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Apparent False Positive Histoplasmin Latex Agglutination Tests in Patients with Tuberculosis

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Serum specimens from patients admitted to a respiratory disease hospital were examined by the histoplasmin latex agglutination, the complement fixation, and the agar gel immunodiffusion tests. Of 300 sera examined, 21 (7.0%) gave an apparent false positive reaction at a dilution of 1:16 or greater. Fourteen (66%) of the 21 patients studied had culturally proven tuberculosis. One patient each had a diagnosis of hypertensive cardiovascular disease with congestive heart failure, infection with atypical mycobacteria (Runyon group III), chronic pneumonitis secondary to gunshot wound, and pulmonary abscess of unknown etiology; two had bronchogenic carcinoma; and one serum specimen came from an apparently healthy employee. The results of the histoplasmin latex agglutination test should be interpreted with caution, particularly if only one serological determination has been made and the titer is low.

There are three major limitations that are frequently encountered by clinicians in obtaining serological confirmation of histoplasmosis. These are the nonspecific reactions, the long interval between onset of illness and the detection of antibody by present laboratory methods, and the limited access to testing facilities. Because of these reasons, the patient may be subjected to prolonged hospitalization pending a serological diagnosis in the absence of histological or cultural evidence of histoplasmosis.

The histoplasmin latex agglutination (HLA) test, which may readily be performed in a hospital laboratory, can overcome the latter two obstacles. The HLA test is simple to perform and yields results after overnight incubation. The antibody that is measured rises relatively early in the acute phase of the disease. However, the specificity of this test is not well established.

Because it is available commercially in a kit form, the HLA test is widely used in hospital and public health laboratories. However, the true diagnostic value of this test has been questioned by some. The purpose of this study was to compare the relative diagnostic and prognostic value of the HLA, immunodiffusion (ID), and complement fixation (CF) tests for histoplasmosis. This paper describes false positive HLA reactions in patients who apparently did not have histoplasmosis.

MATERIALS AND METHODS

The State Park Health Center is a 200-bed respiratory disease hospital operated by the South Carolina Department of Health and Environmental Control. The majority of patients have tuberculosis. Patients and new employees admitted during October and November 1969 and February through May 1972 were tested with the HLA test. During these two time periods, 36 new employees were hired and 312 patients were admitted to the hospital. A total of 300 serum specimens were submitted to the laboratory for examination. None of the patients had an admitting diagnosis of histoplasmosis.

The histoplasmin latex kit (consisting of latex sensitized with histoplasmin and a positive control serum) was obtained from a commercial company (Colab Laboratories, Inc.) and has been in use in this laboratory for several years. The procedure recommended by the manufacturer is as follows: "Serial two-fold dilutions of the serum to be tested are made in seven 13 × 75 mm tubes using 0.85% saline. An eighth tube containing saline only is used as an antigen control. Histoplasmin latex antigen is added to each tube. The tubes are shaken vigorously and incubated in a 37 C water bath for two hours. After shaking again, they are refrigerated overnight. The following morning, the tubes are centrifuged at 2,500 rpm for three minutes and read. The tubes containing 4+ agglutination with a clear supernatant are considered positive. A known positive control is included with each series." The manufacturer states that a titer of 1:16 is suggestive of active infection.

All examinations were performed by one of us. The sera were also tested for H and M bands by the ID described by Heiner (5). Those sera that gave a positive HLA test were also examined by the CF method with mycelial-form and whole yeast-form antigen (4). ID and CF antigens were obtained from the Center for Disease Control.

RESULTS

The results of our findings are presented in Table 1. Of the 300 sera examined, 29 were positive in the HLA test. Twenty-one specimens had an HLA titer of 1:16 or greater (two had a titer of 1:64, ten had a titer of 1:32, and nine had a titer of 1:16). The remaining eight patients had a titer of less than 1:16. None of the 29 sera with a positive HLA test had antibodies in the CF test to either mycelial- or whole yeast-form antigens or produced H or M bands in the ID test. Of the 21 patients with an HLA antibody titer of 1:16 or greater, 14 had a diagnosis of tuberculosis based on a positive intradermal skin test with PPD-S (5 tuberculin units), radiological and clinical symptoms compatible with tuberculosis, and isolation of *Mycobacterium tuberculosis* from the patient.

The essential clinical information on the seven nontuberculous patients is shown in Table 2. One patient each had a diagnosis of hypertensive cardiovascular disease with congestive heart failure, infection with atypical mycobacteria (Runyon group III), chronic pneumonitis secondary to gunshot wound, and pulmonary abscess of unknown etiology; two had bronchogenic carcinoma. The seventh serum was from an apparently healthy 21-year-old, white female inhalation therapist without signs, symptoms, or subsequent development of respiratory disease.

TABLE 1. *HLA titers in 300 patients without evidence of histoplasmosis*

HLA titer	Patients with a positive HLA test			ID bands[a]
	No.	Percent	Cumulative percent	
0	271			295 Neg 4 ND 1 M
1:4	2	0.7	9.7	0
1:8	6	2.0	9.0	0
1:16	9	3.0	8.0	0
1:32	10	3.3	4.0	0
1:64	2	0.7	0.7	0

[a] Neg, Negative; ND, not done; M, M band only.

DISCUSSION

In the absence of a single, well-defined serological test for histoplasmosis and in the absence of the isolation of the causative organism, the most useful information can be gained by using three serological tests for this fungus infection: HLA, CF, and ID. The advantages of the HLA test are: (i) it is readily performed in any laboratory; (ii) it is rapid; (iii) it can be performed on anticomplementary serum; and (iv) there is an early rise in antibody titer in acute histoplasmosis. Disadvantages of the HLA test are: (i) it does not detect the disease in chronic histoplasmosis due to the transitory nature of the antibody; and (ii) there is the presence of nonspecific agglutinins in low titers.

This paper describes data relevant to the latter disadvantage. The HLA test, to detect infection with *Histoplasma capsulatum*, was described in 1958 by Carlisle and Saslaw (2). Experimentally infected monkeys and dogs and immunized rabbits were used as a source of positive serum. A subsequent study using human sera showed that results with the HLA test correlated with those obtained with the collodion agglutination test for histoplasmosis (7). In that study, "normal" serum was obtained from those samples submitted for syphilis serology. Of 204 normal sera, one exhibited a 4+ agglutination to the HLA test; however, a positive reaction in the collodion test suggested to the authors (7) that this serum may have been from a patient with histoplasmosis. Examination of 208 serum specimens from patients hospitalized with various diseases other than histoplasmosis (diseases not specified) demonstrated only one specimen (0.5%) that showed a 4+ reaction in the HLA test.

Evaluation of a commercially prepared antigen consisting of latex particles sensitized with histoplasmin showed that the HLA test was

TABLE 2. *Clinical status of seven nontuberculous patients with a positive HLA test*

Patient	Age	Race[a]	Sex	HLA titer	Disease state
L.M.	47	N	F	1:4	Hypertensive cardiovascular disease; congestive heart failure
C.S.	46	N	M	1:4	Bronchogenic carcinoma
G.H.B.	58	W	M	1:16	Pulmonary infection with atypical mycobacteria (Runyon group III)
E.C.	26	N	M	1:32	Chronic pneumonitis
M.C.	73	W	M	1:32	Pulmonary abscess, etiology unknown
M.D.	21	W	F	1:32	Healthy employee
E.T.	72	N	M	1:64	Bronchogenic carcinoma

[a] N, Negro; W, white.

reasonably specific for histoplasmosis (6). Examination of 50 sera from cases of nonmycotic diseases (diseases not specified) did not demonstrate any positive reactors. Bennett examined sera from 208 patients in a large university medical center located in Missouri, using the commercial HLA kit (1). By correlating HLA titers with the clinical illness, he determined that a titer of 1:32 or greater was a significant indicator of infection with *H. capsulatum*. Using this criterion, he found only five false positive reactions. Two were attributed to a positive histoplasmin skin test before phlebotomy, leaving three (1.5%) as unexplained false positive reactions.

If the titer of 1:16 is considered suggestive of active histoplasmosis, as described in the literature accompanying the commercial antigen (3), then 21 (7.0%) of the sera we examined could be considered to be false positive reactions. The criteria for establishing a false positive reaction were: (i) absence of CF antibody; (ii) absence of H or M bands in the ID test; (iii) absence of signs or symptoms compatible with histoplasmosis; and (iv) a well-documented alternative diagnosis. Of the 21 patients with an HLA titer of 1:16 or greater, 14 (66%) had culturally proven infection with *M. tuberculosis*, six had other systemic disease processes, and one was an apparently healthy individual. It is relevant here that in a subsequent investigation by this laboratory of the serological aspects of experimental histoplasmosis in cattle, it was found that inoculation with *M. tuberculosis* caused a false positive HLA test (8).

Since the present study was retrospective, none of the patients with a positive HLA test were studied sufficiently to absolutely exclude a diagnosis of histoplasmosis. Intradermal skin tests with histoplasmin and sputum cultures were performed on some but not all of the persons in this group. Criteria for the absence of histoplasmosis were: (i) a well-documented alternative diagnosis; (ii) lack of conversion in two other serological tests for histoplasmosis; (iii) a response to treatment with the specific therapy for the disease that was diagnosed; and (iv) negative histoplasmin skin tests and/or negative cultures for *H. capsulatum* in those patients so studied.

The temporal association of antituberculous therapy in the patients with a diagnosis of tuberculosis and the patient's recovery in the absence of evidence to indicate a diagnosis of histoplasmosis suggest that the HLA titers reported in this paper were due to limited specificity of the antigen in low titers. Despite more specific exclusion of histoplasmosis in these patients, these data concerning the specificity of the HLA test are useful because they extend the studies of previous investigations.

Fungal serology is not a substitute for clinical judgment, but it has an important role as essential laboratory data to be considered in establishing a diagnosis when histological evidence or isolation of the etiological agent cannot be attained. The most reliable information from any serological test is obtained from observing changing titers of multiple specimens. The HLA test, when interpreted with caution, particularly if the titer is low, is a useful diagnostic tool.

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