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Sensitivity, Specificity, and Predictive Value of Anti-Candida Serum Precipitin and Agglutinin Quantification: Comparison of Counterimmunoelectrophoresis and Latex Agglutination

THOMAS H. DEE,* GUY M. JOHNSON, AND CAROL S. BERGER†

Section of Infectious Disease, Department of Medicine, University of Wisconsin-Mt. Sinai Medical Center, Milwaukee, Wisconsin 53233

Received 11 August 1980/Accepted 16 January 1981

Many serological techniques have been developed to aid in the discrimination of significant candidiasis from other clinical states. Serum anti-candida precipitin and agglutinin quantification by counterimmunoelectrophoresis and latex agglutination were statistically evaluated as to their respective ability to accomplish this discrimination. Forty-five serum specimens from 16 patients with documented disease and 2 with presumptive disease and 81 specimens from 70 control patients were studied. The control population consisted of patients with candida colonization, transient candidemia, bacteremia, other systemic mycoses, and healthy medical personnel. The two techniques were compared as to sensitivity, specificity, and predictive value of a positive and a negative test. Using a precipitin or agglutinin titer of $\geq$1:8 as the criterion for a positive test, we obtained the following results: counterimmunoelectrophoresis, 78, 97, 88, and 94%, respectively; latex agglutination, 94, 50, 33, and 97%, respectively.

Antemortem diagnosis of significant candidiasis continues to present a diagnostic dilemma. Many serological tests have been developed and evaluated as diagnostic adjuncts. In recent years, two methods, counterimmunoelectrophoresis (CIE) and latex agglutination (LA), have received considerable attention (1). Quantification of serum anti-candida precipitins and agglutinins by these techniques has been shown by some investigators to provide a reliable adjunct for the discrimination of significant disease from other clinical states (2, 3, 6-9).

This paper reports a comparison of the quantification of precipitins and agglutinins by these two techniques with respect to sensitivity, specificity, and predictive value of a positive and a negative test.

MATERIALS AND METHODS

Sera. Forty-five serum specimens were obtained from 16 patients with documented significant candidiasis and 2 with presumptive cases. Predisposing factors and underlying conditions were as follows: multiple surgical or other invasive procedures, multiple or prolonged courses of antimicrobial agents, steroids, or other immunosuppressive agents, indwelling vascular or urinary tract catheters, parenteral hyperalimentation, diabetes mellitus, acute leukemia, Hodgkin's disease, and other malignancies. These specimens were designated at group I. Eighty-one specimens were obtained from 70 cases of candida colonization, transient candidemia, documented bacteremia, other systemic mycoses, and healthy medical personnel. These specimens were designated group II. Criteria for documented and presumptive significant candidiasis, candida colonization and transient candidemia, bacteremia, other systemic mycoses, and healthy medical personnel have been described previously (2, 3).

Precipitin titer. Serum anti-candida precipitin titer quantification was performed by CIE as previously described (2, 3). The antigen used in all CIE studies was a commercially available whole-cell extract previously described (Hollister-Stier Laboratories, Downers Grove, Ill.). Each lot of antigen was made to a specific protein nitrogen content, and its activity was measured by box titration against prepared rabbit anti-candida antiserum of known precipitin titer. Serial twofold dilutions of test serum were placed in the anodal wells. Each dilution was run against six serial twofold dilutions of antigen placed in the cathodal wells. CIE was continued for 90 minutes at 25 mA, and the plates were then read, placed in 0.9% NaCl solution overnight at 4°C, and read again the next morning. The precipitin titer was that final twofold dilution of serum which gave a positive precipitin reaction with any of the antigen dilutions. Positive and negative controls were employed in each run. As previously reported, a precipitin titer of 1:8 is considered significant (2, 3). This titer was used as the cutoff value for a positive test in the statistical evaluation of the method.

Agglutinin titer. The LA test was performed by the method of Stickle et al. (9). A standardized suspension of latex particles (Difco Laboratories; 0.8-μm

† Present address: 4526 E. Mercer Lane, Phoeniz, AZ 85028.

particles, diluted 1:1.5 in glycine-buffered saline without bovine serum albumin) was prepared and sensitized with an equal volume of the concentration of candida antigen (Centers for Disease Control candida whole-cell extract, lot no. 100377) which gave a maximum agglutination reaction in box titrations against rabbit *Candida albicans* antiserum. A 1:8 dilution of antigen in glycine-buffered asline was found to be most sensitive. Results were read immediately. The final dilution of serum giving an agglutination reaction of 1+ or greater was recorded as the agglutinin titer. Glass pipettes and centrifuge tubes were used throughout the procedure since plastic labware was found to interfere with the reaction. Positive and negative controls were employed with each test. An LA titer of 1:8 was considered positive (5, 6, 8, 9).

Statistical evaluation of CIE and LA. The sensitivity, specificity, and predictive value of a positive and a negative test were calculated for each method from the following formulas:

Sensitivity = (TP × 100)/(TP × FN), specificity = (TN × 100)/(TN + FP), predictive value, positive test = (TP × 100)/(TP + FP), and predictive value, negative test = (TN × 100)/(TN + FN) (6), where TP represents true-positive tests; FP represents false-positive tests, TN represents true-negative tests, and FN represents false-negative tests.

RESULTS

Reciprocal values of the anti-candida precipitin and agglutinin titer quantifications are summarized in Tables 1 and 2. In cases in which multiple specimens were obtained, the peak value for each is presented and used for statistical evaluation. It is of interest that in such cases, peak precipitin and agglutinin titers were obtained in the same specimen. Fourteen of 18 (78%) group I cases had precipitin and agglutinin titers ≥1:8. Three of four cases with precipitin titers <1:8 had agglutinin titers ≥1:8. One case had both values of <1:8. In group II, 31 of 49 cases (63%) of candida colonization or transient candidemia or both had LA titers ≥1:8; only 2 cases of this group had CIE titers of this magnitude. Two of four cases of bacteremia and two of five cases of other systemic mycoses had LA titers ≥1:8; none had CIE titers this high. Normal personnel were uniformly <1:8 by both techniques. Sensitivity, specificity, and predictive value of a positive and a negative test for the two techniques were found to be as follows: CIE—78, 97, 88, and 94%, respectively; LA—94, 50, 33, and 97%, respectively.

TABLE 1. *Peak values of serum anti-candida precipitins and agglutinin titers for group I*

Titer	
CIE	LA
4	32
8	8
8	32
8	128
32	1,024
16	1,024
8	128
8	32
4	64
16	128
8	512
64	2,048
1	N[a]
8	64
8	128
8	64
8	32
4	256

[a] ND, None detected.

DISCUSSION

There exists a large volume of literature pertaining to candida mycoserology, much of it contradictory and confusing (1). Few references are extant in which there has been direct comparison and statistical evaluation of the various methods. Merz et al. (8) evaluated a number of agglutination and precipitin tests for significant candidiasis. These included three methods of agar gel diffusion, three methods of CIE, two whole-cell agglutination tests, and LA. The whole-cell agglutination and LA tests were quantified, with titers of ≥1:80 and ≥1:8, respectively, being considered positive. Precipitin titers were not determined. Their patient population consisted of individuals with proven, presumptive, or probable candidiasis. Healthy individuals and patients with other systemic mycoses served as controls. The results of this study indicated that a CIE technique employing the Hollister-Stier antigen gave the best overall performance with respect to sensitivity and specificity.

Harding et al. (4) evaluated whole-cell agglutination, agar gel diffusion, and CIE in a patient population consisting of proven or deep systemic candidiasis, probable deep or systemic disease, and superficial infection or colonization. No control population was studied. Precipitin titers were not determined. Their results indicated that whole-cell agglutination was more sensitive than agar gel diffusion or CIE but that the latter tests were more specific and provided a greater predictive value for a positive test. Our CIE results compared favorably with theirs with respect to sensitivity, specificity, and predictive value of a positive and a negative test (78, 97, 88, and 94% versus 83, 96, 87, and 95%, respectively).

Kozinn et al. (6) have recently evaluated agar gel diffusion, CIE, LA, and whole-cell aggluti-

TABLE 2. *Peak values of serum anti-candida precipitin and agglutinin titers for group II*[a]

Titer									
Candida colonization/transient candidemia				Bacteremia		Other systemic mycoses		Healthy personnel	
CIE	LA	CIE	LA	CIE	LA	CIE	LA	CIE	LA
2	16	8	64	1	16	ND	4	ND	4
2	8	1	8	2	8	ND	1	ND	ND
1	8	2	32	4	4	ND	2	1	ND
4	16	2	2	1	4	ND	8	1	ND
2	32	1	2			ND	256	2	ND
4	8	2	64					ND	ND
4	4	2	4					ND	ND
2	4	ND	ND					ND	ND
2	8	1	4					ND	ND
4	32	2	32					ND	ND
1	8	1	2					ND	ND
1	1	2	64					ND	ND
2	8	4	32						
1	16	2	64						
1	4	1	32						
2	32	2	64						
1	2	4	64						
ND	2	1	32						
1	2	1	4						
ND	ND	2	32						
2	32	2	8						
4	ND	2	4						
1	4	2	32						
16	256								
2	64								
4	128								

[a] ND, None detected.

nation tests. Their population consisted of patients with proven and presumptive systemic disease and a control group primarily composed of hospitalized medical and surgical patients. The antigen used for the agar gel diffusion and CIE tests was a whole-cell sonicate extract. LA and whole-cell agglutinin titers of ≥1:8 and ≥1:160, respectively, were considered positive. Precipitin titer quantification was not done. Their results showed that agar gel diffusion, CIE, and LA were similar as to sensitivity, specificity, and efficiency. Comparison of our CIE technique with that used in the Kozinn study indicates that our technique, although less sensitive (78 versus 88%), was somewhat more specific (97 versus 88%). The results with the LA technique revealed great discordance with regard to sensitivity (94 versus 81%) and specificity (50 versus 89%).

Our results indicate that although LA was more sensitive than CIE (94 versus 78%), CIE provided greater specificity (97 versus 50%) and a greater predictive value for a positive test (88 versus 33%). Both tests showed similar predictive values for a negative test. These findings differ from the previous studies and may be explained by the following facts. First, we included in our study a population of individuals with candida colonization, transient candidemia, and bacteremia. As can be seen from Table 2, 33 of 53 patients (62%) in these subgroups had LA titers of ≥1:8. In the original article by Stickle et al. (9), 63% of the sera from patients considered "least suspicious" for the presence of systemic disease had LA titers of ≥1:8. Inclusion of these patients appears to have affected the specificity and predictive value of a positive test. In none of the three previous studies mentioned above was this type of population investigated. Two of five patients with other systemic mycoses (invasive pulmonary aspergillosis and phycomycete endocarditis) had titers of this magnitude. Secondly, only sera with precipitin titers of ≥1:8 were considered positive for statistical evaluation, resulting in the lower sensitivity rate for CIE, as previous studies had only evaluated a positive test without precipitin quantification.

Thus, in our hands, CIE with precipitin titer quantification provided better discrimination of significant disease from other clinical states and

should be considered the serological method of choice.

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