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

S J Bourke, N F Lightfoot*
Chest Unit, Newcastle General Hospital, University of Newcastle upon Tyne;
*Public Health Laboratory Service, Newcastle upon Tyne, UK

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)
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.28

Laboratory diagnosis
Traditionally, diagnosis of infection is made by the identification of the micro-organism by culture techniques applied to specimens taken from symptomatic patients, or by the detection of an antibody response to the organism. The evidence implicating a suspected pathogen in a disease process is strengthened if no alternative pathogen is isolated, if culture tests become negative as the patient recovers, and if the organism is not isolated from asymptomatic subjects. Newer techniques, such as the direct demonstration of the organism by immunofluorescence or the detection of specific DNA sequences of the organism, are being applied in an effort to improve sensitivity and speed of laboratory diagnosis for several infections. The serological diagnosis of infection is based on detecting the immune response to an infecting agent; typically, in a first infection with a particular organism IgM antibodies are produced initially followed a few days later by IgG antibodies. The IgM antibody level declines as recovery occurs, but the IgG level declines more slowly and IgG antibodies are usually detectable for many years so that seroepidemiological studies can estimate the prevalence of previous infection in a population.

Diagnosis of *C. pneumoniae* infection has proved particularly difficult and four main diagnostic techniques have been applied (box 1): serological antibody tests,14,27 cell culture,14,26 antigen detection,26,28 and identification of specific DNA sequences by the polymerase chain reaction (PCR).26,27 Techniques used for detecting the closely related species, *C. psittaci* and *C. trachomatis*, have been less effective when applied to *C. pneumoniae*, and problems have arisen in differentiating between the three chlamydial species. Diagnosis of *C. psittaci* infection has relied upon the demonstration of an antibody response by the complement fixation (CF) test. This test, however, uses the chlamydial genus lipopolysaccharide antigen and so cannot differentiate between chlamydial species, and it is now clear that many patients diagnosed as having psittacosis on the basis of a positive CF test were, in fact, infected with *C. pneumoniae*.30 Furthermore, the CF test is positive in less than 33% of patients who have other evidence of *C. pneumoniae* infection.2 Type-specific microimmuno- fluorescent (MIF) antibody tests are sensitive in detecting *C. pneumoniae* infection and can differentiate between species but are technically difficult and available only in specialist laboratories.27 Importantly, the MIF test allows differentiation of IgG and IgM antibodies. Positive IgM antibody tests probably indicate current infection, but IgG titres may relate to previous rather than current infection. Debate continues over the serological criteria for diagnosing acute infection, but a fourfold or greater rise in IgM or IgG antibody between acute and convalescent serum samples is usually considered diagnostic and, in some studies, a single IgM titre of $\geq 1:16$ or IgG titre of $\geq 1:512$ has been accepted as evidence of acute infection. The high prevalence of antibodies to *C. pneumoniae* in the general population and cross reactions between chlamydial species may give confusing results,32 and specific antibiotic treatment may suppress the antibody response.32

*C. pneumoniae* is difficult to isolate by culture although several of the well established cell lines (e.g. HeLa 229 or Hep 2) can be used. Cell culture is a specialist technique which is not available in all laboratories but is still considered by many to be the "gold standard". Various techniques have been used for diagnosis by antigen detection including direct fluorescent antibody staining (DFA) and enzyme immunoassay (EIA).26,28 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).26 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.32 Fifteen (19.8%) of the asymptomatic subjects had antibody levels considered to be diagnostic of acute infection by current criteria— that is, IgG titre $\geq 1:512$ or IgM $\geq 1:16$ — 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

---

**Laboratory diagnosis of *C. pneumoniae* infection**

<table>
<thead>
<tr>
<th>Serological antibody tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement fixation test (genus antigen)30</td>
</tr>
<tr>
<td>Microimmunofluorescence test (species specific)27</td>
</tr>
<tr>
<td>Isolation in cell culture14,26</td>
</tr>
<tr>
<td>HeLa 229 cell cultures</td>
</tr>
<tr>
<td>Hep 2 cell cultures</td>
</tr>
<tr>
<td>Antigen detection</td>
</tr>
<tr>
<td>Direct immunofluorescence test26</td>
</tr>
<tr>
<td>Enzyme immunoassay28</td>
</tr>
<tr>
<td>Polymerase chain reaction26,27</td>
</tr>
</tbody>
</table>
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 has been 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 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 Chirgin *et al.* (17.8%) 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 serious 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 re-activation 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...
C pneumoniae respiratory infection
Community acquired pneumonia
Hospital acquired pneumonia
Pneumonia in the immunocompromised
Pharyngitis
Sinusitis
Otitis
Bronchitis
Flu-like illness
Exacerbation of asthma
Exacerbation of chronic obstructive pulmonary disease
Asymptomatic infection

Box 2

erythromycin, although the patient’s wheeze gradually improved over several months. Emire et al isolated C pneumoniae from 13 (11%) of 118 children with acute exacerbations of asthma, but only three had serological evidence of acute infection. Treatment with macrolide antibiotics resulted in an improvement in the clinical course of their reactive airways disease. Cases of newly diagnosed asthma following C pneumoniae infection have been reported, leading some authors to suggest that this organism may have a role in the natural history of reactive airways disease. Whether this role is restricted to exacerbating pre-existing asthma, or whether the organism plays a more fundamental aetiological part by inducing bronchial hyperreactivity, is unclear.

C pneumoniae would appear to be only a rare cause of exacerbations of COPD. Of 44 patients with acute exacerbations of COPD studied by Beaty et al, only two (5%) had serological evidence suggesting acute infection with C pneumoniae, but in neither case was the organism isolated from the nasopharynx. In this study the organism was not isolated from the oropharynx of 97 subjects in a stable phase of COPD. Similarly, Blasi et al found that only five of 142 patients with exacerbations of COPD had serological evidence of recent infection with C pneumoniae and the organism was not isolated from any of these patients. Thus, C pneumoniae appears to be an uncommon pathogen in patients with COPD.

Transmission of C pneumoniae
Outbreaks of C pneumoniae have occurred in universities, schools, military institutions, and within families, suggesting direct person-to-person spread of infection. Seroepidemiological studies show that the prevalence of antibodies to C pneumoniae is low in children under the age of 10 years, increases in teenagers, and is highest in adults and the elderly. Yamazaki et al reported the apparent spread of infection between two children within a family where one child persistently had the organism isolated from her nasopharynx over a period of three months. Where epidemics of C pneumoniae infection have occurred in closely confined populations such as among military trainees, the attack rate was 60–80 per 1000 men and spread of infection seemed to be relatively slow so that it took about six months for the epidemic to run its course even in this closely confined population.

There is no evidence of an avian or other animal reservoir of infection and little is known of the survival of the organism in the environment. Falsey et al showed that the organism remained viable on a formica counter top for 30 hours and in tissue paper for 12 hours. Measurable quantities of C pneumoniae were transferred from these surfaces to hands, but survival time on hands was limited to 10–15 minutes. C pneumoniae survived small particle aerosolisation well and was infectious to mice by both direct inoculation and aerosolisation. Apparent acquisition of C pneumoniae infection occurred during a laboratory accident due to centrifuge malfunction, and transmission of infection during this accident is likely to have been by aerosol inhalation. Thus, several mechanisms of transmission may be important in the transfer of infection from person to person.

Extrapulmonary infection
A number of extrapulmonary features have been associated with C pneumoniae infection (box 3). Socan et al reported a case of pneumonia and lymphocytic meningonecephalitis in which both the cerebrospinal fluid and throat washings gave positive results on direct immunofluorescent testing with C pneumoniae specific monoclonal antibodies, and Michel et al reported a case of lumbosacral meningoradiculitis associated with C pneumoniae infection. Guillain-Barré syndrome has also occurred as a sequel to serologically diagnosed acute C pneumoniae infection. In a study of 70 patients with reactive arthritis five (7%) had serological evidence of C pneumoniae infection and these patients demonstrated C pneumoniae specific lymphocyte proliferation in synovial fluid. Wesslen et al reported a fatal case of myocarditis in which high IgM and IgG antibodies to C pneumoniae were found in the serum, and necropsy tissue samples from the heart and lungs gave positive results by PCR for C pneumoniae. Marrie et al reported a case of endocarditis which was thought probably to be due to C pneumoniae on the basis of serological tests, although the organism was not detected in tissue obtained at surgery from the cardiac valve. Several studies have shown an intriguing relationship between C pneumoniae infection and atheromatous disease of the coronary and carotid arteries. Initially, evidence of this association was based upon seroepidemiological studies in which patients with coronary artery disease were more likely to have antibodies or immune complexes to C pneumoniae than control subjects. More recently, however, the organism has been found in atheromatous plaques of coronary arteries at necropsy using immunocytochemistry or PCR techniques. Saikku et al showed that 68% of patients with

Extrapulmonary C pneumoniae infection
Reactive arthritis
Meningonecephalitis
Meningoradiculitis
Guillain-Barré syndrome
Myocarditis
Endocarditis
Coronary artery atheroma
Carotid artery atheroma

Box 3
an acute myocardial infarction and 50% of patients with chronic angina undergoing angiography had IgG antibodies at a titre of ≥1:128 or IgA antibodies at a titre of ≥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. Grayston 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 Gaydost 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.