Diagnosis of cryptococcal meningitis by cytologic methods: An old technique revisited

Article abstract—In six patients with cryptococcal meningitis, diagnosis was made by routine cytologic examination of cerebrospinal fluid (CSF). A seventh patient had a false-positive cryptococcal antigen titer, and no organism was seen on CSF examination. That patient had herpes simplex encephalitis on brain biopsy. Cytologic examination is recommended whenever cryptococcal meningitis is suspected.

NEUROLOGY 30: 102-105, January 1980

P.C. Bernad, M.D., W.M. Szyfelbein, M.D., H.D. Weiss, M.D., and E.P. Richardson, Jr., M.D.

Meningeal infection with *Cryptococcus neoformans* can now be treated successfully.[1-c] Prompt, accurate diagnosis is therefore imperative. The classical method of identifying the organism in cerebrospinal fluid (CSF) is India-ink preparation. This method is simple but not always reliable. Only a 50% positive yield can be expected, and false-positive diagnoses are frequent.[2,3] Serologic assay for the detection of cryptococcal antigen and antibody has increased the accuracy of diagnosis,[4] but false-positive results occur with aged antisera and in the presence of rheumatoid factor.[5] Culture of the organism may take up to 1 week. Our experience has indicated that routine cytologic examination of CSF can quickly and reliably provide the diagnosis.

Methods. The records at the Massachusetts General Hospital were reviewed from 1971 to 1979 in order to find unequivocal cases of cryptococcal meningitis. The cytologic examinations were performed on six patients in the following manner: A 3- to 4-ml sample of CSF was filtered by gravity, without suction, through a cellulose acetate polymerized membrane-filter with 5-μm perforations (Millipore Corporation, Bedford, MA). In some instances the test tube was additionally rinsed with a few milliliters of physiologic saline solution to obtain the last remaining cells in the specimen. The filter with the adhering cells was fixed for 2 minutes in 95% ethanol and then stained by the Papanicolaou method.[7] Occasionally, supplemental staining with mucicarmine demonstrated the capsular polysaccharide of the organisms more clearly. Other special stains such as periodic acid-Schiff (PAS) and Grocott's silver methenamine were also used.[8,9] Mixing the CSF sample with an equal volume of 50% ethanol made it possible to preserve the sample in case of an anticipated delay of some hours between the time of collection of CSF and the time of actual examination.

Results. *Cryptococcus neoformans* was found on cytologic preparation collected from six patients (figures 1 and 2). In five of these patients, the initial cytology report failed to note any organism, primarily because the laboratory was not alerted to the possibility of *Cryptococcus neoformans* infection and concentrated on looking for malignant cells. The seventh patient, although looking clinically as if he may have had cryptococcal meningitis and having positive serology, failed to demonstrate any cryptococci on multiple cytologic preparations. A subsequent serology on his CSF was negative; the error was attributed to aged antisera. A subsequent brain biopsy established the diagnosis of herpes simplex encephalitis. In cases of verified cryptococcal meningitis, the organisms were scattered and appeared as single spheroids ranging from 2 to 15 μm in diameter. The presence of clear halos around the cytoplasmic membrane gave the impression of a double capsule. Rare budding was also observed.

The clinical cytologic correlations and treatment as well as the follow-up are summarized in the table. In two of the six patients, the cytologic preparations revealed the microorganisms before clinical relapse was evident. Treatment with amphotericin B or 5-fluorocytosine eradicated the fungi from the CSF. Postmortem examination in the two patients who died showed the organisms in the pia-arachnoid.

Discussion. Although our experience indicates that routine Millipore filter preparation of CSF stained with the Papanicolaou method is sufficient for accurate recognition of *Cryptococcus neoformans*, there are other valid methods such as the newer technique of cytocentrifuged smear and the older technique of sedimentation. Some laboratories prefer only a formalin fixative with plain hematoxylin stain, and they obtain equally good results.

Patients with malignant reticuloendothelial neoplasms or patients receiving immunosuppressive medications are susceptible to cryptococcal infection.[10] As increasing numbers of patients are being treated successfully for malignant disease or kept alive after organ transplantation, the chances of infections of the central nervous system increase. Early diagnosis of infection is imperative so that available treatment may be started as soon as possible. In addition to its reliability and ease of

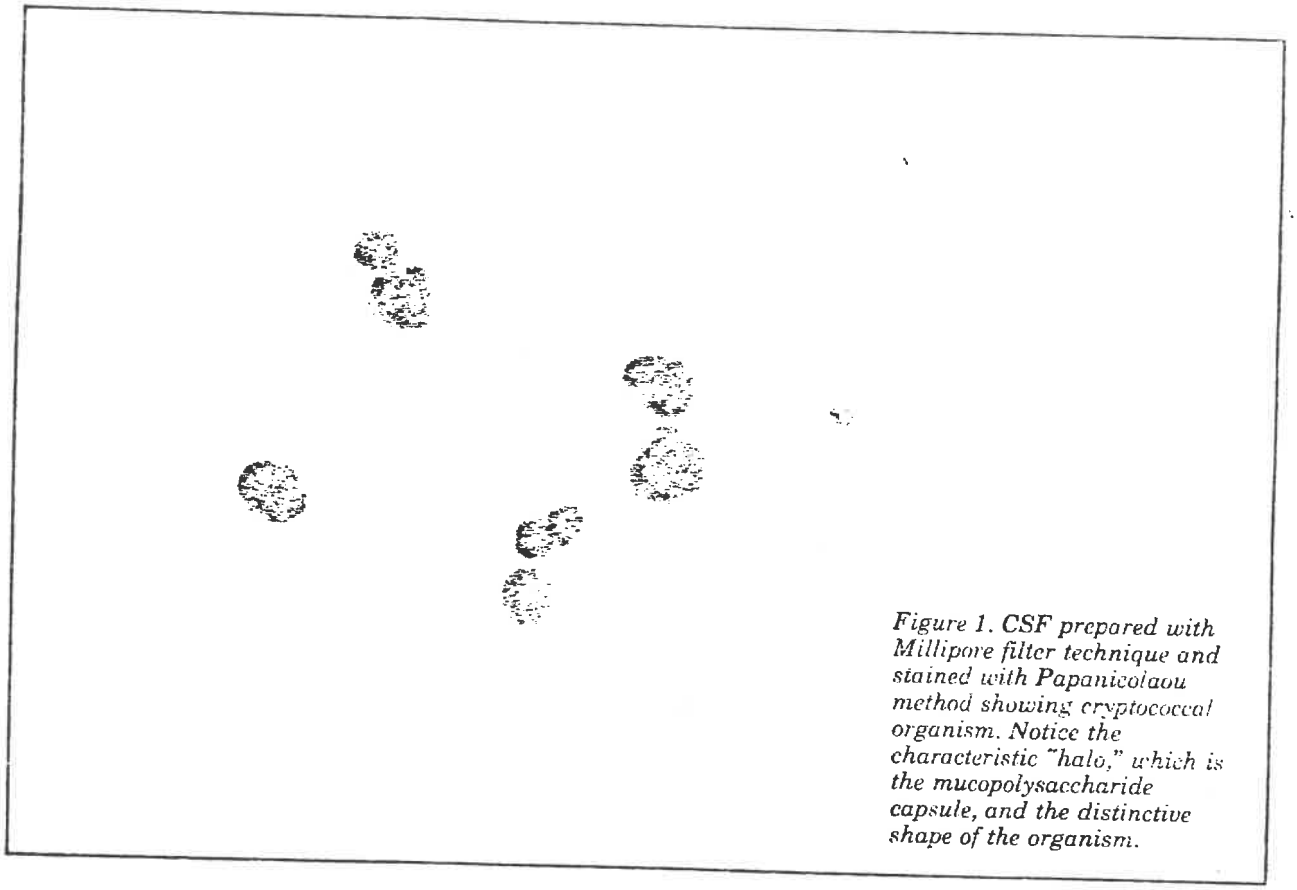

Figure 1. CSF prepared with Millipore filter technique and stained with Papanicolaou method showing cryptococcal organism. Notice the characteristic "halo," which is the mucopolysaccharide capsule, and the distinctive shape of the organism.

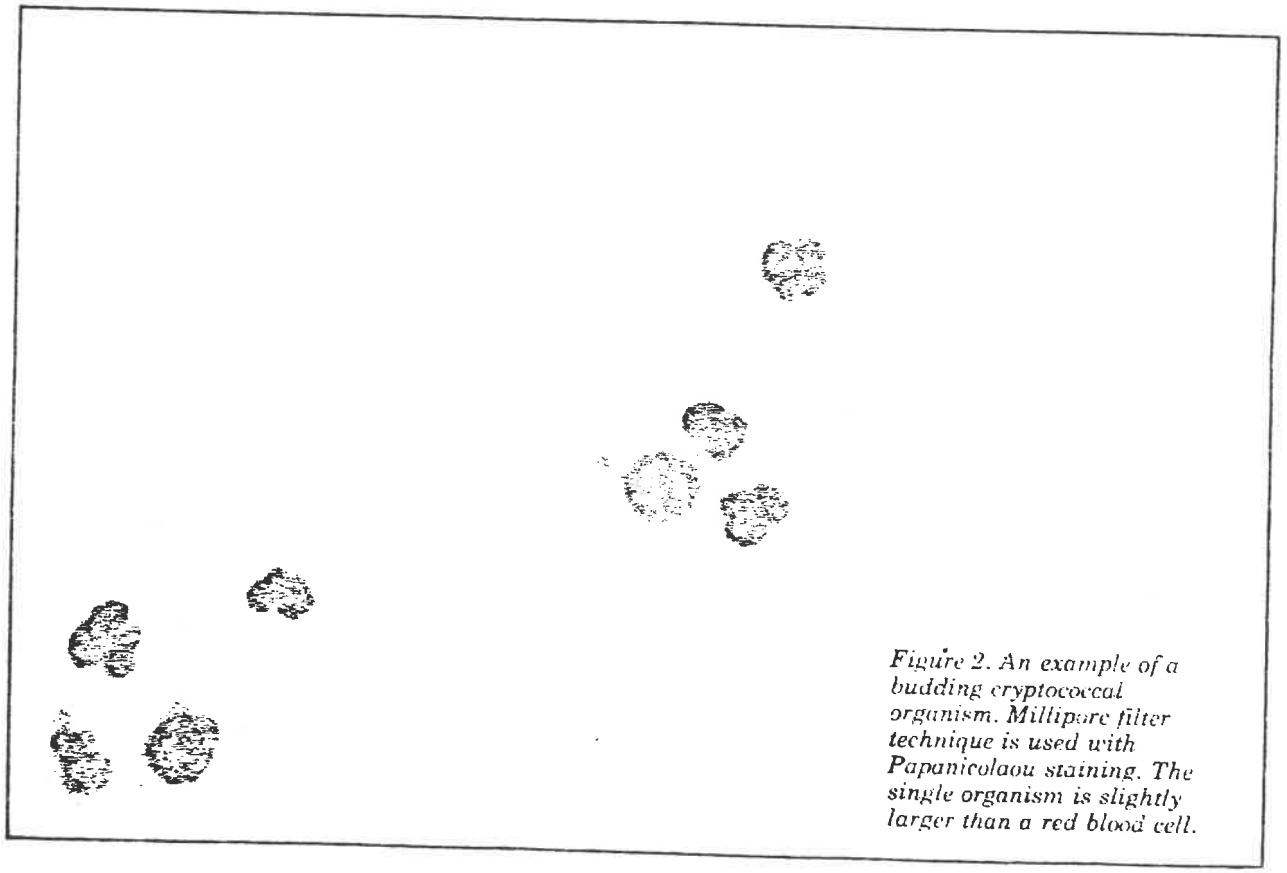

Figure 2. An example of a budding cryptococcal organism. Millipore filter technique is used with Papanicolaou staining. The single organism is slightly larger than a red blood cell.

Table. Clinical-cytologic correlations in cryptococcal meningitis

Patient	Age	Sex	Associated condition	Prior treatment	Summary of cases: Culture	Titer	Cytology	Treatment	Follow-up
1	20	M	None	None	+	1:64	+	Amphotericin B 5-Fluorocytosine	4.6 years No recurrence
2	56	M	None	None	+	1:16	+	Amphotericin B	2 years No recurrence
3	15	F	Kidney transplant Roussy-Levy syndrome	Azathioprine Prednisone Actinomycin D	+	Not reported	+	Amphotericin B	2.4 years No recurrence
4	73	F	Chronic lymphocytic leukemia Adenocarcinoma of sigmoid	Splenectomy	+	1:512	+	Amphotericin B	1.6 years No recurrence
5	32	M	Hodgkin's disease	Nitrogen mustard Vincristine Procarbazine Prednisone	+	Not done	+	Amphotericin B	Died; autopsy confirmed diagnoses
6	61	M	Lymphocytic lymphoma Progressive multifocal leukoencephalopathy	Cyclophosphamide Vincristine	+	1:4096	+	Amphotericin B 5-Fluorocytosine	Died; autopsy confirmed diagnoses
7	30	M	None	None	−	False + (not reported)	−	Amphotericin B (2 days adenine arabinoside)	At 2 years, found to have herpes simplex encephalitis; recovered

use, cytologic examination is of value in the follow-up of patients under treatment.[11-14]

Cytologic examination of CSF in suspected cryptococcal meningitis has the advantage of providing the most rapid and least expensive method of establishing the diagnosis. We advise its regular use in any clinical situation where an infection of this kind is a possibility.

Addendum. Since submitting the manuscript, we have seen a 39-year-old woman with cryptococcal meningitis confirmed by cytology, serology, and culture. The patient's primary diseases were angioimmunoblastic lymphadenopathy and Sjögren syndrome.

From the James Homer Wright Pathology Laboratories of the Massachusetts General Hospital and the Department of Pathology and Neurology-Neuropathology, Harvard Medical School, Boston.

Accepted for publication May 3, 1979.

Address reprint requests to Dr. Bernad, Medical Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, Bldg 10, Room 10D18, Bethesda, MD 20205.

References

1. Spickard A, Butler WT, Andole V, et al: The improved prognosis of cryptococcal meningitis with amphotericin B therapy. Ann Intern Med 58:66-83, 1963

2. Butler WT, Alling DW, Spickard A, et al: Diagnostic and prognostic value of clinical and laboratory findings in cryptococcal meningitis. N Engl J Med 270:59-67, 1964
3. Kunz LJ: Diagnosis of cryptococcal meningitis. N Engl J Med 285:1488, 1971
4. Goodman JS, Kaufman L, Koenig MG: Diagnosis of cryptococcal meningitis. N Engl J Med 285:434-435, 1971
5. Bennett JE, Bailey JW: Control for rheumatoid factor in the latex test for cryptococcosis. Am J Clin Pathol 56:360-365, 1971
6. Bennett JE: Cryptococcosis. In Hoeprich P (Editor): Infectious Diseases. Ed 2. New York, Harper & Row Publishers, Inc, 1977
7. Oehmichen M: Cerebrospinal Fluid Cytology. Philadelphia, WB Saunders Company, 1976
8. Luna LG (Editor): Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. Ed 3. New York, McGraw-Hill Book Company, 1960
9. Saigo P, Rosen PP, Kaplan MH, et al: Identification of *Cryptococcus neoformans* in cytologic preparation of cerebrospinal fluid. Am J Clin Pathol 67:141-145, 1977
10. Kaplan MH, Rosen PP, Armstrong D: Cryptococcosis in a cancer hospital. Cancer 39:2265-2274, 1977
11. Jameson B, Wells DG: Cytologic diagnosis of cryptococcal meningitis. N Engl J Med 286:1267, 1972
12. Jequier M, Dufresne JJ: Diagnosis of cryptococcal meningitis. N Engl J Med 286:785-786, 1972
13. Dufresne JJ: Cytologie pratique du liquide cephalorachidien. Documenta Geigy (Basle). 1972
14. Tourtellotte WW, Itabashi H, Tucker RP, et al: Cerebrospinal fluid cytology. Trans Am Neurol Assoc 93:288-299, 1968

Multiple sclerosis and narcolepsy/cataplexy in a monozygotic twin

Article abstract—Symptoms of narcolepsy/cataplexy developed in a monozygotic twin at the age of 56 years, 25 years after the onset of multiple sclerosis. The diagnosis of narcolepsy/cataplexy was confirmed by polygraphic recordings demonstrating sleep-onset periods of rapid eye movements (REM), increase in REM time per 24 hours, and disturbed nocturnal sleep. Frequent cataplectic attacks were almost completely controlled by clomipramine. These symptoms may constitute one of the paroxysmal syndromes in multiple sclerosis. The discordancy for multiple sclerosis is attributed to a submaximal risk factor in the HLA system and a strong environmental factor in only one of the twins.

NEUROLOGY 30: 105-108, **January 1980**

Harald Schrader, M.D., Ole B. Gotlibsen, Tech. EEG, and Gunstein N. Skomedal, M.D.

Narcolepsy is rare in multiple sclerosis (MS).[1] Of the few reported cases, some fulfill criteria for unequivocal diagnosis of MS and narcolepsy/cataplexy in the same patient.[2-4] In two of these documented cases[4] narcolepsy appeared after the onset of MS, suggesting a causal relationship. In no case, however, has there been confirmation of narcolepsy by polygraphic recordings.

We now describe such a case.

Case report. This woman was born after an uneventful pregnancy, the last of 12 siblings and the second-born of monozygotic twins. She was well until age 31, when her right hand became clumsy and her gait unsteady. Three weeks later the right hand was weak and for 2 weeks she had decreased visual acuity, scintillations, and impaired color vision. Her condition improved to some extent during the next weeks. She was examined 4 months after onset. Visual acuity and fields, pupillary light reflexes, and funduscopy were all normal. She was slightly dysarthric. There was nystagmus on gaze to the left and a few nystagmoid jerks on gaze to the right. There was slight weakness of the right arm and leg with hypesthesia in the hand, trunk, and leg on the right, with sensory level at the costal arch and the middle of her thigh. Position sense was impaired in the right toes. Stereognosis was impaired in both hands. Stretch reflexes were brisker on the left. Plantar responses were extensor bilaterally. Abdominal reflexes were absent. There was ataxia of gait and of the right arm. The cerebrospinal fluid (CSF) contained seven lymphocytes/3.2 cu mm, but protein was not determined. The Wassermann reaction was negative.

She was confined to bed for 1 year, but later could walk alone. Until age 56, the disease was benign with minor exacerbations and remissions; her symptoms were unsteadiness of gait, incoordination, and myoclonic movements of the thighs that often preceded micturition. She developed nocturia, diurnal frequency, and urge incontinence. At age 56 she had herpes zoster in the T-6 dermatome.

For years she slept for 1 to 2 hours each afternoon. During the rest of the day she was awake. After age 56, nocturnal sleep decreased with many awakenings. At first these awakenings seemed to be related to nocturia, but later she had leg cramps or awoke spontaneously. She began to sleep more during the day and had short attacks of sleep while sitting in a chair, reading, or looking at TV. Warm rooms induced sleep attacks. She napped up to five times a day for about 15 minutes, and awoke when touched or called. She felt refreshed after these sleep attacks and continued to take her usual 1- to 2-hour afternoon nap. Caffeine was ineffectual in preventing sleep attacks. Ephedrine, 50 mg twice a day, resulted in a moderate decrease of daytime sleepiness but did not influence the nocturnal sleep disorder.

At age 57 she had the first episode of generalized weakness. Attacks were elicited mostly by laughter, but also by anger or surprise. Some attacks occurred without overt emotional stimuli. While standing or walking, she would fall to the ground and remain powerless for about 2 minutes. During most attacks she could move her arms slightly, and often felt some twitching in her face. She