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 mannann, 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, proven 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, 5 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 IA; n = 28) and those who were immunocompetent (group IB; 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 Enolase ELISA Immuglobulin 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 ± 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
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 Enolasa 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 Enolasa 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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REFERENCES
1. Asensio, A., R. Canton, J. Vaque, J. Rossello, and J. L. Arribas. 2002. Etiology of hospital-acquired infections in Spanish hospitals (EPINE, 1990-1999). Med. Clin. 118:725–730.
2. Edmond, M. B., S. E. Wallace, D. K. McClish, M. A. Pfaller, R. N. Jones, and R. P. Wenzel. 1999. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin. Infect. Dis. 29:239–244.
3. Garcia-Ruiz, J. C., M. C. Arilla, P. Reguera, M. Quindos, A. Alvarez, and J. Ponton. 1997. Detection of antibodies to Candida albicans germ tubes for diagnosis and therapeutic monitoring of invasive candidiasis in patients with hematologic malignancies. J. Clin. Microbiol. 35:3264–3267.
4. Guinovarcz, O., S. Gillespie, K. Lee, B. J. Vande, J. Hu, S. Messer, L. Herwaldt, M. Pfaffer, and D. Diekema. 2003. Attributable mortality of nosocomial candidemia, revisited. Clin. Infect. Dis. 37:1172–1177.
5. Jones, J. M. 1980. Kinetics of antibody responses to cell wall mannans and a major glycoprotein antigen of Candida albicans in rabbits and humans. J. Lab. Clin. Med. 96:845–860.
6. Kami, M., U. Machida, K. Okuzumi, T. Matsumura, S. S. Mori, A. Hori, T. Kashima, Y. Kanda, Y. Takaue, H. Sakamaki, H. Hirai, A. Voneyama, and Y. Mutou. 2002. Effect of fluorocytosine prophylaxis on fungal blood cultures: an autopsy-based study involving 720 patients with haematological malignancy. Br. J. Haematol. 117:40–46.
7. Kozinn, P. J., C. L. Tsachdjian, P. K. Goldberg, W. P. Protzmann, D. W. Mackenzie, J. S. Remington, S. Anderson, and M. S. Selig. 1978. Efficiency of serologic tests in the diagnosis of systemic candidiasis. Am. J. Pathol. 78:893–898.
8. Mason, A. B., H. R. Buckley, and J. A. Gorman. 1993. Molecular cloning and characterization of the Candida albicans enolase gene. J. Bacteriol. 175:2632–2639.
9. Mitsutake, K., S. Kohno, T. Miyazaki, H. Miyazaki, S. Maesaki, and H. Koga. 1994. Detection of Candida albicans antibody in patients with candidiasis. J. Clin. Lab. Anal. 8:207–210.
10. Osterosky-Zeichner, L., B. D. Alexander, D. H. Kett, J. Vazquez, P. G. Papas, F. Sacki, P. A. Ketchum, J. Wingard, R. Schiff, H. Tabara, M. A. Finkelman, and J. H. Rex. 2005. Multicenter clinical evaluation of the (1–3)-beta-D-glucan assay as an aid to diagnosis of fungal infections in patients with cancer. J. Clin. Microbiol. 43:654–659.
11. Pazos, C., M. D. Moragues, G. Quindos, J. Pontón, and A. del Palacio. 2006. Diagnostic potential of detection of (1–3)-beta-D-glucan and antibodies to Candida albicans germ tubes for diagnosis and therapeutic monitoring of invasive candidiasis in neutropenic adult patients. J. Clin. Microbiol. 44:209–215.
12. Philip, A., Z. Odabasi, G. Matsuishi, V. L. Paetznick, S. W. Tan, J. Warmington, J. H. Rex, and L. Osterosky-Zeichner. 2005. Syscan3, a kit for detection of anti-Candida antibodies for diagnosis of invasive candidiasis. J. Clin. Microbiol. 43:4834–4835.
13. Pontón, J., M. D. Moragues, and G. Quindos. 2002. Non-culture based diagnostics, p. 395–425. In R. A. Calderone (ed.), Candida and candidiasis. American Society for Microbiology, Washington, DC.
14. Prella, M., J. Bille, M. Pugnal, F. Duvósín, M. Cavassini, T. Calandra, and D. Marchetti. 2005. Early diagnosis of invasive candidiasis with mannan antigenemia and antimanann antibodies. Diagn. Microbiol. Infect. Dis. 51:95–101.
15. Quindos, G., M. D. Moragues, and J. Pontón. 2004. Is there a role for antibody testing in the diagnosis of invasive candidiasis? Rev. Iberoam. Micol. 21:10–14.
16. Sendi, B., M. Tabouret, J. L. Poirot, D. Mathieu, J. Fruit, and D. Pouliant. 1999. New enzyme immunoassays for sensitive detection of circulating Candida albicans mannan and antimanann antibodies: useful combined test for diagnosis of systemic candidiasis. J. Clin. Microbiol. 37:1510–1517.
17. Strockbine, N. A., M. T. Largen, S. M. Zweibel, and H. R. Buckley. 1984. Identification and molecular weight characterization of antigens from Candida albicans that are recognized by human sera. Infect. Immun. 40:75–81.
18. van Deventer, A. J., H. J. van Wiet, C. H. Hop, and W. H. Goessens. 1994. Diagnostic value of anti-Candida enolase antibodies. J. Clin. Microbiol. 32:4323–4325.
19. Wahyuningsih, R. H., J. Freisleben, H. G. Sonntag, and P. Schnitzler. 2000. Simple and rapid detection of Candida albicans DNA in serum by PCR for diagnosis of invasive candidiasis. J. Clin. Microbiol. 38:3016–3021.
20. Walsh, T. J., J. W. Hathorn, J. D. Sobel, W. G. Merz, V. Sanchez, S. M. Maret, H. R. Buckley, M. A. Pfaller, R. Schauele, and C. Silva. 1991. Detection of circulating Candida enolase by immunosassay in patients with cancer and invasive candidiasis. N. Engl. J. Med. 324:1026–1031.

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

| Population (no. of subjects) | No. of patients* | Sensitivity | Specificity | Positive Predictive value (%) | Diagnostic accuracy (%) |
|-----------------------------|----------------|------------|------------|-----------------------------|------------------------|
| Immunocompetent (51)        | 19             | 82.6       | 78.6       | 76                          | 84.6                   | 80.4                   |
| Immunocompromised (47)      | 15             | 78.9       | 89.3       | 83.3                        | 86.2                   | 85.1                   |
| Total population (98)       | 34             | 81.0       | 83.9       | 79.1                        | 85.5                   | 82.7                   |

* TP, true positive; TN, true negative; FP, false positive; FN, false negative.