Failure of Serology in Diagnosing Chlamydial Infections of the Female Genital Tract

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Chlamydia trachomatis was recovered from 20% (36/180) of women attending a venereal disease clinic. All infected women had chlamydial antibodies in their serum and cervical secretions. However, the background rates of chlamydial antibody in chlamydia-negative women were very high. Measurement of antibodies in serum (complement fixation or immunoglobulin G [IgG] and IgM by microimmunofluorescence) or cervical secretion (IgG, IgM, IgA or secretory IgA classes) did not result in predictive values of >92%. It is concluded that the detection of chlamydial antibodies in serum or cervical secretions cannot be substituted for agent isolation in diagnosing these infections.

Chlamydia trachomatis is currently recognized as one of the most common sexually transmitted pathogens, with an expanding clinical spectrum which rivals that of gonococcal infections (10). Because of the high prevalence of chlamydial infections there is a great need for sensitive and readily applicable procedures to diagnose these infections. The historic technique of demonstrating intracytoplasmic inclusions in Giemsa-stained conjunctival, urethral, or cervical scrapings was supplanted in the 1960s by yolk-sac isolation and shortly thereafter by tissue culture procedures. Because the tissue culture techniques are not readily available to many clinics, efforts have been made to refine assays of chlamydial antibodies as a means of diagnosing chlamydial infections.

The complement fixation (CF) test, a genus-specific test, has not been useful in diagnosing the localized genital tract infections (11). The microimmunofluorescence (Micro-IF) test developed by Wang and Grayston has proven to be a sensitive and specific assay for antichlamydial antibodies (14). Application of this test in selected cases (e.g., of men having a first attack of urethritis) has shown that it can be useful in diagnosing recently acquired chlamydial infection (1). Unfortunately, the high prevalence of chlamydial antibodies in sexually active individuals has made it difficult to demonstrate changing antibody levels in paired acute- and convalescent-phase sera (8, 9). Some workers have suggested that measurement of antibodies to chlamydiae in genital tract secretions might provide a useful diagnostic tool (2, 12). Our experience does not support that contention. We report here the results obtained in measuring antibodies against chlamydiae in cervical secretions and in sera collected from women attending a venereal disease clinic.

MATERIALS AND METHODS

Population studied. The women screened in this survey attended the Contra Costa County Venereal Disease Clinic in Richmond, Calif. Many were named as contacts of men diagnosed as having gonorrhea. One hundred eighty women were tested in this study. All were to be tested for chlamydial infection of the cervix. The first 84 were tested by CF test and by Micro-IF test for serum immunoglobulin G (IgG) antibodies. In addition to these tests, the second group of 96 women also had Micro-IF test for serum IgM antibodies and had cervical secretions tested for antibodies of the IgG, IgA, and secretory IgA classes. Discrepancies in numbers represent lost tubes or specimens.

Chlamydia isolation. Specimens were collected by vigorous rotation of calcigate swabs against the walls of the endocervical canal. The swabs were placed into a tissue culture medium (minimal essential medium plus 10% fetal calf serum supplemented with glutamine and glucose and containing 20 µg of gentamicin, 100 µg of vancomycin, and 4 U of amphotericin B per ml). The specimens were transported to the laboratory, either on the day of collection or after overnight storage in a refrigerator. They were then shaken with glass beads and inoculated into iododeoxyuridine-treated McCoy cells following the procedure of Wentworth and Alexander (16). After centrifugation for 1 h at 2,800 × g, the specimens were incubated for approximately 65 h at 37°C. After staining with iodine, the slides were examined microscopically for inclusions.

Serology. The CF test was performed with the boiled phenolized 6BC antigen (9). The Micro-IF procedure was the simplified method of Wang et al., using pooled antigens (15). Staining reagents for the direct immunofluorescent assay were fluorescein-conjugated goat anti-human IgG, IgM, IgA, and secretory piece (purchased from Hyland Laboratories). Cervical secre-
tions, collected by placing a Weckcel cellulose sponge at the os and allowing it to become saturated, were placed in a tube containing phosphate-buffered saline diluent. Sponges had been precalibrated and were eluted to yield an initial dilution of 1:10. None of the women was menstruating when the specimens were collected. None of the specimens was grossly contaminated with blood, but presence of blood was not rigorously excluded.

Results were analyzed by use of $\chi^2$. Sensitivity, specificity, and predictive value were calculated by standard procedures (11).

RESULTS

Chlamydial isolation results. C. trachomatis was recovered from 20% of all the women (36/180) and from 36.7% (22/60) of women with gonorrhea. Gonococci were recovered from 35% (61/174) of the women.

Serum antibodies. The results of the serological tests for Chlamydia are presented in Table 1. As expected, the CF test was a relatively insensitive indicator of chlamydial infection; 26% (9/34) of chlamydia-positive women had CF titers $\geq$ 1:16 compared to 23% (32/139) of chlamydia-negative women. All women yielding chlamydiae had detectable Micro-IF IgG antibody levels, but this 100% rate was not statistically significantly different from the 87% sero-positive rate for the chlamydia-negative women (121/139). IgM antibodies were much less prevalent, being found in only 6 of the 19 (32%) chlamydia-positive women compared to 14 of the 75 (19%) chlamydia-negative women.

Antibodies in cervical secretions. As with the serum antibodies, all chlamydia-positive women had chlamydial antibodies of the IgG class in their cervical secretions. This 100% rate (19/19) was statistically significantly higher ($P < 0.05$) than the 71% rate (55/77) observed in chlamydia-negative women. Although the IgM, IgA, and secretory IgA antibody rates were higher for chlamydia-positive women, the differences were not statistically significant.

The sensitivity, specificity, and predictive values of these tests as applied to serum and cervical secretions are presented in Table 2. None of the tests yielded results that would suggest them as useful diagnostic or screening tests. The high background levels of IgG antibody detected in the Micro-IF test brought specificity (identification of true negatives) to less than 30% in serum or secretions even though the sensitivity (detection of true positives) was 100%. The tests with relatively high specificity, such as the CF test or Micro-IF test for IgM antibodies, showed poor sensitivity, indicating they missed too many positives. Although the prevalence of chlamydial infection is 20% of the clinical population, none of these serological tests has a predictive value above 32%.

DISCUSSION

Although chlamydial isolation procedures are not routinely available, it is abundantly clear from these results that the CF or Micro-IF tests applied to serum or secretions do not provide a useful diagnostic tool that could supplant isolation of the organism. The prevalence of antibody observed in the serum of these venereal disease clinic patients was relatively high, but our results do not differ substantially from results obtained with other similar populations (6–8). The 89% prevalence of chlamydial antibodies probably reflects cumulative exposure to these agents in a high-risk venereal disease population. It is unlikely that serological tests would be of greater benefit in diagnosing chlamydial infection in other populations. Women attending this clinic had a relatively high infection rate (20%) for chlamydia, and the predictive value of a test increases with the prevalence of infection. Thus, these tests would not have a greater predictive value in populations having lower prevalence of infection unless the background rate of antibody decreased more than proportionately. Since it appears that chlamydial infection is common in sexually active populations, it is unlikely that

<table>
<thead>
<tr>
<th>Table 1. Serological tests for Chlamydia trachomatis in women</th>
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<tbody>
<tr>
<td>Test (IgA)</td>
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<tr>
<td>Serum (IgA)</td>
</tr>
<tr>
<td>Micro-IF (IgM)</td>
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<td>Micro-IF (IgG)</td>
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$^a$ P < 0.05 when compared to chlamydia-negative women.

**Table 2. Sensitivity, specificity, and predictive value of serological tests for Chlamydia trachomatis**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
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</thead>
<tbody>
<tr>
<td>Serum</td>
<td>26.5</td>
<td>77.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Micro-IF (IgG)</td>
<td>100.0</td>
<td>12.9</td>
<td>21.9</td>
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<tr>
<td>Micro-IF (IgM)</td>
<td>31.6</td>
<td>81.3</td>
<td>30.0</td>
</tr>
<tr>
<td>Cervical secretions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micro-IF (IgG)</td>
<td>100.0</td>
<td>28.6</td>
<td>25.7</td>
</tr>
<tr>
<td>Micro-IF (IgM)</td>
<td>26.3</td>
<td>85.7</td>
<td>31.2</td>
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<tr>
<td>Micro-IF (IgA)</td>
<td>63.2</td>
<td>53.2</td>
<td>25.0</td>
</tr>
<tr>
<td>Micro-IF (secretory IgA)</td>
<td>47.4</td>
<td>71.4</td>
<td>29.0</td>
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$^a$ Prevalence of infection, 20%.
this would prove to be the case in the populations most needing testing for chlamydial infections. In a study of female college students, McCormack and colleagues found a 4.6% prevalence of chlamydial infection, whereas only 13% of the total population had antibodies in their cervical secretions (5). Here then is a lower-risk population with a lower rate of infection and a lower rate of cervical antibody than the one we studied (77% with IgG antibody). In these college women, the sensitivity and specificity of cervical secretion antibody would approach 90%, but as a function of the lower infection rate the predictive value of a positive test would still only be about 30%, similar to our findings. Other studies from the same laboratory indicated that in a more sexually experienced population, a 40% level of cervical antibody was found with only a 2% active infection rate (McCormack, personal communication). From these results and other studies performed on tears in ocular chlamydial infections (4), it seems likely that antibodies in cervical secretions are essentially a reflection of circulating antibody. In our study, cervical IgG antibody was significantly associated (P < 0.001) with serum IgG antibody, since 73/74 (99%) of the women with cervical IgG antibody were also seropositive compared to 14/22 (64%) of cervical antibody-negative women. The same held true for IgM and IgA antibodies (data not shown).

It must be noted that statistically significant associations of chlamydial antibody with recovery of chlamydiae could be demonstrated for both cervical secretions (Table 1) and serum antibody, e.g., serum IgG antibodies could be associated with chlamydiae by increasing the dilution levels used to establish a positive result. However, there is a loss in the sensitivity of the test as the specificity is increased by more rigorous criteria. Although antibodies could be associated, in a statistical sense, with the presence of the organism, the ultimate utility of a serological test is its predictive value (probability that a positive test indicates infection) (3). Given the 20% prevalence rate, one could randomly choose one in five women in this clinic as having a chlamydial infection; if the entire battery of serological tests were added, the best result would not increase the chances of correctly identifying a chlamydial-infected woman to one in three. It seems unlikely that these tests can be used for diagnosing chlamydial infections (or even as a screening test for them except in highly selected populations).

In our opinion, the only currently available useful test is direct isolation of the agent. Sensitivity of tissue culture isolation procedures is not known, but based on the results of repeated screening tests it is probably 80 to 90%. However, isolating the agent diagnoses infection and has a predictive value of 100%. Since chlamydial infections are highly prevalent, can be treated, and are associated with significant morbidity, it would seem that addition of chlamydial isolation capabilities is warranted in any major medical facility treating patients who have sexually transmitted diseases.

ACKNOWLEDGMENTS
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LITERATURE CITED