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Chlamydophila pneumoniae

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Abstract

Chlamydophila pneumoniae infection is ubiquitous. It accounts for 10% of community-acquired pneumonias and 5% of cases of pharyngitis, bronchitis and sinusitis in both immunocompetent and immunocompromised hosts. It is also involved in exacerbations of chronic bronchitis and asthma. Moreover, *C. pneumoniae* has been reported as a possible cause of atherosclerosis and central nervous system disorders. The current reference standard for serological diagnosis of acute infection is microimmunofluorescence testing, although molecular detection techniques may well become reference diagnostic tests in the near future. Tetracyclines and erythromycin show good *in vitro* activity, and so far have been the most commonly employed drugs in the treatment of *C. pneumoniae* infection. New macrolides, ketolides and fluoroquinolones are other potentially effective drugs. This review analyses the most recent data concerning the involvement of *C. pneumoniae* in human diseases.

Keywords: *Chlamydophila pneumoniae*, diagnostic methods, epidemiology, review, treatment

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Introduction

In 1989, the previously labelled *Chlamydia* strain TWAR was recognized as a third species of the *Chlamydia* genus and was named *Chlamydia pneumoniae* [1]. In 1999, a new taxonomic classification was proposed, renaming the bacterium as *Chlamydophila pneumoniae* [2]. The proposal was not universally accepted, and both names are currently in use by different authors. This agent is an obligate intracellular bacterium that is present in two developmental forms: infective elementary bodies, and reproductive reticulate bodies. Chlamydiae multiply within membrane-bound vacuoles in eukaryotic host cells but are unable to generate ATP and are therefore dependent on the host cell ATP deposits for all energy requirements. Moreover, they are incapable of *de novo* nucleotide biosynthesis, and depend on host nucleotide pools.

Epidemiology

C. pneumoniae infection is ubiquitous, with an antibody prevalence of 50% by age 20 years and 70–80% at age 60–70 years. It is a cause of community-acquired pneumonia (CAP), pharyngitis, bronchitis, sinusitis, exacerbations of chronic bronchitis and asthma. Moreover, *C. pneumoniae* has been reported as a possible cause of atherosclerosis and central nervous system (CNS) disorders.

Pneumonia

C. pneumoniae is reported to account for a relatively large number of cases (6–20%) of CAP [3], although data are largely based on serological determinations alone. The clinical course may vary from mild, self-limiting illnesses to severe forms of pneumonia, particularly in elderly patients, and those with coexisting cardiopulmonary diseases. This agent participates in co-infection involving other bacterial agents in approximately 30% of adult cases of CAP [4].

Asthma

An association between asthma and *C. pneumoniae* infection was first put forward by Hahn *et al.* in the early 1990s [5]. A relationship between acute infection with atypical pathogens and acute asthma exacerbations in children has been sought in controlled and uncontrolled studies [6]. The vast majority of studies were concordant in finding an association between atypical bacterial infection and asthma exacerbations. Rates of *C. pneumoniae* infection identified in asthma episodes varied between 4.5% and 25% [6].

Acute atypical infection has been studied in adults with asthma exacerbations [7]. A recent study revealed that patients with acute asthma exacerbation and evidence of

acute infection due to *C. pneumoniae* exhibited more severe functional impairment upon admission. In addition, these patients also showed a slower forced expiratory volume in 1 s (FEV_1) rise during follow-up when compared with the group without acute infection [8].

The relevance of *C. pneumoniae* in the pathogenesis of chronic asthma has also been extensively investigated. Accumulating evidence from seroepidemiological studies has shown that many asthmatics have elevated levels of antibodies to *C. pneumoniae* [9].

Should the role of *C. pneumoniae* infection in asthma patients be proven, *C. pneumoniae* eradication from the airways would become an important aspect of treatment.

Antibiotics exerting activity against atypical bacteria, e.g. macrolides, the ketolide telithromycin, tetracyclines and fluoroquinolones, would be logical candidates. To date, clinical trials in children and adults with asthma have mostly involved macrolides, owing to their favourable tolerability/safety profile and excellent intracellular accumulation characteristics [10–13].

A Cochrane review of macrolide usage in treatment of chronic asthma revealed an overall positive effect on symptoms and eosinophilic markers of inflammation following macrolide therapy [14].

A recent multicentre, double-blind, randomized, placebo-controlled clinical study assessed oral telithromycin as a supplement to standard-of-care treatment for adults with acute asthma exacerbations [15]. Ketolide treatment was associated with statistically significant and clinically substantial benefits. In this population, 61% of patients showed evidence of *C. pneumoniae* and/or *Mycoplasma pneumoniae* infection, and the effect of telithromycin on FEV_1 was statistically significant in patients with documented infection at baseline, but not in those without evidence of infection. However, there were no differences between infection-positive and infection-negative groups in terms of other study outcomes, so that the mechanisms causing benefit remain unclear.

The main limits of the above studies are related to the size of patient populations and the prevalent use of serology as the diagnostic test. There is a need for further well-designed studies to define both the importance of *C. pneumoniae* involvement in acute and chronic asthma and the usefulness of antibiotics in treating this disease.

Chronic obstructive pulmonary disease (COPD)

In patients with COPD, the persistence of microorganisms in the respiratory tract may facilitate access of different pathogens to the lower airways, and long-standing infection might trigger what is traditionally described as the vicious circle of chronic bronchitis. Chronic *C. pneumoniae* infection has been found to be common in cases of chronic bronchitis, and could contribute to disease progression through a toxic effect on bronchial epithelial cells, impairing ciliary function, and increasing chronic inflammation via proinflammatory cytokine production [16,17]. The possibility of chronic colonization with *C. pneumoniae* in patients with COPD is supported by serology, electron microscopy and immunohistochemistry [18,19]. Chronic colonization with *C. pneumoniae* is significantly associated with more severe functional impairment, and colonization is associated with a greater propensity to develop acute exacerbations [20]. Moreover, long-term antibiotic treatment, delivered over a 6-week period, is insufficient to eradicate the organism.

Biological basis for *C. pneumoniae* involvement in asthma and chronic airway inflammation

Current evidence indicates that *C. pneumoniae* gene products (mainly heat shock protein-60), through the activation of transcription factors (notably nuclear factor kappa-B), are responsible for the activation of most cellular elements in bronchial tissue (epithelium, endothelium, monocytes–macrophages, smooth muscle cells), resulting in a cascade of cytokine release and adhesion molecule upregulation, which favours cellular influx into the airways, persistent infection and airway remodelling. Considering that *C. pneumoniae* infection is extremely common in the population, there must be individual predisposing factors involved in associating this infection with an asthma phenotype [21]. Fig. 1 summarizes the interactions between *C. pneumoniae* and the different cell types present in airways. In a recent animal model, mice were inoculated intranasally with *C. pneumoniae* [22]. *C. pneumoniae* infection caused both sustained airway hyperresponsiveness and airway inflammation. This has clinical implications, as changes in airway responsiveness and inflammation status induced by this bacterium may worsen and/or provoke breathlessness in individuals with asthma and COPD.

Atherosclerosis

The first indication that *C. pneumoniae* has an association with atherosclerosis and coronary heart disease dates back to 1986 [23]. This association has been shown by seroepidemiology, immunohistochemistry, PCR, electron microscopy and tissue culture. Animal models of atherosclerosis have been used to study the role of *C. pneumoniae* in the initiation and progression of atherosclerotic disease [24]. The results of some treatment trials using antibiotics for the prevention of cardiovascular events in animal models of atherosclerosis encouraged secondary prevention trials in humans [25,26].

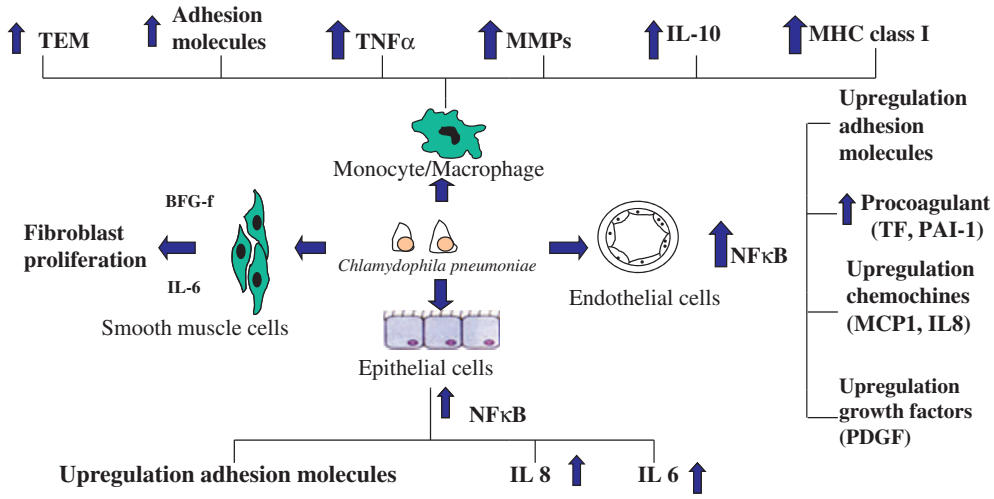

FIG. 1. *Chlamydophila pneumoniae* interaction with different human cell types. BFG, basic fibroblast growth factor; IL, interleukin; MCP, monocyte chemotactic protein; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NFκB, nuclear factor kappa-B; PAI-1, plasminogen activator inhibitor-1; PDGF, platelet-derived growth factor; TEM, transendothelial migration; TF, tissue factor; TNFα, tumour necrosis factor-α.

Small-scale studies indicated that antibiotic treatment may prevent adverse cardiovascular events. However, large clinical trials failed to demonstrate any effect [27,28]. A comprehensive review analysed the epidemiological and experimental evidence accumulated over the last 20 years linking *C. pneumoniae* to atherosclerosis [29]. The authors conclude that, considering present evidence, *C. pneumoniae* is neither sufficient in itself nor necessary to cause atherosclerosis or its clinical consequences in humans. However, *C. pneumoniae* is highly likely to be a modifiable risk factor that may be a target of future therapies.

Multiple sclerosis (MS)

C. pneumoniae is detected with higher frequency in the cerebrospinal fluid of MS patients than in that of neurological controls. The original findings of Sriram *et al.* [30] have been partially replicated by some, but not all, studies [31–34], because of genetic heterogeneity, differences in patient selection, and techniques of DNA extraction or amplification. *C. pneumoniae* infection seems to characterize a subgroup of MS patients with an anticipated onset of disease and more pronounced evidence of CNS inflammation/demyelination [35]. It was also found that *C. pneumoniae*-infected patients who were positive according to PCR had more active lesions than *C. pneumoniae*-infected patients who were PCR-negative, suggesting a role for *C. pneumoniae* in fostering a chronic inflammatory stimulation within the CNS. These findings led to the hypothesis that *C. pneumoniae* might act as a cofactor that is able to fuel already established inflammatory and demyelinating processes and promote more active disease. Published data suggest the need for longitudinal observations, and clinical trials with *C. pneumoniae*-specific antibiotics to clarify the exact role of *C. pneumoniae* infection in MS patients [36].

Alzheimer's disease (AD)

Studies that have used PCR to detect *C. pneumoniae* in the brains of AD patients have yielded conflicting results [37–39]. A recent study demonstrated that brain tissue samples from a high proportion of patients with AD are PCR-positive for *C. pneumoniae*, but those from age-/sex-matched non-AD controls are not. Moreover, the organism is viable within the brains of patients with AD, indicating metabolic activity of the organism in those tissues [40]. A randomized, placebo-controlled, multicentre clinical trial has been performed to determine whether a 3-month course of doxycycline and rifampin can reduce the decline of cognitive function in patients with AD [41]. This study showed significantly less cognitive decline at 6 months in the antibiotic group than in controls. Antibiotic therapy was also associated with less dysfunctional behaviour at 3 months. There was no clear relationship between the results and treatment in terms of eradication of chronic *C. pneumoniae* infection, suggesting that the activity of the two drugs may be related to non-antibiotic effects. None of these observations demonstrates a causal relationship between CNS infection with *C. pneumoniae* and the neuropathogenesis characteristic of AD, but they do open the way to further investigations.

Diagnostic Methods

Reliable diagnosis of infection due to *C. pneumoniae* and investigation of its role in chronic diseases remain difficult because of the paucity of well-standardized and commercially available diagnostic tests that are accurate and reliable (Table 1) [42]. Laboratory methods for the diagnosis of *C. pneumoniae* infection include culture, antigen detection, serology and PCR.

Different cell lines have been evaluated for culturing *C. pneumoniae*, and HL and Hep-2 have been found to yield the best results. So far, successful culture of *C. pneumoniae* has been obtained in a limited number of laboratories. The main problems encountered are easy inactivation during transport, and low yield, often requiring repeated blind passages.

The sensitivity of antigen detection using direct fluorescent antibodies on respiratory specimen smears is estimated to be 20–60% as compared to that of culture; the specificity should approach 95%, but this is highly operator-dependent. This technique is mostly employed for culture confirmation.

The development of PCR technology has brought major advantages to the diagnosis of *C. pneumoniae* infection. It has been successfully employed with respiratory specimens, lung and vascular biopsy specimens, and blood. Several studies have found PCR to be a more sensitive technique than culture [42]. Nested PCR assays involve significant problems with contamination, which may result in the overestimation of disease attributed to *C. pneumoniae*. Real-time assays are reported to have distinct advantages over conventional assays [43]. A multiplex PCR has been developed that allows simultaneous identification of *C. pneumoniae*, *Legionella pneumophila* and *M. pneumoniae* in respiratory specimens [44]. The overall diagnostic utility of PCR techniques is currently hampered by the lack of standardization of extraction procedures, primer definition, etc., and by the limited number of commercially available tests. Some are now marketed in Europe, but none has been approved by the US Food and Drug Administration.

Serology testing for *C. pneumoniae* currently includes microimmunofluorescence (MIF) assays, ELISAs and enzyme immunoassays, each of which exists in a variety of in-house and commercial versions. MIF is considered by the CDC to be the only currently acceptable serological test, and is considered to be the reference standard for serodiagnosis, despite significant limitations [45].

On the basis of available techniques, the most convincing evidence of acute infection is obtained when IgM antibodies or a four-fold rise in IgG antibodies can be shown. However, the need for paired sera to show a four-fold rise in antibody titres is a limitation of the MIF technique.

In primary infections, IgM antibodies appear 2–3 weeks after infection and IgG antibodies appear 6–8 weeks after infection, whereas in cases of re-infection, IgM antibodies may be absent, or of low titre if present, and IgG antibodies appear earlier, within 1–2 weeks after infection.

Recently, a new method for rapid diagnosis of *C. pneumoniae* pneumonia has been published [46]. It is an immunochromatographic test for the detection of *C. pneumoniae*-specific IgM antibodies. The results obtained with serum samples from 140 patients (41 with *C. pneumoniae* pneumonia) using this test were compared with those obtained using two other serological tests (MIF and enzyme immunoassay). The reported sensitivity and specificity of the test were 100% and 92.9%, respectively. However, as it detects only the IgM response, the usefulness of this test may be limited to primary infection.

Treatment

Considering the distinctive characteristics of *Chlamydia* infections, the best therapy should combine high intrinsic drug activity with high intracellular concentrations. Data

TABLE 1. Summary of clinical relevance of diagnostic techniques for the identification of *Chlamydophila pneumoniae*

Technique	Comments	References
Culture	Difficult and time-consuming	[42]
Antigen detection	Suboptimal sensitivity (20–60%), good specificity (95%)	[45]
	Little clinical value	
Serology	MIF is still the reference standard for acute infection diagnosis	[42]
	Paired serum samples are required	[45]
PCR	Variable sensitivity and specificity	[44]
	Some commercial kits available	[43]
	Nested PCR may be prone to contamination	
	Real-time PCR seems to be the most promising technique	
	Multiplex PCR allows testing for multiple pathogens with a single test	
	May become reference diagnostic test in the near future but is still under evaluation	

MIF, microimmunofluorescence.

concerning the *in vitro* activity of antibiotics against *C. pneumoniae* are relatively limited, owing to the difficulty in isolating the agent and in performing susceptibility tests (Table 2).

Tetracyclines and erythromycin show good *in vitro* activity and, so far, have been the most commonly employed drugs in the treatment of *C. pneumoniae* infection. The most active macrolide seems to be clarithromycin [47]. Ketolides have shown good activity against a broad range of respiratory pathogens, including *Mycoplasma* and *Chlamydia* [48].

Fluoroquinolones show good activity. Levofloxacin is more active than ofloxacin and ciprofloxacin. The MIC of moxifloxacin for three strains of *C. pneumoniae* was 0.6 mg/L, and the minimal chlamidiacidal concentration values ranged from 0.06 to 0.125 mg/L [49].

The β-lactam target is the bacterial cell wall, a structure absent in chlamydiae. However, penicillin and ampicillin, although showing no effect on *Chlamydia* viability, can inhibit infectivity [50]. Rifampicin seems to have high activity against *C. pneumoniae*, showing MIC and minimal chlamidiacidal concentration values ranging from 0.005 to 0.01 mg/L.

A newly described antibiotic, rifalazil (a benzoxazinorifamycin), inhibits bacterial DNA-dependent RNA polymerase [51]. MIC values of benzoxazinorifamycins for chlamydiae are in the microgram per litre range.

Eradication of *C. pneumoniae* during first infection is difficult, even with prolonged (up to 3–4 weeks) use of macrolides. The length of treatment may be associated with the long life cycle of *Chlamydia*, and with the possibility of a quiescent phase in the replication of the bacterium. However, data from clinical trials with new macrolides and fluoroquinolones show that clinical cure can be obtained with a shorter course of therapy. Much more complex is the eradication of chronic infection. Prolonged macrolide treatment of 6 weeks or more has been suggested, but definitive proof of its efficacy is lacking. In order to overcome the problem of the quiescent phase, multiple-injection therapy or multiple-drug regimens, including macrolides or ketolides combined with fluoroquinolones, have been employed.

TABLE 2. *In vitro* susceptibility of *Chlamydophila pneumoniae* (data taken from [47–49,51])

Drug	MIC (mg/L)	MCC (mg/L)
Tetracycline	0.05–1	0.05–4
Doxycycline	0.01–0.5	0.12–2
Minocycline	0.016–0.06	0.06–0.125
Erythromycin	0.01–0.5	0.01–0.25
Clarithromycin	0.004–0.25	0.004–0.25
14-OH-clarithromycin	0.03	0.25
Azithromycin	0.01–2	0.06–0.25
Roxythromicin	0.05–2	0.06–2
Telithromycin	0.01–2	0.03–2
Ketolide RU 64004	0.01–0.5	0.01–1
Ciprofloxacin	0.25–4	2–8
Ofloxacin	0.25–2	0.5–2
Levofloxacin	0.125–0.5	0.125–0.5
Lomefloxacin	0.25	–
Fleroxacin	2–8	2–8
Moxifloxacin	0.06	0.125
Gemifloxacin	0.06–0.25	0.06–0.25
Rifalazil	0.00025	0.004

MCC, minimal chlamydiacidal concentration: ranges of values reported in literature (values are given as range for clinical isolates or for type strains of *C. pneumoniae*).

Conclusions

C. pneumoniae is an obligate, intracellular bacterium associated with a wide variety of acute and chronic diseases. *C. pneumoniae* infection is characterized by persistence and immunopathological damage to host target tissues, including the lung. A substantial proportion of lower respiratory tract infections, including pneumonia and exacerbations of chronic bronchitis, have been associated with this pathogen. *C. pneumoniae* is also involved in both acute and chronic asthma, and some data link this agent to new-onset asthma in adults. Over the past 20 years, a variety of studies have investigated a possible link between *C. pneumoniae* infection and atherosclerosis, because of its role in all stages of atherosclerosis, from initial inflammatory lesions to plaque rupture.

This pathogen has been associated with CNS diseases such as MS and AD. Astrocytes, microglia and neurons all serve as host cells for *C. pneumoniae* in the brain of AD patients, and infected cells are found in close proximity to both neuritic senile plaques and neurofibrillary tangles.

The interpretation of all these findings is hampered by the fact that different studies have used not only different diagnostic tools or combinations thereof, but also different diagnostic criteria for making a diagnosis of acute or chronic infection. Most importantly, none of the available methods has been standardized, which has resulted in a wide variation of interlaboratory test performance, even when the same test and the same criteria have been used [42]. The results of these studies prompted several treatment trials, ranging from asthma to atherosclerosis and CNS diseases, none of which gave definitive results. There is a clear need for further well-designed studies to determine both the importance of *C. pneumoniae* involvement in human diseases and the usefulness of antibiotic treatment.

Transparency Declaration

The authors declare no competing interests.

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