行政院國家科學委員會補助專題研究計畫成果報告

侵襲性黴菌感染快速診斷之建立及應用

計畫類別：■個別型計畫　□整合型計畫

計畫編號：NSC90-2314-B-002-303

執行期間：90 年 8 月 1 日至 91 年 7 月 31 日

計畫主持人：張上淳 台大醫學院內科

共同主持人：陳宜君台大醫學院內科

計畫參與人員：戴惠敏

執行單位：台大醫學院內科

中華民國 91 年 12 月 30 日

中文摘要

由於缺乏特定臨床表徵，以及血液培養陽性率的比例偏低，使得念珠菌菌血症或侵襲性念珠菌感染，特別是慢性念珠菌感染的臨床診斷較困難。此外，這些菌種的移生狀態也使得檢驗室的培養預測更形複雜。我們評估一種製備好、針對 mannan 抗原和抗體的聯胺免疫吸附測定（ELISA）。收集的檢體涵括 2000 年三月至 2002 年十一月間，88 名罹患侵襲性念珠球菌感染的病患、22 名念珠球菌移生而未接受抗黴菌藥物治療、69 名未發生念珠球菌感染或移生的病患、以及 7 名健康者，共計 490 支血清檢體。計算每位病人的抗原和抗體測試結果。共計 47 名病人的念珠菌血液培養結果爲陽性。88 名罹患侵入性念珠球菌感染的病人中，31 名（35.2%）爲中性白血球過低，6 名接受類固醇治療、6 名發生腎臟衰竭並/或接受血液透析。罹患侵襲性念珠菌感染之住院病患的死亡率爲 22.7%（20/88），相關死亡率爲 18.2%（16/88）。抗原的整體敏感度僅 29.5%，與急性念珠球菌感染病患之敏感度（19.6%）相比，慢性念珠球菌感染或微生物失敗（microbiological failure）之病患的敏感度較高，分別爲 50.0%和 43.8%，p=0.025。此外，因侵襲性念珠球菌感染而死亡的病患，其抗原測試敏感度較高（56.3%），而出院存活（23.5%）或因其他原因而死亡（25%）之患者的抗原測試敏感度較低（p=0.035）。再者，抗原與抗體合併測試，可使侵襲性念珠球菌感染的敏感度高達 79.5%，且特異性達到 91.8%。此項研究確認血清診斷對於侵襲性念珠菌感染的診斷而言，具有實用價值。

關鍵字：侵襲性念珠菌感染（invasive candidiasis），血清診斷 (serodiagnosis)，聯胺免疫吸附測定（ELISA），mannan 抗原

Summary

The diagnosis of candidemia or invasive candidiasis is difficult due to the lack of specific clinical features and low sensitivity of blood cultures, particularly for patients with chronic invasive candidiasis. Besides, the interpretation of laboratory cultures is complicated by the commensal status of these organisms. A commercial enzyme-linked immunosorbent assay (ELISA) for *Candida* mannan antigen and anti-mannan antibody (Platelia Candida Ag, Platelia Candida Ab, Bio-Rad) were evaluated. From March 2000 through Nov 2002, a total of 490 serum samples were collected on a consultation basis from 88 patients with invasive candidiasis, 22 patients with *Candida* colonization and without antifungal therapy, 69 patients without *Candida* infection or colonization, and 7 healthy

volunteers. The results of antigen and antibody assays were calculated per patients. A total of 47 patients had blood cultures positive of *Candida* spp. Thirty-one of 88 patients with invasive candidiasis (35.2%) were neutropenic, 6 received corticosteroids and 6 had renal failure and/or received dialysis. In-hospital mortality of patients with invasive candidiasis was 22.7% (20/88) and attributable mortality was 18.2% (16/88). The overall sensitivity of antigen assay was 29.5%, and was higher for patients with either chronic invasive candidiasis (50.0%) or microbiologic failure (43.8%) than those with acute invasive candidiasis (19.6%) (P=0.025). Besides, the sensitivity of antigen assay was higher for patients died of invasive candidiasis (56.3%) than those were alive at discharge (23.5%) or died due to other reasons (25.0%) (P=0.035). Furthermore, the combined use of antigen and antibody detection allowed the detection of 79.5% of invasive candidiasis with a specificity of 91.8%. This study confirmed the usefulness of serodiagnosis of invasive candidiasis on a consultation basis. For such a test with consistently high specificity, combined test complement blood cultures for both acute and chronic disseminated candidiasis.

Key words: invasive candidiasis, serodiagnosis, ELISA, mannan antigen

Introduction

The frequency of nosocomial infection due to fungi, particularly *Candida* spp., has increased significantly in the past decade (Beck-Sague et al., 1993; Chen et al., 1997). *Candida* became the leading pathogens of nosocomial blood culture isolates (Chen et al. 1997). Mortality associated with candidemia is high, ranging from 40% to 60% (Wey et al., 1988; Hung et al., 1996). However, studies has shown an error rate of 15-30% in defining a population of candidemic patients who do not received antifungal therapy (Edwards et al., 1997; Hung et al., 1996). The diagnosis of candidemia or systemic candidiasis is difficult due to the lack of specific clinical features. Moreover, interpretation of laboratory cultures is complicated by the commensal status of these organisms and the mere isolation of yeast from clinical materials is of questionable significance. Our prospective study has demonstrated that only 25% of critically ill patients with *Candida* colonization developed invasive candidiasis subsequently (Chen et al., 2001).

Blood culture is the golden standard for the diagnosis of systemic candidiasis, particularly candidemia. However, blood cultures have a reputation for being unreliable in confirming the diagnosis and were cited as being negative in 56% of necropsy proven cases of systemic candidiasis (De Repentigny and Reiss, 1984). Even lysis-centrifugation Isolator system failed to identify 27% of patients with histologically documented systemic candidiasis (Telenti and Roberts, 1989). Depending on the patient population, the method used and the frequency of sampling, blood cultures were positive in 25~82% of leukemic patients with systemic candidiasis (Jones, 1990). Systemic candidiasis can take either an acute or chronic course. Patients with hepatosplenic candidiasis, a representative of chronic disseminated candidiasis, are the least likely to have positive blood cultures, only 8%~32% of cases having positive blood cultures (Thaler et al., 1988; Kauffman et al., 1991; Chen et al., 2003).

A commercial sandwich enzyme-linked immunosorbent assay (ELISA) for *Candida* mannan antigen (Platelia *Candida* Ag, Rio-Rad) and a ELISA assay for anti-mannan antibody (Platelia *Candida* Ab, Bio-Rad) were evaluated. Mannan is the main component of the *Candida* cell wall and a highly immunogenic polysaccharide (?).Previous studies required serially serum samples for determination of usefulness of the ELISA in the diagnosis of systemic candidiasis and the assays become not cost effective. In clinical practice, the assay will be requested at the time when clinically warranted. Therefore, we conducted a prospective study on a consultation basis.

Methods

Study population

From March 2000 through Nov 2002, a total of 490 sera were collected from 179 patients and 7 healthy volunteers. We evaluated these patients on consultation bases due to one of the following clinical situations (1) persistent signs of infections or deteriorated clinical conditions despite apparently appropriate broad-spectrum antibacterial treatment, (2) persistent fever (>96 h) in neutropenic patients with cancer, (3) persistent signs of infection despite apparently appropriate antifungal treatment (>72 h). Results of antimicrobial therapy and post-mortem findings were also included in the results. Clinical data were analyzed prospectively independent of

serological results.

Definitions

The invasive fungal infections (IFIs) in immunocompromised patients were defined according to an international consensus developed by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG definitions) (Ascioglu et al., 2002). The IFIs were classified to proven, probable, and possible to express disease certainty. For those high-risk patients with persistent febrile neutropenia for >96 h refractory to appropriate broad-spectrum antibacterial treatment and response to antifungal therapy were classified as suspected IFI if they did not fulfill the microbiological and clinical criteria of EORTC/MSG definitions. The definite hepatosplenic candidiasis was defined as that imaging studies revealed multiple focal lesions in the liver and/or spleen and a specific diagnosis of IFI was established by the examination of deep-tissue specimens or blood culture (Ascioglu et al., 2002; Chen et al., 2003). Probable infection was defined as that multiple focal lesions were demonstrated in the liver and/or spleen by imaging without evidence of other microbiological infection.

As the EORTC/MSG definitions were applied to immunocompromised patients with cancer and hematopoietic stem cell transplants, we adapted additional criteria of presumed *Candida* infections (Table 1) which was modified from a definition used in our previous prospective study for critically ill patients (Chen et al., 2001). Patients were classified as 'infected' by clinical signs, imaging techniques, cultural isolation of *Candida* spp. and absence of viral and bacterial colonization (Table 1). A patient was considered infected if there was documentation of either a candidemia or a *Candida*l infection requiring the use of systemic antifungal therapy. Patients with *Candida* colonization and persistent signs and symptoms of infection despite apparently appropriate antibacterial therapy were considered to have *Candida* infection. Colonization was defined as cultural detection of *Candida* spp. without relevant signs of infection. Sepsis due to the IFI was defined if patients with the IFI fulfill the criteria of sepsis (systemic inflammatory response syndrome plus evidences of infection). Systemic inflammatory response syndrome (SIRS) was defined according to the criteria of the American College of Chest Physicians/Society of Critical Care

Medicine (ACCP/SCCM) Consensus Conference (Bone et al., 1992).

Microbiologic failure was defined as persistent signs of infection and repeated positive cultures from blood or infectious foci obtained from patients with candidemia or invasive candidiasis despite 72 hr of adequate antifungal therapy. The IFI was considered to be the primary cause of death of patients who died within 7 days following a positive blood culture of *Candida* or presumed onset of the IFI, when no other cause (including the primary disease, other infections and hemorrhage) was identified (Hung et al., 1996). The IFI was considered to be an associated cause of death when it was still present at time of death (as indicated by fever, with or without positive cultures), although another complication (e.g., hemorrhage or a secondary nonfugnal infection) or an uncontrolled underlying disease was also present. Attributable mortality included both. Death was considered unrelated to the IFI when candidemia was cleared or the IFI was improved consistently at time of death (by symptoms, signs and blood culture) and there was another likely cause such as the underlying disease.

Once patients were consulted, serum samples were collected, and the IFIs were defined prospectively and revised according to therapeutic responses and other evidences generated during follow-up periods. The classifications of IFIs were reviewed again at least 2 months later to exclude the possibility of infections due to mycobacteria or other slow-growing microorganisms and defined independently to the results of antigen/antibody assays.

Microbiological methods

Bacteria and fungi were identified and characterized by standard culture methods routinely used in our clinical microbiology laboratory. For specimens that contained numerous species of bacteria or fungi, only the four dominant organisms were selected for analysis. No cultures were done for viruses or *Mycoplasma*. Blood samples were cultured by inoculation into BACTEC fungal medium (Becton-Dickinson Microbiology Systems, Cockeysville, Md, USA) and tested daily for microbial growth by BACTEC 9240 system (BD Biosciences, Sparks, Md., USA). Organisms were identified by germ tube analysis and morphology on cornmeal-Tween 90 agar or, when necessary, by standard biochemical testing with the API 20C system (API BioMerieux Vitek, Inc., Hazelwood, Mo.).

Antigen/antibody detection

The frozen serum aliquots were coded and the test was done blindly. *Candida* antibody titers and antigen titer were determined. The sandwich ELISA was performed according to the manufacture's instructions. Sera were heat-treated (10 min at 100°C) for destruction of the heat-labile manna antigen. Briefly, 300 uL of each serum sample were used in a sandwich ELISA (*Candida* Platelia; Sanofi Diagnostics Pasteur [now Bio-Rad], France). This test utilized an immobilized monoclonal antibody and associates total anti-*Candida* immunoglobulin detection for antigen detection (Sendid et al., 1999, JCM). Each run contained a negative control (Tris-buffer saline alone) and two positive controls, containing 1 and 10 ng of GM per mL. The optical density results were converted into GM concentrations expressed in ng/mL, as deduced from the calibration curve obtained for each run. Positive samples contained a concentration of GM of $\geq$ 1 ng/mL. The results were expressed as index (index <1, negative; index > 1 and < 1.5, doubtful; index > 1.5, positive).

Statistical analysis

Statistical analyses were performed with Statistical Package for the Social Science (SPSS, version 10.0) for Windows (SPSS Inc., Chicago IL, USA). Univariate analysis of categorical variables was done with the chi-square test or Fisher's exact test. All *P* values were two-tailed, and a *P* value <0.05 was considered to indicate statistical significance. Sensitivity, specificity, positive predictive value, negative predictive value and efficiency were calculated as described (Sackett et al., 1985). Data were analyzed according to patient population, disease status, source of Candida isolates, clinical presentation and in-hospital mortality. The true-negative population included healthy volunteers, patients with IFI not caused by a *Candida* spp., and patients with infection other than fungi.

Results

Study subjects

Table 2 shows host factors, source of *Candida* isolates and in-hospital mortality of 88 patients with invasive candidiasis, 22 patients with *Candida* colonization without antifungal therapy, and 76 patients without candidiasis or *Candida* colonization or healthy volunteers. Among 88 patients with invasive candidiasis, 60 patients had proven IFI, 3 had probable IFI, 25 had suspect IFI. A total of 47 patients had blood cultures positive of *Candida* spp. Thirty one of 88

patients (35.2%) were neutropenic, 6 received corticosteroids, and 6 had renal failure and/or received dialysis. In-hospital mortality of patients with invasive candidiasis was 22.7% (20/88) and attributable mortality was 18.2% (16/88). On the other hand, 13 of 22 patients (59.1%) with *Candida* colonization were neutropenic and 9 patients died at discharge (40.9%).

Yield of assays

The sensitivity for 88 patients with invasive candidiasis was 29.5% for antigen assay, 67.0% for antibody assay, and 79.5% for combined test (Table 2). There were no different among proven IFI, probable or suspect IFI. Among 98 patients or healthy volunteers, 8 patients had false positive results (5 patients with *Candida* colonization and 3 patients without invasive candidiasis or *Candida* colonization). However, all these 8 patients received systemic antifungal therapy for proven IFI (2 patients), probable (3 patients) or suspect IFI (3 patients) (Table 3). Thus, the yields of serology were calculated using either 98 subjects as control (model 1) or 90 subjects (model 2, excluding 8 "false-positive"). The sensitivity, specificity, and predictive values of antigen and antibody testing were calculated per patients (Table 4). The combined use of antigen and antibody detection allowed the detection of 79.5% of invasive candidiasis with a specificity of 91.8% (model 1)~100% (model 2).

Variation of sensitivity by different *Candida* spp. or source of isolates

The sensitivity of antigen assay was higher for *C. tropicalis* and *C. glabrata*. On the other hand, the sensitivity of antibody assay was higher for *C. albicans*. Overall, the sensitivities of either antigen, antibody or combined test were no different among different *Candida* spp. or source of isolates.

Variation of sensitivity by different wards, host factors, clinical presentation, course of invasive candidiasis or outcome

The sensitivities of antigen/antibody assays were no different among different wards or host factors (Table 6). The sensitivity of antigen assay was as high as 66.7% for patients with renal failure with or without dialysis. However, the patient number was only 6. Furthermore, the yield of these assays was not higher for patients with sepsis (Table 7). However, the sensitivity of antigen assay was higher for patients with either chronic candidiasis (50.0%) or microbiologic failure (43.8%) than those with acute candidiasis (19.6%) (P=0.025) (Table 7). Besides, the sensitivity of antigen assay was

higher for patients died of invasive candidiasis (56.3%) than those were alive at discharge (23.5%) or died due to other reasons (25.0%) (P=0.035).

Discussion

Factors which influence the sensitivity include the quality of anti-mannan antibodies, the species of infecting *Candida*, the patient's underling condition, the frequency of serum sampling the time of collection relative to disease severity (Matthews, 1996). However, this study did not show significant difference in the sensitivity between different Candida spp., between patients with sepsis or without or among different host factors. Instead, our data showed higher sensitivity in chronic invasive candidiasis or microbiological failure than that in acute infection. Antifungal therapy affects the yield particularly in acute hematogenous candidiasis without complication. Manna is cleared from the blood very rapidly after intravenous infection into rabbits (Bailey et al., 1985). Others have confirmed the transient nature of mannan antigenemia (Kahn et al., 1986; Herent et al., 1992). As the majority of samples were collected after at least one dose of antifungal agent, the sensitivity of antigen assay was only 19.6% in patients with acute course. Mannan assay was positive only in serum samples collected when patients were symptomatic. On the other hand, persistent presence of circulating mannan supported the clinical judgement that patient with candidemia or chronic invasive candidiasis responded poorly to appropriate antifungal therapy despite of negative follow-up blood culture results.

Antibody assay was not considered for diagnosis previously because immunosuppressed patients and critically ill patients may fail to produce antibody and high-level colonization but no infection may produce antibody. However, recent study demonstrated that antibody detection in Candida infection has a sensitivity of 53% and a specificity up to 94% (Sendid et al., JCM 1999). These results also led us to reconsider the diagnostic value of antimannan antibody detection. We have found a specificity of 93.9% and a sensitivity of 67.0% for the ELISA for antimannan antibody detection. The combined tests had a sensitivity and specificity of 79.5% and 100%, respectively.

The objective of this study was to evaluate the implication of the serological assays in clinical practice. Positive results in less immunocompromised hosts with

deteriorated clinical conditions will help physicians to initiate antifungal preemptive therapy in stead of waiting for another positive fungal results or invasive procedures. Thus, serological data are complemented to microbiological and histopathological results and are helpful in decision making. However, negative results alone should not disprove the possibility of invasive fungal infection and prevent from initiation of antifungal therapy as negative predictive values were low and invasive fungal infections other than candidiasis, aspergillosis and cryptococcosis are emerging. Furthermore, epidemiology of invasive fungal infections in less immunocompromised hosts is limited. Prolonged (>3 weeks) use of corticosteroids in previous 60 days is one of the host factors. However, these patients were not limited to patients with cancer and recipients of hematopoietic stem cell transplant. Physicians caring this patient population are less alert of these infectious complications and are hesitate to initiate antifungal therapy empirically or preemptively while histological documentation is prohibited by worsening conditions.

There are frequent clinical situations in the suspect category in which therapy is warranted on empirical grounds. The Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer and Mycoses Study Group of the National Institute of Allergy and Infectious Diseases emphasized that the definitions of invasive fungal infections are intended for use in the context of clinical and/or epidemiological research, not for clinical decision making (Ascioglu et al., 2002).

ACKNOWLEDGMENTS

We thank Dr. for critical review and useful comments. This work was supported by grants (NSC 89－2314－B－002－342 and NSC90-2314-B-002-303)

References

Ascioglu S, Rex JH, de Pauw, Bennett JE, Bille J, Crokaert F, et al., Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Die 2002; 34:7-14.

Bailey JW, Sada E, Brass C, Bennett JE. Diagnosis of systemic candidiasis by latex agglutination for serum antigen. J Clin Microbiol 1985;21:749-52.

Bar W, Hecker H. Diagnosis of systemic *Candida* infections in patients of the intensive care

unit. Significance of serum antigens and antibodies. Mycoses 2002;45:22-8.

Beck-Sague CM, Jarvis WR, the National Nosocomial Infections Surveillance System：Secular trends in the epidemiology of nosocomial fungal infections in the Untied States, 1980-1990. *J Infect Dis* 1993;167:1247-51.

Becker M, de Marie S, Willemse D, Verbrugh HA, Bakker-Woudenberg IA. Quantitative galactomannan detection is superior to PCR in diagnosing and monitoring invasive pulmonary aspergillosis in an experimental rat model. J Clin Microbiol 2000;38:1434-8.

Chen, Y.C., Chang, S.C., Hsieh, W.C., Luh, K.T. (1996). In vitro antifungal susceptibilities of *Candida* species isolated from the blood. *Int J Antimicrob Agents* **7**,217-22.

Chen YC, Chang SC, Sun CC, et al：Secular trends in the epidemiology of nosocomial fungal infections at a teaching hospital in Taiwan, 1981-1993. *Infect Control Hosp Epidemiol* 1997;18:369-75.

Chen, Y.C., Lin, S.F., Liu, C.J., Jiang, D.D.S., Yang, P.C., Chang, S.C. (2001). Risk factors for ICU mortality in critically ill patients. *J Forms Med Assoc* **100**,656-61.

Chen, Y.C., Chang, S.C., Tai, H.M., Hsueh, P.R., Luh, K.T. (2001). Molecular epidemiology of *Candida* colonizing critically ill patients in intensive care units. *J Formos Med Assoc* **100**,791-7.

Chien-Yuan Chen,[1] Yee-Chun Chen,[1] Yao-Chang Chen,[12] Ming-Ching Shen,[12] Chiu- Hwa Wang,[12] Jih-Luh Tang,[1] Woei Tsai,[1] Ming Yao,[1] Shang-Yi Huang,[1] Hwei-Fang, Tien,[1]Hepatosplenic fungal infection in patients with acute leukemia in Taiwan: Incidence, Treatment and Prognosis.

De Repentigny L, Reiss E. Current trends in immunodiagnosis of candidiasis and aspergillosis. Rev Infect Dis 1984;6:301-12.

Edwards, J.E. Jr, Bodey, G.P., Bowden, R.A., Buchner, T., de Pauw, B.E., Filler, S.G., et al. (1997). International conference for the development of a consensus on the management and prevention of severe *Candida*l infections. *Clin Infect Dis* **25**,43-59.

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. J Clin Microbiol 1992;30:2158-64.

Hughes WT, Armstrong D, Bodey GP, et al. 1997 Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Clin infect Dis 1997;25:551-73.

Hung CC, Chen YC, Chang SC, Hsieh WC：Nosocomial candidemia in a university hospital in Taiwan. *J Formos Med Assoc* 1996;95:19-28.

Jones JM. Laboratory diagnosis of invasive candidiasis. Clin Microbiol Rev 1990;3:32-45.

Kahn FW, Jones JM. Latex agglutination tests for detection of *Candida* antigens in sera of patients with invasive candidiasis. J Infect Dis 1986;153:579-85.

Kauffman CA, Bradley SF, Ross SC, Weber DR. Hepatosplenic candidiasis: successful treatment with fluconazole. Am J Med 1991;91:137-41.

Matthews RC. Comparative assessment of the detection of *Candida*l antigens as a diagnostic tool. J Med Veter Mycol 1996;34:1-10.

McNeil, M.M., Nash, S.L., Hajjeh, R.A., Phelan, M.A., Conn, L.A., Plikaytis, B.D., Warnock, D.W. (2001). Trends in mortality due to invasive mycotic diseases in the United States, 1980-1997. *Clin Infect Dis* **33**,641-7.

Members of the ACCP/SCCM Consensus Conference：Definitions for sepsis and organ failure and guidelines for the use of innovative therapies for sepsis. *Crit Care Med* 1992;20:864-74.

Na BK, Song CY. Use of monoclona lantibody in diagnsosi of candidiaiss causd by *Candida* albicnas: detecti onfo circulating aspartyl proteinase antigen. Clin Diagn Lab Immunol 1999;6:924-9.

Rex, J.H., Walsh, T.J., Sobel, J.D., Filler, S.G., Pappas, P.G., Dismukes, W.E., Edwards, J.E. (2000). Practice guidelines for the treatment of candidiasis. *Clin Infect Dis* **30**,662-78.

Robert WF, James AT. Fever and neutropenia – how to use a new treatment strategy? N Engl J Med 1999;341:362-3.

Sackett DL, Haynes B, Tugwell P. Clinical epidemiology. The interpretation of diagnostic data. P. 59-138. Little, Brown & Co., Boston, Mass. 1985.

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. J Clin Microb 1999;37:1510-7

Singh, N (2001). Changing spectrum of invasive candidiasis and its therapeutic implications. *Clin Microbiol Infect* **7**(Suppl 2),1-7.

Singh, N. (2001). Trends in the epidemiology of opportunistic fungal infections: predisposing factors and the impact of antimicrobial use practices. *Clin Infect Dis* **33**,1692-6.

Telenti A, Roberts GD. Fungal blood cultures. Eur J Clin Microbiol Infect Dis 1989;8:825-31.

Thaler M, Pastakia B, Shawker TH, O'Leary T, Pizzo PA. Hepatic candidiasis in cancer patients : the evolving picture of the syndrome. Ann Intern Med 1988;108:88-100.

Van Deventer AJM, Goessens WHF, van Zeijl JH, Mouton JW, Michel MF, Verbrugh HA. Kinetics of anti-mannan antiboides useful in confirming invasive candidiasis in immunocompromised patietns. Microbiol Immunol 1996;40:125-31.

Viscoli, C., Girmenia, C., Marinus, A., Collette, L., Martino, P., Vandercam, B., et al. (1999). Candidemia in cancer patients: a prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). *Clin Infect Dis* **28**,1071-9.

Wey, S.B., Motomi, M., Pfaller, M.A., Woolson, R.F., Wenzel, P.R. (1989). Risk factors for hospital-acquired candidemia: a matched case-control study. *Arch Intern Med* **149**,2349-53.

Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of *Aspergillus* infection in a large cohort of patients undergoing bone marrow transplantation. J Infect Dis 1997;175:1459-66.

Wey SB, Mori M, Pfaller MA, et al : Hospital-acquired candidemia – the attributable mortality and excess length of stay. *Arch Intern Med* 1988;148:2642-5.

Warren, N.G., Hazen, K.C. (1995). *Candida , Cryptococcus*, and other yeasts of medical importance. In Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H. (eds): *Manual of Clinical Microbiology*. 6th ed. Washington, D.C.: ASM Press; p.723-37.

Table 1. Inclusion criteria for patients with suspected *Candida* infection and requiring the use of antifungal treatment

Criterion A. Clinical evidence of infection and warranting therapeutic intervention

1. Signs of sepsis (fulfill at least two criteria of systemic inflammatory response syndrome). Include elevated C-reactive proteins as one of the criteria for patients with "normal" leukocyte counts
2. Persistent fever refractor to appropriate broad-spectrum antibacterial treatment in high-risk patients defined

Criterion B. Microbiological evidence of *Candida* colonization

1. Demonstration of *Candida* colonization by intraocular inspection or by histopathology
2. Cultural isolation of *Candida* spp. from normally sterile sites (blood, i.v. catheter, internal organs)
3. Cultural isolation of *Candida* spp. from two separate sites or tissues

Criterion C. Absence of bacterial, viral or mycobacterial infection

1. No isolation of relevant bacteria, virus or mycobacteria
2. Clinical deterioration despite apparently appropriate antibacterial treatment
3. Response to antifungal treatment without modification of antibacterial treatment

Interpretation

Candida infection was assumed following these criteria if at least one sign for each criterion was fulfilled

Table 2. Classification of study subjects

Patient status (no. of patients)	Results of serology			Isolation of *Candida*			Host factors			In-hospital mortality	
	Candida antigen n (%)	*Candida* antibody n (%)	*Candida* combined n (%)	Blood n (%)	Other sterile site n (%)	Non-sterile site n (%)	Neutropenia n (%)	Steroid n (%)	Acute renal failure and/or hemodialysis	Attributable mortality n (%)	Crude mortality n (%)
Infection (88)	26 (29.5)	59 (67.0)	70 (79.5)	47 (53.4)	4 (4.5)	30 (34.1)	31 (35.2)	6 (6.8)	6 (6.8)	16 (18.2)	20 (22.7)
Proven (60)	22 (36.7)	37 (61.7)	46 (76.7)	47 (78.3)	3 (5.0)	10 (16.7)	16 (26.7)	5 (8.3)	5 (8.3)	12 (20.0)	13 (21.7)
Probable (3)	1 (33.3)	2 (66.7)	3 (100)	0 (0)	0 (0)	2 (66.7)	2 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)
Suspect (25)	3 (12.0)	20 (80.0)	21 (84.0)	0 (0)	1 (4.0)	18 (72.0)	13 (52.0)	1 (4.0)	1 (4.0)	4 (16.0)	7 (28.0)
Colonization (22)	3 (13.6)	3 (13.6)	5 (22.7)	0 (0)	0 (0)	22 (100)	13 (59.1)	2 (9.1)	1 (4.5)	0 (0)	9 (40.9)
Others (76)	1 (1.3)	3 (3.9)	3 (3.9)	0 (0)	0 (0)	0 (0)	34 (44.7)	12 (15.8)	3 (3.9)	0 (0)	16 (21.1)
Total (186)	30 (16.1)	65 (34.9)	78 (41.9)	47 (25.3)	4 (2.2)	52 (27.9)	78 (41.9)	20 (10.7)	10 (5.4)	16 (8.6)	45 (24.2)

Note.

1. Patients were classified according to defined criteria as described in Methods.
2. One patient with probable hepatic candidiasis had positive *Candida* antigen.
3. Three patients with *Candida* colonization had positive *Candida* antigen: disseminated cryptococcosis (1 patient), acute invasive pulmonary aspergillosis (1), and probable fungal pneumonia (1) (see Table 4a).
4. Two neutropenic patients without evidences of *Candida* infection and colonization had positive *Candida* antibody: suspect fungal pneumonia (1), and probable fungal pneumonia (1) (see Table 4a).
5. Among 15 of 79 patients without evidences of *Candida* infection and colonization died, 12 patients died related to invasive fungal infection other than candidiasis.

Table 3. Ten patients who did not fulfill the criteria of *Candida* infection had positive *Candida* antigen and/or antibody (causes of false positive results)

Patient no.	Age (yr)	Sex	Ward	*Candida* antigen	*Candida* antibody	Host factor	Diagnosis of IFI	Pathogen	Isolation of *Candida*	Remark	In-hospital mortality
205	47	M	Hematology	N	P	0	Proven	*Aspergillus*	O	Probable hepatic candidiasis 3 months ago, splenic aspergillosis 2 month ago; drug-induced hepatic failure	Death
22	14	M	Hematology	P	P	1	Proven	*Aspergillus*	O	Fungal sinusitis and pneumonia	Death
83	47	M	Hematology	N	P	1	Probable	*Aspergillus*	No		Death
DOH86-317	57	M	ICU	P	N	0	Proven	*Cryptococcus*	O	Cryptococcal meningitis	Alive
301	72	M	Hematology	P	N	1	Probable	Fungus	O		Death
8	46	M	ICU	N	P	1	Suspect	Fungus	O		Death
401	50	F	Hematology	N	P	1	Suspect	Fungus	No		Death
333	25	F	Hematology	N	P	1	Suspect	Fungus	O		Death

Note. All these 8 patients received amphotericin B. Fungal cultures were performed as clinically indicated. Surveillance cultures for fungi were not performed during this study period. No autopsy or necropsy was performed for patients who passed away soon after collection of serum samples. Therefore, candidiasis can not be excluded definitely in these patients.

Table 4. Sensitivity, specificity, and predictive values for the detection of antigen and anti-mannan antibodies

	Candida antigen		*Candida* antibody		Combination	
Parameter	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Sensitivity (%)	29.5	29.5	67.0	67.0	79.5	79.5
Specificity (%)	95.9	100	93.9	100	91.8	100
Positive predictive value (%)	86.7	100	90.8	100	89.7	100
Negative predictive value (%)	60.2	59.2	76.0	75.6	83.3	83.3
Efficiency (%)	64.5	65.2	81.2	83.7	86.0	89.9

Note.

1. Results are calculated per patient according to the results of an analysis of serum samples from 88 patients with *Candida* infection and serum samples from 98 controls in model 1 and 90 controls in model 2. As *Candida* infection can not be excluded definitely, eight patients with "false positive" *Candida* antigen/antibody results were excluded in the analysis in model 2.

2. Sensitivity = TP/(TP+FN); specificity = TN/(FP+TN); positive predictive value = TP/(TP+FP); negative predictive value = TN/(TN+FN); efficiency = (TP+TN)/(TP+FP+TN+FN) (where TP is true positive FP is false positive, TN is true negative, and FN is false negative).

Table 5. Variation of sensitivity by *Candida* spp. and source of *Candida* isolates*

Candida spp.	Source (No. of patients)	Antigen No. (%)	Antibody No. (%)	Combination No. (%)
C. albicans	All (48)	13 (27.1)	36 (75.0)	39 (81.2)
	Blood (23)	9 (39.1)	15 (65.2)	17 (73.9)
	Others (25)	4 (16.0)	21 (84.0)	22 (88.0)
C. tropicalis	All (14)**	6 (42.8)	6 (42.8)	11 (78.6)
	Blood (10)	6 (60.0)	4 (40.0)	9 (90.0)
	Others (3)	0	1 (33.3)	1 (33.3)
C. parapsilopsis	All (6)	1 (16.7)	3 (50.0)	4 (66.7)
	Blood (6)	1 (16.7)	3 (50.0)	4 (66.7)
	Others (0)	0	0	0
C. glabrata	All (4)	2 (50.0)	2 (50.0)	2 (50.0)
	Blood (2)	2 (100)	2 (100)	2 (100)
	Others (2)	0	0	0

* *Candida* were isolated from blood in 41 patients, from other sterile sties in 4 patients and from nonsterile sties in 26 patients. For patients with *Candida* isolated from nonsterile sties, only those with *Candida* isolated from 2 samples collected indifferent anatomical sites or in different times were included.

** One patient had relapse of hepatic candidiasis due to *C. tropicalis* without microbiologic documentation.

Table 6. Variation of sensitivity by host factors

Parameter (No. of patients)	Antigen No. (%)	Antibody No. (%)	Combination No. (%)
Wards			
Hematology (32)	9 (28.1)	21 (65.6)	26 (81.3)
Intensive care units (34)	9 (26.5)	20 (58.8)	24 (70.6)
Others (22)	8 (36.4)	18 (81.8)	20 (90.0)
Risk factors			
Neutropenia (31)	9 (29.0)	18 (58.1)	25 (80.6)
Corticosteroids (6)	1 (16.7)	3 (50.0)	3 (50.0)
Renal failure and/or dialysis (6)	4 (66.7)	3 (50.0)	5 (83.3)
Others (45)	12 (26.7)	35 (77.8)	37 (82.2)

Anti-mannan antibodies are considered as of low specificity (low titers fail to differentiate colonization from infection) and low sensitivity (low response in immunocompromised patients and critically ill patients).

Table 7. Variation of sensitivity by presentation and clinical course

Parameter (No. of patients)	Antigen No. (%)	Antibody No. (%)	Combination No. (%)
Clinical presentation: sepsis			
No (18)	3 (16.7)	13 (72.2)	15 (83.3)
Yes (70)	23 (32.9)	46 (65.7)	55 (78.6)
Clinical course			
Acute (56)	**11 (19.6)***	37 (66.1)	**39 (69.6)****
Chronic (16)	**8 (50.0)**	12 (75.0)	**16 (100)**
Microbiologic failure (16)	**7 (43.8)**	10 (62.5)	**15 (93.8)**
In-hospital mortality			
Alive (68)	**16 (23.5)**	49 (72.1)	55 (80.9)
Attributory mortality (16)	**9 (56.3)*****	8 (50.0)	13 (81.3)
Other mortality (4)	**1 (25.0)**	2 (50.0)	2 (50.0)

* P=0.025; ** P=0.009; *** P=0.035