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Atherosclerosis associated with *Chlamydia pneumoniae*: Dissecting the etiology

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ABSTRACT

Chlamydia pneumoniae related infections and atherosclerosis are both common entities. Today, the literature presents an increasing amount of data regarding the role of C. pneumoniae in the development and sustainment of atherosclerosis and allowing us to comprehend the molecular mechanisms behind better. The implications of C. pneumoniae in atherogenesis include altered platelet function, hypercoagulability, macrophage dysfunction, vascular smooth muscle proliferation, and increased neutrophilic migration. Therefore, it would not be wrong to implicate that, C. pneumoniae plays important roles in almost every stage of atherogenesis. Furthermore, various serological markers suggestive of active or past C. pneumoniae infection are known to be associated with multiple clinical presentations, such as abdominal aortic aneurysms, subclinical atherosclerosis in young individuals, aggravated atherosclerosis in heterozygous familial hypercholesterolemia. This review, aims to provide detailed insights into the pathophysiological mechanisms of atherogenesis associated with C. pneumoniae and its clinical implications.

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Introduction

Atherosclerosis is a public health issue leading to numerous clinical syndromes and diseases affecting a great majority of the population, including but not limited to coronary heart disease, peripheral artery disease, ischemic stroke, vascular dementia and mesenterial ischemia, constituting the greatest contribution to morbidity and mortality [1]. While the recent developments have shown that atherosclerosis is a complex process, in which many factors acting on the endothelium play various roles, whereby the immune system is responsible for a majority of irreversible changes or widespread evolution of this condition throughout the body, which led to the efforts of identifying specific cell types inhabiting the atherosclerotic niche, which includes but not limited to single-cell approaches [2].

The latest research has identified many antigens implicated in the pathogenesis of atherosclerosis, with the majority of these being modified innate molecules such as oxidized low-density lipoproteins (ox-LDLs), beta-2-glycoprotein-1 (Beta2GP1), lipoprotein (a) (Lp(a)), with some other foreign antigens stemming from bacteria such as some *Porphyromonas spp.* and *Chlamydia pneumoniae* (Cp) [3].

Among these, Cp is an obligate intracellular implicated Gram-negative and exists in two morphological forms: elementary and reticulate bodies. The elementary bodies play an important role in the transmission of the pathogen, which are not metabolically active. Whereas reticulate bodies are active forms with no role in transmissions [4]. Cp is known to induce various epigenetic changes in the cell, including deoxyribonucleic acid (DNA) methylation, gene silencing via microRNAs, and post-translational histone modifications [5].

Cp is a well-recognized cause of atypical community-acquired pneumonia (CAP), responsible for 10% of all CAPs, therefore a common pathogen to encounter in the clinical wards, and it has been of interest as a potential suspect in several immune-mediated diseases, spanning from multiple sclerosis [6] and reactive arthritis to asthma [7]. The exact mode of transmission for Cp is still uncertain but transmission through respiratory secretions, in form of droplet aerosols, has been suspected. This organism is known to survive on laminated countertops for 30 hours and small-particle aerosolization. Enclosed populations, including military personnel, prisoners and nursing home residents constitute significant risk groups for infection with Cp [8].

The role of Cp in the immune changes associated with atherosclerosis has lately become a topic of interest, with some authors suggesting that Cp might act as both an initiator and a driver of chronic inflammation in the atherosclerotic plaque niche [4].

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Cp has been discovered to be involved in a series of atherosclerotic cardiovascular diseases (ASCVD) including coronary artery disease (CAD), carotid artery stenosis, stroke, peripheral artery disease, and aortic aneurysms. This pathogen is proven to exist preferentially in the atherosclerotic lesions both in humans and animal models [4], however the data on how it contributes to the atheroma development or progression is controversial.

Nevertheless, three possible roles can be hypothetically attributed to Cp: first, it might persist in vascular cells as a bystander, therefore, not contributing to the pathogenesis; second, it might initiate atherosclerotic changes; third, it might contribute to the severity of the disease, all of which, however, requiring further investigation and validation [4].

In this review, we aim to summarize the latest literature in order to provide insights into the etiological role of Cp in the development and sustainment of the atherosclerosis, along with emphasizing the molecular pathways implicated. The role of Cp in the development of atherosclerotic lesions is summarized in **Table 1** and depicted in **Figure 1**.

C. pneumoanie as a mere bystander? Probably not

Some authors agree that the relationship between Cp and atherosclerosis might not be as straightforward as often presented, therefore adhering to the fact that it might only play the role of a bystander,

Table 1 | Roles attributed to Chlamydia pneumonia in atherosclerosis.

Stages of atherosclerotic lesion	Chlamydia pneumonia associated changes
Fatty streak formation	Increased uptake of oxLDL by endothelium, by upregulation of LOX-1 [17-19]
Early macrophage reaction and foam cell formation	Promoting trans endothelial migration of monocytes through phosphorylation of tyrosine in vascular endothelial cadherin [25] Induction of foam cell formation from monocytes when LDL-C is present, dependent on TLR2 [12-14] Firmer monocyte adhesion to endothelium, through redistribution of CD44 [27] Increased uptake of oxLDL by macrophages, by upregulation of LOX-1 [17-19]
Continuing immune reaction	Increased macrophage presence and faster lesion progression through activation of IL-17 [47-49] Increased serum levels of IFN-γ, IL-10, TNF-α, sVCAM-1 and soluble E-selectin [51, 54-55] Increased expressions of SAA and VCAM -1 [50]
Fibrous cap formation	Promoting vascular smooth muscle cell migration through JunB-Fra-1/MMP2 pathway [24]
Late thrombosis and embolization	Increased tissue factor formation from the endothelium [31] Increased chemokine expressions on platelets [32] Increased platelet aggregation through, increased P-selection expressions on platelets [33] Stimulation of platelets, leading to ROS production and migration via bloodstream [35] Increased tPA expressions, leading to reduced thrombosis [39] Leading to increased bFGF expressions, associated with symptomatic plaques [51-52]

OxLDL: oxidized low-density-lipoprotein, **LOX-1:** oxidized low-density lipoprotein receptor 1, **LDL-C:** low-density lipoprotein cholesterol, **TLR2:** toll-like receptor 2, **IL-17:** interleukin-17, **IFN- γ:** interferon gamma, **TNF-α:** tumor necrosis factor alpha, **sVCAM1:** soluble vascular cell adhesion molecule 1, **SAA:** serum amyloid A, **VCAM:** vascular cell adhesion molecule, **bFGF:** basic fibroblast growth factor, **ROS:** reactive oxygen species, **JunB:** transcription factor Jun-B, **Fra-1:** Fos-related antigen 1, **MMP2:** matrix metalloprotease 2.

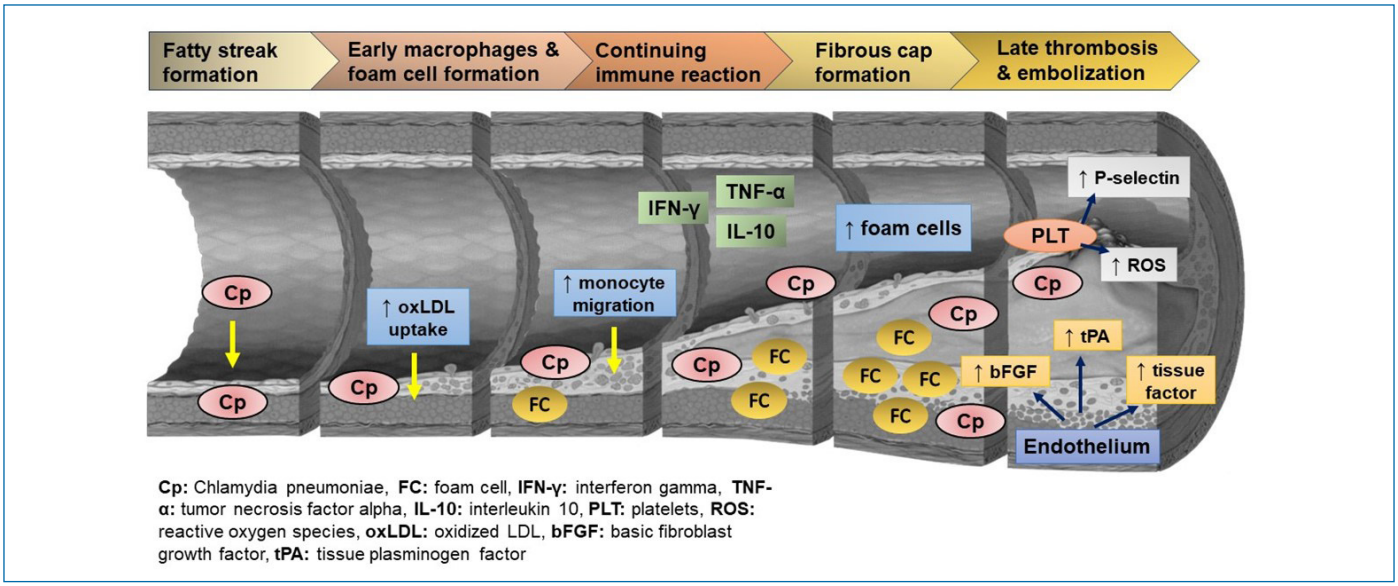

Figure 1 | Chlamydia pneumoniae and atherosclerosis formation.

therefore, not contributing significantly to the pathogenesis [4]. While the subjects included in the epidemiological studies, where Cp seropositivity has been detected, were also carrying many other possible confounding risk factors for atherosclerosis such as smoking, high-cholesterol diet, diabetes, and hypertension [4]. Additionally, the incidence of Cp and arterial disease differs significantly among countries, ranging from 71% in Canada [9] to 0% in Australia [10]. The role of the sample-size, detection methods used and the season of sample collection in contributing to this variety remains unknown [4].

On the other hand, data from basic and translational science have propesed various roles for Cp in the development of atherosclerosis, after spreading from the lungs to reach atherosclerotic lesions via vasculature. First, Cp is known to induce *in-vitro* vascular smooth muscle cell (VSMC) proliferation and lead to the release of atherogenic cytokines in cultured human aortic vascular smooth cells inoculated with Cp [11]. Second, Cp can induce foam cell formation from monocyte-derived macrophages in the presence of LDL-C. These macrophages can enhance cellular oxidation of LDL-C, a reaction independent of the presence of reactive oxygen species (ROS), when Cp is involved. The latter, however, could be prevented by the heat treatment of the bacterium, which rules out the bacterial lipopolysaccharide (LPS) as the culprit for the LDL oxidization, as previously acknowledged [12], leading to the identification of another possible culprit known as chlamydial heat shock protein 60 (HSP60), that is found to be present in 47% of human surgical atherosclerotic tissue specimens [12]. However, it is important to note that, bacterial lipopolysaccharide (LPS) still plays an important role in the formation of foam cells, a hallmark of atherosclerosis [11].

Pathophysiological mechanisms

C. pneumoniae infects monocytes and vascular smooth muscle cells

An earlier study proved that phagocytosed Cp leads to the increased survival of granulocytes, whereas normal granulocytes undergoing apoptosis within 10 hours, while infected ones surviving up to 90 hours, as a result, exploiting these as host cells for multiplication [13]. The internalization of Cp occurs in an opsonin-independent manner [13].

Cp can infect *in-vitro* VSMCs [14]. Approximately two hours after inoculation with Cp, VSMCs start to overexpress toll-like receptor (TLR) 2 mRNA, which belongs to a greater family known as pattern recognition receptors (PRR) [4], while TLR2 only recognizing the organism but not chlamydial LPS or chlamydial heat shock protein 60, which are involved in various atherogenic processes, the latter two are recognized by another TLR, TLR4 [15].

Cp leads to the induction of foam cell formation in monocyte-derived macrophages after internalization, a sign of the early stages of atherosclerosis, when LDL-C is also present. However, LPS of Cp leads to this change only in cells presenting TLR2. On the other hand, lack of TLR4, another toll-like-receptor indicated in the *Escherichia coli* LPS associated foam cell formation, does not interfere with Cp-associated foam cell formation, suggesting that foam cell formation due to Cp is dependent on TLR2 [16].

Both TLR2 and TLR4 have an adapter protein named MyD88 to be activated, delineating this protein as a therapeutic target against chlamydia-associated atherosclerotic changes [15]. Nevertheless, Cp is capable of inducing foam cell formation dependent on and independent of MyD88 pathways, which are both activated by TLR2 and TLR4 [17]. On the other hand, liver X receptors (LXR) are known to downregulate TLR-mediated inflammatory pathways, and their expressions are decreased by Cp, promoting the transformation of macrophages into foam cells [18]. An LXR agonist can, as a result, significantly reduce Cp associated foam cell formation [17].

In *in-vitro* LDL-treated macrophages, Cp upregulates the expressions of both scavenger receptor A1 (SR-A1) and acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1), additionally downregulating the expression of ATP binding cassette transporters (ABCA1 and ABCG1) by exploiting the TLR2-NFκB-miR-33 pathway [19], facilitating cholesterol accumulation and therefore foam cell formation. Peroxisome proliferator-activated receptor gamma (PPARγ) plays an important role in the regulation of the above-mentioned SR-A1, ACAT, ABCA!, and ABCG1. Agonism of PPARγ through rosiglitazone can attenuate Cp-related foam cell formation, whereas antagonism of PPARγ leads to increased foam cell formation. Similar positive effects are produced by PPARα agonist fenofibrate, suggesting PPARα's possible role [20, 21]. Additionally, retinoic acid can suppress foam cell formation due to Cp in hyperlipidemic mice, whereas having no effect on uninfected animals [22]. Another inhibitor of ABCA1 is Cp-induced extracellular IL-1β through exploiting NLRP3 inflammasome, leading to the accumulation of lipids inside cells, which might lead to the investigation of NLRP3 inhibitors as alleviators of Cp-associated accelerated atherosclerosis [23].

On the other hand, inhibition of C-Jun NH2 terminal kinase (JNK1/2) and extracellular signal-regulated kinase (ERK1/2) strongly inhibit foam cell formation due to Cp infection in cultured macrophages, as Cp can not only downregulate PPARγ and PPARα but also downregulate the expressions of JNK1/2, ERK1/2 and p38 mitogen associated protein kinase (MAPK) through phosphorylation, suggesting the role of MAPK-PPARα/γ signal transduction pathway in foam cell formation due to Cp [24, 25].

Infection with Cp leads to the accumulation of mitochondrial ROS, which activates downstream signaling of the JunB-Fra-1/MMP2 pathway, promoting VSMC migration. In $TLR2^{-/-}$ $ApoE^{-/-}$ animal models, downstream activation of this pathway is not possible and Cp infection cannot lead to atherosclerosis [26].

Cp promotes trans-endothelial migration (TEM) of the monocytes into the subendothelial intimal layer, as it increases the permeability of the vascular endothelium and the rate of monocyte TEM while increasing the phosphorylation of tyrosine in vascular endothelial cadherins [27].

Furthermore, lung infection with Cp directly targets white adipose tissue and results in lipolysis, which subsequently result in fatty acid binding protein 4 (FABP4) secretion via endoplasmic reticulum stress. Released FABP4 can be taken up by neighboring adipocytes and contribute to further spread of the inflammation [28].

In addition, infection of fresh monocytes with Cp leads to a slower, uniform and steady rolling on E-selectin and endothelium compared to controls. Infection with Cp leads to the redistribution of CD44, a modulator of rolling functions, resulting in firmer adhesion of these monocytes on endothelial cells [29].

Lectin-like ox-LDL receptor is activated by C. pneumoniae

Oxidized forms of LDL (oxLDL) are known to be very proatherogenic and are one of the main culprits in the atherosclerotic plaque formation. Their receptor-mediated uptake can lead to functional changes in various cells involved in atherosclerotic plaque development, as a result, presenting the oxLDL-LOX-1 axis as a potential future therapeutic target [30]. Cp is able to upregulate the expression of the LOX-1 mRNA in both macrophages and endothelial cells , resulting in an increased uptake of oxidized LDLs [31]. This upregulation requires activation of ERK1/2, however can be inhibited by PPARγ agonists such as rosiglitazone, as Cp is also known to decrease PPARγ expressions in the infected endothelium, eventually leading to

apoptosis [32]. However, the role of statins on this mechanism activated by Cp remains a mystery, requiring further investigation, considering that LOX-1 is normally inhibited by statins [33].

On the other hand, chlamydial glycan plays a crucial role in the Cp-induced upregulation of LOX-1, as prior treatments of organisms with PNGase, which can remove the chlamydial glycan, abolishes this process [31]. Additionally, mitigation of LOX-1 upregulation can be achieved through preincubation of the cells to be inoculated with anti-LOX-1 antibodies, that leads to the prevention of increased adhesion protein expressions such as matrix metalloproteinases (MMP) 1 and 3, which is observed after inoculation with Cp [31].

C. pneumoniae promotes hypercoagulability and altered platelet functions

In-vitro studies conducted with human umbilical vein endothelial cells (HUVEC) infected with Cp showed that endothelial synthesis of tissue factor has been increased via chlamydial factor, which can explain thrombosis associated with Cp infections [34].

Platelets in atherosclerotic patients have significant expressions for various chemokines including CCL3, CCL5, CCL7 and CXCL8 and treatment of platelets with live Cp or chlamydial LPS is known to induce similar increases in the expressions of these chemokines. In addition, these patients had positive sera of anti-Cp antibodies [35].

Chlamydial LPS increases platelet aggregation, leading to thrombotic occlusion of the vessels, as it causes increased P-selection expression on platelets, a process which can be inhibited by interfering with glycoprotein IIb/IIIa via abciximab [36]. Cp IgM is associated with platelet activation and there has been a relationship between P-selectin levels and Cp IgM titers in patients with myocardial infarction with ST-segment elevation, who received thrombolysis [37].

On the other hand, the interaction of Cp with platelets leads to ROS production through protein kinase C. This interaction is eventually associated with the oxidation of LDL particles, which is known to play an important role in the development of ASCVD [38]. These stimulated platelets leading to lipid peroxidation can be released from atherosclerotic lesions into the circulation even after percutaneous coronary interventions [39]. Additionally, in patients undergoing carotid endarterectomy, Cp IgA seropositivity is associated with embolization due to thrombosis but not due to plaques [40].

In patients with ASCVD widely used cyclooxygenase (COX) inhibitors fail to affect platelets stimulated by Cp, however inhibitors of 12-lipoxygenase have effects on these stimulated platelets, antagonizing their activation [41].

Some earlier studies suggest that Cp infection may lead to increased tissue plasminogen activator (tPA) levels in patients with chronic heart disease, which is associated with lower aggregability of platelets, therefore highlighting the increased inflammatory response as the main culprit for the development of atherosclerosis in Cp infection. Nonetheless, treatment with azithromycin in these patients with known Cp infection can also lead to higher levels of tPA [42].

C. pneumonia and apolipoprotein B are likely associated

Apolipoprotein (Apo) B plays a role in the LDL receptor mediated clearance of the LDL-C, as a result, mutations leading to defective or absent ApoB can result in familial hypercholesterolemia [43]. Some studies suggest that patients with Cp IgG and IgM seropositivity have higher mean ApoB levels compared with Cp negative controls [44]. Human ApoB and chlamydial LPS share common antigenic epitopes, and therefore antibodies against Cp might cross-react with ApoB [45].

Another study assessing the treatment with a multi-antigenic construct consisting of ApoB100, HSP60 and outer membrane protein of Cp showed that this treatment is associated with plaque stabilization and reduction of necrosis in plaques, with decreased expressions of MMP9, leading to reduced macrophage apoptosis. The authors suggested that tolerance to these atherogenic peptides causes an increase in regulatory T cells activating M2 macrophages and preventing the proliferation of T lymphocytes, eventually reducing plaque destabilization and inflammation, in an animal model of established atherosclerosis [46]. This approach might be the basis of future vaccines of atherosclerosis, as it causes reduction of early atherosclerotic lesions [47].

IL-17 is a mediator of C. pneumonia associated vascular changes

Cp is known to induce a T-helper-1 (Th1) dominated response, including the induction of proinflammatory cytokines such as IFN-γ, IL-12, and TNF-α [48]. Interleukin 17 (IL-17) is another proinflammatory cytokine, which functions to maintain the integrity of the epithelium and modulate the activity of adipocytes [49]. IL-17$^{-/-}$ mice fed with high-fat are shown to have diminished lesions in aortic sinus plaques and aorta when compared with controls after infection with Cp. They also tend to have slower lesion progression and lesser macrophage presence in the atherosclerotic niche [50].

Infection with Cp leads to increased IL-17 expression in VSMCs in ApoE deficient mice model of hyperlipidemia, which results in VSMC migration via c-Fos/IL-17 signaling [51]. Resveratrol can decrease IL-17 expressions, superoxide anions and the number of foam cells [52].

Endothelial response to C. pneumoniae is multifaceted

Cp leads to increased concentrations of adhesion molecules such as intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM), whose levels correlate with measured serum amyloid A levels, potentially reflecting the extent of endothelial affection, however requiring further investigation [53].

Chronic infection with Cp, on the other hand, leads to the activation of endothelial cells, promoting the production of basic fibroblast growth factor (bFGF) [54], a growth factor implicated in unstable plaque rupture and thrombus formation, leading to symptomatic plaques associated with myocardial infarction [55]. On the contrary, some authors suggest that Cp leads to an in-vitro decrease of platelet derived growth factor receptor beta (PDGFR-β) expression by aortic smooth muscle cells, considering this as a possible inhibiting factor for the development and advancement of atherosclerotic plaques [56], whereas others suggesting that it leads to an increase in PDGF-β, promoting intimal thickening in rabbit models [57].

Seropositivity of IgA antibodies against Cp is associated with increased serum levels of IFN-γ, IL-10, TNF-α, soluble VCAM 1 (sVCAM-1) and soluble E-selectin, however, antibodies targeting Cp's major outer membrane protein are not associated with any increased inflammatory markers [58].

Possible Clinical implications

Chlamydial LPS circulating in serum, which is recognized by TLR4, correlates with the levels of LPS binding proteins, IL-6 and high C-reactive protein levels, in patients with established peripheral artery disease or abdominal aortic aneurysm [59]. Elevated chlamydial LPS levels also are associated with elevated body mass index [60].

In another study, which covered a 3.5 year follow-up, intimal medial thickness did not differ between patients who were seropositive and those who were seonegative for antibodies against chlamydial LPS [61]. In patients with anti-chlamydial IgA antibodies, the frequency of myocardial infarction was found to be lower, and the use of an-

ti-chlamydial antibiotics had no effect on ischemic events [61]. Additionally, seropositivity for chlamydial LPS antibodies were more common in patients with abdominal aortic aneurysms (AAA) but not in those with thoracic aortic aneurysms. Presence of antibodies against chlamydia species cross react with vessel wall antigens in AAA [62].

The presence of IgG antibodies against Cp is associated with an increased risk for acute coronary syndrome (ACS) with an odds-ratio (OR) of 1.62, along with other factors such as diabetes (OR 1.91), hypertension (OR 1.46), prior myocardial infarction, (or 1.78), and elevated troponin-T (OR 12.44) etc. [63]. Circulating Cp DNA is also associated with CAD in men (odds ratio [OR] 3.2, 95% confidence interval [CI] 1.1-8.9), but not in women who were [64].

In patients with heterozygous familial hypercholesterolemia (HeFH), Cp infection is known to be associated with a risk of chronic heart disease, consistent with the animal models of this disease [65]. Atherogenic effects of Cp are potentiated by the high levels of serum cholesterol supporting the hypothesis that Cp plays an aggravating rather than an initiating role in CAD [65].

Lp(a) and fibrinogen levels were found to be higher in patients with Cp seropositivity [66]. Formation of immune complexes containing IgG antibodies against Cp can also enhance the proatherogenic effects of Lp(a) [67].

Circulating Cp DNA can be associated with asymptomatic atherosclerosis in younger normotensive individuals [68]. However Cp DNA is not a reliable marker in high-risk populations such as type 2 diabetic patients [69]. However, Cp seropositivity is not known to be associated with intima media thickness [70]. Additionally, an infection with Cp doesn't play a role in the development of diabetes [71], nevertheless, a positive association between Cp seropositivity and the occurrence of metabolic syndrome is known [72].

Rapamycin, an immunosuppressive and antiproliferative agent, can inhibit in-vivo growth of Cp if applied at the beginning of the chlamydial infection, therefore, rapamycin-eluting stents might be useful in preventing Cp-associated stent restenosis [73]. On the other hand, selective cyclooxygenase (COX) inhibitors have bacteriostatic effects on Cp in *in-vivo* studies, but they can neither prevent infection nor eradicate Cp in affected cells [74].

Although the effect of antibiotics is out of our scope for this review, there are some studies that deserve to be shortly mentioned. Therapy with antichlamydial antibiotics might prove itself useful in patients with peripheral artery disease, accompanied by Cp IgG seropositivity, which has been further associated with shorter walking distance and higher need for revascularization. Roxithromycin, a macrolide, has positive effects on these parameters in these patients [75]. Contrarily, Secondary prevention of atherosclerosis through chlamydia pneumoniae eradication (SPACE Trial) has shown that azithromycin has no significant effect in patients with peripheral artery disease [76]. In an Apo-E deficient animal model of hyperlipidemia, in which atherosclerosis had been accelerated via Cp, antibiotic treatment with azithromycin also had no significant effects [77]. Another microbial agent, rifalazil, provided no significant symptomatic improvement in patients with established peripheral artery disease [78]. However, in another study, including patients with established carotid atherosclerosis who had no improvement under probucol therapy for 24 months, a 12-month combination therapy with levofloxacin, a fluoroquinolone, and probucol, an antioxidant, proved beneficial [79].

Finally, another important concern is that even though Cp seropositivity has been shown in various autoimmune diseases involving many systems, confounding factors such as overall increased proinflammatory immune response might additionally explain the increased prevalence or acceleration of atherosclerosis in these patients, aside from Cp seropositivity [80].

Conclusion

Cp, as a common cause of respiratory tract infections, is a pathogen with which many practitioners are familiar. The effects of Cp on vascular system are of utmost importance due to Cp's enormous clinical impact. Cp gained the attention of many clinicians in the late 20th century through some pioneering work, however the data, as the methods of investigation, have significantly evolved ever since.

Today we know that Cp seems to play an important role in almost every stage of atherosclerotic lesion formation and might interfere with our therapeutic efforts and prove itself resistant to our therapeutic or prophylactic means. Even though an infection with Cp can easily be treated with antibiotics in short-term, the effects on the vascular bed still present itself as an important problem, while even IgG seropositivity, supporting old infections, is associated with vascular complications.

It's important to highlight that there are still many questions requiring answers, including but not limited to the following: Cp's interaction with Lp(a), another rediscovered risk factor for atherosclerosis, colchicine's secondary preventive effects on patients with ASCVD who are seropositive for Cp. As we have summarized here, these are the latest developments in this often overlooked field within atherosclerosis research.

Author contributions

All authors contributed equally and participated to the conception, design, data acquisition and analysis and data interpretation regarding the work presented. All authors contributed to drafting, reviewing and/or revisiting of the presented work and approved its publication.

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References

[1] Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. Circ Res. 2016 Feb 19; 118 (4):535-46.

[2] Eberhardt N, Giannarelli C. How Single-Cell Technologies Have Provided New Insights Into Atherosclerosis. Arterioscler Thromb Vasc Biol. 2022 Mar; 42 (3):243-52.

[3] Milioti N, Bermudez-Fajardo A, Penichet ML, Oviedo-Orta E. Antigen-induced immunomodulation in the pathogenesis of atherosclerosis. Clin Dev Immunol. 2008; 723539.

[4] Khoshbayan A, Taheri F, Moghadam MT, Chegini Z, Shariati A. The association of Chlamydia pneumoniae infection with atherosclerosis: Review and update of in vitro and animal studies. Microb Pathog. 2021 May; 154:104803.

[5] Stein RA, Thompson LM. Epigenetic changes induced by pathogenic Chlamydia spp. Pathog Dis. 2023 Jan; 81.

[6] Arjmandi D, Graeili Z, Mohammadi P, Arshadi M, Jafari Tadi M, Ardekani A, et al. Chlamydia pneumonia infection and risk of multiple sclerosis: A meta-analysis. Mult Scler Relat Disord. 2023 Sep; 77:104862.

[7] Hahn DL. Does the asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS) exist? A narrative review from epidemiology and practice. Allergol Immunopathol. 2022; 50 (6):100-6.
[8] Hammerschlag MR, Kohlhoff SA, Gaydos CA. Chlamydia pneumoniae. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2015;2174-82.e2.
[9] Chiu B, Viira E, Tucker W, Fong IW. Chlamydia pneumoniae, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. Circulation. 1997 Oct; 96 (7):2144-8.
[10] Paterson DL, Hall J, Rasmussen SJ, Timms P. Failure to detect Chlamydia pneumoniae in atherosclerotic plaques of Australian patients. Pathology. 1998 May; 30 (2): 169-72.
[11] Selzman CH, Netea MG, Zimmerman MA, Weinberg A, Reznikov LL, Grover FL, et al. Atherogenic effects of Chlamydia pneumoniae: refuting the innocent bystander hypothesis. J Thorac Cardiovasc Surg. 2003 Sep; 126 (3):688-93.
[12] Byrne GI, Kalayoglu MV. Chlamydia pneumoniae and atherosclerosis: Links to the disease process. Am Heart J. 1999 Nov; 138 (5 Pt 2):S488-90.
[13] van Zandbergen G, Gieffers J, Kothe H, Rupp J, Bollinger A, Aga E, et al. Chlamydia pneumoniae multiply in neutrophil granulocytes and delay their spontaneous apoptosis. J Immunol. 2004 Feb; 172 (3):1768-76.
[14] Li M, Qian M, Kyler K, Xu J. Endothelial-Vascular Smooth Muscle Cells Interactions in Atherosclerosis. Front Cardiovasc Med. 2018; 5 (October):1-8.
[15] Joyee AG, Yang X. Role of Toll-Like Receptors in Immune Responses to Chlamydial Infections. Curr Pharm Des. 2008 Feb 1; 14:593-600.
[16] Cao F, Castrillo A, Tontonoz P, Re F, Byrne GI. Chlamydia pneumoniae–induced macrophage foam cell formation is mediated by Toll-like receptor 2. Infect Immun. 2007 Feb; 75 (2):753-9.
[17] Chen S, Sorrentino R, Shimada K, Bulut Y, Doherty TM, Crother TR, et al. Chlamydia pneumoniae-induced foam cell formation requires MyD88-dependent and -independent signaling and is reciprocally modulated by liver X receptor activation. J Immunol. 2008 Nov; 181 (10):7186-93.
[18] Bobryshev YV, Orekhov AN, Killingsworth MC, Lu J. Decreased expression of liver X receptor-α in macrophages infected with Chlamydia pneumoniae in human atherosclerotic arteries in situ. J Innate Immun. 2011; 3 (5):483-94.
[19] Zhao GJ, Mo ZC, Tang SL, Ouyang XP, He PP, Lv YC, et al. Chlamydia pneumoniae negatively regulates ABCA1 expression via TLR2-Nuclear factor-kappa B and miR-33 pathways in THP-1 macrophage-derived foam cells. Atherosclerosis. 2014 Aug; 235 (2):519-25.
[20] Wu X, Cheng B, Guo X, Wu Q, Sun S, He P. PPARα/γ signaling pathways are involved in Chlamydia pneumoniae-induced foam cell formation via upregulation of SR-A1 and ACAT1 and downregulation of ABCA1/G1. Microb Pathog. 2021 Dec; 161 (Pt B):105284.
[21] Mei C li, He P, Cheng B, Liu W, Wang Y fu, Wan J jing. Chlamydia pneumoniae induces macrophage-derived foam cell formation via PPAR alpha and PPAR gamma-dependent pathways. Cell Biol Int. 2009 Mar; 33 (3):301-8.
[22] Jiang SJ, Campbell LA, Berry MW, Rosenfeld ME, Kuo CC. Retinoic acid prevents Chlamydia pneumoniae-induced foam cell development in a mouse model of atherosclerosis. Microbes Infect. 2008 Oct; 10 (12-13):1393-7.
[23] Tumurkhuu G, Dagvadorj J, Porritt RA, Crother TR, Shimada K, Tarling EJ, et al. Chlamydia pneumoniae Hijacks a Host Autoregulatory IL-1β Loop to Drive Foam Cell Formation and Accelerate Atherosclerosis. Cell Metab. 2018 Sep; 28 (3):432-448.e4.
[24] Cheng B, Wu X, Sun S, Wu Q, Mei C, Xu Q, et al. MAPK-PPARα/γ signal transduction pathways are involved in Chlamydia pneumoniae-induced macrophage-derived foam cell formation. Microb Pathog. 2014; 69-70:1-8.
[25] Kitazawa T, Fukushima A, Okugawa S, Yanagimoto S, Tsukada K, Tatsuno K, et al. Chlamydophilal antigens induce foam cell formation via c-Jun NH2-terminal kinase. Microbes Infect. 2007 Oct; 9 (12-13):1410-4.
[26] Zhao X, Miao G, Zhang L, Zhang Y, Zhao H, Xu Z, et al. Chlamydia pneumoniae Infection Induces Vascular Smooth Muscle Cell Migration and Atherosclerosis Through Mitochondrial Reactive Oxygen Species-Mediated JunB-Fra-1 Activation. Front cell Dev Biol. 2022; 10:879023.
[27] Liu J, Miao G, Wang B, Zheng N, Ma L, Chen X, et al. Chlamydia pneumoniae infection promotes monocyte transendothelial migration by increasing vascular endothelial cell permeability via the tyrosine phosphorylation of VE-cadherin. Biochem Biophys Res Commun. 2018 Mar; 497 (2):742-8.
[28] Kurihara Y, Walenna NF, Ishii K, Soejima T, Chou B, Yoshimura M, et al. Chlamydia pneumoniae Lung Infection in Mice Induces Fatty Acid-Binding Protein 4-Dependent White Adipose Tissue Pathology. J Immunol. 2023 Apr; 210 (8):1086-97.
[29] Evani SJ, Ramasubramanian AK. Biophysical regulation of Chlamydia pneumoniae-infected monocyte recruitment to atherosclerotic foci. Sci Rep. 2016 Jan; 6:19058.
[30] Khan MA, Mohammad I, Banerjee S, Tomar A, Varughese KI, Mehta JL, et al. Oxidized LDL receptors: a recent update. Curr Opin Lipidol. 2023 Aug; 34 (4):147-55.
[31] Campbell LA, Lee AW, Rosenfeld ME, Kuo CC. Chlamydia pneumoniae induces expression of pro-atherogenic factors through activation of the lectin-like oxidized LDL receptor-1. Pathog Dis. 2013 Oct; 69 (1):1-6.
[32] Sun S, Duan X, Wu Q, He Y, Bu X, Ming X, et al. ERK1/2-PPARγ pathway is involved in Chlamydia pneumonia-induced human umbilical vein endothelial cell apoptosis through increased LOX-1 expression. J Recept Signal Transduct Res. 2020 Apr; 40 (2):126-32.
[33] Biocca S, Iacovelli F, Matarazzo S, Vindigni G, Oteri F, Desideri A, et al. Molecular mechanism of statin-mediated LOX-1 inhibition. Cell Cycle. 2015; 14 (10):1583-95.
[34] Fryer RH, Schwobe EP, Woods ML, Rodgers GM. Chlamydia species infect human vascular endothelial cells and induce procoagulant activity. J Investig Med. 1997 Apr; 45 (4):168-74.
[35] Al-Bannawi A, Al-Wesebai K, Taha S, Bakhiet M. Chlamydia pneumoniae induces chemokine expression by platelets in patients with atherosclerosis. Med Princ Pract. 2011; 20 (5):438-43.
[36] Kälvegren H, Majeed M, Bengtsson T. Chlamydia pneumoniae binds to platelets and triggers P-selectin expression and aggregation: a causal role in cardiovascular disease? Arterioscler Thromb Vasc Biol. 2003 Sep; 23 (9):1677-83.
[37] Järemo P. Evidence that Chlamydia pneumoniae affects platelet activity in patients with acute myocardial infarction and ST-segment elevations. Scand J Infect Dis. 2001; 33 (10):747-8.
[38] Kälvegren H, Bylin H, Leanderson P, Richter A, Grenegård M, Bengtsson T. Chlamydia pneumoniae induces nitric oxide synthase and lipoxygenase-dependent production of reactive oxygen species in platelets. Effects on oxidation of low density lipoproteins. Thromb Haemost. 2005 Aug; 94 (2):327-35.
[39] Kälvegren H, Fridfeldt J, Garvin P, Wind L, Leanderson P, Kristenson M, et al. Correlation between rises in Chlamydia pneu-

moniae-specific antibodies, platelet activation and lipid peroxidation after percutaneous coronary intervention. Eur J Clin Microbiol Infect Dis. 2008 Jul; 27 (7):503-11.
[40] Vainas T, Kurvers HAJM, Mess WH, de Graaf R, Ezzahiri R, Tordoir JHM, et al. Chlamydia pneumoniae serology is associated with thrombosis-related but not with plaque-related microembolization during carotid endarterectomy. Stroke. 2002 May; 33 (5):1249-54.
[41] Kälvegren H, Andersson J, Grenegård M, Bengtsson T. Platelet activation triggered by Chlamydia pneumoniae is antagonized by 12-lipoxygenase inhibitors but not cyclooxygenase inhibitors. Eur J Pharmacol. 2007 Jul; 566 (1-3):20-7.
[42] Gabriel AS, Ahnve S, Gnarpe H, Gnarpe J, Martinsson A. Azithromycin therapy in patients with chronic Chlamydia pneumoniae infection and coronary heart disease: immediate and long-term effects on inflammation, coagulation, and lipid status in a double-blind, placebo-controlled study. Eur J Intern Med. 2003 Dec; 14 (8):470-8.
[43] Kayikcioglu M, Tokgozoglu L. Current Treatment Options in Homozygous Familial Hypercholesterolemia. Pharmaceuticals (Basel). 2022 Dec; 16 (1).
[44] Adiloglu AK, Can R, Kinay O, Aridogan BC. Infection with Chlamydia pneumoniae but not Helicobacter pylori is related to elevated apolipoprotein B levels. Acta Cardiol. 2005 Dec; 60 (6):599-604.
[45] Petyaev IM, Zigangirova NA, Tsibezov VV, Ross A, Bashmakov YK. Monoclonal antibodies against lipopolysaccharide of Chlamydia trachomatis with cross reactivity to human ApoB. Hybridoma. 2011 Apr; 30 (2):131-6.
[46] Thota LN, Ponnusamy T, Philip S, Lu X, Mundkur L. Immune regulation by oral tolerance induces alternate activation of macrophages and reduces markers of plaque destabilization in Apobtm2Sgy/Ldlrtm1Her/J mice. Sci Rep. 2017 Jun; 7 (1):3997.
[47] Lu X, Xia M, Endresz V, Faludi I, Szabo A, Gonczol E, et al. Impact of multiple antigenic epitopes from ApoB100, hHSP60 and Chlamydophila pneumoniae on atherosclerotic lesion development in ApobtmSgyLdlrtm1HerJ mice. Atherosclerosis. 2012 Nov; 225 (1):56-68.
[48] Mamata Y, Hakki A, Newton C, Burdash N, Klein TW, Friedman H. Differential effects of Chlamydia pneumoniae infection on cytokine levels in human T lymphocyte- and monocyte-derived cell cultures. Int J Med Microbiol. 2007; 297 (2):109-15.
[49] Liu Y, Ouyang Y, You W, Liu W, Cheng Y, Mai X, et al. Physiological roles of human interleukin-17 family. Exp Dermatol. 2024 Jan; 33 (1).
[50] Chen S, Shimada K, Zhang W, Huang G, Crother TR, Arditi M. IL-17A is proatherogenic in high-fat diet-induced and Chlamydia pneumoniae infection-accelerated atherosclerosis in mice. J Immunol. 2010 Nov; 185 (9):5619-27.
[51] Zheng N, Zhang L, Wang B, Wang G, Liu J, Miao G, et al. Chlamydia pneumoniae infection promotes vascular smooth muscle cell migration via c-Fos/interleukin-17C signaling. Int J Med Microbiol. 2019 Dec; 309 (8):151340.
[52] Di Pietro M, De Santis F, Schiavoni G, Filardo S, Sessa R. Resveratrol in Chlamydia pneumoniae-induced foam cell formation and interleukin-17A synthesis. J Biol Regul Homeost Agents. 2013; 27 (2):509-18.
[53] Tuomainen AM, Hyvärinen K, Ehlers PI, Mervaala E, Leinonen M, Saikku P, et al. The effect of proatherogenic microbes on macrophage cholesterol homeostasis in apoE-deficient mice. Microb Pathog. 2011; 51 (3):217-24.
[54] Prochnau D, Rödel J, Hartmann M, Straube E, Figulla HR. Growth factor production in human endothelial cells after Chlamydia pneumoniae infection. Int J Med Microbiol. 2004 Jul; 294 (1):53-7.
[55] Parma L, Peters HAB, Sluiter TJ, Simons KH, Lazzari P, de Vries MR, et al. bFGF blockade reduces intraplaque angiogenesis and macrophage infiltration in atherosclerotic vein graft lesions in ApoE3*Leiden mice. Sci Rep. 2020 Sep; 10 (1):15968.
[56] Rödel J, Lehmann M, Vogelsang H, Straube E. Chlamydia pneumoniae infection of aortic smooth muscle cells reduces platelet-derived growth factor receptor-beta expression. FEMS Immunol Med Microbiol. 2007 Nov; 51(2):363-71.
[57] Coombes BK, Chiu B, Fong IW, Mahony JB. Chlamydia pneumoniae infection of endothelial cells induces transcriptional activation of platelet-derived growth factor-B: a potential link to intimal thickening in a rabbit model of atherosclerosis. J Infect Dis. 2002 Jun; 185 (11):1621-30.
[58] Schumacher A, Seljeflot I, Lerkerød AB, Sommervoll L, Otterstad JE, Arnesen H. Chlamydia LPS and MOMP seropositivity are associated with different cytokine profiles in patients with coronary heart disease. Eur J Clin Invest. 2005 Jul; 35 (7):431-7.
[59] Vikatmaa P, Lajunen T, Ikonen TS, Pussinen PJ, Lepäntalo M, Leinonen M, et al. Chlamydial lipopolysaccharide (cLPS) is present in atherosclerotic and aneurysmal arterial wall–cLPS levels depend on disease manifestation. Cardiovasc Pathol. 2010; 19 (1):48-54.
[60] Lajunen T, Vikatmaa P, Bloigu A, Ikonen T, Lepäntalo M, Pussinen PJ, et al. Chlamydial LPS and high-sensitivity CRP levels in serum are associated with an elevated body mass index in patients with cardiovascular disease. Innate Immun. 2008 Dec; 14 (6):375-82.
[61] Gerdes VEA, Verkooyen RP, Kwa VIH, de Groot E, van Gorp ECM, ten Cate H, et al. Chlamydial LPS antibodies, intima-media thickness and ischemic events in patients with established atherosclerosis. Atherosclerosis. 2003 Mar; 167 (1):65-71.
[62] Schillinger M, Domanovits H, Mlekusch W, Bayegan K, Khanakah G, Laggner AN, et al. Anti chlamydia antibodies in patients with thoracic and abdominal aortic aneurysms. Wien Klin Wochenschr. 2002 Dec; 114 (23-24):972-7.
[63] Chandra HR, Choudhary N, O'Neill C, Boura J, Timmis GC, O'Neill WW. Chlamydia pneumoniae exposure and inflammatory markers in acute coronary syndrome (CIMACS). Am J Cardiol. 2001; 88 (3):214-8.
[64] Wong YK, Dawkins KD, Ward ME. Circulating Chlamydia pneumoniae DNA as a predictor of coronary artery disease. J Am Coll Cardiol. 1999 Nov; 34 (5):1435-9.
[65] Kontula K, Vuorio A, Turtola H, Saikku P. Association of seropositivity for Chlamydia pneumoniae and coronary artery disease in heterozygous familial hypercholesterolaemia. Vol. 354, Lancet; 1999; 46-7.
[66] Tütüncü NB, Güvener N, Tütüncü T, Yilmaz M, Güvener M, Böke E, et al. Chlamydia pneumonia seropositivity correlates with serum fibrinogen and lipoprotein a levels: any role in atherosclerosis? Endocr J. 2001 Apr; 48 (2):269-74.
[67] Glader CA, Boman J, Saikku P, Stenlund H, Weinehall L, Hallmanns G, et al. The proatherogenic properties of lipoprotein(a) may be enhanced through the formation of circulating immune complexes containing Chlamydia pneumoniae-specific IgG antibodies. Eur Heart J. 2000 Apr; 21 (8):639-46.
[68] Mitusch R, Luedemann J, Wood WG, Berger K, Schminke U, Suter M, et al. Asymptomatic carotid atherosclerosis is associat-

ed with circulating chlamydia pneumoniae DNA in younger normotensive subjects in a general population survey. Arterioscler Thromb Vasc Biol. 2005 Feb; 25 (2):386-91.

[69] Reverter JL, Tàssies D, Alonso N, Pellitero S, Sanmartí A, Reverter JC. Non-detectable Chlamydophila pneumoniae DNA in peripheral leukocytes in type 2 diabetes mellitus patients with and without carotid atherosclerosis. Med Clin. 2012 Jan; 138 (1):11-4.

[70] Mazza A, Bossone E, Gianicolo E, Mazza F, Distante A.[Lack of association between Chlamydia pneumoniae seropositivity and common carotid intima-media thickness in type 2 diabetic patients]. Monaldi Arch chest Dis. 2003 Dec; 60 (4):283-7.

[71] Lutsey PL, Pankow JS, Bertoni AG, Szklo M, Folsom AR. Serological evidence of infections and Type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis. Diabet Med. 2009 Feb; 26 (2):149-52.

[72] Lin CY, Su SB, Chang CC, Lee TM, Shieh JM, Guo HR. The association between Chlamydia pneumoniae and metabolic syndrome in Taiwanese adults. South Med J. 2009 Dec; 102 (12):1203-8.

[73] Yan Y, Silvennoinen-Kassinen S, Leinonen M, Saikku P. Rapamycin can inhibit the development of Chlamydia pneumoniae, which might partly contribute to the prevention of instent restenosis. Cardiovasc Drugs Ther. 2010 Jun; 24 (3):189-95.

[74] Yan Y, Silvennoinen-Kassinen S, Törmäkangas L, Leinonen M, Saikku P. Selective cyclooxygenase inhibitors prevent the growth of Chlamydia pneumoniae in HL cells. Int J Antimicrob Agents. 2008 Jul; 32 (1):78-83.

[75] Krayenbuehl PA, Wiesli P, Maly FE, Vetter W, Schulthess G. Progression of peripheral arterial occlusive disease is associated with Chlamydia pneumoniae seropositivity and can be inhibited by antibiotic treatment. Atherosclerosis. 2005 Mar; 179 (1):103-10.

[76] Vainas T, Stassen FRM, Schurink GWH, Tordoir JHM, Welten RJTJ, van den Akker LHJM, et al. Secondary prevention of atherosclerosis through chlamydia pneumoniae eradication (SPACE Trial): a randomised clinical trial in patients with peripheral arterial disease. Eur J Vasc Endovasc Surg. 2005 Apr; 29 (4):403-11.

[77] Blessing E, Campbell LA, Rosenfeld ME, Chesebro B, Kuo CC. A 6 week course of azithromycin treatment has no beneficial effect on atherosclerotic lesion development in apolipoprotein E-deficient mice chronically infected with Chlamydia pneumoniae. J Antimicrob Chemother. 2005 Jun; 55 (6):1037-40.

[78] Jaff MR, Dale RA, Creager MA, Lipicky RJ, Constant J, Campbell LA, et al. Anti-chlamydial antibiotic therapy for symptom improvement in peripheral artery disease: prospective evaluation of rifalazil effect on vascular symptoms of intermittent claudication and other endpoints in Chlamydia pneumoniae seropositive patients (PROVIDENCE-1). Circulation. 2009 Jan; 119 (3):452-8.

[79] Sawayama Y, Tatsukawa M, Kikuchi K, Maeda S, Ohnishi H, Furusyo N, et al. Effect on carotid atherosclerosis of probucol plus levofloxacin for Chlamydia pneumoniae infection. J Infect Chemother. 2007 Apr; 13 (2):92-8.

[80] Cope AP. Regulation of autoimmunity by proinflammatory cytokines. Curr Opin Immunol. 1998 Dec; 10 (6):669-76.