# Indicators of infection with *Chlamydia pneumoniae* are associated with expansion of abdominal aortic aneurysms

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***Purpose:*** **Chlamydia pneumoniae has been shown to be associated with atherosclerosis, myocardial infarction, and abdominal aortic aneurysms (AAAs). The possible association between AAA expansion and *C pneumoniae* infection was therefore assessed.**

***Methods:*** **Blood samples were taken from patients with an AAA that was considered for surgical repair after having been diagnosed by means of the Chichester aneurysm screening program (UK) as having an initially infrarenal aortic diameter of 3.0 to 5.9 cm. The patients were examined prospectively for as long as 11.5 years (mean, 4.1 years) with ultrasound scanning. Of 110 patients considered for surgery, 90 men and 10 women had blood samples taken. Their IgG and IgA antibodies against *C pneumoniae* were measured by means of a microimmunofluorescence test. Unpaired *t* tests, multiple linear regression analyses, and logistic regression analyses were used for statistical analysis.**

***Results:*** **A total of 44% (95% CI, 31%-55%) of the men with an AAA had an IgA titer of 64 or more, an IgG titer of 128 or more, or both, compared with 10% of the women with an AAA (OR = 7.2; 95% CI, 1.05-160.8). A titer of IgG of 128 or more was significantly associated with higher expansion (5.3 vs 2.6 mm per year), even after adjustment for initial AAA size and age. A significant positive correlation between both IgA and IgG titers and mean annual expansion was observed ($r$ = 0.28; 95% CI, 0.05-0.49; and $r$ = 0.45; 95% CI, 0.24-0.62, respectively), persisting after adjusting for initial AAA size and age. An IgG titer of 128 or more was present significantly more often in cases with an expansion greater than 1 cm annually (adjusted OR = 12.6; 95% CI, 1.37-293).**

***Conclusion:*** **A high proportion of men with an AAA has signs of infection with *C pneumoniae*. The progression of their AAAs was positively correlated with the presence of indicators of *C pneumoniae* infection. (J Vasc Surg 2001;34:212-5.)**

An association between *Chlamydia pneumoniae*, atherosclerosis, and the risk of acute myocardial infarction has been shown by means of seroepidemiological studies,[1-6] and *C pneumoniae* antigen has been detected in atherosclerotic lesions both from coronary and carotid arteries and in abdominal aortic aneurysms (AAAs) in several studies.[7-13] However, the culture of *C pneumoniae* from atherosclerotic lesions has only been observed twice. Two relatively small controlled clinical trials in which patients with ischemic heart disease were randomized into antibiotic treatment or placebo groups were conducted.[14,15] Both trials showed a significant reduction in serious end points for ischemic heart disease in patients who were receiving macrolide. It is not known whether this is caused by the eradication of *C pneumoniae* organisms or by the nonspecific anti-inflammatory effect of the macrolide.[16] The relationship between infection and atherosclerotic disease therefore remains unknown, and the clinical importance of detecting the organism is uncertain.

Surgery is not usually recommended for small AAAs; however, some small AAAs continue to expand to dimensions that carry a high risk of rupture. For this reason, patients with a small AAA are offered regular ultrasonographic scans. This group of patients provides a unique opportunity for studying the progress of the disease and its possible association with signs of chronic *C pneumoniae* infection. In 1998, the Viborg Study[17] in Denmark reported a significant correlation between IgA antibodies and aneurysmal expansion. However, the significance was only noticed with an IgA titer-cutpoint that was lower than the usual titer that indicates recent or present infection. Furthermore, no correlation between titer and expansion rate was noticed. Consequently, to confirm the results of clinically relevant aneurysms with a longer observation period and higher expansion rate, we have studied cases referred for surgical evaluation from the Chichester Screening Study.

## MATERIALS AND METHODS

As part of an ongoing screening program in the Chichester area (UK) that started in 1983, more than 10,000 65- to 80-year-old men and women were invited to undergo B-mode ultrasonographic scanning screening for AAA.[18] The attendance rate was 64%. An AAA was defined as an aortic diameter of 30 mm or greater in the anteroposterior or transverse plane, and 5.8% of the participants had an AAA. Participants identified as having an aortic diameter of 3.0 to 5.9 cm were offered follow-up examina-

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**Table I.** *Chlamydia pneumoniae* seropositivity among men and women with AAAs

| | *Mean age (y)** | *Mean initial AAA size (mm)** | *IgA titer ≥ 64†* | *IgG titer ≥ 128†* | *IgA titer ≥ 64 or IgG titer ≥ 128†* |
|---|---|---|---|---|---|
| Men (n = 90) | 70.1 (4.91) | 45.4 (12.8) | 22% (14%-32%) | 39% (29%-49%) | 44% (31%-55%) |
| Women (n = 10) | 74.0 (5.58)‡ | 56.6 (14.7)‡ | 10% (0.3%-44%) | 10% (0.3%-44%) | 10% (0.3%-44%) |
| OR | | | 2.57 (0.29-58.4) | 5.73 (0.68-128.1) | 7.20 (1.05-160.80)‡ |

*SD listed in parentheses.
†95% CI listed in parentheses.
‡$P < .05$.

tions at 1-year or 3-month intervals, depending on the aortic size, to check for any expansion. Patients with AAAs exceeding 6 cm in diameter or expanding more than 1 cm annually or in whom symptoms attributable to the aneurysm developed were considered for surgery. A total of 110 patients with an AAA that was considered for surgery were seen as outpatients. Of these, 90 men and 10 women had IgG and IgA antibodies against *C pneumoniae* measured by means of microimmunofluorescence tests.[19] Ten cases were excluded because of refusals and errors in the sampling.

Expansion was calculated as the change in the anteroposterior diameter during the whole observation period, transformed to annual units.

SPSS 6.1 software (SPSS Inc, Chicago, Ill) was used as a means of performing unpaired *t* tests and multiple linear regression analyses on the mean annual expansion rates, and $\chi^2$ tests and logistic regression analyses were used as a means of assessing annual expansion rates less or greater than 3, 5, and 10 mm as a dependent variable.

## RESULTS

The prevalence of chlamydial seropositivity in cases varied from 10% to 44%, depending on patient sex and type of antibodies. They are listed and compared in Table I. The presence of infection, defined as an IgG titer of 128 or more or an IgA titer of 64 or more, was significantly more common in men with an AAA than in women with an AAA (OR = 7.20; 95% CI, 1.05-160.8).

For expansion, the observation time varied from 0.5 to 11.5 years (mean time, 4.1 years), and the average annual expansion rate was 3.5 mm (range, 3-16 mm).

By means of bivariate analysis, an IgA titer of 64 or more, an IgG titer of 128 or more, or raised IgA or IgG titers was associated with increased mean annual expansion and an annual expansion rate greater than 3 and 10 mm (Table II). After adjustment for initial AAA size and age, an IgG titer of 128 or more remained significantly associated with mean annual expansion and an annual expansion rate greater than 3 and 10 mm (Table II).

Finally, a positive correlation between both IgA titer and IgG titer and mean annual expansion rate was noticed ($r = 0.28$; 95% CI, 0.05-0.49; and $r = 0.45$; 95% CI, 0.24-0.62, respectively). This correlation remained significant after adjustment for initial AAA size and age.

## DISCUSSION

The microimmunofluorescence test is specific for the *C pneumoniae* species and allows detection of the various immunoglobulin types against *C pneumoniae*. IgA antibody titers usually decline and disappear 3 to 12 months after infection. An elevated level of IgA of 64 or more is thus believed to indicate a recent or present infection.[2] IgG antibodies may persist for some years.[1,20-26] Only IgG titers of 128 or more are thought to indicate recent or present infection.[2] IgM antibodies are not detectable in reinfections and persistent infections. Consequently, we only measured IgA and IgG titers. We would have liked to take repeated measurements, but this was not possible for practical reasons.

Finally, the used titers to define recent or present infections are unvalidated and were based on the opinions from the experts. We are especially concerned about the validity in diagnosing chronic infections. In an attempt to find a better method, nested polymerase chain reaction (PCR) identifying chlamydial DNA in monocytes in peripheral blood has been introduced. Almost all PCR-positive peripheral monocytes are also seropositive, but one third of the seropositive are not PCR-positive. We do not know which method gives the truth. It seems logical that not all chronic infections have permanent bacteremia. Furthermore, Dr Boman, who has taken part in the development of nested PCR and has detected chlamydial DNA in AAA walls, cannot find any cases with PCR-positive peripheral monocytes among these patients with an AAA. Consequently, we believe the definitions we used as indicators of infection are currently as optimal as possible.

Similar serological identification of *C pneumoniae* infection occurred in 44% of our patients with an AAA. Saikku et al[1] found that 50% of 40 men with acute myocardial infarction and 68% of 30 men with angina pectoris, but only 17% of 41 matched control subjects, had raised levels of IgG and IgA antibodies against *C pneumoniae*. The seroprevalence therefore seems to be similar to that seen in men with an AAA.

The Viborg Study[17] reported that a lower IgA titer of 32 or more was associated with aneurysmal expansion, but the study was not able to show any positive correlation between titers and expansion rate, nor could it demonstrate that a titer indicating recent or present infection with *C pneumoniae* was associated with expansion. Our data have suggested that serological signs of recent or present infection were associated with increased aneurysmal expansion and also demonstrated a positive correlation between titers and expansion. This correlation

**Table II.** *Chlamydia pneumoniae* seropositivity compared with mean annual expansion rate and annual expansion rates less or greater than 3, 5, and 10 mm of AAAs in men

| | *IgA titer ≥ 64* | | *IgG titer ≥ 128* | |
|---|---|---|---|---|
| | *Yes (n = 20)* | *No (n = 70)* | *Yes (n = 35)* | *No (n = 55)* |
| Mean annual expansion (mm/y) | 5.4* | 3.2 | 5.3† | 2.6 |
| | *Crude OR* | *Adjusted OR* | *Crude OR* | *Adjusted OR* |
| > 3 mm annual expansion (no, n = 53; yes, n = 37) | 1.93 (0.65-5.71) | 1.70 (0.43-6.78) | 2.83* (1.10-7.33) | 2.37† (1.07-6.75) |
| > 10 mm annual expansion (no, n = 83; yes, n = 7) | 6.22* (1.03-39.8) | 5.07† (1.03-41.6) | 12.6* (1.37-293.0) | 12.3† (1.23-319.2) |

*$P < .05$ (univariate analysis).
†$P < .05$ (multiple analysis adjusting for age and initial AAA size).

suggests that the grade of the immunological response plays a part in the degenerative response and that serological monitoring could be relevant in assessing the disease progression.

In contrast to the Viborg Study,[17] our data showed the strongest correlations between expansion and IgG; the mean annual expansion rate was twice as high in serologically defined cases of infection with an IgG titer of 128 or more. This remained significant after adjusting for age and initial AAA size. This adjustment was made because the IgG seroprevalence increases with age,[27] and the most powerful predictor of aneurysmal expansion is the initial AAA size.[18,28-32] The reason IgG was stronger than IgA could be that the longer observation times made the persistence of the IgG antibodies more relevant. In an earlier paper, we reported the validation of our ultrasonographic scanning measurements of the AAA diameter[33]; the variance of the measurements was 1 to 3 mm, which was similar to that of other studies.[28,34,35] Variation of measurements will always diminish associations, and it could be reduced with the use of computed tomography scans. However, the costs of the screening program would then have increased considerably. The observed AAAs expanded an average of 15 mm. Consequently, the influence of the interobserver variation must have been limited. Because of the interobserver variation and the analysis of clinically relevant expansion, logistic regression analyses were performed with an annual expansion rate greater than 3 mm and more than 10 mm as dependent variables, adjusting for age and initial AAA size. They were all significantly dependent on a titer indicating recent or present infection with *C pneumoniae*, with an adjusted OR varying from 2.8 to 12.6, except for an IgA titer of 64 or more. This was greatest in cases with rapid expansion higher than 1 cm annually. However, the wide CIs caused by few numbers must be kept in mind, because it indicates more uncertain data for this subgroup.

The Viborg Study[17] could not demonstrate an association with clinically important expansion of more than 10 mm annually.[36,37] The reason our study could show such a high OR, whereas the Viborg Study could not, is probably because our sampling was carried out on patients considered for surgery, which includes more cases with rapid expansion than those sampled at the initial diagnosis.

A possible explanation for the correlation between expansion rates and chlamydial antibody titers is that men with more severe atherosclerotic diseases have a higher aneurysmal expansion rate, and the observed higher titers are caused by the severe atherosclerosis.[29,30,38] No prospective studies have been able to demonstrate coexisting atherosclerosis as a risk factor for expansion; the available data suggest the reverse, that lower ankle/brachial systolic blood pressure was associated with a lower aneurysmal expansion rate.[31]

We think our observations suggest that infection with *C pneumoniae* may play a clinically relevant part in the pathogenesis of AAAs.

Macrolide treatment of *C pneumoniae* seemed to improve the outcome of ischemic heart disease in the 2 intervention studies aforementioned.[14,15] However, the numbers were small, and the effect may have been caused by removal of thrombogenic risk factors with eradication of the bacteria or by the nonspecific anti-inflammatory and antioxidative effects of macrolide.[16] Thus, the role of *C pneumoniae* in the pathogenesis of atherosclerosis remains unknown. Our results suggest that the organism takes part in vascular degenerative processes, and macrolide treatment should be considered in cases in which patients are unfit for surgery while we are waiting for the conclusions of randomized intervention trials.

Finally, large prospective studies with serological surveillance including nested PCR-analysis of peripheral blood would be interesting as a means of identifying a priori cases for surgery.

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