Evaluation of Candida Precipitin and Agglutinin Tests for the Diagnosis of Systemic Candidiasis in Burn Patients

IAN A. HOLDER,^{1*} PHILIP J. KOZINN,² AND EDWARD J. LAW†

Departments of Microbiology and Surgery, University of Cincinnati College of Medicine and The Shriners Burns Institute—Cincinnati Unit, Cincinnati, Ohio 45219,¹ and State University of New York, Downstate Medical Center, Brooklyn, New York 11203²

Received for publication 6 April 1977

Candida agglutinin and precipitin tests were performed on a group of burned patients without evidence of systemic candidiasis to determine whether tests could be used to distinguish between colonization and systemic disease, as has recently been reported. There were considerable numbers of false-positive results using both tests. These false-positive results occurred regardless of the candida colonization status of the patient. We conclude that these tests are of little diagnostic value in burn patients.

Disseminated candidiasis has emerged as a major life-threatening complication of burns (6). Renal, pulmonary, cardiac, and cerebral invasion with candida are not uncommon autopsy findings. The diagnosis of disseminated candidiasis by clinical and cultural means is difficult (9). Specific antifungal therapy with either intravenous amphotericin B or oral flucytosine is available. However, the prolonged course of therapy with relatively toxic drugs makes accuracy of diagnosis imperative.

Punch biopsies of the wound give valuable information as to the extent of localized invasion, but cast little light on dissemination (2). Positive blood cultures were found in 11 out of 14 patients with proven systemic candidiasis as well as in patients in whom candidemia was transient (6). The average day of the first positive blood culture was day 61. In contrast, positive blood cultures were found in a minority of 91 proven cases of candidal endocarditis, and dependence on this finding led to an absence of treatment (12).

The value of candida antibody tests for the diagnosis of systemic candidiasis is in dispute. Contradictory reports from reputable laboratories are based to a great extent on the absence of standardized antigens, standardized laboratory techniques, and patient selection (14). Chew and Theus (3) and Pepys et al. (11) used mannan antigens for the precipitin test and had many false positives. Taschdjian et al. (14) used a cytoplasmic antigen and showed a satisfactory efficiency of the precipitin test. In a recent study (5) of 115 high-risk patients for systemic candidiasis, in which disseminated

candidal infection was demonstrated histologically in 69 patients, the agar gel diffusion (AGD) precipitin test had a sensitivity of 94% and a specificity of 85%. In another prospective study of serodiagnosis by six independent laboratories on 100 sera, the precipitin and counter-current immunoelectrophoresis tests had sensitivities and specificities greater than 90% (7). The sera in both studies came from a cross section of patients in general hospitals and did not include any burn patients. However, in a study on burn patients, the germ tube dispersion test was found superior to both the agglutinin and precipitin tests (10).

Although cytoplasmic antigens were used in patients with subacute bacterial endocarditis (1) and those with open heart surgery (8), as well as in patients having uncomplicated candidal vaginitis (13), the AGD precipitin test was often positive in the absence of systemic candidiasis. These disease entities are apparent pitfalls for this test.

This study was undertaken to determine the efficacy of the agglutinin and the AGD precipitin test using the cytoplasmic antigen for the diagnosis of systemic candidiasis in the burn patient. Our goal was to determine whether these tests would be of any value in a population of patients in whom false-positive results have previously been reported and in whom the candida colonization rate is high (10). We also hoped to relate the extent of colonization to the degree of serological response.

MATERIALS AND METHODS

Patients. All acute-phase patients with total-body surface burns of 20% or greater were available for inclusion in the study. Wound, urine, and stool cultures were taken weekly. Cultures of sputum,

† Present address: Division of Surgery, Southern Illinois University School of Medicine, Springfield, IL 62708.

catheters, throat, blood, etc., were obtained when clinically indicated. Once a week, blood was drawn for serological tests. Testing was terminated when the patient had passed the acute phase of burn injury based upon clinical judgment. There were 100 patients in whom culture reports indicated the presence of candida at one time or another. Serological tests were performed on all of these. No patient ever had evidence of systemic candidiasis during the course of the study.

Forty-one patients with three or more sequential serum samples tested were evaluated to compare the results of the precipitin and agglutinin tests. It was felt that having at least three sequential tests would make evaluation of these tests more meaningful.

Second, we attempted to determine the relationship between the extent of candida colonization and the serological test results. For this, we identified two populations from those patients in whom three or more sequential serological tests were performed.

(i) **Extensive colonization.** Fifteen patients were selected in whom *Candida albicans* was isolated from wounds a minimum of four times and from two other sites at least two times. Cultural and serological data were obtained from these patients over periods ranging from 1 to 16 months. Excluding the one patient followed for 16 months (mean testing time, 3.2 months), the minimum number of cultures taken on a patient from this group was 22, with an average of 86 cultures per patient in this group.

(ii) **Occasional colonization.** Ten patients were chosen in whom *C. albicans* was isolated a maximum of four times from wounds and a maximum of two times from any other site. Cultural and serological data were obtained from these patients over periods ranging from 15 days to 2.3 months, with the mean testing time being slightly over 1 month. The minimum number of cultures taken on a patient from this group was 16, with an average of 39 cultures per patient in this group.

Serological tests. All serological tests were performed by Philip J. Kozinn, Maimonides Medical Center, Brooklyn, N.Y. Blood samples were collected from patients; the serum was separated and then frozen at -70°C . Sera were shipped to Philip J. Kozinn by air and were packed in dry ice during shipment.

Precipitin test. This test employed an experimental kit prepared by Schering Corp., Bloomfield, N.J. The kit consisted of 50-mm dishes of buffered (sodium barbital) agarose, 1.9 mm in depth, with eight wells, and an antigen that was the supernatant of an homogenate (Braun MSK homogenizer) of a culture of *C. albicans* serotype A grown for 48 h at 28°C in proteose-peptone broth. The supernatant of the homogenate was filter sterilized and standardized against a panel of known sera.

In the test, 20- μl samples of sera were introduced into the four larger wells, four samples per plate, and 10 μl of the antigen was introduced into each of the four smaller wells. The plates were incubated at room temperature and read at 24 and 48 h. The presence of one or more precipitin lines between antigen and serum wells was interpreted as a positive reaction. All negative sera were retested by the

same procedure before being reported as negative. Positive and negative controls were included in all determinations.

Agglutinin test. This test employed a whole-cell *C. albicans* antigen, serotype A, prepared by H. Hasenclever, National Institutes of Health, Bethesda, Md. Only one test was performed on each sample. As described by Hasenclever and Mitchell (4), serum samples were serially diluted in saline from 1:20 and 1:256 in 0.25-ml volumes. To each tube of diluted serum, 0.25 ml of the whole-cell antigen suspension was added; the tubes were then sealed with Parafilm and incubated in a water bath for 3 h at 37°C . Positive and negative serum controls were included in each test run. The tubes were reincubated at 2 to 8°C for 18 to 24 h. The titer of a positive serum was the highest dilution that produced a visible agglutination. A specimen was regarded as positive if it had a titer of 1:80 or greater.

RESULTS

The results of agglutinin and precipitin tests on 41 patients with three or more sequential serological tests are presented in Table 1. In the agglutinin tests, 66.5% were positive. In 18 of the 41 patients, all of the tests were positive, whereas in 6 of the 41, none of the tests was ever positive. Precipitins were positive in 51% of the tests. Eight of the 41 patients had all of their precipitin tests positive, as opposed to 12 of the 41 where none of the tests was positive. In most cases precipitin and agglutinin tests were run on each serum sample. In some instances, one test was positive but the other was negative. Agglutinins were present and precipitins were absent in 47 of 127 tests, representing 21 of the patients. On the other hand, 10 of 30 sera tested had precipitins in the absence of agglutinins. This occurred in only 5 of the 41 patients.

The results of culture data on patients extensively colonized by *C. albicans* are presented in Table 2. The category "other" includes throat and nasopharyngeal cultures, sputum, and catheter tips.

The results of agglutinin and precipitin tests in this group of patients are shown in Table 3. In 7 of the 15 patients, the agglutinin tests were positive in every test and, in 3 of the patients, none of the tests was ever positive. In patients with both positive and negative tests, no pattern emerged; the negative tests were interspersed randomly among positive tests. Precipitins were positive in every test in only 2 of the 15 patients in this group, whereas they were totally absent in 4 of the 15. Here, as well as with the agglutinins, the negative tests were randomly interspersed among the positive tests.

To contrast this extensively colonized group with a group considerably less colonized, data

TABLE 1. Results of agglutinin (AGG) and precipitin (PPT) tests on 41 patients with cultures positive for *C. albicans*

No.	AGG: no. positive/no. tested	PPT: no. positive/no. tested	Mismatches	
			AGG + PPT -	AGG - PPT +
1	38/38	35/38	3/38	
2	2/5	0/5	2/5	
3	5/6	4/6	1/6	
4	0/5	0/5		
5	0/4	1/4		1/4
6	4/6	3/6	1/3	
7	2/3	1/3	1/2	
8	3/3	3/3		
9	3/3	2/3	1/3	
10	0/6	6/6		6/6
11	3/4	3/4		
12	6/6	6/6		
13	2/3	3/5		1/3
14	5/5	5/7		
15	5/5	5/7		
16	2/3	3/3	1/3	
17	3/3	3/3		
18	1/6	1/6		
19	5/5	5/5		
20	1/11	0/11	1/11	
21	4/8	2/8		
22	0/3	0/3		
23	1/6	2/6		1/6
24	1/6	1/6		
25	2/4	0/4	2/4	
26	2/6	4/6	2/4	
27	0/3	0/3		
28	4/4	3/4	1/4	
29	5/6	4/6	1/6	
30	5/5	5/5		
31	3/3	0/3	3/3	
32	3/3	0/3	3/3	
33	6/6	0/6	6/6	
34	5/5	0/5	5/5	
35	5/5	0/5	5/5	
36	3/3	1/3	2/3	
37	3/3	3/3		
38	7/7	6/7	1/7	
39	3/3	1/3	2/3	
40	3/3	0/3	3/3	
41	0/11	1/11		1/11

were collected on 10 patients in whom candida was cultured only occasionally from one or two sites. The cultural and serological results from these patients are presented in Table 4. In spite of the fact that the patients were only occasionally colonized, 6 of 10 had all of their agglutinin tests positive, whereas in only 2 cases were the tests uniformly negative. Similarly, in four patients the precipitin tests were uniformly positive, whereas in only three were they all negative.

Table 5 presents data comparing serological tests on the extensively colonized group with

the occasionally colonized group. In the extensively colonized group, 73% of the agglutinin tests were positive, with 63% of the precipitin tests positive; in the occasionally colonized group, 63% of agglutinin tests were positive, whereas 57% of precipitin tests were positive.

DISCUSSION

In the absence of histological evidence, systemic candidiasis cannot be proven or disproven in the patients under study. In a previous study at the Shriners Burns Institute (6), there were 14 histologically proven cases of disseminated candidiasis out of 427 hospitalized burn patients or an incidence of 3.5%. However, Table 1 indicates positive agglutinin titers in 66.5% and positive precipitin titers in 51% of the burn

TABLE 2. Culture data from 15 patients extensively colonized by *C. albicans*

No.	No. of positive cultures from:					
	Wounds	Urine	Urine quantitation - range	Stool	Other	Blood
1	79	25	10 ² -10 ⁵	8	11	5
2	11	13	10 ² -10 ⁶	4	10	0
3	7	2	10 ⁵ -10 ⁶	3	1	0
4	6	1	10 ²	3	2	0
5	13	5	10 ² -10 ⁶	5	3	0
6	11	3	10 ² -10 ⁴	3	5	0
7	17	2	10 ²	12	2	0
8	7	1		4	2	0
9	16	0	10 ⁴ -10 ⁶	12	3	0
10	21	5	10 ⁴	4	4	0
11	5	4	Mixed flora	1	7	0
12	8	3	10 ²	0	1	0
13	4	1	Mixed flora	1	4	1
14	16	6	Mixed flora	1	2	0
15	7	1		0	2	1

TABLE 3. Agglutinin and precipitin results on 15 patients extensively colonized by *C. albicans*

No.	Agglutinins: no. positive/no. tested (%)	Precipitins: no. positive/no. tested (%)
1	38/38 (100)	35/38 (92)
2	0/5 (0)	0/5 (0)
3	4/6 (66)	3/6 (50)
4	2/3 (66)	1/3 (33)
5	0/6 (0)	6/6 (100)
6	3/4 (75)	3/4 (75)
7	6/6 (100)	6/6 (100)
8	2/3 (67)	3/5 (60)
9	1/6 (17)	1/7 (17)
10	0/11 (0)	1/11 (9)
11	3/3 (100)	0/3 (0)
12	6/6 (100)	0/6 (0)
13	5/5 (100)	0/5 (0)
14	3/3 (100)	1/3 (33)
15	7/7 (100)	6/7 (86)

TABLE 4. Results of agglutinin and precipitin in 10 patients occasionally colonized by *C. albicans*

No.	Wound	Stool	Other	Urine	Blood	Agglutinin: no. positive/no. tested (%)	Precipitin: no. positive/no. tested (%)
1	2	1	2	0	0	1/2 (50)	1/2 (50)
2	0	2 ^a	0	0	0	3/3 (100)	3/3 (100)
3	1 ^a	1 ^a	0	0	0	2/2 (100)	2/2 (100)
4	2	2 ^a	0	0	0	1/1 (100)	0/1 (0)
5	4	3	0	0	0	2/8 (25)	4/8 (50)
6	1	1	0	0	0	0/3 (0)	0/3 (0)
7	3	1	0	0	0	5/5 (100)	5/5 (100)
8	3	3	0	0	0	3/3 (100)	0/3 (0)
9	4	1	0	0	0	2/2 (100)	2/2 (100)
10	2	1	0	0	0	0/1 (0)	0/1 (0)

^a *Candida* species only.

TABLE 5. Comparison of serological data on burned patients extensively colonized and patients occasionally colonized with *C. albicans*

Group	Agglutinins	Precipitins
Extensively colonized	80/110 (73) ^a	69/110 (63)
Occasionally colonized	19/30 (63)	17/30 (57)

^a Number positive/total tests (percent).

patients. These positive antibody tests must represent more false-positive than true-positive tests.

These results show that in some patients with positive cultures for candida all of the agglutinin tests are positive, whereas in some they are uniformly negative. Similar results were obtained in the precipitin tests; however, twice as many patients (16/41) had uniformly positive agglutinin tests than had uniformly positive precipitin tests (8/41). In addition, half as many patients had uniformly negative agglutinins (6/41) compared with precipitins (12/41). Furthermore, when both tests were performed on the same serum sample, there were a considerably higher number that were positive for agglutinins and negative for precipitins than vice versa. These data from a population of patients with no evidence of systemic disease would tend to indicate that the agglutinin test is more sensitive in detecting antibody and therefore gives more false-positive results than the precipitin test.

Data presented on a group of patients extensively colonized by candida (Table 3) indicates that again the agglutinin test gives more false-positive results than the precipitin test, since 7 of 15 of these patients were uniformly positive in agglutinin tests as opposed to only 2 of 15 in precipitin tests. However, with each test, there were only a few patients whose test results were uniformly negative: 3 of 15 for the agglutinin test and 4 of 15 for the precipitin test. In patients only occasionally colonized by candida,

6 of 10 were uniformly positive by the agglutinin tests, and only 2 of 10 were uniformly negative, whereas precipitin tests were uniformly positive or negative in similar numbers of patients (4 of 10).

It appears, then, that both the agglutinin test and the precipitin test become positive in a large number of burned patients, regardless of extent of exposure to candida. We have previously shown that positive agglutinin and precipitin tests occur in burned patients uninfected with candida (10), and the data presented here indicate that the same thing obtains in patients colonized with candida but without evidence of systemic involvement. Although we observed an increase in the number of positive tests obtained from patients extensively colonized compared with those only occasionally colonized (Table 5), the fact that there was only a 10% increase in positive agglutinin tests and a 6% increase in positive precipitin tests in those respective groups indicates that the increase is very slight considering the vast differences in extent of colonization and the considerably longer duration of testing in the extensively colonized group.

In addition, there appeared to be no apparent relationship between increases in positive serological tests and the site of positive cultures. Patient no. 1 in the extensively cultured group (Tables 2 and 3) had numerous positive cultures in all sites listed and between 90 to 100% of all serological tests positive, whereas patients 2 and 10 had multiple cultures from all sites except blood and had no positive serological tests.

From data presented here and in a previous report (10), we can conclude that large numbers of false-positive candida agglutinin and precipitin tests can be expected in burned patients. These false-positive results occur in these patients regardless of whether the patients are not colonized by candida or are extensively colo-

nized by candida. The false-positive rate increases only slightly with more extensive colonization and is probably not significant. It therefore appears that the candida agglutinin test and the candida precipitin test are of no diagnostic value in this patient population.

We suggest that new serological tests for systemic candidiasis be sought and be given a thorough evaluation in patient populations, such as burned patients, for whom agglutinin and precipitin tests are not valid.

ACKNOWLEDGMENTS

We thank John E. Bennett, National Institutes of Health, Bethesda, Md., for his valuable comments, criticism, and suggestions concerning this study. We thank P. K. Goldberg for his technical assistance.

LITERATURE CITED

1. Bacon, P. A., C. Davidson, and B. Smith. 1974. Antibodies to *Candida* and autoantibodies in subacute bacterial endocarditis. *Q. J. Med.* 172:537-550.
2. Brunch, H. M., G. Nash, J. M. Stein, and R. B. Lindberg. 1972. Studies of the occurrence and significance of yeasts and fungi in the burn wound. *Ann. Surg.* 176:108-110.
3. Chew, W. H., and T. L. Theus. 1967. *Candida* precipitins. *J. Immunol.* 98:220-224.
4. Hasenclever, H. F., and W. O. Mitchell. 1963. Observations of two antigenic groups in *Candida albicans*. *J. Bacteriol.* 82:570-573.
5. Kozinn, P. J., R. S. Galen, C. L. Taschdjian, P. K. Goldberg, W. Protzman, and M. A. Kozinn. 1976. The precipitin test in systemic candidiasis. *J. Am. Med. Assoc.* 235:628-629.
6. MacMillan, B. G., E. J. Law, and I. A. Holder. 1972. Experience with *Candida* infections in the burn patient. *Arch. Surg.* 104:509.
7. Merz, W. G., G. L. Evans, S. Shadomy, S. Anderson, L. Kaufman, P. J. Kozinn, D. W. Mackenzie, W. P. Protzman, and J. S. Remington. Laboratory evaluation of serologic tests for systemic candidiasis. *J. Clin. Microbiol.* 5:596-603.
8. Murray, I. G., H. R. Buckley, and G. C. Turner. 1969. Serological evidence of *Candida* infection after open heart surgery. *J. Med. Microbiol.* 2:463-468.
9. Nathan, P., I. A. Holder, and B. G. MacMillan. Burn wounds: microbiology local host defenses and current therapy. *CRC Crit. Rev. Clin. Lab. Sci.* 4:61-100.
10. Oblack, D., J. Schwarz, and I. A. Holder. 1976. Comparative evaluation of the *Candida* agglutinin test, precipitin test and germ tube dispersion test in the diagnosis of candidiasis. *J. Clin. Microbiol.* 3:175-179.
11. Pepys, M. B., J. A. Faux, J. L. Longbottom, D. S. McCarthy, and F. E. Hargreave. 1968. *Candida albicans* precipitins in respiratory disease in man. *J. Allergy* 6:305-318.
12. Seelig, M. S., C. P. Speth, P. J. Kozinn, C. L. Taschdjian, E. F. Toni, and P. K. Goldberg. 1974. Patterns of candida endocarditis following cardiac surgery: importance of early diagnosis and therapy (an analysis of 91 cases). *Progr. Cardiovasc. Dis.* 17:125-159.
13. Stanley, V. C., and R. Hurley. 1974. *Candida* precipitins in pregnant women: validity of the test systems used. *J. Clin. Pathol.* 27:66-69.
14. Taschdjian, C. L., M. S. Seelig, and P. J. Kozinn. 1973. Serological diagnosis of candidal infections. *CRC Crit. Rev. Clin. Lab. Sci.* 4:19-59.