SHORT REPORT

Polymicrobial candidaemia revealed by peripheral blood smear and chromogenic medium

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Candida spp are the fourth most common group of nosocomial pathogens isolated from patients on medical, surgical, and intensive care wards. Polymicrobial candidaemia has rarely been described. The diagnosis of candidaemia from peripheral blood smears has not been widely reported. This report describes the case of a young woman suffering from Ewing's sarcoma who developed a syndrome of septic shock. Deep fungal infection was diagnosed from a systematic peripheral blood smear and yeasts were isolated within 24 hours. A subculture on CHROMagar® Candida allowed the differentiation and presumptive identification of *Candida tropicalis* and *Candida krusei*. Species identification was confirmed by the ID 32C® system. This report underlines the usefulness of peripheral blood smears in the diagnosis of fulminant deep fungal infections, and of a differential isolation medium in the rapid presumptive identification of clinically important yeast species from clinical samples. This medium is particularly useful for the detection of mixed fungal infections, allowing early and better adapted antifungal treatment.

Fungal pathogens, in particular *Candida* spp, have become a major cause of nosocomial infection.[1 2] An epidemiological study conducted in four Swiss university hospitals showed that *Candida* spp were the fourth most common microorganisms isolated from patients on medical, surgical, and intensive care wards.[3] Predisposing factors for disseminated candidiasis include immunosuppressive chemotherapy, indwelling catheters, multiple antibiotic treatment, and heart or abdominal surgery. Mortality rates for systemic candidiasis are high, ranging from 50% to 80%, despite appropriate treatment.[2]

Because clinical signs are non-specific, the diagnosis of systemic candidiasis remains difficult.[4] Diagnosis is currently based on the isolation of *Candida* spp from normally sterile sites, but such methods lack sensitivity.[5] Many efforts have been made to develop more sensitive approaches. Among these, recent studies have reported that the detection of circulating candida mannan and anti-mannan antibodies has improved sensitivity for the detection of infectious episodes caused by the most pathogenic species of candida.[6 7] The use of molecular diagnostic tools to detect candida nucleic acid sequences has promised both high detection rates and the identification of specific species.[7] Pittet and co-workers proposed the determination of a candida colonisation index to identify patients at high risk of developing deep candida infection.[8] However, a general consensus has not been reached on the usefulness of any of these methods, and no specific method is currently recommended by physicians, except for blood culture and histological examination.

Previous reports have suggested that peripheral blood smears may be useful for the detection of disseminated yeast infection (table 1). The yeast species detected to date in peripheral blood smears include *Histoplasma capsulatum, Cryptococcus neoformans, Candida* spp, *Hansenula anomala, Penicillium marneffei,* and *Rhodotorula* spp.

> ''Because clinical signs are non-specific, the diagnosis of systemic candidiasis remains difficult''

Polymicrobial candidaemia has been described infrequently.[9] However, the isolation and identification methods used routinely could miss mixed yeast cultures.[10] Several cases of mixed species candidaemia were detected using a differential isolation medium containing chromogenic substrates, CHROMagar® Candida,[11] and polymerase chain reaction methods, whereas conventional methods identified a single causative species.

Our report describes the detection of a disseminated coinfection with *Candida tropicalis* and *Candida krusei* in a peripheral blood smear, which was confirmed with CHROMagar Candida, and the speciation of isolates by biochemical tests.

CASE REPORT

A 16 year old girl had a clinical history of Ewing's sarcoma localised on the right femur with medullary metastases (D4 to D6) diagnosed in February 1999. She was treated with five courses of chemotherapy (adriamycin/cyclophosphamide), followed in July by two further courses of mephalan/busulfan, and autologous peripheral blood stem cell transfusion.

At the beginning of September, the patient developed Lyell's syndrome after administration of cotrimoxazole. She was transferred to the paediatric intensive care unit where her condition worsened and cutaneous lesions, which were infected by *Staphylococcus* spp, spread widely. She developed a vein occlusive disease with hepatomegaly, splenomegaly, and major hepatic cytolysis, ascites, and cholostase. She also presented with acute renal insufficiency. Septic shock appeared on 15 September and she died less than 24 hours later, despite the introduction of antifungal treatment with liposomal amphotericin B (Ambisome®; 5 mg/kg/day intravenously).

A white blood cell count performed on 9 September revealed 14 300 cells/mm^3, mostly polymorphonuclear neutrophils (73%). Six days later, on 15 September, the white blood cell count was 35 500 cells/mm^3, with 60% polymorphonuclear neutrophils, 1% metamyelocytes, and 3% myelocytes. On May-Grumwald-Giemsa stained smears, polymorphonuclear neutrophil cells were damaged morphologically, and showed vacuolisation and intracytoplasmic yeast cells (fig 1). The yeast cells were both intracellular and extracellular, and consisted of round or oval blastospores and pseudohyphae. Blood cultures were carried out immediately

Table 1 Review of clinical cases of disseminated yeast infection diagnosed from peripheral blood smears

Ref	No. of cases	Category of patients	Outcome	Isolated species	Antifungal treatment
Berrouane *et al. J Clin Pathol* 1998;**51**:537–8	1	Intestinal obstruction	Death	*Candida albicans*	Fluconazole
Manabe *et al. Rinsho Ketsueki* 1997;**38**:669–73	1	Disseminated neuroblastoma	Death	*Rhodotorula rubra*	–
	1	Langerhan's cell histiocytosis	Death	*Candida guilliermondii*	AMB
Gavinet *et al. J Mycol Med* 1995;**5**:53–5	1	Epidermoidal carcinoma	Death	*Candida glabrata*	AMB
Chao *et al. Bone Marrow Transplant* 1994;**14**:647–9	1	Syngeneic BMT	Alive	*Candida parapsilosis*	Fluconazole
	1	Allogeneic BMT	Alive	*Rhodotorula glutinis*	AMB
Girmenia and Jaalouk. *Eur J Haematol* 1994;**52**:124–5	4	Haematological malignancies	Alive	*C parapsilosis (2), C guilliermondii (1), Hansenula anomala (1)*	–
Supparatpinyo and Sirisantana. *Clin Infect Dis* 1994;**18**:246–7	1	AIDS	Loss of sight	*Penicillium marneffei*	AMB
Casanova-Cardiel and Ruiz-Ordaz. *Rev Invest Clin* 1993;**45**:67–70	4	AIDS	Death	*Histoplasma capsulatum*	None (1), AMB (3)
Marshall *et al. Am J Clin Pathol* 1990;**93**:526–32	2	AIDS	–	*H capsulatum (2)*	–
	2	Prematurity	–	*C albicans (1), C parapsilosis (1)*	–
	2	Intestinal diseases	–	*C albicans (1), Candida tropicalis (1)*	–
Rosti *et al. Haematologica* 1990;**75**:480–1	1	Allogeneic BMT	Alive	*C tropicalis*	Liposomal AMB
Yao *et al. Am J Med* 1990;**89**:100–2	1	AIDS	Death	*Cryptococcus neoformans*	–
Buchman *et al. JAMA* 1988;**260**:2926	1	Longterm total parenteral nutrition	Alive	*C glabrata*	AMB
Kates *et al. Lab Med* 1988;**19**:25	1	Longterm total parenteral nutrition	Alive	*Candida krusei, then C albicans*	AMB
Ossenkoppele *et al. Neth J Med* 1988;**33**:30–2	1	Autologous BMT	Alive	*C parapsilosis*	AMB
Dietriech *et al. Schweiz Med Wochenschr* 1987;**117**:1289–96	1	AIDS	Death	*H capsulatum*	–
Monihan *et al. Arch Pathol Lab Med* 1986;**110**:1180–1	1	CLL and Kaposi's sarcoma	Death	*C parapsilosis*	AMB
Paul *et al. Pediatr Infect Dis* 1986;**5**:274–5	1	AIDS	Death	*H capsulatum*	AMB
Baptist *et al. N Y State J Med* 1985;**85**:664–5	1	AIDS	Death	*H capsulatum*	None
Henochowicz *et al. JAMA* 1985;**253**:3148	1	AIDS	Death	*H capsulatum*	AMB
Macher *et al. Ophthalmology* 1985;**92**:1159–64	1	AIDS	Death	*H capsulatum*	AMB
Wheat *et al. Am J Med* 1985;**78**:203–10	1	AIDS	Death	*H capsulatum*	AMB
Bonner *et al. Arch Intern Med* 1984;**144**:2178–81	1	AIDS	Alive	*H capsulatum*	AMB
Girard *et al. South Med J* 1977;**70**:65–6	1	Metastatic carcinoma	Death	*H capsulatum*	AMB
	1	Hodgkin lymphoma	Death	*H capsulatum*	AMB
Kobza and Steenblock. *BMJ* 1977;**1**:1640–1	1	Intestinal obstruction	Alive	*C albicans*	AMB+5FC
Portnoy *et al. N Engl J Med* 1971;**285**:1010–11	1	Intestinal obstruction	Alive	*C albicans*	AMB+5FC
	1	Malabsorption syndrome	Death	*C albicans*	AMB
Hahn *et al. MO Med* 1973;**70**:249–50	1	CLL	Death	*H capsulatum*	AMB
Silverman *et al. Am J Clin Pathol* 1973;**60**:473–5	1	Intestinal obstruction	Death	*C albicans*	AMB
Jacobs. *JAMA* 1969;**207**:1916	1	Renal transplantation	Death	*H capsulatum*	–
Lopez and Grocott. *Am J Clin Pathol* 1968;**50**:692–4	1	Histiocytosis X	Death	*H capsulatum*	AMB
Holland and Holland. *Am J Dis Child* 1966;**112**:412–21	1	Abdominal distension	Death	*H capsulatum*	AMB
Jobe and Koepke. *Tech Bull Regist Med Technol* 1966;**36**:156–7	1	Anaemia	Death	*H capsulatum*	AMB
Hood *et al. Can Med Assoc J* 1965;**93**:587–92	1	Renal homotransplantation	Death	*H capsulatum*	–
Silverman *et al. Am J Med* 1955;**19**:410–59	1	–	Death	*H capsulatum*	–
Ffrench and Shemoi. *Can Med Assoc J* 1954;**71**:238–41	1	Aplastic anaemia	Death	*C albicans*	–
Parsons and Zarafonetis. *Arch Intern Med* 1945;**75**:1–23	1	–	Death	*H capsulatum*	–
Reid *et al. J Lab Clin Med* 1942;**27**:419–34	1	–	Death	*H capsulatum*	–
Amolsch and Wax. *Am J Pathol* 1939;**15**:477–81	1	–	Death	*H capsulatum*	–
Dodd and Tompkins. *Am J Trop Med* 1934;**14**:127–37	1	Anaemia	Death	*H capsulatum*	–

AMB, amphotericin B; BMT, bone marrow transplantation; CLL, chronic lymphocytic leukaemia; 5FC, 5-fluorocytosine.

on Bactec® 9050 mycosis aerobic medium and yeasts were isolated 24 hours later. Identification of the yeasts was performed using the following standard tests: BICHRO LATEX ALBICANS®, which was negative, and the ID 32C® system, which was uninterpretable, suggesting the possibility of a mixed yeast infection. A subculture on CHROMagar Candida revealed two types of colony: *C tropicalis* (blue with a halo) and *C krusei* (pink). This presumptive identification was confirmed with the ID 32C system.

DISCUSSION

In most reported cases, the observation of fungal elements in peripheral blood smears from patients has allowed an early diagnosis and the initiation of antifungal treatment (table 1). The diagnosis was mainly fortuitous in systematic peripheral blood smears stained with May-Grumwald-Giemsa or Giemsa, often when performing a white blood cell count. Most patients described had haematological malignancies, AIDS, or intestinal obstruction, and the fungaemias in these patients were associated with a high mortality rate (62%). *Histoplasma capsulatum* was involved in 26 of the 52 reported cases of disseminated yeast infection. Among these, 13 cases were in patients with AIDS. The second most common cause of fungaemia was *Candida* spp. Most episodes were seen in patients with haematological malignancies (eight of 14) or intestinal diseases (seven of eight). *Candida albicans* was involved in six of the seven cases of disseminated candidiasis

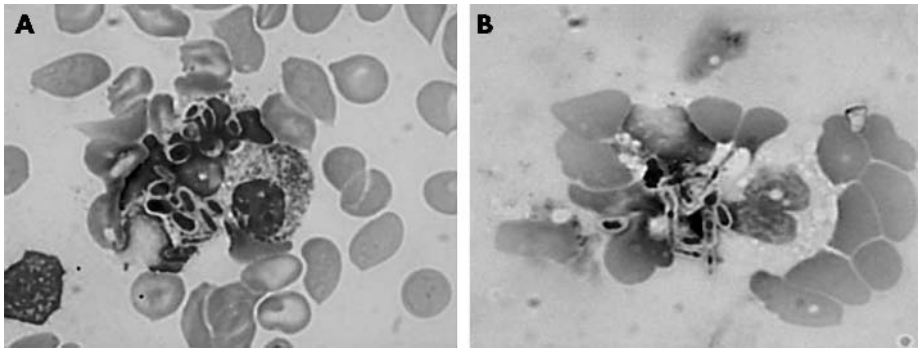

Figure 1 Peripheral blood smear with yeast blastoconidia and pseudohyphae in the cytoplasm of morphopolynuclear cells (May-Grumwald-Giemsa stain; original magnification, ×1000).

Take home messages

- Peripheral blood smears are extremely useful in the diagnosis of fulminant deep fungal infections
- CHROMagar® Candida isolation medium can help in the rapid presumptive identification of clinically important yeast species from clinical samples
- This medium is particularly useful for the detection of mixed fungal infections, allowing early and better adapted antifungal treatment

after severe intestinal disease. In contrast to previous reports, which mentioned only *C albicans* in patients with a history of intestinal obstruction, our report describes a patient with similar symptoms in whom systemic infection was caused by mixed non-*albicans* species. On peripheral blood smears, two morphotypes of yeast were observed: round blastospores, which suggested *C tropicalis*, and small, oval blastospores, which resembled *C krusei*. Two distinct morphotypes could also be observed, namely: oval yeasts and pseudohyphae (fig 1). None of the other reports have described polymicrobial candidaemia, which is an infrequent clinical event.[9] However, routine isolation methods could miss the detection of mixed yeast cultures.[10] In broth based blood culture systems, one species might overgrow the other and prevent its detection. An agar based culture method might facilitate the detection of multiple fungal species when their colony morphologies are different. However, *C tropicalis* and *C krusei* have similar morphotypes characterised by white to cream and butyrous colonies, with a rough texture observed for some *C krusei* strains. The introduction of differential isolation media might help in the detection of mixed fungal infections.[11] CHROMagar Candida medium allowed the identification of two candida species in less time than conventional methods.

> ''Routine isolation methods could miss the detection of mixed yeast cultures''

According to statistical evaluation of CHROMagar Candida for the presumptive identification of commonly isolated yeast species,[12] blue to blue/grey colonies are highly suggestive of *C tropicalis*, whereas dry, flat, rough textured, and spreading colonies with a pale pink colour and white edges are highly suggestive of *C krusei*. In both cases, the positive predictive value was 100%. However, some strains of *C krusei* were not identified because they produced smooth pink/purple colonies with a white edge. Thus, the systematic use of CHROMagar *Candida* helped not only in the presumptive identification of *Candida* spp, but also in detecting the presence of several yeast species in the same sample. This strategy was helpful in the initiation of appropriate antifungal treatment, which targets *Candida* spp according to their antifungal susceptibility. Indeed, in this patient, the *C krusei* and *C tropicalis* strains isolated had a different susceptibility to triazole antifungal agents, whereas most clinical isolates of *C krusei* are resistant to fluconazole.[13]

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