REVIEW

Chlamydia pneumoniae and atherosclerosis

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Abstract

Objective—To review the literature for evidence that chronic infection with *Chlamydia pneumoniae* is associated with atherosclerosis and acute coronary syndromes.

Data sources—MEDLINE and Institute of Science and Information bibliographic databases were searched at the end of September 1998. Indexing terms used were chlamydi*, heart, coronary, and atherosclerosis. Serological and pathological studies published as papers in any language since 1988 or abstracts since 1997 were selected.

Data extraction—It was assumed that chronic *C pneumoniae* infection is characterised by the presence of both specific IgG and IgA, and serological studies were examined for associations that fulfilled these criteria. Pathological studies were also reviewed for evidence that the presence of *C pneumoniae* in diseased vessels is associated with the severity and extent of atherosclerosis.

Data synthesis—The majority of serological studies have shown an association between *C pneumoniae* and atherosclerosis. However, the number of cases in studies that have reported a positive association when using strict criteria for chronic infection is similar to the number of cases in studies which found no association. Nevertheless, the organism is widely found in atherosclerotic vessels, although it may not be at all diseased sites and is not confined to the most severe lesions. Rabbit models and preliminary antibiotic trials suggest that the organism might exacerbate atherosclerosis.

Conclusion—More evidence is required before *C pneumoniae* can be accepted as playing a role in atherosclerosis. Although use of antibiotics in routine practice is not justified, large scale trials in progress will help to elucidate the role of *C pneumoniae*. (Heart 1999;81:232-238)

agent. Chlamydia are unique, obligate, intracellular bacterial pathogens of eukaryotic cells. As they are difficult organisms to culture, much knowledge about *C pneumoniae* infections has come from serological studies, many of which, since 1988, have shown an association between *C pneumoniae* infection and atherosclerosis. However, association does not prove causality and, unfortunately, serological distinction between past and current infection is difficult.

In a general population, infection was absent in children under 5 years, attaining a peak incidence of 9.2% in those aged 5-10 years before falling to 1.5% over the age of 20.² By the age of 20 years, prevalence of specific IgG has already reached 50%, persisting or even increasing with age.³⁻⁵ Seroprevalence is proportional to the product of annual incidence and the duration of seropositivity following infection. As specific IgG antibodies are thought to disappear about three years following primary *C pneumoniae* infection,⁶ an annual incidence of 1.5% should result in a prevalence of 4.5% rather than the observed 50%. One possibility is that there is a high prevalence of chronic infection driving a persistent specific IgG response. This suggests that the optimal time to eradicate *C pneumoniae* infection is around the age of 20 years, when half the population is seropositive and after which the risk of reinfection is relatively low. However, epidemics do occur^{7,8} and IgG titres probably decay more slowly than previously thought. Of 90 students followed up for up to five years, only those with an initial low titre of 1:16 had a geometric mean titre of less than this at five years.⁶ Therefore, a high seroprevalence might indicate a population characterised not by chronic infection needing treatment but by a high past exposure.

The problem is how to prove persisting infection against a background of persisting antibody. In primary respiratory infection, the predominant IgM response is followed by a delayed IgG and a delayed, weak, or absent IgA response. Adult infections are thought to be mainly reinfections characterised by specific IgG and IgA antibody responses without changes in IgM.⁹ Thus, accurate detection of acute infection requires paired samples taken four to six weeks apart and the demonstration of a threefold or more rising titre. Serological criteria for chronic infection are more controversial but persistently raised specific IgA and

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Accepted for publication
20 October 1998

The organism now known as *Chlamydia pneumoniae* was first isolated in 1965 in Taiwan. It was subsequently recognised to be a cause of acute respiratory disease¹ giving rise to the acronym TWAR (Taiwan acute respiratory

IgG is generally accepted as an indication of chronic infection, since IgA is thought to have a much shorter half life than IgG. These uncertainties partly explain why different authors have used different criteria for seropositivity. The picture is further complicated by reports of acute infection without an antibody response,^{10 11} and that few studies of antibody response have used culture and antigen or DNA detection as absolute criteria of current infection. Compounding these problems, serology uses crude, whole *C pneumoniae* antigens, containing epitopes potentially cross reactive with antibodies to other

Chlamydia species,¹² to other Gram negative bacteria,¹³ or even to human heat shock protein as has been described for *Chlamydia trachomatis*.¹⁴ Atherosclerosis is known to be associated with the presence of antibody to human heat shock protein,¹⁵ and the possible confounding effects of such antibody has not been determined in any serological study of *C pneumoniae* and heart disease.

Pathological studies have generally shown that *C pneumoniae* is more common in atherosclerotic than control blood vessels. However, such studies do not show whether infection preceded or followed the development of

Table 1 Studies investigating the serological association between *C pneumoniae* and atherosclerosis

		Association with†			
Study	Case control*	IgG	IgA	ICs	Diagnostic antibody titre‡
<i>Prospective studies</i>					
Saikku (1992) ¹⁹	103 v 103	2.2 (1.1–4.5)	2.6 (1.2–5.2)		IgG ≥ 128 and/or IC or IgA ≥ 64
Miettinen (1996) ²⁰	Cardiac death or MI 162 v 636 (NIDDM) 40 v 1155 (No DM)	6 months before event but not 5 years before 32% v 15%. 2.44 (0.98–6.08)			and/or IC IgG ≥ 128 and IgA ≥ 40
Ossewaarde (1998) ²⁹	Cardiac death or MI 54 v 108	Only in non-diabetic men in East Finland 52% v 34%	No	No	ELISA
Nieto (1997) ³⁰	Cardiac death or MI or angina 256 v 550	2.8 (1.3–5.8) No			IgG ≥ 64
Siscovick (1998) ³¹	Cardiac death, MI or revascularisation 100 v 183 Cardiac death or MI	No			Not stated (abstract)
<i>Cross sectional studies</i>					
Diedrichs (1997) ²¹	131 v 63 CA	66% v 48%	44% v 22%		
Halme (1997) ²²	93 v 115 CA	100% v 61% (in men only)		No	IgG ≥ 128 and/or IgA ≥ 40
Saikku (1988) ²³	70 v 41 MI or stable angina	49% v 15% 5.5 (2.1–14.7)	41% v 10%		IgG ≥ 128 IgA ≥ 32
Cook (1998) ²⁴	176 v 1518 CVA	Acute infection 13.6% v 5.7% 4.2 (2.5–7.1) Chronic infection 32.4% v 12.7% 4.4 (3–6.5)			IgG ≥ 512 or 4 fold IgG rise or IgM ≥ 8 IgG ≥ 64 and ≤ 256 or IgA ≥ 8
Mazzoli (1998) ²⁵	29 v 74 MI	82% v 34%	71% v 14.9%		
Dahlen (1995) ³²	60 v 60	93.3% v 78.3% 3.56 (1–16.1)	No		IgG ≥ 32 IgA ≥ 16
Mendall (1995) ³³	100 v 64 CA	22% v 5% 7.4 (1.7–33.1)	No		IgG ≥ 64 IgA not stated
Blasi (1997) ²⁸	61 v 61 MI	57% v 30% 3.2 (1.5–6.8)	No		IgG ≥ 16 IgA ≥ 16
Thom (1991) ³⁴	461 v 95 CA	22% v 13% 2 (1–4)			IgG ≥ 64
Thom (1992) ³⁵	171 v 120 CA	67% v 56% 2.6 (1.4–4.8)			IgG ≥ 8
Melnick (1993) ³⁶	326 matched pairs. Carotid doppler	73% v 63% 2 (1.2–3.4)			IgG ≥ 8
Patel (1995) ³⁷	83 v 305 Rose questionnaire, ECG	30% v 18% 2.25 (1.1–4.6)			IgG ≥ 64
Thomas (1997) ³⁸	83 v 93 MI/IHD	71.1% v 31.2% 5.4 (2.7–10.9)			IgG ≥ 16
Toss (1998) ³⁹	256 v 190 Unstable angina	Seroprevalence not reported	36% v 19%.		IgG ≥ 16 IgA ≥ 64
Wimmer (1996) ⁴⁰	58 v 52 CVA	No	47% v 23% 1.7 (1.1–2.7)	24% v 8% 2 (1.1–3.8)	IgG ≥ 32 IgA ≥ 16
Leinonen (1990) ¹⁶	42 v 41 MI	No	No	57% v 12% 10 (3–29)	IgG ≥ 128 IgA ≥ 32
Leinonen (1994) ¹⁷	95 v 139 MI	No	No	58% v 26% 4 (2–7)	IgG ≥ 32 and ≤ 128 IgA ≥ 8 and ≤ 32
Weiss (1996) ¹⁸	65 v 28 CA	No	No		All titres considered
Kark (1997) ²⁶	302 v 486 MI	No	No	No	All titres considered
Boman (1998) ²⁷	101 v 52	No	No		
Linnanmaki (1993) ⁴¹	46 v 46 CA	No		41% v 15% 4 (1.4–11)	IgG ≥ 32
Anderson (1998) ⁴²	124 v 97 CA	No			IgG ≥ 16

*Number of cases and controls in final analysis and diagnosis of cases.

†Percentage of cases and controls who are seropositive or who have immune complexes (ICs). Odds ratio and 95% confidence interval. Adjusted figures are shown where reported by the authors.

‡Microimmunofluorescence test unless indicated

CA, coronary arteriograms; CVA, cerebrovascular accident; MI, myocardial infarction; IHD, ischaemic heart disease; NIDDM, non-insulin dependent diabetes mellitus; DM, diabetes mellitus; ELISA, enzyme linked immunosorbent assay.

Figure 1 Age standardised attack rates per 100 000 population in men (A) and women (B) for coronary events (definite and probable myocardial infarction and coronary deaths) in two regions of east Finland and prevalence of *C pneumoniae* IgG.

atherosclerosis. Nevertheless, it might be expected that the distribution of *C pneumoniae* should be related either to the extent or severity of atherosclerosis.

Data sources and data extraction

Serological and pathological studies published as papers in any language since 1988 or as abstracts since 1997 were searched for using the MEDLINE and Institute of Science and Information bibliographic databases. The search was completed at the end of September 1998. Indexing terms used were chlamydi* and heart, chlamydi* and coronary, and chlamydi* and atherosclerosis.

Information abstracted from serological studies included how many immunoglobulin classes were detected for and whether an association with atherosclerosis was found. Pathological studies were examined for information on the extent and distribution of *C pneumoniae* in subjects with atherosclerosis.

Serological studies of *C pneumoniae* and atherosclerosis

Table 1 shows that 21 of 27¹⁶⁻⁴² studies that have investigated *C pneumoniae* and atherosclerosis have reported some sort of positive serological association. However, although all studies measured IgG, only three prospective and 15 cross sectional studies measured IgA. Of the cross sectional studies, five found an association with both immunoglobulins, three with IgG alone, and two with IgA alone. Five studies found no association with either immunoglobulin, although three of these did show an association with circulating chlamydial immune complexes.^{16 17 40} Therefore, the serological evidence that chronic rather than past *C pneumoniae* infection is associated with atherosclerosis is not compelling. In fact, the number of cases in studies which found a positive association with both immunoglobulins¹⁹⁻²⁵ is similar to that in studies which found no association with either,^{16-18 20 26 27} although controls were numerically far greater in the former group (642 and 3069 v 767 and 1382). People with chronic debilitating diseases including

heart failure are predisposed to respiratory tract infections and this may be one reason why people with coronary artery disease are more likely to have experienced *C pneumoniae* infection. Controlling for known confounding factors such as smoking has not always been adequate,⁴³ and it is not known whether there is an association of *C pneumoniae* with heart failure. Of the five prospective studies, three reported a positive association but two of these depended on subset analysis. In one study, seropositivity was associated with coronary events six months but not five years before an event,¹⁹ while in the other an association was seen in non-diabetic men in east but not west Finland and not in diabetic men.²⁰

Acute infections are easier to diagnose and four studies investigated whether antibody titre rises were associated with acute vascular events such as myocardial infarction and cerebrovascular accident. Cook and colleagues²⁴ found an association with cerebrovascular accidents while Saikku and colleagues²³ found an association between rising IgM titres to chlamydial group lipopolysaccharide and myocardial infarction. Blasi and colleagues²⁸ found that 20% of patients with myocardial infarction had a rise in IgG but the proportion in the control group was unknown. No association was seen in the fourth study.¹⁶ Therefore, there is some evidence that acute infection is associated with acute vascular events. If this is true, the incidence of such events should increase during epidemics. East Finland has one of the highest coronary mortality rates in the world but there has been a gradual decrease since the 1970s because of a primary prevention programme. East Finland has also contributed to the World Health Organisation monitoring of trends and determinants in cardiovascular disease (WHO MONICA) project,⁴⁴ and serum samples from this project have been tested to estimate the population prevalence of *C pneumoniae*.⁵ Figure 1 shows the attack rates of coronary events in two regions of east Finland and the seroprevalence of *C pneumoniae*. It can be seen that in the epidemic year of 1987, acute coronary events did not increase.

Pathological studies of *C pneumoniae* in blood vessels

Techniques such as immunocytochemistry (ICC), the polymerase chain reaction (PCR), and culture have provided direct evidence that *C pneumoniae* localises to blood vessels. Generally, ICC finds more evidence for *C pneumoniae* than PCR but it is not known whether this is because of better sensitivity or worse specificity. It is notoriously difficult to obtain perfect ICC with severe atherosclerosis and the concern is that antibodies used in ICC cross react with components of atherosclerotic tissue. However, atherosclerotic tissue is known to contain inhibitors of PCR. These uncertainties make it difficult to estimate the prevalence of *C pneumoniae* in blood vessels, especially as specimens positive by one technique were not necessarily positive by another.⁴⁵ Culture of *C pneumoniae* from blood vessels is technically challenging and had only been managed on two occasions^{46 47} until recently when, following multiple serial passage, the organism was isolated from 11 of 70 atheromatous samples.⁴⁸

Histological evidence of atherosclerosis is ubiquitous⁴⁹ and 11 of 25 studies^{18 45-48 50-69} did not have control vessels (table 2). Only three studies had controls completely matched for age, origin of tissue, and tests used to detect for *C pneumoniae*. Even then, control arteries either had histological evidence of early atherosclerosis,⁵⁰ or came from arteries where adjacent segments had disease.⁵¹ For the other studies, control vessels tended to be numerically smaller and obtained at necropsy from younger subjects.

Although three studies failed to find evidence for *C pneumoniae* in atherosclerotic vessels,^{18 52 53} most have found it in 15–100% of cases. In contrast, it appears to be uncommon in control vessels and only in two studies was *C pneumoniae* as prevalent in control as in diseased vessels.^{54 55} In reality, it is likely that most control vessels, although macroscopically normal, did have early histological disease, which would suggest that *C pneumoniae* is more common in people with severe rather than mild atherosclerosis. There were five studies in which the severity of atherosclerosis was formally graded on a histological basis. In one, *C pneumoniae* was detected in 86% of severe compared with 6% of mild lesions using ICC.⁴⁵ However, when a subset of samples was tested by PCR, discordant results were obtained and the prevalence in non-atherosclerotic or minimally atherosclerotic lesions was said to be quite high.⁷⁰ In a necropsy study of 60 Alaskan natives who died mainly from non-cardiovascular causes (mean age 34.1 years), the Stary classification was used to grade the severity of a segment of the right coronary artery obtained from each subject.⁵⁶ Twenty two subjects were found to have *C pneumoniae* but there was no difference in the severity of their coronary artery segments compared with that of subjects without *C pneumoniae*. In total, 14 of 40 specimens with raised lesions were positive for *C pneumoniae* compared with 7 of 18 specimens with flat lesions. The main interest of this paper was the finding that a high IgG titre of ≥ 256 on average eight years before death was associated with the presence of *C pneumoniae* in coronary arteries, suggesting

Table 2 Studies investigating the presence of *C pneumoniae* in blood vessels

Study	Number of cases/controls* (description of case tissue)	Case and controls positive for <i>C. pneumoniae</i> *		
		PCR	ICC	Other
<i>Controlled studies. Age, tissue, and diagnostic method matched</i>				
Kuo (1995) ⁵¹	18 v 31 (coronary artery)	17% v 0%	39% v 0%	
Maass (1997) ⁵⁰	61 v 39 (carotid endarterectomy)	15% v 0%		
Petersen (1998) ⁶¹	40 v 40 (AAA)	35% v 5%		
<i>Other controls</i>				
Grayston† (1995) ⁶²	5 v 0 (carotid endarterectomy)	60%	100%	
	56 v 6 (archival or necropsy carotid specimens)		57% v 0%	
Ong† (1995) ⁵⁵	32 AAA repairs v 6 patients with normal vascular tissue	44% v 50%	3/8 (38%) v not done	
Jackson (1997) ⁶³	38 v 38 (vascular v non-vascular tissue from necropsy cases)	16% v 0–8%	24% v 5–11%	
Juvonen (1997) ⁶⁴	12 v 9 (abdominal aortic aneurysm)	6/6 v 0/9	100% v 0%	
Kuo† (1997) ⁶⁵	23 v 8 (diseased femoral and popliteal arteries)	48% v 0%		0%‡ v not done
Maass† (1998) ⁴⁸	70 v 17 (coronary atherectomy, failed grafts and other vessels)	30% v 0%		16%‡ v not done
Wong (1998) ⁵⁴	58 v 58 (coronary atherectomy and failed grafts)	39% v 12–30%		
Shor (1992) ⁶⁶	10 v 5 (coronary artery) Cases were highly selected from 1000 arteries		5/7 (71%) v 0/5 (0%)	10/10§ v not done
Kuo (1993) ⁶⁷	20 v 4 (aortic atheroma)		30% v 0%	
Chiu† (1997) ⁵⁸	76 v 20 (carotid and aortic tissue)		71% v 0%	
Muhlestein† (1996) ⁶⁸	90 coronary atherectomies 24 other controls			79% v 4.2%¶
<i>Studies without controls</i>				
Kuo (1993) ⁶⁰	36 (coronary artery)	13/30 (43%)	15/36 (42%)	6/21§ (29%)
Campbell (1995) ⁵⁹	37 (coronary atherectomy)	32%	45%	2/2§
Ramirez (1996) ⁴⁶	12 (coronary artery)	41.7%	41.7%	8%‡ 25%§
Jackson (1997) ⁴⁷	25 (carotid endarterectomy)	24%	8/16 (50%)	4%‡
Blasi (1996) ⁶⁹	51 (AAA)	51%		
Weiss (1996) ¹⁸	72 (coronary atherectomies)	1/50 (2%)		0/22‡§
Davidson (1998) ⁵⁶	60 (coronary artery)	14/60 (23.3%)	20/60 (33.3%)	
Bauriedel (1998) ⁵⁷	32 (carotid and coronary arteries)		47%	
Lindholt (1998) ⁵²	20 (AAA)	0%		
Paterson (1998) ⁵³	30 (carotid and coronary arteries)	0%		
Saldeen (1998) ⁴⁵	60 (coronary arteries)		62%	

*In some cases, more than one specimen was obtained from each patient. In these cases and where it was possible to determine from the papers, results are expressed as positive patients per number of patients.

†Studies where control vessels did not come from age matched subjects or where control subjects were poorly described; ‡culture; §electron microscopy; ¶direct immunofluorescence.

PCR, polymerase chain reaction; ICC, immunocytochemistry; AAA, abdominal aortic aneurysm; IMA, internal mammary artery.

that chronic infection occurs. We also used the Stary grade to assess coronary atherosclerosis and found no association of severity with the presence of *C pneumoniae*.⁷¹ Nevertheless, in another study, *C pneumoniae* was found in diseased segments of carotid artery but not in adjacent segments that were macroscopically normal but had early disease.⁵⁰ Similarly, in a study of young adults, *C pneumoniae* was more common in arteries with atheroma than in arteries with intimal thickening, but was absent in arteries that appeared normal.⁵¹

Few studies have examined the extent of *C pneumoniae* infection in individuals. In a postmortem study where two segments from each coronary artery were examined, we found that in patients with three vessel coronary artery disease, the organism was just as likely to be found in one as in two or three arteries.⁷¹ Similarly, another study found that contiguous segments from diseased coronary arteries or different coronary arteries from the same subject were not all positive for *C pneumoniae*.⁴⁶

In summary, pathological studies have shown that *C pneumoniae* is common in atherosclerotic vessels from a wide variety of sources, including those from young subjects.^{51 72} This would suggest that chronic infection occurs. *C pneumoniae* may be more prevalent in severe compared with mild lesions but its distribution in an individual often does not necessarily match that of atherosclerosis. These findings suggest that *C pneumoniae* exacerbates rather than causes atherosclerosis. Thus, one study using ICC and electron microscopy reported that it was more frequently found in atherectomy samples from patients presenting with acute coronary syndrome than from patients with stable angina.⁵⁷ However, we found using PCR that *C pneumoniae* was just as likely to be found in mildly diseased lesions as in fatal plaques with acute thrombosis, rupture, or haemorrhage.⁷¹ Also, *C pneumoniae* has been found in vessels not usually associated with atherosclerosis, such as the internal mammary artery and saphenous vein.^{54 72}

In vitro, animal, and antibiotic studies

Evidence of the potential pathogenicity of *C pneumoniae* for vasculature comes from four studies of experimental *C pneumoniae* infection in the New Zealand white rabbit.⁷³⁻⁷⁶ These rabbits do not spontaneously develop atherosclerosis until a late stage, but may do so if given cholesterol supplemented diets. Such a model is useful in assessing whether *C pneumoniae* exacerbates or causes atherosclerosis. Intranasal inoculation with *C pneumoniae* in rabbits fed a normal diet was found to induce aortic inflammatory changes consistent with early atherosclerosis,⁷⁵ including foam cells,⁷⁴ in two studies but not in a third.⁷³ The histological changes were early and compatible with inflammation. In the largest study, in rabbits fed a diet supplemented with small amounts of cholesterol, lesion size was significantly increased in infected compared with uninfected rabbits, and these changes could be prevented by azithromycin.⁷⁶

At the cellular level, recent studies indicate how *C pneumoniae* may interact with known risk factors of atherosclerosis to cause atheroma. It is clear that *C pneumoniae* is able to infect most of the cell types involved in atherogenesis, including endothelium, smooth muscle cells, and macrophages.⁷⁷ Intriguingly, a recent study found that human macrophages infected with *C pneumoniae* and incubated with low density lipoprotein were transformed into foam cells, the characteristic cells of early atheroma, within 22 hours.⁷⁸

A key question is whether *C pneumoniae* plays a unique role in the induction of atherosclerosis. Other infectious agents, including *Helicobacter pylori*, periodontal bacteria, and cytomegalovirus, have been implicated.⁴³ All bacteria, including chlamydia, share highly homologous heat shock proteins (HSP) capable of inducing autoimmune antibodies in humans caused by the presence of cross reactive sites on human HSP. Macrophages in atherosclerotic lesions express high concentrations of HSP,⁷⁹ and concentrations of specific antibodies to HSP are increased in the sera of patients with atherosclerosis.¹⁵ One possibility is that the immune response to bacterial infection generally may interact with chlamydial or human HSP in vessel walls, so exacerbating atherosclerosis. A recent study found that chlamydial and human HSP-60 co-localises in atherosclerotic lesions and that both types of HSP could induce TNF α (tumour necrosis factor) and matrix degrading metalloproteinase activity when incubated with mouse macrophages.⁸⁰

There have been two small antibiotic trials where patients with unstable angina or myocardial infarction were given macrolide antibiotics and were followed up.^{81 82} Both reported beneficial effects in terms of reduction of further coronary events. A third study gave doxycycline to men who had had previous coronary artery bypass graft surgery.⁸³ No change in antibody titres or basal nitric oxide production was seen after four months of treatment. Preliminary reports from a fourth antibiotic trial reported no reduction in further coronary events.⁸⁴ Larger studies with longer follow up periods are needed; such trials are either underway or being planned. However, there is debate as to whether *C pneumoniae* can be eradicated and how long treatment should be. One study is planning to give antibiotics for a year.⁸⁵ Macrolide antibiotics have anti-inflammatory as well as antimicrobial properties and these will need to be controlled for. Pending the results of such trials, antibiotic therapy is not indicated.

Conclusion

There is as yet no conclusive evidence that *C pneumoniae* causes atherosclerosis or precipitates acute coronary syndromes. Current knowledge about its distribution in blood vessels, animal studies, and antibiotic trials suggest that it may exacerbate atherosclerosis but these are preliminary results. There is no doubt that *C pneumoniae* is commonly found in blood vessels and it is important to discover

what the effects of infection are. It is too early to know whether antibiotics will play a role but such studies should be targeted at those who are clearly infected. Serology is an unreliable and poorly standardised surrogate for infection, failing in some studies to discriminate between those subjects with and without *C pneumoniae*.⁵⁰⁻⁵⁸ This may be because seropositive subjects in whom *C pneumoniae* was not found had the organism at sites other than those investigated. However, it is clear that *C pneumoniae* infection may occur in the presence of low specific antibody levels.⁵⁹⁻⁶⁰ It has been reported from Malaysia that *C pneumoniae* can be detected by PCR in serum samples,⁸⁶ although this has not been confirmed by other studies.⁸⁷ A promising possibility is the finding that *C pneumoniae* DNA can be detected in circulating white cells by PCR.²⁷⁻⁸⁸ Using this technique, we have found in Southampton an association of coronary artery disease with circulating *C pneumoniae* DNA in men but not women.⁸³ In northern Sweden, in a population with a high seroprevalence of *C pneumoniae* antibody, a high prevalence of circulating *C pneumoniae* DNA was found in both patients and blood donors.²⁷

The potential role of *C pneumoniae* and other infectious agents in coronary artery disease is an exciting and crucial area of cardiac research. Our hypothesis is that infection plays a role in only a subset of patients with coronary artery disease. Nevertheless the impact on public health could be substantial and the challenge to researchers is how to identify those patients most at risk.

Collaborative research in Southampton between molecular microbiology, experimental pathology and the Wessex cardiothoracic units is supported by the British Heart Foundation and the Wessex Cardiac Trust to whom we are most grateful.

- Grayston JT, Kuo CC, Wang SP, et al. A new Chlamydia psittaci strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med* 1986;315:161-8.
- Aldous MB, Grayston JT, Wang SP, et al. Seroepidemiology of Chlamydia pneumoniae TWAR infection in Seattle families, 1966-1979. *J Infect Dis* 1992;166:646-9.
- Kanamoto Y, Ouchi K, Mizui M, et al. Prevalence of antibody to Chlamydia pneumoniae TWAR in Japan. *J Clin Microbiol* 1991;29:816-18.
- Einarsson S, Sigurdsson HK, Magnúsdóttir SD, et al. Age specific prevalence of antibodies against Chlamydia pneumoniae in Iceland. *Scand J Infect Dis* 1994; 26:393-7.
- Karvonen M, Tuomilehto J, Pitkanen J, et al. Chlamydia pneumoniae IgG antibody prevalence in south western and eastern Finland in 1982 and 1987. *Int J Epidemiol* 1994;23: 176-84.
- Patnode D, Wang SP, Grayston JT. Persistence of Chlamydia pneumoniae, strain TWAR micro-immunofluorescent antibody. In: *Chlamydial infections*. Bowie WR, Caldwell HD, Jones RP, et al, eds. Cambridge: Cambridge University Press, 1990:406-9.
- Grayston JT, Mordhorst C, Bruu AL, et al. Countrywide epidemics of Chlamydia pneumoniae, strain TWAR, in Scandinavia, 1981-1983. *J Infect Dis* 1989;159:1111-14.
- Karvonen M, Tuomilehto J, Pitkanen J, et al. The epidemic cycle of Chlamydia pneumoniae infection in eastern Finland, 1972-1987. *Epidemiol Infect* 1993;110:349-60.
- Grayston JT. Chlamydia pneumoniae, strain TWAR. *Chest* 1989;95:664-9.
- Chirgwin K, Roblin PM, Gelling M, et al. Infection with Chlamydia pneumoniae in Brooklyn. *J Infect Dis* 1991;163: 757-61.
- Hyman CL, Augenbraun MH, Roblin PM, et al. Asymptomatic respiratory tract infection with Chlamydia pneumoniae TWAR. *J Clin Microbiol* 1991;29:2082-3.
- Kern DG, Neil MA, Schachter J. A seroepidemiologic study of Chlamydia pneumoniae in Rhode Island: evidence of serologic cross-reactivity. *Chest* 1993;104:208-13.
- Maurin M, Eb F, Etienne J, et al. Serological cross-reactions between Bartonella and Chlamydia species: implications for diagnosis. *J Clin Microbiol* 1997;35:2283-7.
- Domeika M, Domeika K, Paavonen J, et al. Humoral immune response to conserved epitopes of Chlamydia trachomatis and human 60-kDa heat-shock protein in women with pelvic inflammatory disease. *J Infect Dis* 1998;177: 714-19.
- Xu Q, Willeit J, Marosi M, et al. Association of serum antibodies to heat shock protein 65 with carotid atherosclerosis. *Lancet* 1993;341:225-9.
- Leinonen M, Linnanmaki E, Mattila K, et al. Circulating immune complexes containing chlamydial lipopolysaccharide in acute myocardial infarction. *Microb Pathog* 1990;9: 67-73.
- Leinonen M, Mattila K, Kohlmeier L, Saikku P. Chlamydia pneumoniae specific antibodies and immune complexes in German patients with acute myocardial infarction. In: Orfila J, Byrne GI, Chernesky M, et al, eds. *Chlamydial infections. Proceedings of the eighth international symposium on human chlamydial infections*. Bologna: Societa Editrice Esculapio, 1994:209-11.
- Weiss SM, Roblin PM, Gaydos CA, et al. Failure to detect Chlamydia pneumoniae in coronary atheromas of patients undergoing atherectomy. *J Infect Dis* 1996;173:957-62.
- Saikku P, Leinonen M, Tenkanen L, et al. Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki heart study. *Ann Intern Med* 1992;116:273-8.
- Miettinen H, Lehto S, Saikku P, et al. Association of Chlamydia pneumoniae and acute coronary heart disease events in non-insulin dependent diabetic and non diabetic subjects in Finland. *Eur Heart J* 1996;17:682-8.
- Diedrichs H, Schneider CA, Scharkus S, et al. Prevalence of chlamydia antibodies in patients with coronary heart disease. *Herz Kreislauf* 1997;29:304-7.
- Halme S, Syrjala H, Bloigu A, et al. Lymphocyte responses to chlamydia antigens in patients with coronary heart disease. *Eur Heart J* 1997;18:1095-101.
- Saikku P, Mattila K, Nieminen MS, et al. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart-disease and acute myocardial-infarction. *Lancet* 1988;II:983-986.
- Cook PJ, Honeybourne D, Lip GYH, et al. Chlamydia pneumoniae antibody titers are significantly associated with acute stroke and transient cerebral ischemia—the west Birmingham stroke project. *Stroke* 1998;29:404-10.
- Mazzoli S, Tofani N, Fantini A, et al. Chlamydia pneumoniae antibody response in patients with acute myocardial infarction and their follow-up. *Am Heart J* 1998;135:15-20.
- Kark JD, Leinonen M, Paltiel O, et al. Chlamydia pneumoniae and acute myocardial infarction in Jerusalem. *Int J Epidemiol* 1997;26:730-8.
- Boman J, Soderberg S, Forsberg J, et al. High prevalence of Chlamydia pneumoniae DNA in peripheral blood mononuclear cells in patients with cardiovascular disease and in middle-aged blood donors. *J Infect Dis* 1998;178:274-7.
- Blasi F, Cosentini R, Raccanelli R, et al. A possible association of Chlamydia pneumoniae infection and acute myocardial infarction in patients younger than 65 years of age. *Chest* 1997;112:309-12.
- Ossewaarde JM, Feskens EM, DeVries A, et al. Chlamydia pneumoniae is a risk factor for coronary heart disease in symptom free elderly men, but Helicobacter pylori and cytomegalovirus are not. *Epidemiol Infect* 1998;120:93-9.
- Nieto FJ, Folsom A, Sorlie P, et al. Chlamydia pneumonia infection and incident coronary heart disease: the atherosclerosis risk in communities (ARIC) study [abstract]. *Am J Epidemiol* 1997;145:331.
- Siscovick DS, Schwartz SM, Corey L, et al. Antibody to Chlamydia pneumoniae, herpes simplex virus type 1, cytomegalovirus and incident myocardial infarction and coronary heart disease death: the cardiovascular health study [abstract]. *Circulation* 1998;97:2.
- Dahlen GH, Boman J, Birgander LS, et al. Lp (A) Lipoprotein, IgG, IgA and IgM antibodies to Chlamydia pneumoniae and HLA Class II genotype in early coronary artery disease. *Atherosclerosis* 1995;114:165-74.
- Mendall MA, Carrington D, Strachan D, et al. Chlamydia pneumoniae—risk factors for seropositivity and association with coronary heart disease. *J Infect* 1995;30:121-8.
- Thom DH, Wang SP, Grayston JT, et al. Chlamydia pneumoniae strain TWAR antibody and angiographically demonstrated coronary artery disease. *Arterioscler Thromb* 1991;11:547-51.
- Thom DH, Grayston JT, Siscovick DS, et al. Association of prior infection with Chlamydia pneumoniae and angiographically demonstrated coronary artery disease. *JAMA* 1992;268:68-72.
- Melnick SL, Shahar E, Folsom AR, et al. Past infection by Chlamydia pneumoniae strain TWAR and asymptomatic carotid atherosclerosis. *Am J Med* 1993;95:499-504.
- Patel P, Mendall MA, Carrington D, et al. Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors. *BMJ* 1995;311:711-14.
- Thomas GN, Scheel O, Koehler AP, et al. Respiratory chlamydial infections in a Hong Kong teaching hospital and association with coronary heart disease. *Scand J Infect Dis* 1997;104(suppl):30-3.
- Toss H, Gnarpe J, Gnarpe H, et al. Increased fibrinogen levels are associated with persistent Chlamydia pneumoniae infection in unstable coronary artery disease. *Eur Heart J* 1998;19:570-7.

- 40 Wimmer MLJ, Sandmannstrupp R, Saikku P, *et al.* Association of chlamydial infection with cerebrovascular disease. *Stroke* 1996;27:2207-10.
- 41 Linnanmaki E, Leinonen M, Mattila K, *et al.* Chlamydia pneumoniae specific circulating immune-complexes in patients with chronic coronary heart disease. *Circulation* 1993;87:1130-4.
- 42 Anderson JL, Carlquist JF, Muhlestein JB, *et al.* Evaluation of C-reactive protein, an inflammatory marker, and infectious serology as risk factors for coronary artery disease and myocardial infarction. *J Am Coll Cardiol* 1998;32:35-41.
- 43 Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? *Lancet* 1997;350:430-6.
- 44 Salomaa V, Arstila M, Kaarsalo E, *et al.* Trends in the incidence and mortality from coronary heart disease in Finland, 1983-1988. *Am J Epidemiol* 1992;136:1303-15.
- 45 Saldeen TP, Ericson K, Lindquist O, *et al.* Chlamydia and HLA-DR genotypes in coronary atherosclerosis [abstract]. *J Am Coll Cardiol* 1998;31:A272.
- 46 Ramirez JA, Ahkee S, Summersgill JT, *et al.* Isolation of Chlamydia pneumoniae from the coronary-artery of a patient with coronary atherosclerosis. *Ann Intern Med* 1996;125:979-82.
- 47 Jackson LA, Campbell LA, Kuo CC, *et al.* Isolation of Chlamydia pneumoniae from a carotid endarterectomy specimen. *J Infect Dis* 1997;176:292-5.
- 48 Maass M, Bartels C, Engel PM, *et al.* Endovascular presence of viable Chlamydia pneumoniae is a common phenomenon in coronary artery disease. *J Am Coll Cardiol* 1998;31:827-32.
- 49 Tejada C, Strong JP, Montenegro MR, *et al.* Distribution of coronary and aortic atherosclerosis by geographic location, race, and sex. *Lab Invest* 1968;18:509-26.
- 50 Maass M, Krause E, Engel PM, *et al.* Endovascular presence of Chlamydia pneumoniae in patients with hemodynamically effective carotid artery stenosis. *Angiology* 1997;48:699-706.
- 51 Kuo CC, Grayston JT, Campbell LA, *et al.* Chlamydia pneumoniae (TWAR) in coronary-arteries of young-adults (15-34 years old). *Proc Natl Acad Sci USA* 1995;92:6911-14.
- 52 Lindholt JS, Ostergard L, Henneberg EW, *et al.* Failure to demonstrate Chlamydia pneumoniae in symptomatic abdominal aortic aneurysms by a nested polymerase chain reaction (PCR). *Eur J Vasc Endovasc Surg* 1998;15:161-4.
- 53 Paterson DL, Hall J, Rasmussen SJ, *et al.* Failure to detect Chlamydia pneumoniae in atherosclerotic plaques of Australian patients. *Pathology* 1998;30:169-72.
- 54 Wong Y, Thomas M, Gallagher PJ, *et al.* The prevalence of Chlamydia pneumoniae in atherosclerotic and normal blood vessels of patients undergoing redo and first time coronary artery bypass graft surgery [abstract]. *J Am Coll Cardiol* 1998;31:A146.
- 55 Ong G, Thomas BJ, Mansfield AO, *et al.* Detection and widespread distribution of Chlamydia pneumoniae in the vascular system and its possible implications. *J Clin Pathol* 1996;49:102-6.
- 56 Davidson M, Kuo CC, Middaugh JP, *et al.* Confirmed previous infection with Chlamydia pneumoniae (TWAR) and its presence in early coronary atherosclerosis. *Circulation* 1998;98:628-33.
- 57 Bauriedel G, Schmucking I, Hutter R, *et al.* Evidence of Chlamydia pneumoniae in human arteriosclerotic lesions: Insights into its possible pathogenic role [abstract]. *J Am Coll Cardiol* 1998;31:A390-1.
- 58 Chiu B, Viira E, Tucker W, *et al.* Chlamydia pneumoniae, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. *Circulation* 1997;96:2144-8.
- 59 Campbell LA, O'Brien ER, Cappuccio AL, *et al.* Detection of Chlamydia pneumoniae TWAR in human coronary atherectomy tissues. *J Infect Dis* 1995;172:585-8.
- 60 Kuo CC, Shor A, Campbell LA, *et al.* Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries. *J Infect Dis* 1993;167:841-9.
- 61 Petersen E, Boman J, Persson K, *et al.* Chlamydia pneumoniae in human abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 1998;15:138-42.
- 62 Grayston JT, Kuo CC, Coulson AS, *et al.* Chlamydia pneumoniae (TWAR) in atherosclerosis of the carotid-artery. *Circulation* 1995;92:3397-400.
- 63 Jackson LA, Campbell LA, Schmidt RA, *et al.* Specificity of detection of Chlamydia pneumoniae in cardiovascular atheroma—evaluation of the innocent bystander hypothesis. *Am J Pathol* 1997;150:1785-90.
- 64 Juvonen JJ, Tuvonen T, Laurila A, *et al.* Demonstration of Chlamydia pneumoniae in the walls of abdominal aortic aneurysms. *J Vasc Surg* 1997;25:499-505.
- 65 Kuo CC, Coulson AS, Campbell LA, *et al.* Detection of Chlamydia pneumoniae in atherosclerotic plaques in the walls of arteries of lower extremities from patients undergoing bypass operation for arterial obstruction. *J Vasc Surg* 1997;26:29-31.
- 66 Shor A, Kuo CC, Patton DL. Detection of Chlamydia pneumoniae in coronary arterial fatty streaks and atheromatous plaques. *S Afr Med J* 1992;82:158-61.
- 67 Kuo CC, Gown AM, Benditt EP, *et al.* Detection of Chlamydia pneumoniae in aortic lesions of atherosclerosis by immunocytochemical stain. *Arterioscler Thromb* 1993;13:1501-4.
- 68 Muhlestein JB, Hammond EH, Carlquist JF, *et al.* Increased incidence of Chlamydia species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. *J Am Coll Cardiol* 1996;27:1555-61.
- 69 Blasi F, Denti F, Erba M, *et al.* Detection of Chlamydia pneumoniae but not Helicobacter pylori in atherosclerotic plaques of aortic aneurysms. *J Clin Microbiol* 1996;34:2766-9.
- 70 Mehta JL, Saldeen TP, Rand K. Interactive role of infection, inflammation and traditional risk factors in atherosclerosis and coronary artery disease. *J Am Coll Cardiol* 1998;31:1217-25.
- 71 Thomas M, Wong YK, Thomas D, *et al.* Direct detection of C pneumoniae DNA at multiple sites in human coronary arteries post mortem and its relationship to the histological severity (Stary grade) of associated atherosclerotic plaque. In: Stephens RS, Byrne GI, Christiansen G, *et al.*, eds. *Chlamydial infections. Proceedings of the ninth international symposium on human chlamydial infection*. International Chlamydia Symposium, San Francisco, 1998:199-202.
- 72 TaylorRobinson D, Ong G, Thomas BJ, *et al.* Chlamydia pneumoniae in vascular tissues from heart-transplant donors. *Lancet* 1998;351:1255.
- 73 Moazed TC, Kuo CC, Patton DL, *et al.* Experimental rabbit models of Chlamydia pneumoniae infection. *Am J Pathol* 1996;148:667-76.
- 74 Fong IW, Chiu B, Viira E, *et al.* Rabbit model for Chlamydia pneumoniae infection. *J Clin Microbiol* 1997;35:48-52.
- 75 Laitinen K, Laurila A, Pyhala L, *et al.* Chlamydia pneumoniae infection induces inflammatory changes in the aortas of rabbits. *Infect Immun* 1997;65:4832-5.
- 76 Muhlestein JB, Anderson JL, Hammond EH, *et al.* Infection with Chlamydia pneumoniae accelerates the development of atherosclerosis and treatment with azithromycin prevents it in a rabbit model. *Circulation* 1998;97:633-6.
- 77 Gaydos CA, Summersgill JT, Sahney NN, *et al.* Replication of Chlamydia pneumoniae in-vitro in human macrophages, endothelial cells, and aortic artery smooth muscle cells. *Infect Immun* 1996;64:1614-20.
- 78 Kalayoglu MV, Byrne GI. Induction of macrophage foam cell formation by Chlamydia pneumoniae. *J Infect Dis* 1998;177:725-9.
- 79 Schett G, Metzler B, Mayr M, *et al.* Macrophage-lysis mediated by autoantibodies to heat shock protein 65/60. *Atherosclerosis* 1997;128:27-38.
- 80 Kol A, Sukhova GK, Lichtman AH, *et al.* Chlamydial heat shock protein 60 localises in human atheroma and regulates macrophage tumor necrosis factor-alpha and matrix metalloproteinase expression. *Circulation* 1998;98:300-7.
- 81 Gupta S, Leatham EW, Carrington D, *et al.* Elevated Chlamydia pneumoniae antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. *Circulation* 1997;96:404-7.
- 82 Gurfinkel E, Bozovich G, Daroca A, *et al.* Randomised trial of roxithromycin in non Q-wave coronary syndromes: ROXIS pilot study. *Lancet* 1997;350:404-7.
- 83 Sinisalo J, Mattila K, Nieminen MS, *et al.* The effect of prolonged doxycycline therapy on Chlamydia pneumoniae serological markers, coronary heart disease risk factors and forearm basal nitric oxide production. *J Antimicrob Chemother* 1998;41:85-92.
- 84 ACADEMIC (azithromycin in coronary artery disease: elimination of myocardial infection with chlamydia). Meeting report. *Clin Cardiol* 1998;21:429-30.
- 85 Grayston JT. Antibiotic treatment of Chlamydia pneumoniae for secondary prevention of cardiovascular events. *Circulation* 1998;97:1669-70.
- 86 Naidu BR, Ngeow YF, Kannan P, *et al.* Evidence of Chlamydia pneumoniae infection obtained by the polymerase chain reaction (PCR) in patients with acute myocardial infarction and coronary heart disease. *J Infect* 1997;35:199-200.
- 87 Boman J, Petersen E, Persson K, *et al.* Failure to detect Chlamydia pneumoniae DNA by nested PCR in serum samples of patients with infrarenal abdominal aortic aneurysms. In: Stephens RS, Byrne GI, Christiansen G, *et al.*, eds. *Chlamydial infections. Proceedings of the ninth international symposium on human chlamydial infection*. International Chlamydia Symposium, San Francisco, 1998:211-14.
- 88 Wong YK, Dawkins K, Ward ME. Detection of C pneumoniae DNA circulating in the blood of patients attending for cardiac angiography: preliminary clinical and serological correlates. In: Stephens RS, Byrne GI, Christiansen G, *et al.*, eds. *Chlamydial infections. Proceedings of the ninth international symposium on human chlamydial infection*. International Chlamydia Symposium, San Francisco, 1998:227-30.