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Experimental Hematogenous *Candida* Endophthalmitis: Diagnostic Approaches

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Anterior chamber aspiration and vitreous aspiration were evaluated as diagnostic procedures for establishing a specific microbiological diagnosis in the rabbit model of hematogenous *Candida* endophthalmitis. Vitreous aspiration was the most successful procedure, confirming the diagnosis of hematogenous *Candida* endophthalmitis in 62% of eyes with documented intraocular infection. When animals with only the most severe clinical endophthalmitis were considered, vitreous aspiration confirmed the diagnosis in 89% of eyes evaluated. Vitreous aspiration correlated well with the extent of clinical endophthalmitis, as well as with postmortem, whole-vitreous cultures. Gram staining of the aspirate was additive to culture results in confirming the diagnosis. Anterior chamber aspiration was positive in only 1 of 58 eyes evaluated (1.7%). Additionally, muscle biopsy was evaluated in this study as a tool for establishing a microbiological diagnosis in the rabbit model of disseminated candidiasis. Only 2 of 131 biopsy specimens contained detectable *Candida*. Although vitreous aspiration may be associated with ocular complications, in certain clinical settings this procedure may be valuable in establishing the definitive microbiological diagnosis of hematogenous *Candida* endophthalmitis.

Disseminated fungal disease has become much more common in the past two decades. Sophisticated methods of life support, broad-spectrum antibiotics, aggressive surgical intervention, and the increasing use of radiation and chemotherapy in the treatment of malignant diseases have all contributed to this increased incidence (1, 2, 13, 14, 23).

Candida albicans has assumed an important role as the most common fungal pathogen in many hospitals. In our general hospital, *Candida* species have become the sixth most common blood culture isolate. In a nationwide survey of nosocomial infections, the Center for Disease Control reports *Candida* species as the ninth most common nosocomial blood culture isolate (3).

Although the incidence of serious fungal disease is rapidly increasing, antemortem diagnosis of many disseminated fungal diseases is rarely established. Serological studies have been of value in disseminated cryptococcosis (5), coccidiodomycosis (17), and histoplasmosis (11). Serology has been of less value in the diagnosis of other deep-seated fungal infections, and, in particular, the value of serological testing has been limited in *Candida* infections because of both false-positive and false-negative results. Other diagnostic modalities have been of limited usefulness in the setting of disseminated *Candida* infections. Gaines and Remington have estimated that an antemortem diagnosis of disseminated candidiasis was made in time for the initiation of appropriate therapy in only 15 to 40% of cases (10).

The presence of eye involvement in disseminated *Candida* infection has been well established (7, 9, 22). Our laboratory, as well as others, has suggested using clinically detectable endophthalmitis as a marker for disseminated candidiasis. Although retrospective autopsy studies have shown a high correlation of other organ involvement when eye lesions are present, the precise incidence of eye lesions in the settings of disseminated candidiasis and candidemia is not known. Occasionally, *Candida* endophthalmitis occurs without signs of overt dissemination. Several authors have concluded that intravenous narcotics abusers are at high risk for this syndrome (4, 18-21). Although this syndrome has been sporadically reported in the past, several recent case reports (15, 18, 19, 21), as well as our own recent clinical experience, suggest that the incidence of this syndrome is either increasing

or may be higher than previously recognized.

The clinical appearance of *Candida* endophthalmitis, especially in its early development, is not specific. Because of this non-specificity, several methods for definitively identifying a lesion as due to *Candida* have been attempted. These procedures include anterior chamber aspiration, vitreous aspiration, aspiration and biopsy (19), and partial vitrectomy (15, 18).

These procedures have been used in the face of no experimental data and limited clinical experience. Although these few anecdotal cases appear promising, vitreous aspiration is not without serious sequelae, such as retinal detachment, serious intraocular hemorrhage, and the formation of traction bands.

We evaluated vitreous aspiration in the rabbit model of hematogenous *Candida* endophthalmitis (HCE) with the following goals: (i) to establish the frequency with which this procedure would yield positive results and (ii) to identify the clinical setting in which vitreous aspiration would most likely identify the specific pathogen.

Additionally, isolated case reports of skeletal muscle involvement in cases of disseminated candidiasis (6, 12, 16) led to our evaluation of muscle biopsy as a diagnostic tool in the rabbit model of disseminated *Candida* infection.

MATERIALS AND METHODS

Experimental disseminated candidiasis and HCE. Experimental disseminated candidiasis and HCE were established as previously reported (9) by the injection of 5.0×10^5 cells of a clinical isolate of *Candida albicans* dye strain (ATCC 36082) into the marginal ear vein of 30 New Zealand White rabbits. Previous studies have demonstrated minimal mortality from this inoculum size, with an infection rate of approximately 90% (8). Lack of detectable endophthalmitis in uninfected rabbits as well as in rabbits receiving only intravenous brain heart infusion broth (Difco Laboratories, Detroit, Mich.) has been established previously (8).

Clinical ophthalmological examination. On day 3 after infection, both eyes of the first 15 rabbits were dilated with 1% cyclopentolate hydrochloride (Cyclogel, Alcon Laboratories, Ft. Worth, Tex.) and 10% phenyephrine hydrochloride (Neosynephrine; Winthrop Laboratories, New York, N.Y.). The fundi were then examined by using an indirect opthalmoscope (Frigi-Xonix, Shelton, Conn.) for the presence of *Candida* endophthalmitis. Clinical examination was again repeated before sacrifice on day 6 after infection and again on day 21 following infection. Before sacrifice, the extent of intraocular infection was recorded for each eye by using a scale of 0 to 4+ (see Table 1).

Anterior chamber and vitreous aspiration. A total of 14 rabbits were sacrificed at day 6, and 15 were sacrificed at day 21 after infection (1 rabbit died of anesthesia overdosage at day 3). At 6 days after infection, the animals which had undergone muscle biopsy (see below) were sacrificed. After funduscopic examination, animals were intravenously administered 3.5 ml of sodium pentobarbital (Diabutal; Diamond Laboratories, Des Moines, Iowa). Immediately after sacrifice, the anterior chamber of each eye was aspirated with a 1-ml syringe and a 25-gauge needle. Approximately 0.3 to 0.4 ml of fluid was removed. One drop was spread on a clean microscope slide, air dried, and stained with Gram stain. The remainder of the fluid was cultured in brain heart infusion agar (Difco Laboratories).

After the anterior chamber aspiration, the vitreous aspiration was performed in the following manner. The bulbar conjunctiva was separated over the pars plana by using a scalpel with a no. 15 blade. The sclera was then scratched lightly over the pars plana until the globe was entered. Bleeding was controlled with heat cautery. A 20-gauge needle was inserted into the eye, and the needle tip was placed near the center of the globe. Approximately 0.4 ml of vitreous fluid was aspirated. Again, 1 drop was placed on a slide for Gram staining; 0.2 ml was cultured in brain heart infusion agar. The remaining 15 rabbits were sacrificed and evaluated in the same manner 21 days after infection.

Muscle biopsy. On day 3 after infection and following ophthalmological examination as outlined above, the initial 15 rabbits were anesthetized with approximately 1 ml of Diabutal. By using aseptic technique, muscle samples approximately 5 mm wide and 30 mm long were removed from the gastrocnemius, quadriceps femoris, and deltoideus muscles. A piece of each sample approximately 1 mm long was removed and fixed in Formalin for histological preparation. The remaining muscle was weighed, diluted with brain heart infusion broth, and homogenized with a TriR Grinder (TriR Instruments, Rockwell Center, N.Y.). The resulting homogenates were serially diluted in brain heart infusion broth and plated in brain heart infusion agar. Plates were incubated at 37°C for 48 h; colony counts were then performed, and the number of colony-forming units per gram of muscle was determined.

This procedure was repeated after sacrifice at day 6 following infection, with the corresponding contralateral muscles biopsied at that time.

Data analysis. Gram staining of both the anterior chamber fluid and the vitreous aspirate was evaluated without knowledge of either the extent of clinical involvement in the eye or the result of cultures of the fluids. Smears were evaluated for a minimum of 15 min before being called negative.

Results of cultures from both the anterior chamber and the vitreous fluid were expressed as colony-forming units per milliliter of the aspirate. Cultures of chorioretina, vitreous, kidneys, and skeletal muscle were expressed as colony-forming units per gram of tissue.

Clinical endophthalmitis was scored on the arbitrary scale outlined in Table 1.

The percentages of samples positive by Gram staining by culture, and by both procedures were compared. In addition, positive samples were correlated with the extent of clinical ocular involvement.

The Formalin-fixed specimens were stained with both hematoxylin and eosin stain and periodic acid-Schiff reagent. Histological sections were examined for a minimum of 15 min before being called negative.

RESULTS

Anterior chamber aspiration. Gram stains of the aspirates from all 29 rabbits were negative. In only 1 of the 59 anterior chamber fluid aspirates was *C. albicans* identified by culture.

Vitreous chamber aspiration. By combining Gram stains with cultural data, vitreous aspiration was diagnostic of intraocular *Candida* infection in 64% of all eyes aspirated at day 6 after infection (Table 1). At 21 days postinfection, 47% of all eyes aspirated and 58% of eyes with documented active infection were positive (Table 2). Gram stain was successful as a diagnostic tool in 50% of eyes aspirated at day 6 (14 of 28) and in 43% of eyes evaluated at day 21 (13 of 30).

At day 6 only 1 of 12 eyes graded from 0 to 2+ was positive by Gram stain, whereas 13 of 20 graded either 3+ or 4+ had identifiable yeast or pseudohyphae on Gram stain evaluation. At 21 days, none of the 11 eyes graded 0 to 2+ had organisms found on Gram stain, whereas 13 of 19 of the eyes clinically graded 3+ or 4+ had positive Gram stains. All 6 eyes at day 6 and 10 of 12 eyes at day 21 which were graded 4+ were positive by Gram stain. Also of note is the fact that many of the pseudohyphae identified on Gram stain seemed to be degenerating (Fig. 1).

Culture of the vitreous aspirates yielded *C. albicans* in 9 of 28 eyes (32%) at day 6 and in 8 of 30 eyes (27%) at day 21. Since six eyes were no longer positive by culture of the whole vitreous at autopsy on day 21, then 8 of 24 eyes (33%) which had detectable disease by culture at that time were positive by aspiration.

Positive cultures of vitreous aspirates correlated most closely with the degree of vitreous involvement as determined by postmortem culture of the remaining vitreous from the aspirated eyes. Of interest is that at day 6 after infection, the most heavily infected eyes were those graded 2+ by the clinical examiner (Table 1). By 21 days following infection, the frequency of positive vitreous aspirate cultures paralleled both the extent of clinical involvement and the extent of infection as determined by postmortem, whole-vitreous cultures (Table 2).

Muscle biopsy. No *Candida* was cultured in any of the 45 biopsies taken 3 days following infection. In 2 of 42 samples taken at day 6 and in none of 45 samples taken at day 21 was *Candida* cultured. Histological sections stained with both hematoxylin and eosin stain and periodic acid-Schiff reagent were positive in only 1 of the 131 sections examined, and that sample corresponded with one of the two positive cultures.

TABLE 1. *Vitreous aspiration in the diagnosis of HCE at day 6*

Extent of clinical endophthalmitis[a]	Vitreous aspiration		Avg no. of organisms recovered from vitreous at autopsy	No. with correct diagnosis by vitreous aspiration
	No. with positive Gram stain	No. with positive culture		
$0–1^+$	1/5 (20)[b]	0/5 (0)	3.3×10^1	1/5 (20)
2^+	0/7 (0)	4/7 (57)	4.2×10^2	4/7 (57)
3^+	7/10 (70)	2/10 (20)	3.4×10^2	7/10 (70)
4^+	6/6 (100)	3/6 (50)	2.5×10^2	6/6 (100)
Total	14/28 (50)	9/28 (32)	2.9×10^2	18/28 (64)

[a] 1^+, A single small lesion; 2^+, one to five lesions scattered through the chorioretina, each less than one disk diameter in size; 3^+, large lesions (greater than one disk diameter in size) with associated vitreous haze; 4^+, massive lesions, with nearly complete vitreous involvement.

[b] Numbers in parentheses are percentages.

DISCUSSION

This study was designed to evaluate anterior chamber and vitreous aspiration as diagnostic tools in the rabbit model of HCE. Additionally, muscle biopsy was evaluated in the diagnosis of disseminated candidiasis in the rabbit model of that disease. Of the modalities evaluated, vitreous aspiration was the most successful.

TABLE 2. *Vitreous aspiration in the diagnosis of HCE at day 21*

Extent of clinical endophthalmitis[a]	Vitreous aspiration		Avg no. of organisms recovered from vitreous at autopsy	Correct diagnosis by vitreous aspiration
	No. with positive Gram stain	No. with positive culture		
$0–1^+$	0/7 (0)[b]	0/7 (0)	3.7×10^1	0/7 (0)
2^+	0/4 (0)	1/4 (25)	1.6×10^1	1/4 (25)
3^+	3/7 (43)	1/7 (14)	8.3×10^2	3/7 (43)
4^+	10/12 (83)	6/12 (50)	1.1×10^3	10/12 (83)
Totals				
All eyes	13/30 (43)	8/30 (27)	6.3×10^2	14/30 (47)
Infected eyes	13/24 (54)	8/24 (33)	8.0×10^2	14/24 (58)

[a] 1^+, A single small lesion; 2^+, one to five lesions scattered through the chorioretina, each less than one disk diameter in size; 3^+, large lesions (greater than one disk diameter in size) with associated vitreous haze; 4^+, massive lesions, with nearly complete vitreous involvement.

[b] Numbers in parentheses are percentages.

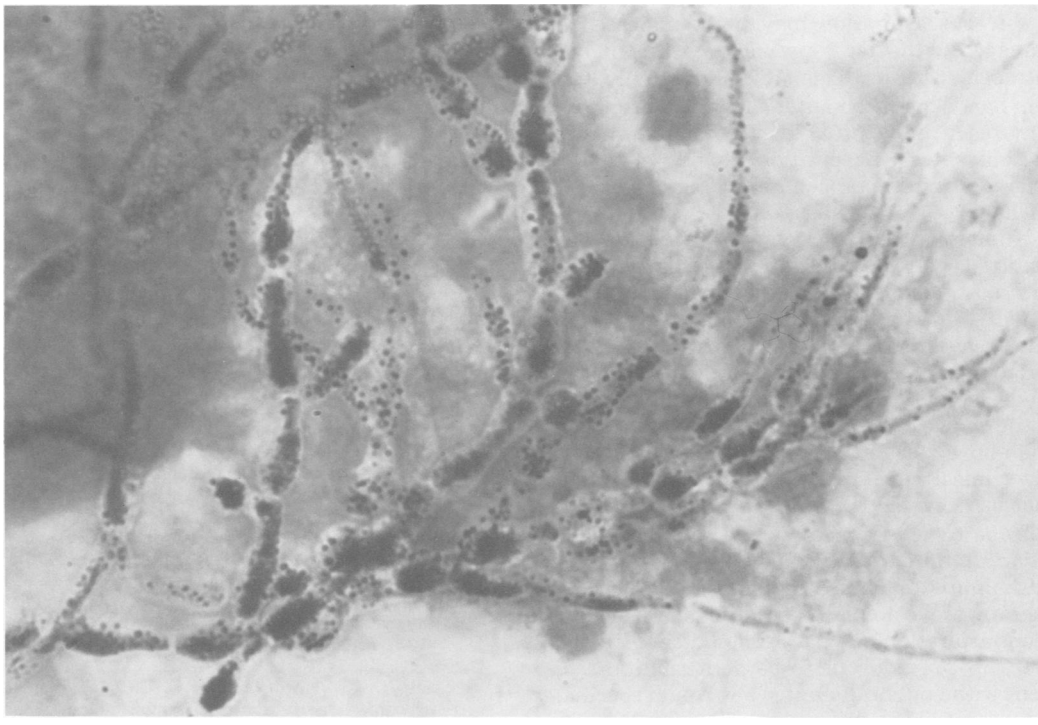

FIG. 1. *Degenerating pseudohyphae of C. albicans recovered from vitreous aspirate. (Gram stain; approximately 850×).*

The combination of Gram staining and culturing of the vitreous aspirate led to the identification of *Candida* in 18 of 28 eyes (64%) with active infection at day 6 and in 14 of 24 eyes (58%) with documented intraocular infection at day 21. If the data are evaluated by examining the eyes with the most severe clinical endophthalmitis (i.e., those graded 3+ or 4+ by the clinical examiner), a diagnosis could be established in 68% of eyes aspirated at day 6 (13 of 19) and in 81% (13 of 16) at day 21. In the rabbits with the most severe involvement (i.e., those graded 4+) *Candida* could be identified in all 6 of the eyes with severe involvement at 6 days postinfection and in 10 of 12 (83%) at 21 days postinfection. In the eyes graded 4+, then, vitreous aspiration was positive in 16 of 18 eyes (89%).

Although the clinical ophthalmoscopic examination correlated well with the results obtained from vitreous aspiration at the time of the final sacrifice (21 days postinfection), results from the first sacrifice (day 6) were not so closely correlated with the clinical examination. At the time of the first sacrifice, 42% of eyes with limited clinical involvement (i.e., those graded 0 to 2+ by the clinical examiner) had positive vitreous aspirates. Since previous studies have shown that a clinically detectable lesion of HCE is primarily composed of masses of inflammatory cells with limited numbers of organisms present (8), one might speculate that the high percentage of positive vitreous aspirate cultures in eyes with limited clinical involvement at the time of the initial sacrifice may be due to a relative lack of development of the inflammatory response, which results in a greater facility for recovering the organisms.

As one might suspect, positive vitreous cultures also correlate with the *Candida* burden in the vitreous at the time of aspiration, as determined by postmortem, whole-vitreous cultures.

If the data from the rabbit model are extrapolated to the human setting, careful indirect ophthalmoscopy for the purpose of detecting the severity of intraocular involvement may help identify those patients in whom vitreous aspiration is most likely to be diagnostic of HCE. Gram stain and culture of the vitreous aspirate were additive in establishing the diagnosis of HCE. In several instances pseudohyphae were identified on smears which appeared to be degenerating (Fig. 1). This observation may, at least in part, account for the occurrence of positive Gram stains in the face of negative cultures of the vitreous aspirates.

Vitreous aspiration may be complicated by damage to the lens, retinal detachment, and intraocular hemorrhage (18). Additionally, traction bands may form after the procedure, which may result in eventual loss of vision. For these reasons, vitreous aspiration should not be considered a routine procedure for the diagnosis of HCE.

Numerous cases of isolated HCE have been reported, and the association of this syndrome with intravenous drug abuse has been well documented in the literature (4, 15, 18–21). Situations may arise which threaten loss of sight in which a firm microbiological diagnosis must be made. Isolation of the causative agent may assist in the choice of appropriate antimicrobial agents and allows for appropriate sensitivity testing of the clinical isolate. One author has suggested that vitreous biopsy may contribute to host defense in the eye by removing a large part of the *Candida* burden in the eye (18). Massive endophthalmitis or failure of empiric antimicrobial therapy are examples of situations in which vitreous aspiration may be a useful diagnostic tool.

If this procedure is to be performed in a patient with endophthalmitis, one would speculate that careful localizing procedures utilizing an operating microscope should be employed and potentially may increase the yield of the aspiration.

Anterior chamber aspiration was additionally evaluated as a diagnostic tool in HCE and was found to be of limited value.

Although muscle biopsy was not found to be a useful diagnostic tool in the rabbit model of disseminated candidiasis, recent clinical reports have suggested that in patients with disseminated candidiasis, this procedure may be of value (12). Interestingly, both patients reported in the latter study, as well as one of the previously reported patients (6), were neutropenic patients with leukemia. Rabbits used in this study presumably had intact host defense mechanisms. We are currently repeating this experiment in neutropenic rabbits.

On the basis of these studies as well as the initial clinical experience, we conclude that further evaluation of vitreous aspiration in the appropriate clinical setting is justified. Further evaluation of muscle biopsy in the appropriate setting awaits further laboratory assessment.

LITERATURE CITED

1. **Bernhardt, H. E., J. C. Orlando, and J. R. Benfield.** 1972. Disseminated candidiasis in surgical patients. Surg. Gynecol. Obstet. **134**:819–825.
2. **Bodey, G. P.** 1966. Fungal infections complicating acute leukemia. J. Chronic. Dis. **19**:667–687.
3. **Center for Disease Control.** 1977. National nosocomial infections study report: annual summary, 1974. Center for Disease Control, Atlanta, Ga.
4. **Cherubin, C. E.** 1967. The medical sequelae of narcotic addiction. Ann. Intern. Med. **67**:23–32.
5. **Diamond, R. D., and J. E. Bennett.** 1974. Prognostic factors in cryptococcal meningitis. Ann. Intern. Med. **80**:176–180.
6. **Diggs, C. H., G. M. Eskenasy, J. C. Sutherland, and P. H. Wiernik.** 1976. Fungal infection of muscle in acute leukemia. Cancer (Brussels) **38**:1771–1772.
7. **Edwards, J. E., Jr., R. Y. Foos, J. Z. Montgomerie, and L. B. Guze.** 1974. Ocular manifestations of candida septicemia. Review of seventy-six cases of hematogenous candida endophthalmitis. Medicine (Baltimore) **53**:47–75.
8. **Edwards, J. E., Jr., J. Z. Montgomerie, R. Y. Foos, V. K. Shaw, and L. B. Guze.** 1975. Experimental hematogenous endophthalmitis caused by *Candida albicans*. J. Infect. Dis. **131**:649–657.
9. **Fishman, L. S., J. R. Griffin, F. L. Sapico, and R. Hecht.** 1972. Hematogenous candida endophthalmitis, a complication of candidemia. N. Engl. J. Med. **286**: 675–681.
10. **Gaines, J. D., and J. S. Remington.** 1972. Systemic candidiasis—a diagnostic challenge. Calif. Med. **116**:8–17.
11. **Kleger, B., and L. Kaufman.** 1972. Detection and identification of diagnostic *Histoplasma capsulatum* precipitates by counterelectrophoresis. Appl. Microbiol. **26**: 231–238.
12. **Kressel, B., C. Szewczyk, and C. U. Tuazon.** 1978. Early clinical recognition of disseminated candidiasis by muscle and skin biopsy. Arch. Intern. Med. **138**: 429–433.
13. **Krick, J. A., and J. S. Remington.** 1976. Opportunistic invasive fungal infections in patients with leukemia and lymphoma. Clin. Haematol. **5**:249–310.
14. **Louria, D. B., D. P. Stiff, and B. Bennett.** 1962. Disseminated moniliasis in the adult. Medicine (Baltimore) **41**: 307–337.
15. **Peyman, G. A., D. W. Vastine, and J. G. Diamond.** 1975. Vitrectomy in exogenous candida endophthalmitis. Albrecht von Graefes Arch. Klin. Exp. Ophthalmol. **197**:55–59.
16. **Schaberg, A., J. A. Hildes, and J. C. Wilt.** 1955. Disseminated candidiasis. Arch. Intern. Med. **96**:112–117.
17. **Smith, D. W., M. T. Saito, and S. A. Simons.** 1956. Pattern of 39,500 serologic tests in coccidioidomycosis. J. Am. Med. Assoc. **160**:546–552.
18. **Snip, R. C., and R. G. Michaels.** 1976. Pars plana vitrectomy in the management of endogenous candida endophthalmitis. Am. J. Ophthalmol. **82**:699–704.
19. **Stone, R. D., A. R. Irvine, and G. R. O'Connor.** 1975. Candida endophthalmitis: report of an unusual case with isolation of the etiologic agent by vitreous biopsy. Ann. Ophthalmol. **7**:757–762.
20. **Sugar, H. S., G. H. Mandell, and J. Shaley.** 1971. Metastatic endophthalmitis associated with the injection of addictive drugs. Am. J. Ophthalmol. **71**:1055–1058.
21. **Vastine, D. W., W. Horsley, S. B. Guth, and M. F. Goldberg.** 1976. Endogenous candida endophthalmitis associated with heroin use. Arch. Ophthalmol. **94**:1805.
22. **Weinstein, A. J., E. H. Johnson, and R. C. Moellering.** 1973. Candida endophthalmitis. A complication of candidemia. Arch. Intern. Med. **132**:749–752.
23. **Young, R. C., J. E. Bennett, G. W. Geelhoed, and A. S. Levine.** 1974. Fungemia with compromised host resistance. Ann. Intern. Med. **80**:602–612.