The study was approved by the regional ethics committee, and all patients and controls gave their informed, written consent to participate. Blood samples were drawn at inclusion in a fasting condition, and serum was prepared within 1 h and kept frozen at -70°C until analysis.

Laboratory methods

All blood samples were analysed using the following methods. *Chlamydia pneumoniae* MIF immunoglobulin A (IgA) and IgG (Labsystems, Helsinki,

Finland) is a species-specific test where *C. pneumoniae* elementary bodies are used as antigen. All samples were screened at a titre of 1:16, then evaluated at serial two-fold dilutions up to 1:128 for IgA and 1:512 for IgG. A positive IgA test was defined as IgA $\geq$ 32, while a positive IgG was defined as titre $\geq$64. The *Chlamydia* IgA and IgG rELISA (Medac, Hamburg, Germany) is a recombinant enzyme immunoassay for detection of the genus-specific LPS antibodies, in which, according to the manufacturer, seropositivity is defined as IgA $\geq$ 50 and IgG $\geq$ 100. The Pyloriset EIA-A III and EIA-G III (Orion Diagnostica, Espoo, Finland) is an enzyme immunoassay for the detection and measurement of *H. pylori* IgA and IgG, seropositivity is defined as $\geq$ 20 ul ml for both. Adhesion molecules were measured using the Human soluble ICAM-1 Parameter, the Human soluble VCAM-1 Parameter, and the Human soluble E-Selectin Parameter (all supplied by R&D Systems Europe, Abingdon, UK). Because Medac's rELISA test does not discriminate between the *Chlamydia* species, the patients who were either IgA or IgG positive in Medac's rELISA test were also tested by the Medac *Chlamydia trachomatis* pELISA IgA and IgG, which detects species-specific antibodies to *C. trachomatis*.

Statistical methods

Results were analyzed statistically using SPSS FOR WINDOWS, version 9.0. The Pearson χ^2 test was used to compare the number of seropositives in the two study groups. The independent *t*-test was used when comparing means of sCAMS between seropositive and seronegative individuals. Because there is no general agreement in the literature about what cut-off levels should be applied when performing MIF [25], we also compared seropositives and seronegatives at one cut-off level above and one below the chosen limit. To evaluate if *H. pylori* and/or *C. pneumoniae* antibodies were independent predictive factors for the sCAMs, seropositivity was introduced, together with established coronary risk factors, into a multiple linear regression model. Lipids and blood pressure were excluded as risk factors in the CHD population because of medication, but were included in multivariate analysis concerning the healthy controls alone.

In line with suggestions by Rothman [26], to avoid the chance of discarding biological rationale, we have not included corrections for multiple comparisons. In all the statistical analyses a two-tailed significance level <0.05 was regarded as statistically significant.

RESULTS

The baseline characteristics of the two study groups are given in Table 1. The mean age was 55 years (27–68), and 18% were women. Of the CHD patients, 176 (91%) were on statin treatment at the time of sampling and 187 (97%) were on medication with either beta-blockers, calcium antagonists or angiotensin-converting enzyme (ACE) inhibitors. As a consequence these patients had significantly lower levels of blood pressure, and of total and low-density lipoprotein cholesterol compared to their matched controls.

The numbers of *C. pneumoniae* and/or *H. pylori* seropositive individuals in the study populations

Table 1 Baseline characteristics in CHD patients vs. healthy controls

	CHD patients ($n=193$)	Healthy controls ($n=193$)	*P*
Diabetes type 2	7.3%	3.6%	0.116
Treated hypertension	37.8%	8.3%	<0.001
Medication			
statins	91.0%	1.6%	<0.001
beta-blockers	94.8%	1.0%	<0.001
ACE inhibitors	16.6%	2.1%	<0.001
calcium antagonists	7.3%	0.5%	0.001
diuretics	4.7%	1.0%	0.032
warfarin	9.8%	0	<0.001
aspirin	90.7%	0.5%	<0.001
Smokers at inclusion	21.8%	25.9%	0.339
Ex-smokers	59.1%	32.1%	<0.001
BMI (kg/m^2)	27.2 (3.9)	25.8 (3.2)	<0.001
Blood pressure			
systolic	125 (17)	135 (17)	<0.001
diastolic	79 (10)	83 (8)	<0.001
Total cholesterol (mmol/L)	4.76 (0.95)	5.69 (0.96)	<0.001
HDL cholesterol (mmol/L)	0.97 (0.30)	1.39 (0.41)	<0.001
Tot chol/HDL	5.29 (1.81)	4.44 (1.52)	<0.001
LDL cholesterol (mmol/L)	3.05 (0.91)	3.72 (0.89)	<0.001
Triglycerides (mmol/L)	1.71 (0.99)	1.26 (0.70)	<0.001

For continuous variables mean values (SD) are given.
P gives the two-tailed significance of the difference between CHD patients and healthy controls. Pearson χ^2 test was used to compare categorical variables, while an independent *t*-test was used to compare continuous variables between the two study groups.

Table 2 Number (%) of *Chlamydia pneumoniae* or *Helicobacter pylori* IgA- and IgG-seropositive individuals among the CHD patients and the individually matched healthy controls, with two different serological methods for detection of *Chlamydia* antibodies

	CHD patients, *n* (%)	Healthy controls, *n* (%)	*P*
C. pn MIF IgA ≥ 32	63 (32.6)	62 (32.1)	0.913
C. LPS rELISA IgA ≥ 50	79 (40.9)	62 (32.1)	0.072
C. pn MIF IgG ≥ 64	119 (61.7)	108 (56.0)	0.255
C. LPS rELISA IgG ≥ 100	119 (61.7)	97 (50.3)	0.024
H. pylori IgA ≥ 20 U/ml	107 (55.4)	87 (45.1)	0.042
H. pylori IgG ≥ 20 U/ml	86 (44.6)	77 (39.9)	0.354

Cross tabs with Pearson χ^2 test was used to compare the number of seropositives between the study groups. *P* values are two-tailed and refer to differences between the groups.
C.pn: *Chlamydia pneumoniae.*
C.LPS: *Chlamydia* lipopolysaccharide.

are shown in Table 2. A significant difference in *Chlamydia* LPS IgG ($P = 0.024$) and *Helicobacter* IgA seropositivity ($P = 0.042$) was observed between the CHD patients and the healthy controls. The difference in *Chlamydia* LPS IgA between the study groups was not statistically significant at the cut-off level chosen, but a significantly higher proportion of the CHD patients had *Chlamydia* LPS IgA > 100 when compared to the healthy controls (24% in the CHD group vs. 14% among controls, $P = 0.014$, data not shown). The numbers of *C. pneumoniae* MIF IgA and IgG seropositives in the two study groups were similar whatever cut-off level was chosen.

The levels of sCAMs were then compared between *C. pneumoniae* seropositive and seronegative individuals within each study group. IgA and IgG serological status was evaluated separately. In the healthy individuals there was no association between *Chlamydia* serology and the levels of sCAMs, whereas significant differences were observed with *Chlamydia* LPS IgA serology in the CHD group, as shown in Table 3. In CHD patients, *Chlamydia* LPS IgA antibody at a titre $\geq 1:50$ was significantly associated with sICAM-1 and E-selectin levels ($P = 0.044$ for both). *C. pneumoniae* IgA seropositivity measured by MIF technique or *C. pneumoniae* IgG seropositivity with any of the two methods used, did not reveal any statistically significant differences in the inflammatory markers at any cut-off level. When *Chlamydia* LPS IgA seropositivity (≥ 50) was introduced into a linear regression model together with smoking habits, diabetes and body mass index (BMI) in CHD patients, *Chlamydia* LPS IgA seropositivity was still an independent predictive factor for sICAM-1 ($P = 0.011$) together with smoking ($P = 0.004$), while the other factors were non-significant. E-selectin in the CHD patients was significantly predicted by *Chlamydia* LPS IgA seropositivity ($P = 0.038$) and BMI ($P = 0.032$).

Table 3 Levels of soluble CAMs in relation to Chlamydia serology in patients with CHD

	sICAM-1	sVCAM-1	E-selectin
MIF IgA ≥ 32	321 (126)	608 (250)	47.5 (20.6)
MIF IgA < 32	328 (138)	650 (183)	47.9 (22.5)
LPS IgA ≥ 50	352 (179)*	657 (226)	51.5 (24.4)*
LPS IgA < 50	308 (87)	622 (193)	45.1 (19.5)
MIF IgG ≥ 64	321 (110)	633 (231)	48.1 (23.2)
MIF IgG < 64	333 (166)	641 (164)	47.2 (19.6)
LPS IgG ≥ 100	318 (111)	646 (217)	47.7 (20.6)
LPS IgG < 100	337 (164)	620 (191)	47.8 (23.8)

Mean values (SD) in ng/mL are given.
*$P < 0.05$ for titres of LPS IgA ≥ 50 as compared with <50, using independent *t*-test.

Among the LPS-positive CHD patients, 9% were *C. trachomatis* IgA positive, vs. 5% among the healthy controls, and for IgG the percentages were 21 vs. 18, respectively. These differences were not statistically significant. No significant differences in the sCAMs were observed between *C. trachomatis*-positive and -negative individuals (data not shown).

C. trachomatis and H. pylori IgA or IgG status was not associated with any differences in the measured inflammatory markers in either of the two study groups (data not shown). The mean value of sICAM-1, however, was significantly higher in the CHD patients with *Chlamydia* LPS IgA and *H. pylori* IgA seropositivity compared to the patient group that was negative for both ($P = 0.034$) (Table 4, Figure 1). A stepwise increase in E-selectin was also observed when adding *H. pylori* IgA seropositivity to *Chlamydia* LPS IgA seropositivity, but no statistically significant

Table 4 Levels of soluble CAMs in CHD patients according to their *Chlamydia* LPS IgA and *Helicobacter* IgA status

	n	sICAM-1	sVCAM-1	E-selectin
1: LPS IgA$^-$/Hel IgA$^-$	55	300 (85)	634 (220)	45.4 (19.8)
2: LPS IgA$^+$/Hel IgA$^-$	31	321 (94)	582 (184)	51.0 (26.6)
3: LPS IgA$^+$/Hel IgA$^+$	48	372 (215)*	705 (239)**	51.9 (23.2)

Mean values (SD) in ng/mL, n = number of patients in three combinations).
LPS IgA: *Chlamydia* lipopolysaccharide IgA; Hel IgA: *Helicobacter pylori* IgA.
Independent *t*-test was used to compare levels of sCAMs between the subgroups based upon combined *Chlamydia* LPS and *H. pylori* serological status.
* $P < 0.05$ when compared to combination 1.
** $P < 0.05$ when compared to combination 2.

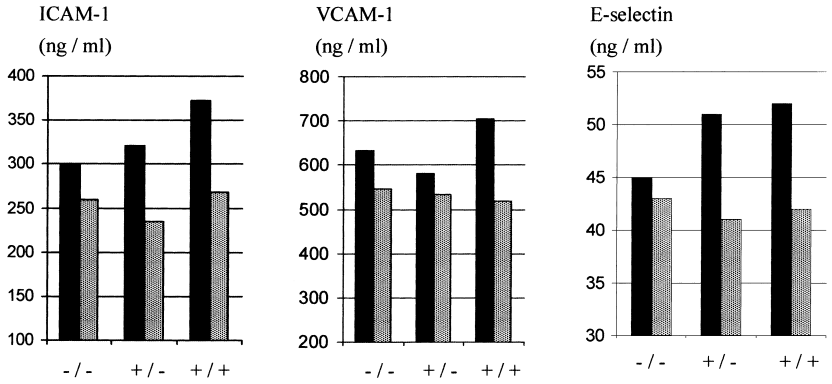

Figure 1 Mean levels of sICAM-1, sVCAM-1 and E-selectin in the CHD patients and their matching healthy controls according to *Chlamydia* LPS IgA and *Helicobacter* IgA status. −/−, *Chlamydia* LPS IgA negative, *Helicobacter* IgA negative; +/−, *Chlamydia* LPS IgA positive, *Helicobacter* IgA negative; +/+, *Chlamydia* LPS IgA positive, *Helicobacter* IgA positive; solid bars, CHD patients; stippled bars, matched healthy controls.

differences between the groups were obtained. The mean sVCAM-1 level in CHD patients was not significantly altered by *Chlamydia* LPS IgA seropositivity, but higher levels of sVCAM-1 were observed in CHD patients with IgA seropositivity to both agents when compared to the individuals with *Chlamydia* LPS IgA seropositivity alone ($P = 0.018$). In the healthy individuals, single or double seropositivity did not influence the level of sCAMs (Figure 1).

DISCUSSION

In the present study we found a significant positive association between *Chlamydia* IgA LPS seropositivity and the levels of sICAM-1 and E-selectin in patients with coronary heart disease. *H. pylori* antibodies alone were not associated with any increase in soluble cell adhesion molecules, but we observed elevated levels of sVCAM-1 in patients who were IgA positive to both microorganisms compared to those that were only *Chlamydia* IgA positive. In the healthy individuals there was no association between serology and the levels of sCAMs.

To our knowledge, it has not previously been shown that seropositivity to *C. pneumoniae* in CHD patients is associated with elevated endothelial inflammatory markers. These observations may indicate that *C. pneumoniae* is more than an innocent bystander in the vessel wall and that *C. pneumoniae*, and possibly also *H. pylori*, may play active roles in vascular inflammation and the development of atherosclerosis in humans. However, to justify this hypothesis, these results need to be confirmed by others.

Serology is a diagnostic tool with obvious limitations. It does not discriminate properly between a chronic, persistent infection and a past and cured one. Serology measures the humoral immunological response, and enables us to conclude that the seropositive individual has once been infected with the microbe. The antibody measurements cannot indicate the outcome of the initial infection, whether the microbe was killed or if a persistent, low-grade infection still exists. The high prevalence of *Chlamydia* antibodies, even in the healthy population, and the relatively small differences between the study groups indicate that this infection is fairly common and does not in general

lead to symptomatic atherosclerotic disease. Better tools are needed to differentiate those people with ongoing, low-grade infection from the ones with simply a serological marker of past and cured disease. It remains to be shown if future microbiological or immunological methods reveal greater differences in soluble inflammatory markers between positive and negative individuals than those revealed in the present study, based upon serology.

The association between seropositivity and elevated levels of sCAMs was observed only in CHD patients and not in the healthy controls, indicating that the raised levels of sCAMs were not associated with the infection *per se*. Higher levels of sCAMs in the *Chlamydia* LPS IgA-positive CHD patients might reflect that *Chlamydia* LPS contributes to the endothelial inflammatory response in patients with atherosclerotic disease. Whether this is a cause or a result of their coronary atherosclerosis, is not possible to assess from the present results.

The association between *C. pneumoniae* antibodies and sCAMs was observed only with IgA. It has been suggested that a high IgA titre reflects chronic infectious activity better than IgG does [27], but this is still controversial [25]. The fact that IgG seropositivity is generally more prevalent than IgA might, however, indicate that IgG more than IgA is a marker of prior infection.

Only *Chlamydia* LPS IgA measured by rELISA was associated with elevated sCAMs in the present study. An obvious difference between Medac's rELISA and Labsystems MIF, is that the MIF technique detects antibodies that are specific for *C. pneumoniae* while the *Chlamydia* LPS antibodies are common for *C. pneumoniae*, *C. trachomatis* and *C. psittaci*. To exclude *C. trachomatis* as the antigen source of human *Chlamydia* LPS antibody production, we analyzed the LPS-positive sera for *C. trachomatis*-specific major outer membrane protein (MOMP) antibodies. *C. psittaci* is probably far less prevalent than the other two *Chlamydia* species [28,29] and less likely to be the inductor of the immune response in these patients.

No association between *C. trachomatis*-specific antibodies and sCAMs was found, making it less probable that the differences in sCAMs observed with *Chlamydia* LPS seropositivity was caused by this agent. Besides, *C. pneumoniae* is the only *Chlamydia* species that has been demonstrated within the lesions in the arterial wall, and is therefore more likely than the other *Chlamydia* species to induce a vascular endothelial inflammatory response.

It was not possible to investigate the sensitivity and the specificity of the two tests included in this study, because the definition of 'true positives' cannot be assessed in patients without clinical evidence of an overt infection, and biopsy material was not available. Other investigators, however, have compared the sensitivity of the MIF test with isolation of *C. pneumoniae* in cell culture or detection with polymerase chain reaction. Good agreement, as well as low detection rates, have been reported in different patient groups [30]. Even though the MIF test is regarded as species-specific, both genus [30,31] and species cross-reactivity [32] have been reported. Nevertheless, the MIF test has generally been regarded as the reference standard internationally.

The antigen used in the genus-specific *Chlamydia* LPS rELISA test is a recombinant LPS containing two *Chlamydia*-specific epitopes and two epitopes which are shared between Chlamydial LPS and the LPS in *Salmonella minnesota* RE-chemotype, an enterobacterial plasmid-transformed laboratory mutant [33–35]. The RE-chemotype LPS is shown not to cross-react with the wild-type enterobacterial LPS [34,35]. Persson and Haidl demonstrated a 100% sensitivity and 99% specificity of Medac's *Chlamydia* LPS rELISA when compared with MIF in patients with atypical pneumonia [30]. However, comparative results between the different serological methods, and also between the rELISA test and isolation in cell culture, differ in the literature [24,36]. In the study of Peerson and Haidl possible cross-reactions between *C. pneumoniae* and *Mycoplasma pneumoniae* were also demonstrated with both methods, but at a very low rate [30]. These data show that even if the probability of cross-reactivity to other bacteria is less than with earlier tests, it cannot be completely ruled out with the methods used in the present study.

Recently, the surface structure of *C. pneumoniae* elementary bodies, the infectious forms of the microbe, has been characterized. *C. pneumoniae* is shown to express LPS as well as MOMP epitopes on the surface [37]. If infectious elementary bodies are found in the circulation or are liberated from infected cells in the atheroma, it is likely that the surface exposed components act as major antigens stimulating the immune response [38]. Free LPS

may also be present as the result of LPS release from the elementary bodies [39] and contribute to the immune stimulation.

Our results do not support the hypothesis that *H. pylori* has an independent role inducing vascular inflammation. On the other hand, CHD patients with IgA antibodies to this microbe in addition to *Chlamydia* LPS IgA antibodies, had the highest levels of sCAMs (Figure 1). In CHD patients with an increased endothelial inflammatory response, a systemic influence from *H. pylori* infection might tend to aggravate it. These findings agree with those from the study by Anderson et al. [40], demonstrating that seropositivity to both *C. pneumoniae and H. pylori*, but not to one agent alone, predicted higher levels of C-reactive protein and increased risk for CHD and myocardial infarction.

In general, the design of the present study can only point to associations and therefore only allows assumptions regarding causality.

In conclusion, increased titres of *Chlamydia* LPS IgA antibodies were associated with statistically significantly elevated levels of sICAM-1 and E-selectin in CHD patients, but not in healthy individuals. This may indicate that *C. pneumoniae* or the immunological response to the microbe induce vascular inflammation. Moreover, in CHD patients, *H. pylori* IgA antibodies, when present in addition to *Chlamydia* LPS IgA, seemed to predict an even larger increase in sICAM-1 and sVCAM-1, indicative of aggravation of the endothelial inflammatory response.

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REFERENCES

1. Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; 340: 115–26.
2. Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am J Pathol* 1992; 140: 665–73.
3. Davies MJ, Gordon JL, Gearing AJ *et al.* The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM and E selectin in human atherosclerosis. *J Pathol* 1993; 171: 223–9.
4. Hwang SJ, Ballantyne CM, Sharett AR *et al.* Circulating adhesion molecules VCAM-1, ICAM-1, and E selectin in carotid atherosclerosis and incident coronary heart disease cases: The Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 1997; 96: 4219–25.
5. Morisaki N, Saito I, Tamura K *et al.* New indices of ischemic heart disease and aging: studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hypercholesterolemia and ischemic heart disease. *Atherosclerosis* 1997; 131: 43–8.
6. Blann AD, Seigneur M, Steiner M, Miller JP, McCollum CN. Circulating ICAM-1 and VCAM-1 in peripheral artery disease and hypercholesterolaemia: Relationship to the location of atherosclerotic disease, smoking, and in the prediction of adverse events. *Thromb Haemost* 1998; 79: 1080–5.
7. Schumacher A, Arnesen H, Seljeflot I, Sommervoll L. Increased levels of soluble markers of endothelial dysfunction in patients with atherosclerotic heart disease. *Scand J Clin Lab Invest* 2002; 62: 59–68.
8. Saikku P, Leinonen M, Mattila K *et al.* Serological evidence of an association of a novel chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 1988; 2: 983–6.
9. Thom DH, Wang SP, Grayston JT *et al.* Chlamydia pneumoniae strain TWAR antibody and angiographically demonstrated coronary artery disease. *Arterioscler Thromb* 1991; 11: 547–51.
10. Patel P, Mendall MA, Carrington D *et al.* Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors. *BMJ* 1995; 311: 711–14.
11. Rathbone B, Martin D, Stephens J, Thompson JR, Samani NJ. Helicobacter pylori seropositivity in subjects with acute myocardial infarction. *Heart* 1996; 76: 308–11.
12. Hoffmeister A, Rothenbacher D, Wanner P *et al.* Seropositivity to Chlamydial lipopolysaccharide and Chlamydia pneumoniae, Systemic inflammation and stable coronary artery disease. *J Am Coll Cardiol* 2000; 35: 112–18.
13. Grayston JT, Kuo CC, Campbell LA, Benditt EP. Chlamydia pneumoniae, strain TWAR and atherosclerosis. *Eur Heart J* 1993; 14(Suppl. K): 66–71.
14. Taylor-Robinson D, Thomas BJ. Chlamydia pneumoniae in atherosclerotic tissue. *J Infect Dis* 2000; 181(Suppl. 3): 437–40.

15. Ouchi K, Fujii B, Kudo S *et al.* Chlamydia pneumoniae in atherosclerotic and nonatherosclerotic tissue. *J Infect Dis* 2000; 181(Suppl. 3): 441–3.
16. Gaydos C, Summersgill JT, Sahney NN, Ramirez JA, Quinn TC. Replication of Chlamydia pneumoniae in vitro in human macrophages, endothelial cells, and aortic artery smooth muscle cells. *Infect Immun* 1996; 64: 1614–20.
17. Quinn TC, Gaydos CA. In vitro infection and pathogenesis of chlamydia pneumoniae in endovascular cells. *Am Heart J* 1999; 138: 507–11.
18. Kalayoglu MV, Byrne GI. Induction of macrophage foam cell formation by Chlamydia pneumoniae. *J Infect Dis* 1998; 177: 725–9.
19. Kaukoranta-Tolvanen SSE, Ronni T, Leinonen M, Saikku P, Laitinen K. Expression of adhesion molecules on endothelial cells stimulated by Chlamydia pneumoniae. *Microb Pathog* 1996; 21: 407–11.
20. Krüll M, Klucken AC, Wuppermann FN *et al.* Signal transduction pathways activated in endothelial cells following infection with Chlamydia pneumoniae. *J Immunol* 1999; 162: 4834–41.
21. Farsak B, Yildrir A, Akyön Y *et al.* Chlamydia pneumonia and Helicobacter pylori are present only in human atherosclerotic plaques but not the healthy segments. *Eur Heart J* 1999; 20(Suppl.): 9(Abstract).
22. Malnick SD, Goland S, Kaftoury A *et al.* Evaluation of carotid arterial plaques after endarterectomy for Helicobacter pylori. *Am J Cardiol* 1999; 83: 1586–7, A8.
23. Blasi F, Denti F, Erba M *et al.* Detection of Chlamydia pneumoniae but not Helicobacter pylori in atherosclerotic plaques of aortic aneurysms. *J Clin Microbiol* 1996; 34: 2766–9.
24. Schumacher A, Lerkerød AB, Seljeflot I *et al.* Chlamydia pneumoniae serology – the importance of methodology in patients with coronary heart disease and healthy individuals. *J Clin Microbiol* 2001; 39: 1859–64.
25. Siscovick DS, Schwartz M, Caps M, Wang SP, Grayston JT. *Chlamydia pneumoniae and* atherosclerotic risk in populations: the role of seroepidemiology. *J Infect Dis* 2000; 181(Suppl. 3): 417–20.
26. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990; 1: 43–6.
27. Saikku P, Leinonen M, Tenkanen L *et al.* Chronic *Chlamydia pneumoniae* infection as a risk factor for Coronary Heart Disease in the Helsinki Heart Study. *Ann Int Med* 1992; 116: 273–8.
28. Koivisto AL, Isoaho R, Von Hertzen L *et al.* Chlamydial antibodies in an elderly Finnish population. *Scand J Infect Dis* 1999; 31: 135–9.
29. Freidank HM, Vögele H, Eckert K. Evaluation of a new commercial microimmuno-fluorescence test for detection of antibodies to Chlamydia pneumoniae, Chlamydia trachomatis and Chlamydia psittaci. *Eur J Clin Microbiol Infect Dis* 1997; 16: 685–8.
30. Persson K, Haidl S. Evaluation of a commercial test for antibodies to the chlamydial lipopolysaccharide (Medac^{TM}) for serodiagnosis of acute infections by *Chlamydia pneumoniae* (TWAR) and *Chlamydia psittaci*. *APMIS* 2000; 108: 131–8.
31. Maurin M, Eb F, Etienne J, Raoult D. Serological cross-reactions between Bartonella and Chlamydia species: Implications for diagnosis. *J Clin Microbiol* 1997; 35: 2283–7.
32. Ozanne G, Lefebvre J. Specificity of the micro-immunofluorescence assay for the serodiagnosis of Chlamydia pneumoniae infections. *Can J Microbiol* 1992; 38: 1185–9.
33. Holst O, Broer W, Thomas-Oates JE, Mamat U, Brade H. Structural analysis of two oligosaccharide biphosphates isolated from a recombinant strain of Escherichia coli F515 (Re chemotype) expressing the genus-specific epitope of Chlamydia lipopolysaccharide. *Eur J Biochem* 1993; 214: 703–10.
34. Fu Y, Bauman M, Kosma P, Brade L, Brade H. A synthetic glycoconjugate representing the genus specific epitope of chlamydial lipopolysaccharide exhibits the same specificity as its natural counterpart. *Infect Immun* 1992; 60: 1314–21.
35. Brade L, Brunnemann H, Ernst M *et al.* Occurrence of antibodies against chlamydial lipopolysaccharide in human sera as measured by ELISA using an artificial glycoconjugate antigen. *FEMS Immunol Med Microbiol* 1994; 8: 27–42.
36. Kutlin A, Isomura N, Emre U, Roblin PM, Hammerschlag MR. Evaluation of chlamydia immunglobulin M (IgM), IgG and IgA rELISAs Medac for the diagnosis of Chlamydia pneumoniae infection. *Clin Diagn Lab Immunol* 1997; 4: 213–16.
37. Wolf K, Fischer E, Mead D *et al.* Chlamydia pneumoniae major outer membrane protein is a surface-exposed antigen that elicits antibodies primarily directed against conformation-dependent determinants. *Infect Immun* 2001; 69: 3082–91.
38. Christiansen G, Boesen T, Hjernø K *et al.* Molecular biology of Chlamydia pneumoniae surface proteins and their role in immunopathogenicity. *Am Heart J* 1999; 138: S491–5.
39. Birkelund S, Lundemose AG, Christiansen G. Immunoelectron microscopy of lipopolysaccharide in Chlamydia trachomatis. *Infect Immun* 1989; 57: 3250–3.
40. Anderson JL, Carlquist JF, Muhlestein JB, Horne BD, Elmer SP. Evaluation of C-reactive protein, an inflammatory marker, and infectious serology as risk factors for coronary artery disease and myocardial infarction. *J Am Coll Cardiol* 1998; 32: 35–41.