

Increased Specificity of Antibody Detection in Surgical Patients with Invasive Candidiasis with Cytoplasmic Antigens Depleted of Mannan Residues

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In this study, it was shown that the diagnostic accuracy of antibody detection by a counterimmunoelectrophoresis technique could be improved by using cytoplasmic antigens depleted of mannan residues. The specificity of the counterimmunoelectrophoresis increased from 28.6 to 78.6% when cytoplasmic antigens depleted of mannan were used, while the sensitivity slightly decreased from 80 to 70%.

The frequency of invasive candidiasis as a major cause of morbidity and mortality among hospitalized patients has increased. An antemortem diagnosis of invasive candidiasis is very difficult because there is no specific clinical picture. Although isolation of *Candida* spp. from clinical specimens and serodiagnostic tests for antigen and antibody detection may help in the diagnosis, the following problems are inherent. Tests for the detection of circulating *Candida* antigens lack sensitivity and have been controversial (3, 4, 13, 14, 19, 21), while antibody detection systems mainly detect antibodies against *Candida* cell wall antigens, which are ubiquitous in the sera of patients with systemic or superficial candidiasis (12, 16). To obtain a more specific test, investigators have evaluated the usefulness of measuring antibodies to cytoplasmic antigens (8, 10, 24, 26, 27). The hypothesis behind these studies was that cytoplasmic antigens are exposed only during invasive infection and are therefore predictive and specific for invasive candidiasis (6, 23, 25). In the present study, we evaluated the antibody response against cytoplasmic antigens in the presence and absence of mannan residues in the sera of surgical patients with either invasive or superficial candidiasis. On the basis of clinical findings and culture results, these surgical patients were subdivided into two groups according to earlier-defined criteria (22). Group I consisted of 30 patients with proven invasive candidiasis. Group II consisted of 14 patients with

superficial *C. albicans* infection. From October 1982 until January 1991, patients who fulfilled the defined criteria (22) were included in this study. Serum samples from the patients were collected at weekly intervals. The majority of the patients underwent major abdominal surgery. Three serum samples per patient, when available, were included for serological evaluation. Besides antibody detection, the same set of sera was used for the detection of *Candida* antigens with the Cand-Tec test (Ramco Laboratories Inc., Houston, Tex.) (2, 5). The first sample was defined as the serum obtained at the time of the first microbiological evidence of invasive candidiasis. Correlations between antibody titers of the three serum samples obtained from each patient were assessed by using the Intraclass correlation coefficient (r_i). The capacity to discriminate between invasive and superficial candidiasis was evaluated by the Mann-Whitney test. Logistic regression was used to evaluate the serological assays simultaneously, with regard to their discriminating capacities. The preparation of the cytoplasmic antigen of *C. albicans* for counterimmunoelectrophoresis (CIE) (17) has been described elsewhere (11) and will be referred to as the M(+) antigen. Precipitating antibodies were scored before and after the gels were stained with Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, Mo.). The cytoplasmic antigen depleted of mannan was prepared from the lyophilized M(+) antigen by removing mannan determinants

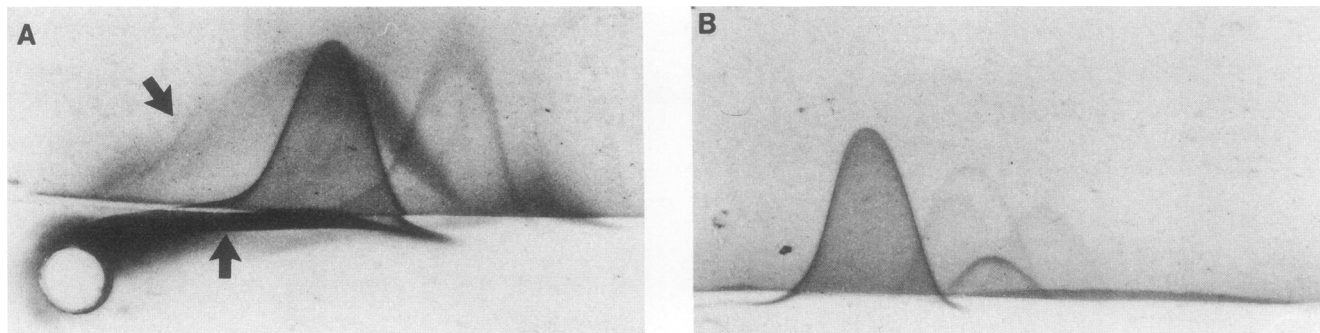


FIG. 1. CIE demonstrating antibodies against the M(+) (A) and M(-) (B) antigens. Antibodies against mannan are indicated by arrows.

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TABLE 1. Percentage of positive titers by M(+) CIE for patients with invasive (group I, $n = 13$) or superficial (group II, $n = 6$) candidiasis from whom three serum samples were available

Serum sample no.	% of positive titers by M(+) CIE ^a			
	Without staining		With staining	
	I	II	I	II
1	77.0	60.0	46.2	80.0
2	92.4	60.0	61.6	60.0
3	92.3	60.0	84.7	20.0

^a Cutoff values for positive titers were ≥ 2 without staining and ≥ 4 with staining.

by affinity chromatography on concanavalin A-Sepharose 4B (Pharmacia LKB, Uppsala, Sweden) (7) and will be referred to hereafter as the M(-) antigen. Before CIE, the M(-) antigen was examined for remaining mannan by crossed immunoelectrophoresis (XIE) (1). When the M(+) antigen was electrophoresed against a patient's serum by XIE, different precipitins were observed (Fig. 1A). As indicated by the arrow, very strong but indistinct precipitins could be observed, but very sharp but less-intense precipitins could also be observed. By tandem XIE, the large precipitin appeared to be identical to a purified immunodominant antigen, which had enolase activity (data not shown). When the M(-) antigen was used in the XIE, the strong but indistinct precipitins due to mannan residues disappeared (Fig. 1B) (25). Also, with the commercial mannan kit (Pasteur Diagnostica, Marnes-la-Coquette, France), the M(-) antigen did not react. However, in order to correlate the antibody titers obtained by the CIE by using the M(-) antigen [M(-) CIE] with those by the CIE by using M(+) antigen [M(+) CIE], the concentrations of antigens other than mannan were equalized by titration in such a way that the areas under the curves for six of the same precipitins of both antigens were the same when electrophoresed against a rabbit hyperimmune serum ($5 \mu\text{l}/\text{cm}^2$; DAKO, Glostrup, Denmark) by XIE. This titration actually resulted in equal protein concentrations of $100 \mu\text{g}/\text{ml}$ for both the M(+) and M(-) antigens, as used in the particular CIE. For M(+) CIE, antibody titers of 1:2 and 1:4 or higher were regarded as positive without and with staining, respectively. For M(-) CIE, antibody titers of 1:1 and 1:2 or higher were regarded as positive without and with staining, respectively. A serum dilution of 1:4 or higher was regarded as a positive antigen titer in the Cand-Tec assay.

The M(+) CIE results showed that for patients with invasive candidiasis, there was an increase in the percentage of positive antibody titers in the three serum samples examined (Table 1). For patients with a superficial candida infection, the titers were either stable (without staining) or decreased (with staining) with time. Antigen titers measured by the Cand-Tec, however, decreased from 50 to 36% in the same sera from patients with invasive candidiasis or remained stable in the sera from patients with superficial candidiasis.

Despite these increases in titers as determined by M(+) CIE (for patients from whom three serum samples were available, $n = 13$), statistical analysis of the sera from all patients ($n = 30$) showed no significant differences with time. The titers for serum samples 1, 2, and 3 of each patient appeared strongly correlated without ($r_1 = 0.76$), and with ($r_1 = 0.78$) staining. Therefore, to evaluate the capability of the CIE to discriminate between invasive and superficial candi-

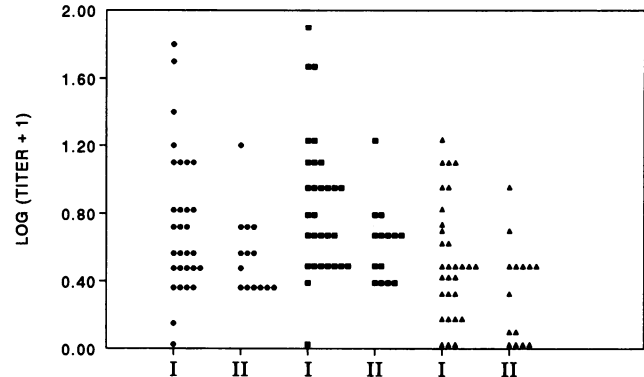


FIG. 2. Distribution of the mean log(titer + 1) obtained for each patient with invasive (group I) or superficial (group II) candidiasis. ●, M(+) CIE without staining; ■, M(+) CIE with staining; ▲, Cand-Tec.

diasis, the mean titers for serum samples 1, 2, and 3 were used after logarithmical transformation. Figure 2 shows the distribution of the mean logarithm(titer + 1) obtained for each patient.

On the basis of statistical analysis, it was observed that the M(+) CIE test without staining could not discriminate between groups I and II (Mann-Whitney, $P = 0.09$). However, significant differences were found between groups I and II ($P = 0.04$) when the gels were stained before scoring. Generally, higher values were obtained for group I. At a cutoff value of 1:4, M(+) CIE with staining had a sensitivity of 66.7% and a specificity of 57.2%. The Cand-Tec assay, on the other hand, did not discriminate between the two groups ($P = 0.12$).

By M(-) CIE, titers were lower (or absent) than those by M(+) CIE for all serum samples from patients with either invasive or superficial candidiasis (Table 2). These results confirm the findings of Matthews and Burnie (18), who found that a negative response in an antibody test may be due to the small number of antigens applied and therefore not always due to the inability of some patients to produce antibodies (20). For the patients from whom three serum samples were available ($n = 12$), an increase in positive titers between the first and the second serum samples was observed by M(-) CIE with and without staining (Table 3). However, the mean values of titers for serum samples 1, 2, and 3 for all patients did not significantly differ. Again, the serum titers were transformed as mentioned before and are

TABLE 2. Percentage of patients with invasive (group I, $n = 30$) or superficial (group II, $n = 14$) candidiasis with the indicated mean titer value for each test

Test	% of patients with indicated titer in group:					
	I			II		
	0-1	2	≥ 4	0-1	2	≥ 4
M(+) CIE						
Without staining	6.7	30.0	63.3	0.0	50.0	50.0
With staining	3.3	26.6	70.0	0.0	42.9	57.1
M(-) CIE						
Without staining	56.7	20.0	23.3	85.7	14.3	0.0
With staining	30.0	40.0	30.0	35.7	57.1	7.1

TABLE 3. Percentage of positive titers by M(-) CIE for patients with invasive (group I, $n = 12$) or superficial (group II, $n = 6$) candidiasis from whom three serum samples were available

Serum sample no.	% of positive titers by M(-) CIE ^a			
	Without staining		With staining	
	I	II	I	II
1	50.0	20.0	50.0	20.0
2	66.7	20.0	83.4	20.0
3	66.7	20.0	66.6	20.0

^a Cutoff values for positive titers were ≥ 1 without staining and ≥ 2 with staining.

depicted in Fig. 3. By statistical analysis, the mean levels of titers obtained by M(-) CIE without staining were significantly higher for invasive candidiasis than for superficial candidiasis, irrespective of a chosen cutoff value ($P = 0.005$). At a cutoff value of 1:1 for a positive titer, the test had a sensitivity of 70% and a specificity of 78.6%. M(-) CIE with staining, on the other hand, could not discriminate between groups I and II ($P = 0.06$). From the results given in Table 4, there is an actual increase in the specificity of the test, by removing mannan, from 28.6 to 78.6% and from 57.2 to 85.7% by CIE without and with staining, respectively. These results are in accordance with those of Jones, who also found that the use of more antigens, including mannan antigens, led to low specificities in antibody detection assays.

It was further shown that during the first two weeks after the first clinical signs of invasive candidiasis, titers increased, leading to higher sensitivities of the test, confirming the findings of Kostiala et al. (15). Increasing titers over time in our data, however, were not significant because of the large number of patients for whom only one or two successive serum samples were available.

Despite the fact that the M(-) CIE had a diagnostic value, the test has only moderate sensitivity. This is because nine patients (30%) with invasive candidiasis did not have an antibody response against the M(-) antigen. From six of these nine patients, only one or two serum samples were available, because of early death, within 6 to 16 days after the clinical onset of disease. From the 21 patients who did produce antibodies against the M(-) antigen, 8 died despite antibody production. However, 13 of the 14 patients who

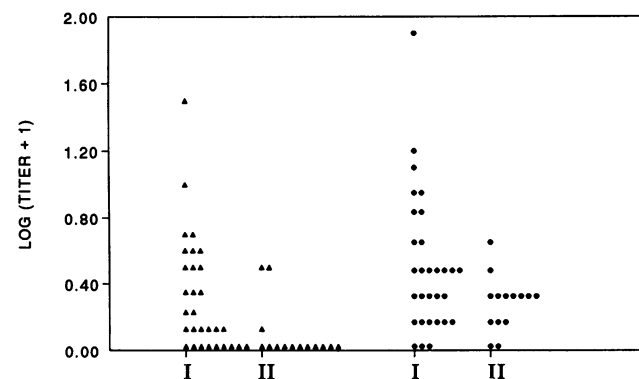


FIG. 3. Distribution of the mean $\log(\text{titer} + 1)$ obtained for each patient with invasive (group I) or superficial (group II) candidiasis. ▲, M(-) CIE without staining; ●, M(-) CIE with staining.

TABLE 4. Sensitivity and specificity of the different tests determined at a chosen cutoff value by using the mean $\log(\text{titer} + 1)$ value for groups I and II

Test	Cutoff value	Sensitivity (%)	Specificity (%)	P value ^a
Cand-Tec	≥ 4	26.7	85.7	0.46
M(+) CIE				
Without staining	≥ 2	80.0	28.6	0.43
With staining	≥ 4	66.7	57.2	0.19
M(-) CIE				
Without staining	≥ 1	70.0	78.6	0.0037
With staining	≥ 2	50.0	85.7	0.0013

^a The P value was determined by the Fisher exact test by comparing the percentages of patients with a titer above cutoff levels for groups I and II.

died produced antibodies against the M(+) antigen. Perhaps these patients produced antibodies against mannan initially but were not able or did not have the time to develop an antibody response to the M(-) antigen, including the 46/47-kDa antigen. Matthews and Burnie have suggested that antibodies against the 46/47-kDa antigen might be protective (18).

Staining of the precipitated complexes led to a general increase in the sensitivity of the test for both groups I and II. In order to maintain the discriminative capacity of the test, higher cutoff values were needed. Eventually, because of this procedure, the sensitivity of the test with staining decreased in comparison with the sensitivity of the test without staining. The data obtained with the Cand-Tec test were not as favorable as claimed in other studies (5, 9). Discrepancies may be due to the different selection criteria used for determining invasive candidiasis, since our criteria were more restrictive. Logistic regression analysis of our data did not show any improvement when the antigen and antibody results were combined.

In conclusion, we believe that for patients with surgical problems without hematological malignancies, the M(-) CIE can assist the clinician in the diagnosis of invasive candidiasis at an early stage of infection. Further study is obviously needed to assess the use of the M(-) CIE for immunocompromised patients.

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