New Enzyme Immunoassays for Sensitive Detection of Circulating Candida albicans Mannan and Antimannan Antibodies: Useful Combined Test for Diagnosis of Systemic Candidiasis

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Received 29 October 1998/Returned for modification 2 December 1998/Accepted 25 January 1999

Two standardized enzyme immunoassays for the serological diagnosis of candidiasis were developed. The first one detects antimannan antibodies, while the second one detects mannan with a sensitivity of 0.1 ng/ml. These tests were applied to 162 serum samples retrospectively selected from 43 patients with mycologically and clinically proven candidiasis caused by Candida albicans. Forty-three serum samples were positive for mannan, and 63 had significant antibody levels. Strikingly, only five serum samples were simultaneously positive by both tests. When the results were analyzed per patient, 36 (84%) presented at least one serum positive by one test. For 30 of them, positivity by one test was always associated with negative results by the other test for any of the tested sera. For six patients whose sera were positive for either an antigen or an antibody response, a balance between positivity by each test was evidenced by kinetic analysis of sera drawn during the time course of the infection. Controls consisted of 98 serum samples from healthy individuals, 93 serum samples from patients hospitalized in intensive care units, and 39 serum samples from patients with deep mycoses. The sensitivities and specificities were 40 and 98% and 53 and 94% for mannanemia or antibody detection, respectively. These values reached 80 and 93%, respectively, when the results of both tests were combined. These observations, which clearly demonstrate a disparity between circulation of a given mannan catabolite and antimannan antibody response, suggest that use of both enzyme immunoassays may be useful for the routine diagnosis of candidiasis.

Yeasts of the genus Candida have been recognized as important agents of hospital-acquired infections. They have become the fourth most common isolate recovered from blood cultures in the United States (23). Similarly, the rates of candidemia have increased substantially in Europe as well (69, 70). Candidal infections occur on both medical and surgical services, but approximately half of them occur in surgical intensive care units. Depending on the hospital ward, the mortality rate attributable to candidemia ranges from 40 to 60% (46, 73). Difficulties in establishing an early and specific diagnosis of candidal infection are among the recognized reasons for such high mortality rates. The difficulties for clinical diagnosis lie in the absence of specific clinical signs (1, 4). Difficulties for biological diagnosis lie in the opportunistic character of yeasts. Their presence in normally colonized body sites of immuno-compromised patients does not prove infection, and they are rarely isolated from infected deep organs or tissues including blood (43, 52, 55). Efforts have been made to find either antibodies against Candida albicans molecules or Candida-derived molecules whose presence in patient sera could indicate deep-tissue invasion. Tests have been developed to detect C. albicans proteins (35, 39, 71), metabolites (62), DNA (5, 15, 63), and polysaccharides. In this regard, a sensitive biochemical test for the detection of glucan, a major structural polysaccharide of the cell wall, has been made commercially available, and promising data from a large number of centers have been documented with a large number of serum samples from patients (29, 39, 40, 42). Like glucans, mannans are major components of the C. albicans cell wall, making up to 7% of the cell dry weight (26a). By contrast to glucans, mannans are noncovalently bound at the cell wall surface and are highly immunogenic (17). They correspond to a large and complex repertoire of mannopyranose units linked by either α-1,6, α-1,3, α-1,2, or β-1,2 linkages (61). Among these units, oligomannose sequences corresponding to epitopes specific for human and animal antibodies, either polyclonal or monoclonal, have been identified; antibody recognition depends on both the type of linkage connecting the mannose units and the length of the mannose chain (17, 19, 22, 32, 47, 61, 65). These epitopes may also be shared by the glycosidic moiety of a large number of different mannoproteins or glycolipids, reinforcing the quantitatively major character of mannose residues in C. albicans cells (64, 65). The use of mannan antigenemia (mannanemia) detection for the immunodiagnosis of systemic candidiasis was suggested by Weiner and Coats-Stephen (72) about two decades ago. Attempts to improve the immunological detection of mannan involved the use of immune complex dissociation by heating sera before performance of the test and the use of monoclonal antibodies that react with defined epitopes (21, 22, 53). These efforts resulted in standardization and a high level of specificity. These tests, however, like the commercially available Pastorex Candida, still lack sensitivity due to the rapid clearance of the antigen from patients’ sera and the test format (latex agglutination) (21, 37, 39, 50).

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In contrast to mannanemia detection, tests based on anti-
mannan antibody detection have been used less and less in the
clinical diagnostic mycology laboratory because they have been
described both as poorly specific and as poorly sensitive. The
reasons for the poor specificity and sensitivity could be attrib-
uted to the elevated antibody titers in heavily colonized but
uninfected hospitalized patients (44) and the possible lack of
anti-mannan antibody response in infected immunocompromised patients
(24). Although antimannan antibody and mannan antigenemia were
used singly, to our knowledge, simultaneous assays for both
targets in the same sera have not been performed when clinical
infection was confirmed by isolation of Candida species.

Thus, in this study, sera from patients with documented can-
didiasis were tested for the presence of mannanemia and anti-
mannan antibodies. To facilitate the combined detection of
both mannan and its antibodies, we (i) developed a double-
sandwich enzyme immunoassay (EIA) using the monoclonal
antibody used in the Pastorex Candida, with increased sensi-
tivity, and (ii) developed an EIA for the simultaneous detec-
tion of antibodies. A total of 162 serum samples from 43
hospitalized patients were retrospectively selected because of the
presentation of clinical and mycological evidence of deep-
seated candidiasis caused by C. albicans and were assessed by the
methods that we developed for the presence of mannan-
emia and antimannan antibodies. Our data demonstrate that the
developed EIA format increases the detection limit of mannan with increased sensitivity without adversely affecting the test specificity. A striking finding in this study is the obser-
vation that serum samples with a high mannanemia response had a low (undetectable) levels of antimannan antibodies and vice versa. This finding was consistent among patients in gen-
eral and for a given patient during the time course of the
disease.

MATERIALS AND METHODS

Patients. Between January and December 1995, 162 serum samples were
retrospectively collected in two different university hospitals from 43 patients (16
females and 27 males [mean age, 56 ± 17 years]) with proven candidiasis. The average number of serum samples per patient in this group was 3.7 ± 2 (Table 1).

The following criteria were used for the retrospective selection rules for the
laboratory and clinical files were examined: (i) positive culture of specimens from
normally sterile sites (blood, bile, pericardial fluid, liver biopsy, drain, and wound
specimens) for C. albicans; (ii) availability of serum samples obtained within a
range of 1 week before and 2 weeks after positive cultures; (iii) the presence of
risk factors (cancer and chemotherapy, abdominal surgery, AIDS, major health
problems requiring hospitalization in intensive care units [ICUs], and use of
broad-spectrum antibiotics, indwelling intravascular catheters, and hyperalimen-
tation; and (iv) the presence of an infectious syndrome (namely, fever) that did
not respond to antibacterial therapy but that did respond to antifungal therapy.

Control sera. Three groups of control sera were included in this study. (i)
Group 1 comprised 93 serum specimens from 23 hospitalized patients (7 females
and 16 males [mean age, 45 ± 12 years]) without evidence of invasive candidiasis.
This group of patients was enrolled in a prospective study conducted in an ICU of
Lille University Hospital for 6 months, the study was designed for the assess-
ment of risk factors for nosocomial candidiasis. These patients were under
clinical and mycological survey for periods ranging from 1 to 74 days (mean, 12
days). Samples of blood, oral swabs, urine, and stools were collected biweekly.
We selected 23 patients. For 19 patients Candida colonization was documented in
at least one body site, but there was no proven, probable, or even suspected
Candida tissue invasion. In four patients, candidal colonization was not detected.

(ii) Group 2 consisted of 39 serum samples from patients with deep mycoses not
caus ed by Candida. Twenty-two serum samples were retrospectively selected from
12 patients with invasive pulmonary aspergillosis (severe neutropenic pa-
tients with persistent fever, despite treatment with broad-spectrum antibacterial
agents, and pulmonary infiltrates that developed on the chest roentgenogram).
Invasive aspergillosis was also confirmed by the detection of Aspergillus galacto-
mannan in sera. Three of 12 patients included in this group were infected with
human immunodeficiency virus (HIV). Thirteen patients (one serum sample
from each patient) were diagnosed with cryptococcal meningitis. Cryptococcal
infection was confirmed by isolation of Cryptococcus neoformans from cerebro-
spinal fluid as well as detection of circulating antigen by the Pastorex Crypto latex
agglutination test (Sanofi Diagnostics Pasteur, Paris). All patients were inves-
tigated for pulmonary disease, characterized by dyspnea, cough, and fever
accompanied by abnormal chest radiographs. In each case, the diagnosis of
pneumonia was confirmed by the presence of P. carinii cysts in bronchoalveolar lavage.

(iii) Group 3 consisted of 98 serum samples from 98 healthy blood donors.
EIA detection of anti-<i>C. albicans</i> mannan antibodies in human sera. Microtiter
plates were sensitized in an industrial setting with C. albicans cell wall mannan.
The solution was prepared from C. albicans strain NW32 grown in standard
conditions used for the chemical and immunological analysis of this molecule (12).
EIA was performed with BEP III automate (Behring Laborato-
ries, Paris, France). For the serological diagnostic procedures already in use for
Candida serology or other antimannan detection tests (51), each set of tests
included a standard dilution which consisted of a serial twofold dilution of a pool
of patients’ sera that strongly reacted with yeast mannans. These standard dilu-
tions were aliquoted and stored at −30°C. For individual sera, 100 μl of serum was
diluted 1/8,000 was applied to each well, and the plate was incubated for 1 h at
37°C. After washing, 100 μl of horseradish peroxidase-conjugated anti-human
immunoglobulin was then added, and the plates were incubated for 1 h at 37°C.
After washing, the result was revealed by 3,3′,5,5′-tetramethylbenzidine solution.
The absorbance at a λ of 450/620 nm was measured. The results were reported in arbitrary units (AU) in relation to the results on the standard curve (Fig. 1).

Detection of mannanemia. Two procedures were used to detect mannanemia.

The first method used the commercially available latex agglutination test
Pastorex Candida (Sanofi Diagnostics Pasteur) and was performed according to
the manufacturer’s instructions. Three hundred microliters of patient sera was
denatured with 100 μl of EDTA treatment solution, and the mixture was
boiled for 3 min and centrifuged at 10,000 × g for 10 min. Forty microliters of superna-
tant was mixed in a plate well with 10 μl of latex particles.

The second method used to detect mannanemia was a Sandwich immunoassay that we developed by using the same monoclonal antibody (monoclonal antibody EBC1A1) used to
the sensitize latex particles in the Pastorex Candida test. The minimal epitope of this monoclonal antibody has been shown to correspond to α-linked manno-
pectase of the C. albicans VW32 mannan acid-stable domain; this epitope is also
present on numerous C. albicans mannoproteins (22). Microtiter plates were
sensitized with monoclonal antibody EBCA1 in an industrial setting. Fifty mi-
croliters of supernatant, obtained from patient serum and treated as described
above, was mixed in a plate well with 50 μl of horseradish peroxidase-conjugated
EBCA1. After incubation for 90 min at 37°C, the plates were washed intensively
and the reaction was revealed by 30 min of incubation in darkness with 200 μl of
tetramethylbenzidine solution. The optical density was read at a λ of 450/620 nm on
a PR2100 reader (Sanofi Diagnostics Pasteur). Reactions were performed in
duplicate. Each experiment included a calibration curve for a pool of normal human
serum supplemented with concentrations of mannan of 0.1 to 27 ng/ml (Fig. 2).

Statistical analysis. Data were analyzed with the SAS program. Sensitivity,
specificity, and predictive values were calculated as described previously (54).
The true-negative population included blood donors, noninfected hospitalized patients,
and patients with deep mycoses not caused by a Candida sp.

RESULTS

Standardization of tests. For each experiment for antimannan
antibody detection, individual sera were tested in duplicate.
The repeatability of the optical density (OD) values on a single
microtiter plate corresponded to a coefficient of variation (CV) of <10%. As controls, each new set of experiments comprised
sera from four patients exhibiting graded antimannan antibody
levels, from 0.0 to 1.0 mg/ml. The interseries reproducibility obtained with these sera
was examined after transformation of the OD through the
standard curve as described above. This corresponded to a
CV of <5% (n = 5). Concerning circulating mannan antigen
detection, preliminary experiments showed that the sensitivity of
detection was 0.1 ng/ml (the study was performed with sequen-
tial dilutions of a negative serum supplemented with 27 ng of
mnan). In this study we considered 0.5 ng/ml to be the cutoff
level since 99% of the controls had mannanemia values that
were less than this value. The mannan concentrations in the

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tested sera were determined from the mean of the OD by using the sigmoid model curve provided with the reader. The intra-assay reproducibility expressed as the mean CV for the two control serum samples provided with the kit (negative and positive) was 3.32% (n = 5). Interseries reproducibility for control sera and test samples mannan concentrations showed a CV of <4%.

Mannan concentrations and antimannan antibody titers in patient and control sera. Figure 3 shows the results obtained with the 162 serum specimens drawn from the 43 patients with candidiasis tested by the EIA for the detection of both antibodies and circulating mannanemia. Individual antigenemia values (expressed in nanograms per milliliter) were plotted as a function of the antimannan antibody response roughly distributed according to the following four categories: A, the antibody response is weak or absent (<10 AU); B, the antibody response is moderately above the cutoff level (between 10 and 20 AU); C, the antibody response is medium (between 20 and 40 AU); and D, the antibody response is strong (>40 AU). Sixty-three (37%) of the serum specimens had antibody titers

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**TABLE 1. Underlying diseases, culture data, and results of antigen and antibody testing for patients with candidiasis**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Hospital ward</th>
<th>Underlying condition</th>
<th>No. of serum specimens</th>
<th>Site of C. albicans isolation</th>
<th>Peak antigen concn (ng/ml) by EIA</th>
<th>Maximum latex agglutination assay titer</th>
<th>Peak antibody response (AU) by EIA</th>
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<td>Blood</td>
<td>3.50</td>
<td>0</td>
<td>12 (7)^e</td>
</tr>
<tr>
<td>40</td>
<td>F</td>
<td>72</td>
<td>Surgery</td>
<td>Small intestine fistula</td>
<td>3</td>
<td>Blood</td>
<td>2.00</td>
<td>1</td>
<td>53 (9)</td>
</tr>
<tr>
<td>41</td>
<td>M</td>
<td>65</td>
<td>Urology</td>
<td>Vesical carcinoma</td>
<td>5</td>
<td>Drain</td>
<td>1.74</td>
<td>0</td>
<td>57 (21)</td>
</tr>
<tr>
<td>42</td>
<td>F</td>
<td>62</td>
<td>ICU</td>
<td>Peritonitis</td>
<td>7</td>
<td>Drain</td>
<td>3.50</td>
<td>1</td>
<td>80 (13)</td>
</tr>
<tr>
<td>43</td>
<td>M</td>
<td>67</td>
<td>ICU</td>
<td>Septic shock</td>
<td>6</td>
<td>Drain</td>
<td>8.53</td>
<td>2</td>
<td>83 (6)</td>
</tr>
</tbody>
</table>

^a M, male; F, female.

^b Boldface indicates positive results.

^c CML, chronic myelocytic leukemia.

^d PL, pleural liquid.

^e Values in parentheses are antimannan antibody titer detected in the same sample which presented a peak of antigenemia.
that exceeded 10 AU and only 43 (25%) displayed antigenemia values greater than 0.5 ng/ml (25%). Strikingly, only 5 of 162 serum specimens concomitantly had circulating mannan and significant antimannan antibodies.

The results obtained for each of the 43 individual patients are summarized in Table 1. Table 1 indicates the maximal antigenemia and/or antibody response obtained for at least one of the retrospectively available serum samples. Patients have been classified into four groups according to the results observed by both tests. The first group (patients 1 to 7; 16%) corresponds to sera in which it was not possible to detect either antigens or antibodies; it is worthy of note that for three of the patients, only one sample was available. In the second group (patients 8 to 19; 28%), antigenemia was detected without evidence of an antibody response during the survey. In the third group (patients 20 to 37; 37%), no antigenemia was detectable but a significant antibody response was evidenced; for groups 2 and 3 which represented 66% of the patients, the striking disparity, i.e., lack of detectable antigen in the presence of significant amount of antibodies and vice versa, is evident. This was also observed for all sera drawn from a single patient during the survey. In the fourth group (patients 38 to 43; 13%), both antigenemia and antibody detection tests were positive. However, with the exception of five serum specimens (two drawn from patient 38 and one each drawn from patients 40, 41, and 42), we never observed positivity by both tests for the same serum sample.

The complementation of the antigen and antibody detection tests was indeed evidenced when considering the kinetics of both parameters with sera drawn from individuals during the time course of the disease. Figure 4 shows the results corresponding to the mannanemia and antimannan antibody levels observed for patients 39 and 43 (see also Table 1). For patient 39 (Fig. 4a), a high mannan concentration was early detected in the serum available 1 week before the isolation of \textit{Candida} from the patient’s blood (see arrow). However, it became negative 5 days later and remained under the threshold limit thereafter (<0.5 ng/ml). At the time of positive antigenemia, the antibody response was negative and sharply increased to reach a maximum within 5 days, with a concomitant decrease in mannanemia. For patient 43 (Fig. 4b), 3 weeks prior to the isolation of \textit{C. albicans}, the antibody response was around the cutoff point (11%). Then, the antibody response gradually decreased during the following 3 weeks. During the same period, a strong antigenemia peak was observed with a single serum sample 10 days before the mycological isolation. The decrease in mannanemia preceded the onset of a strong antibody response concomitantly with mycological detection.
test increased from 28 to 40% by using an EIA format instead of a latex agglutination assay format. This increase in sensitivity did not reduce the specificity. As can be seen from the analysis of individual data, the combined use of EIA detection of antimannan antibodies and EIA detection of mannanemia allowed the detection of 80% of retrospectively mycologically proven deep Candida infections with a specificity of 93%.

**DISCUSSION**

Numerous approaches for the serological diagnosis of candidiasis have concentrated on the detection of C. albicans-derived molecules. These molecules were detected either on the basis of their antigenicity or through biochemical-enzymatic procedures. More recent progress has been made on the latter methods, and kits are commercially available for the detection of arabinotol (62) and glucans (29, 39, 40, 42), whereas PCR-based tests for Candida DNA detection are routinely performed in some laboratories (5, 15, 63). Immunological detection of C. albicans protein antigens of 47 and 48 kDa have represented promising advances (35, 39, 71), but the use of the commercially available assay (Directigen; Becton Dickinson) for the detection of the 48-kDa vacuolar enolase has been limited by its cost. In contrast, the Cand Tec latex agglutination test has been widely used as the first commercially available antigen detection test (3, 19, 31, 67); the still unknown nature and function of the target antigen have nonetheless impeded its further development. An interesting feature of the serological detection of C. albicans-derived antigens in patient sera, in contrast to the detection of nonimmunogenic molecules, is that the detected molecules may elicit an antibody response in infected patients. Depending on the pathophysiological importance of the antigen, joint consideration of patient antigenemia and antibody response can provide insight into the evolution of the infection. Such diagnostic strategies are commonly used in virology for the serological survey of either HIV or hepatitis B virus infections. These methods involved the kinetics of the serum antibody response to the p24, gp41, and gp120 of the hepatitis B surface, core, and e antigens, respectively, for the detection of antigenemia (14). Surprisingly, with the exception of the use of the approach for the detection of the 47-kDa antigen, which has been shown both to circulate and to elicit protective antibodies (36), such an approach has never been applied to the serological diagno-

### TABLE 2. Results of antibody and antigen testing for control populations

<table>
<thead>
<tr>
<th>Group or clinical diagnosis</th>
<th>No. of patients</th>
<th>No. of serum samples</th>
<th>No. of serum samples positive for the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mannanemia of &gt;0.5 ng/ml</td>
</tr>
<tr>
<td>Healthy blood donors</td>
<td>98</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>ICU-hospitalized patients without invasive candidiasis</td>
<td>23</td>
<td>93</td>
<td>1 (0.68)**</td>
</tr>
<tr>
<td>Invasive pulmonary aspergillosis</td>
<td>12</td>
<td>22</td>
<td>1 (1.45)</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>13</td>
<td>13</td>
<td>1 (0.57)</td>
</tr>
<tr>
<td>P. carinii pneumonia</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values in parentheses are the titers observed for the same sample which was antigen positive.*
sis of candidiasis. This is particularly true for the mannan, the major immunogen of the *C. albicans* cell wall, which for two decades has been shown to induce antibodies in humans and to circulate in patients’ sera (34). These diagnostic approaches have considered mannan to be a single molecule, but they failed to take into consideration its chemical and immunological complexity. Within the mannan, a large number of chemically defined sequences of manno residues have been identified (61). Depending on the type of linkage between the manno residues and the mannosyl chain length, mannan-derived oligomannosides have been shown to be involved in such basic processes as inhibition of lymphoproliferation (41), binding to epithelial cells and macrophages (6, 7, 16, 33, 59), induction of cytokines and arachidonic acid derivatives (2, 9), and induction of protective or nonprotective antibodies in animal models (19).

Polyclonal and then monoclonal antibodies have been used to detect circulating mannan in patients’ sera by either EIA (53), radioimmunoassay (72), latex agglutination (3, 18, 37), or coagglutination (30). Some of these epitopes have been preliminarily characterized, as for monoclonal antibodies AF1 and SB2, which have been shown to correspond to β-1,2-linked manno-oligosaccharide units of the mannan acid-labile domain (11, 50). In the present study, we have used monoclonal antibody EBCA1, which is used to sensitize the latex particles involved in the commercially available test Pastorex *Candida*. Recent studies have shown that the monoclonal antibody EBCA1 minimal epitope was among a mixture of mannopentaoses present in the mannan acid-stable domain: an α-1,2-linked isomer and an isomer in which the fifth manno was α-1,6 linked to the reducing unit of manno-α-1,2-tetraose (22). Therefore, monoclonal antibody EBCA1 epitopes fit with the more general structure Manα1-(2Manα1)α-1,2-Manα1-(2Manα1)α-1,2-Manα1 (where *n* is ≥0), a type of mannopranose chain that has been proposed to correspond to antigenic factor 1 (27, 61), which is ubiquitous in yeast species (66). The monoclonal antibody EBCA1 epitope has also been shown to be expressed on the glycan moiety of a large number of *C. albicans* mannoproteins which, if released, can be detected in patient sera. Previous studies on antigen detection with monoclonal antibody EBCA1 by the Pastorex latex agglutination test have shown a good specificity but a poor sensitivity (21, 39, 50). Therefore, we decided to increase the sensitivity of the test by developing an EIA format instead of a latex agglutination assay format. By using this method, the detection limit has been improved up to 0.1 ng of mannan per ml. As expected, this resulted in an increase in sensitivity which allowed us to detect antigenemia in 40% of the patients, whereas with the Pastorex system antigenemia could be detected in only 28% of the patients. This increase in sensitivity was not detrimental to specificity since only 3 of the 150 control serum specimens were positive; none of them was from healthy blood donors, 1 was drawn from a hospitalized colonized patient, and 2 were from patients infected with *Aspergillus fumigatus* and *C. neoformans*, respectively. Although no mycological evidence of candidiasis was found for these patients, the possibility that they could be infected or coinfected with *C. albicans* could not be completely ruled out. When the sensitivity of EIA is compared to that of Pastorex for each serum sample from patients with candidiasis, in general, values lower than 1.5 ng/ml failed to give a positive Pastorex test result. This resulted, however, in a limited gain in overall sensitivity since 43 serum samples were positive by EIA, whereas 35 were positive by Pastorex. Consideration of these results for each serum sample is more disappointing than those for each patient, but this illustrates one of the major limitations of mannanemia detection tests, which lies in the transient character of antigen circulation (25, 52). As a consequence, sensitivity is a function of the number of serum samples available from each patient. In this study, sensitivity of mannanemia detection dropped from 40 to 11% if data for patients for whom only one serum sample was available are considered. Several mechanisms have been proposed to explain this observation, among which we can find the quick degradation of manno oligomers by serum mannosidases (10) or the binding of the manno oligomers to soluble serum proteins (mannose binding protein C3) (20, 26, 60) or membranous receptors of phagocytes (16, 33, 59). However, the present study demonstrates that the detection of a given mannan catabolite in sera from candidiasis patients is inversely correlated to the presence of antimannan antibodies. Whether this phenomenon is restricted to the epitope that was detected (22) (the epitope has structural similarities to *C. albicans* mannan-derived O-linked oligomannosides that inhibit lymphocyte proliferation (41)) or fits with the more general phenomenon of antigen clearance by immune complexes remains to be established. These results also led us, like others recently (68), to reconsider the diagnostic value of antimannan antibody detection. We have found a specificity of 94% and a sensitivity of 53% for the EIA for antimannan antibody detection. It must be stressed that this sensitivity was calculated by including data for patients for whom the diagnosis was established by mannanemia detection. The combined tests had a sensitivity and specificity of 80 and 95%, respectively. With regard to the ability of both tests to diagnose *C. albicans* infection early in the course of infection, conclusions can be drawn only from data for the available sera due to the retrospective character of this study. For 18 of the 43 patients included in the study, at least one serum sample was available before mycological evidence of infection was gained. When considering the results for these sera, six patients presented with mannanemia (in the absence of significant antimannan antibody levels), nine patients presented with significant antimannan antibody levels (in the absence of mannanemia), and sera from the remaining three patients were negative by both tests. Evidence of antigenemia and an antibody response was gained an average of 6.2 and 7.3 days, respectively, before mycological isolation of *C. albicans*. However, this study is limited to patients infected with *C. albicans* only and patients infected with non-*C. albicans Candida* species were not included. Preliminary results obtained with sera from patients infected with *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* which, together with *C. albicans*, account for more than 95% of hospital *Candida* infections, are encouraging. This observation is not surprising since these different species of the *Candida* genus share both the EBCA1 epitope distribution on their mannan and mannoproteins (22) (for antigen detection tests) and a high level of cross-antigenic mannan reactivity (for antibody detection tests) (61). This study has shown that the combined performances of antigen and antibody detection by these tests were similar, irrespective of the immunosuppression status of the patient and the service. Evaluation of the utility of both tests in prospective studies enrolling large numbers of patients at risk for candidiasis (73) is necessary.

**ACKNOWLEDGMENTS**

We are grateful to Mahmoud A. Ghannoum and Donald Mackenzie for helpful suggestions on the manuscript. We thank also Gabriel Reboux (Besançon) for providing the sera from patients with cryptococcal meningitis and Laurence Richard and Nadine François for expert technical assistance.

This work was supported by a grant from the “Programme Hospitalier de Recherche Clinique du Ministère des Affaires Sociales, de la Santé et de la Ville.”
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