Mannose in Body Fluids as an Indicator of Invasive Candidiasis

THOMAS P. MONSON† AND KATHERINE P. WILKINSON

Veterans Administration Medical Center and Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72206

Received 2 March 1981/Accepted 12 June 1981

Using gas-liquid chromatography, we measured free mannose in the serum of six nondiabetic patients with autopsy-proven invasive candidiasis. In all patients serum mannose concentrations were higher than mannose levels found in serum from normal adults and children or from patients with catheter-associated candidemia, mucosal candidiasis, and other mycoses. Spinal fluid from two patients with Candida meningitis showed increased free mannose as compared to seven non-inflammatory spinal fluid samples. However, free mannose in the serum of poorly controlled diabetics (blood glucose of ≥300 mg/dl) did overlap concentrations in patients with invasive candidiasis. In vitro culture of Candida albicans demonstrated increasing concentrations of mannose associated with growth of the organism. We conclude that physical and chemical assay for mannose in body fluids may be a useful technique to assist in the diagnosis of invasive candidiasis.

Severe disease produced by Candida species has become an increasing problem during the last decade, although its association with compromised hosts has been recognized for well over a century (2). High-dosage antibiotic, steroid, and immunosuppressive therapy has significantly contributed to this development (12) and will likely continue to do so. Complicating recognition of this clinical problem is the proclivity of Candida to colonize or cause mild infection of the mucous membranes and skin. Thus, aggressive procedures are often required to obtain a definite diagnosis of invasive candidiasis.

Serological techniques involving Candida antibody detection have been evaluated as diagnostic aids, but the results and the interpretations have been conflicting (4, 5). Serological procedures for detection of circulating microbial polysaccharides have been shown to be clinically useful in several settings (7, 17). Studies utilizing gas-liquid chromatography have suggested that patients with invasive candidiasis may be distinguished by having elevated serum concentrations of D-arabinitol (10, 18) and that increased serum mannose concentrations are present in patients with candidemia (14). In an attempt to confirm the association of elevated serum mannose concentrations with invasive candidiasis, we have used gas-liquid chromatography to measure the concentration of free or unbound body fluid mannose in control populations and in patients with different types of Candida infection. We have also demonstrated the in vitro production of free mannose by culture of Candida albicans.

(These findings were presented in part at the Southern Section meeting of the American Federation for Clinical Research, New Orleans, La., 26 January 1978.)

MATERIALS AND METHODS

Sera from the following subjects were studied: 121 adult male and female blood donors and 23 normal children aged 1 to 19 years served as normal controls; 2 subjects had intravenous catheter-associated candidemia; 42 had mucosal vaginal candidiasis; 6 had systemic candidiasis; 2 subjects had blood cultures and lung aspirate positive for Candida tropicalis, respectively; 7 had untreated non-Candida mycotic infections; 4 patients had disseminated breast carcinomas; 11 patients were poorly controlled diabetics; and 33 were miscellaneous intensive care unit patients. Cerebrospinal fluid (CSF) from six children and one adult, obtained for the purpose of excluding meningitis but showing normal parameters, was also evaluated.

The diagnosis of invasive candidiasis was based on histological evidence of infection with yeastlike organisms resembling Candida in autopsy or biopsy tissue, other than skin or mucosal surfaces (six cases). Catheter-associated candidemia was diagnosed on the basis of fever with positive blood cultures, which promptly remitted in both patients with removal of the catheter. In one case, the catheter was cultured and grew C. albicans. The diagnosis of vaginal candidiasis was based on typical clinical presentation together with a KOH preparation of vaginal exudate demonstrating organisms consistent with Candida. Patients with blastomycosis (two), histoplasmosis (two), dissemi-
nated aspergillosis (one), and cryptococcal meningitis (two) were diagnosed after isolation of the organism from appropriate specimens.

An isolate of C. albicans originally obtained from a patient with systemic candidiasis was maintained in yeast phase on Sabouraud agar. For in vitro studies, the organism was inoculated into 1 liter of Eagle minimal essential medium which had been supplemented with nonessential amino acids (GIBCO, Grand Island, N.Y.). Subcultures and Gram stains were done to confirm purity of the culture. Samples of 10 ml were removed at 42, 48, 72, 96, and 120 h. With a Coleman model 9 nephelo colorimeter (Coleman Instruments, Maywood, Ill.) optical density of the broth culture was determined in duplicate. After determination of optical density, the organisms were separated from the supernatant by centrifugation at 7,000 x g for 30 min at 4°C; the supernatant was then passed through a 0.45-μm pore size membrane filter (Millipore Corp., Bedford, Mass.). The resulting filtrate was stored at −30°C before analysis by gas-liquid chromatography.

Unbound mannose was determined by gas-liquid chromatography of the aldononitrile derivative as described previously (15). A Hewlett-Packard gas-liquid chromatograph equipped with dual flame ionization detectors was used (Hewlett-Packard, Avondale, Pa.). Coiled 1.8-m glass columns (2 mm ID) were packed with 2% neo-pentyl-glycol-succinate on 80/100-mesh Chromosorb W (HP). With hydroxylamine hydrochloride and acetic anhydride with pyridine used as the solvent, the derivative was prepared, dried, and then reconstituted in trichloromethane with 3 μl injected onto the column. Assays were performed in duplicate. The area under the mannose curve was integrated and converted to micrograms of mannose per milliliters of serum by using a standard curve. Day-to-day reproducibilities of between 2 and 12% for mannose were attainable for quantities of 100 to 900 ng. The sensitivity was such that 10 mg/liter could be detected in a 0.1-ml sample of serum.

RESULTS

The in vitro production of mannose in broth culture of C. albicans is shown in Fig. 1. There was no measurable mannose at the time of culture inoculation, but mannose concentrations in the broth supernatant increased concomitantly with growth of the organism as represented by the optical density of the broth. The appearance of mannose was noted without significant transformation to the mycelial phase.

The mannose concentration for the different control groups is summarized in Fig. 2. Serum from normal blood donors (mean, 1.3 μg/ml), normal children (mean, 0.0 μg/ml), and patients with vaginal candidiasis (mean, 0.3 μg/ml) had low concentrations. Although we studied only two patients with catheter-associated candidemia (mean, 36.1 μg/ml), their values were readily distinguishable from patients with invasive candidiasis. Clinical characteristics, including renal function, of the patients with invasive candidi-
revealed *C. tropicalis* together with *Francisella tularensis*. Serum mannose at the time of aspiration was 32.5 μg/ml. The patient died shortly thereafter.

Concentrations of mannose found in the seven "normal" CSF specimens ranged from 0 to 10.5 μg/ml with a mean value of 3.1 μg/ml (data not shown). Two of our six patients had histological and cultural evidence for *C. albicans* meningitis. In one of these (patient no. 6) the serum concentration of free mannose was considerably less (49.4 μg/ml) than in our other five patients, while CSF mannose was modestly elevated. This may reflect the autopsy findings that only the meninges were involved with the *Candida* infection. Patient no. 4, our other patient with central nervous system candidiasis but with higher serum and CSF mannose values than no. 6, had grossly more extensive central nervous system involvement with multiple *Candida* brain abscesses in addition to meningitis.

Serum from patients with non-*Candida* mycoses showed mannose values that were less than those seen with systemic candidiasis (mean, 16.8 μg/ml).

Evidence has been presented showing increased concentrations of total blood mannose (including glycoproteins) in patients with breast carcinoma (16). We measured free serum mannose in four patients with metastatic breast carcinoma and found concentrations to be only slightly above those of normal blood donors (mean, 8.2 μg/ml).

Sera were obtained from 32 consecutive patients admitted to the Veterans Administration Medical Center intensive care unit. This group was comprised primarily of patients having ischemic heart disease, renal insufficiency, and respiratory failure with or without pulmonary infection. These patients were receiving a variety of pharmacological agents including anti-arrhythmic drugs and antimicrobial agents. All four patients with renal insufficiency had mannose concentrations less than 20 μg/ml and did

### Table: Serum Mannose Concentrations

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Mannose Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Invasive candidiasis</td>
<td>32.5</td>
</tr>
<tr>
<td>2. Normal children</td>
<td>3.1 (N=11)</td>
</tr>
<tr>
<td>4. Mucosal candidiasis</td>
<td>3.1 (N=11)</td>
</tr>
<tr>
<td>5. Catheter candidiasis</td>
<td>3.1 (N=11)</td>
</tr>
<tr>
<td>6. Intensive care unit patients</td>
<td>3.1 (N=11)</td>
</tr>
<tr>
<td>7. Breast cancer patients</td>
<td>3.1 (N=11)</td>
</tr>
<tr>
<td>8. Other fungal infections</td>
<td>3.1 (N=11)</td>
</tr>
<tr>
<td>9. Ketoacidosis patients</td>
<td>3.1 (N=11)</td>
</tr>
</tbody>
</table>

### Figure 2: Serum Mannose Concentrations

Fig. 2. Serum mannose concentrations in patients with invasive candidiasis and in control groups. 1, Invasive candidiasis; 2, normal children; 3, blood donors; 4, mucosal candidiasis; 5, catheter candidiasis; 6, intensive care unit patients; 7, breast cancer patients; 8, other fungal infections; 9, ketoacidosis patients.
### Table 1. Summary of clinical, microbiological, and pathological data in patients with invasive candidiasis

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Sex</th>
<th>Underlying conditions</th>
<th>Predisposing therapy*</th>
<th>C. albicans isolation</th>
<th>Renal function with matching mannose value</th>
<th>Organs involved at autopsy</th>
<th>Range of mannose concn (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>M</td>
<td>Cushing's syndrome</td>
<td>A, S</td>
<td>+ + +</td>
<td>NA/27</td>
<td>Lung, bowel, thyroid, myocardium</td>
<td>Serum, 29.8–82.3</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>M</td>
<td>Leukemia</td>
<td>A, C</td>
<td>+</td>
<td>13/0.7</td>
<td>Liver, spleen, lymph nodes, small bowel</td>
<td>Serum, 77.4</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>M</td>
<td>Leukemia</td>
<td></td>
<td>+</td>
<td>25/1.6</td>
<td>Lung, spleen, prostate</td>
<td>Serum, 116.8</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>F</td>
<td>Leukemia</td>
<td>A, C</td>
<td>+ + +</td>
<td>123/2.0</td>
<td>Brain, liver, kidney, spleen, heart</td>
<td>Serum, 296.3; CSF, 489.7</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>M</td>
<td>Renal failure</td>
<td>S</td>
<td>Peritoneal; CSF</td>
<td>94/6.5</td>
<td>Lung</td>
<td>Serum, 49.3–85.4</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>M</td>
<td>Myeloma</td>
<td>A, C</td>
<td>CSF</td>
<td>51/2.3</td>
<td>Meninges</td>
<td>Serum, 49.4; CSF, 33.8–43.2</td>
</tr>
</tbody>
</table>

* A, Antibiotics; S, corticosteroids; C, cytotoxic chemotherapy.
* BUN, Blood urea nitrogen; Cr, creatine clearance. NA, Not available.
not overlap with patients having systemic candidiasis. With the exception of one patient in diabetic ketoacidosis, the intensive care patients had a mean maximum mannose concentration of 13.4 μg/ml of serum. Because the patient in ketoacidosis was found to have a serum mannose concentration of 46 μg/ml, we carried out a preliminary evaluation of serum from 30 diabetic patients. Only in poorly controlled diabetic patients (glucose of ≥300 mg/dl) were mannose concentrations found which overlapped values found in candidiasis patients. Hemoglobin A1C values were not available for these patients. None of our patients with invasive candidiasis was known to be diabetic.

DISCUSSION

The presence of circulating Candida antigen was first reported by Axelsen and Kirkpatrick in a patient with chronic mucocutaneous candidiasis (1). Using anti-Candida antibodies coupled to agarose, Fischer et al. (6) reported the detection of a Candida polysaccharide from the sera of 6 of 23 children with chronic mucocutaneous candidiasis. Miller and co-workers (14) noted a unique gas chromatograph "fingerprint" in patients with blood cultures positive for C. albicans. They suggested that the unique peaks were derivatives of mannose, consistent with the high percentage of mannose-containing compounds known to be present in the cell wall of C. albicans (3). Kiehn and colleagues (10) have used gas chromatography to describe D-arabinitol in the sera of patients with invasive candidiasis. They found 15 of 20 patients to have concentrations greater than 1 μg/ml, although patients with renal insufficiency and colonization with Candida could have elevated values. Roboz and co-workers reported similar results, detecting concentrations of arabinitol of ≥1.2 μg/ml in 9 of 11 sera from patients diagnosed as having invasive candidiasis (18).

Other workers (13, 23) have used hemagglutination inhibition to demonstrate the presence of circulating mannans in 30 to 60% of patients with invasive candidiasis. The enzyme-linked immunosorbent assay and radioimmunoassay have been used to demonstrate Candida antigen in human serum. Warren et al. reported that blastospore antigens could be detected in the sera of two patients with invasive candidiasis (21), and Stevens et al. reported 12 of 19 patients to have measurable levels of a soluble cytoplasmic protein antigen (20). Segal and colleagues used an enzyme-linked immunosorbent assay-inhibition technique to assay for mannan antigen in seven patients with evidence of candidiasis at autopsy (19). Weiner and Coats-Stephens have developed a radioimmunoassay for Candida mannan and detected antigenemia in 6 of 14 patients with invasive candidiasis (22).

Kerkering and co-workers have demonstrated a Candida polysaccharide antigen in eight patients with invasive disease by using counter-immunoelectrophoresis (9), whereas Lehmann and Reiss were unable to detect Candida peptidoglucomannan in six patients with systemic candidiasis by using immunodiffusion, counter-immunoelectrophoresis, and enzyme-linked immunosorbent assay (11).

We present data that quantitation of free mannose identified all six patients with invasive candidiasis. Five of the six tissue-proven cases had markedly elevated serum concentrations, and two patients had increased CSF concentrations. Although a larger variety and number of CSF specimens must be evaluated, our present data suggest that increased free CSF mannose concentrations may accompany Candida involvement of the central nervous system.

Of the two patients with less definite evidence for invasive candidiasis, one had elevated concentrations of mannose which decreased with amphotericin B therapy and the return of peripheral neutrophils. This is consistent with the known antigenic similarity of C. tropicalis and C. albicans (8).

The mannose assay also qualitatively distinguished patients with mucosal infection and catheter colonization from those with invasive disease. It should be noted, however, that only two patients with catheter candidiasis were evaluated. Patients with renal insufficiency did not have mannose values overlapping those of patients with candidiasis. Likewise, there was no correlation between mannose concentration and renal insufficiency in the patients described in Table 1. Thus far, poorly controlled diabetics are the only patients who have been found to have elevated mannose concentrations similar to those of patients with systemic candidiasis. No evidence of infection with Candida or other fungi could be found in these diabetic patients.

As a physicochemical assay, our method avoids some problems inherent with immunoassays for antigen. Wheat and co-workers (24) have recently reported their inability to consistently demonstrate antigenemia in patients with staphylococcal endocarditis by using a radioimmunoassay, presumably a result of preexisting antibodies forming immune complexes. Preexisting antibodies to Candida sp. might have a similar effect on sensitivity of immunoassays for Candida antigen, although acid-heat extraction of serum before assay may improve sensitivity.
A physicochemical assay such as we have described measures a relatively simple compound thought to derive from the Candida cell wall, whereas immunoassays measure more complex antigens. Thus, specificity is often less than immunoassays, and precautions must be taken to remove unwanted sources of antigen (in this case, mannose) during sample preparation. Even then, some degree of overlap with other patient populations may not be avoidable, as with our poorly controlled diabetics.

Our data suggest that further effort is justified in developing physicochemical assays for microbial products. Such procedures might represent a supplementary or alternative method for existing techniques of antigen detection.

ACKNOWLEDGMENT

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LITERATURE CITED