Chlamydia pneumoniae: defining the clinical spectrum of infection requires precise laboratory diagnosis

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Introductory article

Diagnostic utility of PCR-enzyme immunoassay, culture, and serology for detection of C pneumoniae in symptomatic and asymptomatic patients

CA Gaydos, PM Roblin, MR Hammerslag, CL Hyman, JJ Eiden, J Schachter, TC Quinn

To assess the utility of PCR-enzyme immunoassay (EIA) for diagnosis of acute infection with C pneumoniae, we compared tissue culture, PCR-EIA, direct fluorescent-antibody (DFA) stain, and serology in studies with 56 patients with respiratory symptoms and 80 asymptomatic persons. Thirty five patients were positive by either culture or PCR-EIA, and 101 were negative by both assays. Thirty specimens from symptomatic patients and one from an asymptomatic patient were culture positive; 23 of these were also PCR-EIA positive. Of the eight culture-positive, PCR-EIA-negative specimens, five were DFA negative and three were DFA positive. Four additional specimens were culture negative and PCR-EIA positive; of these, three were DFA positive and one was DFA negative. When we used culture-and/or DFA-positive results as a reference or "gold standard", the sensitivity and specificity of PCR were 76-5 and 99-0% respectively. When we used PCR- and/or DFA-positive results as the reference, the sensitivity of culture was 87-5%. On the basis of single acute serum specimens, only 8 of these 35 patients had diagnostic antibody titres. Of the asymptomatic patients, 75% had immunoglobulin G or immunoglobulin M antibody to C pneumoniae; 15 (18-8%) of these had antibody levels considered to be diagnostic of acute infection. This multicentre study indicates that culture and/or PCR-EIA is more reliable for prompt diagnosis of C pneumoniae infection than single-point serology alone. (J Clin Microbiol 1994;32:903–5)

Chlamydia pneumoniae is an obligate intracellular Gram negative bacterium which was first identified as a respiratory pathogen in 1986, when Grayston et al isolated the organism from throat swabs of students with acute respiratory symptoms. Infection with C pneumoniae seems to be extraordinarily common, with seroepidemiological studies showing that more than 50% of adults worldwide have antibodies to this organism. It has been implicated as a cause of about 10% of cases of community acquired pneumonia and has been associated with the full range of respiratory tract infections including pharyngitis, otitis media, bronchitis, and exacerbations of asthma and chronic obstructive pulmonary disease (COPD). Infection with C pneumoniae has also been associated with extrapulmonary diseases such as reactive arthritis, Guillain-Barré syndrome, meningoccephalitis, and atheromatous disease of the coronary and carotid arteries. However, as with many other respiratory pathogens, asymptomatic infection and a chronic carriage state have been described for C pneumoniae so that the clinical relevance of infection in some circumstances has been questioned. The intense research stimulated by the identification of C pneumoniae has greatly advanced our knowledge of the importance of this pathogen, but efforts to define the true clinical spectrum of infection have been hampered by difficulties in laboratory diagnosis. The introductory article by Gaydos et al is therefore timely.
in outlining the range of diagnostic techniques which are currently being used and in describing the application of newer techniques to both symptomatic and asymptomatic subjects.  

### Laboratory diagnosis of C. pneumoniae infection

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<th>Laboratory technique</th>
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<td>Complement fixation test (genus antigen)</td>
<td>Used to detect antibodies to C. pneumoniae genus.</td>
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<td>Microimmunofluorescence test (species specific)</td>
<td>Used to identify species-specific C. pneumoniae antigens.</td>
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<td>Isolation in cell culture</td>
<td>Cells infected with C. pneumoniae can be isolated in culture.</td>
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<td>HeLa 229 cell culture</td>
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<td>Polymerase chain reaction</td>
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Recent developments in the diagnosis of C. pneumoniae infection have used PCR to detect minute amounts of C. pneumoniae DNA. Gaydos et al describe an adaptation of the PCR technique in which it is combined with enzyme immunoassay (PCR-EIA). They report the identification of C. pneumoniae in nasopharyngeal specimens of 56 patients with respiratory symptoms by PCR-EIA and compare their results with those obtained by cell culture, DFA, and serology. They also report the findings from a control group of 80 asymptomatic subjects. They confirm the results of other studies of a high prevalence of antibodies to C. pneumoniae in the general population with 75% of the asymptomatic subjects having IgG or IgM antibodies to the organism. Fifteen (18.8%) of the asymptomatic subjects had antibody levels considered to be diagnostic of acute infection by current criteria - that is, IgG titre ≥ 1:512 or IgM ≥ 1:16 - yet only one of these subjects had the organism identified on nasopharyngeal swabs. This casts doubt upon the diagnostic criteria applied to a single point acute serology sample, although asymptomatic (that is, subclinical) infection is a possible explanation. Thirty five subjects gave positive results either by culture or PCR-EIA, but only eight of these had antibody titres considered diagnostic of acute infection. Perhaps not surprisingly this study suggests that single point serological tests lack sensitivity and specificity for diagnosing infection. Unfortunately this study did not include additional convalescent serological testing and it is likely that this would have improved diagnostic accuracy. Thirty of the 56 patients with respiratory symptoms had C. pneumoniae detectable on nasopharyngeal swabs by PCR-EIA, DFA, or tissue culture in Hep 2 cells. Only one of 80 asymptomatic subjects had C. pneumoniae detected from nasopharyngeal swabs, suggesting that asymptomatic carriage of the organism was rare in this population. Using culture and/or DFA as the "gold
standard" for identifying *C pneumoniae* infection, PCR-EIA had a sensitivity of 76-5% and a specificity of 99-0%.

This study illustrates the range of techniques which are being applied in the diagnosis of *C pneumoniae* infection. No conclusions can be drawn about the incidence of infection, but it is clear that a single serological test is difficult to interpret, and molecular methods such as PCR-EIA are as specific as culture in demonstrating the presence of the organism and almost as sensitive. Diagnostic differences between the various techniques for detecting *C pneumoniae* are not yet fully resolved.

**C pneumoniae as a respiratory pathogen**
The strongest evidence establishing *C pneumoniae* as a respiratory pathogen comes from studies in which the organism was isolated from patients with acute respiratory symptoms in association with the development of an antibody response. These criteria were met in the original report by Grayston et al in 1986, in which the organism was isolated from eight of 13 students with acute respiratory disease who also had serological evidence of *C pneumoniae* infection. Further reports have confirmed the pathogenicity of this organism. Augenbraun et al, for example, reported a case of pneumonia with pleural effusion in a 19 year old man in which *C pneumoniae* was isolated from the nasopharynx and pleural fluid in association with an antibody response. Similarly, in a study of 54 patients with either pneumonia or acute bronchitis 12 were found to have serological evidence of *C pneumoniae* infection; the organism was isolated from seven of the 12 and identified in a further two patients by PCR in the absence of any other identifiable pathogen. In other circumstances the clinical relevance of *C pneumoniae* infection has been less clear. In an investigation of an outbreak of an acute upper respiratory illness among military conscripts in Norway, *C pneumoniae* was isolated from throat swabs of five of 30 patients but this was not accompanied by serological evidence of acute infection, and the outbreak was largely attributable to an adenovirus which was isolated from throat swabs from 18 of the patients in association with antibodies to adenovirus. It is likely that in this situation the isolation of *C pneumoniae* simply indicated a chronic carriage state which was not relevant to the pathogenesis of the acute illness, although a role as a co-pathogen cannot be entirely excluded. Of 91 patients with lower respiratory tract infections studied by Chirgwin et al 17 (18-7%) had evidence of *C pneumoniae* infection, but the association of the organism with the disease was unclear in some cases. Only three of eight patients who were culture positive had serological evidence of acute infection and two patients remained culture positive over a 12 month follow up period. In two patients there was co-infection with *Haemophilus influenzae* and *Streptococcus pneumoniae*, which were thought to be the more likely causes of the illness. Gnape et al isolated the organism from throat swabs of 11 of 234 healthy subjects, suggesting an endemic prevalence rate of 4-7%. Hyman et al described how two laboratory workers were apparently infected with *C pneumoniae* during an accident due to centrifuge malfunction. The organism was cultured from throat swabs taken from both subjects five days after exposure and was still present in one worker 20 weeks later. Neither subject developed symptoms or an acute antibody response, suggesting that subclinical infection and a chronic carriage state had occurred. Thus, although *C pneumoniae* is clearly an important respiratory pathogen, identification of the organism is not in itself sufficient to implicate it in a disease process and its clinical role in some circumstances is uncertain.

**Clinical features of respiratory infection**
The illness described by Grayston et al in the original report of *C pneumoniae* infection was a mild pneumonia preceded by pharyngitis. Although no patient required admission to hospital, many suffered a prolonged relapsing illness even in this group of healthy young adults. *C pneumoniae* may, however, cause severe pneumonia and respiratory failure. Marrie et al found that 18 (6%) of 301 adult patients admitted to hospital with community acquired pneumonia had serological evidence of *C pneumoniae* infection. Six of these patients, who had pre-existing severe chronic disease, had a severe illness and two died. *C pneumoniae* has also been reported as a cause of hospital acquired pneumonia. However, the exact role of *C pneumoniae* in these hospital-based studies is uncertain since diagnosis was based on serological tests alone, without attempts at isolating the organism, and other potential aetiological agents were identified. It is possible that *C pneumoniae* acted as a co-pathogen or that infection represented reactivation of quiescent infection in the lungs of very sick patients. Augenbraun et al cultured *C pneumoniae* from bronchoalveolar lavage fluid of five (10%) of 50 patients with human immunodeficiency virus infection, four of whom had other pulmonary pathogens. This suggested that *C pneumoniae* was present in the lungs of these patients, though it was not necessarily causing their acute respiratory illness. However, Clark et al reported a case of severe pneumonia in an HIV infected man in which direct fluorescent antibody test for chlamydia on bronchial washings was positive and a fourfold rise in IgG MIF antibody to *C pneumoniae* was demonstrated. Detailed testing of bronchial washings failed to identify any other organism. In a study of 593 patients with serological evidence of *C pneumoniae* infection, many of whom were treated in general practice, 50% had a diagnosis of pneumonia, 28% bronchitis, 10% "flu-like" illness, 4% "upper respiratory infection", 4% pharyngitis, 2% sinusitis, and 1% otitis media. *C pneumoniae* has therefore been implicated in the pathogenesis of infections of variable severity in a variety of different circumstances at all levels within the respiratory tract (box 2).

*C pneumoniae* has also been associated with exacerbations of airways disease. Allegra et al found that seven of 74 patients with exacerbations of asthma had serological evidence of acute *C pneumoniae* infection and in two of these seven patients the organism was identified on pharyngeal swabs. Hammerschlag et al described a patient without any preceding history of asthma who developed wheeze with airways obstruction during *C pneumoniae* infection which required a prolonged course of prednisolone. In this patient *C pneumoniae* was persistently isolated from the nasopharynx for an 11 month follow up period despite treatment with...
Transmission of C. pneumoniae

Outbreaks of C. pneumoniae have occurred in universities, military schools, and within families. Direct epidemiological studies show that the prevalence of antibodies to C. pneumoniae increases in children under 50 years of age. There is no evidence of an outbreak in this confined population. However, the rate of infection among military trainees was relatively low, so that it took about eight months for the epidemic to occur. Some studies suggest that C. pneumoniae can be transmitted from person to person, but there is no evidence of person-to-person transmission in a confined population. In the apparent case of person-to-person transmission, the organism was isolated from the tonsils of both patients.

Extrapolaryngeal infection

A number of extrapulmonary features have been attributed to C. pneumoniae infection. Acute arthritis with reactive arthritis of the C. pneumoniae was reported in a case of Guillain-Barré syndrome. In another case, a patient with C. pneumoniae infection developed a skin eruption that was classified as a dermatitis. In a third case, a patient with C. pneumoniae infection developed a necrotizing meningitis that was treated with steroids. In a fourth case, a patient with C. pneumoniae infection developed a meningitis that was treated with antibiotics.

Immunofluorescent antibody (IFA) titers were determined for C. pneumoniae in the sera of patients with reactive arthritis and in the sera of patients with reactive arthritis-like disease. The results were positive in 68% of patients with reactive arthritis and in 68% of patients with reactive arthritis-like disease. The titers were positive in 68% of patients with reactive arthritis and in 68% of patients with reactive arthritis-like disease. The titers were positive in 68% of patients with reactive arthritis and in 68% of patients with reactive arthritis-like disease. The titers were positive in 68% of patients with reactive arthritis and in 68% of patients with reactive arthritis-like disease.
an acute myocardial infarction and 50% of patients with chronic angina undergoing angiography had IgG antibodies at a titre of $\geq 1:128$ or IgA antibodies at a titre of $\geq 1:32$ to C pneumoniae compared with 17% of control subjects. Several other researchers studying different patient groups using various different methodologies have tended to confirm and extend these initial observations. However, there are inherent difficulties in relying on seroepidemiological studies to assess this association because of the high prevalence of antibodies to C pneumoniae in the general population, and it is particularly difficult to establish the independence of C pneumoniae as a risk factor and to exclude potential confounding factors. Thus, Thom et al. found that the association between C pneumoniae infection and coronary artery disease was limited to smokers, with an odds ratio of 3.5 in smokers and 0.8 in subjects who had never smoked. Furthermore, the association was weaker and not statistically significant when cases were compared with subjects with proven normal coronary angiograms rather than with general population controls. An association between C pneumoniae and atheromatous disease need not necessarily be causal, of course. It might, for example, be that smokers, who are known to be at greater risk of coronary artery disease, are also more likely to develop C pneumoniae infection. The general consistency of results from seroepidemiological studies gives impetus to further research of this association but is not yet convincing in establishing a causal relationship.

Evidence of an association between C pneumoniae and atheromatous disease has, however, been considerably strengthened by the identification of the organism in atheromatous plaques but not in normal arteries using immunocytochemistry and PCR techniques. In a study of 36 necropsy samples Kuo et al. identified C pneumoniae in atheromatous plaques in 15 of 36 cases studied by immunocytochemistry tests and in 13 of 30 cases studied by PCR techniques. Paradoxically, the six patients with the highest antibody titres to C pneumoniae in their serum did not have the organism detected in atheromatous plaques. This suggests that the serological response may not be the best way of examining this association since a high antibody titre will usually be found after recent infection but will decay with time. Associations between coronary artery disease and other infections, including Helicobacter pylori and viruses of the herpes group, have also been suggested. There are several mechanisms by which infection could potentially contribute to atherogenesis. These include a direct effect of infection on endothelial cells, alteration in serum lipid metabolism, elevation of serum fibrinogen levels, or the formation of circulating toxins or immune complexes which could elicit an inflammatory response when deposited in vessel walls.

### Treatment

As with C. psittaci and other "atypical" organisms, tetracycline and macrolide antibiotics form the basis of treatment of C pneumoniae infection. In vitro studies of the susceptibility of the organism to antibiotics show that clarithromycin has the lowest minimum inhibitory concentration overall, and the macrolides and tetracycline are more active than the quinolones. Gravston et al found that the clinical response to antibiotics was often slow with persistence of symptoms and frequent clinical relapse requiring further antibiotics. Furthermore, eradication of the organism from the airways may be difficult to achieve. Hammerschlag et al showed that three of nine patients with C pneumoniae infection continued to have positive cultures for the organism over an 11 month period despite treatment with tetracycline. Such persistence of the organism might act as a reservoir for spread of infection and could potentially play a part in the pathogenesis of some of the chronic disease associated with C pneumoniae. Specific antibiotic treatment may suppress the antibody response and this may be an important factor when using serological tests to study the exact role of this organism in clinical disease.

### Conclusions

We have learnt much about C pneumoniae over the past decade. Its importance as a respiratory pathogen has been definitively established by studies which have isolated the organism from the respiratory tract in the presence of acute respiratory disease and an associated serological response. As with other respiratory pathogens it is now clear that asymptomatic infection and a chronic carriage state may occur. This has made it more difficult to be certain of the exact clinical role of C pneumoniae in certain circumstances, since detection of the organism is not in itself sufficient to implicate it in a disease process. Initial research studies relied heavily on serological tests for detecting C pneumoniae infection,
but there are inherent difficulties in this approach because of the complex nature of the antibody response to infection and reinfection, and because of the high prevalence of antibodies in the general population. The development of newer techniques, such as those reported by Gaydos et al, are likely to provide greater diagnostic accuracy.26 Because of the difficulties in laboratory diagnosis of C pneumoniae a routine diagnostic service for this pathogen is not currently available in the UK. What is needed now is a prospective research study of respiratory tract infection, both in community and hospital practice, using a range of newer techniques for detecting C pneumoniae, whilst also testing for the full spectrum of other respiratory pathogens. Meanwhile doctors will need to keep C pneumoniae in mind when choosing empirical antibiotic treatment for respiratory tract infections.