Relationship between peripheral arterial occlusive disease (PAOD) and chronic *Chlamydophila* (*Chlamydia*) *pneumoniae* infection

A meta-analysis

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Summary

We carried out a meta-analysis of observational case-control studies published before May 2004 to assess the degree of association between *Chlamydophila pneumoniae* (Cp) infection and PAOD. A search of the Medline database was performed using atherosclerosis and "Chlamyd* pneumoniae" as keywords. Strict criteria were applied for the selection of case studies, which had to be studies of Cp seroprevalence or of Cp detection in patients versus controls. Forty-three published studies that met these criteria were selected. An association between PAOD and Cp was revealed by immunohistochemical analysis (*OR*=15.4, 95%CI=5.0–46.9) and nested PCR studies of arterial biopsies (*OR*=4.3, 95%CI=1.8–10), by PCR study of non-arterial samples (*OR*=2.9, 95%CI=1.2–7.0), by other direct-detection tests (*OR*=16.7, 95%CI=7.0–39.8), and by ELISA and MIF tests to detect high IgG (*OR*=2, 95%CI=1.1–3.5 and *OR*=1.7, 95%CI=1.0–2.9, respectively) and IgA (*OR*=1.9, 95%CI=1.1–3.4 and *OR*=1.5, 95%CI=1.1–2.0, respectively) titers. No significant association was found in simple PCR studies of arterial biopsies, MIF tests to detect low IgG titers or IgM, or ELISA studies to detect IgM. According to this review, the association between Cp infection and PAOD depends on the analytical method adopted. Establishing a relationship between Cp and PAOD will require a case-control study with an adequate number of cases and samples that uses a combination of direct and indirect techniques to identify the presence of the bacterium in different types of sample from the same subjects, correlating the results with the activity of the disease.

Keywords

Bacteria, atherosclerosis, clinical / epidemiological studies

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Introduction

In recent years, there has been considerable research into the inflammatory phenomena (1) that occur in atherosclerosis (AT). Besides classic risk factors, the possible role of chronic infection by *Helicobacter pylori*, *Chlamydophila* (*Chlamydia*) *pneumoniae* (Cp), or cytomegalovirus has been proposed. These may participate in the genesis of atherothrombosis by maintaining a state of chronic inflammation, as demonstrated in many cross-sectional and prospective studies (2). Cp infection has been one of the most widely researched factors, using various laboratory tests. Studies relating Cp infection to vascular risk can be grouped into those offering epidemiological evidence and those offering pathophysiological evidence. The former category includes association studies using serology (3) or bacteria demonstration (4) and, more recently, clinical studies on the progression of AT under the influence of the infection or a specific treatment, such as the antibiotic used in secondary prophylaxis (5, 6). Studies in the pathophysiological group offer powerful arguments supporting an association between Cp infection and increased vascular risk (7), based on evidence of participation by Cp in the capture and oxidation of LDL, activation of proinflammatory transcription factors related to atherogenesis and proliferation of smooth muscle cells, and on the demonstration that hypocholesterolemic therapy with statins reduces the inflammatory response to Cp (8). Nevertheless, it remains controversial

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Studies	Localization Of Disease Cases / Controls	Adjusted	Cases (N°) / Controls (N°)	Methodology
Alakärppä (4)	AA / hA	No	8 / 3	Direct
Al-Amro (10)	Ath A / Arteries	No	43 / 28	Direct
Berger (11)	Ath A / Arteries	No	60 / 51	Direct
Chiu (12)	Carotid / Arteries	No	76 / 20	Direct
Cook (3)	Stroke / Stroke-free	No	176 / 1518	Indirect
Elkind (13)	Stroke / Stroke-free	No	89 /89	Indirect
Farsak (14)	Carotid + aA / hA	Internal	36 / 39	Direct
Freidank (15)	Carotid / Healthy subjects + Other diseases	Yes	60 / 109	Direct
Gil-Madre (16)	Stroke / Stroke-free	Yes	91 / 112	Indirect
Glader (17)	Stroke / Stroke-free	Yes	97 / 197	Indirect
Goo (18)	AA + aA /hA	No	5 / 2	Direct
Gutiérrez (19)	Ath A / Arteries	Yes	85 / 50	Direct
Gutiérrez-Fernández (20)	Ath A / Arteries	No	66 / 50	Indirect
Juvonen (21)	AA / hA	Yes	12 / 9	Direct
Kalayoglu (22)	Ath A / Arteries	No	25 / 18	Indirect
Kol (23)	Carotid / hA	No	19 / 7	Direct
Kuo (24)	Aa / hA	Yes	21 / 4	Direct
LaBiche (25)	Carotid / Carotid	No	37 / 57	Direct Indirect
Lehto (26)	Ath A / Arteries	No	215 / 1158	Indirect
Lin (27)	Ath A / Arteries	No	200 / 24	Direct
Linares-Palomino (28)	Carotid / Arteries	Yes	26 / 50	Direct Indirect
Maass (29)	Ath A / hA	No	92 / 10	Direct
Maeda (30)	Carotid / Carotid-free	Yes	197/ 2439	Indirect
Melnick (31)	Carotid / Carotid-free	Yes	326 / 326	Indirect
Müller (32)	Stroke / Stroke-free	No	193 / 368	Direct
Neureiter (33)	Carotid / Carotid-free	Yes	20 /40	Direct
Ong (34)	Ath A / Arteries	No	35/8	Direct
Ong (35)	Carotid / Carotid-free	Internal	41 / 41	Direct
Ouchi (36)	Ath A / Arteries	No	30 /110	Direct
Ramos (37)	aA / hA	Internal	147 / 98	Direct
Rassu (38)	Ath A / Arteries	Internal	72 / 13	Direct
Rose (39)	aA /hA	No	58 / 2	Direct
Sessa (40)	Carotid / Carotid	No	18/ 33	Direct Indirect
Shi (41)	aA/ hA	No	10 / 23	Direct
Tanne (42)	Stroke / Stroke-free	Yes	134 / 134	Indirect
Tarnacka (43)	Stroke / Stroke-free	No	44 / 115	Direct
Taylor-Robinson (44)	Ath A / Arteries	Internal	24 /27	Direct
Van der Ven (45)	Ath A / Arteries	No	67 / 29	Indirect
Vécsei (46)	Ath A / Arteries	Yes	14 / 14	Indirect
Vécsei (47)	Ath A / Arteries	Yes	14 / 14	Direct Indirect
Virok (48)	MCA / MCA	No	15 / 4	Direct
Wimmer (49)	Stroke / Stroke-free	Yes	58 / 52	Indirect
Wolf (50)	Ath A / Ath A-free	Yes	106 / 116	Indirect

Localization of the Disease:
Carotid: Atherosclerotic carotid; Carotid-free: Carotid free from atherosclerosis; aA: Atherosclerotic aorta; hA: Healthy aorta; AA: Aortic Aneurysm; MCA: Middle cerebral artery; Ath A: Atherosclerotic Arteries; Arteries: Different non-atherosclerotic arteries; Control samples were atherosclerosis-free biopsies, largely from cadavers.
The "Adjusted" column relates to the comparability of the groups:
No: the clinical characteristics of cases and controls were not controlled for. Internal: control samples are from healthy areas in the same patients. Yes: at least one major risk factor for atherosclerosis was controlled for.

Table 1: Studies with an explicit methodology and defined control group that analyzed the relationship between Cp and PAOD: localization of the disease, adjustment or not of study population, number of samples studied, and direct or indirect nature of the method used to detect the infection.

whether there is a causal relationship between chronic Cp infection and PAOD. Finally, several meta-analyses have evaluated the relationship of Cp with coronary disease (9). However, most of the numerous studies performed allow no definitive conclusions to be drawn that can be applied in the treatment of patients with PAOD.

With this background, a review was conducted of all internationally available studies published before May 2004 that addressed the relationship between PAOD and Cp using defined material and methods. Their quality was assessed and a meta-analysis of their results was performed in order to determine the conclusions available to date on the relationship between the bacterium and the disease. The aim of the systematized and comprehensive analysis of these results was to establish the current state of knowledge as a guide to future research efforts.

Material and methods

An open search of the MEDLINE data base using the keywords "atherosclerosis" and "Chlamyd* pneumoniae" retrieved 793 articles published before May 2004. A subsequent selection was made of 43 articles published in English, Spanish, or French that analyzed the relationship between Cp and PAOD using a described methodology and a defined control group (3, 4, 10–50) (Table 1). A search of the references of the selected articles confirmed that no studies had been missed. Because of the wide diversity of the studies, they were stratified according to the laboratory test used prior to the meta-analysis.

The meta-analysis has a qualitative and a quantitative component. The former is an epidemiologic description of the articles, considering the individual studies as the research subject. The latter corresponds to a statistical pooling of results, reporting estimations of the *Odds Ratio* (*OR*) with a weighting of estimations of individual studies, giving the 95% confidence interval (CI) of the *OR*. The weighting is expressed as the percentage weighting of the study with respect to the weighting of all the articles under consideration. The DerSimonian-Laird method was used to pool values reported by the studies (51) because it provides global estimations that are less affected by their heterogeneity. This heterogeneity was measured using the Mantel-Haenszel (Qexp) test. Possible publication biases were examined using the Rosenthal tolerance index (52) (RI), which assigns a higher value for lower publication bias. There was considered to be no relationship between exposure to Cp and presence of PAOD when the confidence interval included unity (53). Finally, the Newcastle-Ottawa (NOS) (54) scale was used for the quality assessment (Table 2). This scale measures the quality of the article according to three main criteria: *selection*, considering the adequacy of case selection, representativeness of cases, and definition and selection of controls; *comparability*, focused on the comparability of cases and controls on the basis of the design or analysis; and *exposure*, related to the ascertainment of exposure

for cases and controls and the non-response rate. A maximum score of four stars is possible for *selection*, two stars for *comparability*, and three stars for *exposure*.

Results

Nested PCR in arterial biopsies (Table 3)

Most of these studies used carotid artery samples (15, 19, 25, 28, 29, 35, 40). The high Rosenthal Index obtained (RI=161) indicates that publication bias was low. The *ORs* obtained were not homogeneous (Qexp=30.2, p=0.001). Despite the wide variability among the studies, the global *OR* was 4.3 (95%CI=1.8–10, p=0.0006), demonstrating a clear relationship between PAOD and exposure to the bacterium. We highlight the quality of the studies by Freidank et al. (15), Gutiérrez et al. (19), and Linares-Palomino et al. (28), which showed a significant Cp-PAOD association, and that of the study by LaBiche et al. (25), which did not.

Simple PCR in arterial biopsies (Table 4)

The bacterium was not detected in controls in any of these studies and was not detected in any cases in two of them (39, 47). A study by Juvonen et al. (21) of abdominal aortic aneurysms showed the highest *OR* [247], followed by a study by Farsak et al. (14) (*OR*=31.3) of biopsy samples of carotid and abdominal aorta. The *ORs* obtained by these studies were not homogeneous (Qexp=12.2, p=0.01, RI=13). The overall estimated *OR* was 3.7 (95%CI=0.2–59.2, p=0.3), indicating no association between PAOD and Cp exposure. The studies of highest quality were those by Vécsei et al. (47), Farsak et al. (14), and Juvonen et al. (21), with the last two showing a significant association.

Other PCR studies (Table 4)

These were performed in non-arterial samples using various laboratory tests. Overall, 22.3% of PAOD cases were Cp-positive versus 10.3% of controls. Only two studies associated exposure to the bacterium with the disease: one by Al-Amro et al. (10), which obtained the highest *OR* but had the lowest weighting, and another by Sessa et al. (40) in mononuclear cells. The studies with highest weighting were by Müller et al. (32), which showed the lowest *OR*, and Berger et al. (11). Taken together, the studies reported an *OR* of 2.9 (95%CI=1.2–7.0, p=0.01), affirming an association between PAOD and exposure to the bacterium. There was a wide heterogeneity among these studies (Qexp=11.9, p=0.01). The Rosenthal Index (RI=20) was high given the number of studies assessed.

Immunohistochemical studies (Table 5)

Most of the immunohistochemical studies used anti-elemental body (EB) or anti-major outer membrane protein (MOMP) antibodies. Most studies obtained a similar percentage of positive results among PAOD cases, which was 10-fold higher than among controls. The study by Juvonen et al. (21) obtained the highest *OR* but was given the lowest weighting. Studies by Neureiter et al. (33), Ouchi et al. (36) and Shi et al. (41) also showed high *ORs* and were given a high weighting. Taken together, the studies reported an estimated *OR* of 15.4 (95%CI=5.0–46.9, p=0.00000), showing a very strong association between PAOD and Cp expo-

Table 2: Newcastle – Ottawa Quality Assessment Scale.
Case-control studies.

Selection
1) Is the case definition adequate? a) yes, with independent validation* b) yes, e.g., record linkage or based on self reports c) no description
2) Representativeness of the cases a) consecutive or obviously representative series of cases* b) potential for selection biases or not stated
3) Selection of controls a) community controls* b) hospital controls c) no description
4) Definition of Controls a) no history of disease (endpoint)* b) no description of source Comparability
Comparability
1) Comparability of cases and controls on the basis of the design or analysis a) study controls for age* b) study controls for any additional factor*
Exposure
1) Ascertainment of exposure a) secure record (e.g., surgical records)* b) structured interview blinded to case/control status* c) interview not blinded to case/control status d) written self report or medical record only e) no description
2) Same method of ascertainment for cases and controls a) yes* b) no
3) Non-response rate a) same rate for both groups* b) non-respondents described c) different rates and no designation
Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Table 3 Statistical calculation of the results and quality assessment of the nested PCR studies in biopsies.

Studies: Gene / Sensitivity-IFU-Testing	Cases	Controls	Statistical Analysis		Quality (Score)		
	% Positive	% Positive	*OR*	95% CI	S	C	E
Freidank (15).*pstI* gene / 0.01	16.7	0	21.0	1.2–368.0	***	**	***
Gutiérrez (19).*pstI* gene / NA	56.5	12	9.5	3.6–24.7	**	**	***
LaBiche (25).*pstI* gene / NA	13.5	15.8	0.8	0.2–2.7	***		***
Linares-Palomino (28).*pstI* gene / NA	69.2	12	16.5	5.0–54.3	***	**	***
Maass (29).*pst* gene / NA	15.2	0	3.8	0.2–69.9	**		***
Ong (34).*ompA* gene / 10	42.8	50	1.0	0.0–51.6	**		***
Ong (35).*ompA* gene / 0.001	0	0	0.7	0.1–3.5	**	**	***
Rassu (38).*ompA* gene / NA	76.4	53.8	2.7	0.8–9.3	*	*	***
Sessa (40).*pstI* gene / NA	44.4	30.3	1.8	0.5–6.0	**		***
Shi (41). CP*I*-*CP2* & *CPC*-*CPD* primers / NA	10	0	7.4	0.2–198.8	*		***
Taylor-Robinson (44).*ompA* gene / 10	62.5	0	89.7	4.8–1649.2	*	**	***
Virok (48).*ompA* gene / NA	33.3	0	4.7	0.2–104.5	*		***
Events rate(%)	**37.7**	**9.9**	*OR*=4.3 (95%CI=1.8–10, p=0.0006)				

NA: not available, *OR*: odds ratio, CI: confidence interval, S: selection, C: comparability, E: exposure.

Table 4: Statistical calculation of the results and quality assessment of other PCR studies.

Studies	Cases	Controls	Statistical Analysis		Quality (Score)		
Simple PCR of Arterial Biopsy: Gene/Sensitivity Testing	% Positive	% Positive	OR	95%CI	S	C	E
Farsak (14).*RNA16s* gene / NA	27.7	0	31.3	1.7–557.8	***	**	***
Juvonen (21).*ompA* gene / NA	100	0	247.0	4.3–14106.0	**	*	***
Lin (27).*pst1* gene / NA	3.5	0	1.9	0.1–34.2	**		***
Rose (39).*pst1* gene / NA	0	0	0.0	0.00–2.0	**		**
Vécsei (47).*RNA16s* gene / NA	0	0	1.0	0.0–53.8	**	**	***
Events rate(%)	**7.5**	**0**	*OR*=3.7 (95% CI=0.2–59.2, p=0.3)				
Other PCR of Non-Arterial Samples: Gene/Material/Sensitivity Testing	**Cases**	**Controls**	**Statistical Analysis**		**Quality (Score)**		
	% Positive	% Positive	OR	95% CI	S	C	E
Al-Amro (10).pst1 gene Nested/ Lymphocytes/ NA	53.5	3.6	31.0	3.8–249.5	**		***
Berger (11).*RNA16s* gene/Leukocytes / 10 cp	17.1	13.7	1.5	0.5–4.3	***		**
Müller (32).*53-kDa* protein gene Nested/ Mononuclear cells/ NA	10.4	7.9	1.3	0.7–2.4	***		***
Sessa (40).*pst1* gene Nested/ Mononuclear cells/NA	72.2	30.3	5.9	1.6–21.3	**		***
Sessa (40).*pst1* gene Nested / Lymph nodes / NA	33.3	18.2	2.2	0.6–8.4	**		***
Events rate (%)	**22.3**	**10.3**	*OR*=2.9 (95% CI=1.2–7.0, p=0.01)				

NA: not available, *OR*: odds ratio, CI: confidence interval, S: selection, C: comparability, E: exposure.

Table 5: Statistical calculation of results and quality assessment of studies using direct detection methods other than PCR in arterial biopsies.

Non-Immunohistochemical Studies: Test	Cases	Controls	Statistical Analysis		Quality (Score)		
	% Positives	% Positives	OR	95% CI	S	C	E
Alakärppä (4).DNA-DNA Hybridization	62.5	0	11.0	0.4–84.3	*		**
Neureiter (33).Western-blot	65	2.5	72.4	8.1–645.4	**	*	**
Ouchi (36). Isolation	0	0	3.6	0.0–186.3	**		**
Ramos (37).EM	31.3	0	90.2	5.4–1484.8	*	**	**
Rose (39).Immunofluorescence	81	0	20.6	0.9–460.2	**		**
Rose (39).EM	0	0	0.1	0.0–7.2	**		*
Shi (41).DNA-DNA Hybridization	50	4.3	22.0	2.0–232.1	*		**
Shi (41).Southern-blot	30	0	9.8	0.4–219.2	*		*
Tarnacka (44).ELISA. IC LPS	93.2	48.7	14.4	4.2–49.1	***		**
Events rate (%)	**46**	**14.4**	*OR*=16.7 (95%CI=7.0–39.8, p=0.00000)				
Immunihistochemical Studies: Antibodies	**Cases**	**Control**	**OR**	**95% CI**	**S**	**C**	**E**
	% Positive	% Positive					
Chiu (12).anti-EB	71	0	99.3	5.7–1713.6	*		**
Goo (18).anti-MOMP	80	0	15.0	0.4–524.5	***		**
Juvonen (21) .anti-EB	66.7	0	475.0	8.6–26188.2	**	*	***
Juvonen (21).anti-LPS	100	0	35.8	1.6–769.1	**	*	***
Kol (23).anti-HSP60	47.4	0	13.5	0.6–271.0	**		**
Kuo (24).anti-EB	33.3	0	4.6	0.2–98.4	**	*	**
Neureiter (33).anti-EB	50	10	9.0	2.3–34.8	**	*	**
Ouchi (36).anti-EB	46.7	1.8	47.2	9.8–227.5	**		**
Rassu (38).anti-MOMP	66.7	83.3	0.4	0.0–3.6	*	*	**
Shi (41).anti-MOMP	30	0	21.9	1.0–474.9	*		**
Events rate(%)	**60.1**	**4.7**					

IC: immunocomplex, ME: electronic microscope, HSP: heat shock protein, MOMP: major outer membrane protein, LPS: lipopolysaccharide, OR: odds ratio, CI: confidence interval, EB: elemental body, S: selection, C: comparability, E: exposure.

sure. The *ORs* were not homogeneous among the studies (Qexp=18.4, p=0.04). The Rosenthal Index value (RI=192) indicated a low publication bias. The highest quality study was that by Juvonen et al. (21).

Studies using other direct detection methods (Table 5)

Overall, 46% of PAOD cases were positive versus 14% of controls, with similar sample sizes. Only the study by Ouchi et al. (36) failed to detect the bacterium in either cases or controls. Two studies (37, 39) used electronic microscopy, and we highlight the high number of positive cases found by Ramos et al. (37). Overall, the estimated *OR* was 16.7 (95%CI=7.0–39.8, p=0.00000) with a strong association between PAOD and Cp exposure. The study by Ramos et al. (37) showed the highest *OR* followed by that of Neureiter et al. (33). The *ORs* of the studies were homogeneous (Qexp=9.8, p=0.3), and they showed a high Rosenthal Index (RI=142). The highest quality studies were those by Neureiter et al. (33), Ramos et al. (37), and Tarnacka et al. (44).

Determination of low IgG titers by Microimmunofluorescence test (MIF) (Table 6)

Overall, the percentage of positive cases (69.2%) was similar to that of positive controls (61%). Only two studies indicated association, one by Sessa et al. (40), with the highest *OR* but lowest weighting, and another by Linares-Palomino et al. (28), who found an *OR* of 7.1. The study with highest weighting in this group was that by Melnick et al. (31). The overall estimated *OR* was 1.4 (95%CI=0.8–2.5, p=0.2), indicating no association between PAOD and Cp exposure. The *ORs* of the studies were not homogeneous (Qexp=21.3, p=0.001, RI=36). The quality of the studies was generally high, and they considered various factors in cases and controls. The studies of highest quality were by Glader et al. (17), Linares-Palomino et al. (28), and Melnick et al. (31).

Determination of high IgG values by MIF (Table 6)

These studies used serum diluted to a titer of 1/128 except for that by Cook et al. (3) (1/512). The sample sizes of cases and controls were generally large, and the studies by Cook et al. (3) and Lehto et al. (26) had the largest number of controls. Sessa et al. (40) reported the strongest association, with an *OR* of 3.6. The overall estimated *OR* was 1.7 (95%CI=1.0–2.9, p=0.02) but the *ORs* were not homogeneous (Qexp=46.8, p=0.0000). The Rosenthal Index value indicated a low publication bias (RI=97). Regarding the quality, almost all of the studies compared various factors between cases and controls.

Table 6: Statistical calculation of results and quality assessment of studies detecting IgG with MIF.

Studies: Serum Dilution (Low)/Strain	Cases	Controls	Statistics Analysis		Quality (Score)		
	% Positive	% Positive	*OR*	95% CI	S	C	E
Elkind (13).16 / AR39	80.9	83.1	0.8	0.4–1.8	***		**
Glader (17).32 / IOL207	79.4	86.3	0.6	0.3–1.1	***	**	**
LaBiche (25).16 / AR39	83.3	76.4	1.5	0.5– 4.5	***		**
Linares-Palomino (28).32 / TW183	69.2	24	7.1	2.4–20.4	***	**	**
Melnick (31).32 / AR39	47.5	40.2	1.3	0.9–1.8	***	**	**
Sessa (40).32 / NA	94.4	60.6	11.0	1.3–93.3	**		**
Wimmer (49).32 / IOL207, Parola, Kajaani 6	74.1	76.9	0.8	0.3–2.0	**	**	**
Events rate(%)	**69.2**	**61**	*OR*=1.4 (95% CI=0.8–2.5, p=0.2)				
Studies: Serum Dilution (High) Strain	**Cases**	**Controls**	**Statistical Analysis**		**Quality (Score)**		
	% Positive	% Positive	*OR*	95% CI	S	C	E
Cook (3).>512 / NA	13.6	5.7	2.6	1.6–4.2	***		**
Glader (17).>128	50.5	51.8	0.9	0.5–1.5	***	**	**
LaBiche (25).>128	61.1	56.4	1.2	0.5–2.8	***		**
Lehto (26).>128 / Kajaani 6	11.2	8	1.4	0.9–2.3	***		**
Melnick (31).>128	10.1	11	0.9	0.5–1.5	***	**	**
Sessa (40).>128	61.1	30.3	3.6	1.0–12.0	**		**
Events rate(%)	**32**	**14.3**	*OR*=1.7 (95% CI=1.0–2.9, p=0.02)				

NA: not available, *OR*: odds ratio, CI: confidence interval, S: selection, C: comparability, E: exposure.

Table 7: Statistical calculation of results and quality assessment of studies detecting IgA or IgM with MIF.

IgA Studies: Serum Dilution/Strain	Cases	Controls	Statistical Analysis		Quality (Score)		
	% Positive	% Positive	*OR*	95% CI	S	C	E
Elkind (13). 16	46.1	30.3	1.8	1.0–3.6	***		**
Gil-Madre (16). 32 / TW183	43.9	30.3	1.8	1.0–3.2	**	**	**
Glader (17). 16	45.4	43.1	1.0	0.6–1.7	***	**	**
LaBiche (25). 16	75	72.7	1.1	0.4–2.9	***		**
Lehto (26). 40	9.3	5.6	1.7	1.0–2.9	***		**
Sessa (40). 16	72.2	36.4	4.5	1.3–15.9	**		**
Wimmer (49). 16	20.7	23.1	0.8	0.3–2.1	**	**	**
Events rate(%)	**32.6**	**16.2**	*OR*=1.5 (95% CI=1.1–2.0, p=0.004)				
IgM Studies: Serum Dilution	**Cases**	**Controls**	**Statistical Analysis**		**Quality (Score)**		
	% Positive	% Positive	*OR*	95%CI	S	C	E
Elkind (13). 16	0	0	1.0	0.0–50.9	***		**
LaBiche (25). 16	0	1.8	0.5	0.0–12.5	***		**
Wimmer (49). 40	6.2	0	8.0	0.4–160.5	**	**	**
Events rate (%)	**1.7**	**0.5**	*OR*=1.4 (95% CI=0.3–6.2, p=0.6)				

NA: not available. *OR:odds ratio*, CI:confidence interval, S:selection, C:comparability, E:exposure.

Determination of IgA by MIF (Table 7)

Overall, the percentage of positive findings in cases (32.6%) was two-fold that in controls (16.2%). The study by Sessa et al. (40) reported the strongest association with an *OR* of 4.5. The studies with highest weighting were those by Glader et al. (17), Lehto et al. (26), and Gil-Madre et al. (16), with 22.8%, 20.8%, and 18.1%, respectively. Overall, the *OR* was 1.5 (95%CI=1.1–2.0, p=0.004). The *ORs* were not homogeneous (Qexp=7.7, p=0.2, RI=26). Finally, the study of highest quality was that by Glader et al. (17).

Determination of IgM by MIF (Table 7)

In general, few positives were obtained. No association was found between Cp exposure and PAOD (*OR*=1.4, 95%CI=0.3–6.2, p=0.6). The *ORs* of the studies were homogeneous (Qexp=1.8, p=0.7, RI=4), and the studies were generally of good quality.

Determination of IgG by ELISA (Table 8)

Overall, the percentage of positive findings among cases (64.1%) was very similar to that among controls (53.6%). The largest sample was studied by Maeda et al. (30), with 2439 controls and 197 cases. The studies by Linares-Palomino et al. (28) and Gutiérrez-Fernández et al. (20) showed high *ORs* of 17.5 and 7.9, respectively. The overall estimated *OR* was 2 (95%CI=1.1–3.5, p=0.01), showing an association between PAOD and Cp exposure. The *ORs* of the studies were not homogeneous (Qexp=53, p=0.0000). The Rosenthal Index (RI=124) indicated a low publication bias. The studies of highest quality were those by Linares-Palomino et al. (28), Maeda et al. (30), and Tanne et al. (42).

Determination of IgA by ELISA (Table 8)

We highlight the sample size used by Maeda et al. (30), with 2,439 controls and 197 cases. The percentage of positive findings in cases was 65.2% versus 35.9% in controls. Although only three studies indicated an association, those by Maeda et al. (30), Wolf et al. (50), and van der Ven et al. (45), had high weightings of 16.8%, 14.9%, and 12%, respectively. The overall estimated *OR* was 1.9 (95%CI=1.1–3.4, p=0.01) but the studies were not homogeneous (Qexp=32.2, p=0.0000). The Rosenthal Index indicated a low publication bias (RI=143). The studies of highest quality were by Maeda et al. (30) and Tanne et al. (42).

Determination of IgM by ELISA (Table 8)

The percentage of positives in cases (3.6) and controls (5.3) was similar. No association was reported between PAOD and Cp exposure (*OR*=0.7, 95%CI=0.1–3.5, p=0.7). The *ORs* of the studies were homogeneous (Qexp=0.2, p=0.9), and their quality was satisfactory.

Discussion

The present study was prompted by the absence of any published meta-analysis on the relationship between PAOD and Cp. A search of the literature revealed four meta-analyses related to this issue (55–58). Three of them addressed the association between Cp and coronary disease (55–57) and the fourth focused on Cp and clinical manifestations of atherosclerosis (58). The results obtained were variable because the studies used different

Table 8: Statistical calculation of results and quality assessment of studies detecting IgG, IgA, or IgM with ELISA.

Igg Studies: Antigen/ Manufacturer	Cases	Controls	Statistical Analysis		Quality (Score)		
	% Positive	% Positive	*OR*	95% CI	S	C	E
Gutiérrez-Fernández (20). EB / Vircell	80.3	34	1.2	0.5–3.1	**		**
Gutiérrez-Fernández (20). LPS / Medac	24.2	20	7.9	3.4–18.3	**		**
Kalayoglu (22).EB/inhouse	28	11.1	3.1	0.5–17.2	**		**
Linares-Palomino (28). EB / Vircell	76.9	16	17.5	5.3–57.2	***	**	**
Maeda (30). EB / Hitazyme	48.2	53.5	0.8	0.6–1.0	***	**	**
Tanne (42).EB / Savyon	85.8	82.1	1.3	0.6–2.5	***	**	**
van der Ven (45). Proteins / Labsystems	89.5	69	3.8	1.2–11.7	**		**
Vécsei (46). Proteins / Labsystems	78.6	85.7	1.6	0.2–11.7	**	**	**
Vécsei (46).LPS / Medac	85.7	78.6	0.7	0.1–3.3	**	**	**
Vécsei (47). Proteins / Labsystems	42.9	50	1.0	0.1–6.0	**	**	**
Vécsei (47).LPS / Medac	78.6	78.6	0.6	0.0–4.3	**	**	**
Wolf (50). EB / Savyon	66	54.3	1.6	0.9–2.8	***	*	**
Events rate (%)	**64.1**	**53.6**	OR=2 (95% CI=1.1–3.5, p=0.01)				
IgA Studies: Antigen	**Cases**	**Controls**	**Statistical Analysis**		**Quality (Score)**		
	% Positive	**% Positive**	**OR**	**95% CI**	**S**	**C**	**E**
Gutiérrez-Fernández (20). LPS	28.8	26	1.1	0.5–2.6	**		**
Maeda (30).EB	71.1	34	4.7	3.4–6.5	***	**	**
Tanne (42). EB	81.3	77.6	1.2	0.6–2.2	***	**	**
van der Ven (45). Proteins	73.1	51.7	2.5	1.0–6.2	**		**
Vécsei (46). Proteins	42.8	35.7	1.3	0.2–6.1	**	**	**
Vécsei (46). LPS	21.4	28.6	0.6	0.1–3.8	**	**	**
Vécsei (47). Proteins	50	35.7	1.8	0.4–8.1	**	**	**
Vécsei (47). LPS	28.6	35.7	0.7	0.1–3.5	**	**	**
Wolf (50). LPS	67	28.4	5.1	2.8–9.0	***	*	**
Events rate(%)	65.2	35.9	OR=1.9 (95% CI=1.1–3.4, p=0.01)				
IgM Studies: Antigen	**Cases**	**Control**	**Statistical Analysis**		**Quality (Score)**		
	% Positive	**% Positive**	**OR**	**95% CI**	**S**	**C**	**E**
Vécsei (46). Proteins	0	0	1.0	0.0–53.8	**	**	**
Vécsei (46). LPS	7.1	14.3	0.4	0.0–5.7	**	**	**
Vécsei (47). Proteins	0	0	1.0	0.0–53.8	**	**	**
Vécsei (47). LPS	7.1	7.1	1.0	0.0–17.7	**	**	**
Events rate (%)	**3.6**	**5.3**	*OR*=0.7 (95% CI=0.1–3.5, p=0.7)				

OR: odds ratio, CI: confidence interval, EB: elemental body, LPS: lipopolysaccharide, S: selection, C: comparability, E: exposure.

markers of chronic infection. PAOD shares many risk factors with coronary disease because they are both clinical manifestations of underlying atherosclerotic disease. However, the pattern of risk factors has been reported to differ for each clinical manifestation (58). Consequently, it is difficult to compare our findings with those obtained by other authors. In relation to the influence that the localization of the disease may have on the outcomes, it is unlikely to be important because AT of large vessels is a generalized disease, although a lower presence of the bacterium has been reported in the contents of aortic aneurysms in comparison with the wall, due to a lesser cellularity of the sample studied (19). Therefore, wide variations found in study results are more likely to be caused by differences among the tests used.

PCR studies

PCR can be considered the gold standard test for Cp investigations but must be performed with great care to reduce the number of false positives and negatives (59). The nested form of the assay improves the specificity and sensitivity to detect DNA. Our evaluation of the PCR studies was structured according to the clinical sample and methodology utilized. Nested PCR assays of arterial biopsies showed a relationship between the infection and the disease but simple PCR studies did not, which may be explained by the greater sensitivity of the former. It is thought that AT may be produced by chronic Cp infection in which the bacteria have low replication and little DNA presence (7). Therefore, studies of the relationship between disease and infectious agent require tests that can detect a small number of DNA copies, even just one copy. However, the results obtained using nested PCR were widely heterogeneous, probably because of the different methodologies used in the studies. Although only three different primer pairs were used in these 12 studies, the same pairs were used under different PCR conditions, explaining differences in sensitivity, which was assessed in four of these studies.

A significant relationship between Cp presence and PAOD was found in the leukocyte and lymph node samples. This may indicate that the cases have a larger circulating bacterial load versus controls and that the bacteria can reach the arterial wall. In fact, an infection model was recently described that explains how the bacterium may pass from white cells to the artery wall (60). There were differences in the white cell types analyzed and the primer pairs, which may explain the heterogeneity among these studies. The overall quality of these studies was acceptable. We highlight the low comparability between studies that related the bacterium to PAOD and those that did not.

Non-PCR direct tests

The methodology used by this group was widely heterogeneous, with very different sensitivities, specificities, and predictive values. Only one out of the ten immunohistochemical studies did not show any relationship. A relationship between Cp and PAOD was ruled out in two of the nine studies that used non-immunohistochemical direct tests. Despite this, a significant relationship was found overall, with a high *OR*. Variability in study results was, paradoxically, especially wide among immunohistochemical studies that reported a low level of significance, possibly because of the use of different monoclonal antibodies (anti-EB, anti-MOMP, anti-LPS and anti-HSP60). Although the other direct tests were very heterogeneous methodologically, their results were more homogeneous, perhaps because of the small population size. The overall quality of the studies was acceptable. The comparability among studies was not very high, neither in the studies that found an association nor in those that did not.

Antibody investigation

This section includes tests that use protein antigens and/or lipopolysaccharides (LPS) of the external membrane. The former largely contain species- and type-specific epitopes, whereas the latter have no antigenic specificity and are even present in different families of bacteria. Nevertheless, family-specific components have been localized. The elemental bodies used in laboratory tests largely contain proteins, whereas reticular bodies additionally have LPS. Finally, species-specific peptides derived from protein antigens have been synthesized, as have family-specific LPS. In the course of the disease, anti-LPS antibodies initially arise, followed by anti-protein antibodies that persist throughout life. IgG, IgA, and IgM antibodies can be investigated. IgG antibodies are produced earlier when the systemic nature of the disease is greater, as in severe Cp infections, and they are always present at the end of the clinical process. The isolated presence of IgG can only be interpreted as a previous antigenic contact with the exception of the biological phenomena of seroconversion or IgG elevation between two samples, when it is associated with current disease. If only one sample is available, current disease can perhaps be suspected when levels are especially high. The presence of IgM or IgA is associated with current disease, and the former is only present in primary infections (61).

Various laboratory tests have been used to detect the above antibodies. Complement-fixation uses antigens common to the family that are derived from complete inclusions replete with reticular and elemental bodies. From a technical standpoint, it has inadequate sensitivity to diagnose the disease in children, no value in seroprevalence studies, and false negative results are obtained in clinical recurrences. MIF, which uses LPS-purified elemental bodies, is the reference test and has demonstrated a variable specificity and very high sensitivity. In most cases, the reading or interpretation of results is easy and sample dilution can also be automated. Complement-fixation and MIF are economical in reagent costs but not in personnel time. ELISA is an indirect test that uses species-specific antigens of complete, purified elemental bodies or recombinant antigens. It is sensitive and can be automated, although the cost of reagents is higher than in complement-fixation or MIF.

The studies in this meta-analysis used ELISA or MIF to detect IgG, IgA, or IgM. Only the detection of IgA or high titers of IgG showed a relationship between Cp infection and PAOD. These findings are consistent with reports that most individuals have antigenic contact with Cp at some point of their lives and that re-infections are the norm, accounting for the absence of IgM and presence of IgA in chronic infections (62). Finally, all of these studies showed a wide heterogeneity of results with the exception of those that determined IgM and IgA (the latter by MIF). Variability in serum sample dilutions and in the method used by commercial laboratories may account for this heterogeneity, although only small antigenic differences have been found among strains used (61). Antibody studies were generally of a higher quality than most of the studies selected for the meta-analysis, and almost all of them compared various factors between cases and controls. However, the studies of highest quality showed the lowest values for an association between PAOD and Cp.

In conclusion, the finding or not of an association between PAOD and Cp depends on the method employed. Most of the laboratory tests established a relationship between PAOD and Cp, with a variable probability. No relationship was found using the less sensitive simple PCR, and IgM detection showed no positive relationship with the disease, possibly because the infection was not primary. The literature on this issue lacks a case-control study with adequate sample size that uses various microbiological techniques on the same subjects and samples and that correlates the results with the activity of the disease. Until this is undertaken, no definitive conclusion can be drawn about this association.

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