

Impact of Serological Methodology on Assessment of the Link between *Chlamydia pneumoniae* and Vascular Diseases

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We assessed the impact of five serologic tests on the link between *Chlamydia pneumoniae* and abdominal aortic aneurysms (AAA). The results of the tests were inconsistent. Agreement among the five tests was generally poor. Detection of the link between *C. pneumoniae* and AAA depends on the serologic methodology chosen.

Seroepidemiologic studies that investigated the link between *Chlamydia pneumoniae* and vascular diseases have reported inconsistent results, ranging from a strong link to no link at all (3). This discrepancy might be due to methodological factors (15). Two recent studies have demonstrated that the detection of a link between *C. pneumoniae* and coronary artery disease depends on the choice of serologic methods (6, 15). The serologic link between *C. pneumoniae* and vascular diseases has been assessed by microimmunofluorescence tests (MIF) and enzyme-linked immunosorbent assays (ELISA). However, these serologic tests lack sufficient reliability and standardization (4). A poor agreement among the results of these tests has been reported (6, 13, 15).

In this case-control study, we investigated whether the choice of serologic tests influences the detection of a link between *C. pneumoniae* and abdominal aortic aneurysms (AAA). Moreover, we evaluated the agreement among the results of these tests.

The study population used was previously described (9). Patients with AAA and healthy controls were included and matched by age and sex. Serum samples were tested for the presence of *C. pneumoniae* immunoglobulin G (IgG) antibodies by five serologic tests, i.e., the *Chlamydia* MIF IgG (MRL Diagnostics, Cypress, Calif.), the *Chlamydia* IgG SeroFIA (Savyon Diagnostics Ltd., Ashdod, Israel; Savyon MIF), the *Chlamydia* IgG rELISA (Medac Diagnostica, Hamburg, Germany), the SeroCP IgG (Savyon Diagnostics Ltd.; Savyon ELISA), and the Elegance *C. pneumoniae* IgG ELISA (Bioclone, Marrickville, Australia).

The MRL MIF uses, as the antigen, purified *C. pneumoniae* (strain TW 183) elementary bodies (EB) diluted in 3% yolk sac to add contrast to the background. According to the manufacturer's product information, the EB are purified by removing the genus-specific lipopolysaccharide (LPS). The Savyon MIF and the Savyon ELISA also use purified *C. pneumoniae* (strain TW 183) EB as the antigen. The Medac rELISA uses a recombinant LPS fragment as the antigen. For the Bioclone

ELISA, purified *C. pneumoniae* outer membrane protein complexes are used as the antigen. All tests were performed and interpreted in a blinded fashion by the same technician, according to the manufacturers' instructions.

TABLE 1. Baseline characteristics of patients with AAA and control subjects^a

Characteristic	No. of patients	No. of control subjects	P value
Age (yr) (range)	69 (45–85)	67 (44–83)	NS ^c
Male/female	81/7	81/7	NS
Aneurysm			
Under control	35		
Operated	53		
Elective	40		
Symptomatic	7		
Ruptured	6		
Family history of aneurysm	16	9	NS
History			
Myocardial infarction	26	9	<0.01
Cerebrovascular disease	9	4	NS
Pulmonary embolism	2	3	NS
Chronic obstructive pulmonary disease	24	13	NS
Peripheral vascular disease	19	4	<0.01
Diabetes mellitus	6	4	NS
Smoking			
Pack-yr of cigarette smoking ^b (±SD)	34 (±33)	23 (±22)	<0.01
Medication			
Antihypertensive drugs	48	28	<0.01
Cholesterol-lowering drugs	28	8	<0.001
Nonsteroidal anti-inflammatory drugs	37	17	<0.01
Mean systolic blood pressure (mm Hg) (SEM)	159 (2.4)	158 (2.6)	NS
Mean diastolic blood pressure (mm Hg) (SEM)	85 (1.2)	87 (1.2)	NS
Mean cholesterol (mmol/liter SEM)	5.7 (1.13)	5.7 (0.10)	NS

^a Patients ($n = 88$) and controls ($n = 88$) were matched by age and sex.

^b A pack-year was defined as smoking 20 cigarettes/day for 1 year.

^c NS, not significant.

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TABLE 2. Association between *C. pneumoniae* seropositivity and AAA according to five different tests^a

Test	IgG titer or index value	No. of AAA patients	No. of controls	Odds ratio (95% CI) ^b
MRL MIF	IgG \geq 1:16	85	71	6.8 (2.0–24.1)
	IgG \geq 1:512	24	21	1.2 (0.6–1.8)
Savyon MIF	IgG \geq 1:64	79	79	1.0 (0.4–2.7)
	IgG \geq 1:512	21	20	0.9 (0.5–1.8)
Medac rELISA	IgG \geq 1:100	46	48	0.9 (0.5–1.7)
	IgG \geq 1:400	23	18	1.4 (0.7–2.8)
Savyon ELISA ^c	Index > 1.10	67	71	0.8 (0.4–1.6)
Bioclone ELISA ^d	Index > 1.10	84	85	0.7 (0.2–3.4)
	Index > 3.0	20	16	1.3 (0.6–2.8)

^a Eighty-eight patients and 88 controls were tested.

^b CI, confidence interval.

^c For the Savyon ELISA, high titers were not found.

^d According to test kit instructions, an index value of > 3 corresponds to an IgG titer of >1:512.

We used odds ratios and a 95% confidence interval for estimating the relative risk. Kappa (κ) values were used to assess the agreement among the tests. The following guidelines were used in the interpretation of κ : if κ was <0.2, agreement was poor; if κ was 0.21 to 0.4, agreement was fair; if κ was 0.41 to 0.6, agreement was moderate; if κ was 0.61 to 0.8, agreement was good; and if κ was 0.81 to 1.0, agreement was very good (1). *P* values of <0.05 were considered statistically significant.

The study population included 88 patients with AAA and 88 healthy controls. The characteristics of the study population are shown in Table 1. The results of the five tests were inconsistent (Table 2). In the patient group, seropositivity rates varied from 52% (46 of 88 patients) with the Medac rELISA to 97% (85 of 88) with the MRL MIF. In the healthy controls, a similar variation was found: 55% (48 of 88 controls) were positive with the Medac rELISA compared to 97% (85 of 88) with the Bioclone ELISA.

The MRL MIF was the only test that demonstrated a significant link between *C. pneumoniae* and AAA. The other four tests failed to demonstrate any link. However, the MRL MIF

also failed to demonstrate a link when higher IgG titers were used as cutoffs. Although the results of the two MIF used in our study were read by the same technician, there was poor agreement between the two tests. This implies that in addition to the subjective reading of MIF results, other factors may contribute to the disagreement among results of *C. pneumoniae* serologic tests. The test procedure, the type of antigen, the antigen's purity, and the concentration of the antigen may also account for poor agreement among the results of these serologic tests (5). The link between low titers of *C. pneumoniae* IgG and AAA, demonstrated by the MRL MIF, might be the result of a cross-reaction to the antigen used in the test from sources other than *C. pneumoniae* (8). These sources, either infectious or noninfectious, might be associated with AAA and confound the association between *C. pneumoniae* and AAA.

The agreement among the results obtained by the five serologic tests was generally poor (Table 3). Inter- and intralaboratory variations and a poor agreement among results of serologic tests of *C. pneumoniae* have also been demonstrated by others (6, 13, 15). Ranges of agreement from 59 to 90% have been reported (13, 15). Hoymans et al. (6) found poor agreement between results of the MIF and the Medac rELISA, but three other ELISA showed moderate to good agreement in results with the MIF (6).

There is evidence that *C. pneumoniae* serologic tests are less specific than previously realized (5, 8, 11). Cross-reactivity between *C. pneumoniae* and *Chlamydia* species in the MIF has been demonstrated (8, 11). This is probably due to a lack of LPS removal from the EB during antigen preparation (11). It is also possible that *Chlamydia*-like microorganisms, *Bordetella pertussis* and parvovirus, cause serologic antigenic cross-reactivity with *C. pneumoniae* (6, 7, 10, 12, 14).

Our results support the findings of recent studies which have shown that methodology has an important impact on whether a link is found between *C. pneumoniae* and vascular diseases (6, 15). This indicates that methodological factors are partly responsible for the conflicting results in the literature concerning the role of *C. pneumoniae* in the development of vascular diseases (2, 3).

This study shows that the detection of a serologic link between *C. pneumoniae* and AAA depends on which test is used

TABLE 3. Agreement of κ values between the serological results of the five different tests for the patient group and the healthy controls

Group and test	IgG titer or index value	κ value ^a			
		Savyon MIF	Medac rELISA	Savyon ELISA	Bioclone ELISA ^b
Patients					
MRL MIF	IgG \geq 1:16	0.12	0.02	0.14	0.55
Savyon MIF	IgG \geq 1:64		0.12	0.45	0.42
Medac rELISA	IgG \geq 1:100			0.09	0.05
Savyon ELISA	Index > 1.10				0.26
Controls					
MRL MIF	IgG \geq 1:16	0.11	0.06	0.12	0.25
Savyon MIF	IgG \geq 1:64		0.09	0.55	0.47
Medac rELISA	IgG \geq 1:100			0.15	0.08
Savyon ELISA	Index > 1.10				0.25

^a κ expresses the agreement between the tests regarding nominal scale variables (positive and negative results).

^b The Bioclone ELISA had an index value of >1.10.

to measure *C. pneumoniae* antibodies. Further studies should focus on optimizing and standardizing *C. pneumoniae* serologic methods.

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