ANTIBODIES AGAINST ANTIGENS OF *CANDIDA ALBICANS* IN PATIENTS WITH FUNGAEMIA AND BACTERAEEMIA, STUDIED BY ELISA, PRECIPITATION, PASSIVE HAEMAGGLUTINATION AND IMMUNOFLUORESCENCE TECHNIQUES

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SUMMARY. Antibodies against commercially available antigens of *Candida albicans* were assayed in 54 sera from 24 patients with fungaemia and in 66 sera from 33 patients with bacteraemia. In patients with persistent fungaemia, antibody was found during the week after the fungus was first cultured from the blood, but peak titres did not usually occur until the end of the second week. A significant rise of titre in *C. albicans* infection was observed in 50% of paired sera tested by passive haemagglutination (PHA), indirect immunofluorescence (IF) and Ouchterlony immunodiffusion (ID). The same percentage was obtained by counterimmunoelectrophoresis (CIE) against candida metabolic antigens, whereas it was increased to 88% when somatic antigens were used. Enzyme-linked immunosorbent assay (ELISA) demonstrated a rise of titre in 25, 75 and 50% of sera in IgM, IgG and IgA assays, respectively. Sera from patients with transient fungaemia demonstrated persistent antibody titres.

In paired sera from patients with bacteraemia, ID and CIE titres were low (≤ 4). There was an increase of candida antibodies in 0–9% of patients by ELISA, ID or CIE and in 18–21% by PHA or IF. Clinically significant fungaemia was most reliably differentiated serologically from bacteraemia by CIE S-antigen and ELISA IgG assays.

INTRODUCTION

The diagnosis of systemic mycoses is difficult. Blood cultures yield negative results in most cases and a positive finding may stem from transient fungaemia or contamination. A biopsy with histological evidence is needed for a decisive diagnosis (Kozinn *et al.*, 1978), and cases with positive blood cultures alone should be considered presumptive. Because *Candida* antibodies are found in subjects without active infection, serological diagnosis may be unreliable (Cobb and Parrat, 1978; Kozinn *et al.*, 1978; Kaufman, 1980).

In immunocompromised hosts, deep organ mycoses occur but are rare. Septic episodes caused by yeasts are more often seen in patients with intravenous catheters, and form a potential source for dissemination. In the present study, the sera of patients from whom fungi were isolated by blood culture were tested for antibodies against *Candida albicans* by comparing commercial methods recommended for routine clinical use with an enzyme-linked immunosorbent assay (Drouhet, 1973; Müller, 1976; Kaufman, 1980; Kostiala and Kostiala, 1981). As controls, sera from persons with bacterial sepsis were similarly studied.

MATERIALS AND METHODS

Patients. Table I shows the clinical data of 24 adult patients in the University Central Hospital with fungaemia. One or several of the known factors predisposing to fungal diseases

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TABLE I
Predisposing factors in 24 patients with fungaemia

	Number of patients
Basic disease	
carcinoma	5
haemorrhagic pancreatitis	4
ulcerative colitis	3
burns	3
diabetes mellitus	2
renal transplant	2
leukaemia	1
duodenal ulcer	1
mesenteric-vein thrombosis	1
adhesive ileus	1
uraemia	1
Intravenous catheter	21
Broad-spectrum antibiotics	20
Corticosteroids	7
Cytostatics	3

(Kostiala, Kostiala and Kahanpää, 1979) were found in each of these patients and 20 had required surgery. The majority had an intravenous catheter and were being treated with broad-spectrum antibiotic, whereas seven and three received corticosteroid and cytostatic drugs, respectively. Of these patients, 14 were males and 10 females and the mean age was 45 years (range 15–74). Of the patients with bacteraemia, 16 were males and 17 females and the mean age was 49 years (range 8–79).

Sera. From the 24 patients with fungaemia, 54 sera were studied. Paired sera comprised a sample obtained within the first week of the diagnosis of fungaemia (sample I) and another obtained 1–2 weeks later (sample II). Samples from some patients that were obtained later or before diagnosis were also studied. In addition, 66 similarly paired sera from 33 patients with bacteraemia were assayed.

Passive haemagglutination (PHA) and indirect immunofluorescence (IF). Commercial kits (*Candida albicans* HA and IF Antigen Hoffmann-LaRoche Diagnostica, Basel, Switzerland) were used. Titres of ≥ 320 in PHA and ≥ 160 in IF were considered positive (Müller, 1976). By these criteria 3% of 100 blood donors had antibodies.

Ouchterlony immunodiffusion (ID). Serial twofold dilutions of sera in saline against a *Candida albicans* extract (1 in 10, w/v, lot G13821M, Hollister-Stier, Berkeley, Ca, USA) were studied by ID as described earlier (Kostiala and Kostiala, 1981). Titres of ≥ 1 were considered positive. ID (sample I) was positive for 3% of blood donors.

Counterimmunoelectrophoresis (CIE). Somatic (S) and metabolic (M) antigens of *C. albicans* (Pasteur Institute, Paris, France) were used in CIE as previously reported (Kostiala and Kostiala, 1981) to determine titres in serial twofold dilutions of sera made in saline. As a control, a positive reference serum (Anti-*Candida albicans* Serum, Pasteur Institute) with a titre of 32 against S and 4 against M antigens was used. Titres of ≥ 1 were considered positive. Of sample I from blood donors, 5% reacted against S antigen (highest titre 4) and 1% against M antigen.

Enzyme-linked immunosorbent assay (ELISA). The technique has been described (Kostiala and Kostiala, 1981). In brief, *Candida albicans* antigen (1 in 10, w/v, lot G13821M, Hollister-Stier, Berkeley, Ca, USA) was diluted 1 in 1000 in 0.05M carbonate buffer, pH 9.6, and used to coat wells in microtitration plates. Sera were tested in duplicate, diluted 1 in 500, 1 in 10 000 and 1 in 5000 in phosphate-buffered saline, pH 7.4, containing Tween 20 (PBS-T) 0.05% for IgM, IgG and IgA assays respectively. Alkaline-phosphatase-conjugated swine antibodies against human IgM, IgG or IgA (Orion Diagnostica, Helsinki) at a dilution of 1 in 500 in PBS-T were used to detect the bound antibodies. To this end, a substrate solution (Phosphatase

Substrate, Sigma Chemical Co., St Louis, Mo, USA) at 1 mg/ml in carbonate buffer containing 0.02% (w/v) $MgCl_2$ was added and the enzymatic reaction was stopped by 2.5M NaOH after incubation for 1 h at 37°C. The absorbance was measured in a Titerik Multiskan photometer (Flow Laboratories, Eflab Oy, Helsinki) at 405 nm, in antigen-coated wells treated with PBS-T without serum as a blank. To minimise day-to-day variation in the absorbance values, the results were expressed as ELISA units, i.e., as the percentage absorbance of that given by the same reference serum dilution studied simultaneously. This positive serum exhibited a *C. albicans* PHA and an IF titre of 640, an ID titre of 16, and reacted positively in CIE against S and M antigens of *C. albicans* at a titre of 8 and 2, respectively. In ELISA the mean unit + 2 SD given by sera from 100 blood donors was taken as a limiting value, i.e., units > 40 in IgM, and > 20 in IgG and IgA assays were defined as positive reactions, and a doubling of a positive unit was considered significant. Of blood donors' sera, 6% showed reactivity in the ELISA IgM (range of units 0–80; mean $\pm$ SD 14 ± 13), 5% in IgG (range 0–55; mean $\pm$ SD 6 ± 8), and 6% in IgA assay (range 0–34; mean $\pm$ SD 6 ± 7). None of the sera was positive in all three assays, while 86% were negative.

Blood cultures. Aerobic and anaerobic blood-culture bottles containing 50 ml of brain-heart-infusion broth with supplements (Hemobact, Orion Diagnostica) were each inoculated with 5 ml of blood. If a yeast was grown from one or both bottles, the culture was considered positive. Concordant growth of bacteria in both bottles or in several samples was regarded as significant bacteraemia.

RESULTS

Candida antibodies in patients with fungal septicaemia

The figure and table II show our findings in five and nine clinically septic patients respectively. No other cause of fever was revealed, except that most patients had 2–4 blood cultures positive for yeasts; in three patients from whom only one blood culture was obtained, the same organism was also isolated from the tip of an intravenous catheter.

Candida albicans was the causative agent in the majority (10/14) of cases (figure and table II). In serum samples (I) the numbers of patients with antibodies to *C. albicans* ranged from three (33%) in the ELISA IgG assay to 7 (78%) in ID. Serum samples (II) yielded a greater number of positive test results with the lowest incidences in the ELISA IgM assay and IF (six patients, 67%, positive). In ELISA IgA and CIE M antigen and by PHA assays, 7/9 (78%) of serum samples (II) had antibodies to *C. albicans*. The highest numbers, however, were detected by the ELISA IgG, CIE S-antigen and ID assays, 8/9 (89%), 9/9 (100%) and 9/9 (100%) samples respectively. A change from a negative to a positive ELISA unit or a doubling of a positive unit occurred in 2/8 (25%), 6/8 (75%) and 4/8 (50%) of the paired sera for IgM, IgG and IgA assays respectively. In PHA and IF the number of patients with a fourfold rise in titre was 4 (50%). In ID a significant rise was found in 4/8 (50%) sera, while 7/8 (88%) and 4/8 (50%) demonstrated a rise in CIE titres against S and M antigen respectively. None of the serum samples (II) showed a significant fall of titre.

Among the patients with clinically significant candidaemia two were infected with *C. parapsilosis*. In one from whom only the first sample (I) had been drawn, all the tests except for the CIE M-antigen assay were negative while in another from whom only sample II was taken, all assays were positive except those by ELISA IgM and CIE M antigen. The first and only serum sample from a patient infected with *C. tropicalis* did not react in any of the tests with *C. albicans* antigens. In paired serum samples from a patient with *Torulopsis glabrata* fungaemia, the ELISA IgG units changed to a positive value concordantly with a significant rise in PHA, IF and ID titres and the development of reactivity towards M antigen in CIE (table II).

Three patients were studied before they developed fungaemia, and two of these had candida antibodies. Samples taken several weeks after the diagnosis of fungaemia showed lower values than those at 10–14 days (fig.).

Candida antibodies during persistent *C. albicans* fungaemia

Multiple (2–10) blood cultures yielding *C. albicans* were obtained from six additional septic patients, and serological tests were done on the same days that the positive blood cultures were

FIGURE—*Candida* antibodies in patients 1–5 with *C. albicans* sepsis. Enzyme-linked immunosorbent assay (ELISA) for IgM = ▲, IgG = *, IGA = Δ. Passive haemagglutination (PHA) = ○; indirect immunofluorescence (IF) = ●. Immunodiffusion (ID) = ■. Counterimmunoelectrophoresis (CIE) with somatic antigen S = □ and metabolic antigen M = ■. ↑ = Isolation of *C. albicans* from blood.

TABLE II
Candida antibodies in nine patients with clinically significant fungaemia

Patient no.	Species isolated	Serum sample no.*	ELISA units* for			PHA titres*	IF titres*	ID titres*	CIE titres* against	
			IgM	IgG	IgA				S	M
1.	<i>C. albicans</i>	I; II	87; 134	13; 40	121; 290	320; 1280	40; 80	1; 8	0; 16	0; 4
2.	<i>C. albicans</i>	I; II	56; 67	49; 107	205; 349	640; 640	160; 1280	4; 16	2; 8	1; 8
3.	<i>C. albicans</i>	I; II	13; 17	2; 5	3; 9	20; 80	320; 640	1; 2	1; 1	1; 1
4.	<i>C. albicans</i>	I; II	14; 38	7; 22	25; 36	40; 160	20; 80	2; 1	1; 8	0; 0
5.	<i>C. albicans</i>	II	95	72	123	640	1280	32	16	16
6.	<i>C. parapsilosis</i>	II	14	63	31	320	640	2	1	0
7.	<i>C. parapsilosis</i>	I	6	0	0	10	10	0	0	1
8.	<i>C. tropicalis</i>	I	10	8	6	160	40	0	0	0
9.	<i>T. glabrata</i>	I; II	4; 20	8; 32	5; 11	160; 640	80; 320	1; 4	0; 0	0; 1

S = somatic, M = metabolic antigen of *C. albicans*.

* See *Materials and methods* for timing of serum samples and definition of units and titres.

TABLE III
Candida antibodies in eight sera from six patients studied during persistent *C. albicans* fungaemia and in paired sera from four patients after transient fungaemia

Clinical condition	Serum sample no.*	ELISA units,* range (and percentage positive) for			Titre,* range (and percentage positive) by				
		IgM	IgG	IgA	PHA	IF	ID	S	M
Persistent candidaemia	...	1-66 (13)	0-34 (38)	0-72 (50)	20-264 (50)	10-160 (13)	0-2 (25)	0-4 (75)	0-4 (50)
Transient fungaemia	I	6-71 (25)	2-6 (0)	1-12 (0)	40-160 (0)	10-20 (0)	0	0	0
	II	5-42 (25)	2-5 (0)	1-11 (0)	40-160 (0)	10-40 (0)	0	0	0

S=somatic, M=metabolic antigen of *C. albicans*.
* See *Materials and methods* for timing of samples, definition of units and titres, and criteria for positive values.

TABLE IV
Candida antibodies in pairs of sera from 15 patients with gram-positive and 18 with gram-negative bacteraemia

Serum samples no.*	ELISA units,* range (and percentage positive) for			Titre*, range (and percentage positive) by				
	IgM	IgG	IgA	PHA	IF	ID	S	M
I	0-92 (12)	0-64 (24)	0-109 (30)	<10-640 (36)	<10-640 (21)	0-4 (33)	0-4 (48)	0-2 (36)
II	0-97 (21)	0-66 (30)	0-117 (33)	<10-640 (64)	<10-640 (30)	0-4 (48)	0-4 (65)	0-1 (45)
Percentage of pairs showing: rise of value† fall of value	9 0	6 3	6 0	18 3	21 15	6 0	6 9	0 0

S=somatic, M=metabolic antigen of *C. albicans*.

* See *Materials and methods* for timing of samples, definition of units and titres, and criteria for positive values.

† Change from a negative to a positive unit or doubling of a positive unit in the ELISA, fourfold rise of titre in PHA and IF, change from a negative to a positive reaction or a fourfold rise of titre in ID and CIE. For fall of values, corresponding converse changes.

obtained. One or several of the methods revealed candida antibodies in all but one patient. Table III shows that the tests that most often detected antibodies were CIE S antigen (six sera positive out of eight), PHA, ELISA IgA and CIE M antigen assays (four sera positive out of eight).

Candida antibodies in patients with transient fungaemia

Paired sera from four patients with one positive blood culture due to contamination from the skin or transient fungaemia were also studied. Unlike the patients with persistent fungaemia, they were not treated with antifungal drugs. The organism isolated was *C. albicans* from two patients, and *Cryptococcus albidus* and *Rhodotorula rubra* from one patient each. Two serum samples from the patient with *Cr. albidus* showed antibodies to *C. albicans* antigens in the ELISA IgM assay but otherwise the serological findings were negative throughout as shown (table III).

Candida antibodies in sera of patients with bacteraemia

Paired sera from 33 patients with bacteraemia were examined for *C. albicans* antibodies. Fifteen of the patients had sepsis due to a gram-positive organism, these being *Staphylococcus aureus* (5), *Streptococcus pneumoniae* (5) or other streptococci (5) and 18 patients had gram-negative sepsis caused by *Neisseria meningitidis* (5), *Escherichia coli* (5), *Klebsiella pneumoniae* (5) or *Haemophilus influenzae* (3). The findings for candida antibodies were essentially similar in gram-positive and gram-negative bacteraemia, and the results are combined in table IV. Candida antibodies with low to moderately high values were demonstrated in sera from these patients. The number positive in the first sample of each pair ranged from 12% in the ELISA IgM assay to 48% in the CIE S-antigen test. Slightly but not significantly higher percentages were found in all the second samples; however, the change was most marked in PHA and IF, by which a fourfold rise of titre was observed in 18% and 21% of sera respectively. By the other methods, 0–9% of sera showed a rise of values. Half the methods revealed a fall of values which was most frequently seen in IF (15% of sera). The positive values and rises of candida antibodies could not be specifically ascribed to any of the causative agents of bacteraemia.

DISCUSSION

In this study, peak titres of antibodies to *C. albicans* were found within the second week after the diagnosis of fungal septicaemia. However, these patients often have severe longstanding basic illnesses and may have candida antibodies before the diagnosis of fungaemia. Thus the information given by a single serum sample is insufficient for estimating the presence of fungal invasion, but a significant rise of antibody in paired sera has diagnostic value. By ELISA, it was shown that rises occurred especially in IgG and also in the IgA antibodies. Because biopsies were not done, it is not known whether or not organ involvement occurred; however, persistent fungaemia was considered a clinically important cause of septic episodes. Constant antibody levels in paired sera, on the other hand, may help to distinguish a blood-culture contamination or transient fungaemia from true infection with repeated isolation of yeasts over several days. Most of the patients with multiple blood cultures positive for *C. albicans* demonstrated antibodies in serum samples drawn simultaneously, indicating that the antibodies did not protect against the growth of this organism.

In the eight medically important *Candida* species (Hurley, 1967), common and specific antigens have been found intracellularly and on the cell surface (Biguet, Tran van Ky and Andrieu, 1962; Müller and Kranenberg, 1968; Sticle *et al.*, 1972; Segal *et al.*, 1975; Axelsen, 1976). *T. glabrata* is known to be antigenically related to *C. albicans* (Hasenclever and Mitchell, 1960; Müller and Kranenberg, 1968; Sticle *et al.*, 1972), and cross reactions between *Candida* species and *Cryptococcus* have also been demonstrated (Müller and Kranenberg, 1968; Kaufman, 1980). Of the present patients tested with *C. albicans* antigens, one infected with *T. glabrata* and another with *C. parapsilosis* fungaemia showed antibodies.

Up to 47% of single serum samples from patients with bacteraemia have been shown to react with candida antigens in CIE tests (Dee and Rytel, 1975). This was confirmed in the present study, especially with the CIE S-antigen assay. However, the titres were low; none exceeded 4, which was seen in the majority of patients with *C. albicans* fungaemia. This accords with the findings of Dee and Rytel (1977) and Myerovitz *et al.* (1979) who stressed the diagnostic importance of single CIE and ID titres equal to or higher than 8.

In our findings, another difference between persistent fungaemia and bacteraemia was seen in paired serum samples. While in the majority of cases of fungaemia the titres rose significantly, in only a minority of sera from patients with bacteraemia was this found. Even a fall of values occurred in some sera from patients with bacteraemia, but not from those with fungaemia. In bacteraemia, a significant rise of titre occurred most often in PHA and IF. The reason for the positive values in bacteraemia may be the presence of cross-reactive antigens in yeasts and bacteria (Aksoycan and Le Minor, 1960), or a present infection by yeasts. The latter, however, was not clinically shown or suspected, although it is difficult to exclude inapparent superficial candidal proliferation, i.e., in the mouth of patients receiving antibiotic treatment for bacteraemia. This could most readily be reflected in antibody titres against surface components of *Candida* which are measured by PHA and IF (Müller, 1976). It may be concluded that, by the use of commercially available antigens, clinically significant fungaemia was most reliably differentiated serologically from bacteraemia by ELISA IgG and CIE S-antigen assays.

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