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Diagnostic Value of Anti-*Candida* Enolase Antibodies

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An immunodominant antigen with enolase enzyme activity was purified and used for the development of an assay to detect antibodies directed against this antigen in sera from patients with either invasive candidiasis or *Candida* colonization. The Au enzyme-linked immunosorbent assay established with the *Candida* enolase antigen was able to discriminate significantly between invasive candidiasis and colonization in both immunocompetent and immunodeficient groups of patients. The test had a sensitivity of 50% and a specificity of 86% in the immunocompetent patient group. In the immunodeficient patient group, a sensitivity of 53% and a specificity of 78% were established. Antibody levels determined by a counterimmunoelectrophoresis assay with the same set of sera resulted in a better sensitivity for sera from the immunocompetent patient group but a lower specificity, i.e., 80 and 29%, respectively. The counterimmunoelectrophoresis assay of sera from the immunodeficient patient group was not able to discriminate significantly between invasive candidiasis and colonization. With the use of more serum from each patient, the sensitivity of the antibody detection assays increased, while the specificity was maintained. The increase, however, was not statistically significant. Combining the results of the antibody assays with antigen titers obtained by the Cand-Tec assay did not improve the predictive value with respect to invasive candidiasis, as determined by multivariance regression analysis. Furthermore, it was demonstrated by performance of Western blots (immunoblots) that sera from patients as well as a rabbit antiserum cross-reacted with the *Candida* enolase and baker's yeast enolase enzyme. However, by tandem crossed immunoelectrophoresis it was demonstrated that the antibodies were directed toward different epitopes of the antigen.

The use of antibacterial, immunosuppressive, and cytotoxic drugs increases the incidence of lethal invasive candidiasis in hospitalized patients (13). Despite many attempts to develop reliable serological tests, the laboratory diagnosis of deep-seated *Candida* infection still remains a problem (3, 11). Until now, antibody detection tests with crude antigens have produced suboptimal sensitivities and/or specificities (11). However, the detection of antibodies directed against specific antigens seems promising. Matthews et al. (17) demonstrated that the presence of antibodies against an immunodominant antigen of *Candida albicans* with a molecular mass of 47 kDa correlated with invasive infection. Since cytoplasmic antigens are released during invasive infection, it is conceivable that cytoplasmic antigens applied in an antibody detection assay could discriminate between invasive candidiasis and colonization. Recently, Zöller et al. (23) reported in a mixed immunocompromised and immunocompetent patient population a sensitivity and a specificity of 81.5 and 96.4%, respectively, for an antibody detection assay that made use of the partially purified 47-kDa antigen. In the present study, we purified an immunodominant antigen of *C. albicans* and used it to develop an enzyme-linked immunosorbent assay (ELISA) which discriminates positively between invasive candidiasis and colonization. Since several contradictory results have been published concerning the presence or absence of enolase activity in the immunodominant antigen, we also analyzed the purified antigen by Western blot (immunoblot) analysis and tandem crossed immunoelectrophoresis (XIE) and subsequently tested it for enolase activity.

To determine whether the kinetics of the antibody response is more important than the antibody titer itself, three successive serum samples from each patient, when available, were included for serological evaluation. The results obtained by the ELISA were compared with titers of antibodies directed against a cytoplasmic antigen-containing mannan [M(+) antigen] and to antibody titers directed against a cytoplasmic antigen depleted of mannan and glycoproteins [M(−) antigen] as determined by a counterimmunoelectrophoresis (CIE) technique. Besides antibody detection, the same serum samples were analyzed for circulating antigens by the Cand-Tec assay.

MATERIALS AND METHODS

SDS-PAGE and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed as described previously (19). In brief, proteins were separated electrophoretically in a discontinuous system as described by Laemmli (15). The electrophoresis system consisted of a 10% running gel and a 4% stacking gel, and the proteins were subsequently transferred to a polyvinyldifluoride membrane (Immobulon-P; Millipore) by the method of Towbin et al. (20). For immunoblotting, diluted rabbit antiserum raised against a crude antigen prepared from *C. albicans* CBS 5982 serotype A (Centraal Bureau voor Schimmelcultures, Delft, The Netherlands) and patient sera were used. Blots (bands) were developed with alkaline phosphatase-conjugated anti-rabbit immunoglobulin G (IgG) or anti-human IgG, respectively, by using 5-bromo-4-chloro-3-indol phosphate (Sigma Chemical Co., St. Louis, Mo.) and nitroblue tetrazolium (Sigma) as substrate.

Purification of the immunodominant *Candida* antigen. For

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purification procedures *C. albicans* CBS 5982 serotype A was grown for 48 h at 37°C in 2 liters of minimal medium (11.7 g of yeast carbon base per liter 10 g of D-glucose per liter, 20 g of Bacto Peptone [Difco Laboratories, Detroit, Mich.] per liter). Cells were harvested by centrifugation and were washed twice in distilled water. The final pellet was dissolved in 42 ml of ice-cold distilled water and was then disrupted with glass beads (diameter, 2 mm) for 15 min in a Braun shaker (B. Braun, Melsungen, Germany) under continuous CO_2 cooling. The suspension was spun down at 40,000 × *g* at 4°C for 20 min. Supernatants were stored overnight at −80°C, and after being thawed they were centrifuged at 40,000 × *g* for 20 min at 4°C. Supernatants were brought to 50% saturation by adding a saturated solution of $(NH_4)_2SO_4$. Further saturation to 67% was obtained by adding solid $(NH_4)_2SO_4$ to the supernatant. The solution was then centrifuged at 40,000 × *g* at 20°C for 20 min, and solid $(NH_4)_2SO_4$ was added to the supernatant until 100% saturation was accomplished. After 20 min at room temperature, the solution was further centrifuged at 40,000 × *g* at 20°C for 20 min. The pellet was resuspended in a Tris buffer (0.067 M; pH 7.5) and was desalted by using a Sephadex G25 column (Pharmacia LKB, Uppsala, Sweden). The protein-containing fraction was further purified by fast-performance liquid chromatography (FPLC) on a Highload Q-Sepharose high performance column (FPLC HQSHP; Pharmacia) by using a 0 to 10% gradient of 2 M NaCl in 0.067 M Tris buffer (pH 7.5). Gradients were created by using 325 ml (1 to 3%) or 150 ml (3 to 10%) of 2 M NaCl at a flow rate of 4 ml/min. Aliquots of each fraction were analyzed by SDS-PAGE, and the enolase activity in each fraction was determined. Samples containing enolase activity were pooled and exchanged with a 20 mM piperazine buffer (pH 6.0) on a Sephadex G25 column. The pooled fraction was loaded on an FPLC Mono P column (H 5/20; Pharmacia), and proteins were separated by a linear gradient from pH 6 to 4.5 in polybuffer.

Fractions containing enolase activity were analyzed by SDS-PAGE, pooled, and exchanged with a concanavalin A starting buffer (0.02 M Tris, 0.1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ [pH 7.4]) before they were applied to the concanavalin A-Sepharose column (Pharmacia) in order to remove mannan by the method of Ellsworth et al. (5). Removal of mannan was confirmed by a negative result in the mannan kit of Pasteur (Diagnostics Pasteur, Marnes-la-Coquette, France). Finally, the immunodominant antigen containing enolase activity was stored at −80°C in 0.01 M Tris buffer (pH 7.5).

Enolase activity. During the purification procedure, fractions were analyzed for enolase activity by the method of McAlister and Holland (18).

ELISA. Optimal concentrations of the antigen, sera, and conjugate were predetermined by checkerboard titrations with known positive and negative sera obtained by Western blotting. Highly activated polystyrene microtiter plates (Nunc Immunoplate Maxisorp F96) were coated with 1.5 μg of *Candida* antigen in 50 μl of coating buffer (0.13 M sodium carbonate and 0.03 M hydrogenated sodium carbonate [pH 9.6]) per well and were subsequently incubated overnight at 4°C. The plates were washed twice with 200 μl of TBS-block (0.01 M Tris, 0.15 M NaCl [pH 7.6], 0.1% gelatin) per well and were then incubated with 200 μl of TBS-block per well at 37°C for 2 h. The plates were then drained. Sera were diluted twofold in TBS (0.01 M Tris, 0.15 M NaCl [pH 7.6]) starting at a dilution of 1:200. A total of 50 μl of each serum dilution was added to each well, and the plates were subsequently incubated at 37°C for 60 min. After three cycles of washing with TBSt (TBS with 0.04% [wt/vol] gelatin and 0.05% [vol/vol] Tween 20), 50 μl of an alkaline phosphatase-labelled goat anti-human IgG conjugate (diluted 1:1,000 in TBSt [Sigma]) was added to each well; this was followed by incubation for 1 h at 37°C. A 100-μl volume of acid enzyme substrate solution (9.7% diethanolamine, 3 mM NaN_3, 5 mM $MgCl_2 \cdot 6H_2O$, 1 mg/ml *p*-nitrophenylphosphate [Sigma]) was added after three cycles of washing with TBSt, and the plates were incubated at 37°C in the dark. Color development was stopped after 30 min of incubation by adding 100 μl of 3 M NaOH to each well. The optical densities at 405 nm were determined by using an automated plate reader (Bio-Tek Instruments, Inc., Winooski, Vt.), and it was assumed that the results of the ELISA, expressed as optical densities at 405 nm of the reference serum, might fluctuate a maximum of 10%. A cutoff point was determined by analysis of 60 serum samples from healthy individuals of different age groups. The cutoff point defined the upper 5% of this population as positive by the test. Patient sera were regarded as positive when the results obtained by the *Candida* enolase ELISA were above this cutoff point at a serum dilution of 1:200. Sera with positive test results were diluted twofold until the test results became negative, a dilution of 1:200 was regarded as undiluted. Titers were defined as the highest serum dilution with a positive test result.

Tandem XIE. Tandem XIE was performed as described by Axelsen et al. (1). Briefly, glass plates (5 by 5 cm) coated with 1% agarose (Sigma) in barbital buffer (pH 8.6; $\mu = 0.02$) were used. The *Candida* enolase antigen (0.1 mg/ml) was subjected to tandem electrophoresis (10 V cm^{-1}) with the purified bakers' yeast enolase antigen (20 mg/ml; Sigma) in the first dimension. The run in the second dimension against a hyperimmune rabbit serum (7.5 μl/cm^2) (Dako, Glostrup, Denmark) or patient serum (10 μl/cm^2) was performed for 18 h at 2 V cm^{-1}. After electrophoresis, gels were washed first in 0.9% NaCl overnight and then in distilled water for 1 h. Finally, the gels were bound to agarose gelbond film (Bio-Rad Laboratories, Richmond, Calif.) and were stained with Coomassie brilliant blue R-250 (Sigma).

Standard serological tests. Antibody responses against cytoplasmic antigens in the presence or absence of mannan were performed as described previously (21). Briefly, titers of antibodies against M(+) antigen and M(−) antigen were measured by CIE and are referred to as M(+) CIE and M(−) CIE, respectively. Titers of circulating antigens were determined by the Cand-Tec assay (Ramco Laboratories Inc., Houston, Tex.) (2, 4) according to the directions of the manufacturer. A serum dilution of 1:4 or higher was regarded as a positive titer.

Patient sera. The patient sera included in the present study were collected between October 1982 and January 1991. Patients were divided into two groups on the basis of clinical findings and culture results. Group I consisted of 76 patients with confirmed invasive candidiasis. The criteria for this diagnosis were based on obtaining positive *C. albicans* cultures from tissue biopsy specimens or needle aspirates of normally sterile body cavities or at autopsy. One or more positive blood cultures were not adequate to classify a patient as having invasive candidiasis.

Group II consisted of 32 *C. albicans*-colonized patients. Colonization was established by the presence of several positive *C. albicans* cultures of samples from different parts of the gastrointestinal tract in the absence of visible lesions.

On the basis of the immune status of the patients, both groups were subdivided into patients with immunodeficien-

cies caused by therapy or underlying diseases and patients without immunodeficiencies caused by therapy or underlying diseases and patients without immunodeficiencies. The criteria for immunodeficiency were histologically confirmed neoplasms, kidney function disorders requiring dialysis, treatment with corticosteroids, total-body irradiation, or the use of immunosuppressive or cytotoxic drugs. The majority of the patients without signs of immunodeficiencies underwent major abdominal surgery. None of these patients had hematological malignancies or had received treatment with corticosteroids, immunosuppressive or cytostatic drugs, or total-body irradiation. On the basis of these criteria, the group I patients were divided into 46 patients with signs of immunodeficiency (group IA) and 30 immunocompetent patients (group IB); the 32 colonized patients were divided into those with signs of immunodeficiency (group IIA; n = 18) and those who were immunocompetent (group IIB; n = 14).

Not enough serum was available from some patients to be analyzed by all serological assays. Therefore, sera from only 41 patients with invasive candidiasis and 18 colonized patients could be evaluated by the M(+) CIE and the M(−) CIE, and sera from 44 patients with invasive candidiasis and 18 colonized patients could be evaluated by the ELISA.

Three serum samples from each patient, when available, were included for serological evaluation. The sera were obtained at weekly intervals. The first serum sample was defined as that obtained at the time of the first microbiological evidence of invasive candidiasis.

Statistical analysis. Correlations between the three serum samples obtained from each patient were assessed by using intraclass correlation coefficients (r_i). The capacity to discriminate between invasive candidiasis and colonization was evaluated by the Mann-Whitney test. The percentage of patients in groups I and II with titers above the cutoff levels were compared by Fisher's exact test. A significance level of $P = 0.05$ (two-sided) was used. Logistic regression was used as the multivariable analysis to evaluate simultaneously, the discriminating capacities of the various serological assays.

RESULTS

Isolation and characterization of the immunodominant antigen. Samples obtained during different stages of the purification procedure were examined by SDS-PAGE and Western blotting (Fig. 1). The enzyme activities in the pooled fractions obtained during the different purification steps were determined in an enolase assay. After each purification step, the specific activity of the immunodominant protein increased (Table 1). Five milligrams of pure *C. albicans* enolase could be recovered from a 2-liter culture.

Immunologic characterization of the *C. albicans* enolase antigen. By using the techniques SDS-PAGE, Western blotting, and tandem XIE, the *C. albicans* enolase was compared with the baker's yeast enolase. The baker's yeast enolase had a molecular mass of approximately 49 kDa, and the *Candida* enolase had a molecular mass of about 45 kDa (Fig. 2). These findings were confirmed by Western blotting, in which the applied polyclonal rabbit anti-*C. albicans* antiserum cross-reacted with the baker's yeast enolase. The antibodies of some of the patients with deep-seated candidiasis also cross-reacted with the baker's yeast enolase, as determined by Western blotting (Fig. 3). This cross-reactivity was further analyzed by performing tandem XIE with patient sera as well as rabbit serum. The observed cross-reactivities on Western blots must have been due to antibodies directed toward different epitopes, because the precipitated line formed by the *Candida* enolase antigen crossed the precipitated line formed by the baker's yeast enolase (Fig. 4).

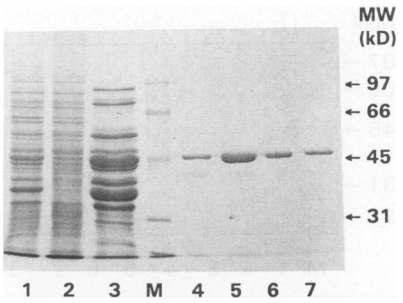

FIG. 1. SDS-PAGE of pooled fractions obtained during the antigen purification procedure. Lanes: 1, supernatant after shaking on a Braun shaker; 2, supernatant after 67% $(NH_4)_2SO_4$ saturation; 3, dissolved pellet after 100% $(NH_4)_2SO_4$ saturation; 4, *Candida* enolase fraction after FPLC HLQSHP; 5, *Candida* enolase fraction after FPLC Mono P; 6, pooled fraction after concanavalin A-Sepharose chromatography; and 7, pooled *Candida* enolase fraction used in the ELISA. M, molecular mass markers are indicated on the right (in kilodaltons).

Immunocompetent patient group. In an earlier study, we scored by Western blotting the quantitative and qualitative aspects of patient sera against the immunodominant antigen (19). To study the diagnostic accuracy of specific antibodies directed toward the immunodominant *Candida* enolase antigen, sera from patients in different groups were examined in an ELISA. Only IgG antibody titers were determined.

Antibodies directed toward the *Candida* enolase antigen studied in the immunocompetent patient group showed increased antibody titers over time. However, the increase in antibody titers with time was not significant. Since these titers were strongly correlated (r_i = 0.72), the mean titers of serum samples 1, 2, and 3 from each patient were calculated, after logarithmic transformation, and were used to discriminate between invasive candidiasis (group IB) and colonization (group IIB). Figure 5 shows the mean logarithm(titer + 1) for each patient in groups IB and IIB. A cutoff value of ≥2 for anti-*Candida* enolase antibody levels gave the best sensitivity and specificity, i.e., 50 and 86%, respectively (Table 2). When sera from healthy individuals were used as the negative control group, the specificity increased to 100%.

Antibody titers determined by the M(+) CIE, which were

TABLE 1. Enolase activities of different fractions during the *Candida* enolase antigen purification procedure

Sample no.[a]	Total amt of protein (mg)	Total activity (units)	Sp act (units/mg)	Relative purification	Yield (%)
1	900	1,050	1.17	1	100
2	210	347	1.65	1.4	33
3	50	149	2.98	2.6	14.2
4	17	96	5.65	4.8	9.1
5	14	93	6.64	5.7	8.9
6	5	61	12.2	10.4	5.8

[a] Sample numbers: 1, supernatant after shaking on a Braun shaker; 2, dissolved pellet after 100% $(NH_4)_2SO_4$ saturation; 3, *Candida* enolase fraction after FPLC HLQSHP; 4, *Candida* enolase fraction after FPLC Mono P; 5, pooled fraction after concanavalin A-Sepharose chromatography; and 6, pooled *Candida* enolase fraction used in the ELISA.

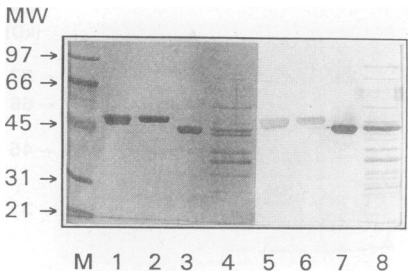

FIG. 2. Comparison of molecular masses obtained by SDS-PAGE and antigenicity by Western blotting of baker's yeast and *Candida* enolase enzymes. Lanes: 1 and 5, baker's yeast enolase; 2 and 6, baker's yeast enolase after Mono Q Sepharose column chromatography; 3 and 7, *Candida* enolase antigen; 4 and 8, partially purified *Candida* extract. Lanes 1 to 4 were stained with Coomassie brilliant blue, and lanes 5 to 8 were incubated with hyperimmune rabbit serum. M, molecular mass markers are indicated on the left (in kilodaltons).

conducted on the same sera from each patient from groups IB and IIB, gave a better sensitivity but a lower specificity, while the M(−) CIE also had a better sensitivity but a specificity comparable to that of the enolase ELISA at the indicated cutoff values (Table 2).

When the mean logarithm(titer + 1) of antigen from the serum samples from all patients was calculated, a sensitivity of 27% and a specificity of 86% were established.

When calculating the sensitivity and specificity for the first serum sample obtained from each patient group from which three serum samples were available, a sensitivity of 47% and a specificity of 71% were established. However, if the mean value for three serum samples for the same selected group of patients was calculated, the sensitivity decreased to 21% and the specificity was 100%. In both situations, the Cand-Tec did not discriminate between invasive candidiasis and colonization ($P = 0.33$ and $P = 0.53$, respectively). Three serum samples were available from 13 immunocompetent patients with invasive candidiasis and 6 immunocompetent colonized patients.

Multivariate analysis showed that the antibody assays—i.e., the *Candida* enolase ELISA, or the M(+) CIE and the antigen detection assay (Cand-Tec)—did not improve the discriminative capacity relative to that of the M(−) CIE.

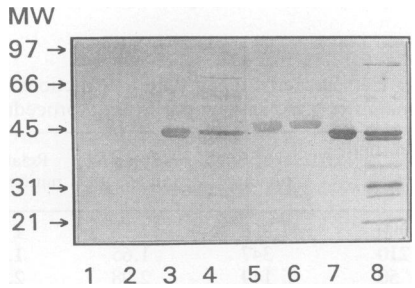

FIG. 3. Western blots of baker's yeast enolase and *C. albicans* enolase obtained by exposure to sera from two patients with invasive candidiasis. Lanes: 1 and 5, baker's yeast enolase; 2 and 6, baker's yeast enolase after Mono Q Sepharose column chromatography; 3 and 7, *Candida* enolase antigen; 4 and 8, partially purified *Candida* extract. Lanes 1 to 4 and 5 to 8 were incubated with sera obtained from two patients with invasive candidiasis, respectively. Numbers on the left are molecular masses (in kilodaltons).

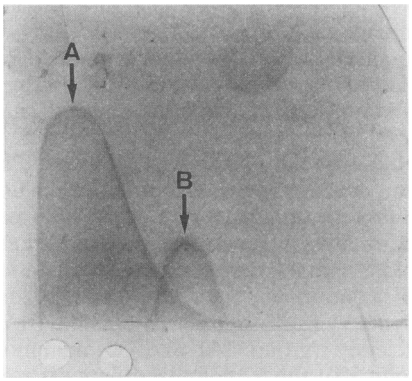

FIG. 4. Tandem XIE demonstrating rabbit antibodies directed toward the *Candida* enolase (arrow A) and the baker's yeast enolase (arrow B).

Immunodeficient patient group. In comparison with the titers in sera from the immunocompetent patient group, anti-*Candida* enolase antibody titers in the sera of immunodeficient patients did not increase significantly over the 2-week interval of observation. Again, the titers in serum were transformed as mentioned above, and the mean logarithm(titer + 1) values are depicted in Fig. 6. Statistical analysis of these data showed that the mean logarithm(titer + 1) levels obtained by the *Candida* enolase ELISA were significantly higher for patients with invasive candidiasis than patients colonized with *C. albicans* ($P = 0.03$; Mann-Whitney test) without a chosen cutoff value. At a cutoff value of ≥1, the test had a sensitivity of 53% and a specificity of 78% (Table 3). Again the specificity of the assay increased when sera from healthy individuals were used as the negative control group (Table 3). Although the increase in antibody titers with time was not significant, the sensitivity of the test for a mean of three serum samples increased from 42 to 54% for the first serum sample obtained from each patient, while the specificity remained constant (78%).

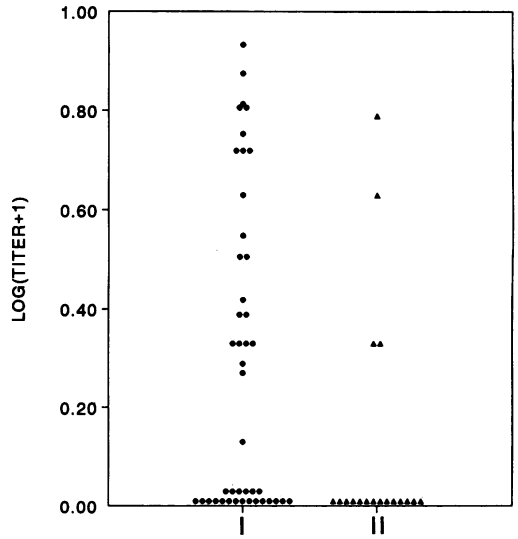

FIG. 5. Distribution of the mean logarithm(titer + 1) of anti-*Candida* enolase antibodies in the sera of each immunocompetent patient with invasive candidiasis (I) or *Candida* or colonization (II).

TABLE 2. Sensitivities and specificities of the different tests determined at a chosen cutoff value by using the mean logarithm(titer + 1) values obtained for groups I and II of the immunocompetent patient group

Serological assay	Cutoff value	Sensitivity (%)	Specificity (%)	*P* value[a]
M(+) CIE	≥2	80	29	0.4
M(−) CIE	≥1	70	79	0.004
Enolase ELISA	≥2	50	86	0.02
Enolase ELISA	≥2	50[b]	100[b]	3.3×10^{-9}
Cand-Tec	≥4	27	86	0.46

[a] P values were determined by the Fisher exact test by comparing percentages of patients in groups IB and IIB whose sera had titers above the cutoff levels.
[b] Sera from healthy individuals were used to calculate the sensitivity, the specificity, and the *P* value.

TABLE 3. Sensitivities and specificities of the different tests determined at a chosen cutoff value by using the mean logarithm(titer + 1) values obtained for groups I and II of the immunodeficient patient group

Serologic assay	Cutoff value	Sensitivity (%)	Specificity (%)	*P* value[a]
M(+) CIE	≥2	69	39	0.79
M(−) CIE	≥1	31	83	0.35
Enolase ELISA	≥1	53	78	0.046
Enolase ELISA	≥1	53[b]	95[b]	2.0×10^{-8}
Cand-Tec	≥4	54	78	0.026

[a] P values were determined by the Fisher exact test by comparing percentages of patients in groups IA and IIA whose sera had titers above the cutoff levels.
[b] Sera from healthy individuals were used to calculate the sensitivity, the specificity, and the *P* value.

The results obtained by the M(+) CIE, M(−) CIE, and Cand-Tec antigen detection assays in the immunodeficient patient group are depicted in Table 3. The *Candida* enolase ELISA was the only antibody detection assay that was able to discriminate between invasive candidiasis and colonization. By multivariate analysis, it was found that the antigen detection assay Cand-Tec was somewhat better at discriminating invasive candidiasis and colonization. Combining the results of Cand-Tec with the those of *Candida* enolase ELISA did not improve the discriminative capacity relative to that of the Cand-Tec assay alone.

DISCUSSION

It is known from the literature and from our own investigations that antibody responses against the 47-kDa antigen are frequently found in the sera of patients with invasive candidiasis (17, 19). In order to develop a quantitative assay, we purified the immunodominant antigen and coated it onto ELISA plates in an attempt to try and detect specific antibodies directed against this antigen. Furthermore, the purified antigen was characterized by different techniques to solve the enigma as to whether we were dealing with the 47-kDa immunodominant antigen or an enolase antigen or whether both characteristics were gathered into the same antigen.

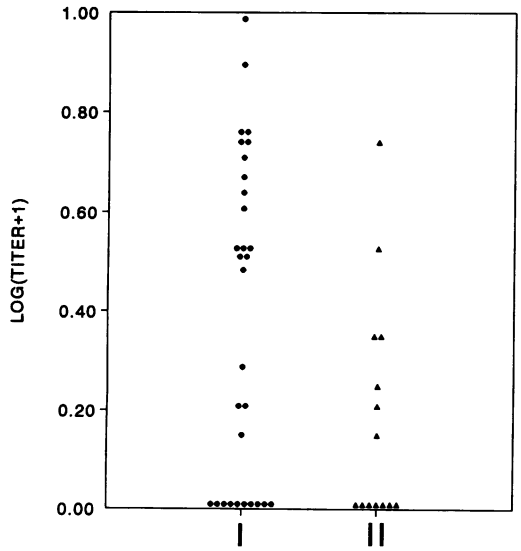

FIG. 6. Distribution of the mean logarithm(titer + 1) of antibodies directed toward the *Candida* enolase in the sera of immunodeficient patients with invasive candidiasis (I) or colonization (II).

From the results that we observed, we must conclude that the molecular mass of the immunodominant antigen is 45 kDa and that our purified immunodominant antigen also has enolase activity. These findings have also been reported by others (7, 12, 16, 22). In accordance with the results found by Mason et al. (16), we also conclude from the results of the immunoblot analysis that antigenic cross-reactivity exists between our 45-kDa antigen and a commercial baker's yeast enolase. Recently, Kortekangas-Savolainen et al. (14) also demonstrated by Western blotting the cross-reactivity of IgE antibodies directed toward a 48-kDa band of *Saccharomyces cerevisiae*, a 46-kDa band of *C. albicans*, and the purified baker's yeast enolase (14). However, from our tandem XIE results we concluded that these cross-reacting antibodies are directed against different epitopes, which was also reported by Franklyn et al. (7).

The *Candida* enolase antibody ELISA was reproducible, as measured by day-to-day variation tests, and was specific for antibodies directed against *Candida* enolase antigen, as determined by inhibition experiments (data not shown). The *Candida* enolase antibody ELISA had a diagnostic value at a cutoff point of ≥2 in the immunocompetent patient group, with an acceptable specificity (86%) but a low sensitivity (50%). Multiple serum samples were necessary to maximize detection, as observed by the fact that the sensitivity increased slightly, from 47 to 50%, when more than one serum sample was used to determine the diagnostic accuracy of the test, whereas the specificity was maintained (86%). In this immunocompetent patient group, an increase in specificity was observed when compared with the data obtained by the M(+) CIE and, to a lesser extent, the M(−) CIE. Despite the use of a more sensitive detection assay, i.e., ELISA as opposed to the CIE, the increase in specificity of the enolase test was counteracted by the loss of sensitivity, i.e., a decrease from 80 to 50%, compared with that of the M(+) CIE.

For the immunodeficient group, the *Candida* enolase ELISA showed significantly higher titers in the sera of patients with invasive candidiasis than in the sera of patients colonized with *C. albicans*. However, the sensitivity of the assay for sera from the immunodeficient patient group was also low (53%), with a specificity of 78%. In the immunodeficient patient group, patients with leukemia did not respond at all with antibodies directed toward the *Candida* enolase

antigen. Consequently, when all leukemic patients in both subgroups IA and IIA were excluded from the immunodeficient patient group for analysis, the assay's sensitivity (64%) as well as specificity (90%) increased ($P = 0.0036$; Fisher exact test).

Greenfield et al. (9) also reported a low sensitivity (25%) and a high specificity (97.8%) for their assay, which was based on a purified 54-kDa major cytoplasmic antigen. On the other hand, Zöller et al. (23) reported a sensitivity of 81.5% and a specificity of 96.4% for their ELISA with 47- to 29-kDa *C. albicans* antigens. The difference between their data and our own is probably due to the different criteria used for patients with invasive candidiasis as well as the fact that patients with immunodeficiencies and immunocompetent patients were regarded as a homogeneous group. The use of different cutoff values, which is necessary for the correct evaluation of the titers obtained for immunocompetent patients and immunodeficient patients, also explains the different interpretation of the results obtained for these groups.

The Cand-Tec assay had a good discriminating capacity for sera from the immunodeficient patient group. However, the sensitivity and specificity of the Cand-Tec assay were not as favorable as those determined by other investigators (4, 8); the sensitivity of the test for sera from both patient groups (IA and IB) was low, while the specificity was acceptable. Discrepancies may have been due to different selection criteria used for determining invasive candidiasis. The test is probably more useful during the early stage of disease, since additional sera from each patient, which were obtained later in the course of the invasive *Candida* infection, did not improve the diagnostic sensitivity of the test. This was also found by Herent et al. (10).

Previously reports of antibody detection assays with crude antigens for the detection of deep-seated candidiasis always showed that the tests suffered from suboptimal sensitivities or specificities (11). The lack of specificity is due to the fact that most healthy individuals already have antibodies directed against cell wall components of *C. albicans* (6). Therefore, it is conceivable that the use of a purified, well-defined antigen rather than a crude extract containing cell wall polysaccharide provides a more specific test for systemic candidiasis. Since it has already been demonstrated that the enolase antigen is released during the progression of infection and that it elicits a specific antibody response (17), it would be conceivable that this particular antigen could be applied in an antibody detection assay. In the present study, we demonstrated that the detection of antienolase antibodies results in a discriminative test with a low sensitivity but an acceptable specificity. Because of its good specificity, results of this particular test, in contrast to previously described assays, support the clinician in making a decision whether to start with toxic antifungal agents. Whether antienolase antibodies can be used in monitoring the response to treatment and in determining the duration of therapy must be investigated.

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