BREAKTHROUGH INVASIVE DISSEMINATED CRYPTOCOCCAL FUNGEMIA IN A PATIENT RECEIVING MICAFUNGIN THERAPY: A CASE REPORT

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ABSTRACT
Micafungin is an echinocandin-class antifungal agent that is extremely used in the recent era due to minimal toxicity and broad-spectrum activity, unfortunately, has limited ability against Cryptococcus neoformans. Since it’s inherently resistant, indeed disseminated invasive cryptococcosis is becoming an essential issue in echinocandin recipient with impaired immunity. We present a patient with systemic lupus erythematosus and miliary tuberculosis, who developed a fungal infection with invasive cryptococcal fungemia during empirical micafungin therapy. To the best our knowledge, breakthrough disseminated cryptococcosis has not been reported, as seen in our patient. This case highlighted that Cryptococcus neoformans infection should be kept in mind as a possible breakthrough infection while receiving echinocandin, especially in patients with cellular immunodeficiency.

KEYWORDS invasive fungal infection, micafungin, Cryptococcus neoformans

Introduction
Cryptococcus neoformans (C. neoformans) is one of the common opportunistic agents in invasive fungal infection among immunocompromised patients such as acquired immunodeficiency syndrome (AIDS) patients or another impair cell-mediated immunity host, for example, those receiving immunosuppression, high dose corticosteroid, and chemotherapy [1-2]. There were a few reports of breakthrough cryptococcal infection during echinocandins therapy occurring in immunocompromised patients. A review of breakthrough invasive mycoses showed that 2.4% patients developed non-Candida albicans and aspergillosis throughout exposure to echinocandins [3]. On the other side, cases of co-infection with tuberculosis and cryptococcosis are very rarely reported even in patients with impaired cell-mediated immunity [4]. The purpose of this case was to describe a possible case of breakthrough C. neoformans infection during empirical micafungin therapy in systemic lupus erythematosus (SLE) patient on steroid therapy with miliary tuberculosis.

Case Report
A 34-year-old Indonesian female was admitted due to prolonged fever for two weeks. She had a history of (1) SLE and treated initially with methylprednisolone 1 mg/kg BW for 4 weeks and then maintained with methylprednisolone 16 mg once daily; (2) lung tuberculosis one year earlier and treated with first-line tuberculosis drugs for six months; and (3) herpes zoster three weeks before admitted. General examination was normal unless the temperature of 38°C. Chest radiology demonstrated widespread small nodular opacities throughout both lungs, presumed to be miliary tuberculosis—laboratory findings described in table 1. SLE treatment with 16 mg methylprednisolone once daily was continued, first-line tuberculosis drugs of isoniazid 200 mg/day, rifampicin 450 mg/day, ethambutol 750 mg/day, and pyrazinamide 1,000 mg/day were started. On the 4th day of admission, the patient’s condition deteriorated, as she experienced shortness of breath, cough with productive sputum,
| Parameters                   | Admission | Day 4  | Day 13 | Normal Value           |
|------------------------------|-----------|--------|--------|------------------------|
| Hemoglobin                   | 12.40     | 11.10  | 7.20   | 11.70-15.50 gr/dl      |
| Hematocrit                   | 37.10     | 33.70  | 23.20  | 35.00-47.00%           |
| Red Blood Cells              | 4.62      | 4.18   | 2.78   | 3.80-5.20 x 10⁶/µl     |
| White Blood Cells            | 9.06      | 7.52   | 8.71   | 3.60-11.00 x 10³/µl    |
| Basophil                     | 0.00      | 0.00   | 0.00   | 0-1%                   |
| Eosinophil                   | 0.00      | 0.00   | 0.00   | 1-3%                   |
| Band neutrophil              | 2.00      | 2.00   | 3.00   | 2-6%                   |
| Segment neutrophil           | 89.00     | 89.00  | 88.00  | 50-70%                 |
| Lymphocyte                   | 7.00      | 6.00   | 6.00   | 25-40%                 |
| Monocyte                     | 2.00      | 3.00   | 3.00   | 2-8%                   |
| Platelets                    | 249.00    | 166.00 | 126.00 | 150.00-440.00 x 10³/µl|
| ESR                          | 58.00     | 71.00  | 72.00  | 0-20 mm/hours          |
| MCV                          | 80.30     | 80.60  | 83.50  | 80.000-100.000 fl      |
| MCH                          | 26.80     | 26.60  | 25.90  | 26.00-34.00 pg         |
| MCHC                         | 33.40     | 32.90  | 31.00  | 32.00-36.00 g/dl       |
| SGOT/AST                     | 16.00     | 33.00  | 32.00  | 0-32 UI/ml             |
| SGPT/ALT                     | 15.00     | 9.00   | 7.00   | 0-33 UI/ml             |
| Albumin                      | -         | 1.67   | -      | 3.50-5.20 g/dl         |
| Ureum                        | 56.00     | 49.00  | 68.00  | < 50.00 mg/dl          |
| Creatine                     | 1.27      | 0.85   | 0.82   | 0.5-1.1 mg/dl          |
| eGFR                         | 51.20     | 81.40  | 84.80  | ml/mnt/1.73 m²         |
| Blood Random Glucose         | 105.00    | 293.00 | -      | < 200.00 mg/dl         |
| Na                           | 134.00    | 125.00 | -      | 137-145 mmol/l         |
| K                            | 4.20      | 4.50   | -      | 3.6-5.0 mmol/l         |
| Cl                           | 97.00     | 10.30  | -      | 98-107 mmol/l          |

**Abbreviations:** ESR erythrocyte sedimentation rate, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC Mean corpuscular hemoglobin concentration, ALT alanine aminotransferase, AST aspartate aminotransferase, SGOT serum glutamic oxaloacetic transaminase, SGPT serum glutamic pyruvic transaminase
high fever, nausea and vomiting. Results of the haematological panel were presented in table 1. Blood gas analysis showed pH 7.410, PO2 62 mmHg, PCO2 18 mmHg, O2 saturation 93.4%. She was transferred to the intensive care unit with acute respiratory distress syndrome and anuria. Empirical antibiotic therapy of intravenous cefopodoxime/ sublactam of 500 mg/500 mg twice daily was started after aerob micro-organism and fungi cultures for blood, urine, and sputum, and also sputum acid-fast bacilli direct examination were obtained. The cultures showed negative results for bacteria and fungi, but the direct examination of sputum was positive for acid-fast bacilli. Empiric micafungin was started on the 7th day of admission as her condition did not improve despite results of bacteria and fungi cultures.

Re-culture examination was obtained of the 12th day of admission. Unfortunately, the patient demise of the 15th day of admission with sepsis and multiple organ dysfunctions. The result of this culture came out after the patient’s demise and revealed C. neoformans (figure 1) for blood and Stenotrophomonas maltophilia for sputum. Species differentiation and antimicrobial susceptibility testing were performed by an automated method from VITEX-2 Compact (Biomérieux, France). The minimum inhibitory concentrations (MICs) and susceptibility for those fungi were 4 µg/mL for fluconazole, 1 µg/mL for amphotericin B, < 1 µg/mL for 5-flucytosine, and no result for micafungin.

**Discussion**

C. neoformans is an opportunistic mycosis agent that causes life-threatening infections in immunocompromised populations that caused high morbidity and mortality to affected patients [2]. A review found that the overall hospital mortality rate of 56.1%, with no significant difference between patients receiving antifungal agents vs. untreated (56.9% vs. 54.8%, p = 0.86) [5]. In other side, autoimmune disease was an independent risk factor for cryptococcal fungemia (adjusted OR = 9.3, [95% CI 1.1 - 135.7], p = 0.038). This finding illustrated that the functional impairment of T helper cells in SLE played a role as a risk factor for cryptococcal fungemia and mortality [6]. Prolonged and high dose therapy of corticosteroids was also known as a risk factor for cryptococcal fungemia [7-8]. Another review also found that invasive fungi such as Cryptococcus spp., Aspergillus spp., and Candida spp. were associated to early-stage SLE disease that used high-dose corticosteroid therapy with a mortality rate of 53% [9-10].

Fungal bloodstream infections were infrequent as a study in Mexico City found that positive blood culture-based diagnosis was only 11% of cases with C. neoformans identified in 21.2% of cases as the third most commonly found fungi after Candida spp. (43.9%) and Histoplasma capsulatum (27.3%) [2]. Similarly with the previous study in Thailand that was found in 14.1% of cases [5]. The empirical choice of antifungal therapy should be based on a host’s susceptibility to specific fungal pathogens. The best antifungal therapeutic strategy against C. neoformans is limited to single or in the combination of amphotericin B (and its liposomal derivatives), 5-flucytosine andazole agents [8, 11]. Although the fungicidal combination is recommended,azole agents, such as fluconazole or voriconazole, is the most reasonable in countries with a high incidence of cryptococcosis [8]. This is related to the unavailability of 5-flucytosine, and the toxicity and need for clinical and laboratory monitoring for amphotericin B [12-13]. Emerging resistance to antifungal drugs has been reported, as a study showed increased fluconazole resistance with 0.6% isolates had MICs of ≥16 µg/ml [12]. These MICs were comparable to 4% of C. neoformans isolates from a study in Taiwan [14]. Whilst other studies concluded that there no changes in susceptibility for the past 15 years, although fluconazole is widely used globally [12, 14]. MICs testing using the microdilution method from the European Committee on Antibiotics Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI) are the gold standard to determine antifungal susceptibility for Cryptococcus [14]. Therefore, the thorough understanding and epidemiological perspective of this disease would be helpful in the prompt diagnosis and therapeutic strategies.

It is reasonable to suspect that cryptococcal fungemia in this patient was acquired from colonization or contamination of the central venous catheter (CVC). However, CVC-related fungemia due to C. neoformans was infrequent, and only C. lauritii and Candida parapsilosis fungemia have been documented. It’s affiliated to configure biofilm on the surface of medical equipment and polystyrene plates. The formation of fungal biofilms depends on the supporting surface characteristics as well as growth in the biofilm phase making the fungal cells less vulnerable to potential environmental pressures, including antimicrobials [1, 15-16]. Other possibility was the reactivation of dormant cryptococcal lung lesions as consequences of impaired cell-mediated immunity enhanced by corticosteroid immunosuppressive therapy. Tuberculosis and cryptococcosis co-infection only a few cases had been reported even in patients with impaired cell-mediated immunity [4,7]. In addition, only two reports have been published in wherein the clinical presentation of C. neoformans was a breakthrough invasive disseminated cryptococcosis (Table 2).

The echinocandins are the latest antifungal drugs in clinical therapy, which act as non-competitive inhibitors of β-1,3-glucan synthase, a major structural component of the fungal cell wall. The drugs in this class are caspofungin, micafungin and anidulafungin. It is known that echinocandins have a good fungicidal activity against most Candida species and fungistatic activity for Aspergillus species, but unfortunately less effective against C. neoformans, Zygomycetes, Fusarium or Scedosporium. The FKS genes encoded β-1,3-glucan synthases in different fungal species. Echinocandin resistance has been attributed to specific mutations leading to amino acid substitutions in two different regions of these genes (Hot spot 1 and 2 or HS1 and HS2) that caused decreased affinity to the target in fungal cell wall. Cryptococcus neoformans is inherently resistant to echinocandins [16]. Although content of glucan in C. neoformans cell wall is low, it has a single Fks1 gene that is essential for viability. The amino acid Leu538 of HS1 and Candida spp. were associated to early-stage SLE disease that used high-dose corticosteroid therapy with a mortality rate of 53% [9-10].

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target enzyme insensitivity or enzyme activity. Resistance is likely due to common microbial drug resistance mechanisms, i.e. the β-1,3-glucan synthase resistance to caspofungin; caspofungin is excluded from cells, and the target by ATP-binding cassette (ABC) transporters or other mechanisms; or caspofungin is degraded either extra- and/or intracellularly [21].

One study discovered that C. neoformans reshapes cell wall and capsule composition during infection to produce titan cell. It was presumed that this phenomenon likely is the mechanism to escape recognition by, and allow modulation of, the host immune system as well as resistance to echinocandins. A study in mice found that there were morphology changes in the initiation and maintenance of C. neoformans infections. During infection, its cell had variable cell body size and capsule size. These structural and morphological characteristics allow C. neoformans to evade and modulate the host immune system As C. neoformans inhaled into the lungs, it differentiates into cells with different size and morphology, including the production of large titan cells [22].

The cell wall of C. neoformans is composed of α- and β-glucans which are polysaccharides of glucose monomers. The α-glucans are essential for capsule attachment to the C. neoformans cell body. Disruption of α-1,3-glucan production produces cells that shed capsule material into the environment and lack the surface capsule normally attached to the cell body [22]. Titan cells have a thick and highly cross-linked capsule attached to their cell wall [23]. Thus, the reduction of glucose content of the titan cell wall is unlikely due to decreased of α-glucans as capsule attachment appears to be unaffected.

The cell wall of C. neoformans also contains two types of β-glucans; β-1,3-glucans and β-1,6-glucans. The proportion of each β-glucan type varied on the cryptococcal strain, growth condition, and exposure to caspofungin [24]. The specific function of β-1,6-glucan in the C. neoformans cell wall is not well understood, but disruption of its production results in enlarged, more diffuse, and more permeable capsules than wild type cells [25]. In vivo titan cells have large cross-linked capsules with reduced permeability, suggesting that β-1,6-glucans are either unaffected or increased [23]. The β-glucans are recognized by the Dectin-1 receptor of host immune cells. The low level of glucan in cell walls and a thick capsule reduce the surface exposure of β-glucans on in vivo C. neoformans cells, thus enabling the Dectin-1 receptor to make direct contact with β-glucans. Direct contact between the Dectin-1 receptor and the microbial surface is required for the activation of the receptor and subsequent signalling [26]. The low level of glucan in cell walls could also...
Table 2 Comparison of previously case reported with current our report

| Author          | Year | Age | Gender | Diagnose on admission                                      | Empirical antimicrobial          | Outcome                                      |
|-----------------|------|-----|--------|-------------------------------------------------------------|----------------------------------|----------------------------------------------|
| Suzuki K, et al | 2008 | 67  | male   | Systemic lupus erythematosus on methylprednisolone           | Meropenem, micafungin             | Cryptococcal meningitis, pulmonary aspergillosis, and patient died |
| Lee WS, et al   | 2011 | 77  | male   | Chronic obstructive pulmonary disease, diabetic nephropathy, and renal kidney disease | Teicoplanin, ceftriaxone, micafungin | Cryptococcal fungemia, cryptococcoci, and patient died on day 40 |
| Present case    | 2020 | 34  | female | Systemic lupus erythematosus on methylprednisolone, miliary tuberculosis | Cefoperazone/sulbactam, first-line tuberculosis drugs, micafungin | Cryptococcal fungemia, and patient died on day 12 |

explain why echinocandins’ effect on β-1,3-glucan synthase does not disrupt the cell walls. During infection, titan cells confer protection from phagocytosis to the entire cryptococcal population [22]. A large amount of mannose was detected in the titan cell wall, and it could be components of cell wall mannos, mannoproteins, or capsule components trapped in the cell wall during extraction. Mammoproteins located in the inner layer of the C. neoformans cell wall are non-structural cell wall components that will migrate to the cell surface and secreted into the environment. It was proposed that the increased production of specific mammoproteins could protect from phagocytosis [27]. Previous studies of capsule structure of C. neoformans on different anatomical location showed differences in capsule structure of cells grown in vitro and those isolated from the lungs, spleen, kidney, liver, heart and brain of infected mice [28].

Conclusion

Ethical Consideration
Written informed consent was obtained from the legal guardian(s) for publication of this case report and accompanying images.

Authors’ contributions
All of the authors contributed equally from conception and design, data analysis and interpretation, drafting and approved the final manuscript of the case report.

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Conflict of Interest
There are no conflicts of interest to declare by any of the authors of this study.

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