Utility of touch preparation for rapid diagnosis of cutaneous mucormycosis

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Cutaneous mucormycosis is an aggressive infection that must be treated rapidly. Touch preparation is an easy diagnostic tool, rarely reported in the literature and underused by physicians. We present a case of an immunocompromised patient with cutaneous mucormycosis diagnosed using touch preparation, highlighting the usefulness of this technique.

CASE REPORT

A 46-year-old diabetic man 7 months status post-liver transplantation for hepatitis C cirrhosis was transferred to Columbia University Medical Center, New York, NY, for management of septic shock. His course was complicated by Cytomegalovirus viremia, renal failure requiring continuous venovenous hemofiltration, and vancomycin-resistant Enterococcus bacteremia. Dermatology was consulted on hospital day 10.

A 6-×-7-cm centrally necrotic ulceration with violaceous borders and surrounding erythema was located on the upper aspect of the abdomen (Fig 1). Laboratory evaluation revealed alkaline phosphatase of 507 U/L (reference range: 3-96 U/L), total bilirubin of 13.2 mg/dL (reference range: 0.3-1.3 mg/dL), direct bilirubin of 7.9 mg/dL (reference range: 0.0-0.4 mg/dL), indirect bilirubin of 5.3 mg/dL (reference range: 0.2-0.9 mg/dL), albumin of 1.8 g/dL (reference range: 3.5-5.5 g/dL), and blood glucose of 309 mg/dL (reference range: 70-100 mg/dL). Blood cultures were negative. Cytomegalovirus viral load by polymerase chain reaction was 37,140 IU/mL (range of detection: 137-9,100,000 IU/mL) and 1,3-beta-D-glucan was greater than 500 pg/mL.

Two 4-mm punch biopsy specimens from the necrotic edge of the ulceration were each split into 2 pieces: half for tissue culture and half stained with hematoxylin-eosin. Before sending the specimens to the laboratory, a touch preparation was performed by gently smearing the biopsy specimen onto a glass slide. The slide was allowed to dry and then stained with Swartz Lamkins fungal stain (Delasco, Council Bluffs, IA), which contains 2.5% potassium hydroxide, 0.04% sodium benzoate, 0.21% dioctyl sulfosuccinate, and 0.5% Parker blue ink pure powder that stains hyphae dark blue. Touch preparation demonstrated many broad nonseptate hyphae branching at wide angles (Fig 2). A preliminary diagnosis of angioinvasive fungal infection was made within 45 minutes of initial consultation based on the necrotic ulceration and nonseptate hyphae on the touch preparation in an immunocompromised host, and intravenous amphotericin B was started. The next day, the patient’s ulcer was surgically debrided.

Hematoxylin-eosin stain of both skin biopsy specimens demonstrated hyphal organisms throughout

Fig 1. Cutaneous mucormycosis. A 6-×-7-cm necrotic ulceration with violaceous borders and a surrounding halo of erythema located superior to the liver transplantation scar.
the dermis, subcutaneous fat, and surrounding blood vessels on low power, and nonseptate widely branching hyphal elements infiltrating blood vessels on high power (Fig 3). Periodic acid–Schiff stain was also positive for hyphal organisms. *Rhizopus oryzae* was identified on culture.

Over the next 3 days, the area was debrided twice more, with the final debridement demonstrating fungal organism at the edges of the debridement and invading cartilage. The patient’s family decided to withdraw care, and the patient passed away 12 days after initial diagnosis of primary cutaneous mucormycosis.

**DISCUSSION**

Primary cutaneous mucormycosis in the immunocompromised patient may start as an indurated reddish purple plaque. Vascular infarction from angioinvasive infection causes cutaneous necrosis and ulceration.\(^1\) If not treated early, *Mucor* invades fascia, muscle, tendon, bone, and ultimately can cause widespread bloodstream dissemination. A large case series found 44% of cutaneous infections were complicated by deep extension or dissemination; the mortality of disseminated disease was 96%.\(^2\)

Among Zygomycetes, the majority associated with human disease are of the order *Mucorales*, including *Rhizopus* species, *Mucor*, *Rhizomucor*, *Absidia*, *Apophysomyces*, *Saksenaea*, *Cunninghamella*, *Cokeromyces*, and *Syncephalastrum* species.\(^3\) *Rhizopus oryzae* is the most common cause of mucormycosis, responsible for approximately 70% of all cases.\(^4\) *Rhizopus oryzae* has several virulence factors involved in pathogenesis including the ability to acquire unbound iron from the host via siderophores and the ability to adhere to and invade blood vessels through interaction with the glucose-regulated protein (GRP78) receptor on endothelial cells.\(^5\)

Diagnosis of mucormycosis is made by identifying organisms in tissue section and in confirmatory culture. The characteristic microscopic appearance of zygomycosis includes wide, ribbonlike, hyaline, aseptate hyphae with wide angle (45-90 degrees) branching. Hyphae are often not well preserved and may appear as “crinkled cellophane.”\(^5\) Invasion of blood vessels by hyphae is often seen in *Mucorales* infections. *Rhizopus* species have a globose (round) sporangium and may be differentiated based on the appearance of unbranched sporangiophores and nodal rhizoid production.\(^3\) Tissue cultures may often be falsely negative because of destruction of fragile hyphal fragments by laboratory processing.\(^3\) Recent studies have shown that polymerase chain reaction–based techniques on histologic specimens may be useful in genus identification when cultures are negative.\(^5\) Histopathology may be rapidly processed but culture results may not be available for several days, and timely treatment intervention is necessary to avoid fatality in this aggressive disease.

Touch preparations have been described extensively in the literature as a diagnostic tool for solid and hematologic malignancies. The usefulness of touch preparation for rapid diagnosis of invasive cutaneous infections has been reported in only 6 cases (Table 1).\(^5\)-\(^10\) However, 3 of the reports (including the current case) were from residents trained at the same hospital program in this simple technique with reproducible success. Multiple stains can highlight fungal organisms including but not limited to potassium hydroxide, periodic acid–Schiff, Gomori methenamine silver, and even Gram stain. In our case the use of a touch preparation with Swartz Lamkins (Delasco) (which contains
Potassium hydroxide allowed for identification of fungal organisms even before tissue culture and pathology results were available, permitting early intervention. Demonstration of the organism in tissue biopsy specimen and confirmation of the species by culture still remain the gold standard for diagnosis of opportunistic infection. Dermatologists should become familiar with a touch preparation because it is easy to perform and may allow for the rapid bedside diagnosis of mycotic and protozoan infections in acutely ill immunocompromised patients.

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**Table I.** Review of touch preparations of skin biopsy specimens for diagnosis of fungal and protozoal infection

| Study                  | Infection                        | Primary disease                  | Stain                        |
|------------------------|----------------------------------|----------------------------------|------------------------------|
| Berger et al, 1987     | *Leishmania* organisms           | None                             | Wright-Giemsa                |
| Held et al, 1988       | *Candida tropicalis*             | Acute myelogenous leukemia, neutropenic | Gram                        |
| Held et al, 1988       | *Candida albicans* and *Candida tropicalis* | Acute myelogenous leukemia, neutropenic | Potassium hydroxide          |
| McGovern et al, 1995   | *Rhizopus arrhizus*             | African trypanosomiasis          | Wright-Periodic acid—Schiff   |
| Rubin and Grossman, 2004 | *Cryptococcus* species       | Chronic lymphocytic leukemia     | Eosin and methylene blue     |
| Patel et al, 2009      | *Rhizopus oryzae*               | Liver transplant recipient       | Swartz Lamkins (Delasco)     |
| Current case           | Rhizopus oryzae                  | Liver transplant recipient       | Swartz Lamkins (Delasco)     |