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Diagnosis of Systemic Candidiasis by Latex Agglutination for Serum Antigen

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Three latex agglutination test procedures for detecting *Candida* antigen in human serum were compared in a retrospective study of 69 patients and 20 normal volunteers. Untreated human serum was reacted with two different latex reagents; one reagent also was reacted with serum treated with protease and heat. The test procedure with treated serum was best, detecting serum antigen in 17 of 21 patients (81%) with disseminated candidiasis. Judging by autopsy-proven cases, there was an increase in positive test results in the last 2 weeks of life. When untreated sera were tested with this reagent, only 3 (14%) of the 21 patients with disseminated candidiasis had detectable antigen in serum. A subset of these same sera was tested by a commercial latex reagent (*Candida* Detection System lot C001; Ramco Laboratories, Inc., Houston, Tex.) and untreated serum. Of 18 patients with disseminated candidiasis, 5 (28%) had at least one positive serum. Sera from patients with less severe clinical forms of candidiasis were usually negative regardless of the test procedure used. With one exception, sera from control patients were negative or were positive only in sera containing rheumatoid factor. Latex agglutination tests for *Candida* spp. in treated serum may prove to be a useful procedure for the rapid diagnosis of severe disseminated candidiasis.

A variety of methods have been used to demonstrate *Candida* antigen in the sera of patients with disseminated candidiasis (2–5, 7, 9, 10). Despite the potential clinical value of antigen testing in candidiasis, few of the published methods have been accepted outside of their originating institutions, and many favorably reported tests are no longer being performed. This enigma arises in part from the fact that the most promising tests have required methods that are too complex for the rapid analysis of single specimens in an ordinary diagnostic laboratory. For this reason, several laboratories are currently focusing their attention on latex agglutination (LA) tests, and at least two such test kits are available commercially. One of these kits (*Candida* Detection System; Ramco Laboratories, Houston, Tex.) involves a method reported to be successful in clinical diagnosis (3). The following study describes our experience with this test kit and compares these results with our own National Institutes of Health (NIH) test. The two procedures differed in the manner of producing reagent antibodies and in treating the serum before testing.

MATERIALS AND METHODS

Test procedures. *Candida* antiserum was raised in New Zealand rabbits by the intravenous injection of 2×10^8 cells of heat-killed serotype A *C. albicans* five times per week until the slide agglutination titer was 1:1,024. The slide agglutination test was performed at room temperature with rotation for 10 min at 160 rpm and equal volumes of serum and *C. albicans* cells in a 2% suspension (packed-cell volume). Serum immunoglobulin G (IgG) was obtained by the caprylic acid method (8), yielding a solution with an agglutination titer of 1:1,024 and a globulin concentration of 1.8 mg/ml. A suspension of latex particles (Difco Laboratories, Detroit, Mich.) was adjusted to an optical density of 0.3 at 650 nm in borate-buffered saline (0.036 M borate, 0.15 M NaCl) and incubated at 37°C for 1 h with an equal volume of IgG antibody diluted 1:2,000 in borate-buffered saline (LA reagent). Normal rabbit IgG (1.6 mg/ml) was prepared in the same manner and used at 1:1,000 to coat the same dilution of latex (LC reagent). Optimal IgG dilutions were selected by checkerboard titrations with mannan for antibody to *Candida* and with rheumatoid factor-positive serum for the normal IgG.

A procedure designed to dissociate antigen-antibody complexes was done as follows. Serum (300 μl) was mixed with 30 μl of protease (subtilisin A, 10 mg/ml; Miles Laboratories, Inc., Elkhart, Ind.), incubated for 1 h at 37°C, and placed in a boiling water bath for 10 min. Approximately 150 μl of supernatant was removed after centrifugation at 12,800 × *g* for 10 min. All sera were tested with our LA reagent by using both untreated and protease-treated sera. For routine screening, sera and supernatants were diluted 1:2, 1:4, and 1:8 in borate-buffered saline with 0.1% bovine serum albumin (Miles), and 30 μl was rotated with an equal volume of latex reagent for 10 min at 160 rpm in 14-mm glass wells (A. H. Thomas, Philadelphia, Pa.). Macroscopic agglutination was recorded only as present or absent. Twofold further serum dilutions were made of sera or supernatants that were positive at 1:8.

Control for rheumatoid factor was done by testing all positive specimens with LC reagent. The sensitivity of LA reagent was assessed for each run by testing borate-buffered saline–bovine serum albumin containing *Candida* mannan at final concentrations for 0.3, 0.6, 1.25, and 2.5 ng/ml. The mannan was prepared from *C. albicans* B311, type A, by the Peat method (6) and contained 0.81% N by micro-Kjeldahl digestion and nesslerization. The LA test for antigen was considered satisfactory if agglutination occurred at a mannan concentration of 1.25 ng/ml. LC reagent was tested periodically for positivity with rheumatoid factor-containing serum. Known positive and negative sera were used as

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TABLE 1. LA tests for *Candida* antigen: comparison of commercial test on untreated serum with NIH test on protease-treated serum

Patient group	No. of sera tested	No. of patients	No. of positive results by following test procedure:			
			Ramco		NIH	
			Sera	Patients	Sera	Patients
1. Disseminated candidiasis	45	18	6	5	18	13
2. Candidemia	5	3	0	0	1	1
3. Endophthalmitis	1	1	0	0	0	0
4. Esophagitis	2	2	1	1	0	0
5. Oral candidiasis	1	1	0	0	0	0
6. Neutropenic controls	5	4	0	0	0	0
7. Normal controls	20	20	0	0	0	0

controls for the protease-heat procedure. An additional control was sometimes employed and consisted of 90% normal human serum containing mannan at a final concentration of 5 ng/ml. This mannan-containing serum was required to agglutinate with the LA reagent after the serum was treated and diluted 1:2.

Some sera were also tested with the commercial LA reagent. We received several kits for antigen detection from Ramco. Lot C001 was selected for use because the LA reagent reacted with the positive control serum provided by the manufacturer. The other kits appeared less reactive but were not studied in detail. It is our understanding that the antibody for this test was raised in rabbits injected subcutaneously with killed *C. albicans* in adjuvant as reported by Gentry et al. (3). Sera were diluted for this test in glycine-buffered saline provided by the company. No LA control reagent was provided with lot C001.

Patient population. A total of 89 people and 277 sera were studied. The sera and clinical data from four patients with *Candida* spp. endophthalmitis were provided by Jack Edwards, Harbor General Hospital, Torrance, Calif. Sera from two patients with disseminated candidiasis were provided by Philip Pizzo, National Cancer Institute, Bethesda, Md. Seven groups of patients were studied. Group 1 consisted of 21 patients with disseminated candidiasis. The diagnosis was made by biopsy (4 patients) or by autopsy (12 patients); persistent positive blood cultures not attributed to an intravenous catheter and occurring during intense neutropenia (2 patients); positive blood and bone marrow cultures from one patient with acquired immune deficiency syndrome and on intraoperative cultures of peritoneal fluid in two patients. Two additional patients with disseminated candidiasis were excluded from group 1 because sera had been collected more than 21 days before autopsy diagnosis and the mycosis was judged to have started later. Group 2 consisted of five patients with candidemia, presumably catheter induced. Four patients had resolving neutropenia; they were treated successfully by catheter removal (one case) or by 10 to 14 days of intravenous amphotericin B (four cases). The five patients in group 3 had candidemia and endophthalmitis. Three of these had documented candidemia and retinal lesions; the other two were diagnosed only by ophthalmoscopic examination and clinical course. The 10 patients in group 4 showed *Candida* esophagitis on autopsy (2 patients), or endoscopic brushing or biopsy (8 patients). Seven of these were neutropenic, and five received amphotericin B intravenously for at least 1 week. Group 5 consisted of six neutropenic patients with oral candidiasis. The 22 patients in group 6 had fever with neutropenia, and the question of disseminated candidiasis had arisen. No evidence of candidiasis was subsequently found. However, the eventual diagnoses in this group included six patients with aspergillosis, two with histoplasmosis, and one with trichosporosis. Of the six aspergillosis patients, two had mixed fungal infections, one with mucormycosis and the other with trichosporosis. Group 7 consisted of 20 normal volunteers.

Test interpretation. The decision was made to call a test positive only when both the 1:2 and 1:4 dilutions showed agglutination. This provided an extra measure of assurance that borderline agglutination reactions could be interpreted unequivocally. Extracts or sera reacting with the normal globulin were called false-positive reactions. Only one protease-treated serum agglutinated with the LC reagent. Two sera from one patient with lupus erythematosus reacted with both the LC and Ramco reagents before protease treatment but were negative after protease treatment.

RESULTS

Untreated serum. Our LA test was specific but relatively insensitive in the diagnosis of systemic candidiasis when used with untreated patient serum. Five sera from four patients were reactive with the LA reagent and negative by LC. Three patients had disseminated candidiasis, and one had a clinical diagnosis of *Candida* endophthalmitis. The only serum remaining positive after protease-heat treatment exhibited a fall in titer from 1:32 to 1:8. The Ramco LA test, also used with untreated serum, was positive with seven sera from six patients (Table 1). Among the group 1 patients, the test was positive in 3 of 11 patients infected with *C. albicans* and in 2 of 6 patients infected with *C. tropicalis*, but it was negative in 1 patient diagnosed histologically. The test was less sensitive than our LA test with the same sera treated with protease. Only three sera from two patients with disseminated candidiasis were positive by the Ramco test and negative with our LA reagent and treated serum. The Ramco reagent did react with two additional sera, but these sera also reacted with our LC reagent, indicating the presence of rheumatoid factor. Of two sera from two patients with esophagitis, one was positive at 1:4 by the Ramco kit and negative when tested by our protease-heat procedure. Titers obtained with the Ramco kit are compared in Fig. 1 with titers from the NIH reagent and treated serum.

Treated serum. The results with our LA reagent and all

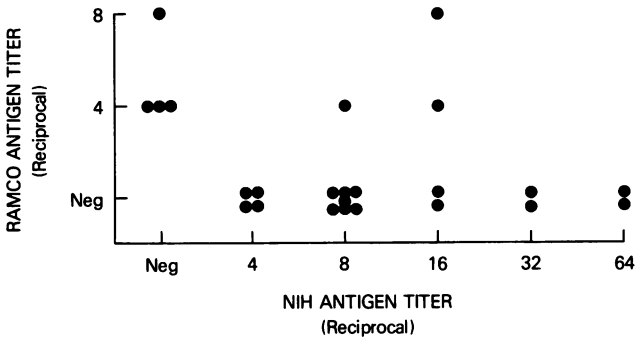

FIG. 1. Comparison of titers in sera positive by either our LA test with treated serum or by the commercial kit with untreated serum.

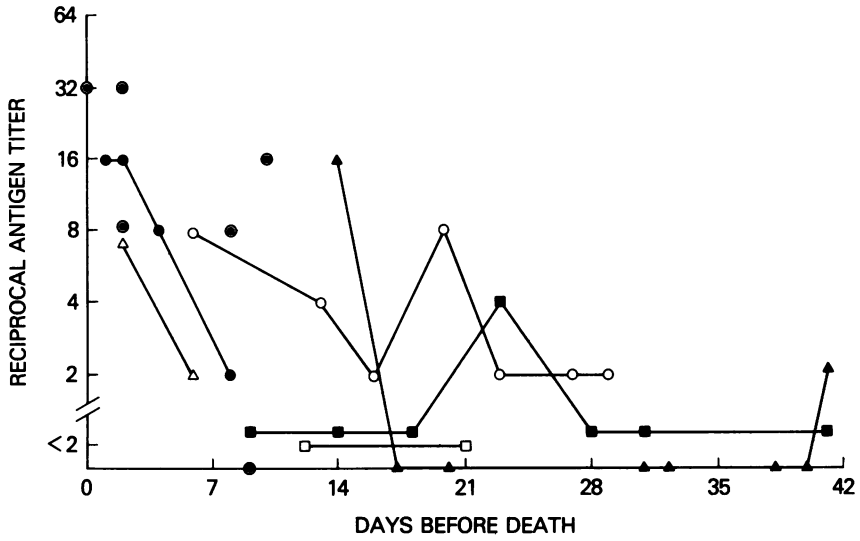

FIG. 2. NIH LA test with 36 treated serum antigen titers from 12 patients with autopsy evidence of disseminated candidiasis. Lines connect serial specimens from six different patients. The circled dots represent single specimens from six individual patients.

277 sera treated with protease-heat are shown in Table 2. The only false-positive result occurred in one patient with aplastic anemia, aspergillosis, and autopsy-proven cytomegalovirus infection of the lung, esophagus, kidney, and adrenal gland. Ten other sera from this patient were negative, including one taken 3 days earlier and one taken 4 days later.

Results with sera from 12 patients in group 1 who had autopsy confirmation of disseminated candidiasis are presented in Fig. 2, which relates titer to day of death. All patients studied in the week before death had antigenemia. Less than 50% had titers of 1:4 or greater in the second week before death, and before that time, only two sera were positive (≥1:4).

To assess the reproducibility of the test, 25 sera were tested on different days by different people using the same reagents. Two sera gave negative tests with both observers. Of the 23 positive tests, 16 were within one twofold dilution, 5 were within two dilutions, and 2 were greater than two dilutions.

The effect of *Candida* species was examined in antigen-positive group 1 patients, 14 of whom were infected with *C. albicans* and 6 with *C. tropicalis*; 5 had positive sera, the maximum reciprocal titers being 4, 4, 8, 16, and 64. These titers compared favorably with those from disseminated *C. albicans* infections, suggesting that *C. tropicalis* infection did not result in consistently less detectable serum antigen.

TABLE 2. LA test for *Candida* antigen in treated serum

Patient group	No. of sera positive/ no. tested	No. of patients positive/ no. tested	No. of patients with highest reciprocal titer of:				
			4	8	16	32	64
1. Disseminated candidiasis	32/76	17/21	1	8	5	2	1
2. Candidemia	3/11	3/5	2	0	0	0	1
3. Endophthalmitis	0/12	0/5	0	0	0	0	0
4. Esophagitis	5/38	4/10	2	1	1	0	0
5. Oral candidiasis	0/54	0/6	0	0	0	0	0
6. Neutropenic controls	1/66	1/22	1	0	0	0	0
7. Normal controls	0/20	0/20	0	0	0	0	0

Composite results. The best of the three test systems examined was clearly the one in which protease-treated serum was employed. In regard to the question of using several test systems for improved diagnosis, only one patient with candidiasis had antigen detected by the Ramco test alone. One patient had antigen detected exclusively by our LA reagent with untreated sera. All other patients with positive tests by the Ramco reagent or our LA test with untreated serum also had a positive test on the same serum or on some other serum, treated with protease-heat, from that patient. There was no tendency for any one of the test procedures to give positive results earlier in the course of candidiasis, thereby allowing earlier diagnosis. The use of the protease-heat procedure alone seemed to be indicated.

Because the sera were tested retrospectively in this study, the clinical impact of these tests could not be fully evaluated. Most important, a retrospective trial makes it difficult to determine whether any of the tests can provide diagnostic information early enough to improve therapeutic outcome.

DISCUSSION

The single most successful test was our LA reagent and serum treated with protease-heat, which detected infections in 81, 60, and 40% of the patients in groups 1, 2, and 4, respectively (Table 2). Of these 41 positive sera, only 1 was positive without treatment, although three patients with negative treated sera had untreated sera that were positive by LA and negative by LC. The control normal serum with added mannan was negative with our LA reagent before protease-heat treatment. It is assumed that protease-heat treatment released the protein-bound antigen. The heat and protease stability of the serum antigen being detected and the sensitivity of our test to about 1.25 ng/ml are consistent with the hypothesis that mannan was the antigen being detected.

The LA test was rapid and had adequate specificity and reproducibility. The most disappointing features were the low titers (Table 2) and the evidence of serum antigen at a time that appeared to be relatively late in the course of the

infection (Fig. 2). There was also no firm correlation between the severity of infection and the antigen titer. Endophthalmitis patients were negative except for one patient with a 1:8 titer on untreated serum. Low or absent antigen levels in serum could not be attributed to infection with *C. tropicalis* rather than *C. albicans*, because antigen was detected in five of six disseminated *C. tropicalis* infections. The influence of serotype could not be evaluated because the isolates were not retained.

In meningitis caused by encapsulated bacteria or the encapsulated fungus *Cryptococcus neoformans*, the antigen concentration in cerebrospinal fluid parallels disease severity very well. Serum antigen titers are generally lower than cerebrospinal fluid titers in meningitis and are often negative in nonmeningeal infections caused by the same organisms. Blood concentrations of *Candida* antigens might be expected to be low in nonmeningeal infections caused by an unencapsulated fungus. In addition, *Candida* mannan is cleared from the blood very rapidly after the antigen is injected intravenously into rabbits (unpublished results). This is in contrast with the prolonged circulation of injected cryptococcal polysaccharide (1). Mannan clearance is probably rapid because of mannose-mediated endocytosis in the reticuloendothelial system and because laboratory preparations are low in molecular weight (about 10,000). The LA test as performed here appears to be near the limit of its sensitivity. For routine diagnostic use, careful attention to reagent stability and positive controls would be essential in ensuring the reliability of the test procedure.

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LITERATURE CITED

1. **Bennett, J. E., and H. F. Hansclever.** 1965. *Cryptococcus neoformans* polysaccharide: studies of serologic properties and role in infection. J. Immunol. **94**:916–920.
2. **de Repentigny, L., and R. Reiss.** 1984. Current trends in immunodiagnosis of candidiasis and aspergillosis. Rev. Infect. Dis. **6**:301–312.
3. **Gentry, L. O., I. D. Wilkinson, A. S. Lea, and M. F. Price.** 1983. Latex agglutination test for detection of *Candida* antigen in patients with disseminated disease. Eur. J. Clin. Microbiol. **2**:122–128.
4. **Lew, M. A., G. R. Siber, D. M. Donahue, and F. Maiorca.** 1982. Enhanced detection with an enzyme-linked immunosorbent assay of candida mannan in antibody-containing serum after heat extraction. J. Infect. Dis. **145**:45–56.
5. **Meckstroth, K. L., E. Reiss, J. W. Keller, and L. Kaufman.** 1981. Detection of antibodies and antigenemia in leukemic patients with candidiasis by enzyme-linked immunosorbent assay. J. Infect. Dis. **144**:24–32.
6. **Peat, S., W. J. Whelan, and T. E. Edwards.** 1961. Polysaccharides of baker's yeast. Part IV. Mannan, J. Chem. Soc. (London) **1**:29–34.
7. **Segal, E., R. A. Berg, P. A. Pizzo, and J. E. Bennett.** 1979. Detection of *Candida* antigen in sera of patients with candidiasis by an enzyme-linked immunosorbent assay-inhibition technique. J. Clin. Microbiol. **10**:116–118.
8. **Steinbeck, M., and R. Audram.** 1969. The isolation of IgG from mammalian sera with aid of caprylic acid. Arch. Biochem. Biophys. **134**:279–284.
9. **Stevens, P., S. Huang, L. S. Young, and M. Berdischewsky.** 1980. Detection of *Candida* antigenemia in human invasive candidiasis by a new solid phase radioimmunoassay. Infection **8**(Suppl. 3):334–338.
10. **Weiner, M. H., and M. Coats-Stephen.** 1979. Immunodiagnosis of systemic candidiasis: mannan antigenemia detected by radioimmunoassay in experimental and human infections. J. Infect. Dis. **140**:989–993.