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# Circulating Candida Antigens and Antibodies: Useful Markers of Candidemia

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**To investigate the utility of the 48-kDa antigen from *Candida albicans* in its commercial form (Directigen; Becton Dickinson) and three other serodiagnostic methods (detection of one antigen by Pastorex Candida [Sanofi Diagnostics Pasteur] and detection of immunoglobulin G [IgG] and IgM antibodies to *C. albicans* blastoconidia [bioMerieux]) for diagnosis of invasive *Candida* infection, we conducted a prospective clinical trial among 10 patients with candidemia (group 1), 30 patients colonized by *C. albicans* (group 2), 20 patients with bacteremia (group 3), and 20 subjects without clinical or microbiological evidence of infection. The Directigen system was positive for at least one serum sample each from eight patients in group 1. In groups 2, 3, and 4, it was positive for only three patients. There was no reaction to the Pastorex system in any of the patients infected with or colonized by *C. albicans* or in the non-*Candida*-carrying controls. The IgG antibody concentration oscillated between 100 and 800 (mean, 510 ± 268) IU/ml for the patients in group 1. In this group, eight patients had IgG antibody levels of >400 IU/ml. The percentages of persons with IgG antibody levels of >400 IU/ml in groups 2, 3, and 4 were 43.3, 0, and 0, respectively. Specific IgM antibody was present in all group 1 patients but not in those in groups 2, 3, and 4. The sensitivity and specificity of the Directigen test were 65 and 97.1%, respectively. For the Pastorex test, the sensitivity was 0%. The sensitivity of IgG antibodies was 80%, with a specificity of 81.4%, while the IgM antibodies were 100% specific and sensitive. Both the positive and negative predictive values of specific IgM antibodies appeared to be superior to those of the other three tests.**

Invasive candidiasis is difficult to diagnose and is the cause of substantial morbidity and mortality in immunosuppressed patients or in those superinfected with the fungus (12). The only currently available, reliable diagnostic aid for detection of invasive candidiasis is open biopsy of deep tissue. Blood cultures are frequently negative with proven deep visceral candidiasis, while patients with central lines or otherwise colonized are positive (11, 20). Among the systems available for detection of fungemia by *Candida* spp. are an immunodiagnostic assay for antibodies to mannoprotein and mannan or for the antigens themselves (12) and detection of serum arabinitol or mannose by gas-liquid chromatography (3, 10, 15). An immunodominant cytoplasmic 48-kDa antigen (*Candida* enolase antigen) has recently been identified and determined to be present in many patients with invasive candidiasis (1, 22). To investigate the utility of this 48-kDa antigen in its commercial form (Directigen; Becton Dickinson) and three other serodiagnostic reagents for invasive *Candida* infection (the detection of one antigen and the two remaining antibodies), we conducted a prospective clinical trial among patients with suspected candidemia.

Eighty patients at high risk for disseminated candidiasis were studied. Two serum samples from each were evaluated for the presence of *Candida albicans* antigens by two methods and for the presence of both immunoglobulin G (IgG) and IgM antibodies to *C. albicans* blastoconidia. Patients were divided into the following four groups: 1, 10 patients with proven first-time *C. albicans* sepsis as evidenced by three positive blood cultures (Becton Dickinson) (Table 1); 2, 30 patients colonized by *C. albicans* (colonization was defined as the presence of *C. albicans* isolated from mucosal surfaces only when there was no evidence of deep invasive infection); 3, 20 patients with bacteremia and no evidence of *Candida* infection; 4, 20 subjects who were considered to have no clinical or microbiologic evidence of infection. When the hemoculture was positive (groups 1 and 3) and when the subject showed no signs of fever (groups 2 and 4), two serum samples were collected (separated by 48 h) and frozen at −70°C. The following detection methods were used: Directigen (liposome immunoassay for detection of the *C. albicans* 48-kDa protein antigen); Pastorex Candida (latex particles conjugated to an anti-mannan monoclonal antibody; Sanofi Diagnostics Pasteur), and Candida-Spot IFA (uses a *C. albicans* blastoconidial clone VW32 slide and fluorescein-labelled anti-IgG or -IgM human globulin; bioMerieux). Results for IgG were expressed as reciprocal final titers (initial dilution, 1/100). Standardization of this test was achieved by use of a pool of sera from patients with candidiasis (bioMerieux). IgG concentrations of >400 IU/ml are considered indicative of candidemia by the manufacturer. IgM antibody was determined at an initial dilution of 1/10 and expressed as either positive or negative. The identity of IgM was confirmed with anti-IgG Absorbent RF (Behring Institute).

The Directigen system was positive for at least one serum sample from each of the patients in group 1, except for patients 3 and 10 (Table 2). In groups 2, 3, and 4, it was positive only four times (three patients) (Table 2). There was no reaction to the Pastorex system for any of the infected or colonized patients (groups 1 and 2) or for the non-*Candida*-carrying controls (groups 3 and 4) (Table 2). The IgG

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TABLE 1. Information about patients with candidemia (group 1)

| Patient | Final outcome | Underlying disorder |
|---|---|---|
| 1 | Cure | Chronic pancreatitis |
| 2 | Cure | Bronchopneumonia |
| 3 | Cure | Duodenostomy |
| 4 | Death | Neoplasm |
| 5 | Death | Premature birth |
| 6 | Cure | Neoplasm |
| 7 | Cure | Neoplasm |
| 8 | Cure | Neoplasm |
| 9 | Cure | Neoplasm |
| 10 | Cure | Neoplasm |

antibody concentration oscillated between 100 and 800 (mean, 510 ± 268) IU/ml for patients in group 1. In this group, eight patients (80%) had IgG antibody levels of >400 IU/ml, the positivity threshold suggested by the manufacturer. The percentages of IgG antibody levels that were >400 IU/ml in groups 2, 3, and 4 were 43.3, 0, and 0, respectively. Specific IgM antibody was present in all group 1 patients but not in those from groups 2, 3, and 4 (Table 2). The sensitivity and specificity of the tests were calculated for each patient. The sensitivity and specificity of the Directigen test were 65 and 97.1%, respectively. For the Pastorex test, the sensitivity was 0%. The sensitivity of IgG antibodies was 80%, with a specificity of 81.4%, while the IgM antibodies were 100% specific and sensitive. While the negative predictive value of specific IgG antibodies appeared to be excellent, both the positive and negative predictive values of specific IgM antibodies appeared to be superior to those of the other three tests (Table 3).

The diagnosis of invasive candidiasis is extremely difficult both clinically and microbiologically, and the role of attendant candidemia is often hard to discern (12). To shed light on this problem, some serodiagnostic methods based upon detection of antigens or antibodies to *C. albicans* have been proposed (1–10, 12, 14–22). Although commercial kits are

TABLE 2. *C. albicans* antigen and antibody results for patients with candidemia (group 1), *Candida* colonization (group 2), or bacteremia (group 3) and healthy subjects (group 4)

| Group (no. of patients)[a] | No. of samples positive/total | | IgG concn (IU/ml)[b] | No. of samples IgM positive/total |
|---|---|---|---|---|
| | Directigen | Pastorex | | |
| 1 (10) | | | | |
| 1 | 2/2 | 0/2 | 800 | 2/2 |
| 2 | 1/2 | 0/2 | 200 | 2/2 |
| 3 | 0/2 | 0/2 | 400 | 2/2 |
| 4 | 2/2 | 0/2 | 800 | 2/2 |
| 5 | 2/2 | 0/2 | 100 | 2/2 |
| 6 | 1/2 | 0/2 | 400 | 2/2 |
| 7 | 2/2 | 0/2 | 400 | 2/2 |
| 8 | 2/2 | 0/2 | 800 | 2/2 |
| 9 | 1/2 | 0/2 | 800 | 2/2 |
| 10 | 0/2 | 0/2 | 400 | 2/2 |
| 2 (30) | 0/60 | 0/60 | 545 ± 140 | 0/60 |
| 3 (20) | 1/40 | 0/40 | 100 ± 50 | 0/40 |
| 4 (20) | 3/40 | 0/40 | 110 ± 60 | 0/40 |

[a] Values for individual patients in group 1 and for all of the patients in groups 2 to 4 are shown.
[b] Mean or mean ± standard deviation.

TABLE 3. Sensitivity, specificity, and positive and negative predictive values, calculated per patient, of Directigen, Pastorex, and IgG and IgM antibodies to *C. albicans*

| Test | % Sensitivity | % Specificity | Positive predictive value (%) | Negative predictive value (%) |
|---|---|---|---|---|
| Directigen | 65 | 97.1 | 76.5 | 95.1 |
| Pastorex | 0 | | | |
| IgG antibody | 80 | 81.4 | 38.1 | 96.6 |
| IgM antibody | 100 | 100 | 100 | 100 |

available, tissue invasion by *C. albicans* cannot be reliably detected by testing for the presence of a specific antigen (12). These include the latex test for mannan detection and agglutination with liposomes to detect the 48-kDa cytoplasmic protein antigen. The sensitivity of both tests is improved when serial assays using multiple consecutive sera are used. Methods involving antibodies also appear to be more effective when performed in series. For example, a negative finding with hemagglutination-based antibody tests rules out the possibility of *C. albicans* infection (12). Our findings indicate that concentrations of *C. albicans* blastoconidium-specific IgG antibody levels higher than 400 IU/ml are observed in the majority of patients with disseminated candidiasis. Perhaps of equal importance is the high predictive value of a negative result. We also demonstrated that *C. albicans* blastoconidial IgM antibodies showed very high sensitivity and specificity for detection of invasive candidiasis. We must consider, however, the fact that these patients had not suffered any other previous candidemia; thus, the efficiency of this test is limited to a first-time infection. It would be interesting to study specific IgM production during reinfection and the duration of these IgM antibodies should they appear. Several new comparative reports have proposed the clinical utility of investigations concerning the cytoplasmic *C. albicans* 48-kDa antigen (22) and detection of the *C. albicans* mannan antigen by latex (13) in subjects with or without invasive candidiasis. We have compared the reliability of four methods (two antigen detection and two antibody detection methods) for the diagnosis of disseminated candidiasis. The cytoplasmic antigen detection kit (Directigen) had moderate sensitivity and high specificity in our population. Walsh et al. (22) obtained values similar to ours (sensitivity, 64%; specificity, 96%), but these values increased in cases of invasive candidiasis. In our study, the Pastorex kit could not detect the *Candida* mannan antigen and did not show adequate sensitivity for diagnosis in either the disseminated-infection or colonized patient group. Nevertheless, Herent et al. (13) recommended this test because it was moderately sensitive, although it was difficult to recognize its value considering the complexity of the results. In addition, in this study a clear evaluation of the kits was further hindered by the absence of clearly defined patient and control populations. Several factors may have contributed to the false-negative determinations of invasive candidiasis in our study (group 1, patients 3 and 10), such as low concentrations of the *Candida* antigen, antibody-mediated clearance of the antigen, and infrequent sampling. Our results showed that the timing of serum collection and the number of samples were particularly important in obtaining a positive result. Specimens were obtained from both patients when the hemocultures were positive, but by that time the antigen could have cleared. It is apparent from our results that two antigen-negative serum samples do not

exclude a diagnosis of candidiasis. We therefore suggest that a large number of specimens be analyzed. Processing should involve a maximum of one freeze-thaw cycle before testing, since repeated cycles of freezing and thawing are known to denature and diminish detectable antigen activity.

In conclusion, when we compared four methods, two involving antigen detection and two involving antibody detection, for serodiagnosis of invasive candidiasis, the most useful markers in patients with first-time *C. albicans* sepsis proved to be *C. albicans* blastoconidial IgM antibodies. Given the apparent complementarity of detection of candidemia and production of IgM antibodies, to adequately detect invasive candidiasis multiple serum samples must be obtained for an antibody assay, either with each set of blood cultures or later. In patients with suspected candidemia, investigation of IgG and IgM antibodies and the 48-kDa antigen is proposed. The cost of this multiple testing may perhaps be contained by limiting testing to those patients considered to be at high risk for invasive candidiasis.

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