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Detection of the *Candida* Antigen Mannan in Cerebrospinal Fluid Specimens from Patients Suspected of Having *Candida* Meningitis

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Cerebrospinal fluid samples from five patients from which *Candida* cells were cultured were tested for the presence of mannan. Samples from four patients categorized as having proven candidosis reacted positively. Samples from the remaining patient and from patients with other central nervous system infections were negative. Detection of mannan may be valuable in the diagnosis of *Candida* meningitis.

Meningitis caused by *Candida* infection is a rare and difficult-to-diagnose infectious disease. A substantial proportion is found among very-low-birth-weight neonates (13), but *Candida* meningitis has been found also in human immunodeficiency virus-infected patients (8, 30) and patients with ventricular shunts or after lumbar puncture (5, 31). The most frequent symptoms are headache, photophobia, nuchal rigidity, and delirium, but an indolent course is also possible. The cerebrospinal fluid (CSF) usually shows a pleocytosis predominated by neutrophils and mononuclear cells, an elevated protein concentration, and a normal or low glucose concentration. The sensitivity of CSF cultures is low because the number of fungal cells in the CSF is small. Therefore, large volumes, preferably more than 5 ml, should be cultured (23). A delay in diagnosis and subsequent treatment is considered to be associated with a poor prognosis (4). Additionally, the significance of a positive culture from the CSF may be unclear. Contamination of the CSF sample may occur because of colonization of the skin or when cultures have been taken from external reservoirs that contain CSF. Several non-culture-based methods have been developed for diagnosing invasive fungal infections of the CNS, such as cryptococcal meningitis (10, 16, 18) and CNS aspergillosis (32, 33). Similarly, a *Candida* cell wall component, mannan, has been used as a target for serological tests. Although the detection of circulating mannan was found to be of limited value in the diagnosis of invasive candidosis, detection of mannan in CSF could be a valuable tool for diagnosing CNS candidosis. Here, we report on five patients who were treated for CNS candidosis and for whom we evaluated the diagnostic value of mannan antigen detection in CSF.

Case 1. A 4-year-old girl was admitted to our hospital because of a relapse of acute nonlymphatic leukemia for which she had been treated with chemotherapy in the previous 6 months. Treatment with fluconazole was started because ultrasound examination of the abdomen showed dense lesions in the liver that raised suspicion of chronic disseminated candidosis and *Candida albicans* was cultured from CSF samples. More CSF samples were taken, and all of them grew *C. albicans*. Because of a clinical and microbiological failure of fluconazole, therapy was switched to amphotericin B lipid complex. The fever initially resolved but later recurred. Other CSF samples, collected during amphotericin B lipid complex therapy, again grew *C. albicans*. Eventually, she died of hypovolemic shock due to a hemorrhage. Autopsy was not permitted.

Case 2. A 60-year-old male was admitted to our hospital because of an organic psychosyndrome. No signs of meningism were present. He was treated outside The Netherlands with prednisolone and immuran for suspected lupus erythematosus. In the preceding months, the course of his disease was complicated by a bowel ischemia that required extensive surgery, abdominal abscesses, and a *Candida* endophthalmitis of his left eye, for which a vitrectomy was performed and oral fluconazole was started. Culture of a CSF sample yielded *C. albicans*. Treatment with fluconazole and flucytosine was started. Repeated CSF cultures still grew *C. albicans*; therefore, fluconazole was replaced with amphotericin B. After an additional 2 weeks of treatment, the patient was discharged while still showing signs of an organic psychosyndrome. Cultures of CSF samples collected in the course of his treatment remained sterile, but chemistry still was suggestive of meningitis. Eventually, he was lost to follow-up.

Case 3. A 48-year-old male with dermatomyositis and treated with prednisone and immuran, was readmitted to the hospital with fever, dysarthria, persistent uveitis, and headache. Culture of a CSF sample yielded *C. albicans*, *C. parapsilosis*, and *C. guilliermondii*. Repeat cultures were positive for *C. albicans* and *C. parapsilosis*. Treatment with fluconazole was started. The patient was discharged from the hospital while undergoing oral fluconazole therapy, but he had to be readmitted several weeks later with a clinical relapse of meningitis. Despite sterile CSF cultures, chemical analysis was suggestive of infection. Fluconazole was replaced with amphotericin B and flucytosine for 2 weeks, which resulted in a clinical im-

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TABLE 1. Results of detection of *Candida* mannan in CSF compared with culture

Case no. or group	Classification[a]	Culture result[b] (no. positive/total)	Mean OD (range)	Interpretation[c] (no. positive/total)
1	Proven infection	4/4	2.999[d]	3/3
2	Proven infection	2/5	0.571 (0.489–0.779)	7/7
3	Proven infection	3/9	1.721 (1.057–2.935)	4/4
4	Proven infection	1/2	3.500[d]	2/2
5	No infection	1/6	0.297 (0.259–0.331)	0/3
A[e]	Control	0/28	0.264 (0.211–0.365)	0/28
B	Control	10/10	0.163 (0.112–0.228)	0/10
C	Control	0/16	0.211 (0.160–0.270)	0/16

[a] Based on criteria adapted from reference 3.
[b] Culture for yeasts.
[c] Cutoff value = 0.440.
[d] ODs out of range.
[e] Control group: A, patients with clinically suspected bacterial meningitis and negative fungal cultures; B, patients with cultures positive for *C. neoformans* and CSF positive for cryptococcal antigen; C, patients CSF positive for *Aspergillus* antigen.

provement and a decrease in CSF neutrophils. The patient was discharged while undergoing therapy with oral fluconazole combined with flucytosine and remained free of clinical symptoms.

Case 4. A dysmature and premature female neonate (birth weight, 700 g; born at 26 weeks of gestation) was admitted to the neonatal intensive care unit. She became septic on day 4; therefore, antibiotic therapy was started. A sepsis workup was performed that included a lumbar puncture on day 11. Culture of the CSF sample obtained showed *C. albicans*. Intravenous fluconazole was started. Three days later, she deteriorated; therefore, fluconazole was replaced with liposomal amphotericin B. Microscopy of CSF showed yeast cells, but cultures remained sterile. Gradually, the clinical condition improved. Eventually, she could be transferred to a pediatric ward in stable condition.

Case 5. A 76-year-old female was admitted because of a relapse of a retroauricular basal cell carcinoma. The tumor was surgically removed, and an intraspinal catheter was placed in order to facilitate monitoring of the pressure in the subarachnoid space. Postoperatively, the patient appeared to have a left-sided hemiparesis, a facialis paresis, and dysarthria but no signs of meningism. A culture of CSF collected shortly after surgery remained sterile. A second postoperative CSF sample grew *C. albicans*. Chemical analysis showed no evidence of infection besides an elevated protein level. Repeat CSF collection showed a decrease in the protein level, and cultures were sterile. Her neurological deficits were already improving. Nevertheless, treatment with fluconazole was started. Cultures of two additional CSF samples remained sterile. After 14 days of treatment, she was transferred to a nursing home. She died several months later because of complications of local tumor growth. Retrospectively, the positive CSF culture was considered to be contaminated.

CSF samples were processed at the microbiology laboratory within 8 h of collection. They were centrifuged at 10,000 × *g* for 10 min. The sediment was stained with Gram's stain, methylene blue, and calcofluor white or Uvitex 2B for direct microscopy. In addition to bacterial cultures, the CSF sediment was plated onto Sabouraud glucose medium, inoculated into Sabouraud broth, and incubated at 30°C for 3 weeks. Blood samples were cultured by using the BacTec system (Becton Dickinson, Cockeysville, Md.). To test the specificity of the mannan detection in CSF, three control groups were used. Group A included 28 CSF samples from patients with clinically suspected bacterial meningitis and negative fungal cultures. Group B included 10 CSF samples from seven patients with culture-proven cryptococcal meningitis and a positive cryptococcal antigen test result. Group C included 16 CSF samples from 10 patients with CNS aspergillosis that were positive for *Aspergillus* antigen.

Mannan detection. Serum and CSF samples were stored at −80°C before use. Detection of mannan was performed with a commercial enzyme-linked immunosorbent assay (ELISA) (Platelia Candida; Bio-Rad, Marnes-La-Coquette, France) in accordance with the manufacturer's instructions. The mean optical density (OD) and standard deviation were calculated for the 28 control CSF samples in group A. The cutoff value for a positive CSF sample was defined as the mean OD plus five times the standard deviation. For serum, a standard curve was constructed by using standard sera with 0.25, 0.5, 1.0, and 2.0 ng/ml. The OD was interpreted as follows: <0.25 ng/ml was considered negative, ≥0.25 and <0.5 ng/ml was considered intermediate, and >0.5 ng/ml was considered positive.

***Aspergillus* antigen detection.** A commercial sandwich ELISA (Platelia Aspergillus) was used to detect the *Aspergillus* antigen galactomannan. ODs were measured at 450 and 625 nm. The ratio of the ODs of the sample mixture and the weakly positive sample were calculated for each sample, and a ratio larger than 0.6 was considered positive, as has been described previously (32).

The results of culture and mannan detection in the CSF samples are shown in Table 1. Of CSF samples from control patients with bacterial meningitis (group A), six were culture positive (*Pseudomonas aeruginosa*, *Neisseria meningitidis*, *Staphylococcus epidermidis*, *S. aureus*, an unspecified gram-positive rod, and a gram-negative rod). *Cryptococcus* antigen was detected in all 10 CSF samples from patients with *Cryptococcus* meningitis (group B), and *Aspergillus* antigen was detected in all 16 samples from patients with CNS aspergillosis (group C). However, none of these control samples showed reactivity with the mannan ELISA, indicating absence of cross-reactivity of the mannan ELISA with *Cryptococcus* and *Aspergillus* antigen or with other putatively present microbial antigens (Table 1).

Mannan was detected in the CSF in all four cases of proven CNS candidosis. In the fifth patient, CNS candidosis was unlikely and mannan was not detected.

Mannan or mannoprotein is the immunodominant surface antigen of *C. albicans* serotypes A and B. Although this antigen is known to circulate in blood during systemic infection (22), the results of antigen detection in serum have been variable, with sensitivities ranging from 0 to 100% and specificities ranging from 88 to 100%, depending on the patient category tested, the type of assay used, and the number of samples collected (3, 7, 9, 17, 20, 24–26, 28, 29, 34). To our knowledge, detection of mannan in CSF has been used once before in the diagnosis and follow-up of treatment of *Candida* meningitis (14). Antigen was detected in CSF samples from four of the five patients described in this report. These patients can be categorized as having proven invasive *Candida* infection, according to consensus definitions (2). For yeast infections, these criteria probably can also be applied to patient categories other than cancer and hematological malignancies, as described in cases 2 to 5. Although our patients probably acquired meningitis hematogenously, not a single blood culture grew yeast. In the first two patients, circulating antigen was not detected in blood at the time of mannan detection in the CSF. Although the kinetics of mannan in CSF are unknown, we think that leakage of mannan from the circulation to the CSF is unlikely on the basis of these observations.

Only one of a series of five CSF samples from the fifth patient showed *C. albicans* in culture, yet chemical analysis showed improvement compared with the analysis performed on the first sample. Similar patterns have been observed in cases with shunts or other devices in the subarachnoid space, in which there appeared to be no meningitis and in which only a single CSF sample was positive for yeasts (11). An explanation for the repeatedly negative results of the ELISA performed on the CSF samples of this patient thus could be that there was no meningitis but a contamination of the single CSF sample that grew *C. albicans*. Therefore, these results suggest that the detection of mannan in CSF may help to differentiate between *Candida* meningitis and contamination. This is supported by the observed high specificity (100%) of the test used here. The mannan ELISA consequently showed no reactivity with samples from patients with infections of the CNS caused by bacteria or *Cryptococcus* or *Aspergillus* spp.

Detection of fungal antigens in CSF has been described for cryptococcal infections of the CNS (21) and has value in monitoring the response to treatment of patients with AIDS-associated cryptococcal meningitis (27). Previously, our group described a case of *Aspergillus* meningitis in which the *Aspergillus* antigen galactomannan was detected 45 days before culture became positive and the titer declined when effective therapy was instituted (32). Likewise, detection and monitoring of mannan in CSF could be useful in *Candida* meningitis, although the value of monitoring mannan levels in relation to clinical response to treatment remains unclear since the number of samples tested was too limited to draw conclusions. One important issue is whether detection of mannan can also be used to diagnose CNS infections caused by non-*C. albicans* species. Although *C. albicans* is one of the most frequently isolated species in CNS infections caused by *Candida* spp. (4), other species, like *C. tropicalis*, (12), *C. lusitaniae* (19), *C. parapsilosis* (6), and *C. glabrata* (1), have also been encountered. Although the antimannan antibody EBCA-1, which is used in the ELISA, is directed against an epitope derived from *C. albicans* mannan, this epitope has also been described in other *Candida* species like *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* (15). ELISA reactivity with mannan derived from these species may therefore be expected. However, the lower limit of detection and therefore the sensitivity may be different for each species, which already has been observed in another ELISA (25).

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