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Detection of Antibodies to *Candida albicans* Germ Tubes for Diagnosis and Therapeutic Monitoring of Invasive Candidiasis in Patients with Hematologic Malignancies

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We prospectively investigated the ability of detection of antibodies to *Candida albicans* germ tubes (CAGT) to diagnose invasive candidiasis in 95 consecutive admissions of 73 patients with hematologic disorders undergoing intensive chemotherapy. The episodes were divided into three groups according to clinical and microbiological diagnosis. Group 1 comprised eight admissions of eight patients with invasive candidiasis. Group 2 comprised 42 admissions of 34 patients without evidence of invasive candidiasis. Group 3 comprised the remaining 45 admissions of 37 patients with febrile episodes which were not diagnosed by microbiological culture. Antibodies to CAGT were detected in 87.5% of group 1 patients. Detection of antibodies to CAGT in patients with *Candida* fungemia was delayed somewhat relative to the time the blood culture was positive, but antibodies to CAGT were detected earlier than a diagnosis was made in patients with deep-tissue candidiasis. Sera from 2 admissions in group 2 and 12 admissions in group 3 revealed antibodies to CAGT. At a titer of ≥1:20, detection of antibodies to CAGT had a sensitivity of 87.5%, specificity of 95.2%, positive predictive value of 77.8%, and negative predictive value of 97.6%. Antibodies to CAGT were usually detected before beginning of empiric antifungal therapy. Titers of antibodies to CAGT were maintained in most patients who died but declined and eventually disappeared in the patients who survived. Since antibodies to CAGT were detected in all patients with tissue-proven invasive candidiasis but negative by blood culture, detection of antibodies to CAGT complemented blood cultures for diagnosis and therapeutic monitoring of patients with hematologic malignancies and invasive candidiasis.

Invasive *Candida* infections are a growing problem among immunocompromised patients. The diagnosis of invasive candidiasis is difficult because there are no specific clinical manifestations and conventional microbiological methods usually lack of both sensitivity and specificity (8). Consequently, therapy is often initiated late in the course of the infection, resulting in substantial morbidity and mortality (11).

During the last few years, different innovative approaches and methods have been used for diagnosis of invasive candidiasis. These techniques include the detection of fungal nucleic acids by PCR (9), detection of (1-3)-β-D-glucan and D-arabinitol (12, 24), detection of cell wall and cytoplasmic antigens (5, 6, 25), and detection of antibodies directed against different *Candida* antigens (22, 27). Nevertheless, each technique has limitations, and none of them have found widespread clinical use (14). Detection of antibodies in patients with invasive candidiasis has two main limitations: (i) they may not be useful diagnostically, since antibody titers can be high in patients who are only colonized (4), and (ii) the antibody response may be delayed, reduced, or absent in immunocompromised patients (3). However, encouraging results in sensitivity and specificity have been obtained with the detection of antibodies to *Candida albicans* germ tubes (CAGT), even in immunocompromised patients with invasive candidiasis (16–20, 23). Since one of the drawbacks of antibody detection is the low or nonexistent antibody response in immunocompromised patients, we have conducted a prospective study to evaluate the usefulness of detection of antibodies to CAGT for the diagnosis and therapeutic monitoring of invasive candidiasis in patients with hematologic disorders.

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MATERIALS AND METHODS

Patients. Over a period of 31 months, we prospectively studied 95 consecutive admissions of 73 patients (42 males, 31 females) with hematologic disorders admitted to the Hematology Service of the Hospital de Cruces, Baracaldo, Spain, to undergo intensive chemotherapy. Most patients had hematologic disorders secondary to malignancies. The average age of the patients was 52.8 years (range, 14 to 84 years).

According to clinical and microbiological diagnosis, patients and sera were divided into three groups. Group 1 comprised 87 sera from eight admissions of eight patients with invasive candidiasis (average, 10.8 sera per patient admission). Patients were considered to have invasive candidiasis if a *Candida* species was recovered from blood or normally sterile nonmucosal tissue. This classification includes fungemia and deep-tissue candidiasis (24). The clinical characteristics of these patients are shown in Table 1. Group 2 comprised 566 sera from 42 admissions of 34 patients without evidence of invasive candidiasis (average, 13 sera per patient admission). Seventy percent of patients in this group had a diagnosis of acute leukemia or chronic leukemia in blast crisis. The remaining patients had a diagnosis of lymphoproliferative malignancies and severe aplastic anemia. Febrile episodes observed in this group were diagnosed by microbiological culture. Patients were considered colonized if a *Candida* species was recovered from antemortem surveillance or bronchoalveolar lavage fluid if neither criterion required for the diagnosis of invasive candidiasis was present. Group 3 comprised the remaining 45 admissions of 37 patients (351 sera; average, 8.0 sera per patient admission). Fifty-seven percent of patients included in this group had a diagnosis of acute leukemia or chronic leukemia in blast crisis. Twenty-seven percent of patients had a diagnosis of lymphoproliferative malignancies and severe aplastic anemia. The remaining patients had miscellaneous diagnoses.

TABLE 1. Patient information and antibody results for group 1

Patient	Underlying condition	Site(s) of *Candida* isolation	Species	No. of sera with CAGT/total no. of sera	Antibody titers[a]		Day antibodies to CAGT were detected[b]
					CAGT	CAB	
1	Acute leukemia	Blood	*C. parapsilosis*	2/13	0–20	20–1,280	16
2	Acute leukemia	Liver, spleen	*C. parapsilosis*	9/26	0–40	80–20,480	−126
3	Acute leukemia	Blood	*C. krusei*	2/19	0–80	160–5,120	4
4	Anaplastic lymphoma	Liver, spleen, kidney, lung	*C. albicans*	3/3	20	160–320	−10
5	Acute leukemia	Blood	*C. albicans*	0/19	0	320–2,560	NA
6	Hemolytic anemia and colorectal surgery	Blood	*C. albicans*	2/2	160–10,240	20,480–327,680	0
7	Anaplastic lymphoma	Liver, spleen	*C. albicans*	2/3	0–20	80–320	−35
8	Acute leukemia	Liver, kidney, lung	*C. albicans*	2/2	80	2,560–2,560	−120

[a] Reciprocal titers.
[b] Indicated as the days before (negative numbers) or after the diagnosis of invasive candidiasis. NA, not applicable.

Patients in this group included febrile episodes which were not diagnosed by microbiological culture.

Sera and microbiological surveillance. Serum samples were prospectively collected twice weekly, numbered, and sent to the Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco. Cultures of blood and sterile body fluids were obtained as clinically indicated. Surveillance cultures of throat, urine, and rectum were collected weekly. Blood was cultured by the BACTEC method (NR 660; Becton Dickinson, Baltimore, Md.), and the rest of the specimens were plated onto Sabouraud glucose agar containing chloramphenicol. Yeast isolates were identified by germ tube formation (21) and by carbohydrate assimilation patterns (21).

***Candida* serology.** *C. albicans* serotype A (NCPF 3153) was grown as germ tubes or blastospores in medium 199 (Sigma, St. Louis, Mo.), as described elsewhere (16). Washed germ tubes and blastospores were used in the preparation of immunofluorescence slides (17). Briefly, to each well of Teflon-coated, 12-well immunofluorescence slides, 10^6 cells suspended in 10 μl of phosphate-buffered saline (PBS) were added, air dried, rinsed in phosphate-buffered saline, and dried with a jet of air.

The sera were adsorbed with heat-killed blastospores of the same strain to remove the antibodies against the blastospore cell wall surface and allow the detection of antibodies to CAGT. The adsorbing suspension was prepared by heating the blastospores at 60°C for 2 h. After three washes in saline, the organisms were counted in a hemacytometer and suspended at 10^{10} cells per ml. The method for adsorption of the sera has been described previously (17). Briefly, sera were adsorbed four times by mixing equal volumes (100 μl) of the adsorbing suspension and sera. Adsorption was performed with rotation by placing the tubes on a turntable which inverted the tubes repeatedly for 2 h at room temperature, and then the organisms were removed by centrifugation. Adsorptions were repeated with supernatants twice at room temperature for 2 h and once more at 4°C for 18 h. Supernatants from the last adsorption (final dilution, 1:16) were adjusted to 1:20 and used for antibody detection.

Anti-*C. albicans* antibodies were detected in both adsorbed (antibodies to CAGT) and nonadsorbed (antibodies to *C. albicans* blastospores [CAB]) sera by an indirect immunofluorescence antibody assay as described elsewhere (17). Briefly, 10 μl of the serum or its serial dilution was applied to each well of the immunofluorescence slides and incubated at 37°C for 30 min. Then the slides were washed and incubated with fluorescein-conjugated goat anti-human immunoglobulin (Sigma) at 37°C for another 30 min. The slides were washed again and examined with a microscope equipped for epifluorescence. The highest dilution of the serum showing fluorescence on the entire *C. albicans* cell wall surface was considered the titer.

Statistics. Sensitivity, specificity, efficiency, and positive and negative predictive values were calculated as described by Kozinn et al. (10). StatView 512+ statistical software for Apple Macintosh was used for computing the statistics. The chi-square test was used for comparison of variables. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Antibodies to CAGT were detected in sera from seven of eight group 1 patients (87.5%). Sera from patients with hematologic malignancies had maximum antibody titers ranging from 1:20 to 1:80, but the serum from the only nonimmunocompromised patient included in group 1 showed a much higher titer (1:10,240). In four patients, invasive candidiasis was diagnosed only by autopsy. These patients had multiple negative blood cultures, but all of them showed antibodies to CAGT (Table 1). However, the sera from one of the four patients with fungemia did not show antibodies to CAGT. This patient developed an invasive aspergillosis by *Aspergillus terreus*. Three patients were infected by non-*C. albicans* species. However, no differences in the titer of antibodies to CAGT were observed between sera from patients infected by *C. albicans* and those infected by non-*C. albicans* species. The relationship between serological and microbiological diagnoses of invasive candidiasis was studied with the group 1 patients. Detection of antibodies to CAGT in sera from patients with *Candida* fungemia was delayed somewhat relative to the time the blood culture was positive (mean, 6.6 days; range, 0 to 16 days) (Table 1). However, antibodies to CAGT were detected earlier than a diagnosis was made with sera from patients with deep-tissue candidiasis (mean, 73 days; range, 10 to 126 days) (Table 1). All sera from group 1 patients showed antibodies to CAB, with maximum antibody titers ranging from 1:320 to 1:327,680.

Group 2 included admissions without evidence of invasive candidiasis. Twenty-eight of the admissions developed febrile episodes which were diagnosed by microbiological culture: 71% were bacteremias, 18% were pneumonias, and the remaining episodes were miscellaneous infections. None of them were caused by *Candida*. Sera from two admissions were positive for antibodies to CAGT (Table 2). These patients were colonized by *C. tropicalis* and *C. albicans*, and their sera had maximum titers of antibodies to CAGT ranging from 1:20 to 1:160. *Candida* colonization was also detected in 10 other patients, but their sera were negative for antibodies to CAGT. Sera from 14 admissions of patients included in group 2 showed no febrile episodes and no antibodies to CAGT. All sera from group 2 admissions showed antibodies to CAB, with maximum antibody titers ranging from 1:20 to 1:20,480.

Group 3 sera originated from 45 admissions with febrile episodes which were not diagnosed by microbiological culture. Sera from 12 admissions of 10 patients showed antibodies to CAGT with maximum titers ranging from 1:20 to 1:640 (Table 2). *Candida* colonization was detected in 13 admissions, and 8 of them had antibodies to CAGT. All sera from group 3 admissions showed antibodies to CAB, with maximum titers ranging from 1:20 to 1:81,920. Since it is possible that some of group 3 patients had undetected candidiasis, these patients were considered nonevaluable for purposes of assessing the detection of antibodies to CAGT as a diagnostic tool.

The ability of detection of antibodies to CAGT to discriminate between group 1 and group 2 patients was assessed. When a cutoff of ≥1:20 was used, the sensitivity, specificity,

TABLE 2. Patient information and antibody results for group 2 and 3 patients with antibodies to CAGT

Patient	Underlying condition	Site of *Candida* isolation	Species	No. of sera with CAGT/total no. of sera	Range of antibody titers[a]	
					CAGT	CAB
Group 2						
1	Acute leukemia	Oral mucosa	*C. tropicalis*	22/35	0–80	160–20,480
2	Acute leukemia	Oral mucosa	*C. albicans*	7/19	0–160	160–10,240
Group 3						
1a[b]	Severe aplastic anemia	Oral mucosa	*C. albicans*	2/8	0–20	320–2,560
1b[b]		Oral mucosa	*Candida* spp.	6/7	0–80	80–1280
2	Acute leukemia	Oral mucosa	*C. albicans*	14/29	20–80	160–81,920
3a[b]	Blast crisis of chronic myelogenous leukemia	Oral mucosa	*C. albicans*	5/5	20	640–2,560
3b[b]		NA[c]	NA	1/1	20	5,120
4	Non-Hodgkin's lymphoma	NA	NA	18/19	0–640	2,560–81,920
5	Colorectal carcinoma	NA	NA	3/3	20–40	2,560–5,120
6	Chronic myelomonocytic leukemia	Oral mucosa	*C. albicans*, *C. tropicalis*	8/8	160–320	10,240–20,480
7	Acute leukemia	Oral mucosa	*C. albicans*	2/2	40–40	1,280–5,120
8	Myelofibrosis (idiopathic)	NA	NA	2/12	0–20	640–1,280
9	Acute leukemia	Oral mucosa, sputum	*C. albicans*	6/6	20–40	1,280–10,240
10	Blast crisis of chronic myelogenous leukemia	Oral mucosa	*C. glabrata*	3/4	0–40	640–1,280

[a] Reciprocal titers.
[b] "a" and "b" refer to the first and second admissions of the same patient.
[c] NA, not applicable.

and positive and negative predictive values were 87.5, 95.2, 77.8, and 97.6%, respectively.

Considering the three groups together, antibodies to CAGT were detected in sera from 21 episodes in 19 patients. This represents a serological prevalence of antibodies to CAGT of 22.1%. Since the antifungal therapy was initiated without knowing the serological data from the patients, we examined the relationship between detection of antibodies to CAGT and the antifungal therapy, as well as the timing of detection of antibodies to CAGT relative to beginning of antifungal therapy. Antifungals were administered in 76.2% of admissions of patients with antibodies to CAGT, compared with 51.3% of those with no antibodies to CAGT ($P < 0.05$). Antibodies to CAGT were detected in sera from 10 of 16 (62.5%) admissions from the former group 16.3 (mean) ± 4.4 days before beginning of antifungal therapy. In the remaining six patients, antibodies to CAGT were not detected until 11.3 (mean) ± 5.8 days after beginning of antifungal therapy.

The relationship between mortality and maintenance of titers of antibodies to CAGT was also assessed. Ten of 19 patients with antibodies to CAGT in their sera died. In 8 of the 10 patients, the titers of antibodies to CAGT were maintained until death. Conversely, the titers of antibodies to CAGT declined and eventually disappeared in the sera from the nine patients who survived.

DISCUSSION

The antibody response in a patient with invasive candidiasis is directed against a variety of *C. albicans* antigens (15). Among them, cell wall antigens seem to be very important since they are on the fungal cell surface. Antibodies against a group of these antigens, the mannans, have been shown to be ubiquitous in human sera (7), making difficult their use in serodiagnosis. The data presented in this study are in agreement with previously published data (18) since antibodies to CAB, which are essentially antimannan antibodies, were detected in all the groups studied. However, detection of antibodies to CAGT was useful for the diagnosis of patients with hematologic malignancies. The sensitivity of the technique in our patient population was 87.5%, due to one false-negative result in a patient included in group 1 who had a concomitant invasive aspergillosis. The antifungal treatment begun in this patient 26 days before the blood culture yielded *C. albicans* may have reduced the fungal burden and the antibody response producing a false-negative result. The specificity of the test was 95.2% since two group 2 patients had antibodies to CAGT. However, these patients may have had invasive candidiasis, since they were colonized by *C. albicans* and *C. tropicalis*, respectively, and received amphotericin B for possible clinically occult invasive candidiasis. The colonization of mucosal surfaces by *C. tropicalis* in neutropenic patients has been shown to be a good predictor of the development of invasive candidiasis (26).

The results presented in this study show that the patients with hematologic malignancies produce low but detectable titers of antibodies to CAGT which can be used for diagnosis of *Candida* infections. In fact, titers of antibodies to CAGT have been detected in bone marrow transplant recipients (23). In addition to the high values of sensitivity and specificity reached in the present study, detection of antibodies to CAGT allowed early recognition of *Candida* infections, especially in patients with tissue-proven invasive candidiasis, where the serological diagnosis was obtained several days before the microbiological diagnosis was made. In patients with fungemia, diagnosis of the infection by blood culture may be slightly earlier than by se-

rology. However, due to the peculiar pathogenesis of invasive candidiasis in hematologic-malignancy patients treated with cytotoxic drugs, detection of antibodies to CAGT may present some advantages over blood culture. The infection may be difficult to detect by conventional techniques in early stages, since the fungal cells have to invade the gut wall and its capillaries and veins to reach the liver (8). This organ acts as a strong barrier to dissemination because its macrophages are very efficient in removing *Candida*, and therefore detection of *Candida* spp. in peripheral blood culture of these patients is difficult (8). Since antibodies to CAGT were detected in sera from all patients with tissue-proven invasive candidiasis but negative by blood culture, detection of antibodies to CAGT seems to complement blood cultures for diagnosis and therapeutic monitoring of patients with hematologic malignancies and invasive candidiasis.

Distinguishing *Candida* colonization from invasive candidiasis may be difficult in febrile neutropenic patients, and the clinician is frequently forced to administer antifungals empirically, with the duration of therapy being determined by arbitrary criteria or by patient's subsequent progress (13). Therefore, a marker of *Candida* infection would be useful in monitoring the response of patients with hematologic malignancies to antifungal therapy for invasive candidiasis. Detection of antibodies to CAGT may be such a marker, since the administration of amphotericin B and fluconazole resulted in decreasing titers of antibodies to CAGT in sera from patients who survived to the infection, suggesting that the antifungal treatment, as well as the recovery of the patient's leukocyte counts, cleared the fungal infection. Disappearance of antibodies to CAGT after successful antifungal treatment has also been described for a patient with intervertebral candidiasis (18). On the other hand, maintenance of titers of antibodies to CAGT was associated with a fatal outcome. Moreover, antibodies to CAGT were detected before empiric amphotericin B treatment was started in 62.5% of admissions.

The prevalence of invasive candidiasis in patients with hematologic malignancies has been established in different autopsy surveys, and it ranges between 2 and 46% (1, 2). If antibodies to CAGT are a good marker of invasive candidiasis, the prevalence of these antibodies in a given patient population should be similar to the prevalence of *Candida* infection found in necropsy studies in the same population. Interestingly, the serological prevalence of antibodies to CAGT in our patient population was 22.1%, which is within the range reported by Bodey et al. (2).

In conclusion, detection of antibodies to CAGT appears to be a useful marker for the diagnosis and therapeutic monitoring of the patients with hematologic malignancies and invasive candidiasis studied. This test may also be useful for the diagnosis of invasive candidiasis in other immunocompromised patients such as human immunodeficiency virus-infected individuals (19). However, studies in a larger group of patients are needed to confirm this conclusion.

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