MYCOLOGY

Combined detection of mannanaemia and anti-mannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic *Candida* species

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A novel strategy for the diagnosis of systemic candidosis was evaluated, based on the combination of two enzyme immunoassays that detect a candida oligomannoside repetitive epitope expressed in large amounts by *Candida albicans* (Platelia Candida Ag), and antibodies against *C. albicans* mannan, the major cell-wall immunogen in which this epitope is present (Platelia Candida Ab). Sera were selected retrospectively from intensive care and haematology patients with clinically suspected systemic candidosis, and from whom *Candida* spp. had been isolated from normally sterile sites. Of the 21 patients infected with *C. albicans*, 13 had positive antigenaemia and 14 had a positive antibody response, including eight patients who were antigenaemia negative. The sensitivity of the combined tests was 100%. In patients infected with *C. glabrata* (*n* = 12) or *C. tropicalis* (*n* = 10), the sensitivity was 83% and 80%, respectively. For the remaining patients, infected with *C. parapsilosis* (*n* = 10), *C. krusei* (*n* = 8) or *C. kefyr* (*n* = 2), the sensitivity of the combined tests was 40%, 50% and 50%, respectively. At least one of the serological tests was positive before yeast growth occurred in 60% of patients for whom a serum sample was available before blood culture sampling. An increase in serological test positivity to >80% was observed for sera obtained around the date of positive culture, irrespective of the *Candida* species isolated. These results suggest that regular serological monitoring for both mannanaemia and anti-mannan antibodies in at-risk patients may contribute to the early diagnosis of candidosis.

Introduction

Several *Candida* species are pervasive pathogens capable of causing systemic infection in critically ill and severely immunocompromised patients [1–4]. In the 1980s, *Candida* species were reported to be the seventh most common nosocomial pathogens hospital-wide, ranking fourth in intensive care units (ICUs) where they accounted for c. 10% of all bloodstream infections [5–7]. This general incidence has continued in the past decade in the USA [8], as well as in Europe [9–12]. Despite the high morbidity and mortality associated with candidosis, no decrease in the incidence of this infection has yet been achieved. Recently, much attention has focused on the increased prevalence of infections caused by non-*albicans Candida* spp. and the emergence of azole resistance in these yeasts [13, 14]. The proportion of non-*albicans* species causing systemic candidosis has been reported to be 46% overall [15–17]. The greater pathogenicity of *C. albicans*, *C. glabrata* and *C. tropicalis* compared with the other species has been documented, and these species account for >80% of all systemic candida infections. In contrast, despite an increase in relative prevalence of *C. parapsilosis* infections, in neonatal patients and patients with intravascular lines, candidosis caused by this species as well as those caused by *C.
krusei are less frequent, transient and generally associated with a better prognosis [18, 19]. Irrespective of the causative species, systemic candidosis is difficult to diagnose clinically and microbiologically. In this respect, continuous efforts are being made to develop methods for the detection of candida DNA [20–23] or metabolites, whose presence in patients’ sera could indicate deep tissue invasion [24–26]. Like glucans, mannans are major components of the candida cell wall. However, in contrast to glucans, mannans are bound non-covalently at the cell-wall surface and are highly antigenic [27]. Since the initial description of the presence of mannans in patients’ sera by Wiener and Yount [28], concerted efforts have been made to increase the sensitivity of serological tests for mannan detection. Despite progress in this area, mannan detection is still associated with low sensitivity, mainly due to the transient nature of mannanemia. Despite the lack of sensitivity of these tests, the more recent generation of mannan detection tests is generally considered to be specific [29]. Detection of anti-mannan antibodies has been recommended as an early diagnostic procedure [30] but is associated with low sensitivity, due to the absence of an antibody response in immunocompromised patients, and low specificity, due to the presence of anti-mannan antibodies in sera from colonised, uninfected patients [30, 31]. In addition to serological procedures, a strategy has recently been proposed to survey at-risk patients to identify those in whom pre-emptive therapy would be justified. This proposed to survey at-risk patients to identify those in colonised, uninfected patients [30, 31]. In addition due to the presence of anti-mannan antibodies in sera in immunocompromised patients, and low specificity, due to the absence of an antibody response to mannan detection tests is generally considered to be specific [29]. Detection of anti-mannan antibodies has been recommended as an early diagnostic procedure [30] but is associated with low sensitivity, due to the absence of an antibody response in immunocompromised patients, and low specificity, due to the presence of anti-mannan antibodies in sera from colonised, uninfected patients [30, 31]. In addition to serological procedures, a strategy has recently been proposed to survey at-risk patients to identify those in whom pre-emptive therapy would be justified. This strategy consists of the determination of a colonisation index (CI), defined as the ratio of the number of non-blood distinct body sites colonised by Candida spp. to the total number of distinct body sites tested. A threshold of ≥0.5 discriminates between infected and colonised patients [3, 32–36]. According to some of these studies, the sensitivity and specificity of this procedure were 100% [35].

In the current study, a strategy for the serological diagnosis of candidosis was developed based on four points: (i) an increase in sensitivity of a previous test (Pastorex Candida) that detects a repetitive oligomannose epitope specific to monoclonal antibody (MAb) EBCA1, expressed on a large number of C. albicans molecules; (ii) the detection of antibodies to mannan, the major C. albicans immunogen which expresses, among other molecules, the MAb EBCA1 epitope; (iii) the development of standardised immunoassays, with cut-off values determined with appropriate controls, which can be automated and better adapted to the large number of at-risk patients in large university hospitals [37]; and (iv) the monitoring of both antigenaemia and antibody response in individual patients, following the routine diagnostic strategies used for other infectious diseases like hepatitis B and HIV infection [38]. In a preliminary study, the sensitivity and specificity of the combined tests were shown to be 80% and 93%, respectively, when used to test serum samples collected from 43 patients with systemic candidosis caused by C. albicans [37]. These tests are now marketed as Platelia Candida Ag and Platelia Candida Ab, respectively.

MAb EBCA1 recognises sequences of α-linked oligomannoses, comprised of more than four residues, present in a large number of mannoproteins extracted from C. glabrata and C. tropicalis, and to a lesser extent C. parapsilosis and C. krusei [39]. Thus, it seemed likely that Platelia Candida Ag would also detect antigens of non-albicans Candida spp. The objective of this study was to extend the preliminary results obtained in patients infected with C. albicans and explore this possibility by examining a cohort of representative patients infected with different Candida spp. Patients with clinical and mycological evidence of systemic candidosis were selected retrospectively from three French university hospitals. For each patient, the relationship between the time each Platelia test became positive and the time of positive culture from a normally sterile site was investigated.

Materials and methods

Patients

A total of 204 sera from 63 patients with proven candidosis was obtained retrospectively between Jan. 1992 and Jan. 2000 from three French university hospitals. Twenty-one patients were infected with C. albicans and 42 were infected with non-albicans Candida spp.: C. glabrata (12 patients), C. tropicalis (10), C. parapsilosis (10), C. krusei (8) and C. kefyr (2). The mean number of serum samples per patient was 3.2 (SD 3) (Table 1). The following criteria were applied retrospectively to laboratory and clinical files as selection rules: (i) positive culture for Candida spp. from normally sterile site (blood, bile, pericardial fluid, liver biopsy specimen, drain, wound); (ii) availability of serum samples within a range of 1 week before to 2 weeks after positive culture; (iii) analysis of patients’ charts, with special attention to risk factors, i.e., cancer and chemotherapy, malignant haemopathies, abdominal surgery, AIDS, major health problems requiring hospitalisation in ICUs, broad-spectrum antibiotics, indwelling intravascular catheters, hyperalimentation; and (iv) infectious syndrome (fever) not responding to antibacterial therapy but responding to antifungal therapy.

Methods

A summary of the methods used is shown in Fig. 1.

Detection of anti-mannan antibodies (Platelia® Candida Ab; BioRad Laboratories). Microtitration plates were sensitised with C. albicans cell-wall mannan [37]. Mannan is a major, non-covalently bound, cell-wall surface antigen, visualised by electron microscopy with periodic acid-thiocarbohydrazide-silver proteinate staining [40] (Fig. 1a), and is extracted and purified from
### Table 1. Patient information, culture data and results of antibody an antigen testing in the study population

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Hospital ward</th>
<th>Number of serum specimens</th>
<th>Candida sp. isolated</th>
<th>Site of isolation</th>
<th>Peak antigen concentration (ng/ml) by EIA</th>
<th>Peak antibody response (AU) by EIA</th>
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<td>59</td>
<td>F</td>
<td>48</td>
<td>Infectious Dis.</td>
<td>5</td>
<td>krusei</td>
<td>KT</td>
<td>0.11 (4)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td>31</td>
<td>Infectious Dis.</td>
<td>2</td>
<td>krusei</td>
<td>Blood</td>
<td>0 (27)</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>M</td>
<td>76</td>
<td>Surgery</td>
<td>1</td>
<td>krusei</td>
<td>Spleen</td>
<td>0 (3)</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>F</td>
<td>75</td>
<td>Surgery</td>
<td>3</td>
<td>kefyr</td>
<td>Spleen</td>
<td>5 (80)</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>M</td>
<td>56</td>
<td>Surgery</td>
<td>2</td>
<td>kefyr</td>
<td>Abscess</td>
<td>0 (4.8)</td>
<td></td>
</tr>
</tbody>
</table>

M. male; F. female; CSF. cerebrospinal fluid; BUC. blood, urine and cutaneous samples. Bold type indicates positive results. Values in parentheses are antimannan antibody titre detection in the same sample which presented a peak of antigenaemia.
candida cells by autoclaving [41] (Fig. 1b). The mannan involved in the test was prepared from *C. albicans* strain VW32 grown in bioreactors [42]. As revealed by polyacrylamide gel electrophoresis (PAGE) and electrotransfer, this mannan appeared as a highly polydispersed molecule when stained with concanavalin A, a lectin which reacts with α-mannosyl residues [43] (Fig. 1c, lane 1). The primary and secondary antibody binding steps, as shown in Fig. 1d, have been described previously [37]. The reactions were performed with the BEP III Automate (Dade-Behring Laboratories, France). Each set of tests included a standard curve...
which consisted of serial two-fold dilutions of a pool of sera strongly reacting with yeast mannan (Fig. 1e). The absorbance was measured at $\lambda = 450/620$ nm. Results were reported in arbitrary units (AU) determined in relation to the standard curve; 10 AU were considered to indicate candidosis [37].

Detection of mannanemia (Platelia® Candida Ag; BioRad Laboratories). This test involves MAb EBCA1, used previously in a latex agglutination test (Pastorex Candida; BioRad Laboratories, Marnes-La-Coquette, France). MAb EBCA1 recognises an epitope present in mannan (Fig. 1), and binding of the antibody almost superimposes concanavalin A staining (Fig. 1c, lane 2). Because of the repetitive nature of the epitope on *C. albicans* molecules [43], the assay involves MAb EBCA1 as both a captor and detector antibody (Fig. 1f) [37].

Serum samples (300 $\mu$l) from patients were denatured with 100 $\mu$l of EDTA treatment solution, boiled for 3 min and centrifuged for 10 min at 10 000 $g$. Then 50 $\mu$l of supernate were mixed in EBCA1-coated wells with 50 $\mu$l of horseradish peroxidase-conjugated EBCA1. After incubation for 90 min at 37°C, the plates were washed thoroughly and the reaction was revealed after incubation for 30 min in the dark with tetramethylbenzidine solution 200 $\mu$l. The optical density was read at $\lambda = 450/620$ nm on a PR2100 reader (BioRad Laboratories). All reactions were performed in duplicate. Each experiment included a calibration curve, which involved a pool of normal human sera supplemented with known concentrations of mannan (0.1–27 ng/ml; Fig. 1g).

**Results**

The results obtained for the 63 patients are summarised in Table 1. Patients were divided into six groups, depending on the *Candida* sp. involved. The maximum mannanemia or anti-mannan antibody response, or both, observed in at least one of the serum samples from each patient is shown.

**Results for each serum sample**

Fig. 2 shows the individual values for the 408 tests performed on the 204 serum specimens obtained from the 63 patients with candidosis. Each antigenaemia value, expressed in ng/ml, is represented either by a closed or open circle for positive or negative antibody responses, respectively. Antigenaemia values were plotted as a function of the date of serum sampling. This method of expressing the results shows the nature of the serum samples in relation to the date of positive mycological examination (PME) corresponding to day 0, but does not take into account the *Candida* sp. isolated. This information is given in Table 2, in relation to the date of PME. Fig. 3 shows the cumulative sensitivity of each test and of the combination of tests, for each serum sample and for each species, as a function of the time of serum sampling. The graph has been split in two parts.
according to the relative prevalence of nosocomial infection caused by Candida spp. i.e., Fig. 3a shows the results for patients infected with the predominant pathogenic Candida spp. (C. albicans, C. glabrata, C. tropicalis; c. 80% of all clinical isolates). (b) Results of sera obtained from patients infected with other yeasts. PME (day 0) indicates the date of isolation of a Candida sp. from normally sterile sites.

Table 2. Results obtained on serum samples available in relation to positive mycological examination (PME) and Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Ag+</th>
<th>Ab+</th>
<th>Ag+/Ab+</th>
<th>Ag+/Ab–</th>
<th>Ag–/Ab+</th>
<th>Ag–/Ab–</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>46</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>31</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>72</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. krusei</td>
<td>21</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. Sensitivity (per serum sample) of antigen (■) and antibody (□) detection tests, and the combination (□) as a function of time of serum sampling. (a) Results of sera from patients infected with the main pathogenic Candida spp. (C. albicans, C. glabrata, C. tropicalis; c. 80% of all clinical isolates). (b) Results of sera obtained from patients infected with other yeasts. PME (day 0) indicates the date of isolation of a Candida sp. from normally sterile sites.

Results for each patient

A previous study with a large number of control sera showed that the specificity of combined mannann and anti-mannan antibody detection by EIA was 93% [37]. Of the 21 patients infected with C. albicans in the current study, 13 had positive tests for antigenaemia and 14 had a positive antibody response, including eight patients who were negative for antigenaemia. The sensitivity of the combined tests was 100%. Of the 12 patients infected with C. glabrata, seven had positive antigenaemia, but in three the lack of detectable antigen was associated with the presence of a significant titre of antibodies (>18 AU). Both tests remained constantly negative for two patients. Therefore, in this series the combined sensitivity of both tests was 83%. Of the 10 patients infected with C. tropicalis, seven had positive antigenaemia and six had positive antibody tests. Both tests were negative in two patients for whom only one serum sample was available. Thus, the sensitivity of the combined tests in this series of C. tropicalis infection was 80%. Twenty patients were infected with C. parapsilosis, C. krusei and C. kefyr, and the combined sensitivities of the tests were 40%, 50% and 50%, respectively.

Although the numbers of serum samples per patient vary from one to 26, some information can be derived from this study about the kinetics of antigenaemia. Persistence, or at least constantly positive antigen tests, mycological isolation, thereafter decreasing slowly. The onset of an antibody response was delayed slightly when compared with antigenaemia and reached a maximum 1 week after mycological detection. On the day of mycological detection, the cumulative sensitivity of both tests was maximum and reached 85%. Fig. 3b represents the results obtained with sera from patients infected with C. parapsilosis, C. krusei or C. kefyr. The performance of the tests in detecting these latter species was not as powerful as for the earlier species. Nevertheless it was possible to see a similar trend of increased sensitivity of both tests around the date of positive mycological investigations.

Table 2. Results obtained on serum samples available in relation to positive mycological examination (PME) and Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of samples examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>46</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>31</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>72</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>29</td>
</tr>
<tr>
<td>C. krusei</td>
<td>21</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>5</td>
</tr>
</tbody>
</table>

For each species, at a given time: Ag+, Ab+, Ag+/Ab+ and Ag–/Ab– represent the number of sera exhibiting positive antigenaemia (without antibody); positive antibody response (without antigenaemia); positive antigen and antibody response; and negative results with both tests, respectively.
was observed (patient 37) for a maximal period of 50
days during which 15 samples were drawn. This patient
(hospitalised in a haematology ward) never developed
an antibody response during this period. For some
patients, disappearance or appearance of antigenaemia
was observed within 3-day intervals. In general, the
more samples that were available the more chance
there was of observing positive antigenaemia. For C.
albicans, C. tropicalis and C. glabrata infections, the
mean number of serum samples for patients who
displayed at least one antigen-positive test was 4.2 (SD
5.4) versus 2.7 (SD 1.9) for patients for whom no
positive test was observed. For infections caused by C.
albicans, C. tropicalis or C. glabrata, comparison of
the results for each patient according to the ward where
they were being treated (ICU or haematology) gave the
following results: sensitivity of Platelia Candida Ag
was 55% and 90%, respectively, and sensitivity of Platelia Ab was 30% and 44%, respectively.

Discussion

Because of the absence of specific clinical signs and
symptoms, systemic candidosis remains a diagnostic
and therapeutic challenge [29, 44, 45]. Diagnosis is
generally supported by a compendium of clinical [46],
radiological [47–49] and mycological evidence [50].
Efforts made to include the results of serological tests
in this compendium have generally failed, despite
consistent improvements in the sensitivity and specific-
ity of these tests, particularly those detecting candida-
derived molecules such as D-arabinitol [26], glucans
[25] and mannan [24, 51]. By changing the format of
the test for the detection of mannan from a latex
agglutination test (Pastorex) to an EIA (Platelia Ag),
the sensitivity of mannan detection has been increased
10-fold. This has lead to an increase in sensitivity per
patient from 28% to 40% with no loss of specificity, as
evaluated with a large number of control sera including
93 sera from ICU patients [37]. The specificity of
Platelia Ab, evaluated under the same conditions, was
94% when a cut-off value of 10 AU was used, as
described previously [37]. This specificity is acceptable
and contrasts with the general current negative opinion
about antibody detection tests [29]. We believe that this
opinion is based on a misunderstanding of the usage
and limits of these tests, and this point needs further
discussion. In a pioneering study in 1971 on systemic
candidosis in patients with acute leukaemia, the authors
came to the following conclusion ‘We recommend
serial determination of anti-Candida agglutinating
antibody titres in groups of patients known to have
increased susceptibility to fungal infections as this is
the most reliable means for diagnosing visceral
candidiasis’ [30]. Following the observation that an
antibody response developed as candida colonisation in
hospitalised patients increased [31, 52], confidence in
the specificity of these tests decreased. Despite several
studies demonstrating the usefulness of anti-Candida
antibody detection tests for diagnosis [53, 54], these
tests are seldom requested by physicians in clinical
practice. The reason given is the absence of clear-cut
discrimination between infected and colonised patients.
However, studies conducted either in ICUs [36, 55] or
clinical haematology wards [33, 56] have firmly estab-
lished colonisation as an independent risk factor for
systemic candidosis. This makes sense in relation to
the natural pathophysiology of systemic candidosis,
which is a dynamic process. Most cases of systemic
candidosis are endogenous in origin with a continuum
from colonisation to infection [34, 45]. When defining
patient categories to determine the cut-off values for
antibody detection tests, the question is at which stage
should patients be considered? Depending on whether
the aim is to provide an early diagnosis or a firm
diagnosis, the cut-off value will be a compromise
between sensitivity and specificity. Similarly, consider-
ing that colonisation is a risk factor and that the
development of antibody response parallels colonisa-
tion, why should an antibody response not be taken
into consideration in clinical surveys? Prospective
studies are now underway to assess the possible link
between a candida antibody response and CI predictive
values.

Together with the antibody detection test, a test for the
detection of antigenaemia was also evaluated, which
detected the epitope expressed on the antigen used for
antibody detection accounting, at least in part, for the
complementary nature of both tests. On the one hand, a
strong antibody response should result in immune
complexes leading to a more rapid clearance of
antigen, whereas, on the other hand, high circulating
antigen levels, which complex antibodies in vivo, may
prevent the detection of antibodies.

In the present study, the clinical usefulness of mannan-
related antigen and anti-mannan antibody detection
tests was confirmed in 21 new episodes of C. albicans
infection. The performance of these tests was preserved
when infections caused by C. glabrata and C. tropicalis
were also considered. When the 43 infectious episodes
caued by C. albicans, C. glabrata and C. tropicalis
were combined, the overall sensitivity for the simul-
taneous detection of circulating mannan and anti-
mannan antibodies was 91%. However, in the case of
infections caused by C. parapsilosis, C. kefyr and C.
krusei (c. 20% of all infections) the contribution of
both tests to the diagnosis was poor. Despite the
availability of serum samples from the majority of
patients, the sensitivity of the Platelia Candida Ag test
was only 30% for infections caused by these latter
species. Differences in sensitivity of antigen detection
depending on the Candida species is consistent with
the nature of the candida mannose epitope recognised
by MAb EBCA1 used in the test. This MAb recognises
an epitope present in large amounts on numerous
mannoproteins of C. albicans (most of those stained by
concanaavalin A, a lectin specific for terminal α-D-
mannopyranosyl residues) [43]. This property is important when considering the sensitivity of the test (i.e., the more epitope that is present, the more chance it has of being detected) and also for development of a two-site immunoenzymetric assay (previously sandwich ELISA) which requires the repetitive expression of epitopes on molecules. The MAb EBCA1 epitope was also found in large amounts on mannoproteins of C. glabrata, C. tropicalis and C. guilliermondii, but was present to a lesser extent on mannoproteins of C. krusei, C. kefyr and C. parapsilosis [39, 57].

Non-significant or low antibody titres were detected in sera from patients infected with C. parapsilosis, C. kefyr and C. krusei. The sensitivity of the Platelia Candida Ab test was only 20% in patients infected with these species. This can be explained by the low level of cross-reactivity between C. albicans serotype A mannan used as the antigen and the natural immunogen. The structure and antigenicity of candida mannan have been elucidated through a series of excellent studies conducted in Japan. It has been demonstrated that Candida strains exhibit different antigenic factors in their mannan depending on the species. Each of these factors correspond to sequences of mannose residues recognised by rabbit polyclonal IgG and listed as 1, 4, 5, 6, 8, 9, 11, 13, 13b, 34 IgGs. Combination of these IgGs is species-specific [58]. Based on this serological classification, the mannan used as the antigen in the Platelia Candida Ab test displays serum factors 1, 4, 5 and 6 [27]. At least three of these major antigenic factors are shared by C. tropicalis and C. glabrata, and this may explain the high level of cross-reactivity with sera obtained from patients infected with these Candida spp. Only factor 1 is shared between C. albicans, C. parapsilosis, C. kefyr and C. krusei, and this could explain the poor performance of Platelia Candida Ab in detection of infection with these species. However, the reason for the disparities in antibody and antigen detection in patients infected by the latter species is unknown, although the possibility of mixed candida infection, which occurs in clinical practice, cannot be excluded [59, 60].

As far as the ability of the two tests to diagnose candida infection early is concerned, conclusions can only be drawn from the sera available because of the retrospective nature of this study. For 25 of the 63 patients included in this study, at least one serum sample was available before mycological evidence of infection was obtained. Eight of these patients had detectable mannanemia (in the absence of significant anti-mannan antibodies), three had significant anti-mannan antibody levels (in the absence of mannanemia), and one had both antigen and antibodies. The sera from the 13 remaining patients were negative in both tests. Evidence for antigaemia and an antibody response was obtained on average 1 week (8.12 SD 3.8 days) before isolation of Candida spp. Thus, the use of both tests allowed an early diagnosis in c. 50% of patients for whom at least one serum sample was available before mycological isolation. Considering the high mortality rate (40–60%) associated with candida infection [45], the prognosis could have been improved for 16 patients by early diagnosis and rapid institution of antifungal therapy. As shown by the increased sensitivity of the tests observed around the day of mycological detection, and the relationship evidenced between the number of available sera per patient and positivity of tests, it is anticipated that this number could be increased by regular serological sampling [51]. Complementation of the tests was observed in whatever clinical service at-risk patients were hospitalised. Haematology patients are known to be unable to develop an antibody response during periods of cytopenia, when candida infection may only be demonstrated by antigen detection and most of the sera available from these patients were drawn during the cytopenia period, which explains the higher proportion of positive antigen tests. However, before and after this period, antibodies are produced during infection and antigen detection tests become negative. This phenomenon is particularly evident in the follow-up of patients who have developed candida infection during a prolonged hospital stay [61]. In ICUs, many factors such as poor nutrition, trauma, hypotension, therapy with steroids or cyclosporine, ischaemia and reperfusion have been related to transitory immunosuppression or damage to the integrity of the gastrointestinal mucosa [10]. This could also explain the complementation of antigen and antibody detection tests observed consistently in these patients during candida infection.

Therefore, in both haematology and ICUs, the combined use of Platelia Ag and Ab tests led to a relatively good sensitivity. To date, all negative controls – including sera from hospitalised, colonised, uninfected patients and patients with non-candida infections, such as aspergillosis or cryptococcosis – have shown that these tests have good specificity.

Diagnosis of systemic candidosis is difficult [62, 63]. Development of infection is based on a complex and poorly understood pathophysiology which depends on both pathogenicity determinants of Candida spp. or strains, the primary illness of the patient and medicosurgical practices designed to control it. Modern trends to manage this problem clinically consist of assessing risk factors prospectively [45, 64]. Blood cultures and mycological surveillance of colonisation have been proposed as microbiological contributions to diagnosis [10]. The present study suggests that inclusion of serological tests in the clinico-biological survey of at-risk patients may be a useful contribution to the diagnosis.

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