Value of the Concomitant Use of Complement Fixation and Immunodiffusion Tests in the Diagnosis of Coccidioidomycosis

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Complement fixation titers of 1:2 and 1:4 with coccidioidin are not always of diagnostic significance. The concurrent use of the complement fixation and immunodiffusion tests is an effective means for specific serologic diagnosis of coccidioidomycosis in patients with low levels of complement-fixing antibodies. In routine testing, it is recommended that only sera that are negative for complement-fixing antibodies at 1:8 but positive in the immunodiffusion test with coccidioidin be selected for titration at the 1:2 and 1:4 levels.

It has been emphasized that complement fixation (CF) titers of 1:2 and 1:4 with coccidioidin may be significant and indicative of Coccidioides immitis infection (5, 8). Assuming this to be factual, some serologists recommend that initial dilutions of 1:2 be used for coccidioidomycosis serological tests. This could present problems to laboratories when CF tests for blastomycosis and histoplasmosis are performed in conjunction with those for coccidioidomycosis. Such laboratories generally use minimal serum dilutions of 1:8 because many nonspecific reactions can occur with Histoplasma capsulatum and Blastomyces dermatitidis antigens at serum dilutions below 1:8, and because sera from coccidioidal infections show intensive cross-reactions with such antigens (1). To further complicate matters, blastomycosis, coccidioidomycosis, and histoplasmosis are frequently clinically and roentgenologically indistinguishable. If a minimal serum dilution of 1:8 is used, patients with primary coccidioidomycosis that give 1:2 or 1:4 CF titers may be overlooked. This problem could be eliminated by initiating all CF tests at the 1:2 dilution. However, this could also result in false coccidioidomycosis diagnosis due to nonspecific CF reactions or residual antibodies.

This study was designed to assess the frequency with which false-positive results are encountered in the coccidioidomycosis CF test with sera tested in low dilutions and to determine the value of using immunodiffusion (ID) tests concurrently with the CF test to improve the accuracy of the serodiagnosis of coccidioidomycosis.

MATERIALS AND METHODS

Human sera. Sera from suspected and culturally proven cases of coccidioidomycosis, histoplasmosis, and other systemic mycotic infections received by the Immunology Diagnostic Laboratory of the Mycology Division, Center for Disease Control, were tested for fungal antibodies by the standardized Laboratory Branch microcomplement fixation test routinely used at the Center for Disease Control (9) and the micro-ID test (6). The final diagnoses on patients from whom the test sera were taken were obtained from the patient’s physicians.

Antigens. Four antigens were used in each Laboratory Branch microcomplement fixation test: a suspension of merthiolated, intact yeast-form cells of H. capsulatum; a soluble mycelial filtrate antigen, histoplasmin; a suspension of ground, yeast-form B. dermatitidis cells; and a soluble, mycelial filtrate antigen of C. immitis, coccidioidin (2). Three concentrated antigens were used in the ID test: a yeast-form filtrate antigen of B. dermatitidis, coccidioidin, and histoplasmin (6).

CF tests. Sera were titrated by the micro-modification of the Laboratory Branch microcomplement fixation test (9). In these tests five 50% units of complement were used. The antigen-antibody-complement mixture was incubated for 15 to 18 h at 4 C. Sera demonstrating 30% hemolysis or less at a particular dilution were considered positive. The initial 1:2 dilutions of heat-inactivated sera were prepared by using conventional pipettes and were transferred to the microplates. Ensuing dilutions were made with microloops.

ID tests. These tests were carried out with one-eighth-inch (ca. 0.31 cm) Plexiglas matrices, each of which contained 17 seven-well patterns. The technique and media used are those published previously (6).

RESULTS

Of 1,000 sera received for routine fungus serological tests in the Mycology Division during the study period, 87 reacted with coccidioidin in the CF test at any titer. Sixty-five of the 87 (75%) sera showed titers of 1:8 or less (Table 1), and only 40 (62%) of these were from
Twenty-three antigens with patients suffering from coccidioidomycosis. The latter ble from diseases other than coccidioidomycosis.

Fourteen sera were ID positive and CF negative or anticomplementary (Table 3). Of these, 12 were from cases of coccidioidomycosis and 2 were from patients with no evidence of coccidioidomycosis. No information was available on coccidioidin skin test reactions in the latter patients or whether they had ever resided or traveled in the coccidioidomycosis endemic areas.

The problem of cross-reactions with the B. dermatitidis, C. immitis, and H. capsulatum antigens in the CF test cannot be overemphasized (Table 4). Low- and high-titered coccidioidomycosis case sera may cross-react with the heterologous antigens, and conversely sera from other systemic mycotic infections as well as from patients with bacterial infections or malignancies may cross-react with coccidioidin. In contrast to CF reactions with coccidioidin, the ID reactions appear to be highly specific. Coccidioidin ID tests on an additional 49 blastomycosis and 47 histoplasmosis case sera were negative.

**DISCUSSION**

Although many coccidioidomycosis case sera demonstrate CF titers ranging from 1:2 to 1:8, positive results at these dilutions have been obtained with specimens from patients known not to have coccidioidomycosis (3, 7). In the present study 38% of the specimens reacting with coccidioidin in the 1:2 to 1:8 range came from patients with no evidence of coccidioidomycosis. In 1965, Huppert and Bailey (4) reported that the ID test, a recommended screening test for coccidioidomycosis, was as sensitive as the CF test, but that its specificity awaited further evaluation. Our studies indicate that the ID test is highly specific. Except for the two cases noted in Table 3, no cross-reactions were detected in numerous tests with well-documented case sera from patients with heterologous mycotic and bacterial infections and other diseases (Table 4). We recommend that the ID test be run in parallel with the CF test. Our data indicate that without exception sera positive in the CF test in the 1:2 to 1:8 range and also positive in the ID test reflect

**TABLE 1. Comparative accuracy of CF titers ranging from 1:1 to 1:8 and immunodiffusion coccidioidin reactions**

| Serologic reactions | No. of sera | No. of cases confirmed by culture and/or histopathology | % |
|---------------------|-------------|---------------------------------------------------------|---|
| CF +               | 31          | 31                                                      | 100 |
| ID +               | 34          | 9                                                       | 26 |
| AC*                | 12          | 10                                                     | 91 |
|                    | 2           | 2                                                      | 100 |

a Anticomplementary.

**TABLE 2. Diagnostic significance of CF titers ranging from 1:1 to 1:8 in comparison with immunodiffusion coccidioidin reactions**

| Serologic reactions | No. of cases | No. cross-reacting | Clinical diagnosis or comments |
|---------------------|--------------|--------------------|-------------------------------|
| CF +                | 30           | 2                  | Coccidioidomycosis            |
| ID +                | 1            | 0                  | Patient normal*               |
| +                   | 9            | 1                  | Coccidioidomycosis            |
| +                   | 15           | 12                 | Histoplasmosis                |
| +                   | 1            | 1                  | Blastomycosis and candidiasis |
| +                   | 1            | 1                  | Tuberculosis                  |
| +                   | 1            | 1                  | Pneumococcal pneumonia        |
| +                   | 1            | 1                  | Staphylococcal empyema*       |
| +                   | 1            | 1                  | Acute alcoholic gastritis      |
| +                   | 1            | 1                  | Coccidioidomycosis excluded*  |
| +                   | 1            | 0                  | Central nervous system malacia |
| +                   | 1            | 1                  | Macular degeneration          |
| +                   | 1            | 1                  | Pulmonary carcinoma           |
| +                   | 1            | 0                  | Regional enteritis            |

a Coccidioidomycosis diagnosed in past.

**TABLE 3. Diagnostic significance of immunodiffusion reactions with coccidioidin**

| Serologic reactions | No. of cases | Clinical diagnosis or comments |
|---------------------|--------------|-------------------------------|
| CF -                | 8            | Coccidioidomycosis            |
| +                   | 1            | Arthritis*                    |
| -                   | 1            | Central chorioretinitis       |
| +                   | 1            | Patient normal*               |
| -                   | 1            | Reticulum cell sarcoma        |
| AC*                 | 2            | Coccidioidomycosis            |

a Coccidioidomycosis diagnosed in past.

b Anticomplementary.
TABLE 4. Comparative CF titers and immunodiffusion reactions of selected coccidioidomycosis and other case sera with B. dermatitidis, C. immitis, and H. capsulatum antigens

| Case serum            | CF titer* | ID reaction |
|-----------------------|-----------|-------------|
|                       | H B C     | H B C       |
| Coccidioidomycosis    |           |             |
|                       | 2 2 16    | 0 0 0 +     |
|                       | 0 0 0 1   | 0 0 0 +     |
|                       | 16 0 16   | 0 0 0 +     |
|                       | 32 128 512| 256 0 0 +   |
| Blastomycosis         |           |             |
|                       | 16 64 0   | 0 0 +       |
|                       | 8 16 0    | 0 0 +       |
|                       | 0 0 8     | 0 0 +       |
| Histoplasmosis        |           |             |
|                       | 16 64 16 2+0| 0 0 + |
|                       | 16 64 128| 16 0 0 +   |
|                       | 0 8 8 8   | 0 0 0 0    |
| Acute alcoholic gastritis | 16 64 0 8 0 0 | 0 0 0 0 |
| Staphylococcal empyema | 0 0 0 2 0 0| 0 0 0 0 |
| Macular degeneration  | 0 0 2 2 0 0| 0 0 0 0 |
| Pneumococcal pneumonia| 2 4 2 2 0 0| 0 0 0 0 |
| Pulmonary carcinoma    | 0 0 16 8 0 0| 0 0 0 0 |
| Tuberculosis           | 2 2 2 2 0 0| 0 0 0 0 |

* End-point dilution factor.
* (H) histoplasmin; (Y) yeastform H. capsulatum; (B) yeastform B. dermatitidis antigens; (C) coccidioidin.

active or recent C. immitis infections (Table 1). Reactions in the CF positive-ID negative category must be carefully interpreted. Of 34 sera in the category, 25 were from non-coccidioidomycosis cases, and 20 of these demonstrated cross-reactions in the CF test with H. capsulatum and/or B. dermatitidis antigens (Table 2). Of the 40 low-titered CF-positive coccidioidomycosis case sera, only three showed cross-reactions and nine were negative for precipitins. It appears that the presence of precipitins or the absence of cross-reactions in sera CF positive with coccidioidin are important qualities to consider in establishing a positive diagnosis of coccidioidomycosis (Table 2). It is also apparent from the data that of 45 case sera with positive ID reactions, 43 were from proven cases of coccidioidomycosis. Cross-reactions are frequently associated with coccidioidomycosis case sera whether they demonstrate positive or negative ID reactions. When cross-reactions are encountered, a prudent diagnosis should rest upon the results of serological tests performed with a battery of antigens (Table 4) and examination of serum specimen for titer changes. Our data indicate no cross-reactivity in ID tests between the histoplasmin H and M antigens, the B. dermatitidis precipitinogens, and coccidioidin. The ID tests clearly show less cross-reactivity or nonspecific reactivity than the CF tests.

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