Review Article

Chlamydophila Pneumoniae Infection and Cardiovascular Disease

Rajnish Joshi, Bidita Khandelwal, Deepti Joshi[1], Om Prakash Gupta[2]

Departments of Medicine, and [1]Pathology, Sikkim Manipal Institute of Medical Sciences, Gangtok, [2]Department of Medicine, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India

Abstract

Atherosclerosis is a multifactorial vascular inflammatory process; however, the inciting cause for inflammation remains unclear. Two decades ago, *Chlamydophila pneumoniae* (formerly *Chlamydia pneumoniae*) infection was proposed as a putative etiologic agent. We performed a PubMed search using the keywords *Chlamydia* and atherosclerosis in a Boolean query to identify published studies on *C. pneumoniae* and its role in atherogenesis, and to understand research interest in this topic. We found 1,652 published articles on this topic between 1991 and 2011. We analyzed relevant published studies and found various serological, molecular, and animal modeling studies in the early period. Encouraged by positive results from these studies, more than a dozen antibiotic clinical-trials were subsequently conducted, which did not find clinical benefits of anti-*Chlamydophila* drug therapy. While many researchers believe that the organism is still important, negative clinical trials had a similar impact on overall research interest. With many novel mechanisms identified for atherogenesis, there is a need for newer paradigms in *Chlamydophila*-atherosclerosis research.

Keywords: Atherosclerosis, *Chlamydophila pneumonia*, Cardiovascular disease, Infection

Address for correspondence: Dr. Rajnish Joshi, Department of Medicine, Sikkim Manipal Institute of Medical Sciences, Gangtok, India.
E-mail: rjoshimgims@gmail.com

Introduction

Atherosclerosis is a leading cause of global mortality and morbidity, among noncommunicable disorders.[1] Atherosclerosis leads to coronary artery disease (CAD), cerebrovascular disease (CVD), and peripheral vascular disease (PVD), which have a wide clinical spectrum including stable or unstable angina (UA), acute myocardial infarction (AMI) or sudden cardiac death (SCD), transient ischemic attacks (TIA), ischemic strokes, vascular dementias, intermittent claudication, and gangrene. Further, atherosclerosis is responsible for a large burden of chronic kidney disease (CKD) and hypertensive heart diseases. At the population level, nine risk factors [smoking, history of hypertension, diabetes, waist/hip ratio, dietary patterns, physical activity, consumption of alcohol, blood apolipoproteins (Apo), and psychosocial factors] account for more than 90% of the population-attributable risk.[2] Despite these strong associations, interactions between these risk factors and an underlying inciting cause remain inadequately explained.[3]

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A number of studies have found that inflammation of the vessel wall is an important mechanism responsible for initiation, progression, sclerosis, erosion, and rupture of atherosclerotic plaques.[4] A low-grade infection either by a virus (cytomegalovirus or human herpes viruses) or a bacterium, [*Helicobacter pylori* or *Chlamydophila pneumoniae* (formerly *Chlamydia pneumoniae*)] has been suggested as a possible etiology for this inflammatory activity,[4] For the last two decades, *C. pneumoniae* has been the strongest candidate organism. *C. pneumoniae* is an ubiquitous, obligate intracellular Gram-negative bacterium, and is a common respiratory pathogen.[5] It has been shown that *C. pneumoniae* infects human mononuclear cells, and this has also been demonstrated after respiratory challenge in animal studies.[6] These infected mononuclear cells transmigrate into circulation [peripheral blood mononuclear cells (PBMC)] and secondarily infect endothelial cells

by cell-to-cell transmission of *C. pneumoniae*. This event could trigger a series of immunological phenomenon leading to atherosclerosis[6] [Figure 1]. Further, it has been demonstrated in animal studies that *C. pneumonia* infection reduces high-density lipoprotein (HDL) levels and intra-plaque hemorrhages.[7] Another mechanism for development of atherosclerosis is mediated through expression of heat shock proteins (HSP) on the endothelium. HSPs are normally located in the endothelium, and have a protective function. Anti-HSP60 antibodies induce endothelial damage, and smooth muscle proliferation. *C. pneumonia* infection leads to production of antibodies as bacterial HSP has a sequence homology with human HSP. Thus, molecular mimicry between human HSP60 and bacterial 60 k-Da HSP contributes to atherosclerosis.[8]

Studies in animal models have isolated *C. pneumoniae* from coronary,[9-11] carotid, [12,13] and peripheral arteries.[14] These isolation studies triggered a debate if association of *C. pneumoniae* infection and atherosclerosis is causal, or if the infectious agent is merely an innocent bystander.[15-17] In a landmark study, Hu and coworkers[18] demonstrated that *Chlamydophila* infection could induce atherogenesis in low-density lipoprotein (LDL)-knockout mice, only in the presence of a high-cholesterol diet. This experiment further lent credence to the hypothesis that infection and hypercholesterolemia are essential causal components leading to atherogenesis. The current article reviews *Chlamydophila* atherosclerosis literature with reference to causal significance of this association and traces investigator interest in this hypothesis from the last two decades.

Materials and Methods

We performed a PubMed search to identify studies about the role of *C. pneumoniae* in atherosclerosis. We used a Boolean query [(*C. pneumoniae*) and (atherosclerosis OR coronary OR stroke OR peripheral vascular disease OR cerebral OR hypertension OR diabetes)] and identified a total of 1,668 studies. We classified published literature by year of publication and described three distinct study designs: (A) Seroepidemiological studies; (B) Molecular studies; and (C) Clinical trials. We excluded animal and cell culture studies and have elaborated human studies alone. We included serology studies, which had used either a case-control study design at a defined cross section in time, or studies nested in well-defined community cohorts. Molecular studies included those that demonstrated *C. pneumoniae* DNA in circulating mononuclear cells or in vascular tissue using electron microscopy, molecular diagnostics, immunochemistry, or cell line culture techniques. Clinical trials included in the description were randomized control trials, which aimed at evaluating reduction in mortality or cardiovascular events with antibiotic therapy in patients with early atherosclerotic disease.

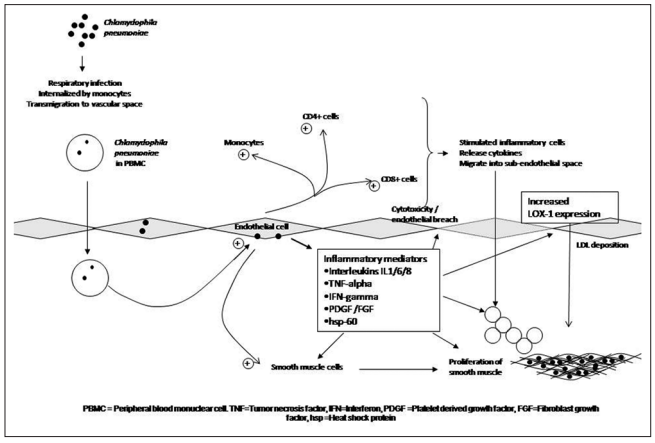

Figure 1: Postulated inflammatory pathways and role of *Chlamydophila pneumoniae* in causation of atherosclerosis

Results

The chronologic distribution of studies evaluating *Chlamydophila*-atherosclerosis association shows a peak in the year 2000, and a gradual decline in number of publications since the year 2003 [Figure 2]. Published literature has three overlapping periods, with most serology studies being published in the first decade (1991-2001) followed by accumulation of molecular evidence. Between 2003 and 2006, negative results in large clinical trials heralded a gradual decline in research publications pursuing this hypothesis.[19] Subsequently, molecular studies have continued to demonstrate presence of *Chlamydophila* antigens in atheromatous tissue, and researchers have argued that failed antibiotic trials do not mean that the hypothesis is refuted. The seroepidemiological, molecular, and clinical trial evidence for and against this hypothesis is detailed below.

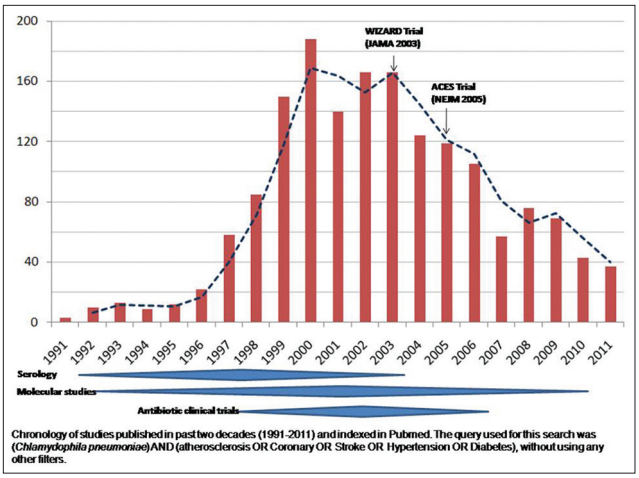

Figure 2: Number of PubMed indexed articles (n=1668) by year of publication on *Chlamydophila pneumoniae* infection and atherosclerosis

Seroepidemiological studies

Various seroepidemiological studies tested the hypothesis of association between exposure to *C. pneumoniae* and cardiovascular outcomes. Two broad study designs have tested this hypothesis. First, case-control studies with a cross-sectional design are hospital- or community-based studies, where exposure is determined in cases, after the outcome had already taken place. Second, case-control studies nested in well-defined community cohorts or prospective case-control studies, where exposure is determined from serum samples, which had been stored prior to occurrence of the outcome. These later studies characterize temporal relationship between exposure and outcome.

Case-control studies: Cross-sectional design

Of a total of 47 studies identified, 44 were included in two systematic reviews[20,21] [Table 1], and a majority of them (23 out of 44) reported an unequivocal positive association. Another 16 studies reported a non-significant positive outcome [odds ratio (OR) for *C. pneumoniae* seropositivity was greater than 1.0, but the 95% confidence interval (CI) crossed 1, while remaining five studies reported negative point estimates (with OR of less than 1.0)]. The range of point estimates was wide (0.7-17.0), representing a considerable heterogeneity in the results. The results of 29 studies included in the review by Bloemankamp *et al*.[20] were pooled using the random effects model, and the weighted pooled OR was 2.0 (95% CI 1.5-2.6).

Most of the earlier studies had evaluated immunoglobulin (Ig) G antibodies, which signify past infection. Subsequently, IgA antibodies were shown to be better associated with the presence of *C. pneumoniae* in the atheromatous tissue, signifying a persistent infection. Different studies showed a higher prevalence of IgA antibodies among patients with established athermanous disease as compared to healthy controls.[22-24] The point estimates risk ratios in these studies were higher and confidence intervals significant even after adjusting for confounders.[25-29]

Case-control studies: Prospective design

These cohorts from which cases and controls were sampled had been followed up for 3-15 years [Table 2]. Eighteen studies with this design were summarized in the two systematic reviews,[20,21] and only two of them reported a positive association of *C. pneumoniae* seropositivity with cardiovascular events [Osserwade *et al*. (OR 2.8 95% CI 1.3-5.8)[30] and Roivainen *et al*. (1.7 95% CI 1.2-2.5)[31]]. The point estimates were equivocal in nine studies, and negative in seven others. The weighted pooled odds of 15 of these studies reviewed by Bloemankamp *et al*.[20] was 1.1 (95% CI 0.8-1.4). In a meta-regression of these studies, the regression coefficient was estimated to be -0.04 (-0.08, -0.01) for every additional year of follow-up of the cohort, implying that for every additional one year of follow-up, the point estimate in these studies was lowered by 0.04. The accuracy of serology for detection of *Chlamydophila* has also been variable across studies, and at different cut-offs [Table 3].

Molecular studies

Circulating C. pneumoniae DNA

It has been hypothesized that PBMC positive for *C. pneumoniae* DNA detected by polymerase chain reaction (PCR) techniques[32] could be considered as a surrogate marker for chronic *C. pneumoniae* infection. Case only studies (which have reported the prevalence of *C. pneumoniae*–positive DNA in different population subgroups) and case-control studies (where cases include patients with atherosclerosis-associated cardiovascular conditions and healthy controls) have been used to determine risk estimates. Smeija *et al*.[32] published a systematic review of these studies. A few[13,33-37] additional studies have appeared since the publication of this review. The prevalence of *C. pneumoniae* DNA in PBMC ranges from 2.5% to 46.2% in healthy blood donors and other healthy individuals who were studied in prevalence studies (in one study, all medical student controls were negative). The pooled odds of presence of *C. pneumoniae* DNA in PBMC as a risk factor for cardiovascular events was 2.03 (95% CI 1.34-3.08).[32]

Identification of C. pneumoniae in vascular tissue

More than 40 studies have used various techniques (immunocytochemistry techniques were used to detect the presence of specific antibodies or antigens in tissue aspirates, PCR, and *in situ* hybridization to demonstrate the presence of *C. pneumoniae* DNA; electron microscopy was used to demonstrate the intact bacterium and cell culture to isolate viable *Chlamydophila* organisms[4]) to determine the presence of *C. pneumoniae* in vascular tissues. The results of these studies have been compiled by Boman *et al*.,[4] and in the 2679 specimens analyzed by all authors by different techniques, immunocytochemistry (676 specimens), electron microscopy (97 specimens), and PCR (2294 specimens) showed positive results in 49.7%, 39.1%, and 24.3% cases, respectively. There was a wide heterogeneity in the results across studies [Table 4], and only 7.3% of the 451 specimens were culture positive.

Mechanism of C. pneumoniae–induced atherosclerosis

It has been hypothesized that respiratory infections by *C. pneumoniae* may result in hematogenous spread through PBMC. The organism has effects on endothelium and vascular smooth muscle cells,

Table 1: Case-control studies that evaluated the relationship between exposure to *Chlamydophila pneumoniae* seropositivity and cardiovascular risk in a cross-sectional manner

First Author (yr) *n* (case/control)	Cases	Controls	Adjustment factors	OR (95% CI)
Blasi (1997)[54] 122 (61/61)	Admitted patients (>65 yr) with AMI	Blood donors	Age, sex, smoking	3.2 (1.2-6.8)
Thomas (1997)[55] 176 (83/93)	Patients hospitalized with CAD or AMI or patients undergoing cardiac catheterization	Patients hospitalized for other reasons	Age, sex	5.4 (2.8-10.4)
Kark (1997) 790 (302/488)	Patients admitted with first AMI	Individuals living in Jerusalem, randomly selected from the Population Registry	Age, sex	0.9 (0.7-1.3)
Gabriel (1998)[56] 238 (136/102)	Admitted patients with AP	Blood donors without symptoms CAD	None	1.5 (0.8-2.8)
Gabriel (1998)[56] 248 (146/102)	Admitted patients with AMI	Blood donors without symptoms CAD	None	1.9 (1.0-3.6)
Boman (1998)[57] 153 (101/52)	Patients admitted for CAD who underwent coronary angiography	Blood donors	None	1.7 (0.5-5.8)
Mazzoli (1998)[58] 103 (29/74)	Patients with AMI admitted to Intensive Care Unit	Blood donors	None	17.0 (4.7-61.6)
Cook (1998)[59] 1694 (176/1518)	Patients admitted with stroke or TIA	Patients submitted to the ER with acute nonpulmonary, noncardiovascular disorder	None	3.8 (2.7-5.2)
Anderson (1998)[60] 345 (219/126)	Patients undergoing angiography with>60% stenosis in one of the major coronary arteries	Patients undergoing angiography with no stenosis	None	1.1 (0.7-2.0)
Anderson (1998)[60] 309 (112/197)	Patients with AMI admitted to Intensive Care Unit	Patients undergoing angiography with no stenosis	None	1.6 (0.8-3.2)
Miyashita (1998)[61] 320 (160/160)	Patients admitted with a diagnosis of AMI	Patients who attended the same hospital for other complaints	None	2.4 (1.3-5.8)
Cellesi (1999)[62] 199 (150/49)	Patients who underwent coronary angiography with a stenotic lesion>50%	Individuals who underwent coronary angiography without coronary lesions	Age, sex, cardiovascular risk factors, alcohol	0.8 (0.3-2.0)
Cellesi (1999)[62] 206 (150/56)	Patients who underwent coronary angiography with a stenotic lesion>50%	Individuals without a history of heart disease	Age, sex, cardiovascular risk factors, alcohol	1.2 (0.5-2.6)
Shimada (1999)[63] 152 (123/29)	Patients with CAD (stenosis>50% in one of the major coronary arteries)	Patients who underwent coronary angiography angiography without coronary lesions	None	2.3 (0.9-5.2)
Sessa (1999)[64] 148 (98/50)	Admitted patients with AMI	Subjects without clinical signs or symptoms of cardiovascular or pulmonary disease	Age, sex	2.3 (1.1-4.6)
Sessa (1999)[64] 130 (80/50)	Admitted patients with AP or AMI	Subjects without clinical signs or symptoms of cardiovascular or pulmonary disease	Age, sex	2.5 (1.2-5.1)
Abdelmouttaleb (1999)[65] 316 (142/74)	Patients with stenosis (>50%) in one of the major coronary arteries	Patients with stable valvular heart disease and volunteers with no evidence for CAD	None	0.8 (0.5-1.5)
Altman (1999)[66] 362 (159/203)	Patients with clinical history of severe CAD or with obstructive peripheral arterial disease (PAD)	Patients with mechanical heart valve protheses without CAD	None	1.4 (0.9-2.2)
Korner (1999)[23] 340 (275/35)	Patients with significant stenosis (>70%) in one of the peripheral arteries, carotid arteries, or coronary arteries	Patients without significant stenosis	None	3.4 (2.0-6.0)

(*Contd...*)

Table 1: Cont...

First Author (yr) *n* (case/control)	Cases	Controls	Adjustment factors	OR (95% CI)
Leowaltana (1999)[67] 358 (243/115)	Patients with CAD admitted for coronary angiography (blood donors)	Blood donors	None	1.2 (0.8-2.0)
Kaykov (1999)[68] 228 (130/98)	Patients with CAD (MI or unstable AP)	Blood donors	Age, sex	6.1 (3.4-10.8)
Nobel (1999)[69] 116 (58/58)	Patients with AMI or proven AP (occlusion or stenosis>70% of at least one major coronary artery)	Blood donors	Age, sex	0.7 (0.3-1.5)
Kontula (1999)[70] 96 (28/68)	Familial hypercholesterolemia patients with AMI or coronary artery bypass grafting	Patients without CAD	None	5.7 (2.1-15.8)
Elkind (2000)[71] 178 (89/89)	A random selection of patients with first ischemic stroke from the North Manhattan Stroke Study	Blood donors selected by means of random digit dialing, without stroke	Age, sex, smoking, cardiovascular risk factors	2.6 (0.9-7.8)
Romeo (2000)[72] 103 (54/49)	Patients with stable CAD admitted	Patients who underwent coronary angiography without CAD	Age	2.0 (0.9-4.5)
Romeo (2000)[72] 105 (56/49)	Admitted patients with unstable CAD	Patients who underwent coronary angiography without CAD	Age	1.3 (0.6-2.9)
Glader (2000)[73] 228 (77/151)	Participants of the Vasterbotten Intervention Program or of the WHO MONICA Project with a first MI	Participants of the Vasterbotten Intervention Program or of the WHO MONICA Project who did not have a first MI	Age, sex, smoking, cardiovascular risk factors	0.9 (0.5-1.6)
Hoffmeister A (2000)[74] 791 (312/479)	Patients with CAD (50% stenosis in one of the major coronary arteries)	Blood donors from the associated Red Cross blood bank	Age, sex, smoking, cardiovascular risk factors	1.0 (0.6-1.6)
Ammann (2000)[75] 42 (21/21)	Patients with CAD admitted for PTCA	Blood donors	Age, Sex	2.0 (0.5-7.5)
Jantos (2000)[76] 752 (489/263)	Patients undergoing coronary angiography with>50% stenosis	Patients undergoing angiography without CAD	Age, Sex, Cardiovascular risk factors	1.8 (1.2-2.8)
Mendis (2001)[77] 91 (41/50)	Patients with AMI	Healthy controls	None	1.08 (0.37-3.12)
Mendis (2001)[77] 80 (30/50)	Patients with stable CAD	Healthy controls	None	2.1 (0.63-7.19)
Bloemenkamp (2002)[78] 871 (228/643)	Young women with PAD	Healthy age-matched women	None	2.0 (1.3-3.1)
Serology studies using IgA antibodies				
Shimada (2001)[26] 707 (507/200)	Patients with CAD	Healthy age-matched controls	Cardiovascular risk factors	1.59 (0.88-2.87)
Shimada (2001)[26] 467 (267/200)	Patients with AMI	Healthy age-matched controls	Cardiovascular risk factors	2.67 (1.32-5.38)
Kinjo (2003)[27] 1585 (618/967)	Patients with AMI	Healthy age-matched controls	None	1.75 (1.04-2.67)
Voorend (2004)[28] 91 (41/50)	Patients with acute ischemic stroke	Healthy age-matched controls	Cardiovascular risk factors	2.80 (1.1-1.71)
Piechowski (2007)[29] 197 (94/103)	Patients with acute ischemic stroke	Healthy age-matched controls	Age, Sex	8.95 (4.44-18.07)
Hasan (2011)[79] 90 (50/40)	Patients with acute ischemic stroke	Healthy age-matched controls	None	3.18 (1.12-9.04)
Rai (2011)[80] 99 (51/48)	Patients with acute ischemic stroke	Healthy age-matched controls	Cardiovascular risk factors	4.72 (1.61-13.83)

The results of many of these studies have been abstracted from a published meta-analysis[20]; AMI: Acute myocardial infarction; AP: Angina pectoris; CAD: Coronary artery disease; CSA: Chronic stable angina; PAD: Peripheral arterial disease; PTCA: Percutaneous transluminal coronary angiography; TIA: Transient ischemic attack.

Table 2: Prospective case-control studies that evaluated the relationship between *Chlamydophila pneumoniae* seropositivity and cardiovascular risk

First author (yr)	Participants	Study design	*Chlamydophila pneumoniae* IgG titer cut off (technique)	Outcome/Case definition	Maximum Follow-up duration (yrs)	RR/OR (95% CI)
Miettinen (1996)[81]	Cohort of diabetics (*n*=798) and Non-diabetics (*n*=1195) in 45-64 years of age	Cohort study	≥1/128 MIF	Angina pectoris (Questionnaire)	7	Diabetics 0.89 (0.55-1.44) Men 1.18 (0.68-2.04) Women Non-diabetics 1.83 (0.90-3.73) Men
Ossewaarde (1998)[30]	Cases>65 years of age who developed coronary heart disease (*n*=54) Controls>65 years of age free of coronary heart disease (*n*=108)	NCC Cohort of elderly men	≥1/32 MIF	Angina pectoris (Rose questionnaire)	5	2.76 (1.31-5.81)
Ridker (1999)	Cases: Normal at baseline, but developed AMI during follow-up (*n*=343) Controls: Remained normal throughout follow-up (*n*=343)	NCC Physician health study	≥1/256 MIF	AMI as determined by hospital records, death certificates, and autopsy records	12	0.8 (0.4-1.3) Matched on age and smoking status, and adjusted for cardiovascular risk factors
Glader (1999)[82]	Cases: Normal at baseline, but developed ischemic stroke (*n*=101) Controls: Age, sex, and area of residence matched controls (*n*=201)	NCC Community intervention study	≥1/32 and≥1/16 MIF	Ischemic stroke as determined by CT scan	11	0.4 (0.2-0.9) titers≥1/32 0.9 (0.5-1.6) titers≥1/16
Nieto (1999)[83]	Cases: Those who developed CAD during follow-up (*n*=240) Controls: Those who remained healthy (*n*=540)	NCC Atherosclerosis risk cohort	≥1/64 MIF	CAD as determined by questionnaire, follow-up visits, and death records	5	1.2 (0.7-2.1) Adjusted for age, race, gender, and cardiovascular risk factors
Strachan (1999)[25]	Cohort of men age 45-59 years followed up for cardiovascular events (*n*=1773)	Cohort study	≥1/16 MIF (IgA)	CAD as determined by hospital and death records	13	1.07 (0.75-1.53) Adjusted for age, smoking, socioeconomic status, and cardiovascular risk factors
Fagerbreg (1999)[84]	Cohort of treated hypertensive men (*n*=152)	Cohort study	≥1/512 MIF	Cardiovascular death, fatal and non-fatal AMI, and Stroke	6.5	2.69 (1.04-6.97)
Danesh (2000)[85]	Cases: Healthy men who had fatal or non-fatal CAD during follow-up *n*=319) Controls: Frequency matched to age and place of residence to cases (*n*=793)	NCC British national heart study	Top tertile of the titers in cases and controls, respectively>213 ELISA	CAD as determined by hospital and death records	15	1.30 (0.90-1.86) Adjusted for Age, smoking, residence, and adult socioeconomic status

(*Contd...*)

Table 2: Cont...

First author (yr)	Participants	Study design	*Chlamydophila pneumoniae* IgG titer cut off (technique)	Outcome/Case definition	Maximum Follow-up duration (yrs)	RR/OR (95% CI)
Wald (2000)[86]	Cases: Those who died due to cardiovascular cause (*n*=647) Controls: Those who survived follow-up period (*n*=1294)	NCC BUPA cohort	Different levels MIF	Cardiovascular death	15.6	1.24 (0.99-1.71) Highest point estimate at IgG antibody level≥20 units
Siscovick (2000)[87]	Cases: Those who developed AMI or cardiovascular death during follow-up, age>65 years (*n*=213) Controls: Healthy controls age>65 years age, sex matched (*n*=405)	NCC Cardiovascular health study	≥1/8 MIF Different titers evaluated	Cardiovascular death or fatal or non-fatal AMI determined by interview and clinical data	3.5 to 5.5	1.1 (0.7-1.8) Adjusted for smoking cardiovascular risk factors and years of education
Roivainen (2000)[88]	Cases: Those who developed AMI or cardiovascular death during follow-up (*n*=241) Controls: Treatment allocation and place of residence matched controls (*n*=241)	NCC Helsinki heart study	>2 MIF	AMI or Cardiovascular death	8.5	1.88 (1.10-3.21) (1-2 vs 3rd quartile) 1.36 (0.84-2.22) (1-2 vs 4th quartile)
Johnsen (2005)[89]	Cases: Those who were normal at baseline and developed ischemic stroke during follow-up (*n*=254) Controls: Age and sex matched normal individuals (*n*=254)	NCC Danish diet cancer and health follow-up study	≥1/16 MIF	Imaging proved Ischemic infarcts	5.1	1.54 (0.96-2.47) Adjusted for smoking, cardiovascular risk factors, and education status
Serology studies using IgA antibodies						
Strachan (1999)[25]	Cohort of healthy men, aged 45-59 years (*n*=1774)	Cohort	MIF	Mortality	13	1.07 (0.75-1.53) incident IHD 1.83 (1.17-2.85) CVD mortality 1.50 (1.10-2.04) all-cause mortality
Volanen (2006)[90]	Cohort of healthy children followed up of 7-11 years of age (*n*=199)	Cohort STRIP trial	>45 EAU ELISA	Intima media thickness	4	Abdominal aorta intima media thickness 3.32 (1.15-9.57)
Sakurai (2010)[91]	209 individuals who died of CAD with equal number of age matched controls, sampled from JACC cohort (*n*=39242)	NCC JCC	MIF	Mortality due to CAD	13	4.09 (0.86-19.4)

Many of these studies have been described in previous systematic reviews[20,21] NCC: Nested case control study in a defined cohort; MIF: Microimmunofluorescence; ELISA: Enzyme linked immunosorbent assay; CAD: Coronary artery disease; AMI: Acute myocardial infarction; IHD: Ischemic heart disease

mediated through cytokines [interleukin (IL)-1, IL6, IL8, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, platelet-derived growth factor (PDGF), HSP60, etc) resulting in upregulation of inflammation,[38] endothelial

Table 3: Accuracy of serology with presence of *Chlamydophila pneumoniae* infection

First author Year (*n*)	Serology test used	Reference standard	TP	FP	FN	TN	Sn (95% CI)	Sp (95% CI)
Maass 1998[92] (158)	MIF titer>512	Tissue PCR	7	12	27	112	21 (10-37)	90 (84-94)
	MIF titer>16	Tissue PCR	34	100	0	24	100 (91-100)	19 (13-27)
Kaul 2000[93] (46)	MIF titer>32	PBMC PCR	11	17	6	12	65 (41-84)	41 (25-59)
Berger 2000[94]	MIF titer>64	73% patients were seropositive (*n*=130) 25% of them had *Chlamydophila* DNA PCR-positive plaque (*n*=130) 20% had *Chlamydophila* DNA PCR-positive in PBMCs (*n*=60)						

(Adapted from Boman *et al*. 2002)[4] MIF: Microimmunofluorescence; PCR: Polymerase chain reaction; TP: True positives; FP: False positives; FN: False negatives; TN: True negatives; Sn: Sensitivity; Sp: Specificity

Table 4: Detection of *Chlamydophila pneumonia* in atheromatous tissue specimens

Technique	Total samples positive/ analyzed	Positivity (%)	Range of positivity across studies (%)	Number of entirely negative studies (Total specimens in these studies)	Number of entirely positive studies (Total specimens in these studies)
Atheromatous tissue specimens (43 studies, 2679 specimens)					
Immunocytochemistry–anti-*Chlamydophila pneumoniae* antibody	336/676	49.7	19-100	-	1 (12)
Immunocytochemistry–*C. pneumoniae* antigen	202/443	45.6	14-100	-	1 (10)
Polymerase chain reaction	558/2294	24.3	0-100	1 (23)	1 (6)
Electron microscopy	38/97	39.1	0-100	1 (22)	3 (10)
Culture	33/451	7.3	0-16	6 (115)	-
In situ hybridization	1/60	1.6	0-10	1 (50)	-
Normal tissue specimens (23 studies, 637 specimens)					
Immunocytochemistry–anti-*C. pneumoniae* antibody	10/312	3.2	0-6	10 (118)	-
Immunocytochemistry–*C. pneumoniae* antigen	12/90	13.3	0-50	6 (64)	-
Polymerase chain reaction	25/548	4.5	0-50	9 (167)	-
Electron microscopy	0/3	0	0	2 (3)	-
Culture	0/2	0	0	1 (2)	-
In situ hybridization	0/2	0	0	1 (2)	-

These are the pooled results from 42 studies published between 1992 and 2000 (adapted from Boman *et al*. 2002)[4]

apoptosis,[39] and vascular smooth muscle proliferation. These events are likely precursors of atheroma formation. In the last five years, additional signaling mechanisms [mediated through Interferon regulatory factors 3 and 7, toll-like receptor (TLR)-2/4, IL-8, Intercellular Adhesion Molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, extracellular signal-regulated kinase (ERK)-1/2, nuclear factor-kappa B (NF-kB), IL-23, IL-6, IL-1beta, transforming growth factor (TGF)-beta, and chemokine (C-C motif) ligand (CCL-20)][40-42] have been postulated to have a role in the initiation and progression of atheromatous lesions. *C. pneumoniae* also has a role in lipid accumulation in the vessel wall by upregulating lecithin-like oxidized LDL receptors (LOX-1) in both endothelial and vascular smooth muscle cells.[43,44] In the same period, four studies[45-48] did not find convincing evidence of presence of *Chlamydophila* genome in the atheromatous tissue, and argued in favor of alternative mechanisms. However, it is likely that *C. pneumoniae* infection may only be providing an initial trigger and is transient rather than persistent. The mechanism mediated through molecular mimicry between human and bacterial HSP60 and production of anti-HSP antibodies is attractive, as it strengthens 'hit-and-run' hypothesis for the organism.

Clinical trials

Various Clinical trials evaluated whether eradication of *C. pneumoniae* is beneficial in the secondary prevention of cardiovascular events. The evidence that such a therapy could be useful had come from animal studies, where administration of high-dose azithromycin for 10 weeks was associated with a reduction in intimal thickening.

Initial studies reported between 1997 and 2001 (Gupta *et al.*, ACADEMIC, ROXIS, CLARIFY, and Leowattana *et al.*) had fewer subjects, shorter duration of antibiotic therapy, and shorter durations of follow-up. Subsequently, larger trials were launched (WIZARD, ACES, ANTIBIO, AZACS, and PROVE-IT) where antibiotics were administered for 3 months to 2 years, and duration of follow-up was 1-4 years [Table 5]. These later trials had greater statistical power to detect smaller differences between the intervention and non-intervention arms. In a meta-analysis summarizing the evidence from 19,217 patients in 11 randomized controlled trials, the point estimates for mortality reduction and reduction in secondary cardiovascular event were 1.02 (95% CI 0.89-1.16) and 0.92 (95% CI 0.81-1.04), respectively.[49]

Discussion

There is an ongoing debate whether the association between *C. pneumoniae* infection and cardiovascular outcomes is causal or the organism is merely an innocent bystander. The main arguments supporting a causal role are: biological plausibility and a consistent finding that atherosclerosis associated with vascular inflammation, is inducible by *C. pneumoniae* in laboratory experiments. The arguments against causality are poor association between seropositivity and cardiovascular outcomes, lack of consistency in demonstration of *C. pneumoniae* in vascular tissue, and failed attempts to show benefits of eradication of the organism. It, however, may be argued that we may be constrained by traditional Koch's postulates in an attempt to prove causality. In case of *C. pneumonia*, it is likely that the organism is not a singular causal factor in atherogenesis or its progression, its presence transient after an initial trigger, and eradication a failed aim because the persistence of the organism in atheromatous tissue and penetration of drugs are both questionable.

Seroepidemiological studies are useful in framing initial hypothesis, but these also have a major limitation. An important assumption in seroepidemiological studies is that the presence of anti-*C. pneumoniae* antibodies is a surrogate measure of chronic *C. pneumoniae* infection. This assumption may not hold true as there is a poor correlation between serology and detection of *C. pneumoniae* in vascular tissues [Table 3]. At low titers, serology had a poor specificity, and the number of false-positive results was high. At high titers, serology had a poor sensitivity but a high specificity.[4] Anti-*Chlamydophila* antibodies are measured using microimmunofluoroscence (MIF) technique, which needs an experienced microscopist to interpret the results.[4] Different studies have used in-house tests, using a variable cut-off to define positivity, and this approach is prone for misclassification error.[50] Owing to these limitations, there is a need for a better serological test to define chronic *Chlamydophila* infection.[15,51]

Infection due to *Chlamydophila* species is common, has seasonal variations, and different species exhibit antigenic cross-reactivity.[51] This leads to a high background prevalence of seropositivity as well as presence of *C. pneumoniae* DNA in PBMC. This diminishes the strength of association between evidence of past infection and atherosclerotic diseases.[52] Further, age, smoking status, and socioeconomic status are potential confounders in the relationship between exposure to

Table 5: Randomized control trials that studied the impact of antibiotic therapy on subsequent cardiovascular events

Study (year)	Number of participants	Intervention	Follow-up duration (mo)	Mortality OR (95% CI)	Combined events OR (95% CI)
Gupta *et al.* 1997[95]	60	AZ 500 mg×3 d	18	0.49 (0.03-8.22)	0.60 (0.16-2.30)
ACADEMIC 1999[96]	302	AZ 500 mg×3 d Then weekly×90 d	24	1.28 (0.34-4.85)	0.93 (0.41-2.11)
ROXIS 1999[97]	202	RX 300 mg×30 d	6	0.38 (0.07-2.01)	0.63 (0.22-1.85)
CLARIFY 2001	148	CL 500 mg×85 d	12	4.17 (0.46-38.24)	0.31 (0.14-0.70)
ISAR-3 2001[98]	1010	RX 300 mg/d×21 d	12	-	1.08 (0.92-1.26)*
Leowattana *et al.* 2001[99]	84	RX 150 mg×30 d	3	0.95 (0.06-15.75)	0.21 (0.02-1.27)
STAMINA 2002[100]	218	AZ 500 mg×3 d	12	0.96 (0.27-3.42)	0.64 (0.34-1.19)
ANTIBIO 2003[101]	868	RX 300 mg×42 d	12	1.10 (0.63-1.91)	1.21 (0.87-1.68)
WIZARD 2003[102]	7722	AZ 600 mg×3 d Then weekly x 77 d	14	0.93 (0.75-1.14)	0.97\ (0.81-1.16)
AZACS 2003[103]	1439	AZ 500 mg×1 d Then 250 mg×4 d	6	0.79 (0.46-1.39)	-
ACES 2005[104]	4012	AZ 600 mg×12 mo	14	1.08 (0.82-1.37)	1.01 (0.81-1.25)
PROVE-IT 2005[105]	4162	GX 400 mg 10 d a week	24	1.30 (0.89-1.16)	0.93 (0.77-1.13)
CLARICOR 2005[106]	4373	CL 500 mg×14 d	36	1.27 (1.03-1.54)	1.15 (0.99-1.34)

Data for many trials have been abstracted from a meta-analysis of randomized controlled trials[49]; * Angiographic restenosis. AZ: Azithromycin; CL: Clarithromycin; RX: Roxithromycin, GX: Gatifloxacin

C. pneumoniae and atherosclerosis. Systematic reviews[20,21] have revealed that studies that had adequately adjusted for confounders had a lower point estimate as compared to studies where adjustment was inadequate [1.1 (0.8-1.7) vs. 1.9 (1.2-3.0)]. In a meta-regression,[20] the regression coefficient for the degree of adjustment was -0.10 (-0.23, 0.02), implying that for every additional degree of adjustment, the point estimate is lowered by 0.1.

Despite reasonable molecular and serologic evidence, antibiotic trials failed to improve clinical outcomes. While this is a strong evidence against the *Chlamydophila*-atherosclerosis hypothesis, and may indicate an absence of the organism from either circulation or atheromatous plaques. The lack of protective effect in these antibiotic trials has been a major setback for the *C. pneumoniae*–atherosclerosis hypothesis, and calls for a reappraisal of its pathological mechanisms. Four main arguments are proposed to counter these negative results. First, the organism is difficult to eradicate and refractory to current anti-*Chlamydophila* antibiotics. Second, most patients in antibiotic trials had advanced atheromatous lesions that had already reached an irreversible stage. Third, the bacterium might be acting by a hit-and-run mechanism, in which case secondary prevention strategies are unlikely to be beneficial. Finally, the presence of other causal factors, such as hypercholesterolemia may be essential for the organism to induce atherosclerosis, and hence this mechanism may operate in specific subpopulations. Researchers argue that negative antibiotic trials should not put a premature end to *C. pneumoniae*–atherosclerosis hypothesis,[7,19] rather these should stimulate research into newer treatment strategies targeting *Chlamydophila*-specific proteins and machinery directly involved in their survival, replication, and maintenance.[53]

Infectious disease etiology for atherosclerosis is an attractive hypothesis, as it can cause a paradigm shift in preventive strategies. Current etiologies have led to primary and secondary prevention strategies targeting multiple risk factors, and reinforcing positive and negative behaviors is an intensive life-long process.[107,108] On the contrary, a single infectious etiology can stimulate research into vaccine development, and has the potential for better prevention and cure. In recent years, same paradigm shift has been adopted for cervical cancers. However, with multiple-candidate organisms, the most promising of these failing trials, there has never been a setback. Newer approaches to understand its pathogenesis and identifying a successful clinical application must continue.

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