

## Detection of Candida Serum Precipitins by Counterimmunoelectrophoresis: an Adjunct in Determining Significant Candidiasis

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We report our experience with the use of counterimmunoelectrophoresis for the detection and quantitation of candida serum precipitins in 164 patients. Group I consisted of 24 patients with significant candidiasis; group II consisted of 97 patients with either colonization or transient candidemia; and group III consisted of 43 subjects with noncandida systemic mycoses, bacterial infections, and normal controls. Prospective studies were done in ten patients. Double immunodiffusion was performed in all cases. Ratios of counterimmunoelectrophoresis precipitin detection were significantly different between groups I and II and groups I and III ( $P < 0.001$ ). Precipitin titers of 1:8 or greater were found more often in group I as compared to group II or III ( $P < 0.001$ ). All prospectively studied patients who developed significant candidiasis had peak precipitin titers of  $\geq 1:8$  (>fourfold titer increase) during the period of observation. Quantitation of candida serum precipitins by counterimmunoelectrophoresis is helpful in diagnosing significant candidiasis.

Candidiasis continues to be an infection of great significance in hospitalized patients, especially the compromised host (3, 4, 9, 10). A major problem in diagnosis is the differentiation of significant infection from colonization or transient blood stream invasion by this organism (2, 5, 7). Recently we reported the correlation between serum candida precipitin titers by counterimmunoelectrophoresis (CIE) and positive double immunodiffusion (DID) tests in patients with significant candidiasis (6). This paper details our continued experience with CIE in this regard. In addition, it presents data on 10 patients who were studied prospectively with serial antibody determinations.

### MATERIALS AND METHODS

**Selection of patients.** The study population consisted of 21 patients with documented significant candidiasis (group IA), 3 patients with strongly suspected significant candidiasis (group IB), and 97 patients with candida colonization or transient candidemia (group II). Group III consisted of 9 patients with noncandida systemic mycoses, 20 patients with bacterial infections, and 14 normal controls.

The criteria for determining significant candidiasis are detailed elsewhere (6). They include clinical evaluation, culture and histological examination of biopsy or autopsy specimens, cultures of wounds and

abscesses, transtracheal aspirates with appropriate chest roentgenograms, and repeatedly positive blood cultures unrelated to indwelling vascular cannulas. Diagnoses in this group were (number of cases in parentheses): wound abscess (four), intra-abdominal abscess (five), pneumonitis (four), persistent candidemia (four), and one case each of invasive esophagitis, endocarditis, endophthalmitis, and pneumonitis with abscesses of the liver, spleen, kidney, and thyroid. Twelve patients in this group received antimycotic chemotherapy (amphotericin B, 5-fluorocytosine, or both). Five of these patients succumbed to infection (42%). Postmortem examination revealed no evidence of candidiasis in four of these patients. The one patient in whom candidiasis was found had expired within 48 h of initiation of therapy. Nine patients did not receive therapy. Eight of these patients died (89%). Postmortem examination was performed in five patients; candida tissue invasion and abscesses were found in all.

Three patients were strongly suspected of having significant candidiasis (group IB). Because of our inability to obtain appropriate antemortem diagnostic data or postmortem examinations, a definite diagnosis could not be made. None of these patients received antimycotic chemotherapy, and two died.

The criteria for determination of colonization or transient candidemia and the predisposing conditions have been previously described (6). These criteria include transiently positive urine, sputum, mucocutaneous, or blood cultures associated with intravenous catheters, indwelling Foley catheters, hyperalimentation, or multiple and/or prolonged courses of antimicrobial therapy. In addition, one

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patient with chronic mucocutaneous infection is included. No clinical evidence of significant candidiasis was present in this group. Four patients in this group received antimycotic chemotherapy (amphotericin B); only one patient received more than 200 mg. No autopsied patient in this group had evidence of significant candidiasis, including one patient who had received therapy.

Criteria for the diagnosis of noncandida systemic mycoses, significant bacterial infection, and the normal controls have been previously described (6). The cases of noncandida systemic mycoses included two cases each of invasive pulmonary aspergillosis and *Torulopsis glabrata* fungemia and one case each of pulmonary blastomycosis, rhodotorula fungemia, cryptococcal meningitis, coccidioidal meningitis, and phycocytetes endocarditis. Bacterial infection consisted of one or more cases of bacteremia with *Proteus mirabilis*, *Escherichia coli*, *Herellea vagincola*, *Serratia* sp., *Bacterioides fragilis*, *Salmonella typhimurium*, *Streptococcus viridans*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Neisseria meningitidis*. One case of culture-positive pulmonary tuberculosis was included. No evidence of significant candidiasis was found in any autopsied group III patient.

All study patients had at least one serum specimen examined for candida serum precipitins by CIE and DID. In addition, 10 patients were studied prospectively with three or more determinations during the period of observation. These patients were considered "at risk" and were so studied to evaluate the usefulness of serial precipitin titer measurements in helping to differentiate significant infection from colonization. Five of the patients developed significant candidiasis (group I), and five did not (group II).

Candida serum precipitin detection and precipitin titers were determined by CIE, and DID was performed as previously described. We observed in our initial study that heating sera at 56°C for 30 min did not alter the precipitin titer; thus, this step was omitted in our subsequent determinations (6). In addition, all DID precipitin-positive sera were tested against rabbit candida antiserum for observation of a line(s) of identity with the prepared antiserum (13). The antigen employed in all studies was a commercially available whole cell extract, as previously described (Hollister-Stier Laboratories, Inc., Downers Grove, Ill.) (6). This preparation contained 31,000 protein nitrogen units per ml in the 1:10 dilution supplied by the company.

## RESULTS

**Precipitin detection.** The rates of precipitin detection for each group are summarized in Table 1. In group I, 22 of 24 patients (92%) had candida serum precipitins detected by CIE, and 21 of 24 (88%) were precipitin positive by DID. Two patients in this group had no detectable precipitins by either method. Fifty of 97 (52%) group II patients were precipitin positive by CIE, and two (2%) were positive by DID. In group III, 12 of 43 (28%) patients were positive

by CIE, and none were positive by DID. Statistical analysis of precipitin detection by CIE showed a significant difference between groups I and II ( $\chi^2 = 13.15$ ,  $P < 0.001$ ) and groups II and III ( $\chi^2 = 23.61$ ,  $P < 0.001$ ).

**Serum precipitin titers.** The precipitin titers of CIE-positive patients in each group are summarized in Fig. 1. These titers include the peak precipitin titer of each patient studied prospectively (vide supra). Table 2 depicts the ratio of each group with respect to precipitin titer of 1:8 or greater. Seventeen of 22 (77%) precipitin-positive group I and three of 50 (6%) precipitin-positive group II patients had precipitin titers of this magnitude. No group III patient had a titer this high. Statistical comparison of groups I and II showed a significant difference ( $\chi^2 = 38.71$ ,  $P < 0.001$ ).

**Prospective study.** Five group I and five group II patients were studied prospectively.

TABLE 1. Sera positive for candida precipitins by CIE and DID

Group	No. tested	Positive by CIE		Positive by DID	
		No.	%	No.	%
I	24	22	92	21	88
A	21	19	90	18	85
B	3	3	100	3	100
II	97	50	52	2	2
III	43	12	28	0	0

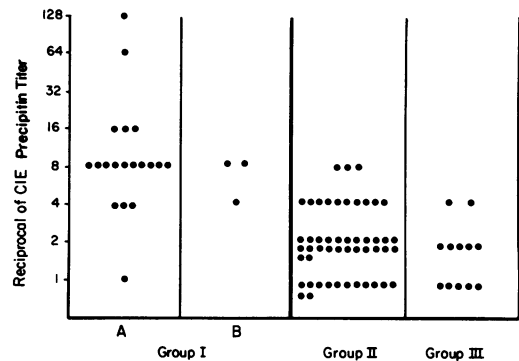


FIG. 1. Distribution of candida serum precipitin titers by CIE of patients in groups I, II, and III.

TABLE 2. Sera with CIE precipitin titers  $\geq 1:8$

Group	Positive by CIE	Titer $\geq 1:8$	
		No.	%
I	22	17	77
A	19	15	79
B	3	2	67
II	50	3	6
III	12	0	0

Each group I patient attained a serum precipitin titer of 1:8 or greater during the study period. Individual peak titers were 1:128, 1:64, 1:16, 1:8, and 1:8. These values represent a more than fourfold increase in precipitin titer. Three patients received antimycotic chemotherapy. Two of these patients demonstrated a decreased titer during therapy. One of the patients expired. Postmortem examination revealed no evidence of significant candidiasis, and death was attributed to other causes. The third patient had an initial decrease in precipitin titer, but subsequent determinations showed a persistent titer of 1:8 until death. No autopsy was obtained. However, this patient had antemortem documentation of candida endocarditis (6). The two group I patients who did not receive antimycotic chemotherapy had rising titers. Both expired. One patient was autopsied; candida pneumonia and candida abscesses of the liver, spleen, kidney, and thyroid were found. The courses of three group I patients studied are depicted in Fig. 2 through 4.

No group II patient achieved a precipitin titer greater than 1:4 during the period of observation. Peak individual titers were 1:4 and 1:2; three patients had no detectable serum precipi-

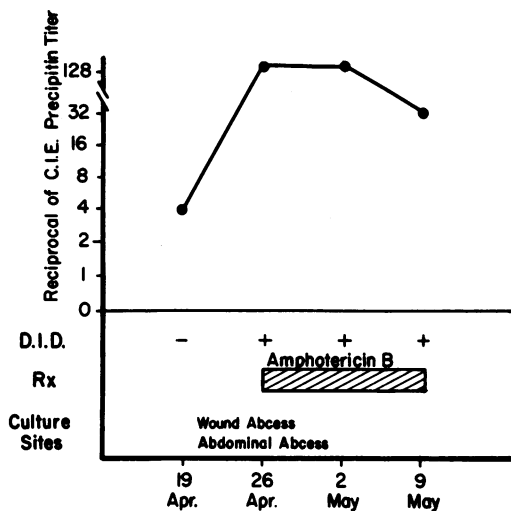


FIG. 2. A 50-year-old female with metastatic cervical carcinoma had multiple pelvic and abdominal surgical procedures and prolonged antibiotic therapy. The initial CIE precipitin titer was 1:4, and DID was negative. Drained wound and abdominal abscesses were culture positive for *Candida albicans*. Repeat CIE precipitin titer was 1:128, and DID was positive. During therapy with amphotericin B, CIE precipitin titer decreased to 1:32. Previously culture-positive sites were negative. The patient expired unexpectedly. Postmortem examination revealed no evidence of significant candidiasis.

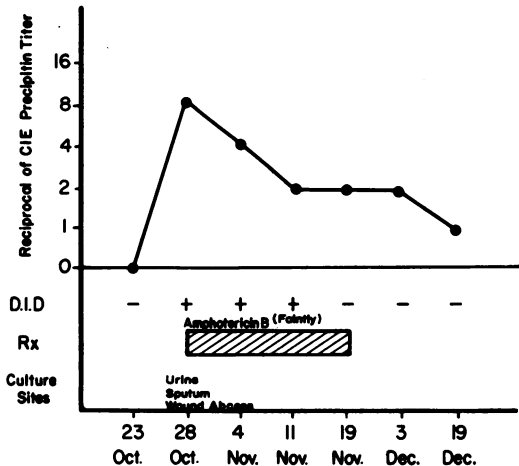


FIG. 3. A 72-year-old male with multiple abdominal surgery had prolonged antibiotic therapy and intravenous and urethral catheters in place. Initial serum was negative for detectable precipitins by CIE and DID. Subsequent cultures of urine, sputum, and wound abscess were positive for *C. albicans*. A second serum specimen had a CIE precipitin titer of 1:8 and was positive by DID. During amphotericin B therapy the culture sites cleared, the CIE precipitin titer decreased, and DID became negative. The patient survived.

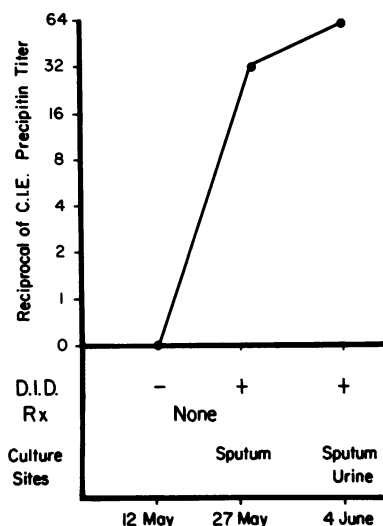


FIG. 4. A 27-year-old female with drug-induced aplastic anemia had prolonged antibiotic therapy. Initial serum specimen was negative for precipitins by CIE and DID. The sputum and urine became culture positive for *C. albicans*. Subsequent serum specimens demonstrated a rising precipitin titer by CIE and were positive by DID. No antimycotic chemotherapy was initiated. The patient expired. Postmortem exam showed candida bronchopneumonia and abscesses of the liver, spleen, kidney, and thyroid.

tins. All five patients had candida isolated from the urine and/or sputum or from wound exudate. Four patients in this group expired. Two were autopsied. No evidence of significant candidiasis was found.

### DISCUSSION

Candidiasis is the most common fungal infection in compromised hosts, a situation made more ominous by the difficulty in establishing antemortem diagnosis (2-5, 7, 9, 10). Although the DID technique for candida serum precipitin detection has been shown to be of great specificity, it may take from 24 to 96 h for a positive reaction to be discernible (8, 13-15). Remington et al. (12) demonstrated the detection of candida serum precipitins by CIE in patients with significant candidiasis. We demonstrated that, by determining serum precipitin titers by CIE, significant candidiasis could be reasonably differentiated from other clinical states if the titer obtained was 1:8 or greater. Furthermore, the time required for results was significantly reduced (6).

The rates of precipitin detection by CIE were significantly different between groups I, II, and III. Although statistical analysis was not applied, it is obvious that precipitin detection by DID was far more specific. The two group I patients without detectable serum precipitins by either method deserve comment. Both patients were initially studied when clinically preterminal. Postmortem examinations revealed significant candidiasis in both. Determination of serum immunoglobulins by the radial immunodiffusion technique of Mancini showed both to have decreased values for immunoglobulin G (IgG), IgA, and IgM (Tri-Partigen, Behring Diagnostics, American Hoechst Corp., Somerville, N.J.). One group I patient had a low titer of serum precipitins by CIE (undiluted serum only) and none detectable by DID. Serum immunoglobulins were found to be decreased in this patient also. Diffuse candida tissue invasion was found at autopsy.

Serum precipitin titers were determined in all CIE precipitin-positive patients. Precipitin titers of 1:8 or greater were found to be significantly different in comparing groups I and II. Three group II patients who had precipitin titers 1:8 were positive by DID also. One patient had chronic cutaneous candida infection with associated thyroiditis. Taschdjian et al. (14) have described positive DID precipitin reactions in patients with chronic mucocutaneous infections associated with autoimmune endocrinopathies but without evidence of deep tissue invasion. The second patient had an aortic

value replacement and developed colonization of the oropharyngeal mucosa postoperatively. Murray et al. (11) have reported difficulty with interpretation of serological tests for candida in open heart surgery patients. In their series, 22% of such patients developed positive precipitin and agglutinin tests postoperatively without evidence of significant candidiasis. Recently, Bacon et al. (1) have reported a high incidence of positive precipitin and agglutinin tests for candida in patients with culture-proven subacute bacterial endocarditis. Over 50% of the patients studied had positive tests. These positive tests were associated with positive results for autoantibodies such as antinuclear factors and smooth muscle antibody. During antimicrobial therapy for subacute bacterial endocarditis, the rate of positive candida serological tests and autoantibodies decreased. The authors cited no evidence of the presence of significant candidiasis and suggested that the positive serological tests may be related to non-specific stimulation of humoral immune mechanisms related to the underlying disease.

The value of prospective determinations of candida serum precipitin titers by CIE in patients at risk is suggested by our observations. Although a small number of patients were studied, the results indicate that those patients with significant candidiasis developed higher precipitin titers than those with colonization only. Furthermore, measuring precipitin titer changes during therapy may be of prognostic value. A larger patient population must be studied before any definite conclusions can be reached in these areas.

The results of this study demonstrate the usefulness of determining candida serum precipitin titers by CIE in the diagnosis of significant candidiasis. A serum precipitin titer of 1:8 or greater correlates well with a positive precipitin test by DID and provides good presumptive evidence for the presence of significant candidiasis in a patient at risk. This technique does have limitations, however. It may not be useful in patients who have reduced levels of serum immunoglobulins or in clinical states in which nonspecific hyperactivity of the humoral immune system exists. The results of initial prospective studies are encouraging, but further work is required.

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