Eur J Clin Microbiol Infect Dis (2001) 20:864–870
DOI 10.1007/s100960100629

ARTICLE

H. Yera · B. Sendid · N. Francois · D. Camus
D. Poulain

Contribution of Serological Tests and Blood Culture to the Early Diagnosis of Systemic Candidiasis

Published online: 13 December 2001

Abstract The isolation of *Candida* species from a single blood culture is considered sufficient evidence for the initiation of systemic antifungal therapy. However, blood cultures still lack sensitivity. Previous reports have suggested that the combined serological detection of mannanemia and anti-mannan antibodies may be useful for the diagnosis of systemic candidiasis caused by *Candida albicans* (specificity and sensitivity 93% and 80%, respectively). In this study, serological tests to detect *Candida albicans* mannan and *Candida albicans* antibodies (Platelia *Candida* Antigen and Antibody tests; Bio-Rad, France) were applied retrospectively to a series of patients with at least one *Candida*-positive blood culture and from whom at least one serum sample, taken before or on the day of blood culture, was available. Forty-five patients were selected, including 23 infected by *Candida albicans*, 4 by *Candida glabrata*, 9 by *Candida tropicalis*, 5 by *Candida parapsilosis*, and 4 by *Candida krusei*. Serological tests were positive in 73% of patients at least 2 days, and in some patients, up to 15 days before blood cultures became positive. These data suggest that serological surveillance of at-risk patients using the Platelia *Candida* tests could result in earlier initiation of antifungal therapy, especially when used in conjunction with blood cultures. In this way, more efficient management of nosocomial infections caused by *Candida* species can be achieved.

H. Yera · B. Sendid · N. Francois · D. Camus · D. Poulain (✉)
Laboratoire de Parasitologie-Mycologie, CHRU,
Faculté de Médecine Pôle Recherche, Place de Verdun,
59045 Lille Cedex, France
e-mail: dpoulain@univ-lille2.fr
Tel.: +33-3-20-62-34-15, Fax: +33+3-20-62-34-16

B. Sendid · D. Poulain
Equipe Inserm 9915,
Laboratoire de Mycologie Fondamentale et Appliquée,
Faculté de Médecine Pôle Recherche, Place de Verdun,
59045 Lille Cedex, France

Introduction

Fungi have emerged as an increasingly frequent cause of nosocomial infections worldwide. In a survey of positive blood cultures performed in the mid-1980s in the USA, *Candida* species ranked fifth in terms of overall incidence and represented the fourth most common group of nosocomial pathogens isolated from the intensive care unit (ICU) [1]. A similar high incidence of candidemia was also reported in European hospitals. An incidence of 0.71 episodes per 10,000 patient-days was reported in 1995 [2], and in 1999 an epidemiological study conducted in four Swiss university hospitals showed that *Candida* species were the fourth most common isolate from both medical and surgical services [3]. Furthermore, the general increase in the incidence of candidemia has been accompanied by an increase in the impact of systemic *Candida* infections. Depending on the type of hospital ward, attributable mortality rates for candidemia range from 40 to 60% [4, 5], and death can occur as early as 48 h after the detection of *Candida* in the bloodstream [6]. In addition to mortality, candidemia is usually associated with considerable morbidity. When only survivors were considered, the median length of hospital stay was 30 days longer for infected patients compared to controls [7], and extra costs for patients who survived were estimated to be more than 40,000 U.S. dollars per patient [8]. Recently, attention has focused on the increasing prevalence of infections caused by non-*albicans Candida* species and the related emergence of azole resistance in species such as *Candida glabrata* [9, 10]. In 1995, these species accounted for 46% of *Candida* infections overall [11, 12, 13].

The diagnosis of systemic candidiasis is difficult because clinical signs are nonspecific [14, 15]. Isolation of *Candida* species from a single blood culture is now considered to be sufficient evidence for the immediate initiation of systemic antifungal therapy [16]. However, it is recognized that blood cultures lack sensitivity, widely reported to be less than 50% [17, 18], particularly for deep-seated infections, and usually take several days to

Table 1 Characteristics of patients with systemic candidiasis, and serum samples collected at each center

Characteristic	Lille (*n*=27)	Paris (*n*=13)	Dijon (*n*=5)	Total (*n*=45)
Male	16	8	2	26
Female	11	5	3	19
Mean age in years	57	56	60	58
Intensive care unit	14	1	0	15
Hematology ward	5	1	5	11
Surgery ward	8	9	0	17
Infectious diseases ward	0	2	0	2
No. of serum samples before the day of blood culture sampling	40	12	14	66
No. of serum samples on the day of blood culture sampling	11	8	4	23
No. of serum samples after the day of blood culture sampling	25	11	12	48

become positive [19]. Detection of *Candida* DNA in blood or serum samples by polymerase chain reaction has generated a great deal of interest over the past few years, and promising results have recently been published for the detection and identification of *Candida* DNA by polymerase chain reaction in febrile patients with hematological malignancies [20]. Other serological techniques for the detection of markers of infection are generally considered to have two major drawbacks: detection of anti-*Candida* antibodies fails to discriminate between disseminated and superficial candidiasis, and *Candida*-derived metabolite and antigen detection lacks sensitivity.

Cell wall mannan is one of the major *Candida* antigens that circulate during infection. Mannan is bound noncovalently in the cell wall and is shed easily into serum, where it is highly immunogenic [21]. The observation of a balance between mannan epitope circulation and anti-mannan antibody response in patients' serum has lead to the idea that the combined detection of mannanemia and anti-mannan antibodies by enzyme immunoassay (EIA) may be a useful diagnostic procedure. The sensitivity and specificity of this procedure were both found to be greater than 80% in patients infected by *Candida albicans* or other pathogenic *Candida* species [22]. These tests have been marketed as Platelia *Candida* Antibody and Platelia *Candida* Antigen (Bio-Rad, France).

This study evaluated the contribution of the Platelia tests in combination with blood cultures to the establishment of an early diagnosis of systemic candidiasis.

Materials and Methods

Patients

This retrospective study involved hospitalized patients presenting risk factors for deep-seated *Candida* infection and for whom candidiasis was suspected on clinical grounds. These patients had an infectious syndrome resistant to antibiotic therapy, justifying a request by physicians for blood culture and/or serological tests. Between January 1995 and December 1999, 132 patients presented with *Candida*-positive blood cultures in the University Hospital, Lille. Of these patients, 27 could be included in this study on the basis of the availability of at least one serum sample drawn on the day of blood culture sampling or during the 15 days preceding sampling. The number of blood cultures that were negative during the same period was also recorded for each patient.

To increase the number of patients in the study, 18 patients from two other French university hospitals, selected in the same way, were also included (13 patients from Hôpital Saint Antoine, Paris, hospitalized between January 1990 and December 1999, and 5 patients from the University Hospital, Dijon, hospitalized between January 1998 and December 1999). Details of these patients and the serum samples are shown in Table 1. A total of 137 serum samples were collected, including 66 taken before the day of blood culture sampling and 23 taken on the day of blood culture. An average of two serum samples per patient was obtained. All sera were stored at –20°C and tested in a blinded fashion.

Serological Tests for Detection of Circulating *Candida albicans* Antigens and Anti-*Candida albicans* Antibodies

Each serum sample was tested by three methods: two methods for the detection of anti-*Candida albicans* antibodies and one for the detection of *Candida albicans* mannan.

Enzyme Immunoassay for Detection of *Candida albicans* Mannan

The Platelia *Candida* Antigen test (Bio-Rad, France) has been described previously [22]. This test employs the same monoclonal antibody, EBCA1, as that used in the Pastorex *Candida* latex agglutination test. The epitope recognized is the alpha-linked mannopentaose of *Candida albicans* VW32 mannan. The monoclonal antibody was coated onto wells of a microtiter plate. Each serum sample was treated with EDTA and heat, and 50 µl of supernatant was then added to wells containing EBCA1 antibodies coupled to peroxidase. After incubation and washing, the presence of circulating mannan was revealed by the development of a colored enzymatic reaction whose intensity was proportional to the mannan concentration. A standard dilution curve allowed the determination of the serum mannan concentration in ng/ml. The specificity and sensitivity of mannan detection were 98% and 40%, respectively [22].

Enzyme Immunoassay for Detection of Anti-*Candida albicans* Mannan Antibodies

The Platelia *Candida* Antibody test (Bio-Rad) has also been described previously [22]. Briefly, cell wall mannan from *Candida albicans* VW32 was fixed to the wells of microtiter plates. Serum (100 µl) diluted 1/8,000 was added to each well and the plates incubated. After washing, peroxidase-conjugated anti-human immunoglobulins were added, which bound to the anti-mannan antibodies present in the serum. The immunological reaction was revealed by the development of a colored enzymatic reaction whose intensity was proportional to the antibody concentration. A standard dilution curve allowed the determination of anti-mannan antibody concentration in arbitrary units (AU). The specificity and sensitivity of anti-mannan antibody detection were 94% and 53%, respectively [22].

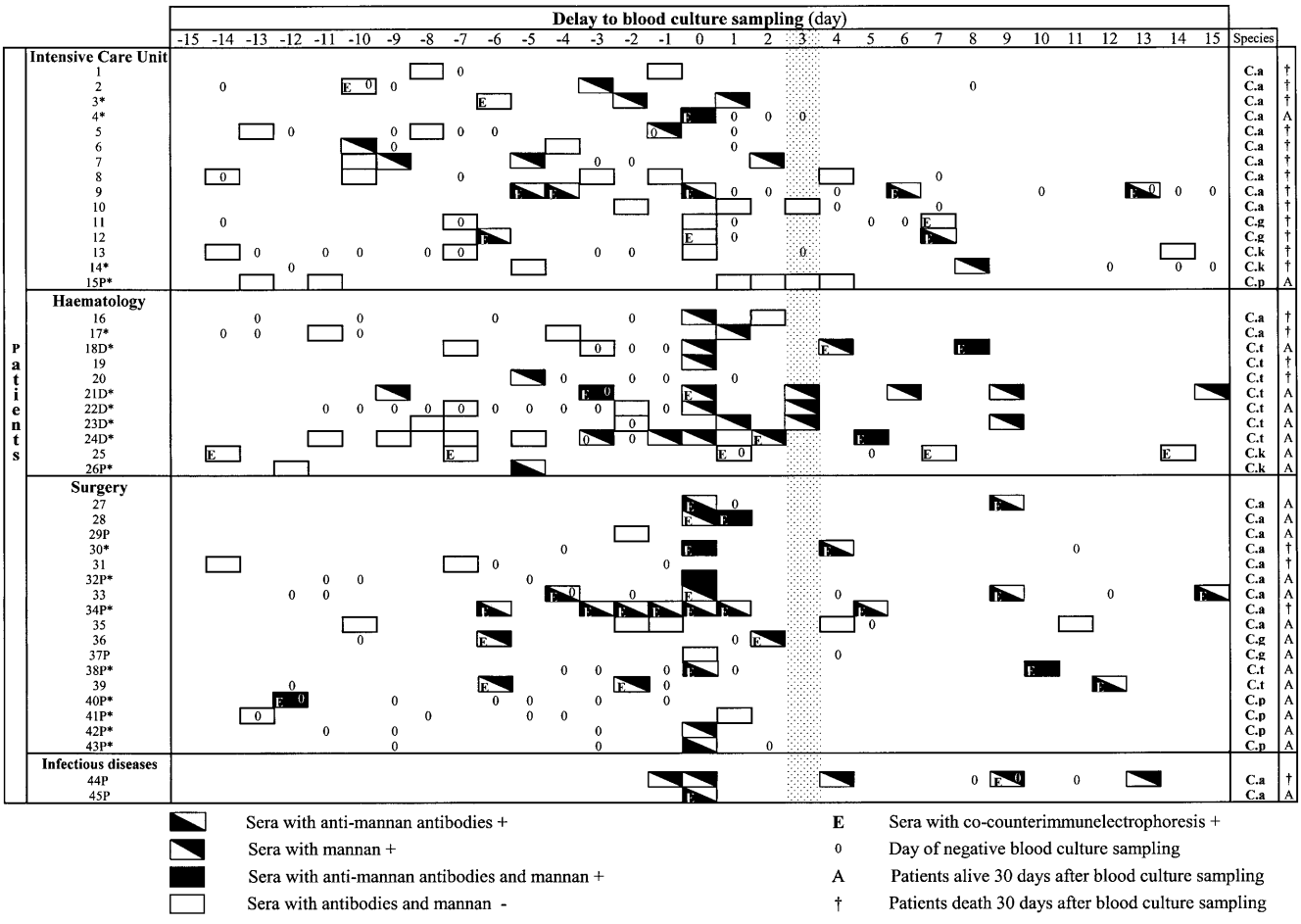

Fig. 1 Serological surveillance of patients at risk of candidiasis in relation to day of blood culture sampling (0). C.a., *C. albicans*; C.t., *C. tropicalis*; C.p., *C. parapsilosis*; C.g., *C. glabrata*; C.k., *C. krusei*; P., Paris; D., Dijon; *, patients common with Sendid et al. [36]

Combination of the two EIA tests gave a specificity and sensitivity of 93% and 80%, respectively [22].

Co-Counterimmunoelectrophoresis

This serological technique has been used in our laboratory for the past 20 years to detect precipitins in the serum of patients with candidiasis. The antibodies in serum coprecipitate with a major precipitin arc produced by antiserum to *Candida albicans*. The test was developed and described by Poulain et al. [23, 24]. Briefly, 20 µl of test serum and 20 µl of reference serum were deposited at the anode on a cellulose acetate membrane. At the cathode, 15 µl of somatic antigen prepared from *Candida albicans* VW32 was deposited at an equal distance from the sera. Electrophoresis was performed for 2 h 15 min at 6.3 V/cm. Precipitins were observed after staining the membrane with Coomassie blue. Immunological specificity was expressed as continuity of the major precipitin system in the control serum with the precipitin line produced by the test serum. In cases in which two serum samples from the same patient are tested side by side, evolution of antibody levels can be estimated from the distance and intensity of the precipitin arc relative to the serum deposit. Confirmed immunological candidiasis was defined by intense and specific reaction obtained with reference serum. The specificity and sensitivity of co-counterimmunoelectrophoresis were 100% and 69%, respectively [24].

Blood Culture

Different automated blood culture systems were used, depending on the hospital where the patient was being treated. In the University Hospital, Lille, the Bio Argos system (Diagnostics Pasteur, France) was used by the bacteriology laboratory, which then transferred the positive yeast culture to our mycology laboratory. In the mycology laboratory, until November 1999, blood samples were mixed with an anticoagulant and cultured immediately upon receipt. After November 1999, the Bactec 9050 mycosis aerobe system (Becton Dickinson, USA) was used. In the Hôpital Saint Antoine, Paris, and in the University Hospital, Dijon, the BacT/Alert system (Organon Teknika, USA) and the Bactec 9240 mycosis aerobe system (Becton Dickinson) were used, respectively. A total of 166 blood cultures were recorded.

Results

Figure 1 shows the results of the serological tests performed on the 137 serum samples from the 45 patients included in the study. Patients were classified according to the service where they were hospitalized and the *Candida* species isolated from blood. In this series, the prevalence of *Candida* species isolated was as follows: *Candida albicans*, 51% (*n*=23); *Candida tropicalis*, 20% (*n*=9); *Candida parapsilosis*, 11% (*n*=5); *Candida glabrata*, 9% (*n*=4); and *Candida krusei*, 9% (*n*=4). *Candida albicans* was the most frequently isolated species (100% of infectious disease cases, 67% in ICU, 53% in

surgery), except in hematology wards, where *Candida tropicalis* represented two-thirds of the isolates due to the inclusion of patients infected during a microepidemic in the Dijon hospital.

The results in Fig. 1 show the date of serum sampling in relation to the date of positive blood culture sampling. This shows that, during the 30-day period covered by the study (15 days before blood culture and 15 days after), 35 (78%) patients had at least one positive serological test. Eighteen patients had a positive test before blood culture sampling, 13 patients on the day of blood culturing, and four patients during the succeeding days. In total, 31 (69%) patients had a positive serological test and 14 (31%) had a negative serological test on or before the day of blood culture. Among these latter patients, two seroconverted on day 1. The mean delay between the date of blood culture and the day when blood cultures became positive was 3 days (grey line, Fig. 1) [25]. When this delay was taken into consideration, at least one serological test was positive in 33 (73%) patients before blood cultures became positive.

The Platelia *Candida* Antigen test was positive in 24 patients between day 15 before and the day that blood cultures became positive (mean, 6 days before blood cultures became positive). Antibodies were detectable in 21 patients between day 17 before and the day that blood cultures became positive (mean, 7 days before blood cultures became positive). Twelve patients had both positive antigen and positive antibody tests before blood cultures became positive.

A few discrepancies (22/137 sera) were observed between the Platelia *Candida* Antibody test and co-counterimmunoelectrophoresis (Co-CIE). Only three patients (26, 32, and 43) had a positive Platelia Antibody test in the absence of positive Co-CIE, whereas in the other cases (patients 2, 3, 11, 25, 36, and 44) Co-CIE was positive alone. However, with the exception of patients 11 and 25, who were infected by *Candida glabrata* and *Candida krusei*, all patients had antigen in their serum simultaneously or a few days later.

The basis for inclusion of the 45 patients was the observation of one positive blood culture. However, a single blood culture only was positive for each patient out of the 166 tested (Fig. 1). Within 2 weeks before positive blood cultures were obtained, a total of 82 blood samples from 28 patients (minimum 1, maximum 10, mean 3, per patient) were cultured, all of which were negative. A total of 33 patients (73%) presented a positive serological test before blood cultures became positive; among these, 10 (patients 2, 5, 6, 7, 20, 21, 24, 33, 39, and 40) had at least one negative blood culture taken at the time when a serological test was positive. The earliest positive serological test was the Platelia *Candida* Antigen test (8/10 patients). Thirty-nine blood samples from 22 patients with evidence of candidemia were also cultured, and all were negative.

The overall kinetics of serological tests becoming positive per patient are presented in Fig. 2. This figure demonstrates that at 5 days before blood samples for culture were obtained, 14 of 30 (47%) patients for whom sera were available had already given a positive serological test. On the day of blood culturing, 31 of 45 (69%) patients had at least one positive serological test. Antibodies were, in general, detected first, and antigenemia appeared gradually, even in patients who first presented with an antibody response that subsequently disappeared. At the time blood cultures became positive, and later, the population of patients with positive serological tests was distributed into three approximately equal groups: patients having positive antibody detection tests alone; patients having positive antigen detection tests alone; and patients for whom both tests were positive (usually not simultaneously). However, these kinetics were different depending on the wards on which the patients were hospitalized. As shown in Fig. 1, all hematology patients presented at least one positive serological test before blood cultures became positive. Among them, nine (82%) had positive antigen tests versus four who had positive antibody tests. Similarly, in the ICU, the antigen detection test was positive more frequently than the antibody detection tests before blood cultures became positive (40% vs. 33%, respectively). In contrast, in the surgical services, a predominance of antibody response over mannanemia was observed before blood cultures became positive (65% vs. 47%, respectively).

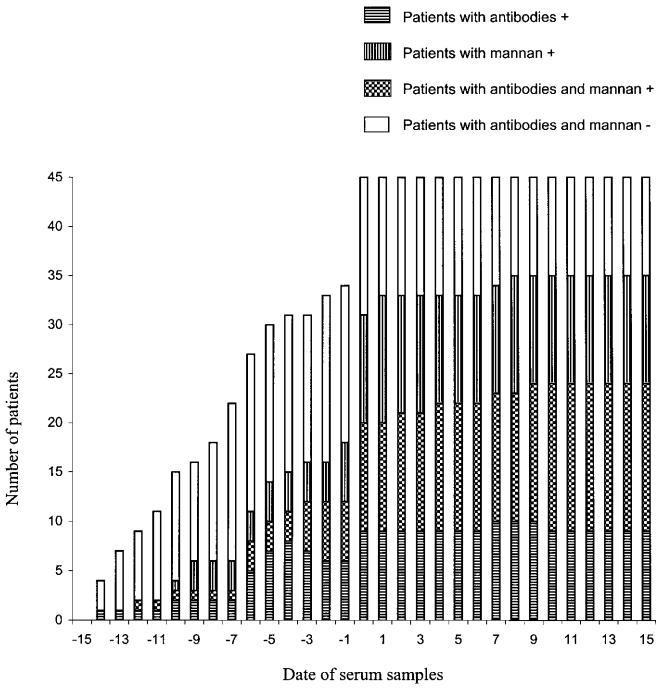

Fig. 2 Evolution of positive serological tests in relation to day of blood culture sampling

When the results of the serological tests were related to the *Candida* species involved, serological tests were positive before blood cultures became positive in 74% of patients infected by *Candida albicans* and in 73% of patients infected by other *Candida* species (9/9 patients infected by *Candida tropicalis*, 3/5 infected by *Candida*

parapsilosis, 2/4 infected by *Candida glabrata*, and 2/4 infected by *Candida krusei*). The overall mortality of patients at 30 days was 47% (Fig. 1) and was variable depending on the hospital service: 87% in the ICU, 36% in hematology, and 18% in surgery. In the ICU and hematology, where mortality rates were higher, 11 of 12 patients infected by *Candida albicans* died. In services where mortality rates were lower (surgery and infectious diseases), only 36% of deaths were observed in patients infected with *Candida albicans*. Among the 24 patients who survived, 16 displayed an antibody response, while only 8 of 21 patients who died had a positive antibody test. Due to the balance between mannanemia and antimannan antibody response, this suggests that persistence of antigenemia in the absence of antibody production may be linked to an unfavorable prognosis.

Discussion

Yeasts of the genus *Candida* are a major cause of systemic infection in hospitalized patients receiving intensive medical care, particularly on ICU and hematology wards [4, 13]. The diagnosis of systemic candidiasis is difficult due to nonspecific clinical signs and the natural commensal status of these opportunistic pathogens [26]. A single isolation of *Candida* from blood was initially considered to reflect transient candidemia, and isolation of *Candida* from three successive positive blood cultures taken 48 h apart was recommended before *Candida* septicemia could be considered and treated [27, 28]. Although the possibility of candidemia may still exist, several recent studies have shown that isolation of *Candida* from a single blood culture was associated with a mortality rate of 40–60%, which was directly attributable to yeast infection and was linked to important extra hospital costs for patients who survived [8, 29, 30]. Current recommendations are the initiation of antifungal therapy following isolation of *Candida* from a single blood culture [16]. However, it is also agreed that blood cultures lack sensitivity, and due to the risk associated with systemic candidiasis, several physicians recommend empirical treatment based on a compendium of clinical signs, risk factors, and assessment of colonization, while others recommend systemic chemoprophylaxis using azole antifungal agents, despite the possible risk of generating resistant strains. Rationalization of this situation, which has resulted in regularly increasing hospital charges without any apparent decrease in the incidence of systemic candidiasis, is not easy in the absence of biological tests that prove the pathogenic development of *Candida*.

Several methods for the detection of yeast nucleic acids in serum or blood by polymerase chain reaction have given promising results [20, 31]; this method is more sensitive than blood culture and allows identification of the species involved. Alternative biological tests to diagnose systemic candidiasis consist of either the detection of other *Candida*-derived molecules (proteins, polysaccharides [mannan or glucans]) and metabolites, or the detection of antibodies against proteins or mannan [32]. In a recent study, the combined detection of antimannan antibodies and mannan antigen in patients' sera by EIA was shown to be useful for the diagnosis of systemic candidiasis. These tests, which are standardized, automated, and easy to perform, can be applied to large series of sera and are available commercially [22].

In this study, the question of complementing blood culture techniques with serological tests for the rapid diagnosis of candidiasis has been addressed in a series of patients with *Candida*-positive blood cultures. The range of *Candida* species isolated from blood was generally similar to that reported elsewhere [33, 34], with differences in prevalence due, in part, to the inclusion of five patients from microepidemic in a hematology ward in Dijon, France. Strain typing of these isolates is currently underway. The type of infected patient, who originated mainly from hematology wards, surgical services, and ICUs, is also representative of at-risk patients in large university hospitals. Under such conditions, 73% of patients were observed to display at least one positive serological test before blood cultures became positive.

Among patients for whom at least one serum sample was available before a blood culture was positive, detectable mannan and/or antibodies were found in almost half (47%) as early as 5 days before blood culture sampling and in more than two-thirds (69%) on the day of blood culture sampling. The medium time intervals between the observation of a positive serological test and a positive blood culture were 7 days for *Candida* antibody detection and 6 days for *Candida* antigen detection. As observed previously [22], the association of antigen and antibody detection on a given serum sample increased the chance of a positive test, although the sequence of events depended on the patients' predisposing condition. Non-neutropenic patients (mainly in surgery) tended to present first with positive antibody tests, followed by positive antigenemia and *Candida* isolation from the bloodstream. In contrast, patients from hematology services tended to present with antigenemia first followed by an antibody response whose onset was dependent on the period of release aplasia. Due to their complementarity, these tests may be useful in the clinical mycology laboratory for serological surveillance of patients irrespective of their degree of immunity or the stage of evolution of candidiasis [22, 35, 36]. When the specificity of these combined tests was explored previously, it was found to be reasonably high (93%) [22], and the present study demonstrates that the routine use of these tests would increase the sensitivity of as well as reduce the time to biological diagnosis of systemic candidiasis. Results from these tests may be obtained within 24 h of serum samples being taken.

Due to the general concomitance between the peak of antigenemia and positive blood cultures, it is suggested that an increase in blood culture positivity could be achieved if blood cultures are taken when the Platelia *Candida* antigen test is positive. Together, these elements would contribute to the earlier initiation of anti-

fungal chemotherapy, thereby reducing mortality or the duration of hospital stay.

In this study, the mortality rate among patients 30 days after blood culture sampling was 47%, which is similar to that reported previously [4, 5]. Whether this rate could have been reduced among the 33 patients who had presented at least one positive serological test before blood cultures became positive is impossible to say and has to be established by further prospective studies. During the 30-day period covered by the study, 35 of 45 (78%) patients presented at least one positive serological test. To date, retrospective studies with the Platelia *Candida* tests have involved a total of 341 serum samples from 106 different patients [22, 35, 36]. From these studies, it has been concluded that the sensitivity of the Platelia tests ranged from 80 to 100% for infections caused by the most pathogenic *Candida* species, namely *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*, which account for over 80% of all nosocomial *Candida* infections [11]. For species such as *Candida krusei* and *Candida parapsilosis*, the sensitivity ranged from 40 to 50%. Our results from this series of patients were similar. The sensitivity of the Platelia tests was 78% for infections caused by the most pathogenic *Candida* species. For *Candida krusei* and *Candida parapsilosis*, the sensitivity was 44%.

Mortality was mainly associated with *Candida albicans* infection. Although the number of patients is too small to draw any definitive conclusions, there appeared to be an association between a positive anti-mannan antibody response (generally in the absence of detectable antigen) and a favorable outcome, irrespective of the species involved. Whether this is attributable to the patients' immune status or to protective antibodies is impossible to say in the absence of mortality directly attributable to yeast infection. Moreover, mannan itself is a complex repertoire of epitopes [37], some of which have been shown to induce protective antibodies and some not [38, 39] in experimental models. The use of purified oligomannosides would be necessary to assess the pathophysiological significance of these epitopes.

The specificity of the Platelia tests was assessed previously as 93% using a large series of relevant control sera, including sera from hospitalized colonized patients, patients with deep mycoses not caused by *Candida*, and healthy blood donors [22]. The sensitivity per patient in this series was 73% before a single blood culture become positive. However, in contrast to previous reports in which only positive blood cultures were considered [40], we have included the number of blood cultures that were negative before the positive sample referred to was taken. Although this number varies depending on the individual patient, these results demonstrate the lack of sensitivity of blood cultures, whose positivity is recommended for initiation of treatment. For 10 such patients, at least one blood culture was negative (and up to 6 blood cultures were negative) and serological tests were positive before a positive blood culture was obtained. A representative example is patient no. 40 (Fig. 1), for whom both antigen and antibody tests were positive in the single serum available 12 days before a positive blood culture was obtained, although 6 blood cultures drawn in between were negative.

The results of the present study suggest that the inclusion of regular serological surveillance (every 3 days) for mannanemia and anti-*Candida albicans* antibodies in patients would complement blood cultures for the early detection of candidiasis in at-risk patients. Co-CIE antibody detection did not confirm the positive Platelia *Candida* antibody tests in all patients. This serological technique was less sensitive than the combined detection of mannanemia and anti-mannan (69% vs. 80%). However, Co-CIE antibody detection was useful for the diagnosis of systemic candidiasis in patient no. 25, in whom it was the only positive serological test before blood cultures became positive. Prospective studies are necessary to determine whether this approach would be medically and economically beneficial to the control of current acute problems linked to the development of nosocomial *Candida* infections.

Acknowledgements The authors thank L. Richard for her valuable technical assistance. They are grateful to the late Dr. J. Fruit for her helpful advice and attention, and to Dr. U. Hopwood for help in editing the manuscript.

References

1. Jarvis WR, Martone WJ: Predominant pathogens in hospital infections. Journal of Antimicrobial Chemotherapy (1992) 29, Supplement A:19–24
2. Voss A, Kluytmans JA, Koeleman JG, Spanjaard L, Vandenbroucke-Grauls CM, Verbrugh HA, Vos MC, Weersink AY, Hoogkamp-Korstanje JA, Meis JF: Occurrence of yeast bloodstream infections between 1987 and 1995 in five Dutch university hospitals. European Journal of Clinical Microbiology & Infectious Diseases (1996) 15:909–912
3. Harbarth S, Ruef C, Francioli P, Widmer A, Pittet D: Nosocomial infections in Swiss university hospitals: a multi-centre survey and review of the published experience. Swiss-Noso Network. Schweizerische Medizinische Wochenschrift (1999) 129:1521–1528
4. Wenzel RP: Nosocomial candidemia: risk factors and attributable mortality. Clinical Infectious Diseases (1992) 20:1531–1534
5. Pittet D, Li N, Wenzel RP: Association of secondary and polymicrobial nosocomial bloodstream infections with higher mortality. European Journal of Clinical Microbiology & Infectious Diseases (1993) 12:813–819
6. Fraser VJ, Jones M, Dunkel J, Storfer S, Medoff G, Dunagan WC: Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality (see comments). Clinical Infectious Diseases (1992) 15:414–421
7. Vincent JL, Anaissie E, Bruining H, Demajo W, El-Ebiary M, Haber J, Hiramatsu Y, Nitenberg G, Nystr öm PO, Pittet D, Rogers T, Sandven P, Sganga G, Schaller MD, Solomkin J: Epidemiology, diagnosis and treatment of systemic *Candida* infection in surgical patients under intensive care. Intensive Care Medicine (1998) 24:206–216
8. Debra AG, Sondra JS, Robert JF: Cost analysis of *Candida* infection among surgical intensive care unit patients. Clinical Drug Investigation (1996) 12:176–180
9. Klepser ME, Ernst EJ, Pfaller MA: Update on antifungal resistance. Trends in Microbiology (1997) 5:372–375

10. Nguyen MH, Peacock JE, Morris AJ, Tanner DC, Nguyen ML, Snydman DR, Wagener MM, Rinaldi MG, Yu VL: The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance. American Journal of Medecine (1996) 100:617–623
11. Pfaller MA: Nosocomial candidiasis: emerging species, reservoirs, and modes of transmission. Clinical Infectious Diseases (1996) 22, Supplement 2:89–94
12. Rex JH, Rinaldi MG, Pfaller MA: Resistance of *Candida* species to fluconazole. Antimicrobial Agents and Chemotherapy (1995) 39:1–8
13. Wingard JR: Importance of *Candida* species other than *Candida albicans* as pathogens in oncology patients. Clinical Infectious Diseases (1995) 20:115–125
14. Armstrong D: Problems in management of opportunistic fungal diseases. Reviews of Infectious Diseases (1989) 11, Supplement 7:1591–1599
15. Bodey GP: Candidiasis. Pathogenesis, diagnosis and treatment. Raven Press, New York (1993) pp 407–408
16. Edwards JE, Bodey GP, Bowden RA, Buchner T, de Pauw BE, Filler SG, Ghannoum MA, Glauser M, Herbrecht R, Kauffman CA, Kohno S, Martino P, Meunier F, Mori T, Pfaller MA, Rex JH, Rogers TR, Rubin RH, Solomkin J, Viscoli C, Walsh TJ, White M: International conference for the development of a consensus on the management and prevention of severe candidal infections. Clinical Infectious Diseases (1997) 25:43–59
17. Odds FC: Candida and candidiasis. A review and bibliography. Baillière-Tindall, London (1988) pp 206–222
18. Reiss E, Morrison CJ: Nonculture methods for diagnosis of disseminated candidiasis. Clinical Microbiology Reviews (1993) 6:311–323
19. Morris AJ, Byrne TC, Madden JF, Reller LB: Duration of incubation of fungal cultures. Journal of Clinical Microbiology (1996) 34:1583–1585
20. Morace G, Pagano L, Sanguinetti M, Posteraro B, Mele L, Equitani F, D'Amore G, Leone G, Fadda G: PCR-restriction enzyme analysis for detection of *Candida* DNA in blood from febrile patients with hematological malignancies. Journal of Clinical Microbiology (1999) 37:1871–1875
21. Fukazawa Y: Antigenic structure of *Candida albicans*. Immunochemical basis of the serologic specificity of the mannans in yeast. Immunology Series (1989) 47:37–62
22. Sendid B, Tabouret M, Poirot JL, Mathieu D, Fruit J, Poulain D: New enzyme immunoassays for sensitive detection of circulating *Candida albicans* mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. Journal of Clinical Microbiology (1999) 37:1510–1517
23. Poulain D, Pinon JM: Diagnosis of systemic candidiasis: development of co-counterimmunoelectrophoresis. European Journal of Clinical Microbiology (1986) 5:420–426
24. Poulain D, Fruit J, Fournier L, Dei Cas E, Vernes A: Diagnosis of systemic candidiasis: application of co-counterimmunoelectrophoresis. European Journal of Clinical Microbiology (1986) 5:427–434
25. Murray PR, Hollick GE, Jerris RC, Wilson ML: Multicenter comparison of BACTEC 9050 and BACTEC 9240 blood culture systems. Antimicrobial Agents and Chemotherapy (1998) 36:1601–1603
26. Richardson MD, Kokki MH: New perspectives in the diagnosis of systemic fungal infections. Annals of Medicine (1999) 31:327–335
27. Soutter DI, Todd TR: Systemic candidiasis in a surgical intensive care unit. Canadian Journal of Surgery (1986) 2:197–199
28. Ellis CA, Spivack ML: The significance of candidemia. Annals of Internal Medicine (1967) 67:511–513
29. Meunier F: Therapy of systemic candidiasis. Mycoses (1994) 37, Supplement 2:52–55
30. Wey SB, Mori M, Pfaller MA, Woolson RF, Wenzel RP: Hospital-acquired candidemia. The attributable mortality and excess length of stay. Archives of Internal Medicine (1988) 148:2642–2645
31. Loffler J, Hebart H, Sepe S, Schumacher U, Klingebiel T, Einsele H: Detection of PCR-amplified fungal DNA by using a PCR-ELISA system. Medical Mycology (1998) 36:275–279
32. Martinez JP, Luisa Gil M, Lopez-Ribot JL, LaJean Chaffin W: Serologic response to cell wall mannoproteins and proteins of *Candida albicans*. Clinical Microbiology Reviews (1998) 11:121–141
33. Pfaller MA, Jones RN, Doern GV, Sader HS, Messer SA, Houston A, Coffman S, Hollis RJ, and the SENTRY Participant Group: Bloodstream infections due to *Candida* species: SENTRY antimicrobial surveillance program in North America and Latin America, 1997–1998. Antimicrobial Agents and Chemotherapy (2000) 4:747–751
34. Rangel-Frausto MS, Wiblin T, Blumberg HM, Saiman L, Patterson J, Rinaldi M, Pfaller M, Edwards JE, Jarvis W, Dawson J, Wenzel RP: National epidemiology of mycoses survey (NEMIS): variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal intensive care units. Clinical Infectious Diseases (1999) 29:253–258
35. Sendid B, Bonnin A, Lopez J, Tabouret M, Poulain D, Caillot D: Mannan antigen and antibodies in haematological patients with invasive candidiasis. Clinical Microbiology and Infection (2000) 6, Supplement 1:34
36. Sendid B, Poirot JL, Tabouret M, Bonnin A, Caillot D, Camus D, Poulain D: Combined detection of mannanemia and antimannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic *Candida* species. Journal of Medical Microbiology (2001) in press
37. Suzuki S: Immunochemical study on mannans of genus *Candida*. I. Structural investigation of antigenic factors 1, 4, 5, 6, 8, 9, 11, 13, 13b and 34. Current Topics in Medical Mycology (1997) 8:57–70
38. Han Y, Cutler JE: Antibody response that protects against disseminated candidiasis. Infection and Immunity (1995) 63:2714–2719
39. Han Y, Kanbe T, Cherniak R, Cutler JE: Biochemical characterization of *Candida albicans* epitopes that can elicit protective and nonprotective antibodies. Infection and Immunity (1997) 65:4100–4107
40. Herent P, Stynen D, Hernando F, Fruit J, Poulain D: Retrospective evaluation of two latex agglutination tests for detection of circulating antigens during invasive candidosis. Journal of Clinical Microbiology (1992) 30:2158–2164