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Comparison of Enzyme Immunoassay and Gas-Liquid Chromatography for the Rapid Diagnosis of Invasive Candidiasis in Cancer Patients

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Three proposed quantitative markers for candidiasis, arabinitol, mannose, and mannan in serum, are compared in 50 normal blood donors and 38 high-risk patients, 23 with and 15 without invasive candidiasis. Arabinitol concentrations in serum, the arabinitol/creatinine ratio, and mannose concentrations in serum were significantly greater in the 15 patients without candidiasis than in the normal blood donors ($P < 0.05$). The sensitivities and specificities were 26 and 87% for arabinitol, 13 and 93% for the arabinitol/creatinine ratio, and 39 and 87% for mannose. On the other hand, mannan concentrations in serum were <1 ng/ml in normal blood donors and patients without candidiasis ($P = 0.344$), and the sensitivity and specificity were 65 and 100%, respectively. Of 23 patients with proven or probable candidiasis, 16 had mannan levels in serum greater than the mean + 2 standard deviations (0.46 ng/ml) for the 15 controls. In 16 patients with invasive candidiasis and positive blood cultures for the *Candida* spp., only 13 had elevated levels of at least one of the three markers. The arabinitol/creatinine ratio, the mannose level, and the mannan level became elevated an average of 4 days before, 1 day before, and on the same day that the blood cultures were drawn, respectively. Conversely, mannan was detected in the sera of six of seven patients with invasive candidiasis and negative blood cultures. We conclude that the best approach to diagnosing invasive candidiasis involves obtaining blood cultures and carrying out serial assays for mannan in serum.

Invasive candidiasis is a frequent cause of morbidity and mortality in immunocompromised patients, especially those with acute leukemia (4). Autopsy data have shown an increasing incidence of candidiasis, which can be as high as 34% (21). Unfortunately, the diagnosis of this fungal infection remains difficult, and a premortem diagnosis is made early enough for treatment in only 15 to 40% of patients (4). Although isolation of *Candida* spp. from clinical specimens may aid in diagnosis (25), blood cultures are negative in 56% of patients with autopsy-proven disseminated disease (21). Furthermore, serological tests for antibody to the *Candida* sp. are false-negative in 27 to 70% of leukemic patients, probably because of the immunosuppression (7, 10, 12, 20).

Because of these shortcomings, much attention has been directed to tests which directly detect *Candida* spp. antigens or metabolites in body fluids. Among these, three methods have independently been described: (i) detection of circulating antigens such as α-D-mannan (11, 14, 16, 22, 23, 26, 28, 29), the main antigenic polysaccharide component of the *Candida* spp. cell wall, by enzyme immunoassay or radioimmunoassay; detection of cytoplasmic protein antigens by enzyme immunoassay (1) or radioimmunoassay (27); and detection of glycoprotein antigens by latex agglutination (8); (ii) gas-liquid chromatography for the quantitation of arabinitol in serum (5, 9, 13, 24, 31, 32); and (iii) gas-liquid chromatographic quantitation of mannose in serum (15, 18, 19). Although all three techniques seem promising, their sensitivities, specificities, and predictive values have not been compared in immunosuppressed patients at greatest risk of invasive candidiasis. Investigators in many hospitals are currently interested in implementing these new diagnostic methods, and we considered it pertinent to gather data on the relative clinical value of these tests. In this study, we compared mannan, arabinitol, and mannose concentrations in serum samples from normal blood donors and high-risk patients with or without invasive candidiasis.

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MATERIALS AND METHODS

Patients and serum samples. A retrospective study design was used. A total of 50 serum samples from normal blood donors were obtained from the Serum Bank, Division of Host Factors, Centers for Disease Control, Atlanta, Ga. These samples were drawn between 1978 and 1983 and stored at −20°C. Patients hospitalized at Emory University Hospital from 1978 to 1983 and who were at high risk of developing invasive candidiasis were also studied. Unused portions of serum samples drawn from these patients for routine laboratory tests were identified and stored at −20°C. Patients with hematological malignancies who had undergone autopsy and patients with candidemia were selected for further study, and their charts were reviewed. Information was obtained on the underlying disease history, presence and duration of neutropenia (less than 1,000 neutrophils per mm^3), renal function, chemotherapy, radiotherapy, allogeneic bone marrow transplantation, use of corticosteroids and broad-spectrum antibacterial antibiotics, surgery, fungal and

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nonfungal infections, surveillance cultures, antifungal treatment, and autopsy results.

On the basis of this clinical information, patients were divided into the following groups: (i) 13 patients with leukemia or lymphoma and autopsy-proven invasive candidiasis, defined as postmortem histopathological evidence of tissue invasion of at least one deep organ by yeast and pseudohyphae typical of the *Candida* spp.; (ii) 10 patients with probable invasive candidiasis, who had candidemia associated either with neutropenia and a hematological malignancy or with repeated culture of the same *Candida* sp. from a normally sterile site in the setting of postoperative intra-abdominal or intrathoracic sepsis; and (iii) 15 patients with leukemia or lymphoma and no evidence of candidiasis at autopsy.

The mean number of serum samples studied per patient, with the range of samples per patient given in parentheses, for the different patient groups were as follows: autopsy-proven invasive candidiasis, 3.3 (1 to 9); probable invasive candidiasis, 5.4 (1 to 13); and no candidiasis at autopsy, 2.9 (1 to 6). The last samples available for study were obtained within 1 week of death from six of eight patients with probable invasive candidiasis and within 2 weeks of death from all eight patients who died in this group. In the 15 control patients without candidiasis, the last available samples from 10 patients were drawn less than 1 week before death.

Analytical techniques. A code number was assigned to each serum sample, and the assays were carried out without knowledge of the clinical histories of the patients.

Serum samples from normal blood donors were assayed for creatinine with an automated analyzer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). All other creatinine concentration data were obtained from the charts of each patient.

Simultaneous determination of arabinitol and mannose concentrations in serum by gas-liquid chromatography and double-antibody sandwich enzyme immunoassay for mannan concentrations in serum were performed as previously described (3, 23). Adjustment for accumulation of arabinitol in renal insufficiency was made by calculating the arabinitol/creatinine ratio (31), with arabinitol expressed in micrograms per milliliter and creatinine expressed in milligrams per deciliter. Mannose concentrations in serum were adjusted for renal insufficiency by using the following equation: adjusted mannose concentration = measured mannose concentration − 2.41(serum creatinine) + 4.1, as previously described (2).

Statistical methods. The significance of differences between the means was analyzed by the two-tailed Student *t* test (6); the method of Welch (30) was used when population variances were unequal.

RESULTS

Clinical findings. The clinical findings in the patients at high risk of candidiasis who were selected for study are shown in Tables 1 and 2. All patients with autopsy-proven invasive candidiasis or with no evidence of candidiasis at autopsy and 7 of 10 patients with probable invasive candidiasis had a hematological malignancy as the underlying disease. Two of the four patients with chronic leukemia were studied during a blast crisis. Although there was considerable overlap, average survival (in months) from initial diagnosis until death for patients with acute leukemia and proven (10.5) or probable (8.3) invasive candidiasis was less than for patients with acute leukemia and no evidence of candidiasis at autopsy (17.7).

In addition to the underlying disease, most patients had several other predisposing factors (Table 1). Five patients with proven or probable invasive candidiasis had bacteremia

TABLE 1. Clinical findings in 13 patients with autopsy-proven invasive candidiasis, 10 patients with probable invasive candidiasis, and 15 high-risk control patients without candidiasis at autopsy

Conditions	No. of patients[a] with:		
	Autopsy-proven invasive candidiasis	Probable invasive candidiasis	No candidiasis at autopsy
Underlying disease			
Acute myelogenous leukemia	9	6	6
Acute lymphocytic leukemia	3	1	1
Chronic myelogenous leukemia	0	0	3
Chronic lymphocytic leukemia	0	0	1
Lymphoma or Hodgkin's disease	1	0	4
Intra-abdominal or intrathoracic sepsis	0	3	0
Predisposing factors			
Neutropenia lasting more than 1 week	12	7	8
Chemotherapy	13	7	12
Radiotherapy	1	0	3
Corticosteroid therapy	5	2	6
Broad-spectrum antibiotic therapy	13	10	14
Surgery	1	4	3
Allogeneic bone marrow transplantation	4	0	1
Infections other than candidiasis			
Non-fungal infections	3	7	5
Invasive aspergillosis	2	0	2
Candidemia from	6	10	0
C. albicans	0	3	0
C. tropicalis	5	6	0
T. glabrata	0	1	0
C. parapsilosis	1	0	0
Candida maculopapular skin rash	4	2	0
Candida chorioretinitis	0	1	0
Antifungal treatment	7	8	11
Amphotericin B	6	7	4
Ketoconazole	2	4	10
Miconazole	2	2	1
5-Fluorocytosine	0	2	0
Outcome			
Survivors	0	2	0
Deceased	13	8	15

[a] Average age (range) of (i) patients with autopsy-proven invasive candidiasis, 62.5 (22 to 80); (ii) patients with probable invasive candidiasis, 54.8 (25 to 67); (iii) patients with no candidiasis at autopsy, 54.7 (18 to 76). Of the 13 patients with autopsy-proven invasive candidiasis, 11 were male and 2 were female; of the 10 patients with probable invasive candidiasis, 7 were male and 3 were female; and of the 15 patients with no candidiasis, 7 were male and 8 were female.

TABLE 2. Clinical findings and assay results in 13 patients with invasive candidiasis at autopsy

Patient no.	Date of autopsy (mo/day/yr)	Sites where *Candida* was found (species involved)	Date (mo/day/yr) on which blood cultures with *Candida* were drawn	Date (mo/day/yr) of antifungal treatment with:			Serum dates	Concn in serum of:		Arabinitol/creatinine ratio	Concn in serum of:	
				Amphotericin B	Ketoconazole	Miconazole		Creatinine (mg/dl)	Arabinitol (μg/ml)		Mannose (μg/ml)	Mannan (ng/ml)
1	3/10/82	Heart, gastrointestinal tract, lungs, diaphragm, liver, bone marrow (*C. albicans*)					11/3/81	0.8	2.70	3.38	59.27	1.0
							11/10/81	1.4	7.27	5.19	36.02	0.10
							3/7/82	1.1	5.36	4.87	83.16	0.0
							3/8/82	1.5	10.97	7.31	367.44	0.0
							3/9/82	2.5	13.37	5.35	29.86	0.0
2	5/23/82	Lungs (*C. parapsilosis*)	5/5/82	5/5/ 82 to 5/21/82			12/24/81	1.0	1.89	1.89	26.53	0.80
							5/5/82	1.5	5.99	3.99	115.12	2.66
							5/6/82	1.9	7.10	3.74	80.93	0.06
							5/7/82	1.9	3.54	1.86	74.82	0.01
							5/11/82	1.8	6.63	3.68	67.10	0.03
							5/18/82	1.9	2.74	1.44	96.84	3.47
							5/19/82	1.8	3.37	1.87	47.11	0.06
							5/22/82	2.2	4.36	1.98	134.10	1.46
							5/23/82	3.7	2.13	0.58	131.40	0.15
3	11/11/82	Heart, lungs, liver, kidneys, spleen, pancreas, thyroid, adrenals, gastrointestinal tract (*C. tropicalis*)	11/11/82				11/2/82	1.6	0.57	0.36	19.38	0.0
							11/8/82	1.5	2.14	1.43	40.82	3.18
							11/9/82	1.5	3.47	2.31	50.46	1.98
							11/10/82	1.4	0.60	0.43	68.05	4.09
							11/11/82	2.3	11.67	5.07	20.99	0.0
4	4/24/83	Liver, spleen, kidneys		1/17/83 to 4/13/83	12/31/82 to 3/4/83		5/26/82	0.8	0.47	0.59	41.98	0.0
							6/2/82	0.7	0.53	0.76	45.67	0.87
							7/22/82	0.7	0.89	1.27	63.97	0.0
							8/4/82	0.8	1.92	2.40	27.04	0.0
							1/4/83	0.6	1.95	3.25	34.98	0.63
							1/27/83	0.7	1.52	2.17	55.72	2.23
							2/3/83	0.8	0.13	0.16	22.41	0.0
5	8/4/82	Lung, gastrointestinal tract, thyroid (*C. albicans*)					6/2/82	0.9	1.49	1.66	2.85	0.0
							6/8/82	0.7	1.32	1.89	33.84	0.0
							7/22/82	0.7	2.66	3.80	37.73	0.0
							7/27/82	0.6	0.62	1.03	65.03	0.75
							8/4/82	2.0	8.85	4.43	54.81	1.83
6	1/21/83	Gastrointestinal tract					1/18/83	1.8	3.15	1.75	61.25	1.66
							1/19/83	1.8	4.78	2.66	47.37	4.21
							1/20/83	2.1	3.58	1.70	28.20	7.10
							1/21/83	2.2	3.89	1.77	31.25	4.17
7	8/23/82	Lungs, heart, gastrointestinal tract, liver, pancreas, spleen, kidneys (*C. tropicalis*)	8/23/82				8/4/82	0.8	1.01	1.26	32.88	0.0
8	9/7/79	Heart, gastrointestinal tract, kidneys (*C. tropicalis*)	8/26/79, 9/6/79	8/27/79 to 8/30/79		8/30/79 to 9/7/79	8/13/79	1.3	0.36	0.28	11.18	0.10
9	1/26/79	Pleura, gastrointestinal tract, peritoneum (*C. albicans*)		1/21/79 to 1/24/79		1/24/79 to 1/26/79	1/3/79	0.9	0.12	0.13	23.0	0.0
10	1/25/80	Lungs					1/14/80	0.9	0.98	1.09	516.5	0.0
							1/21/80	0.8	1.43	1.79	63.79	6.39
11	12/28/78	Lungs, heart, liver, spleen, kidneys, adrenals, bone marrow (*C. tropicalis*)					12/11/78	2.4	<0.1	<0.1	59.71	0.49
12	5/28/79	Heart, lungs, spleen, pancreas, adrenals, kidneys, gastrointestinal tract, thyroid, brain (*C. tropicalis*)	5/25/79	5/26/79 to 5/28/79			5/8/79	0.9	64.46	71.62	218.0	0.0

Continued on following page

TABLE 2—*Continued*

Patient no.	Date of autopsy (mo/day/yr)	Sites where *Candida* was found (species involved)	Date (mo/day/yr) on which blood cultures with *Candida* were drawn	Date (mo/day/yr) of antifungal treatment with:			Serum dates	Concn in serum of:		Arabinitol/creatinine ratio	Concn in serum of:	
				Amphotericin B	Ketoconazole	Miconazole		Creatinine (mg/dl)	Arabinitol (μg/ml)		Mannose (μg/ml)	Mannan (ng/ml)
13	1/12/79	Heart, lungs, kidneys, spleen, testes, parathyroid, skeletal muscle, adrenals, thyroid (*C. tropicalis*)	1/1/79, 1/4/79, 1/10/79	1/2/79 to 1/12/79			1/11/79	5.5	34.04	6.19	460.61	0.37

with *Corynebacterium* sp. group JK, enterococcus, *Staphylococcus aureus*, or *Flavobacterium meningosepticum* and three patients without candidiasis at autopsy had one or more of *Klebsiella pneumoniae*, enterococcus, group B streptococcus, *S. aureus*, or *Pseudomonas aeruginosa* isolated from blood cultures. Weekly surveillance cultures of sputum, urine, and feces samples were done in patients with hematological malignancies. Six patients with proven invasive candidiasis, five patients with probable invasive candidiasis, and seven patients without candidiasis at autopsy were colonized with *Candida albicans, C. tropicalis,* or *C. parapsilosis* at one or more sites.

Renal function was normal in 50 normal blood donors (mean creatinine concentration in serum, 0.89 mg/dl; standard deviation [SD], 0.15; range, 0.6 to 1.2 mg/dl). However, 7 of 13 patients with proven invasive candidiasis (Table 2), 8 of 10 patients with probable invasive candidiasis, and 6 of 15 patients without candidiasis had renal insufficiency (creatinine concentration in serum of ≥1.5 mg/dl). Most patients with renal insufficiency in all three groups showed a progressive rise in the creatinine concentration in serum during the study period. The mean highest creatinine concentrations in serum (with the range given in parentheses) for all patients in each group were as follows: proven invasive candidiasis, 2.0 mg/dl (0.8 to 5.5 mg/dl); probable invasive candidiasis, 3.9 mg/dl (1.1 to 10.3 mg/dl); no candidiasis, 2.3 mg/dl (0.7 to 5.6 mg/dl).

Arabinitol concentrations in serum. Arabinitol concentrations in sera from 50 normal blood donors, 25 sera from 9 control patients with normal renal function, 19 sera from 6 control patients with renal failure, and all as well as the highest concentrations found in 23 patients with proven or probable invasive candidiasis are shown in Table 3. For patients with invasive candidiasis who developed renal failure during the study period, the highest arabinitol concentrations in serum both during normal renal function and during renal failure are shown.

In normal blood donors, the arabinitol concentration (mean ± SD) was 0.45 ± 0.37 μg/ml. Based on the concentrations in these sera, the upper limit of normal for arabinitol, defined as the mean concentration + 2 SD, was 1.19 μg/ml.

Sera from patients with normal renal function and without invasive candidiasis had increased arabinitol concentrations (mean ± SD = 1.50 ± 1.68 μg/ml) when compared with sera from normal blood donors (0.45 ± 0.37 μg/ml) ($P < 0.003$). In sera from patients without renal failure and with invasive candidiasis, the mean arabinitol concentration ± SD (4.01 ± 13.53 μg/ml) was greater than in sera from patients without renal failure and without candidiasis (1.50 ± 1.68 μg/ml), but this difference was not significant ($P = 0.411$). During normal renal function, only 4 of 15 patients with invasive candidiasis had sera with arabinitol concentrations greater than the mean + 2 SD (4.86 μg/ml) observed in sera from patients without invasive candidiasis and with normal renal function.

In control patients with renal failure, arabinitol concentrations in serum (mean ± SD = 8.09 ± 9.68 μg/ml) were significantly greater ($P < 0.009$) than those in control patients with normal renal function (1.50 ± 1.68 μg/ml). In addition, all as well as the highest arabinitol concentrations in sera of patients with invasive candidiasis and renal failure were greater than those in patients with invasive candidiasis

TABLE 3. Arabinitol concentrations in sera of normal blood donors and patients with and without invasive candidiasis

Category	No. of subjects	No. of sera	Arabinitol concn (μg/ml)[a]			
			Mean ± SD	Median	Range	Ratio[b]
Normal blood donors	50	50	(a) 0.45 ± 0.37	0.37	<0.1–1.58	(h) 0.48 ± 0.39
Patients with candidiasis—all sera	23	97				
Normal renal function[c]	8	22	(b) 4.01 ± 13.53	1.0	<0.1–64.46	4.67 ± 15.00
Renal failure[d]	15	75	(c) 8.38 ± 9.85	5.03	<0.1–48.40	2.97 ± 2.43
Patients with candidiasis—highest concentrations	30	30				
Normal renal function[c]	15	15	(d) 6.68 ± 16.09	2.66	0.12–64.46	7.24 ± 17.89
Renal failure[d]	15	15	(e) 16.61 ± 14.39	9.83	<0.10–48.40	5.05 ± 3.89
Patients at risk with:	15	44				
Normal renal function[c]	9	25	(f) 1.50 ± 1.68	0.92	<0.10–6.14	(i) 2.69 ± 3.74
Renal failure[d]	6	19	(g) 8.09 ± 9.68	4.93	0.39–41.32	(j) 1.92 ± 2.66

[a] *P* values: (a) versus (b), not significant (NS); (a) versus (f), <0.003; (b) versus (f), NS; (f) versus (g), <0.009; (d) versus (e), NS; (b) versus (c), NS; (c) versus (g), NS; (i) versus (j), NS; (h) versus (i) and (j), <0.001.
[b] Arabinitol/creatinine ratio (in micrograms of arabinitol per milliliter/milligrams of creatinine per deciliter).
[c] Creatinine concentration <1.5 mg/dl.
[d] Creatinine concentration ≥1.5 mg/dl.

TABLE 4. Mannose concentrations in sera of normal blood donors and in patients with and without invasive candidiasis

Category	No. of subjects	No. of sera	Mannose concn (μg/ml)[a]		
			Mean ± SD	Median	Range
Normal	50	50	(a) 14.5 ± 11.0	9.98	<0.10–44.91
Candidiasis—all sera	23	97	(b) 67.0 ± 81.5	47.11	<0.10–516.5
Candidiasis—highest concns	23	23	135.31 ± 140.56	68.05	11.18–516.5
At risk	15	44	(c) 37.0 ± 26.3	33.60	<0.10–127.53

[a] *P* values: (a) versus (c), <0.001; (a) versus (b), <0.001; (b) versus (c), <0.001.

and normal renal function, but these differences were not significant. Only 3 of 15 patients with invasive candidiasis and renal failure had sera with arabinitol concentrations greater than the mean + 2 SD (27.45 μg/ml) observed in sera from patients with renal failure but without invasive candidiasis.

In patients without candidiasis and with normal renal function the mean + 2 SD for arabinitol concentration in serum was 4.86 μg/ml, whereas in patients without candidiasis and with renal failure this value was 27.45 μg/ml. With these as upper limits of normal, the overall sensitivity of arabinitol in the sera of patients with proven or probable invasive candidiasis was 26%. The specificity (percentage of patients without invasive candidiasis who did not have elevated concentrations in serum) was 87%.

Arabinitol is excreted by the kidneys, and its clearance is identical to that of creatinine. On this basis, the arabinitol/creatinine ratio has been used to adjust arabinitol concentrations in serum for renal failure, since this ratio is independent of renal function (9). In sera from 50 normal blood donors, the mean arabinitol/creatinine ratio ± SD (in micrograms of arabinitol per milliliter/milligrams of creatinine per deciliter) was 0.48 ± 0.39, and the mean + 2 SD was 1.25. For patients without invasive candidiasis, the mean arabinitol/creatinine ratios ± SD were 2.69 ± 3.74 and 1.92 ± 2.66 for sera with a creatinine concentration of less than and greater than 1.5 mg/dl, respectively. These ratios were not significantly different ($P = 0.43$). The mean arabinitol/creatinine ratio ± SD for all sera from the patients without candidiasis was 2.37 ± 3.33, which was significantly greater ($P < 0.001$) than that for the sera from normal blood donors (0.48 ± 0.39). The mean + 2 SD for the arabinitol/creatinine ratio in patients without candidiasis was 9.03. With this value as the upper limit of normal, the overall sensitivity of this ratio in patients with proven or probable invasive candidiasis was 13% and the specificity was 93%.

Mannose concentrations in serum. Mannose concentrations in sera from 50 normal blood donors, 44 sera from 15 patients without invasive candidiasis, and all as well as the maximum concentrations observed in 23 patients with proven or probable invasive candidiasis are shown in Table 4.

The mean ± SD mannose concentration in normal blood donors was 14.5 ± 11.0 μg/ml. The upper limit of normal for the mannose concentration in serum based on these sera (mean + 2 SD) was 36.6 μg/ml. However, patients without invasive candidiasis had increased mannose concentrations in serum when compared with normal blood donors ($P < 0.001$). The mean mannose concentration ± SD in these control patients without candidiasis was 37.0 ± 26.3 μg/ml, and an upper limit of normal (mean + 2 SD) based on this group of patients was 89.7 μg/ml. Of 23 patients with proven or probable invasive candidiasis, 9 had at least one mannose concentration in serum which exceeded this upper limit (89.7 μg/ml). Thus, the sensitivity of mannose in serum for the diagnosis of invasive candidiasis was 39%. The specificity (percentage of patients without candidiasis who did not have elevated concentrations) was 87%.

Mannan concentrations in serum. Mannan concentrations in serum samples from 50 normal blood donors, 44 samples from 15 patients without invasive candidiasis, and sera from 23 patients with proven or probable invasive candidiasis are shown in Table 5. For the group with invasive candidiasis, all as well as the highest mannan levels are depicted.

Mannan concentrations in sera from 50 normal blood donors (mean ± SD) were 0.04 ± 0.15 ng/ml, which was not statistically different ($P = 0.344$) from those in sera from patients without candidiasis (0.08 ± 0.19 ng/ml). Mannan was completely undetectable in the vast majority of sera from the blood donors or control patients.

The mean + 2 SD mannan concentration in sera from patients without invasive candidiasis was 0.46 ng/ml. With this as the upper limit of normal, 8 of 13 (62%) patients with proven candidiasis and 8 of 10 with probable invasive candidiasis had at least one elevated mannan concentration in serum during the study period. The sensitivity of mannan in serum for the diagnosis of invasive candidiasis was thus 70%. Specificity (percentage of patients without invasive candidiasis whose mannan concentrations in serum did not exceed 0.46 ng/ml) was 87%. If an upper limit of normal of 1.0 ng/ml was used, the sensitivity was 65% and the specificity was 100%.

DISCUSSION

The clinical and microbiological diagnosis of invasive candidiasis in immunosuppressed patients is infrequently achieved premortem. A clear need exists for a sensitive, specific, and rapid method which will allow earlier diagnosis

TABLE 5. Mannan concentrations in sera of normal blood donors and in patients with and without invasive candidiasis

Category	No. of subjects	No. of sera	Mannan concn (ng/ml)[a]		
			Mean ± SD	Median	Range
Normal	50	50	(a) 0.04 ± 0.15	0.0	0.0–0.88
Candidiasis—all sera	23	97	(b) 1.51 ± 3.23	0.32	0.0–20.82
Candidiasis—highest concns	23	23	3.73 ± 5.56	2.23	0.0–0.73
At risk	15	44	(c) 0.08 ± 0.19	0.0	0.0–0.73

[a] *P* values: (a) versus (c), not significant; (a) versus (b), <0.001; (b) versus (c), <0.001.

TABLE 6. Results of assays for serum arabinitol/creatinine, mannose, and mannan in invasive candidiasis caused by different *Candida* spp.

Candida spp. involved (no. of patients)	No. of patients with at least one positive result[a] for:		
	Arabinitol/creatinine	Mannose	Mannan
C. tropicalis (12)	3	4	7
C. albicans (7)	0	4	6
C. parapsilosis (1)	0	1	1
T. glabrata (1)	0	0	0

[a] Defined as arabinitol/creatinine > 9.03 μg per ml/mg per dl; mannose > 89.7 μg/ml; mannan > 0.46 ng/ml.

and prompt administration of antifungal therapy. In this study, we retrospectively compared three proposed quantitative serum markers for the rapid diagnosis of invasive candidiasis. Arabinitol, mannose, and mannan concentrations in sera from normal blood donors and immunosuppressed patients with or without invasive candidiasis were measured. Sera from immunocompromised patients with candidemia who did not undergo autopsy were included because it has been reported that 88% of patients with *C. albicans* and 81% of patients with *C. tropicalis* fungemia occurring in this setting had either disseminated or focal invasive disease due to the fungus (17).

Greater arabinitol concentrations were observed in sera from patients with renal failure than in sera from those with normal renal function, and calculation of the arabinitol/creatinine ratio made the arabinitol concentration independent of renal function. Similar results have been observed by Gold et al. (9). The arabinitol concentrations in sera from normal blood donors (mean ± SD = 0.45 ± 0.37 μg/ml) were similar to those found by Gold et al. in sera from patients with normal renal function without candidiasis (0.56 ± 0.32 μg/ml) (9). However, we found significantly greater concentrations of arabinitol and a higher arabinitol/creatinine ratio in sera from high-risk patients without candidiasis than in sera from normal blood donors. There are two possible explanations for this observation. Heavy colonization by the *Candida* sp. at mucosal sites may have resulted in a production of arabinitol sufficient to cause a significant increase in concentrations. However, the arabinitol/creatinine ratio (mean ± SD) was not greater in sera from patients without invasive candidiasis who were colonized (1.84 ± 2.24) than in sera from those who were not colonized (2.69 ± 4.00). A second possible explanation is that increased arabinitol concentrations in sera of patients without candidiasis resulted from the administration of corticosteroids. de Repentigny et al. demonstrated in uninfected rabbits that cortisone acetate alone increases arabinitol concentrations in serum (2). For our patients without invasive candidiasis, the arabinitol/creatinine ratio was greater ($P < 0.02$) in sera drawn within 1 week of corticosteroid therapy (mean ± SD = 5.35 ± 7.45) than in sera drawn more than 1 week after corticosteroid therapy or sera from patients who did not receive corticosteroids (1.90 ± 1.93). Because of these nonspecific increases in arabinitol concentrations in sera from control patients without candidiasis, the sensitivity and specificity of the arabinitol/creatinine ratio were 13 and 93%, respectively. The sensitivity was far less than that reported by others (64%), although the specificity was comparable (96%) (9).

As for arabinitol concentrations in serum, mannose concentrations in serum were significantly greater for patients without invasive candidiasis than for normal blood donors. Monson and Wilkinson (19) observed that mannose concentrations in sera from intensive care unit patients without candidiasis and in uninfected patients with diabetic ketoacidosis were higher than in sera from normal blood donors. Although several control patients were under intensive care, a single patient without candidiasis had diabetes mellitus but was well controlled at the time of study. Furthermore, patients colonized with the *Candida* sp. without invasive candidiasis did not have significantly greater ($P = 0.576$) mannose concentrations (mean ± SD = 41.38 ± 33.77 μg/ml) than those who were not colonized (33.68 ± 18.96 μg/ml). Our studies in uninfected rabbits showed that corticosteroids alone increase mannose concentrations in serum (2). However, the mean mannose concentration ± SD in sera drawn less than 1 week after corticosteroid administration (27.88 ± 19.98 μg/ml) was not greater than the mean mannose concentration ± SD in sera drawn more than 1 week after corticosteroid therapy or in sera from patients who did not receive corticosteroids (38.45 ± 27.12 μg/ml). Thus, nonspecific increases in mannose concentrations in the sera of control patients without candidiasis were not explained by diabetic ketoacidosis, colonization by the *Candida* sp., or corticosteroid administration but may be similar to the unexplained increases in the mannose concentration in sera observed from intensive care unit patients without candidiasis (19). Higher concentrations of mannose in the sera of control patients without candidiasis than in the sera of normal blood donors produced a sensitivity of only 39% for this test and a specificity of 87%. This stands in contrast to the few (six) patients with invasive candidiasis studied by others (19), who all had higher mannose concentrations in serum than those in sera of control patients, excluding patients with diabetic ketoacidosis. These differences may reflect a larger sample size in the present study.

In contrast to arabinitol and mannose concentrations in serum, mannan concentrations were similar in the sera of normal blood donors and patients without invasive candidiasis. Because of this, mannan in serum was more sensitive (70%) and had a specificity similar (87%) to those of the other two tests. These results are similar to those reported in the literature (11, 14, 16, 22, 26, 28, 29). If a single technique is to be used, quantitation of mannan in serum by enzyme immunoassay appears to be the rapid method of choice for diagnosing invasive candidiasis.

There was no correlation between increased concentrations of mannose and mannan in sera of patients with autopsy-proven invasive candidiasis (Table 2). Mannose and mannan concentrations were simultaneously increased in only 3 sera, whereas mannose was elevated in the absence of mannan in 6 sera and mannan was increased with normal mannose in 16 sera. The hypothesis that mannose might circulate as a metabolic product of mannan also seems improbable when one considers that mannose concentrations (in micrograms per milliliter) are 1,000-fold greater than mannan concentrations (in nanograms per milliliter).

A question of interest is whether arabinitol, mannose, and mannan in serum all give equally good diagnosis of invasive candidiasis caused by the different *Candida* spp. This is especially important because of the increasing incidence of *C. tropicalis* as the etiological agent of this fungal infection (17). Cultures of blood, maculopapular skin rash, or organs involved at autopsy allowed a species diagnosis to be established in 21 of 23 cases of proven or probable invasive candidiasis. The species involved were *C. tropicalis* in 12 cases, *C. albicans* in 7 cases, and *C. parapsilosis* and

Torulopsis glabrata in 1 case each (Table 6). The sensitivity of each of these tests relative to the different *Candida* spp. was comparable, although the numbers were small. It would be interesting to accumulate more data on this point in the future.

A timely diagnosis, obtained early enough for antifungal therapy to be given, is of great importance in the treatment of invasive candidiasis. In 16 patients with proven or probable invasive candidiasis and positive blood cultures, the arabinitol/creatinine ratio first became positive (three patients) from 17 days before to 4 days after the first positive blood culture was drawn (mean, 4 days before). Mannose in serum became positive (seven patients) from 17 days before to 10 days after drawing the first positive blood culture (mean, 1 day before). Finally, mannan in serum was first positive (10 patients) from 3 days before to 4 days after the first positive blood culture was drawn (mean, same day). Because of the delay in processing blood cultures and because all three proposed tests can be completed in 1 day, a more rapid diagnosis can be obtained in some patients by each of these three methods. However, they cannot be used as substitutes for blood cultures, since all three tests were negative in 3 of 16 patients with positive blood cultures and proven or probable invasive candidiasis, two of whom had autopsy-proven infection.

Seven patients with autopsy-proven invasive candidiasis had negative blood cultures, and only three of these seven received antifungal therapy. Six of these seven patients had a positive result in the mannan assay, two had elevated mannose concentrations in serum, and none had a significantly increased arabinitol/creatinine ratio. Both patients with a positive mannose test also had a positive mannan test. Therefore, although these three tests cannot be used as substitutes for blood cultures, a number of patients with invasive candidiasis and negative blood cultures will be identified by the use of the mannan test. The other two tests do not seem to provide added diagnostic sensitivity when used as well as the mannan test in patients with negative blood cultures. Looking at the overall results, 22 of the 23 patients with proven or probable invasive candidiasis would have been identified by positive blood cultures for the *Candida* sp. or a positive mannan test. Considering the mean + 2 SD of 0.46 ng/ml as the upper limit of normal, 16 of 23 (70%) patients with proven or probable invasive candidiasis were considered to have a positive mannan test. Based on these results, it seems reasonable to conclude that the best approach to the diagnosis of invasive candidiasis in immunocompromised patients would involve obtaining blood cultures and carrying out serial assays for mannan in serum. Additionally, the recommendation that every immunocompromised patient with fungemia be treated (17) should probably be extended to those with a positive mannan test. These findings need to be confirmed in prospective studies before the assay for mannan in serum can be recommended for general clinical use.

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