The main risk factors for candidemia are the degree and duration of neutropenia; prior colonization with Candida spp.; mucosal barrier disruption following cytotoxic chemotherapy or irradiation; prolonged use of broad-spectrum antibiotics, particularly glycopeptides; the number of antibiotics received; and mucosal colonization by Candida (46). These risk factors, which serve to identify individuals at high risk of developing candidemia, are shared by a large number of patients. Moreover, the clinical features of systemic candidiasis are nonspecific, making the early diagnosis of systemic candidiasis difficult (38, 45). Histopathology- or culture-based examination of sterile body sites is often not feasible in practice, and for reasons that remain unclear, culture of blood for fungi, even when it is performed daily, has a poor sensitivity (9, 24). As a consequence, the diagnosis of candidemia is generally established at a late stage, or even by autopsy, in a considerable number of cases, which accounts for its poor prognosis (8, 13).

In order to overcome these difficulties, several groups have focused on the development of biological tests based on the detection of either antibodies to Candida proteins or polysaccharides or Candida components such as mannan (32, 48), glucan (28), arabinol (41), or nucleic acids (15, 21) in body fluids under the assumption that these molecules would prove to be early specific markers of disseminated infection. Among these putative markers, mannan is a major component of the
Candida cell wall, both quantitatively and qualitatively. Extensive studies of this polysaccharide have demonstrated its role as a potent modulator of innate and adaptive immunity (24, 31, 36, 40). Mannan induces a strong antibody response toward a large repertoire of oligomannose epitopes. Some of these antibodies may be protective to the host, while others may not. In this context, a new diagnostic approach has recently been proposed by our group, based on the combined detection of mannan and antimannan antibodies in patients at risk of developing candidiasis. This strategy is based on the detection of mannan and antimannan antibodies by two distinct immunoenzymatic assays (the Platelia Candida-specific antigen [Ag] and Platelia Candida-specific antibody [Ab] tests; Bio-Rad Laboratories, Marnes-la Coquette, France). Retrospective studies based on the analysis of more than 500 serum samples from 130 patients with candidemia treated in different hospital wards and 150 control serum samples from patients without candidemia showed that the combined use of these tests had a 93% specificity and an 80% sensitivity in the diagnosis of infections caused by the most pathogenic Candida species. Among the main conclusions of these retrospective studies was the fact that regular serum sampling was critical to achieving an early diagnosis (36, 37, 49).

We recently investigated a pseudoepidemic of Candida tropicalis infections that occurred in a cohort of seven adult neutropenic patients with lymphoblastic or myeloid leukemia undergoing myeloablative treatment. The availability of serial serum samples together with complete clinical and biological records gave us the opportunity to assess the Platelia tests for the detection of infections caused by C. tropicalis, a species emerging as one of the main causative agents of candidemia in patients with malignancies (3, 20).

MATERIALS AND METHODS

Patients. Between June 1998 and December 2000, a cohort of consecutive adult patients with hematological malignancies was enrolled in a prospective study aimed at evaluating the value of serial screening for Aspergillus galactomannan in patients at high risk of invasive aspergillosis. All patients received myeloablative treatment that induced neutropenia (polymorphonuclear leucocyte count, <500/μl). During the neutropenia that followed chemotherapy, patients were hospitalized in single reverse isolation rooms or in laminar air-protected rooms. Chest X rays were systematically taken in these rooms twice a day. During this survey seven patients (four females and three males; mean age, 51.8 ± 14.2 years) developed systemic C. tropicalis infection, confirmed by a positive blood culture and subsequent mycological examination of cerebrospinal fluid or urine specimens, liver or spleen biopsy specimens, cutaneous nodules, or aqueous humor, depending on the clinical symptoms. The clinical and mycological findings are summarized in Table 1, together with the nature, duration, and dose of antifungal treatment. This cluster of C. tropicalis infections probably resulted from a microepidemic, which is under investigation. A total of 83 serum samples were collected from these patients (average number of serum samples per patient, 11.2 ± 5.6). The samples were stored at −80°C, transferred in dry ice, and tested in a blinded fashion.

Controls consisted of 12 febrile neutropenic adult patients hospitalized in the same hematology unit (4 patients with invasive aspergillosis, 4 patients with bacterial sepsis, and 4 patients with no evidence of infection). Four serum samples were collected from each of the control patients before (n = 2), during (n = 1), and after (n = 1) the period of aplasia. Altogether, 48 control serum samples were available for testing.

Myological investigations. Blood samples for culture and specimens from patients infected with fungi were collected according to established clinical guidelines (7). Ten milliliters of blood was inoculated in a BACTEC Plus aerobic/Bottle and incubated for up to 14 days with the BACTEC 9240 blood culture system (Becton Dickinson, Le Pont de Claix, France). Other clinical samples were cultured at 30°C on Sabouraud dextrose agar containing gentamicin (40 mg/liter) and chloramphenicol (0.4 g/liter). The yeast isolates were identified by the presence or absence of germ tube formation at 37°C in human serum and by the API 32C identification system (Bio-Mérieux, Marcy l’Etoile, France) (11).

Detection of anti-C. albicans mannan antibodies in human sera. Antibodies to C. albicans mannan were detected by the commercially available Platelia Candida-specific Ab test (Bio-Rad Laboratories) (36). Microtiter plates were sensitized with C. albicans cell wall mannan, which was extracted and purified from C. albicans strain VW32 grown in bioreactors by standard protocols (10). Enzyme immunoassay reactions were performed with the BIP III automate (Dade-Behring Laboratories, Paris, France). Each set of tests included a standard curve, which was obtained by using serial twofold dilutions of a pool of sera strongly reacting with yeast mannan. For individual serum samples, 100 μl of serum diluted 1/6,400 was applied to each well, and the plate was incubated at 37°C for 1 h. After the plate was washed, 100 μl of horseradish peroxidase-conjugated anti-human immunoglobulin was added, and the mixture was incubated for 1 h at 37°C. After intensive washing, the reactions were revealed by 30 min of incubation in the dark with 200 μl of tetramethylbenzidine solution, and the absorbance (A = 450 and 620 nm) was then measured. The results are reported as arbitrary units (AU) in relation to the standard curve. Ten AU was considered indicative of candidiasis (37).

Detection of mannannanemia. Mannannanemia was detected by the commercially available Platelia Candida-specific Ag test (Bio-Rad Laboratories). This test involves the monoclonal antibody (MAB) designated EBCAI, which recognizes a repetitive epitope present in Candida mannan (42). The minimal epitope of this MAB has been shown to correspond to α-linked mannopentaose of the C. albicans VW32 mannan acid-stable domain. This epitope is also present on numerous C. albicans mannoproteins (18). Because of the repetitive nature of this epitope, MAB EBCAI is used as both the antigen-capture and the detection antibody. Microtiter plates were sensitized with MAB EBCAI in an industrial setting.

Three-hundred microliters of patient serum was denatured with 100 μl of EDTA treatment solution, boiled for 3 min, and then centrifuged at 10,000 × g for 10 min. Fifty microliters of supernatant was mixed in EBCAI-coated wells with 50 μl of horseradish peroxidase-conjugated MAB EBCAI. After incubation for 90 min at 37°C, the plates were washed thoroughly and the reaction was revealed by incubation with 200 μl of tetramethylbenzidine solution for 30 min in the dark. The optical density was read at A450 and 620 nm on a PR2100 reader (Bio-Rad Laboratories). Each experiment included a calibration curve, which was made with a pool of normal human serum supplemented with known concentrations of mannan ranging from 0.1 to 2 ng/ml. Reactions were performed in duplicate. For mannanemia levels exceeding 2 ng/ml (above the range of the calibration curve), a precise determination of the antigen concentration, which is important for patient monitoring, was performed systematically. The test was repeated after dilution of the patient serum 1/5 in negative control serum.

RESULTS

The clinical and antifungal therapy data for the patients with C. tropicalis bloodstream infections are shown in Table 1. Day 0 corresponds to the date when the first blood sample positive by culture was drawn (i.e., an average of 2 to 3 days before fungal growth was obtained). Patients 1 and 3 had concurrent aspergillosis, as suggested by the high serum Aspergillus galac- tomannan titers, computed tomography scans, and isolation of...
Aspergillus fumigatus from bronchoalveolar fluid. Patient 7 also presented with septicemia due to Enterococcus faecium and Staphylococcus epidermidis. All patients with C. tropicalis infection included in this study survived at day 30. Figures 1 to 7 show the evolution of mannanemia, antimannan antibodies, leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Altogether, a mean of eight culture-negative blood samples were collected before the first culture-positive blood sample was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively. As described above, day 0 corresponds to the date when the first blood sample positive by culture was collected. Interestingly, during the same period, significant mannanemia was detected in sera obtained 12, 7, 2, 9, 2, and 2 days before day 0 in patients 1, 2, 3, 4, 6, and 7, respectively. Peaks of mannanemia often coincided with low leukocyte counts, and negative blood cultures for patients 1 to 7, respectively.

### DISCUSSION

Candidiasis is the leading cause of invasive fungal infection in patients with malignant hematological disorders (particularly those with acute myeloid leukemia) and bone marrow transplant recipients (29, 35, 44). Prolonged neutropenia associated with the treatment of hematological malignancies is a major risk factor predisposing these patients to invasive candidiasis (1, 20, 23). The growing incidence of systemic candidiasis has a strong impact on overall rates of mortality (13, 43). Moreover, the increased morbidity as a result of candidemia generates important extra costs related to longer hospital stays and prophylactic, empirical, or curative antifungal treatment (33).
FIG. 1 Kinetics of circulating mannan (●) and antimannan antibodies (○) in sequentially drawn sera from seven adult neutropenic patients (panels 1 to 7, respectively) with systemic candidiasis. The evolution of mannanemia and antimannan antibodies (the levels of which are given in AU) is shown, together with leukocyte (WBC) counts (the bottom part of each figure) and the number of negative blood cultures (vertical bars preceding day 0), as a function of time in relation to day 0, which represents the day on which the first blood sample positive for \textit{C. tropicalis} by culture was taken. The horizontal dotted line represents the cutoff values of the tests (0.5 ng/ml for the mannanemia test and 10 AU for the antimannan antibody test).
FIG. 1—Continued.
While the availability of new antifungal drugs such as echinocandins (39) and new azoles (16) is a promising step toward the development of non-culture-based methods based on the detection of fungal components (28, 37, 48), the presence of circulating epitopes from fungal cells may contribute to the pathophysiology of infection (31). One of the immunomodulatory properties of fungi is the induction of an antibody response that can be used in the diagnosis of fungal infections. The repertoire of epitopes expressed by fungi can vary depending on the species and the stage of infection. However, it is important to note that the antibody response may be down-regulated after the initial infection, which limits its diagnostic value.

In conclusion, the development of new diagnostic tools for fungal infections is crucial for the timely initiation of antifungal therapy. These tools should be specifically designed to identify the species and strain of the infecting fungus, as well as to monitor the response to treatment. The combination of culture-based and non-culture-based methods, such as antigen detection, is likely to provide the most accurate and timely diagnosis of fungal infections.
patients, suggests a common source of infection. The molecular relatedness of the strains is under investigation. All seven patients were included in a prospective evaluation of serum *Aspergillus* galactomannan levels at the time of *C. tropicalis* infection. Serial serum samples were thus available, which allowed a thorough evaluation of the two Platelia tests under the recommended conditions (i.e., screening of a series of samples starting before the onset of infection). Previous studies have reported good specificities and negative predictive values for the Platelia tests (37). These findings were confirmed by including relevant controls (sera from febrile patients treated in the same ward and either infected or not infected with *A. fumigatus* or bacteria).

In all patients except patient 5, significant mannanemia was detected between 12 and 2 days before the first positive blood culture, a period when blood cultures were constantly negative. In patient 5, the first positive Platelia *Candida*-specific Ag test was obtained on day 0. In most patients, significant mannanemia persisted for several weeks. Mannanemia was detected early in patient 4, who finally presented with histologically and culture-proven hepatosplenic candidiasis. In this respect, Prella et al. (M. Prella, J. Bille, M. Pugnale, M. Cavassini, C. Durussel, M. Knaup, B. Duvoisins, M. G. Plassier, and T. Calandra, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 847, 2001) recently reported on a series of 12 patients with confirmed hepatosplenic candidiasis; 11 of these patients presented with positive mannanemia and/or antiman- nan antibodies by the Platelia Candida-specific tests an average of 18 days prior to the appearance of radiological signs. This is an interesting finding, since the diagnosis of hepatosplenic candidiasis is generally difficult to establish.

Patient 4 also presented a sharp peak in antiman- nan antibody titers. However, the antibody response was transient and rapidly became undetectable when the antigen titer again increased at day 0, a profile consistent with immune complex formation. Significant antibody responses were detected in patients 2 and 3 for several weeks. In these two patients, as well as in patients 6 and 7, recovery from asplasia was associated with an increase in antimanman antibody levels. No significant antibody responses were observed in the controls. As a complement to the antigen levels, which were significantly higher during the period of asplasia (*P < 0.02 by Student's *t* test), detection of antibodies at levels that mirror the level of anti- genemia are also indicative of tissue invasion. The results of the present study suggest that the inclusion of regular serological surveillance for mannanemia and anti-*Candida* mannan antibodies in patients would complement blood culture for the early detection of candidiasis in at-risk patients.

Analysis of the contribution of serology to the diagnosis of candidiasis is often hampered by the small number of serum samples available and the irregularity of sampling. The availability of a sample collected before the beginning of infection is also necessary, since rising titers are highly indicative of an increasing fungal burden. The samples collected during the present study met all these requirements. Altogether, the correlation of serological and microbiological findings with clinical and radiological data allowed a precise evaluation of the role of the combined Platelia tests in the detection of *C. tropicalis* systemic infection in neutropenic patients.

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