LETTERS TO
THE EDITOR

Rapid identification of Chlamydia psittaci and TWAR (C. pneumoniae) in sputum samples using an amplified enzyme immunoassay

Recent publications on the importance of the TWAR agent and our own experience of Chlamydia psittaci infections have led us to conduct a prospective study of routine sputum samples from patients with respiratory infections using a commercial enzyme linked immunosorbent assay (EIA) for detecting chlamydia antigen. One hundred and six sputa were received from 100 patients with acute or persisting chest infections and with no known underlying or predisposing condition. Sera were requested whenever possible and were obtained from 30 patients. Three sputa from patients not fulfilling the trial criteria but yielding a pure heavy growth of Staphylococcus aureus were included to confirm the non-reactivity of this organism in this assay.

All sputa were cultured routinely to establish their bacteriological profile. Sputum specimens for Chlamydia detection were tested by the IDEIA Chlamydia test (Novo BioLabs Ltd.) and sera from patients, where available, were tested by routine complement fixation test and by a modification of a previously described enzyme immunoassay (EIA) for Chlamydia group-specific IgM. This modification required a 1 in 400 dilution of serum (100 µl) and a first incubation step of 90 minutes at 37°C. After washing, incubation with antigen took place at 37°C for one hour and conjugate was added for a further one hour at 37°C. Remaining aspects of the test were as previously described. Specimens positive by the EIA antigen test were also tested by direct immunofluorescence for direct visualisation of elementary bodies with characteristic morphology, using a species specific major outer membrane protein (MOMP) reactive monoclonal antibody (SYVA Microtrak) and a genus specific lipopolysaccharide (LPS) monoclonal (Imagen, Novo BioLabs Ltd).

Preparation for antigen EIA detection required mucolysis of sputum in 2% buffered n-acetyl cysteine before washing and centrifugation. The deposit was resuspended in the transport medium provided by the manufacturers. Samples positive by EIA were centrifuged as before, resuspended in PBS, and smears were examined by direct immunofluorescence for both genus and species specific fluorescence.

Results

No clinically important pathogens were detected in 79 of the 106 sputa examined. A variety of other organisms were detected including Haemophilus influenzae (n = 7), Streptococcus pneumoniae (n = 1), S aureus (n = 6), Pseudomonas sp (n = 4), Aspergillus sp (n = 1) and yeasts (n = 1).

Seven sputa from five patients were positive by IDEIA Chlamydia test and also by Imagen Chlamydia direct immunofluorescence but negative by SYVA Microtrak, indicating that the organism was C psittaci or C pneumoniae. In all cases the chlamydia IgM test was positive and in four cases the complement fixation titre was raised, indicating recent chlamydial infection.

One specimen was positive by all three chlamydia tests, indicating C trachomatis infection. Although the complement fixation titre was less than 16, the specific IgM was positive and species antibody was raised.

No other sputum samples were negative by IDEIA Chlamydia test including the three specimens with heavy S aureus growth. All sera from patients other than those mentioned above had undetectable specific IgM and complement fixation titres of < 32.

The laboratory findings of the seven patients with evidence of chlamydial infection are shown in the table.

Laboratory findings of seven respiratory chlamydial infections

<table>
<thead>
<tr>
<th>Case No</th>
<th>Clinical details</th>
<th>Date of sputum sample</th>
<th>Direct immunofluorescence (LPS)</th>
<th>Direct immunofluorescence (MOMP)</th>
<th>Date of serum</th>
<th>Serology</th>
<th>Titre</th>
<th>IgM</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Cough for weeks, du 14.3.89 +0.958 +</td>
<td>12/4 128 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>Chronic cough, 25.5.89 +2 +</td>
<td>1/6 &lt;128 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>Chest infection, 12.5.89 +0.178 +</td>
<td>8/5 &gt;128 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Haemoptysis 20.6.89 +2 +</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cough, no bird 5,7.89 +1.309 +</td>
<td>+</td>
<td>11/7 16 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Chest infection, 31.7.89 +0.279 +</td>
<td>2/8 16 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Chest infection, parrot died 38.7.89 +</td>
<td>7/8 &gt;128 +</td>
<td></td>
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Antigen detection

Diagnostic evaluation of chlamydia using DNA probes

During the past 15 years it has become increasingly evident that patients being immunosuppressed for malignant disease or organ transplantation may develop generalized toxoplasmosis which affects the central nervous system and may result in death. Recent findings have shown that patients with AIDS constitute a further group prone to severe disseminated toxoplasmosis. In such patients toxoplasmosis constitutes a life-threatening opportunistic infection. The current methods for the diagnosis of toxoplasmosis have given dismayingly poor results in such patients and, therefore, there is a need for more sensitive procedures for the early diagnosis of Toxoplasma gondii in body fluids or biopsy tissues.

A good alternative to the serological tests may be the detection of the pathogen's nucleic acids in clinical specimens. Over the past few years the development of recombinant DNA procedures has led to the development of a number of DNA probes for potential use in the diagnosis of pathogens such as Neisseria gonorrhoeae, Plasmodium falciparum, cytomegalovirus and enterotoxigenic Escherichia coli. Recent advances in recombinant DNA technology, and in particular the development of the polymerase chain reaction (PCR), allow certain pathogens to be detected which would not be present in very small numbers in a given sample.

We wanted to develop a DNA based test for the diagnosis of T gondii. We used the PCR (30 cycles) and two oligonucleotide probes (20-mers) based on the published sequence for the toxoplasma P30 gene to amplify toxoplasma DNA. These reactions were performed with purified toxoplasma DNA (0.1 mg) in the presence or absence of human DNA (this corresponds to about 1.5 x 10⁶ leucocytes). On electrophoresis of the reactions on 0.8% (w/v) agarose gels and subsequent staining of the gel with ethidium bromide, amplified product (915 base pairs long) could be seen in those reactions contain-
some clinical samples, including a brain biopsy specimen from a patient with AIDS (unpublished observations). The results were extremely promising and suggest that diagnosis of toxoplasmosis using the PCR and DNA probes may soon become a routine alternative to serological tests.

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Evaluation of 3-fucosyl N-acetylated lactosamine antibody staining in histological assessment of CIN

Expression of 3-fucosyl N-acetylated lactosamine (CD15) antigen by non-keratinising squamous epithelia gives an indication of cell maturity and might therefore prove valuable in improving objectivity of cervical intra-epithelial neoplasia (CIN) grading.1-3 MC2 is a monoclonal antibody raised against human granulocytes4 which recognises the CD15 antigen. In biopsy specimens of normal cervix the squamous epithelium shows a broad zone of staining in suprabasal cells; the depth of this MC2 stained zone progressively reduces with increasing severity of CIN grade.4

Thirty six cervical biopsy specimens were examined by 11 consultant histopathologists: for each case there was a section stained with haematoxylin and eosin and a serial section stained for CD15 by an indirect avidin-biotin immunoperoxidase method. The pathologists initially graded the lesions using the haematoxylin and eosin section; thereafter the sections were regraded, but also now taking into account the CD15 stained section in an attempt to improve the consistency of diagnosis. Details of the proforma used for recording the results and the statistical analysis by κ statistics have been described previously.5 The κ statistics are a measure of overall agreement which do not require any assumption concerning a "gold standard", correct diagnosis. The statistical method includes a correction for the amount of agreement which could be expected by chance alone.

Table 1 presents the κ statistics for agreement on the presence and degree of CIN. When the haematoxylin and eosin stained slides were studied alone overall agreement was fair (κ= 0-40), but this was largely due to good agreement on the CIN 3 and normal categories: the results for the CIN 1 and 2 categories showed poor agreement. Addition of the slide stained with CD15 resulted in slight reduction in the overall level of agreement (κ= 0-34). There was no improvement in the assessment of CIN grades 1 and 2 and there was a slight reduction in the level of agreement on those biopsy specimens which had been reported as normal. The table also gives the κ statistics for agreement on the presence of viral features in the specimens. The level of agreement is poor with the haematoxylin and eosin stained section alone (κ= 0-18) and shows no improvement when the CD15 stained slide is also used in the assessment (κ= 0-14).

In a previous study we showed good agreement among histopathologists in the diagnosis of CIN 3 but not for the lesser grades of CIN.1 In view of the problems associated with interpreting cervical biopsy specimens using only a standard haematoxylin and eosin stained section and the reported suggestion that use of a CD15 stained section might improve discrimination, we had expected that this study would result in improved consistency of reporting. Although there seemed to be an association between a decrease in CD15 staining and increasing severity of CIN, the consistency of grading, unfortunately, did not improve significantly. The reasons for this are uncertain but may reflect a subconscious unwillingness on the part of the pathologist to be swayed diagnostically by a CD15 stained section, preferring instead to rely more on the well accustomed use of the standard haematoxylin and eosin stained section. Alternatively, the localisation of CD15 may not have been sufficiently precise, or the antigen may have been dispersed too irregularly, to permit a critical distinction of the lower grades of CIN, the area of greatest diagnostic difficulty.

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Table 1 Kappa statistics

<table>
<thead>
<tr>
<th>Agreement on CIN categories:</th>
<th>Normal</th>
<th>CIN1</th>
<th>CIN2</th>
<th>CIN3 (+)</th>
<th>Overall</th>
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<td>Haematoxylin and eosin alone</td>
<td>K 0.48</td>
<td>0.08</td>
<td>0.20</td>
<td>0.64</td>
<td>0.40</td>
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<td>Comparison</td>
<td>K 0.33</td>
<td>0.11</td>
<td>0.13</td>
<td>0.66</td>
<td>0.34</td>
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</table>

<table>
<thead>
<tr>
<th>Agreement on viral features:</th>
<th>None</th>
<th>Outwith</th>
<th>Within</th>
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<tbody>
<tr>
<td>Haematoxylin and eosin alone</td>
<td>K 0.19</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>Haematoxylin and eosin and MC2</td>
<td>K 0.17</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Comparison</td>
<td>K 0.79</td>
<td>0.72</td>
<td>0.86</td>
</tr>
</tbody>
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Diagnosis of toxoplasmosis using DNA probes.

D Savva and R E Holliman

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