

Chlamydia pneumoniae Serology: Importance of Methodology in Patients with Coronary Heart Disease and Healthy Individuals

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Most publications on the relationship between infection with *Chlamydia pneumoniae* and coronary heart disease (CHD) propose an association, but negative studies are also reported. Seroepidemiological studies vary in the use of different serological methods, different cutoff limits, different sampling times in relation to acute cardiac events, and different clinical stages of CHD. We wanted to compare three different commercially available methods for measuring *Chlamydia* antibodies to see how the choice of method influenced the prevalence of seropositive individuals in CHD patients and in healthy individuals and to see if sampling time in relation to an acute cardiac event or the stage of atherosclerotic disease influenced the results. Blood samples from 197 CHD patients and 197 individually matched healthy control individuals were tested at baseline and after 6 months; the mean age was 55 years in both groups, and 18% were women. Among the CHD patients, 166 were included at a median of 16 days after an acute cardiac event and 31 had chronic disease with the latest acute event being >3 months earlier. The difference in prevalence of antibodies between the CHD patients and the healthy controls was significant when *Chlamydia* lipopolysaccharide antibodies were measured, while no significant differences between the study groups were observed by the two methods detecting *Chlamydia pneumoniae* major outer membrane protein antibodies. The number of seropositive individuals was quite similar at inclusion and 6 months later, and no significant differences were observed between patients with a recent cardiac event and those with a more remote cardiac event. We conclude that the choice of serological method is of major importance when evaluating a possible relationship between *C. pneumoniae* and CHD.

The old hypothesis that atherosclerosis could be caused by infectious agents has received new attention during the last 15 years, and *Chlamydia pneumoniae* is one of the main pathogens under suspicion. Since Saikku et al. (31) proposed an association between *C. pneumoniae* and coronary heart disease (CHD), many reports from different countries have been published, with diverging results (10, 11, 14, 17, 25, 37). Although some investigations are based on direct immunofluorescence or PCR demonstrating *C. pneumoniae* in situ in the atherosclerotic plaque, most studies are based on serology, using different methods to detect human antibodies against the organism. Two basic methods are used: microimmunofluorescence tests (MIF) or enzyme immunoassays (EIA and ELISA techniques). Some tests detect antibodies to the species-specific major outer membrane proteins (MOMP), and some detect antibodies to the chlamydia lipopolysaccharide (LPS), which is common to *Chlamydia pneumoniae*, *Chlamydia trachomatis*, and *Chlamydia psittaci*. Furthermore, the titer end points used as cutoff values for seropositivity when comparing various groups differ in various studies.

The aim of the present study was to compare three different, commonly used methods for measuring *Chlamydia* antibodies to elucidate how the choice of method influenced the results in

CHD patients and in healthy individuals. We also wanted to evaluate if antibody titers in CHD patients differed according to sampling time in relation to cardiac events compared to the variation pattern over time in healthy individuals, and, finally, we wanted to see if the stage of CHD influenced the serology results.

MATERIALS AND METHODS

Study population. The study population comprised 197 patients with documented CHD and 197 age- and sex-matched healthy controls from the county of Vestfold, Norway. The CHD patients were included at a median of 16 days (minimum, 0 days; maximum, 70 days) after an acute myocardial infarction (AMI) ($n = 74$), hospitalization for unstable angina ($n = 4$), percutaneous transluminal coronary angioplasty ($n = 38$), or coronary artery bypass grafting ($n = 50$). In addition, 31 patients (16%) in the chronic stage of their disease, with more than 3 months since their last acute event, were included. Of the CHD patients, 137 (70%) had suffered a previous myocardial infarction and 103 had an available coronary angiogram. None of the patients had severe heart failure (New York Heart Association class 4).

A total of 400 healthy controls were recruited from four working sites in Vestfold to form a pool of control persons, and for each CHD patient included in the study, one age- and sex-matched control was drawn from this control pool. This apparently healthy individual was included after an interview and a clinical examination by a physician, including exercise electrocardiogram, in the absence of symptoms or clinical evidence of atherosclerotic disease. We also aimed at matching the educational level but ended up with a somewhat higher proportion of persons with postgraduate education in the control group (43.1%) than in the CHD group (33.5%). The mean age in both groups was 55 years (minimum, 27 years; maximum, 68 years), and 18% were women. At inclusion, 22.3% of the CHD patients and 26.4% of the healthy individuals were smokers. The regional ethics committee had approved the study, and all patients and controls had given an informed written consent to participate.

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Laboratory methods. Blood samples were drawn at inclusion and after 6 months, and sera were kept frozen at -20°C until analysis. All the samples ($n = 783$) were analyzed by the following methods. (i) The first was LabSystems (Helsinki, Finland) *C. pneumoniae* MIF immunoglobulin G (IgG), IgA, and IgM, a species-specific test where *C. pneumoniae* elementary bodies are used as the antigen. Seropositivity was defined as IgA ≥ 32 , IgG ≥ 64 , and IgM ≥ 32 . (ii) The second was the LabSystems *C. pneumoniae* IgG, IgA, and IgM EIA, which is species specific and, according to the manufacturer, gives results that are comparable to the results from the MIF test. Consequently, we applied the same seropositivity definitions as used for the first method (IgA ≥ 32 , IgG ≥ 64 , and IgM ≥ 32). (iii) The third was the Medac (Hamburg, Germany) *Chlamydia* IgG, IgA, and IgM rELISA, a recombinant for detection of the genus-specific LPS antibodies. IgA seropositivity is defined as IgA ≥ 50 , IgG seropositivity is defined as IgG ≥ 100 , and IgM seropositivity is defined as IgM ≥ 50 , according to the manufacturer. Because of the differences in specificity level between this test and the other two tests for *Chlamydia* antibodies, the patients who were either IgG, IgA, or IgM positive in the Medac rELISA were also tested by the Medac *C. trachomatis* pELISA IgG and IgA. Specific methods for *C. psittaci* antibodies were not included, because no such ELISA was available and because the prevalence of *C. psittaci* antibodies is probably too low to have a significant influence on the results (7, 16).

All the samples were blinded. One investigator who was otherwise not involved in the study controlled the grouping of samples so that inclusion and 6-month samples from CHD patients and their individually matched healthy controls were analyzed together. Evaluation of the MIF analysis results was always performed by experienced staff members. To evaluate the reproducibility of the results, 25 serum samples were analyzed twice by the three methods described above; the two analyses of a given sample were carried out on two different days but with the same technician and the same equipment, and the samples were blinded between the two times they were tested. Based on these data, we calculated the intra-assay coefficient of variation (CV) for these methods in our laboratory.

Statistical methods. The results were analyzed statistically using SPSS for Windows version 9.0. Crosstabs with the chi-square test were used when comparing the number of seropositive persons in the different groups of individuals, while McNemar's test for paired samples was used to compare the results from the different serological methods applied to the same sera and also to compare the inclusion samples with the results after 6 months in the same individuals. To assess the agreement between the different tests, we used kappa (κ) (nominal scale variables) as proposed by Landis and Koch (1) and Spearman's rank correlation coefficient (ordinal scale) (S_p) comparing pairs of tests. Guidelines for the interpretation of κ are as follows: $\kappa < 0.2$, poor agreement; $\kappa = 0.21$ to 0.4 , fair agreement; $\kappa = 0.41$ to 0.6 , moderate agreement; $\kappa = 0.61$ to 0.8 , good agreement; and $\kappa = 0.81$ to 1 , very good agreement (adapted from reference 1). For reproducibility evaluation, we calculated CV by using the 25 samples analyzed twice: $\text{CV} = \text{SD}/(\sqrt{2})x$, where SD is the standard deviation of the difference between the first and second analysis results and x is the mean value of all the results for one immunoglobulin class measured by one method. Since the variables are not normally distributed and the MIF scale is a ratio scale, the calculation of CV is based on logarithmically transformed values.

RESULTS

Seropositivity in the CHD patients and the healthy controls. The number of *Chlamydia* IgA, IgG, and IgM positives in the inclusion samples from 197 CHD patients, compared to the 197 age- and sex-matched healthy controls, is shown in Table 1. Because there is no general agreement in the literature about which cutoff levels should be applied when performing MIF, the study groups were also compared using one lower and one higher cutoff level than defined in Materials and Methods. By performing antibody analyses with the LabSystems MIF test in our study population, the number of seropositive individuals among the CHD patients was equal to the number in the group of healthy controls, regardless of antibody class or titer. With the Medac rELISA serology, however, there were statistically significant differences between the two groups, with a larger number of IgA positives ($P = 0.074$ with a cutoff of ≥ 50 , $P = 0.014$ with a cutoff of ≥ 100) and IgG positives ($P = 0.033$) among the CHD patients. LabSystems EIA IgA and IgG sero-

TABLE 1. Number of IgA-, IgG-, and IgM-seropositive samples from the CHD patients at inclusion compared to the healthy controls, using three different serological methods and different titers

Test and titer	No. (%) of:		<i>P</i> ^a
	CHD patients	Healthy controls	
LabSystems MIF, IgA ≥ 16	103 (52.3)**	107 (54.3)**	NS
LabSystems MIF, IgA ≥ 32	63 (32.0)**	64 (32.5)**	NS
LabSystems MIF, IgA ≥ 64	41 (20.8)**	32 (16.2)**	NS
LabSystems EIA, IgA ≥ 16	82 (41.6)	59 (29.9)	0.016
LabSystems EIA, IgA ≥ 32	35 (17.8)	25 (12.8)	NS
LabSystems EIA, IgA ≥ 64	7 (3.6)	12 (6.1)	NS
Medac rELISA, IgA ≥ 50	79 (40.1)	62 (31.5)	0.074
Medac rELISA, IgA ≥ 100	46 (23.4)	27 (13.7)	0.014
LabSystems MIF, IgG ≥ 32	145 (73.6)*	142 (72.1)	NS
LabSystems MIF, IgG ≥ 64	120 (60.9)**	110 (55.8)*	NS
LabSystems MIF, IgG ≥ 128	75 (38.1)**	63 (32.0)**	NS
LabSystems EIA, IgG ≥ 30	156 (79.2)	144 (73.1)	NS
LabSystems EIA, IgG ≥ 64	102 (51.8)	98 (49.7)	NS
LabSystems EIA, IgG ≥ 128	56 (28.4)	45 (22.8)	NS
Medac rELISA, IgG ≥ 100	121 (61.4)	100 (50.8)	0.033
Medac rELISA, IgG ≥ 200	80 (40.6)	60 (30.5)	0.035
MIF IgM, ≥ 16	2 (1)	7 (3.6)	NS
Lab EIA, IgM pos ^b	2 (1)	2 (1)	NS
Medac rELISA, IgM ≥ 50	14 (7.1)	15 (7.6)	NS

^a *P* values refer to differences between the two groups. *, $P < 0.05$ when comparing LabSystems MIF and LabSystems EIA seropositivity on the same titer levels within the same patient group. **, $P < 0.01$ performing the same comparison as above.

^b Definition of LabSystems EIA IgM positivity is signal/cutoff units > 1.1 , as defined in the test kit instructions.

positivity was somewhat more frequent in the CHD patients than in the control group, but the differences were statistically significant only when using a cutoff level of IgA ≥ 16 ($P = 0.016$). The IgM levels measured were low in both groups and by all methods, and there was no significant difference between CHD patients and healthy controls.

With the intention of further exploring the reason for the different results between the MIF test and the LPS serology (Medac), we tested all the patients with positive LPS serology to either IgA or IgG ($n = 264$ [144 CHD patients and 120 healthy controls]) by using a species-specific test for *C. trachomatis* to also evaluate this microbe as a possible source of LPS antibodies. For *C. trachomatis* IgA, 8.3% of the LPS-positive CHD patients were IgA positive versus 5.0% of the healthy controls, and for IgG the percentages were 20.9 and 16.7, respectively. These differences were not statistically significant.

As described above, the CHD patients were included after an AMI, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting, hospitalization for unstable angina, or in a chronic stage of their disease. We found no significant difference in the number of IgA- or IgG-seropositive patients between these subgroups. No difference was observed between those with a previous myocardial infarction

TABLE 2. Comparison between Medac rELISA IgA and IgG and Labsystems MIF IgA and IgG in CHD patients and healthy controls^a

Medac rELISA result	No. of patients with Labsystems MIF result of:			
	IgA ⁺ ^b	IgA ⁻	IgG ⁺ ^c	IgG ⁻
IgA ⁺ ^b	110	167		
IgA ⁻	154	352		
IgG ⁺ ^c			287	146
IgG ⁻			165	185

^a Inclusion and 6-month samples together. A total of 783 samples were used.
^b IgA⁺ is defined as IgA ≥ 50 in the Medac rELISA and IgA ≥ 32 in the Labsystems MIF.
^c IgG⁺ is defined as IgG ≥ 100 in the Medac rELISA and IgG ≥ 64 in the Labsystems MIF.

(MI) compared to those who had never suffered an MI. Among the 103 CHD patients who had a coronary angiogram available, we found no correlation between the extent of atherosclerotic disease judged as single-, double-, or triple-vessel disease and the seroprevalence of *C. pneumoniae* antibodies.

Agreement between the tests. In Table 1 it is further apparent that the results of the Labsystems EIA were significantly different from those of the MIF test. Among the CHD patients, 17.8 and 32.0% were IgA positive in the Labsystems EIA and the MIF test, respectively (*P* < 0.01), and in the control group the values were 12.8% and 32.5%, respectively (*P* < 0.01). Comparison of IgG results from the two tests gave similar results, with significant differences between the two tests in both study groups (Table 1).

A comparison between Medac rELISA and Labsystems MIF IgA and IgG seropositivity gave quite similar numbers in the control group (31.5% versus 32.5% for IgA, 50.8% versus 55.8% for IgG). In the CHD group, however, there was a larger number of *Chlamydia* LPS IgA-positive individuals (Medac rELISA IgA) than the number of IgA-positive individuals detected by MIF (40.1% versus 32.0%, *P* = 0.088), while the numbers of IgG-seropositive individuals in the Medac rELISA equaled the number detected by MIF (61.4% versus 60.9%).

To further evaluate the strength of the agreement between the three test methods, we calculated the degree of concordance, κ and S_p , comparing pairs of tests. Since there were no major differences between CHD patients and healthy controls at inclusion or after 6 months, all results were evaluated together. Corresponding results for Medac rELISA and Labsystems MIF are shown in Table 2. The degrees of concordance between these two tests (both tests negative or positive) were only 59.0% with IgA and 60.3% with IgG, i.e., κ = 0.094 for IgA and κ = 0.192 for IgG, indicating poor agreement. Comparing the results along an ordinal scale gave S_p = 0.151 for IgA and S_p = 0.232 for IgG (Table 3). Similar results were obtained when comparing Medac rELISA and Labsystems EIA. MIF and Labsystems EIA agreed in 77.7% of IgA results (κ = 0.426) and in 81.5% of IgG results (κ = 0.628), with S_p = 0.764 for IgA and S_p = 0.787 for IgG, respectively (Table 3).

Because of the disagreement observed between Medac rELISA measuring LPS antibodies and Labsystems MIF measuring the species-specific antibodies, we focused on the individuals with discordant results, paying special attention to the individuals who were seropositive by Medac rELISA and se-

TABLE 3. κ and S_p as measures of agreement and correlation between pairs of the serological methods used^a

Antibody	Labsystems MIF vs EIA		Labsystems EIA vs Medac rELISA		Medac rELISA vs Labsystems MIF	
	κ^b	S_p^c	κ	S_p	κ	S_p
	IgA	0.426	0.764	0.120	0.309	0.094
IgG	0.628	0.787	0.267	0.338	0.192	0.232

^a Inclusion and 6-month samples from CHD patients and healthy controls (*n* = 783 samples) were used.
^b κ expresses the strength of agreement between the tests regarding nominal scale variables (positive and negative results). IgA positivity is defined as MIF IgA ≥ 32, Labsystems EIA IgA ≥ 32, and Medac IgA ≥ 50. IgG positivity is defined as MIF IgG ≥ 64, Labsystems EIA IgG ≥ 64, and Medac IgG ≥ 100.
^c S_p is the rank correlation coefficient when comparing the end titer results from pairs of methods.

ronegative by MIF. At inclusion, 86 individuals had this IgA pattern, and 9.3% of these were *C. trachomatis* IgA positive. Of the 69 individuals who were Medac rELISA IgG positive and MIF IgG negative at inclusion, 23.2% were *C. trachomatis* IgG positive. Thus, in the majority of the individuals with this antibody pattern the discordant serological results could not be explained by *Chlamydia* genus versus species specificity of the tests used.

Antibody titers in relation to sampling time in acute cardiac events. Table 4 summarizes the results obtained by comparing inclusion and 6-month samples from the 197 CHD patients. No significant differences in IgA or IgG seropositivity could be demonstrated by any method or with any cutoff level. Identical results were also obtained for the healthy controls at the two times tested (data not shown).

Reproducibility. The CV values in our laboratory, based on the 25 samples analyzed twice, were 7% for both IgA and IgG by Labsystems MIF, 13 and 5% for Labsystems EIA, and 6 and 2% for Medac rELISA.

TABLE 4. Number of *C. pneumoniae* IgA- and IgG-positive individuals at inclusion and 6 months later

Test and titer	No. (%) of patients		<i>P</i> ^a
	At inclusion	After 6 mo	
Labsystems MIF, IgA ≥ 32	63 (32.5)	65 (33.7)	NS
Labsystems MIF, IgA ≥ 64	41 (20.8)	38 (19.7)	NS
Labsystems EIA, IgA ≥ 32	35 (17.8)	38 (19.7)	NS
Labsystems EIA, IgA ≥ 64	7 (3.6)	10 (5.1)	NS
Medac rELISA, IgA ≥ 50	79 (40.1)	79 (40.1)	NS
Labsystems MIF, IgG ≥ 64	120 (60.9)	108 (56.0)	NS
Labsystems MIF, IgG ≥ 128	75 (38.1)	67 (34.7)	NS
Labsystems EIA, IgG ≥ 64	102 (51.8)	108 (54.8)	NS
Labsystems EIA, IgG ≥ 128	56 (28.4)	58 (29.4)	NS
Medac rELISA, IgG ≥ 100	121 (61.4)	117 (60.6)	NS

^a Significance of the difference between the number of seropositive individuals in the CHD group at inclusion compared to the results after 6 months, using McNemar's test for two related groups.

DISCUSSION

In the present study we could not demonstrate striking evidence of an association between antibodies to *C. pneumoniae* and CHD. However, statistically significant differences were observed for the method detecting *Chlamydia* LPS antibodies, with higher titers of IgA and IgG in CHD patients. Although a positive relationship has been shown in different populations and with different atherosclerotic manifestations (4, 5, 10, 17, 22, 23, 27, 31, 35), other studies could not demonstrate such an association (11, 14, 37). Documentation of the presence of *C. pneumoniae* in the arterial wall is substantial (9, 10, 13, 18, 19, 21, 25), but it is still not clear whether *C. pneumoniae* is an innocent bystander or a causative agent, and there is little or no documentation of a significant correlation between immunohistochemical findings in plaques and the serological detection of antibodies. Large prospective studies have failed to demonstrate an association between antibodies to *C. pneumoniae* and the incidence of future myocardial infarction (29, 30, 34). Various explanations of these diverging serological results have been suggested. Based on the present results, however, mainly methodological considerations will be discussed.

Different serological methods are being used, and the different results may be related to the choice of method. Some investigators have used methods detecting the species-specific MOMP antibodies (4, 11, 14, 22, 23), and some have used methods detecting the genus-specific LPS antibodies (5, 11, 17, 22). The MIF technique has generally been regarded as a "gold standard" for the detection of *C. pneumoniae* antibodies in seroepidemiological studies. However, MIF methods differ from commercial methods to in-house MIF techniques, and the antigen composition varies between tests. In addition, some authors have focused on IgA positivity, some have focused on IgG, and some have used a combination of the two.

The use of several different methods would be no problem if the agreement between the tests is generally high. Our observations regarding the number of seropositives obtained by the different methods suggest that the sensitivity of the MIF method was higher than that of the Labsystems EIA, while the prevalence of seropositive individuals detected by the Medac rELISA was equal to that found by MIF. The only difference in prevalence observed between the last two methods was a slightly higher proportion of CHD patients who were IgA positive by Medac rELISA than by the MIF method, but this difference was not statistically significant.

A more striking observation was the poor agreement between the LPS-based serology (Medac) and the other two methods on the individual level. As shown for Medac rELISA and Labsystems MIF in Table 2, 41% of the individuals were IgA seropositive by one method and seronegative by the other, versus 39.7% for IgG. This observation may be due to technical methodological differences, but it might also reflect differences in the immunological responses among individuals.

The difference in specificity level between the tests is one possible reason why some individuals are seronegative by MIF and seropositive by Medac rELISA. Therefore, we analyzed the LPS-positive sera for *C. trachomatis*-specific MOMP antibodies. As described above, the majority of the discordant results obtained by MIF and Medac rELISA could not be

explained by *Chlamydia* species versus genus specificity of the tests. Because Medac rELISA, in contrast to the earlier complement fixation tests for *Chlamydia* LPS, is based on a *Chlamydia*-specific small fragment of the LPS in the outer membrane instead of the total LPS content, the probability of cross-reactions to other gram-negative bacteria is far lower than with earlier tests (3, 8, 12). We have, however, not included serological testing for other gram-negative bacteria in our study, and the possibility of cross-reactions to LPS from other sources than *Chlamydia* cannot be completely ruled out. Some cross-reactivity between the MOMP of the different *Chlamydia* species might, however, also occur (15, 24, 26), indicating that the MIF method might not be completely specific either.

The fact that some samples were seropositive by Labsystems MIF and seronegative by Medac could be a result of higher sensitivity of the MIF method, but there may also be other explanations for the diverging results than the sensitivity and specificity aspect of the tests. We cannot fully explain why some individuals have a dominance of persisting *Chlamydia* LPS antibodies and others have a dominance of the species-specific *C. pneumoniae* antibodies detected by MIF. Whether this is caused by differences in the properties of the infectious agents, the clinical infection they induce, or the human immunological response remains to be settled. Other investigators have shown that children with respiratory *C. pneumoniae* infection may develop antibodies that are not detected by MIF (2, 6, 20), and it has been proposed that the MOMP is not the dominant protein for immune responses (28, 36). More research is needed to explain this phenomenon and to determine if these differences are of pathogenetic importance.

Our results do not provide sufficient basis for a recommendation about methodology. We conclude that in our study, LPS antibodies were related to atherosclerotic disease while the antibodies detected by MIF were not. Similar disagreement between different seroepidemiological methods used might thus explain the diverging results reported in various studies. However, both positive (4, 9, 10, 17, 22, 23, 27) and negative (11, 14) correlations have been observed by MIF as well as by *Chlamydia* LPS serology, indicating that a methodological aspect is not the entire explanation of the observed discrepancies.

Different titer limits are used in different studies, and most studies classify individuals as seropositive or seronegative based on low titer limits of IgG or IgA; low titers will not distinguish between passed and persistent infection. There is no general agreement in the literature about which titer limit should be regarded as positive. In different publications, the definition of seropositivity varies from IgA ≥ 8 to IgA ≥ 64 and from IgG ≥ 16 to IgG ≥ 128 . One proposed explanation for the negative findings is that a low IgA or IgG titer reflects prior infection but cannot distinguish passed (and cured) infection from a chronic, persistent one (33). According to this hypothesis, one would expect greater differences between the CHD patients and the healthy controls based on IgG or IgA titers instead of just positive or negative results, based on low titer limits. As shown in Table 1, the prevalence of MIF IgA- or IgG-seropositive individuals in the CHD group is the same as the prevalence among healthy individuals when using IgA ≥ 16 or IgA ≥ 32 and IgG ≥ 32 as the criterion for seropositivity. When the numbers of individuals with IgA ≥ 64 and IgG ≥ 64

or IgG ≥ 128 in the two groups were compared, there was a slight tendency toward a larger number in the CHD group, but the difference was not statistically significant. Furthermore, when numbers of individuals with IgA ≥ 128 or IgG ≥ 512 in the two groups were compared (data not shown), there was still no statistically significant difference between the groups (9.1% of CHD patients versus 8.6% of healthy controls with IgA; 9.1% versus 4.6% with IgG).

For Medac IgG serology, the differences between the groups were statistically significant at both IgG ≥ 100 and IgG ≥ 200 , and the significance of the IgA difference was stronger for high titers (IgA ≥ 100). We conclude that our results with MIF serology do not support the hypothesis of higher titers in CHD patients than in healthy individuals, but there seems to be a higher proportion of CHD patients than healthy individuals with high titers of LPS IgA and IgG.

The time interval from an acute event to sampling time differs in different publications, and immunomodulation in relation to an acute cardiac event may alter the antibody titer. Most studies aim at sampling in a stable phase and try to avoid the acute-phase reaction and its possible influence on antibody titers. In our study we measured antibody titers in CHD patients at a median 16 days (minimum, 0 days; maximum, 70 days) after an acute cardiac event and compared the results to those obtained after 6 months. In general, no difference in antibody titers between the two sampling times were found. Therefore, we conclude that serology tested on average 2 weeks or more after the acute event is representative of the patient's antibody status toward *C. pneumoniae*. In other words, if the sampling occurs more than 2 weeks after an acute event, different time intervals until sampling probably cannot explain diverging results in different studies.

Different study populations with different stages of atherothrombotic disease may account for the diverging results. In our study there was no correlation between the extent of atherothrombotic disease as judged from coronary angiograms and the prevalence of *C. pneumoniae* antibodies. No difference was observed between patients with a sustained MI compared to those who had never had an MI. Our results consequently do not support the theory that different stages of stable atherothrombotic disease in the study populations should account for the diverging results between studies. The study population did not enable us to evaluate unstable CHD. However, because of relatively small numbers in the subgroups and the methodological limitations of serology, we cannot entirely rule out an association between *C. pneumoniae* infection and the extent of CHD.

Conclusion. Taken together, the discrepancy between the methods used has important implications for the evaluation of a possible association between *C. pneumoniae* antibodies and CHD. There is no definite answer to the question of which test gives the most reliable results, and the various results on the correlation between seropositivity and CHD obviously represent a problem in deciding which test to use. In our study, the high LPS IgA titer gave the strongest distinction between the population with documented CHD and age- and sex-matched healthy controls. There is, however, a possibility that a high IgA titer to *C. pneumoniae* may be a better marker of persistent infection than the other antibodies measured, as proposed by Saikku et al. in the Helsinki Heart Study almost 10 years ago

(32). *C. pneumoniae* IgA is a less prevalent finding than IgG in the healthy population, and a rise in the IgA titer is a common finding in reinfection. More work has to be done to evaluate the role of serological tests as markers for the possible relationship between infection and atherothrombotic disease. From our experience, it seems mandatory that strict descriptions and evaluations of the methods used for serological studies of *Chlamydia pneumoniae* be undertaken.

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