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EVALUATION OF TWO SEROLOGICAL TESTS FOR THE DIAGNOSIS OF CHLAMYDIAL RESPIRATORY DISEASE

EDWARD G. RUSSELL

Department of Microbiology, Monash Medical Centre, Clayton, Victoria, Australia

Summary

Serological tests for chlamydial infection are one of the most frequently used methods in the diagnosis of atypical respiratory infections. Use of serological tests has implicated chlamydial infections in asthma, arthritis and coronary heart disease, but the specificity of chlamydial serology tests has been questioned. The immunofluorescence test is the most sensitive and specific serological test available for detection of chlamydial antibodies. This study compares two commercially available immunofluorescent antibody tests. The SeroFIA test using purified elementary bodies of *Chlamydia pneumoniae*, *C. psittaci* and *C. trachomatis*, detected 24 cases of acute *C. pneumoniae* infection, whereas the Spot IF test using whole cell antigen of *C. psittaci* and *C. trachomatis*, misdiagnosed 20 of these as psittacosis and missed four cases.

Key words: Respiratory disease, serology, *Chlamydia psittaci*, *Chlamydia pneumoniae*, immunofluorescent antibody test.

Abbreviations: CF, complement fixation; EIA, enzyme immunoassay; IF, immunofluorescent.

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INTRODUCTION

While serological tests for chlamydial infection are often described as being of limited value, they are, of necessity, one of the most frequently used methods in diagnosis of atypical respiratory infections[1] and have been recently advocated as useful in upper genital tract disease.[2] Positive serological tests for chlamydia have been associated with clinical asthma,[3] arthritis[4] and coronary heart disease.[5] In Victoria, most chlamydial respiratory diseases have been diagnosed as sporadic cases of psittacosis[6] on the basis of clinical features supported by complement fixation (CF) serology, however the CF test is known to be only genus specific.[7] A local outbreak of psittacosis in 1994, also confirmed by serological tests, differed epidemiologically from these sporadic cases.[8,9] The 40 to 150 cases of psittacosis notified annually in Victoria have made up the bulk of national reports in the last decade, but the disease is not notifiable in NSW.[10] The most common chlamydia species associated with respiratory disease are *C. pneumoniae* and *C. psittaci*. Serological tests which do not have *C. pneumoniae* antigen may over-diagnose the incidence of psittacosis. This study compares the use of two commercially available immunofluorescent (IF) tests for chlamydial antibodies in assisting with the specific diagnosis of respiratory infection.

MATERIALS AND METHODS

Sera from 34 unrelated patients presenting with clinical pneumonia to the Monash Medical Centre between October 1994 and December 1995 were tested. The sera were from consecutive adult patients with a mean age of 52 years who fulfilled the criteria of having returned as outpatients for a second bleed and of not having antibodies to other commonly tested respiratory pathogens.

The kits evaluated were the Savyon Diagnostics SeroFIA IgG, IgM and IgA kits (Savyon Diagnostics, Beersheva, Israel) and the bioMerieux *Chlamydia psittaci*-Spot IF kit (bioMerieux Vitek, Marcy-l'Etoile, France) using the Fluoline-G and Fluoline-M reagents to test for IgG and IgM, respectively. The initial sera were tested for mycoplasma IgM by EIA and then frozen at −20°C. Subsequent second bleeds taken at a mean of 22 (range 16 to 43) d after the first one, were tested in parallel with the first for antibodies to mycoplasma by particle agglutination, influenza A and B by CF test, a pool of 8 legionella antigens (*Legionella pneumophila* subtypes 1 to 6, *L. longbeachae* and *L. micdadei*) by total IF assay and for anti-chlamydial IgG using the Spot IF test. Duplicate sera held at −70°C were later coded and tested in one run by one operator in the SeroFIA test and the Spot IF test was similarly repeated.

The SeroFIA test is a solid-phase IF assay performed on a 21-well glass slide. Purified elementary bodies of *C. pneumoniae, C. trachomatis* and *C. psittaci* are fixed to the slide wells. Diluted patient sera were placed over these antigens and incubated. After washing, fluorescein-conjugated anti-human antibody was overlaid and fluorescence detected by microscopy. The Spot IF kit is similar but has wells containing egg-grown, inactivated whole cells of *C. trachomatis* and *C. psittaci* only. Test performance and result interpretation was as directed in the kit instructions. Thus past infection was defined by the presence of IgG or IgA in the first serum without IgM or a rise in the convalescent serum and current infection was defined as the presence of IgM or a 4-fold rise in IgG or IgA titre or their first appearance in the second bleed.

Assessment of the agreement of results was based on these definitions. Where the interpretation of the results of both kits agreed completely, the results were classed as concordant. Where the results of one test indicated infection or exposure and the other did not, the results were classed as discordant. Discordant results indicating past infection were classed as non-significant as they did not lead to initiation of antibiotic treatment, whereas discordance with respect to recent or current infection may well have influenced antibiotic treatment and was thus classed as significant.

RESULTS

The results of the serological tests are presented in Table 1. Of the 34 patients, three had no evidence of previous chlamydial infection and seven had evidence of previous but not current infection. The SeroFIA test detected 24 cases of acute *C. pneumoniae* infection, 20 of which showed reactivity to *C. psittaci* with the Spot IF test.

Cases one to 20 had serological evidence of acute *C. pneumoniae* infection which was diagnosed as *C. psittaci* by the Spot IF. Cases 21 to 25 had evidence of previous *C. pneumoniae* infection, but were negative on the Spot IF and

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TABLE 1 Immunofluorescence assay results.

	SeroFIA			Spot IF		
	C.pneumoniae	*C. trachomatis*	*C. psittaci*	*C. psittaci*	*C. trachomatis*	
Cases 1–4	Four-fold increase	Not detected	Not detected	Four-fold increase	Not detected	Discordant
Cases 5–8	Seroconversion	Not detected	Not detected	Seroconversion	Not detected	Discordant
Cases 9–11	Seroconversion	Not detected	Not detected	Four-fold increase	Not detected	Discordant
Case 12	Four-fold increase	Not detected	Not detected	Seroconversion	Not detected	Discordant
Cases 13–20	Four-fold increase	Not detected	Not detected	Seroconversion	Not detected	Discordant
Cases 21–25	Detected	Not detected	Not detected	Not detected	Not detected	Discordance not significant
Cases 26–27	Not detected	Not detected	Not detected	Detected	Not detected	Discordance not significant
Cases 28–30	Not detected	Not detected	Not detected	Not detected	Not detected	Concordant
	IgG/IgM/IgA	IgG/IgM/IgA	IgG/IgM/IgA	IgG/IgM	IgG/IgM	
Case 31 ser 1	64/<32/<32	<32/<32/<32	<32/<32/<32	<32/<32	<32/<32	Significant discordance
ser 2	64/<32/256	<32/<32/<32	<32/<32/<32	<32/<32	<32/<32	
Case 32 ser 1	32/32/<32	<32/<32/<32	<32/<32/<32	32/<32	<32/<32	Significant discordance
ser 2	128/128/32	<32/<32<32	<32/<32/<32	32/<32	<32/<32	
Case 33 ser 1	32/<32/32	<32/<32/<32	<32/<32/32	32/<32	<32/<32	Significant discordance
ser 2	512/32/512	<32/<32/<32	<32/<32/32	64/<32	<32/<32	
Case 34 ser 1	<32/<32/<32	<32/<32/<32	<32/<32/<32	<32/<32	<32/<32	Significant discordance
ser 2	64/<32/128	<32/<32/<32	<32/<32/<32	<32/<32	<32/<32	

Four-fold increase, increase in IgG titre between serum 1 and 2 was four-fold or greater; Seroconversion, IgG titre of <32 in serum 1 and IgG titre of 64 or greater in serum 2; Detected, IgG titre in serum 1 and 2 was 32 or greater; Not detected, IgG titre in sera 1 and 2 (first and second bleeds) was <32; ser 1, first bleed; ser 2, second bleed.

Cases 26 to 27 had evidence of previous *C. pneumoniae* or *C. psittaci* infection, but were negative on the SeroFIA. Cases 28 to 30 had no evidence of previous chlamydial exposure. Cases 31 to 34 had considerable differences in their serology pattern and these are shown in more detail in the table. These four patients had acute *C. pneumoniae* infection which was not diagnosed by the Spot IF. Antibodies to *C. trachomatis* were not detected in any patient sample by either test.

DISCUSSION

The incidence of chlamydial respiratory infection in these patients was high but this was expected as the clinical histories indicated prolonged infection, and the selection criteria eliminated those with other commonly tested respiratory pathogens. While the SeroFIA test indicates that the reactive sera contain antibodies to *C. pneumoniae*, the bioMerieux test showed cross-reacting antibodies despite the fact that it is supposedly specific for *C. psittaci*. A possible explanation for this is that the bioMerieux test, whose antigen is whole inactivated chlamydia cells, is reacting with an antibody or several antibodies made against *C. pneumoniae* and *C. psittaci* but not *C. trachomatis*, and thus giving false-positive reactions. Given that this test dates from at least 1986, before it was appreciated that *C. pneumoniae* may be a widespread respiratory pathogen,[12] it is unlikely that cross-reactions were looked for in the development of the test. Although there are undoubtedly cases of human psittacosis in the local community, it appears that many patients with atypical pneumonia are infected with *C. pneumoniae* rather than the agent of psittacosis.

The IF test, using purified elementary bodies, is the most sensitive and specific serological test available for chlamydial testing. Its availability has been restricted to research laboratories, but commercial products are becoming available.[11] With the availability of species-specific tests, other studies have re-examined supposed *C. psittaci* outbreaks which were diagnosed as such by a variety of serological tests, and found mainly antibodies to *C. pneumoniae*.[13–17] The disagreement in seven cases (21 to 27), where one test (Spot IF five times, SeroFIA two times) showed a static IgG titre, indicating past infection, and the other test indicated no evidence of previous exposure, was probably not clinically significant. However, it appears that the SeroFIA test diagnosed four additional cases (31 to 34) of acute *C. pneumoniae* respiratory disease and this was clinically significant. Tests such as the SeroFIA assay which contain specific *C. pneumoniae* antigen, are necessary for the accurate diagnosis of chlamydial respiratory tract infection.

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Address for correspondence: E.G.R. Department of Microbiology, Southern Health Care Network Pathology Service, Monash Medical Centre, 246 Clayton Road, Clayton, Vic 3168, Australia. E-mail: *e.russell@shcn.com.au*

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