New Microbiologica, 47, 3, 217-226, 2024, ISSN 1121-7138

REVIEW

Updating the relationship of *Chlamydia pneumoniae* with atherosclerotic cardiovascular diseases: a systematic review of reviews

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SUMMARY

Chlamydia pneumoniae is the etiologic agent of respiratory tract infections in humans, including community-acquired pneumonia, and has been associated with atherosclerotic cardiovascular diseases. The present systematic review of reviews aimed at answering important questions on the involvement of *C. pneumoniae* in the pathogenesis of atherosclerosis, its cellular and molecular mechanisms, and whether there is evidence of a causal relationship. The databases PubMed/Medline, Scopus, and Web of Science were searched for all review articles published from 2003 to the end of 2023. A total of 27 reviews, systematic reviews, and systematic reviews with metanalysis were included. Overall, current evidence suggests that *C. pneumoniae* is a biologically plausible candidate for the causation of atherosclerosis, albeit not all the 4 Koch postulates are fulfilled; oxidative stress and inflammation are the most likely pathogenic mechanisms mediated by *C. pneumoniae*. However, it is still unclear how the persistent form, responsible for chronic inflammation, fits into this etiopathogenetic scenario. In the future, the newly-designed transformation systems for the genomic manipulation of *C. pneumoniae* will surely help expand our knowledge on the role of this pathogen in atherogenesis.

Received July 19, 2024 *Accepted* September 20, 2024

INTRODUCTION

Chlamydia pneumoniae is typically responsible for infections of the lower respiratory tract in humans, causing 10-20% of all cases of community-acquired pneumonia. Infection with *C. pneumoniae* is widespread globally, and is frequently asymptomatic, seldom manifesting with mild to moderate symptoms; a respiratory infection by *C. pneumoniae* can develop at a young age, and could be followed by persistence, as highlighted by epidemiological studies, observing a 50% prevalence of anti-chlamydia antibodies by the age of 20 and 80% by the age of 60 to 70 (Gautam and Krawiec, 2024; Grayston, 2000; Grayston *et al.*, 2015). *C. pneumoniae*, an obligate intracellular pathogen, possesses a peculiar growth cycle compared to all other bacteria, and consists of a biphasic form: the extracellular elementary body (EB), capable of invading host cells, and the intracellular reticulate body (RB), actively multiplying within the host cell cytoplasm (Abdelrahman and Belland, 2005; Gitsels *et al.*, 2019). In addition to the typical EB and RB, a third form, non-infectious and unable to replicate in the intracellular milieu, named persistent form, has long been considered a critical factor for chronic infections due to its ability to remain viable within the host cell for a prolonged time. As a consequence, chlamydial persistent forms can evade the defences of the host immune system and are intrinsically resistant to antibiotics, leading to a chronic inflammatory response (Di Pietro *et al.*, 2019; Kozusnik *et al.*, 2024; Panzetta *et al.*, 2018). Numerous *in vitro* studies have described different models of persistence over the years, including exposure to interferon gamma (IFN)-γ or antibiotics (e.g., penicillin and amoxicillin), nutrient deprivation (e.g., lack of essential amino-acids or iron), as well as coinfections with Herpes Simplex Virus type 2 or Toxoplasma gondii (Campbell and Rosenfeld, 2014; Panzetta *et al.*, 2018).

C. pneumoniae can resist the damaging effect of reactive oxygen species (ROS), thriving in a wide array of different host cells, including monocytes/

Key words:
Chlamydia pneumoniae, Persistent Form, Atherosclerotic Cardiovascular Diseases, Risk Factors, Systematic Review.

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macrophages, lymphocytes, vascular smooth muscle cells (VSMCs), and endothelial cells (Gaydos, 2000; J M Kern *et al.*, 2009a; Sessa *et al.*, 2009a).

Over the years, several studies have suggested the involvement of *C. pneumoniae* in the etiopathogenesis of atherosclerosis, the leading cause of death with over 17 million deaths per year worldwide, and the main pathological process underlying cardiovascular diseases (Mendis *et al.*, 2011). Atherosclerosis is a chronic disease characterized by an increased inflammatory state within the arterial vessel, whose development is a continuous process that starts from the injury of the endothelium and ends with the formation of the advanced plaque. Subsequent erosion or rupture of atherosclerotic plaques triggers cardiovascular events that can potentially be fatal.

Saikku and colleagues, in 1988 (Saikku *et al.*, 1988), suggested, for the first time, that *C. pneumoniae* could represent a potential cardiovascular risk factor, as evidenced by the higher prevalence of anti-*C. pneumoniae* antibodies in patients undergoing acute myocardial infarction and chronic heart disease (68%), in comparison to controls (17%). Kuo and colleagues also provided evidence that *C. pneumoniae* could be observed in atherosclerotic coronary plaque tissue biopsies by electron microscopy and immunohistochemistry (Kuo *et al.*, 1993). In the following years, more studies, including original papers and narrative reviews, as well as systematic reviews and metanalysis, analysed the role of *C. pneumoniae* in the pathogenesis of atherosclerotic cardiovascular diseases.

Articles published in the last 20 years were included in this systematic review of reviews, aimed at answering the following questions:

1) is there evidence of the involvement of *C. pneumoniae* in the pathogenesis of atherosclerosis?
2) is there evidence of the cellular and molecular mechanisms by which *C. pneumoniae* may contribute to the atherosclerotic process?
3) is there evidence of a causal relationship between *C. pneumoniae* and atherosclerosis?

MATERIAL AND METHODS

Selection Protocol and Search Strategy

The protocol of this systematic review of reviews was registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the following registration ID: CRD42024565581, and was devised in accordance with the most recent Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement (Aromataris *et al.*, 2015); Zotero citation management software (RRID: SCR_013784) was employed to remove any duplicates, as well as to organize and select the literature records.

The literature search was performed from 2nd to 9th March 2024 in three different databases of international scientific importance, namely PubMed, Web of Science, and Scopus. Suitable studies were selected through the following search strategy: (*Chlamydia pneumoniae*) AND ((cardio*) OR (vascular*) OR (atheros*) OR (aort*) OR (myocard*) OR (coron*)). Truncation filters (*) were introduced to represent any combination of letters. Two independent reviewers (SF, MDP) performed the search, read the titles and the abstracts of all the selected articles. During this multi-step exclusion process, disagreements on the studies were discussed until a consensus was reached. The process was supervised by other investigators (RS, SR), and, finally, the PRISMA flow chart diagram was used for summarizing the selection steps.

Eligibility criteria and exclusion criteria

Only narrative reviews, systematic reviews, and systematic reviews with meta-analyses, focusing on *C. pneumoniae* and atherosclerotic cardiovascular disease association, were identified from 1st January 2003 to 31st December 2023.

The exclusion criteria were narrative reviews, systematic reviews with meta-analyses that (a) had no full text available or was not written in English, and (b) investigated the association between multiple bacteria (bacterial burden) or virus and atherosclerotic cardiovascular diseases or atherosclerosis. Conference abstracts and the systematic review with meta-analyses of diagnostic trials were also excluded.

Data extraction

The following information was extracted by two authors (SF, MDP) independently from all of the articles included in this systematic review of reviews: authors, country, year of publication, journal, type of review (i.e., narrative review, systematic review, systematic review with meta-analysis), aim of the review, conclusions of the authors.

RESULTS

Study Selection and Characteristics

After searching the designated databases, 1199 articles published in the last twenty years (2003-2023) were retrieved. A total of 111 reviews, systematic reviews, and systematic reviews with metanalysis were initially obtained. 27 reviews were further screened and included in the systematic review only after considering the inclusion and the exclusion criteria; the remaining articles were excluded due to their not being relevant to the systematic review or not containing a large section dealing with the aforementioned topics.

Figure 1 reports the search strategy and the flow diagram of the articles selected for this review. *Table 1*

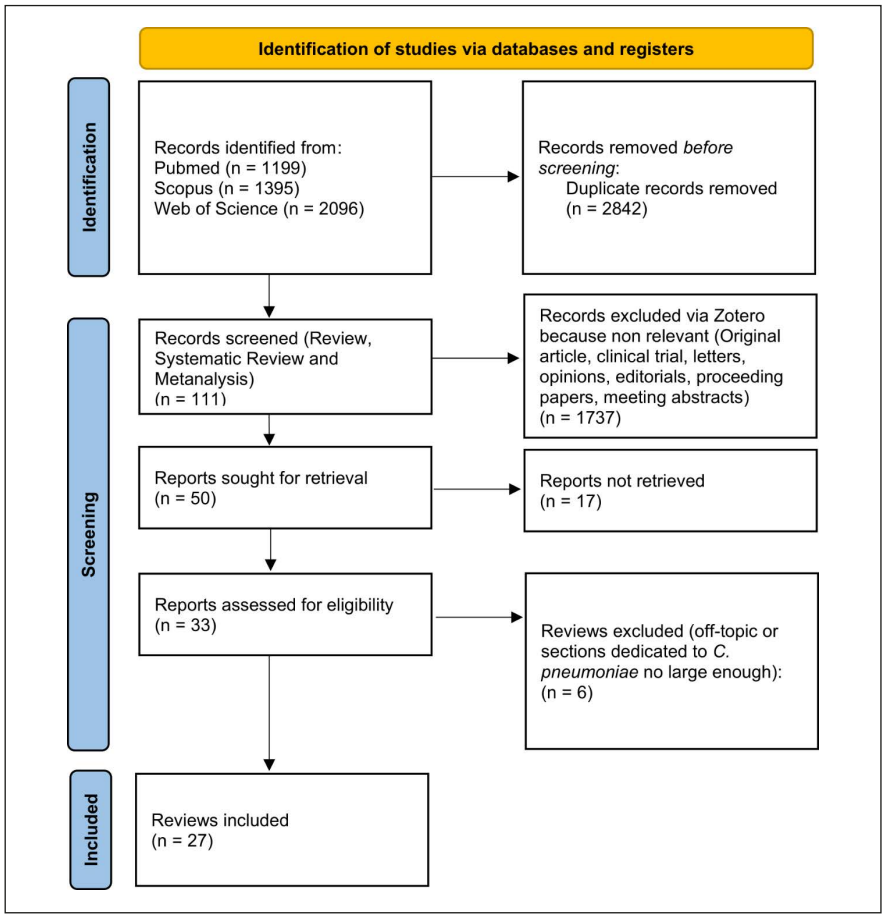

Figure 1
PRISMA flow diagram.

reports the principal characteristics of the review articles contained in this systematic review.
Supplementary Table S1 provide brief extracts from the aim of the study and the main discussion and conclusion of each review article. Briefly, evidence from sero-epidemiological and pathological studies, *in vitro* and *in vivo* studies, as well as human clinical trials, were considered by the included articles. Furthermore, data on the potential molecular and cellular mechanisms underlying the contribution of *C. pneumoniae* in the atherosclerotic process were also retrieved.

Table 1 - *List of the reviews included in the present systematic review.*

#	*References*	*PMID*	*Country*	*Year of publication*	*Journal*	*Type of Review*
1	(Campbell and Kuo, 2003)	PMID: 12652454	USA	2003	Semin Respir Infect.	Narrative review
2	(Higgins *et al.*, 2003)	PMID 12630585	USA	2003	Expert Rev Cardiovasc Ther.	Narrative review
3	(Higgins, 2003)	PMID 15030265	USA	2003	Mayo Clin Proc.	Narrative review
4	(Hirono and Pierce, 2003)	PMID 12841348	USA	2003	Mol Cell Biochem.	Narrative review
5	(Muhlestein, 2003)	PMID: 14624138	USA	2003	Curr Opin Lipidol.	Narrative review
6	(Lindholt *et al.*, 2003)	PMID: 12570733	Denmark	2003	Curr Drug Targets Infect Disord.	Narrative review
7	(Kuo and Campbell, 2003)	PMID: 12456307	USA	2003	Front Biosci.	Narrative review
8	(Anderson and Muhlestein, 2004)	PMID: 15061624	UTAH	2004	Tex Heart Inst J.	Narrative review

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#	*References*	*PMID*	*Country*	*Year of publication*	*Journal*	*Type of Review*
9	(Belland *et al.*, 2004)	PMID: 14706098	USA	2004	Cell Microbiol.	Narrative review
10	(Campbell and Kuo, 2004)	PMID: 15035006	USA	2004	Nat Rev Microbiol.	Narrative review
11	(Tsirpanlis, 2004)	PMID: 15114030	Greece	2004	Kidney Blood Press Res	Narrative review
12	(Andraws *et al.*, 2005)	PMID: 15928286	USA	2005	JAMA.	Systematic review and meta-analysis
13	(de Kruif *et al.*, 2005)	PMID: 15639470	The Netherlands	2005	Cardiovasc Res.	Systematic review
14	(Ieven and Hoymans, 2005)	PMID: 15634945	Belgium	2005	J. Clin. Microbiol.	Narrative review
15	(Liu and Waters, 2005)	PMID: 15991152	USA	2005	Prog Cardiovasc Dis.	Narrative review
16	(Mussa *et al.*, 2006)	PMID: 16765261	USA	2006	J Vasc Surg	Narrative review
17	(Stassen *et al.*, 2008)	PMID: 18276989	The Netherlands	2008	Pharmacol Rep.	Narrative review
18	(Jan Marco Kern *et al.*, 2009)	PMID: 19589286	Austria	2009	FEMS Immunol Med Microbiol.	Narrative review
19	(J M Kern *et al.*, 2009b)	PMID: 19281565	Austria	2009	Clin Microbiol Infect.	Narrative review
20	(Sessa *et al.*, 2009a)	PMID: 19220338	Italy	2009	Int J Immunopathol Pharmacol.	Narrative review
21	(Di Pietro *et al.*, 2009)	PMID: 19309547	Italy	2009	J Biol Regul Homeost Agents.	Narrative review
22	(Chen *et al.*, 2013)	PMID: 24261578	China	2013	BMC Neurology	Systematic review and meta-analysis
23	(Di Pietro *et al.*, 2013a)	PMID: 23877837	Italy	2013	Int J Mol Sci.	Narrative review
24	(Campbell and Rosenfeld, 2014)	PMID: 24711989	USA	2014	Front Cell Infect Microbiol.	Narrative review
25	(Di Pietro *et al.*, 2014)	PMID: 25561227	Italy	2014/2015	Int J Mol Sci.	Narrative review
26	(Khoshbayan *et al.*, 2021)	PMID: 33609645	Iran	2021	Microb Pathog.	Narrative review
27	(Keikha and Karbalaei, 2022)	PMID: 35592534	Iran	2022	New Microbes New Infect	Systematic review and meta-analysis

DISCUSSION

Atherosclerosis is a well-known pathological process that starts with the injury of the endothelial tissue, accompanied by the oxidation and accumulation of low-density lipoprotein (LDL) into vascular cells. This, in turn, activates the pro-inflammatory cascade [interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α], consequently inducing the proliferation of VSMCs. These complex pathological mechanisms spark a series of events that include the formation of foam cells from monocytes/macrophages, followed by the development of the fibrous cap and the thrombus. Henceforth, advanced plaque occurs, leading to cardiovascular diseases, including coronary heart disease, stroke, and peripheral vascular disease.

Several risk factors for atherosclerosis have been identified, including traditional ones such as hypertension, diabetes, dyslipidaemia, and smoking, and non-traditional ones including inflammation and oxidative stress, that can be triggered by infectious agents, such as *C. pneumoniae* (Di Pietro *et al.*, 2009; Gaydos, 2000; Madaudo *et al.*, 2024). Over the last decades, numerous studies have investigated the involvement of *C. pneumoniae* in the pathogenesis of atherosclerotic cardiovascular diseases. From 1988 to 2023, a total of approximately 1800 papers were

published, with the peak in the number of published articles per year reached from 1999 to 2006.
This systematic review summarized the current existing evidence on *C. pneumoniae* relationship with atherosclerotic cardiovascular diseases from published narrative reviews and systematic review with metanalysis to better understand its role in atherogenesis.

Is there evidence of the involvement of C. pneumoniae in the pathogenesis of atherosclerosis?

Several pieces of research suggested the involvement of *C. pneumoniae* in atherogenesis, mainly *in vitro* and *in vivo* studies. *In vitro* studies evidenced the ability of *C. pneumoniae* to contribute to several phases of the atherosclerotic process, such as foam cell formation, VSMCs proliferation and migration, and platelet activation and aggregation, leading to advanced plaque formation, rupture, and consequent acute cardiovascular events (Belland *et al.*, 2004; Campbell and Kuo, 2004, 2003; Higgins, 2003; Jan Marco Kern *et al.*, 2009; Kuo and Campbell, 2003; Mussa *et al.*, 2006; Sessa *et al.*, 2009a; Tsirpanlis, 2004).
In vivo studies have shown the ability of *C. pneumoniae* to disseminate from the lungs to the vascular tissue, probably via peripheral blood monocytes in which it has found a way of surviving (de Kruif *et al.*, 2005; Hirono and Pierce, 2003). Also, different etiopathogenetic properties of *C. pneumoniae*, underlying its involvement in atherogenesis, have been demonstrated in animal models: for example, *C. pneumoniae* is able to initiate atherosclerosis in rabbits receiving a regular diet, and to accelerate the progression of atherosclerotic lesions in mice receiving a high-fat diet (de Kruif *et al.*, 2005; Khoshbayan *et al.*, 2021). Enhanced endothelial dysfunction and lipid accumulation in the aortic sinus, typical changes associated with early and advanced plaques, were observed in different mouse and rabbit models (de Kruif *et al.*, 2005; Khoshbayan *et al.*, 2021; Mussa *et al.*, 2006).
Over the years, further evidence came from numerous observational studies showing an association between anti-*C. pneumoniae* antibodies and cardiovascular diseases, or by the detection of chlamydial DNA in tissue biopsies from atherosclerotic lesions in coronary and carotid arteries, as well as in the aneurysm of abdominal aorta, but not in healthy arteries (Sessa *et al.*, 2009b). Sero-epidemiologic studies have also shown that chronic *C. pneumoniae* infection appears to be associated with dyslipidaemia, an altered serum lipid profile characterized by increased triglycerides and decreased high-density lipoproteins, considered to increase the risk of atherosclerosis (Sessa *et al.*, 1999). Furthermore, in diabetic patients, the presence of *C. pneumoniae* may accelerate the development of atherosclerotic plaques (Rizzo *et al.*, 2012).
A metanalysis, published in 2013, demonstrated a significant correlation between *C. pneumoniae* infection, expressed as anti-*C. pneumoniae* IgG and IgA or the presence of chlamydial DNA in atherosclerotic plaques, and cerebrovascular diseases, via case-control studies (Chen *et al.*, 2013). By including similar study types, a further metanalysis evidenced the relationship between *C. pneumoniae* infection and the occurrence of ischemic stroke (OR: 2.14; CI: 1.9–2.3) (Keikha and Karbalaei, 2022). However, prospective studies, able to investigate temporal relations and the causal relationship between *C. pneumoniae* infection and atherosclerosis, provided inconclusive evidence (Chen *et al.*, 2013; Watson and Alp, 2008).
In this scenario, several key issues have emerged, such as the presence of pre-existing IgG antibodies in a large part of the population, and the focal detection of *C. pneumoniae* in vascular tissues, making it more difficult to study the role of this respiratory pathogen in atherogenesis (Ieven and Hoymans, 2005; J M Kern *et al.*, 2009b; Lindholt *et al.*, 2003; Sessa *et al.*, 2009a).
The multifactorial aetiology of atherosclerosis may also have significantly impacted the heterogeneity in the outcomes of the studies linking *C. pneumoniae* to cardiovascular diseases. For example, environmental factors, such as diet and lifestyle, as well as individual genetic predisposition to chronic diseases, may contribute to a higher risk of developing atherosclerotic cardiovascular diseases as compared to the general population, hiding the impact of *C. pneumoniae* infection in these subjects. Lastly, some genetic polymorphisms have been shown to be significantly associated with *C. pneumoniae* infection and chlamydia-mediated atherosclerosis, including the C(-260)>T polymorphism in the CD14 promoter gene and alterations of the IL8 gene (IL8–251A>T polymorphic genotype) (Almeida *et al.*, 2019; Eng *et al.*, 2003). In particular, the C(-260)>T polymorphism appeared to modulate individual susceptibility to *C. pneumoniae* infection, increasing the odds ratio to 2.08, and the IL8–251A>T polymorphism was mostly associated with valvopathies and a previous exposure to *C. pneumoniae* (Almeida *et al.*, 2019; Eng *et al.*, 2003).

Is there evidence of the cellular and molecular mechanisms by which C. pneumoniae may contribute to the atherosclerotic process?

Numerous evidence in the literature has suggested that oxidative stress and inflammation may be the most likely pathogenetic mechanisms employed by *C. pneumoniae* for contributing to either early or late stages of the atherosclerotic process in all vascular cells (Di Pietro *et al.*, 2014, 2013b; Filardo *et al.*, 2021; Khoshbayan *et al.*, 2021). In particular, *in vitro* findings have demonstrated that *C. pneumoniae*

activated, in endothelial cells, nicotinamide adenine dinucleotide phosphate oxidase (NOX-1, NOX-4, and p22phox), and cyclooxygenase-2 (COX-2), at the same time limiting antioxidant enzymes, including catalase, superoxide dismutase 1 and thioredoxin-1, thus contributing to ROS production and, consequently, to endothelial dysfunction (Kreutmayer *et al.*, 2013). A similar outcome has also been observed after exposure to the heat shock protein-60 (cHSP60) of *C. pneumoniae*, by decreasing endothelial nitric oxide synthase (eNOS) expression and nitric oxide (NO) production through several regulatory mechanisms: for example, interference of eNOS trafficking from the Golgi apparatus to the plasma membrane, mitogen-activated protein kinase (MAPK) activation, and increased oxidative stress (Chen *et al.*, 2009; Mueller and Wolf, 2015). In macrophages, *C. pneumoniae* was able to induce the generation of ROS via the activation of NOX and cytochrome c oxidase by the binding to CD14 receptors and Ca^{2+} influx signalling (Azenabor *et al.*, 2005), leading to LDL oxidation followed by foam-cell formation. In platelets, *Chlamydia* lipopolysaccharide can trigger PKC activity and, in turn, phosphorylates proteins involved in shape change and ROS production, including NO synthase and lipoxygenase (Di Pietro *et al.*, 2014; Kälvegren *et al.*, 2005), resulting in platelet aggregation. In VSMCs, recent evidence has shown that *C. pneumoniae*-mediated increase in ROS production did not depend on NOX activation, but on mitochondrial dysfunction, favouring chlamydial persistence; higher levels of mitochondrial ROS, in turn, activated the expression of JunB Proto-Oncogene (JunB) and FBJ osteosarcoma oncogene-related antigen 1 (Fra-1), promoting VSMCs migration and the following atherosclerotic lesion formation (Zhao *et al.*, 2022).

Regarding inflammation, *C. pneumoniae* was able to trigger and sustain, in macrophages, platelets, endothelial cells, and VSMCs, an increased production of pro-inflammatory cytokines (IL-6, IL-8, IL-17A, and TNF-α), chemokines (monocytes chemoattract protein), and adhesion molecules (endothelial-leukocyte adhesion molecule; intercellular adhesion molecule; and vascular cell adhesion molecule), involved in the initiation, progression, and destabilization of the atherosclerotic plaque (Belland *et al.*, 2004; Campbell and Kuo, 2004; Jan Marco Kern *et al.*, 2009; Khoshbayan *et al.*, 2021; Stassen *et al.*, 2008).

C. pneumoniae could also contribute to chronic inflammation in the arterial wall via the development of chlamydial persistent forms. This hypothesis was supported by the detection of *C. pneumoniae* antigens and/or DNA in atherosclerotic tissue biopsies, without the isolation of the organism (Campbell and Rosenfeld, 2014). Over the years, to confirm this pathogenetic theory, a large number of studies have been performed in the attempt to identify specific biomarkers for *C. pneumoniae* persistent form. In this regard, transcription analyses of different genes involved in early and late chlamydial developmental cycle have been carried out; among them, most studies evaluated the early genes *ompA* and *groEL*, encoding for the chlamydial major outer membrane protein and the heat shock protein-60, respectively, as well as the late genes *hctA* and *hctB*, encoding for proteins involved in the DNA condensation required for RB to EB differentiation, although they failed to reveal a transcriptional profile common to the different persistence models (Di Pietro *et al.*, 2012; Panzetta *et al.*, 2018).

Of great pathological relevance are several immune evasion mechanisms of *C. pneumoniae*, resulting in a chronic infection that is known to contribute to the atherosclerotic process. First, several studies have demonstrated that *C. pneumoniae* can survive in monocytes/macrophages by maintaining a relatively high antioxidant to oxidant ratio, thereby abrogating the bacteria-killing effect of ROS (Azenabor *et al.*, 2006). Second, in macrophages as well as in neutrophil granulocytes, *C. pneumoniae* was able to successfully escape from apoptosis, a programmed cell death considered an important defence mechanism against invading pathogens, via the inhibition of procaspase-3 as well as the increased expression of the antiapoptotic interleukin (IL)-8 and NFkB (Carratelli *et al.*, 2002; J. Kern *et al.*, 2009; van Zandbergen *et al.*, 2004). Lastly, *C. pneumoniae* was evidenced to kill activated human T cells by inducing apoptotic and inflammasome cell death pathways via the activation of caspase 1, 8, 9, and IL-1β production, respectively (Olivares-Zavaleta *et al.*, 2011).

To date, other atherogenic cellular and molecular mechanisms mediated by *C. pneumoniae* have been demonstrated:

1) enhanced foam cell formation by suppressing cholesterol efflux (downregulating the ATP binding cassette transporters ABCA1 and ABCG1) and by increasing the uptake of ox-LDL (upregulating the scavenger receptor A1) in macrophages (Di Pietro *et al.*, 2013a; Wu *et al.*, 2021);
2) *C. pneumoniae* invasion of VSMCs with their consequent migration and acceleration of atherosclerosis through the crosstalk between toll-like receptor 2 and C-X-C chemokine receptor type 4 (Khoshbayan *et al.*, 2021);
3) the interplay between inflammation, oxidative stress, and *C. pneumoniae*-activated immune cells, alongside dyslipidaemia, resulting in the accumulation of intracellular cholesterol, foam cell formation and acceleration of the atherosclerotic process (Di Pietro *et al.*, 2019; Stassen *et al.*, 2008);
4) the secretion of several growth factors, such as the platelet-derived growth factor and the basic fibroblast growth factor in endothelial cells and VSMCs, as well as metalloproteinases (MMP-3,

MMP-10, MMP-12) in monocytes/ macrophages, involved in plaque progression and destabilization (Belland *et al.*, 2004; Campbell and Kuo, 2004). Lastly, *C. pneumoniae* may also contribute to atherosclerotic cardiovascular diseases by acting on systemic inflammation; indeed, a meta-analysis showed significantly higher levels of high sensitivity C-reactive protein, IL-6, and fibrinogen in *C. pneumoniae* IgA seropositive compared to seronegative atherosclerotic patients (Filardo *et al.*, 2015).

Is there evidence of a causal relationship between C. pneumoniae and atherosclerosis or cardiovascular diseases?

Today, there is no causal relationship between *C. pneumoniae* and atherosclerosis or cardiovascular diseases; the 4 traditional criteria in Koch postulates are not entirely fulfilled since *C. pneumoniae* was not found in all cases of disease and was not isolated from the diseased host. Furthermore, few *in vivo* studies have demonstrated the development of atherosclerotic disease as well as the presence of *C. pneumoniae* in the vascular wall following its inoculation into healthy rabbits or mice (Liu and Waters, 2005).

A further issue that could have hidden the role of *C. pneumoniae* in atherosclerosis is the etiopathogenetic contribution of the "infectious burden." This consists of the simultaneous presence of *C. pneumoniae* alongside other pathogens, including *Helicobacter pylori,* human cytomegalovirus, influenza virus and periodontal pathogens, that could contribute to the pathogenesis of atherosclerosis (Higgins, 2003; Rosenfeld and Campbell, 2011; Sessa *et al.*, 2014) through overlapping mechanisms. Those include the production of cytokines, ROS, growth factors, and cellular adhesion molecules (Dengler *et al.*, 2000; Rahbar and Söderberg-Nauclér, 2005; Speir *et al.*, 1994), the induction of systemic inflammation that, in turn, may damage the vascular wall (Jackson *et al.*, 2009; Madjid *et al.*, 2003), and molecular mimicry, that may play a role in the pathogenesis of atherosclerosis (Choi *et al.*, 2011; Kreutmayer *et al.*, 2013; Okada *et al.*, 2007). Furthermore, recent studies evidenced SARS-CoV-2/*C. pneumoniae* coinfection in COVID-19 patients (de Kruif *et al.*, 2005; Frutos *et al.*, 2022; Oliva *et al.*, 2020), opening an interesting pathophysiological scenario in the etiopathogenesis of atherosclerotic cardiovascular diseases; *C. pneumoniae*, acquired early in life, may contribute to the cytokine storm observed in severe COVID-19 disease due to its ability to induce local and systemic chronic inflammation (Costela-Ruiz *et al.*, 2020). Additionally, the oxidative mechanisms related to *C. pneumoniae* may be involved in severe COVID-19 disease. These hypotheses may be supported by evidence that SARS-CoV-2 and *C. pneumoniae* share some cellular and molecular pathways in endothelial dysfunction, thrombus formation, and ROS and proinflammatory cytokine production (Filardo *et al.*, 2021).

The absence of causality between *C. pneumoniae* infection and atherogenesis was also supported by the failure of human clinical antibiotic trials that attempted to demonstrate that anti-chlamydial antibiotics could prevent coronary heart disease, as evidenced by systematic reviews with metanalysis (Andraws *et al.*, 2005; Baker and Couch, 2007). These treatment failures must be taken with caution considering that all subjects enrolled in the antibiotic trials had advanced disease and *C. pneumoniae* is well known to cause asymptomatic infections early in life. As a result, antibiotic treatment may be too late to produce significant clinical effects. Lastly, these negative results from clinical trials may also be related to other factors, such as the refractoriness of chlamydial chronic infection to antibiotics related to the presence of persistent forms as evidenced by *in vivo* studies (Campbell and Rosenfeld, 2014; Jan Marco Kern *et al.*, 2009).

Given the failure of the human anti-microbial trials, alternative strategies for the treatment of *C. pneumoniae* and related atherosclerotic events have been proposed: for example, antioxidant compounds, reducing ROS production or enhancing antioxidant defence systems, novel antibiotics, or other molecules directly targeting chlamydial intracellular mechanisms.

As antioxidants, polyphenol compounds, such as curcumin and resveratrol, have been described as potential inhibitors of NOX-mediated ROS production in the vascular wall. Furthermore, COX-2 inhibitors such as ibuprofen and diclofenac reduced *C. pneumoniae*-induced ROS production in monocytes (Mouithys-Mickalad *et al.*, 2004). Other investigators have focused their attention on lipid lowering drugs such as statins (coenzyme A reductase inhibitors), capable of antioxidant activity alongside the reduction of LDL levels (Antonopoulos *et al.*, 2012). Among the substances able to mimic the biochemical activity of SOD, an antioxidant defence system, Mn (III) tetrakis (4-benzoic acid) porphyrin chloride (MnTBAP) ,was able to stimulate NOS activity in endothelial cells, reducing ROS levels and increasing NO bioavailability (Chen *et al.*, 2009). Similarly, other free radical scavengers, such as sesamol, were also effective in inhibiting the *C. pneumoniae*-mediated proliferation of VSMCs (Fukuoka *et al.*, 2004).

As a novel antibiotic approach, a recent study has shown that muraymycin D2 and its derivatives, inhibitor of MraY, a peptide involved in the biosynthesis pathway of peptidoglycan, possess the ability to eradicate persisting chlamydial forms *in vitro*, potentially reducing the risk of chronic sequelae like atherosclerosis (Löckener *et al.*, 2024). Besides the several treatments against *C. pneumoniae* and related atherosclerotic events, a different approach may be to

prevent chlamydial infection via the development of an effective vaccine. However, the complexity of *C. pneumoniae* and its multi-stage life cycle, alongside the host-pathogen interaction and the multiple immune correlates of protection, has, thus far, hindered research on this topic (Puolakkainen, 2009). Numerous different chlamydial epitopes have been considered as potential targets for vaccine strategies, such as, for example, components of the type III secretion system, as well as surface proteins. Subsequently, more potential targets have been designed through genome-based approaches and *in silico* methodologies, although, to date, the issue of a *C. pneumoniae* vaccine has not yet been resolved (Puolakkainen, 2009).

CONCLUSIONS

Overall, the available evidence suggests that *C. pneumoniae* is a biologically plausible candidate for the causation of atherosclerosis, since the disappointing results of antibiotic trials in humans do not fully exclude that *C. pneumoniae* may possess a role in the development and/or progression of atherosclerosis and, hence, in cardiovascular diseases.

Clearly, the difficulties in isolating, culturing, and genetic manipulation of *C. pneumoniae* have impaired the progression of research; today, an important unsolved concern is the persistence state into the host cell as a potential survival strategy of *C. pneumoniae* in the arterial wall. For years, several research teams worldwide have attempted to shed light on the factors responsible for the onset and maintenance of Chlamydiae persistent forms, focusing on their specific transcriptional profiles.

In the future, the newly designed transformation systems based on engineered plasmids absorption by the chlamydial cell, for the insertion of foreign DNA sequences, as well as novel transposon systems, will surely help expand our knowledge on the cellular and molecular mechanisms involved in *C. pneumoniae* infection (Shima *et al.*, 2018; Yanatori *et al.*, 2021). In particular, these systems will be able to precisely explore the temporal dynamics, the role, as well as the functions, of the countless genes that are differentially expressed during the phases of the chlamydial growth cycle, contributing to discover the elusive mechanisms leading to persistent forms. Furthermore, deciphering the functions of the essential genes underlying host-cell interactions will most likely provide novel targets for the development of an effective vaccine towards Chlamydiae.

Lastly, 16S RNA sequencing and high-throughput sequencing and bioinformatics analyses will allow better identification of the bacteria that may potentially play a role during human atherosclerotic plaque development, providing a more detailed view of the "infectious burden."

Sources of funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Disclosure of interest

All the authors declare that they have no conflicting interest.

References

Abdelrahman Y.M., Belland R.J. (2005). The chlamydial developmental cycle. *FEMS Microbiol Rev.* **29**, 949-959. https://doi.org/10.1016/j.femsre.2005.03.002

Almeida N.C.C., Queiroz M.A.F., Lima S.S., Brasil Costa I., Ayin Fossa M.A., Vallinoto A.C.R., Ishak M. de O.G., Ishak R. (2019). Association of Chlamydia trachomatis, C. pneumoniae, and IL-6 and IL-8 Gene Alterations With Heart Diseases. *Front Immunol.* **10**. https://doi.org/10.3389/fimmu.2019.00087

Anderson J.L., Muhlestein J.B. (2004). Antibiotic trials for coronary heart disease. *Tex. Heart Inst. J.* **31**, 33-38.

Andraws R., Berger J.S., Brown D.L. (2005). Effects of antibiotic therapy on outcomes of patients with coronary artery disease: a meta-analysis of randomized controlled trials. *JAMA.* **293**, 2641-2647.

Antonopoulos A., Margaritis M., Shirodaria C., Antoniades C. (2012). Translating the effects of statins: From redox regulation to suppression of vascular wall inflammation. *Thromb Haemost.* **108**, 840-848. https://doi.org/10.1160/TH12-05-0337

Aromataris E., Fernandez R., Godfrey C.M., Holly C., Khalil H., Tungpunkom P. (2015). Summarizing systematic reviews. *Int J Evid Based Healthc.* **13**, 132-140. https://doi.org/10.1097/XEB.0000000000000055

Azenabor A.A., Muili K., Akoachere J.-F., Chaudhry A. (2006). Macrophage antioxidant enzymes regulate Chlamydia pneumoniae chronicity: Evidence of the effect of redox balance on host–pathogen relationship. *Immunobiology.* **211**, 325-339. https://doi.org/10.1016/j.imbio.2005.12.001

Azenabor A.A., Yang S., Job G., Adedokun O.O. (2005). Elicitation of reactive oxygen species in Chlamydia pneumoniae-stimulated macrophages: a Ca2+-dependent process involving simultaneous activation of NADPH oxidase and cytochrome oxidase genes. *Med Microbiol Immunol.* **194**, 91-103. https://doi.org/10.1007/s00430-004-0223-4

Baker W.L., Couch K.A. (2007). Azithromycin for the secondary prevention of coronary artery disease: a meta-analysis. *Am J Health Syst Pharm.* **64**, 830-836. https://doi.org/10.2146/ajhp060539

Belland R.J., Ouellette S.P., Gieffers J., Byrne G.I. (2004). Chlamydia pneumoniae and atherosclerosis. *Cell. Microbiol.* **6**, 117-127.

Campbell L.A., Kuo C.-C. (2004.) Chlamydia pneumoniae–an infectious risk factor for atherosclerosis? *Nat. Rev. Microbiol.* **2**, 23-32.

Campbell L.A., Kuo C.-C. (2003). Chlamydia pneumoniae and atherosclerosis. *Semin Respir Infect.* **18**, 48-54. https://doi.org/10.1053/srin.2003.50006

Campbell L.A., Rosenfeld M.E. (2014). Persistent C. pneumoniae infection in atherosclerotic lesions: rethinking the clinical trials. *Front. Cell. Infect. Microbiol.* **4**, 34.

Carratelli C.R., Rizzo A., Catania M.R., Gallè F., Losi E., Hasty D.L., Rossano F. (2002). Chlamydia pneumoniae infections prevent the programmed cell death on THP-1 cell line. *FEMS Microbiol Lett.* **215**, 69-74. https://doi.org/10.1111/j.1574-6968.2002.tb11372.x

Chen C., Chai H., Wang X., Lin P.H., Yao Q. (2009). Chlamydia heat shock protein 60 decreases expression of endothelial nitric oxide synthase in human and porcine coronary artery endothelial cells. *Cardiovasc Res.* **83**, 768-777. https://doi.org/10.1093/cvr/cvp150

Chen J., Zhu M., Ma G., Zhao Zhangning, Sun, Z. (2013). Chlamydia pneumoniae infection and cerebrovascular disease: a systematic review and meta-analysis. *BMC Neurol.* **13**, 183.

Choi J., Lee S.-Y., Kim K., Choi B.-K. (2011). Identification of immunoreactive epitopes of the Porphyromonas gingivalis heat shock protein in periodontitis and atherosclerosis. *J*

Periodontal Res. **46**, 240-245. https://doi.org/10.1111/j.1600-0765.2010.01339.x
Costela-Ruiz V.J., Illescas-Montes R., Puerta-Puerta J.M., Ruiz C., Melguizo-Rodríguez L. (2020). SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. *Cytokine Growth Factor Rev.* **54**, 62-75. https://doi.org/10.1016/j.cytogfr.2020.06.001
de Kruif M.D., van Gorp E.C.M., Keller Tymen T., Ossewaarde J.M., ten Cate H. (2005). Chlamydia pneumoniae infections in mouse models: relevance for atherosclerosis research. *Cardiovasc. Res*. **65**, 317-327.
Dengler T.J., Raftery M.J., Werle M., Zimmermann R., Schönrich G. (2000). Cytomegalovirus infection of vascular cells induces expression of pro-inflammatory adhesion molecules by paracrine action of secreted interleukin-1beta. *Transplantation*. **69**, 1160-1168. https://doi.org/10.1097/00007890-200003270-00022
Di Pietro M., De Santis F., Schiavoni G., Filardo S., Sessa R. (2013a). Resveratrol in Chlamydia pneumoniae-induced foam cell formation and interleukin-17A synthesis. *J Biol Regul Homeost Agents*. **27**, 509-518.
Di Pietro M., Filardo S., De Santis F., Mastromarino P., Sessa R. (2014). Chlamydia pneumoniae and oxidative stress in cardiovascular disease: state of the art and prevention strategies. *Int. J. Mol. Sci*. **16**, 724-735.
Di Pietro M., Filardo S., De Santis F., Sessa R. (2013b). Chlamydia pneumoniae infection in atherosclerotic lesion development through oxidative stress: a brief overview. *Int. J. Mol. Sci*. **14**, 15105-15120.
Di Pietro M., Filardo S., Romano S., Sessa R. (2019). Chlamydia trachomatis and Chlamydia pneumoniae Interaction with the Host: Latest Advances and Future Prospective. *Microorganisms*. **7**. https://doi.org/10.3390/microorganisms7050140
Di Pietro M., Schiavoni G., Del Piano M., Shaik Y., Boscolo P., Caraffa A., Grano M., Teté S., Conti F., Sessa R. (2009). Chlamydia pneumoniae and atherosclerosis: the role of mast cells. *J. Biol. Regul. Homeost. Agents*. **23**, 65-69.
Di Pietro M., Tramonti A., De Santis F., De Biase D., Schiavoni G., Filardo S., Zagaglia C., Sessa R. (2012). Analysis of gene expression in penicillin G induced persistence of Chlamydia pneumoniae. *J Biol Regul Homeost Agents*. **26**, 277-284.
Eng H., Chen C., Kuo C., Wu J., Wang C., Lin T. (2003). Association of CD14 Promoter Gene Polymorphism and *Chlamydia pneumoniae* Infection. *J Infect Dis*. **188**, 90-96. https://doi.org/10.1086/375744
Filardo S., Di Pietro M., Diaco F., Romano S., Sessa R. (2021). Oxidative Stress and Inflammation in SARS-CoV-2- and Chlamydia pneumoniae-Associated Cardiovascular Diseases. *Biomedicines*. **9**. https://doi.org/10.3390/biomedicines9070723
Filardo S., Di Pietro M., Farcomeni A., Schiavoni G., Sessa R. (2015). Chlamydia pneumoniae-Mediated Inflammation in Atherosclerosis: A Meta-Analysis. *Mediators Inflamm*. **2015**, 378658. https://doi.org/10.1155/2015/378658
Frutos M.C., Origlia J., Gallo Vaulet M.L., Venuta M.E., García M.G., Armitano R., Cipolla L., Madariaga M.J., Cuffini C., Cadario M.E., Grupo de Trabajo Bacterias Atípicas, S.A. de B.M. y P.C. (SADEBAC), D. de la A.A. de M. (2022). SARS-CoV-2 and Chlamydia pneumoniae co-infection: A review of the literature. *Rev Argent Microbiol*. **54**, 247-257. https://doi.org/10.1016/j.ram.2022.05.009
Fukuoka K., Sawabe A., Sugimoto T., Koga M., Okuda H., Kitayama T., Shirai M., Komai K., Komemushi S., Matsuda K. (2004). Inhibitory Actions of Several Natural Products on Proliferation of Rat Vascular Smooth Muscle Cells Induced by Hsp60 from *Chlamydia pneumoniae* J138. *J Agric Food Chem*. **52**, 6326-6329. https://doi.org/10.1021/jf0351164
Gautam J., Krawiec C. (2024). Chlamydia Pneumonia.
Gaydos C.A. (2000). Growth in vascular cells and cytokine production by Chlamydia pneumoniae. *J Infect Dis*. **181** Suppl. 3, S473-S478. https://doi.org/10.1086/315612
Gitsels A., Sanders N., Vanrompay D. (2019). Chlamydial Infection From Outside to Inside. *Front Microbiol*. **10**, 2329. https://doi.org/10.3389/fmicb.2019.02329
Grayston J.T. (2000). Background and current knowledge of Chlamydia pneumoniae and atherosclerosis. *J Infect Dis*. **181**, Suppl. 3, S402-S410. https://doi.org/10.1086/315596
Grayston J.T., Belland R.J., Byrne G.I., Kuo C.C., Schachter J., Stamm W.E., Zhong G. (2015). Infection with Chlamydia pneumoniae as a cause of coronary heart disease: the hypothesis is still untested. *Pathog Dis*. **73**, 1-9. https://doi.org/10.1093/femspd/ftu015
Higgins J.P. (2003). Chlamydia pneumoniae and coronary artery disease: the antibiotic trials. *Mayo Clin Proc*. **78**, 321-332. https://doi.org/10.4065/78.3.321
Higgins J.P., Higgins J.A., Higgins Patricia M., Ahuja S., Higgins D.L. (2003). Chlamydia pneumoniae and coronary artery disease: legitimized linkages? *Expert Rev. Cardiovasc. Ther*. **1**, 367-384.
Hirono S., Pierce G.N. (2003). Dissemination of Chlamydia pneumoniae to the vessel wall in atherosclerosis. *Mol. Cell. Biochem*. **246**, 91-95.
Ieven M.M., Hoymans V.Y. (2005). Involvement of Chlamydia pneumoniae in atherosclerosis: more evidence for lack of evidence. *J. Clin. Microbiol*. **43**, 19-24.
Jackson L., Britton J., Lewis S.A., McKeever T.M., Atherton J., Fullerton D., Fogarty A.W. (2009). A Population-Based Epidemiologic Study of Helicobacter Pylori Infection and its Association with Systemic Inflammation. *Helicobacter*. **14**, 460-465. https://doi.org/10.1111/j.1523-5378.2009.00711.x
Kälvegren H., Bylin H., Leanderson P., Richter A., Grenegård M., Bengtsson T. (2005). Chlamydia pneumoniae induces nitric oxide synthase and lipoxygenase- dependent production of reactive oxygen species in platelets. *Thromb Haemost*. **94**, 327-335. https://doi.org/10.1160/TH04-06-0360
Keikha M., Karbalaei M. (2022). Potential association between bacterial infections and ischemic stroke based on fifty case-control studies: A systematic review and meta-analysis. *New Microbes New Infect*. **47**, 100980.
Kern J., Maass V., Maass M. (2009). Chlamydia pneumoniae adversely modulates vascular cell properties by direct interaction with signalling cascades. *Thromb Haemost*. **102**, 1064-1070. https://doi.org/10.1160/TH09-06-0348
Kern J.M., Maass V., Maass M. (2009a). Molecular pathogenesis of chronic Chlamydia pneumoniae infection: a brief overview. *Clin Microbiol Infect*. **15**, 36-41. https://doi.org/10.1111/j.1469-0691.2008.02631.x
Kern Jan M., Maass V., Maass M. (2009). Chlamydia pneumoniae-induced pathological signaling in the vasculature. *FEMS Immunol. Med. Microbiol*. **55**, 131-139.
Kern J.M., Maass V., Maass M. (2009b). Molecular pathogenesis of chronic Chlamydia pneumoniae infection: a brief overview. *Clin. Microbiol. Infect*. **15**, 36-41.
Khoshbayan A., Taheri F., Moghadam Majid T., Chegini Z., Shariati A. (2021). The association of Chlamydia pneumoniae infection with atherosclerosis: Review and update of in vitro and animal studies. *Microb. Pathog*. **154**, 104803.
Kozusnik T., Adams S.E., Greub G. (2024). Aberrant Bodies: An Alternative Metabolic Homeostasis Allowing Survivability? *Microorganisms*. **12**. https://doi.org/10.3390/microorganisms12030495
Kreutmayer S., Csordas A., Kern J., Maass V., Almanzar G., Offterdinger M., Öllinger R., Maass M., Wick G. (2013). Chlamydia pneumoniae infection acts as an endothelial stressor with the potential to initiate the earliest heat shock protein 60-dependent inflammatory stage of atherosclerosis. *Cell Stress Chaperones*. **18**, 259-268. https://doi.org/10.1007/s12192-012-0378-7
Kuo C.-C., Campbell L.A. (2003). Chlamydial infections of the cardiovascular system. *Front. Biosci*. **8**, e36-43.
Kuo C.C., Shor A., Campbell L.A., Fukushi H., Patton D.L., Grayston J.T. (1993). Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries. *J Infect Dis*. **167**, 841-849. https://doi.org/10.1093/infdis/167.4.841
Lindholt J.S., Stovring J., Andersen P.L., Henneberg E.W., Østergaard L. (2003). A review of macrolide treatment of atherosclerosis and abdominal aortic aneurysms. *Curr. Drug Targets Infect. Disord*. **3**, 55-63.
Liu C., Waters D.D. (2005). Chlamydia pneumoniae and atherosclerosis: from Koch postulates to clinical trials. *Prog. Cardiovasc. Dis*. **47**, 230-239.
Löckener I., Behrmann L.V., Reuter J., Schiefer A., Klöckner A., Krannich S., Otten C., Mölleken K., Ichikawa S., Hoerauf A., Schneider T., Pfarr K.M., Henrichfreise B. (2024). The MraY Inhibitor Muraymycin D2 and Its Derivatives Induce Enlarged Cells in Obligate Intracellular Chlamydia and Wolbachia and Break the Persistence Phenotype in Chlamydia. *Antibiotics*. **13**, 421. https://doi.org/10.3390/antibiotics13050421
Madaudo C., Coppola G., Parlati A.L.M., Corrado E. (2024). Discovering Inflammation in Atherosclerosis: Insights from Path-

ogenic Pathways to Clinical Practice. *Int J Mol Sci*. **25**, 6016. https://doi.org/10.3390/ijms25116016

Madjid M., Naghavi M., Litovsky S., Casscells S.W. (2003). Influenza and Cardiovascular Disease. *Circulation*. **108**, 2730-2736. https://doi.org/10.1161/01.CIR.0000102380.47012.92

Mendis S., Puska P., Norrving B., World Health Organization, World Heart Federation, World Stroke Organization (2011). Global atlas on cardiovascular disease prevention and control / edited by: Shanthi Mendis ... [*et al.*]. World Health Organization. World Health Organization, Geneva.

Mouithys-Mickalad A., Deby-Dupont G., Dogne J.-M., de Leval X., Kohnen S., Navet R., Sluse F., Hoebeke M., Pirotte B., Lamy M. (2004). Effects of COX-2 inhibitors on ROS produced by Chlamydia pneumoniae-primed human promonocytic cells (THP-1). *Biochem Biophys Res Commun*. **325**, 1122-1130. https://doi.org/10.1016/j.bbrc.2004.10.155

Mueller K.E., Wolf K. (2015). *C. pneumoniae* disrupts eNOS trafficking and impairs NO production in human aortic endothelial cells. *Cell Microbiol*. **17**, 119-130. https://doi.org/10.1111/cmi.12341

Muhlestein J.B. (2003). Antibiotic treatment of atherosclerosis. *Curr. Opin. Lipidol*. **14**, 605-614.

Mussa F.F., Chai H., Wang X., Yao Q., Lumsden A.B., Chen C. (2006). Chlamydia pneumoniae and vascular disease: an update. *J. Vasc. Surg*. **43**, 1301-1307.

Okada T., Ayada K., Usui S., Yokota K., Cui J., Kawahara Y., Inaba T., Hirohata S., Mizuno M., Yamamoto D., Kusachi S., Matsuura E., Oguma K. (2007). Antibodies against heat shock protein 60 derived from Helicobacter pylori: Diagnostic implications in cardiovascular disease. *J Autoimmun*. **29**, 106-115. https://doi.org/10.1016/j.jaut.2007.05.004

Oliva A., Siccardi G., Migliarini A., Cancelli F., Carnevalini M., D'Andria M., Attilia I., Danese V.C., Cecchetti V., Romiti R., Ceccarelli G., Mastroianni C.M., Palange P., Venditti M. (2020). Co-infection of SARS-CoV-2 with Chlamydia or Mycoplasma pneumoniae: a case series and review of the literature. *Infection*. **48**, 871-877. https://doi.org/10.1007/s15010-020-01483-8

Olivares-Zavaleta N., Carmody A., Messer R., Whitmire W.M., Caldwell H.D. (2011). Chlamydia pneumoniae Inhibits Activated Human T Lymphocyte Proliferation by the Induction of Apoptotic and Pyroptotic Pathways. *The Journal of Immunology*. **186**, 7120-7126. https://doi.org/10.4049/jimmunol.1100393

Panzetta M.E., Valdivia R.H., Saka H.A. (2018). Chlamydia Persistence: A Survival Strategy to Evade Antimicrobial Effects in-vitro and in-vivo. *Front Microbiol*. **9**, 3101. https://doi.org/10.3389/fmicb.2018.03101

Puolakkainen M. (2009). Innate immunity and vaccines in chlamydial infection with special emphasis on Chlamydia pneumoniae. *FEMS Immunol Med Microbiol*. **55**, 167-177. https://doi.org/10.1111/j.1574-695X.2008.00519.x

Rahbar A., Söderberg-Nauclér C. (2005). Human Cytomegalovirus Infection of Endothelial Cells Triggers Platelet Adhesion and Aggregation. *J Virol*. **79**, 2211-2220. https://doi.org/10.1128/JVI.79.4.2211-2220.2005

Rizzo A., Paolillo R., Iafusco D., Prisco F., Romano Carratelli C. (2012). Chlamydia pneumoniae infection in adolescents with type 1 diabetes mellitus. *J Med Microbiol*. **61**, 1584-1590. https://doi.org/10.1099/jmm.0.048512-0

Rosenfeld M.E., Campbell L.A. (2011). Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb Haemost*. **106**, 858-867. https://doi.org/10.1160/TH11-06-0392

Saikku P., Leinonen M., Mattila K., Ekman M.R., Nieminen M.S., Mäkelä P.H., Huttunen J.K., Valtonen V. (1988). Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet*. **2**, 983-6. https://doi.org/10.1016/s0140-6736(88)90741-6

Sessa R., Di Pietro M., Santino I., del Piano M., Varveri A., Dagianti A., Penco M. (1999). Chlamydia pneumoniae infection and atherosclerotic coronary disease. *Am Heart J*. **137**, 1116-1119. https://doi.org/10.1016/S0002-8703(99)70371-6

Sessa R., Nicoletti M., Di Pietro M., Schiavoni G., Santino I., Zagaglia C., Del Piano M., Cipriani P. (2009a). Chlamydia pneumoniae and atherosclerosis: current state and future prospectives. *Int. J. Immunopathol. Pharmacol*. **22**, 9-14.

Sessa R., Nicoletti M., Di Pietro M., Schiavoni G., Santino I., Zagaglia C., Del Piano M., Cipriani P. (2009b). Chlamydia pneumoniae and atherosclerosis: current state and future prospectives. *Int J Immunopathol Pharmacol*. **22**, 9-14. https://doi.org/10.1177/039463200902200102

Sessa R., Pietro M. Di Filardo S., Turriziani O. (2014). Infectious burden and atherosclerosis: A clinical issue. *World J Clin Cases*. **2**, 240-249. https://doi.org/10.12998/wjcc.v2.i7.240

Shima K., Wanker M., Skilton R.J., Cutcliffe L.T., Schnee C., Kohl T.A., Niemann S., Geijo J., Klinger M., Timms P., Rattei T., Sachse K., Clarke I.N., Rupp J. (2018). The Genetic Transformation of Chlamydia pneumoniae. *mSphere*. **3**. https://doi.org/10.1128/mSphere.00412-18

Speir E., Modali R., Huang E.-S., Leon M.B., Shawl F., Finkel T., Epstein S.E. (1994). Potential Role of Human Cytomegalovirus and p53 Interaction in Coronary Restenosis. *Science*. **265**, 391-394. https://doi.org/10.1126/science.8023160

Stassen F.R., Vainas T., Bruggeman C.A. (2008). Infection and atherosclerosis. An alternative view on an outdated hypothesis. *Pharmacol. Rep*. **60**, 85-92.

Tsirpanlis G. (2004). Chlamydia pneumoniae and atherosclerosis: no way-out or long way? What about renal failure patients? *Kidney Blood Press. Res*. **27**, 134-142.

van Zandbergen G., Gieffers J., Kothe H., Rupp J., Bollinger A., Aga E., Klinger M., Brade H., Dalhoff K., Maass M., Solbach W., Laskay T. (2004). Chlamydia pneumoniae Multiply in Neutrophil Granulocytes and Delay Their Spontaneous Apoptosis. *The Journal of Immunology*. **172**, 1768-1776. https://doi.org/10.4049/jimmunol.172.3.1768

Watson C., Alp N.J. (2008). Role of Chlamydia pneumoniae in atherosclerosis. *Clin Sci (Lond)*. **114**, 509-531. https://doi.org/10.1042/CS20070298

Wu X., Cheng B., Guo X., Wu Q., Sun S., He P. (2021). 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*. **161**, 105284. https://doi.org/10.1016/j.micpath.2021.105284

Yanatori I., Miura K., Chen Y.-S., Valdivia R.H., Kishi F. (2021). Application of a C. trachomatis expression system to identify C. pneumoniae proteins translocated into host cells. *J Bacteriol*. **203**. https://doi.org/10.1128/JB.00511-20

Zhao X., Miao G., Zhang, L., Zhang, Y., Zhao, H., Xu, Z., Wang, B., Zhang, L., 2022. 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*. **10**. https://doi.org/10.3389/fcell.2022.879023