

Chlamydia pneumoniae and Atherosclerosis: No Way-Out or Long Way?

What about Renal Failure Patients?

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Key Words

Antibodies · Antibiotic trials · Renal failure · Hemodialysis · Vaccine · Confounders

Abstract

Recently, *Chlamydia pneumoniae* is the microorganism frequently implicated in the infection-based inflammatory atherogenous hypothesis. Although in vitro experimental data and initial sero-epidemiologic, pathology-based studies and antibiotic trials supported this interesting hypothesis, later data are conflicting. Some confounding factors are the causes of uncertainty; lacking of standard methods for *C. pneumoniae* detection, co-existence of other atherosclerotic risk factors and anti-inflammatory effects of antibiotics used in clinical trials seem to be the principal ones. Standardization of methodology used, antibiotic trials with a different orientation-design and a vaccine preparation that eventually will be tested in clinical trials with a long follow-up, should provide a definite answer regarding the probability *C. pneumoniae* to be a main, a secondary or an irrelevant factor to atherosclerosis. Studies linking *C. pneumoniae* to inflammation and accelerated atherosclerosis in renal failure patients are accumulated but limitations are similar to the above mentioned.

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Introduction

Among the so-called nontraditional risk factors for atherosclerosis, probably the most intriguing [1] (in terms of simplicity and direct effect) is the infectious one where a microorganism is responsible for the initiation and evolution of atherosclerosis. If anti-microbial treatment is efficient for peptic ulcer because *Helicobacter pylori* is implicated in the pathogenesis of this disease, why can't azithromycin be efficient for coronary heart disease (CHD) treatment?

Fifteen years have passed since the publication of the first serologic study performed in Finland by Saikku et al. [2] who found that patients with CHD were significantly more likely to have serum antibodies against *Chlamydia pneumoniae* than control patients. Since then, hundreds of studies have been published. Despite this large volume of information, we haven't yet a definite answer about a causal relationship between *C. pneumoniae* and atherosclerosis.

In this short review, what is already known is briefly presented, and confounding factors preventing final clarification of this issue are described. What is expected and what will probably be helpful for confirmation of this hypothesis or reconsideration is also discussed. Accumulated studies about *C. pneumoniae* and atherosclerosis in

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renal failure patients are summarized. A number of comprehensive reviews that have been published recently, offer a more detailed approach to this interesting topic and are recommended [3–6].

Experimental Evidence: Quite Strong

Probably the strongest evidence linking *C. pneumoniae* and atherosclerosis comes from in vitro experimental studies. *C. pneumoniae* can infect, activate and replicate in the main cells – monocytes/macrophages, endothelial and smooth muscle cells (SMCs) – involved in the atherosclerosis process [7]. In vitro infection of endothelial cells induces secretion and up-regulation of factors promoting rolling, tethering, attachment and trans-endothelial migration of monocytes [8, 9] while the infection by *C. pneumoniae* of monocytes induces the differentiation of these cells into macrophages [10], essential first steps in atherosclerotic injury. *C. pneumoniae* also induces foam cell formation [11], and thus participates in the earlier lesion of atherosclerosis, ‘fatty streak’ formation [12]. Fibrous plaque (the more advanced atherosclerotic lesion), can be promoted by *C. pneumoniae*; it induces production of SMCs growth factors from infected endothelial cells [13] contributing to SMCs activation, migration and proliferation [14] into the neointima as well as production of extracellular matrix, key events in this second atherosclerotic stage [12]. Inflammation within the vessel wall, present in all stages of atherosclerotic process [15], can be sustained by activation of all cells infected by *C. pneumoniae* and production of pro-inflammatory cytokines [16] (probably via nuclear factor kappa B activation [17, 18]). Infection and stimulation of SMCs, followed by the production of metalloproteinases (that results in degradation of extracellular matrix) [19, 20] as well as upregulation of procoagulant factors in endothelial cells, SMCs and macrophages infected by *C. pneumoniae* [21–25] induce plaque destabilization and thrombus formation [12], the final stage of atheroma evolution, after which clinical consequences become apparent.

More interestingly, recent studies showed that *C. pneumoniae* inhibit apoptosis in monocytes and epithelial cells [26, 27] and secrete factors that downregulate expression of major histocompatibility complex in host cells (complex absolutely necessary for antigen recognition) [28]. Both mechanisms (and the second one might be unique for this pathogen) may help *C. pneumoniae* to escape immunological surveillance, establishing a persistent cellular infection and activation, and possible infection of

new cells [29]. This chronic persistent infection can mediate the development of a chronic disease like atherosclerosis.

In vivo studies in hyperlipidemic or normolipidemic experimental animals, have shown conflicting results [30, 31]. In a number of them it was found that *C. pneumoniae* acts mainly as co-risk factor, accelerating atherosclerosis in hyperlipidemic animals [32–34].

In summary – as mainly in vitro experimental studies have showed – *C. pneumoniae* has the capability to induce key features in the atherosclerotic process. The fact that this microorganism is *capable* of inducing atherosclerosis, doesn’t mean at all that it is also *necessary* for this process to be induced. Other, well-established factors – e.g. oxidized LDL – are efficient and necessary for this process initiation and evolution. If *C. pneumoniae* is the principal (sufficient and necessary) factor in some cases of atherosclerosis induction and evolution, or if it is a co-factor aggravating this process remains to be defined.

Clinical Seroepidemiological Studies: Weak Positive Results

The majority of clinical studies published are based on *C. pneumoniae* serum antibodies (IgG and/or IgA) detection and correlation to clinical, angiographic or laboratory atherosclerotic patient data. Three reports by Danesh et al. [35–37] are indicative. The first one [35], published in 1997, included 18 seroepidemiological studies with 2,700 patients, examining the association of *C. pneumoniae* and CHD or cerebrovascular disease and in the majority of them a positive correlation between seropositivity and atherosclerosis was found (odds ratio, OR, 1.2–>8). Only 3 of 18 studies were prospective; serologic criteria were different and in most of them the determination of serum antibodies was performed once. The second [36] report included 14 other prospective studies with 3169 patients with myocardial infarction or death from CHD published until May 2000. These later studies (as well as those included in the third report) were better adjusted for smoking and other risk factors for CHD (as well as for *C. pneumoniae* infection) than those of the first report and showed a borderline association of serum IgG antibodies against *C. pneumoniae* and atherosclerosis (OR 1.15 [95% CI, 0.97–1.36]). The third [37] report published in 2002, included 10 prospective studies with 2,283 cases and examined correlation between IgA antibodies against *C. pneumoniae* (supposed to be a better index of persistent *C. pneumoniae* infection, because of shorter

serum half-life) and CHD. This later meta-analysis was also weakly positive and of marginal statistical significance (OR 1.25 [CI 95% 1.03–1.53]). Finally, a meta-analysis published in 2003 [38] (including 38 studies published between 1997 and December 2000) is also of interest. The overall OR of prospective case-control studies was lower than the OR of cross-sectional studies (1.1, 95% CI 0.8–1.4 vs. 2.0, 95% CI 1.5–2.6). Moreover, the duration of follow-up in prospective studies was inversely related to the strength of the relation found. Authors of this meta-analysis conclude that their results are probably due to a lack of causality between *C. pneumoniae* and atherosclerosis.

Thus, although initial studies appeared positive, the later ones, better designed and more carefully adjusted for other risk factors, showed a weak positive, of marginal significance, correlation between *C. pneumoniae* seropositivity and atherosclerosis.

***C. pneumoniae* in Atheromatous Vessel Wall: Present but How Frequent and What Does That Mean?**

A large number of studies were performed for *C. pneumoniae* identification in atherosclerotic plaques of coronary arteries or other vessel walls. Methods applied varied (polymerase chain reaction, PCR, immunocytochemical staining, ICC, cell cultures, electron microscopy) and even more varied were the results [4]. Higher percentage of *C. pneumoniae* detection in atheroma was found by ICC (in about half of specimens examined), lower by cell cultures (7% of specimens) [4], while PCR results for *C. pneumoniae* DNA detection within atherosclerotic lesions varied between 0 and 100% [39]. Methodological differences seem to be a major problem. In a recent multi-center comparison trial [39] of DNA extraction methods and PCR assays for detection of *C. pneumoniae* in the vessel wall, positivity rates for the same atheroma specimens examined varied between 0 and 60%. In a comprehensive review Boman and Hammerschlag [4] examined the results of 43 studies with 2664 atherosclerotic specimens from human vessel walls, published between 1992 and 2000, where different methods of *C. pneumoniae* identification were used; considering as criterion of true positivity for *C. pneumoniae* detection, confirmation by the use of two (or more) independent techniques (e.g. PCR and cell cultures) they calculated a prevalence of 15.14% for *C. pneumoniae* presence in atheromatous tissue specimens. This percentage is similar to that found by

Maass et al. [40] (16%) in 70 human atheromata, applying a highly sensitive cell culture method for *C. pneumoniae* identification – the method considered as gold standard for proving viability of obligatory intracellular in host eukaryotic cells microorganisms (though it is also problematic when applied for *C. pneumoniae* identification). PCR was also used for *C. pneumoniae* DNA detection in peripheral blood mononuclear cells (PBMCs) – monocytes have a crucial role in systemic *C. pneumoniae* dissemination from pulmonary tissue to the vessel wall – with results varying between 8.8 and up to 50% [41, 42] and a prevalence of *C. pneumoniae* DNA in PBMCs – as was recently summarized from 18 relevant studies [43] – of 14.3% in atherosclerotic patients.

Thus, although *C. pneumoniae* is present in atherosclerotic vessel wall or circulating monocytes, the true prevalence is unknown. A positivity estimation of 15–16% (of *C. pneumoniae* in arterial wall identified by cell culture/or a combination of methods or of *C. pneumoniae* DNA in PBMCs detected by PCR) in atherosclerotic patients may be high or low, because we don't know how sensitive and specific our methods are and we can not also know, due to the same limitations, if this percentage is significantly higher in atheroma than in normal vessel wall.

***C. pneumoniae* Assays: Need of Standardization**

A frequent finding – with some exceptions [44] – in the above mentioned studies, is that *C. pneumoniae* identification in vessel wall [40, 45] or monocytes [41, 42] (with PCR, cell cultures, ICC or other methods) does not correlate with detection of serum antibodies against *C. pneumoniae*. In a recent study, only 10% of PCR positive healthy blood donors for *C. pneumoniae* DNA in PBMCs were also seropositive while seropositivity was 40% in the PCR-negative group [46]. Moreover, culture-documented infection in adults with pneumonia occurs without any detectable serum antibodies [47]. If we add in these findings the fact that seropositivity in the general population is often nearly 70% [48] (while unusual in children younger than 5 years, it is up to 50% by the age of 20 years and 70–80% by the age of 65 years [3]) and that antibody titers fluctuate over time [4], it is clear that seropositivity, as the diagnostic tool used in the majority of clinical studies for atherosclerosis, is not the most reliable method for *C. pneumoniae* identification.

In a workshop for *C. pneumoniae* diagnostic assay standardization, leading researchers in the field tried to formulate, for the first time, specific recommendations

[49]. It should be mentioned that it has been emphasized that 'no validated serologic marker of persistent or chronic infection, exists in present' a conclusion of great importance for the *C. pneumoniae* atherogenous hypothesis based mainly on the assumption of chronic persistent infection. Microimmunofluorescence is the recommended method for *C. pneumoniae* antibodies detection; using single IgG or IgA titers for determining acute or chronic infection is discouraged, and it is stressed that cell culture remains essential to document *C. pneumoniae* viability, but requires propagation of the isolate or confirmation by PCR and specific recommendations are given for ICC and PCR [49]. Attempts to explain discrepancies in the reported rates of *C. pneumoniae* DNA identification [50], reliability of new assay kits [51] and testing of newer, highly sensitive and specific, methods (e.g. real time PCR [52]) are continuous.

Are We Waiting for a Definite Answer from Antibiotic Trials?

Randomized controlled antibiotic trials for atherosclerotic cardiovascular disease (CVD) yielded to diverse results. Two [53, 54] out of 6 relatively small clinical studies of a short (some days up to 1 month) [54–57] or intermediate (3 months) [53, 58] treatment duration with a macrolide (or a combination of antibiotics, [57]) had negative results regarding primary (CVD) and secondary (anti-*C. pneumoniae* antibodies and/or inflammatory indexes) end points; in one study results were positive [55], in another [56] initially positive and then (after 6 months of follow-up) negative and in a third one [58] vice versa. In the last [57] of the 6 studies mentioned above, a significant (40%) reduction in primary (cardiac) end points but not in secondary ones (antibodies to *C. pneumoniae* and *Helicobacter pylori*) was observed. From one more, relatively small study, results are expected [59].

Recently, the negative results (for primary and secondary end points, including *C. pneumoniae* antibodies) of a larger study, after a short term treatment with azithromycin were published [60]. The largest completed study to date (including 7,724 patients, 83% males with documented myocardial infarction and *C. pneumoniae* IgG >1/16), after a 3-month treatment with azithromycin, yielded negative results for primary (CVD morbidity and all-cause and CVD mortality) and secondary (anti-*C. pneumoniae* titers, inflammatory indexes) end points after a median of 14 months of follow-up [61, 62].

Results of 3 important ongoing large studies are expected. In the first one [63], samples of coronary artery for *C. pneumoniae* detection by PCR are examined, post treatment with azithromycin. In the other two studies (each including 4000 patients) [64, 65] a significantly longer treatment duration (1 and 2 years in azithromycin or a combination of a quinolone and statin) and a relatively long follow-up (2 and 4 years) has been designed.

The main criticism for the studies mentioned above is focused on the antibiotic treatment duration [66]. This criticism is based on the known difficulties of this microorganism eradication [66, 67].

Supposing that large long term treatment studies have positive results, will this be a proof for a causal relationship of *C. pneumoniae* and atherosclerosis? Most probably not. The reason is, mainly, the presence of two confounding factors; first, the well-known anti-inflammatory [68, 69] (and anti-oxidant – anticoagulant, as well [5]) effects of macrolides that probably (as has, already been shown [57]) could, independently, influence primary (atherosclerotic CVD) and secondary (inflammatory) end points in these studies; second, the limited reliability of serology, used as single diagnostic tool for *C. pneumoniae* detection in all (except one [63]) of these studies.

Answering the question 'are antibiotics useful in atherosclerotic CVD treatment' doesn't mean that the question 'is *C. pneumoniae* the cause of – or even an aggravating factor for – atherosclerosis' is also answered. Studies with antibiotic trials have probably the power to confirm or not the first, but not the second hypothesis as well.

What about Patients with Renal Failure?

Cardiovascular disease morbidity and mortality is high in chronic renal failure patients and particularly in those on renal replacement treatment. Traditional CVD risk factors do not fully explain the accelerated atherosclerosis present in these patients [70]. Emerging CVD risk factors might also contribute in this population, at the initiation and progression of this process [70]. Inflammation is among these later factors [71]. The infection based atherogenous hypothesis could be a rational basis explaining both, accelerated atherosclerosis and increased inflammation in these patients.

A number of studies, have examined possible association between *C. pneumoniae* presence and atherosclerosis or/and inflammation in this category of patients (table 1). Worth noting are two more studies; the first one found a higher prevalence of *C. pneumoniae* antibodies in renal

Table 1. Studies correlating *C. pneumoniae* to atherosclerosis – inflammation in renal failure (predialysis, hemodialysis, peritoneal dialysis, transplant) patients

	<i>C. pneumoniae</i> detection	Correlation to atherosclerosis	Correlation to inflammation
Zoccali et al. [72]	IgG	(+) carotid artery echo, in males	(+) CRP, in males
Stenvinkel et al. [73]	IgA, IgG	(+) carotid artery echo -IgA	(+) CRP (IgG)
Zoccali et al. [74]	IgG	(+) carotid artery echo (smokers- high CRP)	(+) CRP (smokers)
Iliescu et al. [75]	<i>C. pneumoniae</i> DNA in PBMCs by PCR	(-) medical history data	(-) CRP
Haubitz et al. [76]	IgA, IgG	(+) risk of CHD and risk of death (72 months follow-up) – IgA	(+) CRP
Song et al. [77]	IgA, IgG	(+) CHD (coronary angiography data) – IgA	NM
Kato et al. [78]	IgA, IgG	(+) carotid artery echo-IgA	(+) IL-6
Stenvinkel et al. [79]	IgA, IgG	(+) carotid artery echo (IgA in progressors after 12 months re-evaluation)	(+) IL-6
Oh et al. [80]	IgG	(+) coronary artery calcification and Carotid artery echo	NM
Muller et al. [81]	<i>C. pneumoniae</i> DNA in PBMCs by PCR	NM	NM
Paniagua et al. [82]	IgG	(+) higher CVD mortality (follow-up 10.2 patient-months)	(+) CRP
Wolf et al. [83]	IgA, IgG	(+) medical history data and CVD mortality (2 years follow-up) – IgA	NM
Tsirpanlis et al. [84, 85]	<i>C. pneumoniae</i> in PBMCs (cell culture and PCR) and IgG	(+) medical history data (patients with <i>C. pneumoniae</i> in PBMCs – not seropositives)	(+) IL-1 and VCAM-1 (patients with <i>C. pneumoniae</i> in PBMCs)
Bellomo et al. [86]	IgA, IgG	(+) CVD events (36 months follow-up)	(+) CRP
Maeda et al. [87]	IgA, IgG	(-) Carotid artery echo	NM
Zoccali et al. [88]	IgA, IgG	(-) CVD and all cause mortality after adjustment for traditional and not traditional CVD risk factors (39 ± 20 months follow-up)	(-) CRP

CRP = C-reactive protein; PBMCs = peripheral blood mononuclear cells; PCR = polymerase chain reaction; CHD = coronary heart disease; NM = not mentioned; IL-6 = interleukin-6; CVD = cardiovascular disease; IL-1 = interleukin-1; VCAM-1 = vascular cell adhesion molecule-1.

failure patients with a myeloperoxidase (an enzyme involved in the production of free radicals) – high expression – genotype [89]; the second one did not detect *C. pneumoniae* (by cell culture and PCR) in the hyperplastic vein segment in hemodialysis patients with thrombosed vascular graft [90].

Limitations (regarding mainly seropositivity unreliability, unknown sensitivity and specificity of the other methods for *C. pneumoniae* detection and the other atherosclerotic CVD risk factors influences) are the same as in studies in atherosclerotic population with normal renal function. Especially the very recently published study by Zoccali et al. [88], indicates that the more careful adjustment for other CVD risk factors (traditional or not), weakens *C. pneumoniae* and atherosclerosis correlation in renal failure patients – as was observed in similar studies in the general population.

Conclusions

Although the *C. pneumoniae*-based atherogenous hypothesis remains attractive (mainly because of the interesting findings from experimental in vitro data), it seems that establishing a causal relationship between this pathogen and atherosclerosis in humans is complex. Despite the fact that clinical studies based on seropositivity provided the initial stimulus for this hypothesis, later the same type of studies weren't helpful for a definite clarification or even confounded it. Studies applying various other methods for the detection of this microorganism in vessel walls or monocytes are also confusing. Antibiotic trials – based on seropositivity and macrolide treatment – are also not probably effective in solving this problem. All these unsuccessful attempts seem to lead to a no way-out regarding confirmation of this interesting hypothesis.

There is an urgent need for standardized *C. pneumoniae* detection methods, with high sensitivity and specificity as well as for clear definitions about diagnostic criteria

Table 2. *C. pneumoniae* and atherosclerosis hypothesis: summary of evidence, initial and later interpretations, confounding factors and expectations in this research field

	Seroepidemiological studies	Pathology-based studies	Clinical antibiotic trials	Experimental studies
Initial findings/interpretation	patients with atherosclerotic CVD have more antibodies against <i>C. pneumoniae</i> than controls	<i>C. pneumoniae</i> is more frequently detected (by PCR, cell culture, etc.) in atherosclerotic than in normal vessel wall or in PBMCs	<i>C. pneumoniae</i> bactericidal antibiotics improved outcome in atherosclerotic patients	in vitro: <i>C. pneumoniae</i> induces and propagates inflammatory atherosclerotic process; in vivo: <i>C. pneumoniae</i> induces atherosclerosis in experimental animals
Later findings/interpretation	prospective studies – adjusted to CVD risk factors: weak positive correlation between <i>C. pneumoniae</i> seropositivity and atherosclerosis	inconclusive results: large discrepancies in positivity rates between/within detection methods	more studies with negative than positive results; seropositivity (in most studies) is not affected by antibiotics	new data about <i>C. pneumoniae</i> atherosclerotic effect; in vivo: <i>C. pneumoniae</i> is co-risk factor for atherosclerosis
Confounding factors	high prevalence of <i>C. pneumoniae</i> antibodies in general population; other CVD (or <i>C. pneumoniae</i> infection) risk factors might have influenced results; single-sample IgA or IgG: unreliable to define chronic infection	lacking of standardized – sensitive and specific – methods for <i>C. pneumoniae</i> detection in healthy or infected tissues.	anti-inflammatory effects of macrolides: possible improvement of CVD outcome independently of their anti-bacterial activity; eradication of <i>C. pneumoniae</i> : problematic; seropositivity: unreliable index	other factors (e.g. oxidized-LDL) are effective and – more – necessary for atherosclerosis induction
What is expected?	definition of serologic criteria for <i>C. pneumoniae</i> persistent infection; improving methods for the detection of antibodies	standardization of methods for <i>C. pneumoniae</i> detection	larger ongoing studies results; studies confirming that <i>C. pneumoniae</i> eradication (detected by reliable methods) independently improves atherosclerotic CVD outcome	further investigation of molecular mechanisms of <i>C. pneumoniae</i> atherosclerotic capability; standardization of experimental in vivo studies
Other probable solutions	replacement of serology by other methods (e.g. <i>C. pneumoniae</i> DNA detection in PBMCs)	application of newer (e.g. real time PCR) or combined (e.g. cell culture and PCR) methods	vaccination of <i>C. pneumoniae</i> -free children: follow-up for atherosclerotic CVD	

CVD = Cardiovascular disease; PCR = polymerase chain reaction; PBMCs = peripheral blood mononuclear cells.

for entities such as ‘chronic persistent infection’ due to this pathogen. Attempts to this direction are continuous, but the way seems to be long. Antibiotic trials could provide valuable data if focused on *C. pneumoniae* detection, before treatment initiation and verification of this microorganism eradication after treatment completion, by more reliable methods than serology. These methods (e.g. real-time PCR) could be applied in PBMCs for *C. pneumoniae* DNA detection. To overcome the confounding factor of anti-inflammatory antibiotic effects, probably other effective antibiotics should be tried (without anti-inflammatory properties) or, more control groups (matched for inflammation level but without detectable *C. pneumoniae*) should be added in future studies. Treatment duration also has to be long.

Probably the most suitable way for the *C. pneumoniae* – atherosclerosis hypothesis verification is vaccination against this microorganism, before first exposure and the

long term follow-up of vaccinated children for atherosclerotic CVD. The coincidence of seropositivity appearance, in the first/second decade of life, with the well-known atherosclerosis initiation in the same decades of life [12, 15], is a strong point that promises reliable conclusions, from such an approach. More information about activation of host immune mechanisms against this agent [91], emerging genomic technologies for vaccine preparation [92] and an already prepared vaccine against this microorganism [93] show that this is a realistic expectation (table 2 summarizes data mentioned in this review).

Although from all the above mentioned data we seem to be in a dead end situation regarding the causal relationship between *C. pneumoniae* and atherosclerosis, a more realistic approach is to recognize that we have a long way ahead of us for this documentation. Eventually, at the end of this long way the definite answer to this interesting hypothesis will not be a simple yes or no. More likely the

answer will be intermediate. Atherosclerosis is a multifactorial disease. That *C. pneumoniae* may be a co-risk factor or a main risk factor in some people (depending on the genetic background or on the contribution of other environmental factors) seems, today, more reasonable.

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References

- Quaschnig T, Wanner C: The role of *Chlamydia* in coronary heart disease—fact or fiction? *Nephrol Dial Transplant* 1999;14:2800–2803.
- Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, Huttunen JK, Valtonen V: Serological evidence of an association of a novel *Chlamydia*, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 1988;ii:983–986.
- Kalayoglu MV, Libby P, Byrne GI: *Chlamydia pneumoniae* as an emerging risk factor in cardiovascular disease. *JAMA* 2002;288:2724–2731.
- Boman J, Hammerschlag MR: *Chlamydia pneumoniae* and atherosclerosis: Critical assessment of diagnostic methods and relevance to treatment studies. *Clin Microbiol Rev* 2002; 15:1–20.
- Higgins JP: *Chlamydia pneumoniae* and coronary artery disease: The antibiotic trials. *Mayo Clin Proc* 2003;78:321–332.
- Ngeh J, Amand V, Gupta S: *Chlamydia pneumoniae* and atherosclerosis – what we know and what we don't. *Clin Microbiol Infect* 2002; 8:2–13.
- Gaydos C, Summersgill J, Sahney N, Ramirez J, Quinn T: Replication of *Chlamydia pneumoniae* in vitro in human macrophages, endothelial cells, and aortic artery smooth muscle cells. *Infect Immun* 1996;64:1614–1620.
- Summersgill J, Molestina R, Miller R, Ramirez J: Interactions of *Chlamydia pneumoniae* with human endothelial cells. *J Infect Dis* 2000;181 (Suppl 3): S479–S482.
- Molestina R, Miller R, Ramirez J, Summersgill J: Infection of human endothelial cells with *Chlamydia pneumoniae* stimulates transendothelial migration of neutrophils and monocytes. *Infect Immun* 1999;67:1323–1330.
- Yamaguchi H, Haranaga S, Widen, Friedman H, Yamamoto Y: *Chlamydia pneumoniae* infection induces differentiation of monocytes into macrophages. *Infect Immun* 2002;70: 2392–2398.
- Kalayoglu M, Byrne G: Induction of macrophage foam cell formation by *Chlamydia pneumoniae*. *J Infect Dis* 1998;177:725–729.
- Lusis A: Atherosclerosis. *Nature* 2000;407: 233–241.
- Coombes B, Mahony J: cDNA array analysis of altered gene expression in human endothelial cells in response to *Chlamydia pneumoniae* infection. *Infect Immun* 2001;69:1420–1427.
- Hirono S, Dibrov E, Hurtado C, Kostenuk A, Ducas R, Pierce GN: *Chlamydia pneumoniae* stimulates proliferation of vascular smooth muscle cells through induction of endogenous heat shock protein 60. *Circ Res* 2003 (published online before print September 18, 2003).
- Ross R: Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115–126.
- Gaydos C: Growth in vascular cells and cytokine production by *Chlamydia pneumoniae*. *J Infect Dis* 2000;181(suppl 3): S473–S478.
- Nissner A, Kaun C, Zorn G, Speidl W, Turel Z, Christiansen G, Pedersen AS, Bickelund S, et al: Polymorphic membrane protein (PMP) 20 and PMP 21 of *Chlamydia pneumoniae* induce proinflammatory mediators in human endothelial cells in vitro by activation of the nuclear factor-kappa B pathway. *J Infect Dis* 2003;188: 108–113.
- Gensay MM, Tamm M, Glanville A, Perruchoud AP, Roth M: *Chlamydia pneumoniae* activates epithelial cell proliferation via NF-kappa B and the glucocorticoid receptor. *Infect Immun* 2003;71:5814–5822.
- Kol A, Sukhova G, Lichtman A, Libby P: Chlamydia heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor- α and matrix metalloproteinase expression. *Circulation* 1998;98:300–307.
- Rodel J, Prochnau D, Prager K, Pentcheva E, Hartmann M, Straube E: Increased production of matrix metalloproteinases 1 and 3 by smooth muscle cells upon infection with *Chlamydia pneumoniae*. *FEMS Immunol Med Microbiol* 2003;38:159–164.
- Fryer R, Schwobe E, Woods M, Rodgers M: *Chlamydia* species infect human vascular endothelial cells and induce pro-coagulant activity. *J Invest Med* 1997;45:168–174.
- Dechend R, Maass M, Gieffers J, Dietz R, Scheidereit C, Leutz A: *Chlamydia pneumoniae* infection of vascular smooth muscle cells and endothelial cells activates NF-kB and induces tissue factor and PAI-I expression: A potential link to accelerated arteriosclerosis. *Circulation* 1999;100:1369–1373.
- Bea F, Puolakkainen MH, McMillen T, Hudson FN, Mackman N, Kuo CC, Campbell LA, Rosenfeld ME: *Chlamydia pneumoniae* induces tissue factor expression in mouse macrophages via activation of Erg-1 and the MEK-ERK1/2 pathway. *Circ Res*. 2003;92:394–401.
- Rupp J, Mass M: Erg-1, a major link between infection and atherosclerosis? *Circ Res* 2003; 16:e78.
- Vehmaan-Kreula P, Puolakkainen M, Sarvas M, Welgus HG, Kovanen PT: *Chlamydia pneumoniae* proteins induce secretion of the 92-kDa gelatinase by human monocyte-derived macrophages. *Arterioscler Thromb Vasc Biol* 2001;21:e1–e8.
- Geng Y, Shane R, Berencsi K, Gonczol E, Zaki MH, Margolis DJ, Trinchieri G, Rook AH: *Chlamydia pneumoniae* inhibits apoptosis in human peripheral blood mononuclear cells through induction of IL-10. *J Immunol* 2000; 164:5522–5529.
- Airenne S, Surcel M, Tuukkanen J, Leinonen M, Saikku P: *Chlamydia pneumoniae* inhibits apoptosis in human epithelial and monocyte cell lines. *Scand J Immunol* 2002;55:390–398.
- Fan P, Dong F, Huang Y, Zhong G: *Chlamydia pneumoniae* secretion of a protease-like activity factor for degrading host cell transcription factors is required for major histocompatibility complex antigen expression. *Infect Immun* 2002;70:345–349.
- Mahony J, Coombes B: *Chlamydia pneumoniae* and atherosclerosis: Does the evidence support a causal or contributory role? *FEMS Microbiol Lett* 2001;197:1–9.
- Campbell LA, Kuo C-C: *Chlamydia pneumoniae* pathogenesis. *J Med Microbiol* 2002;51: 623–625.
- Aalto-Setälä K, Laitinen K, Erkkilä L, Leinonen M, Jauhiainen M, Ehnholm C, Tamminen M, Puolakkainen M, Penttilä I, Saikku P: *Chlamydia pneumoniae* does not increase atherosclerosis in the aortic root of apolipoprotein E-deficient mice. *Atheroscler Thromb Vasc Biol* 2001;21:578–584.
- Blessing E, Campbell LA, Rosenfeld ME, Chouh N, Kuo C-C: *Chlamydia pneumoniae* infection accelerates hyperlipidemia induced atherosclerotic lesion development in C57BL/6J mice. *Atherosclerosis* 2001;158:13–17.
- Ezzahiri R, Nelissen-Vrancken HJMG, Kurvers HAJM, Stassen FRM, Vliegen I, Grauls GELM, van Paul MML, Kitslaar PJEHM, Bruggeman CA: *Chlamydia pneumoniae* (*Chlamydia pneumoniae*) accelerates the formation of complex atherosclerotic lesions in Apo E3-Leiden mice. *Cardiovasc Res* 2002;56: 269–276.

- 34 Blessing E, Campbell LA, Rosenfeld ME, Kuo C-C: *Chlamydia pneumoniae* and hyperlipidemia are co-risk factors for atherosclerosis: Infection prior to induction of hyperlipidemia does not accelerate development of atherosclerotic lesions in C57BL/6J mice. *Infect Immun* 2002;70:5332–5334.
- 35 Danesh J, Collins R, Peto R: Chronic infections and coronary heart disease: Is there a link? *Lancet* 1997;350:430–436.
- 36 Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Wong Y-K, Bernades-Silva M, Ward M: *Chlamydia pneumoniae* IgG titres and coronary heart disease: prospective study and meta-analysis. *BMJ* 2000;321:208–213.
- 37 Danesh J, Whincup P, Lewington S, Walker M, Lennon L, Thomson A, Wong Y-K, Zhou X, Ward M: *Chlamydia pneumoniae* IgA titres and coronary heart disease. *Eur Heart J* 2002; 23:371–375.
- 38 Bloemenkamp DGM, Mali WPTM, Visseren FKJ, van de Graaf Y: Meta-analysis of seroepidemiologic studies of the relation between *Chlamydia pneumoniae* and atherosclerosis: Does study design influence results? *Am Heart J* 2003;145:409–417.
- 39 Apfalter P, Blasi F, Boman J, Gaydos C, Kundi M, Maass M, Makristathis A, Meijer A, Nadrchal R, Persson K, Rotter ML, Tong CYW, Stanek G, Hirschl AM: Multicenter comparison trial of DNA extraction methods and PCR assays for detection of *Chlamydia pneumoniae* in endarterectomy specimens. *J Clin Microbiol* 2001;39:519–524.
- 40 Maass M, Bartels C, Engel P, Mamat U, Sievers H-H: Endovascular presence of viable *Chlamydia pneumoniae* is a common phenomenon in coronary artery disease. *J Am Coll Cardiol* 1998;31:827–832.
- 41 Wong Y, Dawkins K, Ward M: Circulating *Chlamydia pneumoniae* DNA as a predictor of coronary artery disease. *J Am Coll Cardiol* 1999;34:1435–1439.
- 42 Boman J, Soderberg S, Forsberg J, Birgander LS, Allard A, Persson K, Jidell E, Kumlin U, Juto P, Waldenstrom A, Goran W: 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;178:274–277.
- 43 Smieja M, Mahony J, Petrich A, Boman J, Chernesky M: Association of circulating *Chlamydia pneumoniae* DNA with cardiovascular disease: A systematic review. *BMC Infect Dis* 2002;2. <http://www.biomedcentral.com/1471-2334/2/21>.
- 44 Neureiter D, Heuschmann P, Stintzing S, Kolominsky-Rabas P, Barbera L, Jung A, Ocker A, Mass M, Faller G, Kirchner T: Detection of *Chlamydia pneumoniae* but not of *Helicobacter pylori* in symptomatic atherosclerotic carotids associated with enhanced serum antibodies, inflammation and apoptosis rate. *Atherosclerosis* 2003;168:153–162.
- 45 Zamorano J, Garcia-Tejada J, Suarez A, Culebras E, Castanon J, Moreno R, Reguillo F, Gil M, Picazo J, Sanchez-Harguindey L: *Chlamydia pneumoniae* in the atherosclerotic plaques of patients with unstable angina undergoing coronary artery bypass grafting: Does it have prognostic implications? *Int J Cardiol* 2003;90: 297–302.
- 46 Bodetti T, Timms P: Detection of *Chlamydia pneumoniae* DNA and antigen in the circulating mononuclear cell fractions of human and koalas. *Infect Immun* 2000;68:2744–2747.
- 47 Hammerschlag M, Roblin P: Microbiologic efficacy of moxifloxacin for the treatment of community-acquired pneumonia due to *Chlamydia pneumoniae*. *Int J Antimicrob Agents* 2000;15:149–152.
- 48 Muhlestein J: Chronic infection and coronary artery disease. *Med Clin N Am* 2000;84:123–149.
- 49 Dowell S, Peeling R, Boman J, Carlone GM, Fields BS, Guarner J, Hammerschlag MR, Jackson LA, Kuo C-C, Mass M, Messmer TO, Talkington DF, Tondella ML, Zaki and the *C. pneumoniae* Workshop Participants: Standardizing *Chlamydia pneumoniae* assays: Recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Center for Disease Control (Canada). *Clin Infect Dis* 2001;33:492–502.
- 50 Apfalter P, Assadian O, Blasi F, Boman J, Gaydos CA, Kundi M, Makristathis A, Nehr M, Rotter ML, Hirschl AM: Reliability of nested PCR for detection of *Chlamydia pneumoniae* DNA in atheromas: Results from a multicenter study applying standardized protocols. *J Clin Microbiol* 2002;40:4428–4434.
- 51 Chernesky M, Smieja M, Schachter J, Summersgill J, Schindler L, Solomon N, Campbell K, Campbell LA, Cappuccio A, Gaydos C, Chong S, Moncada J, Phillips J, Jang D, Wood BJ, Petrich A, Hammerschlag M, Cerney M, Mahony J: Comparison of an industry-derived LCx *Chlamydia pneumoniae* PCR research kit to in-house assays performed in five laboratories. *J Clin Microbiol* 2002;40:2357–2362.
- 52 Reischl U, Lehn N, Simnacher U, Marre R, Essing A: Rapid and standardized detection of *Chlamydia pneumoniae* using LightCycler Real-Time Fluorescence PCR. *Eur J Clin Microbiol Infect Dis* 2003;22:54–57.
- 53 Anderson JL, Muhlestein JB: The ACADEMIC study in perspective (Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infarction with *Chlamydia*). *J Infect Dis* 2000; 181(suppl 3):S579–S581.
- 54 Leowattana W, Bhuripanyo K, Singhviranon L, Akaniroj S, Mahan N, Samranthin M, Pokum S: Roxithromycin in prevention of acute coronary syndrome associated with *Chlamydia pneumoniae* infection: A randomized placebo controlled trial. *J Med Assoc Thai* 2001; 84(suppl 3):S669–S675.
- 55 Gupta S, Leatham E, Carrington D, Mendall MA, Kaski JK, Camm AJ: Elevated *Chlamydia pneumoniae* antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. *Circulation* 1997;96:404–407.
- 56 Gurfinkel E, Bozovich G, Beck E, Testa E, Livellara B, Mautner B: Treatment with the antibiotic roxithromycin in patients with acute non-Q-wave coronary syndromes: The final report of the ROXIS study. *Eur Heart J* 1999;20: 121–127.
- 57 Stone AFM, Mendall MA, Kaski J-C, Edger TM, Risley P, Poloniecki J, Camm J, Northfield TC: Effect of treatment for *Chlamydia pneumoniae* and *Helicobacter pylori* on markers of inflammation and cardiac events in patients with acute coronary syndromes. South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina (STAMINA). *Circulation* 2002;106:1219–1223.
- 58 Sinisalo J, Mattila K, Valtonen V, Anttonen O, Juvonen J, Melin J, Vuorinen-Markkola H, Nieminen MS; Clarythromycin in Acute Coronary Syndrome Patients in Finland (CLARIFY) Study Group: Effect of 3 months of antimicrobial treatment with clarythromycin in acute non-Q-wave coronary syndrome. *Circulation* 2002;105:1555–1560.
- 59 Reiner Z, CROAATS Study Group: Azithromycin in the secondary prevention of adverse cardiovascular events in *C. pneumoniae* positive post-myocardial infarction patients. Available at: www.icmask.org/icmask-06/abstracts.
- 60 Cercek B, Shah PK, Noc M, Zahger D, Zeymer U, Matetzky S, Mauer G, Mahrer P: Effect of short-term treatment with azithromycin on recurrent ischaemic events in patients with acute coronary syndrome in the Azithromycin in Acute Coronary Syndrome (AZACS) trial: A randomized controlled trial. *Lancet* 2003;361: 809–813.
- 61 Dunne MW: Rationale and design of a secondary prevention trial of antibiotic use in patients after myocardial infarction: The WIZARD (weekly intervention with zithromax [azithromycin] for atherosclerosis and its related disorders) trial. *J Infect Dis* 2000;181(suppl 3): S572–S578.
- 62 O'Connor CM, Dunne MW, Pfeffer MA, Muhlestein JB, Gupta S, Benner RJ, Fisher MR, Cook TD: Azithromycin for the secondary prevention of coronary heart disease events: the WIZARD study: A randomized controlled study. *JAMA* 2003;290:1459–1466.
- 63 Gupta S: *Chlamydia pneumoniae*, monocyte activation and azithromycin therapy in coronary heart disease. Available at: www.INSERM.fr/athero/.
- 64 Jackson LA: Description and status of the Azithromycin and Coronary Events Study (ACES). *J Infect Dis* 2000;181(suppl 3):S579–S581.
- 65 Cannon CP, McCabe CH, Belder R, Breen J, Braunwald E: Design of the Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT)-TIMI 22 trial. *Am J Cardiol* 2002;89:860–861.

- 66 Grayston JT: Antibiotic treatment of atherosclerotic cardiovascular disease. *Circulation* 2003;107:1228–1230.
- 67 Yamaguchi H, Friedman H, Yamamoto M, Yasuda K, Yamamoto Y: *Chlamydia pneumoniae* resists antibiotics in lymphocytes. *Antimicrob Agents Chemother* 2003;1972–1975.
- 68 Labro MT: Anti-inflammatory activity of macrolides: A new therapeutic potential? *J Antimicrob Chemother* 1998;41(suppl B):37–46.
- 69 Culic O, Eracovic V, Parnham MJ: Anti-inflammatory effects of macrolide antibiotic. *Eur J Pharmacol* 2001;429:209–229.
- 70 Zoccali C, Mallamaci F, Tripepi G: Traditional and emerging cardiovascular risk factors in end-stage renal disease. *Kidney Int* 2003;(suppl 85):S105–S110.
- 71 Wanner C, Zimmermann J, Quaschnig T, Galle J: Inflammation, dyslipidemia and vascular risk factors in hemodialysis patients. *Kidney Int* 1997;(suppl 62):S53–S55.
- 72 Zoccali C, Benedetto F, Foca A, Tripepi G, Malatino L, Mallamaci et al, GREED (Cardiovascular risk extended evaluation in dialysis patients) Investigators: C-reactive protein and atherosclerosis in dialysis patients. *Nephrol Dial Transpl* 1998;13:2710–2711.
- 73 Stenvinkel P, Heimbürger O, Jogestrand T, Karnell A, Samuelson A: Does persistent infection with *Chlamydia pneumoniae* increase the risk of atherosclerosis in chronic renal failure? *Kidney Int* 1999;56:2531–2532.
- 74 Zoccali C, Benedetto F, Mallamaci F, Tripepi G, Fermo I, Foca A, Paroni R, Malatino LS, on behalf of the Greed Investigators: Inflammation is associated with carotid atherosclerosis in dialysis patients. *J Hypertens* 2000;18:1207–1213.
- 75 Iliescu E, Fiebig M, Morton A, Sankar-Mistry P: *Chlamydia pneumoniae* DNA in peripheral blood mononuclear cells in peritoneal dialysis patients. *Perit Dial Int* 2000;20:722–726.
- 76 Haubitz M, Brunkhorst R: C-reactive protein and chronic *Chlamydia pneumoniae* infection-long term predictors for cardiovascular disease and survival in patients on peritoneal dialysis. *Nephrol Dial Transplant* 2001;16:809–815.
- 77 Song H, Tasaki H, Yashiro A, Okazaki M, Ioka T, Taniguchi H, Nakashima Y: *Chlamydia pneumoniae* infection and accelerated development of coronary artery disease in patients with chronic renal failure. *Clin Nephrol* 2001;56:346–352.
- 78 Kato A, Odamaki M, Takita T, Maruyama Y, Kumagai H, Hishida A: Association between interleukin-6 and carotid atherosclerosis in hemodialysis patients. *Kidney Int* 2002;61:1143–1152.
- 79 Stenvinkel P, Heimbürger O, Jogestrand T: Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dialysis patients: Association with *Chlamydia pneumoniae* seropositivity. *Am J Kidney Dis* 2002;39:274–282.
- 80 Oh J, Wunsch R, Turzer M, Bahner M, Raggi P, Quercfeld U, Mehls O, Schaefer F: Advanced coronary and carotid arteriopathy in young adults with childhood-onset chronic renal failure. *Circulation* 2002;106:100–105.
- 81 Müller J, Nyvad O, Larsen NA, Lokkergaard N, Pedersen RS, Solling K, Pedersen EB: *Chlamydia pneumoniae* DNA in peripheral blood mononuclear cells in dialysis patients, renal transplant recipients and healthy controls. *Scand J Clin Lab Invest* 2002;62:503–509.
- 82 Paniagua R, Frias Y, de Ventura MJ, Rodriguez E, Hurtado ME, Alcantara G, Vazquez R, Otriz R, Salcedo M, Rios ME, Kaji J, Amato J: C-reactive protein and anti-*Chlamydia pneumoniae* antibodies as risk factors of cardiovascular death in incident patients on peritoneal dialysis. *Perit Dial Int* 2003;23:132–137.
- 83 Wolf SC, Mayer O, Jurgens S, Vonthein R, Schultze G, Risler T, Brehm BR: *Chlamydia pneumoniae* IgA seropositivity is associated with increased risk for atherosclerotic vascular disease, myocardial infarction and stroke in dialysis patients. *Clin Nephrol* 2003;4:273–279.
- 84 Tsirpanlis G, Chatzipanagiotou S, Ioannidis A, Moutafis S, Pouloupoulou C, Nikolaou C: Detection of *Chlamydia pneumoniae* in peripheral blood mononuclear cells: correlation with inflammation and atherosclerosis in haemodialysis patients. *Nephrol Dial Transplant* 2003;18:918–923.
- 85 Tsirpanlis G, Chatzipanagiotou S, Ioannidis A, Ifanti K, Bagos P, Lagouranis A, Pouloupoulou C, Nikolaou C: The effect of viable *Chlamydia pneumoniae* on serum cytokines and adhesion molecules in hemodialysis patients. *Kidney Int* 2003;63(suppl 84):S72–S75.
- 86 Belomo G, Lippi G, Saronio P, Reboli G, Verdura C, Timio F, Timio M: Inflammation, infection and cardiovascular events in chronic hemodialysis patients: a prospective study. *J Nephrol* 2003;16:245–251.
- 87 Maeda N, Sawayama Y, Tatsukawa M, Okada K, Furusyo N, Shigematsu M, Kashiwagi S, Hayashi J: Carotid artery lesions and atherosclerotic risk factors in Japanese hemodialysis patients. *Atherosclerosis* 2003;169:183–192.
- 88 Zoccali C, Mallamaci F, Tripepi G, Parlongo S, Cutrupi S, Benedetto FA, Bonanno G, Semina G, Pasquale F, Rapisarda F, Malatino LS: *Chlamydia pneumoniae*, overall and cardiovascular mortality in end-stage renal disease (ESRD). *Kidney Int* 2003;64:579–584.
- 89 Pecoits-Filho R, Stenvinkel P, Marchlewska A, Heimbürger O, Barany P, Hoff CM, Holmes CJ, Suliman M, Lindholm B, Schalling M, Nordfors L: A functional variant of the myeloperoxidase gene is associated with cardiovascular disease in end-stage renal disease patients. *Kidney Int* 2003;63(suppl 84):S172–S176.
- 90 Zidlewski AW, Stothard DR, Van Der Pol B, Jones RB, Filo RS, Hasbargen JA: Is there a role for *Chlamydia pneumoniae* in hemodialysis vascular access thrombosis? *Am J Kidney Dis* 2000;36:1122–1125.
- 91 Wizel B, Starcher BC, Samten B, Chroneos Z, Barnes PF, Dzuris J, Higashimoto Y, Apella E, Sette A: Multiple *Chlamydia pneumoniae* antigens prime CD8 T cell responses that inhibit intracellular growth of this vacuolar pathogen. *J Immunol* 2002;169:2524–2535.
- 92 Grandi G: Rational antibacterial vaccine design through genomic technologies. *Int J Parasitol* 2003;33:615–620.
- 93 TWARVAX (*Chlamydia pneumoniae* vaccine). Available at www.antexbiologics.com/antexbio/twarvax.phtml.