

Evaluation of a Novel Enzyme-Linked Immunosorbent Assay To Detect Immunoglobulin G Antibody to Enolase for Serodiagnosis of Invasive Candidiasis[∇]

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The performance of a new test to detect antibodies to *Candida albicans* recombinant enolase was investigated in 47 immunocompromised and 51 immunocompetent patients. The sensitivity, specificity, and positive and negative predictive values of the test for the diagnosis of invasive candidiasis were 81.0, 83.9, 79.1, and 85.5%, respectively.

Invasive candidiasis is the most severe clinical presentation of *Candida* infections and a major cause of morbidity and mortality in critically ill and immunocompromised patients. The rate of candidemia has increased substantially in the United States and Europe, ranking as the fourth or fifth most common cause of bloodstream infections (1, 2). Depending on the hospital ward, the mortality rate attributable to candidemia ranges from 49 to 61% (4). The diagnosis of invasive candidiasis is difficult due to the lack of specific clinical features and to the low sensitivity of blood culture for isolation of *Candida* species, especially in patients receiving fluconazole prophylaxis (6).

Detection of fungal DNA by use of PCR (19), (1-3)- β -D-glucan (10, 11), cell wall and cytoplasmic circulating antigens (16, 20), and antibodies against different *Candida* antigens, including mannan, germ tube-specific antigens, and enolase (3, 5, 8, 12–14, 18), have all been investigated for the serodiagnosis of invasive candidiasis, but none has yet achieved broad validation.

In the present study we evaluated the diagnostic potential of a new and commercially available enzyme-linked immunosorbent assay (ELISA) to detect antibodies against *Candida* enolase for the serodiagnosis of invasive candidiasis.

We retrospectively studied 98 different adult hematological cancer or intensive care unit patients at increased risk for invasive candidiasis. Patients were divided into two groups according to their clinical and microbiological diagnostic data. Group I included 42 patients (224 sera) with invasive candidiasis proved by positive blood culture for *Candida* spp. or histopathology. The *Candida* species distribution was as follows: *C. albicans*, 25 of 42; *C. parapsilosis*, 7 of 42; *C. tropicalis*, 3 of 42; coinfection with *C. albicans* and *C. glabrata*, 1; *C. guilliermondii*, *C. utilis*, *C. dubliniensis*, and *C. krusei*, 1 each; and

Candida spp., 2 of 42. Group II was a control group with 56 different adult patients (214 sera) with no clinical or microbiological evidence of invasive candidiasis. Colonization was established by the presence of positive *Candida* cultures from mucosal specimens. On the basis of the immune status of the patients, both groups were subdivided into patients with immunodeficiencies caused by therapy or underlying diseases and patients without immunodeficiency. Group I patients were divided into 19 patients with signs of immunodeficiency (group IA) and 23 immunocompetent patients (group IB). The group II patients were divided into those with signs of immunodeficiency (group IIA; $n = 28$) and those who were immunocompetent (group IIB; $n = 28$). All of the sera were stored at -20°C until use.

Antibodies directed to *C. albicans* recombinant enolase were detected by the commercial *Candida* Enolasa ELISA Immunoglobulin G (IgG) kit (Laboratorios Vircell, Granada, Spain), according to the manufacturer's instructions. Each serum was tested in triplicate. The absorbance at 490 nm was measured in an automated ELISA plate reader (Microplate Autoreader; Bio-Tek Instruments). To avoid run-to-run variations, results were expressed as a relative absorbance index calculated by dividing the absorbance of the sample by the absorbance of a reference serum. The sensitivity, specificity, and positive and negative predictive values were calculated as described by Kozinn et al. (7). Mean values of relative absorbance of groups were compared by using the Student t test (Microsoft Excel); P values of <0.05 were considered statistically significant.

Both immunocompetent and immunocompromised patients produced similar amounts of anti-*C. albicans* enolase antibodies (the mean relative absorbances \pm the standard deviations were 0.9 ± 0.77 and 0.8 ± 0.65 , respectively). The performances of the test were similar in both groups, and the selected cutoff (mean of the relative absorbance plus three times the standard variation of group 2 sera) allowed differentiation between patients with invasive candidiasis and patients without invasive candidiasis in both groups.

The detection of antibodies to the *C. albicans* enolase was slightly more sensitive but less specific for the diagnosis of

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TABLE 1. Diagnostic performance of *Candida* Enolase ELISA IgG with immunocompetent and immunocompromised patients

Population (no. of subjects)	No. of patients ^a				%		Predictive value (%)		Diagnostic accuracy (%)
	TP	TN	FP	FN	Sensitivity	Specificity	Positive	Negative	
Immunocompetent (51)	19	22	6	4	82.6	78.6	76	84.6	80.4
Immunocompromised (47)	15	25	3	4	78.9	89.3	83.3	86.2	85.1
Total population (98)	34	47	9	8	81.0	83.9	79.1	85.5	82.7

^a TP, true positive; TN, true negative; FP, false positive; FN, false negative.

invasive candidiasis in the immunocompetent group of patients than in the immunocompromised group (Table 1). The sensitivity, specificity, and positive and negative predictive values of the test for the diagnosis of invasive candidiasis in the whole population studied were 81.0, 83.9, 79.1, and 85.5%, respectively (Table 1).

In a limited number of patients, the availability of serial serum samples allowed us to investigate whether the detection of antibodies to the *C. albicans* enolase by the *Candida* Enolase ELISA IgG kit anticipated the diagnosis made by blood culture. Interestingly, the detection of antibodies to the *C. albicans* enolase anticipated the blood culture for 10 of 17 patients studied.

Mannan and enolase are probably the most immunogenic antigens of *Candida* (8). Detection of antibodies against extracts containing enolase or purified enolase has been investigated to help in the diagnosis of invasive candidiasis, since they elicit strong humoral responses (9, 12, 17, 18). Published reports have shown that detection of antibodies to purified enolase allows the detection of invasive candidiasis with a sensitivity of 50 to 92.5% and a specificity of 78 to 95% (9, 18). The results presented here confirm these data using recombinant enolase. Since immunocompromised patients have an increased risk for developing invasive candidiasis and they may produce lower antibody titers than immunocompetent patients, we investigated the performance of the test in two patient populations: one immunocompromised and the other immunocompetent. However, the performances of the test were similar in both patient populations. These results are in agreement with those reported by van Deventer et al. (18), who detected anti-enolase antibodies in both immunocompromised and immunocompetent patients, and with those reported by our group detecting antibodies to *C. albicans* germ tubes in both patient populations (15).

In summary, the *Candida* Enolase ELISA IgG kit is useful for the diagnosis of invasive candidiasis, providing an objective, simple, and rapid method. A prospective, multicenter evaluation of this kit is needed to further evaluate its full clinical utility.

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