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Clinical and microbiological characteristics of cryptococcosis in Singapore: predominance of *Cryptococcus neoformans* compared with *Cryptococcus gattii*☆

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SUMMARY

Objectives: To describe the clinical features, treatments, outcomes, and subtype prevalence of cryptococcosis in Singapore.
Methods: All patients with laboratory confirmed cryptococcal infections admitted from 1999 to 2007 to a teaching hospital in Singapore were reviewed retrospectively. Identification and molecular types of *Cryptococcus neoformans* variants and *Cryptococcus gattii* were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Serotypes were inferred with a multiplex PCR method.
Results: Of 62 patients with cryptococcosis, *C. neoformans* var. *grubii* was the predominant subtype (in 95%), affecting mainly immunocompromised hosts (91%) with HIV infection (80%). Patients with HIV were younger (median age 36.5 vs. 49.5 years, $p = 0.006$) and less likely to present with an altered mental status (14% vs. 50%, $p = 0.013$). In contrast, delayed treatment (median 7 days vs. 2 days, $p = 0.03$), pulmonary involvement (58% vs. 14%, $p = 0.03$), and initial treatment with fluconazole (25% vs. 2%, $p = 0.02$) were more common in HIV-negative patients. *C. gattii* was uncommon, affecting only three patients, all of whom were immunocompetent and had disseminated disease with pulmonary and neurological involvement. All *C. gattii* were RFLP type VG II, serotype B and all *C. neoformans* var. *grubii* were RFLP type VN I, serotype A, except for one that was RFLP type VN II.
Conclusion: *C. neoformans* var. *grubii*, subtype VN I, was the predominant subtype in Singapore, infecting younger, mainly immunocompromised hosts with HIV. *C. gattii* was uncommon, causing pulmonary manifestations in older, immunocompetent patients and were RFLP type VG II.

1. Introduction

Cryptococcus neoformans is a basidiomycetous encapsulated yeast with worldwide distribution. After inhalation from environmental sources, this pathogen may result in life-threatening infections in humans commonly affecting the central nervous system or respiratory system.[1]

C. neoformans was previously subclassified into three varieties based upon biochemical differences and into four non-hybrid serotypes according to capsular agglutination reactions: *C. neoformans* var. *grubii* (serotype A), *C. neoformans* var. *neoformans* (serotype D), and *C. neoformans* var. *gattii* (serotype B and C).[2,3]

C. neoformans is the commonest cause of fungal meningitis worldwide.[4,5] *C. neoformans* var. *grubii* is an opportunistic pathogen of immunocompromised patients, with HIV infection, corticosteroid therapy, haematological malignancies, and solid-organ transplantation identified as major risk factors.[6,7] Cryptococcal meningitis is the fourth most common opportunistic infection in patients with HIV, with an estimated one million HIV-associated cryptococcosis cases diagnosed annually worldwide.[5] In Southeast Asia, cryptococcosis is common amongst HIV-infected individuals, with an estimated 120 cases per 1000 HIV-infected individuals per year.[4] The growing size of the immunocompromised patient population from treatment with chemotherapy and biological agents will likely further contribute to the medical importance of cryptococcosis.[6]

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Cryptococcus gattii is now recognized as a separate species from *C. neoformans* due to significant differences in genetic, biochemistry, ecology, and clinical characteristics.[8,9]

C. gattii is associated with the unique environmental source of Australian River and Forest Red Gum trees (*Eucalyptus camaldulensis* and *Eucalyptus tereticornis*), although it has also been cultured from other trees, bird excreta, and soil.[9–11] Mainly restricted to tropical and subtropical regions, a recent large outbreak of *C. gattii* in British Columbia and surrounding areas has highlighted a shift in the geographical distribution.[12,13] Unlike *C. neoformans*, disease due to *C. gattii* is usually observed in patients without significant immunosuppression. Characteristics of *C. gattii* include a higher rate of brain mass lesions, more refractory response to antifungal chemotherapy, and more aggressive and prolonged treatment with increased long-term sequelae and higher mortality.[13–15]

In Singapore, identification to the species level has only recently been implemented in clinical laboratories so the historic distribution of the various serotypes and species is unknown. In addition, no recent data exist on patient characteristics, treatments, and outcomes of cryptococcosis since the onset of the HIV epidemic. To provide this information, our study aimed to describe the epidemiological, clinical, and outcome aspects of patients with cryptococcosis admitted to a teaching hospital from 1999 to 2007.

2. Methods

Tan Tock Seng Hospital is a 1400-bed university teaching hospital in Singapore. We retrospectively reviewed the medical and microbiological records of all patients aged 16 years or older hospitalized from March 1999 to June 2007 with cryptococcal infection. Patient characteristics were extracted from patient records and included demographic characteristics, co-morbidities, clinical presentation, radiological and microbiological evaluation including cerebrospinal fluid (CSF) analysis, antifungal treatment, adjunctive neurosurgery, and clinical outcomes including length of hospital stay, hospital re-admission after discharge, and 30-day mortality.

Cryptococcosis was defined as clinical features consistent with active *Cryptococcus spp* infection and isolation of *Cryptococcus spp* from a normally sterile site. Cryptococcal meningitis was defined as positive CSF culture for *Cryptococcus spp*. Pulmonary cryptococcosis was defined by a clinically compatible presentation with parenchymal infiltrates or nodules demonstrated on chest radiography or computed tomography for which no other cause was apparent and *Cryptococcus spp* isolated from a normally sterile site. Cryptococcaemia was defined as blood cultures positive for *Cryptococcus spp*. Disseminated cryptococcosis was defined as involvement of two or more non-contiguous sites according to the criteria above.

All isolates were originally identified as *C. neoformans* with the API20AUX system (bioMérieux), which could not distinguish *C. neoformans* from *C. gattii*. In this study, polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to distinguish *C. neoformans* variants from *C. gattii*[16] and to place isolates into one of eight possible molecular types: VN I, II, III, and IV, and VG I, II, III, and IV. A multiplex PCR was used to infer the serotype as serotyping reagents are no longer manufactured.[17] The presence of cryptococcal antigen in the blood and CSF was determined with the use of a cryptococcal latex agglutination system (CALAS; Meridian Diagnostic Inc., Cincinnati, OH, USA). Blood cultures were collected and processed with the use of BACTEC and BacT/ALERT systems. The collection and publication of these data was approved by the institutional ethics review board.

2.1. Statistical analysis

Descriptive statistics (means, standard deviations, median, range, frequency counts) were used to describe the distribution of the variables in the study population of HIV-positive and HIV-negative patients. A Chi-square test or Fisher's exact test was used accordingly to evaluate differences in categorical variables. Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for categorical variables. Crude ORs for continuous variables were obtained using simple logistic regression. Stratified analyses were used for death within 30 days after diagnosis and CSF parameters (white blood cell count (WBC), glucose, and protein). All tests were two-tailed and p-values less than 0.05 were considered statistically significant. The statistical analysis was performed using Stata version 10 (StataCorp LP, College Station, TX, USA).

3. Results

A total of 77 cryptococcal isolates were recovered during the 9-year study period. Medical records were available for 62 patients with 69 positive cultures (Table 1). The mean age was 42 ± 12 years (range 17–76 years). Forty-nine patients (79%) were male. The majority (81%) were infected with HIV with CD4 counts <200 cells/mm^3 (median 20 cells/mm^3, range 2–140 cells/mm^3). All four patients with rheumatologic disease had systemic lupus erythematosus (SLE). Three patients had active SLE on chronic steroid therapy, and the remaining patient presented with neuropsychiatric SLE, pulmonary haemorrhage, and cryptococcaemia. None were on immunobiologicals. Five patients had no apparent underlying medical condition, three of whom had *C. gattii*. The most common symptoms were fever (79%), headache (71%), and cough (45%). On admission 45% were febrile.

On physical examination, 12 patients (19%) had meningism. Nine patients had cranial nerve (CN) palsies, the most common being CN VI palsy, which was present in seven patients. Two patients had hemiplegia. Reduced visual acuity was present in four patients and papilloedema in three. Six patients had cutaneous findings including folliculitis (two patients), eczema, pyoderma gangrenosum, Kaposi sarcoma, and herpes zoster (one patient each).

Chest radiographs were abnormal in 28 (45%) patients (Table 2). Of the abnormal chest radiographs, nine showed coin-like opacities, 18 showed infiltrates, and two had pleural effusions. Brain imaging was performed in 61 patients. Meningeal enhancement occurred in eight patients. Multiple space-occupying lesions suggestive of cryptococcoma were present in five patients and single space-occupying lesions in three. Sixty patients had a CSF examination. The median opening pressure on lumbar puncture was 23 cmH_2O (range 4–50 cmH_2O). The median value for the CSF WBC was 4×10^6 cells/l (range 0–342×10^6 cells/l), glucose was 2.5 mmol/l (range 0.1 to 9 mmol/l), protein was 59 mg/dl (range 16–433 mg/dl), and the cryptococcal antigen titre was 512 (range 0–65 536). The median serum cryptococcal antigen titre was 2048 (range 0–262 144). Blood cultures obtained in our hospital were positive for *Cryptococcus spp* in 29 patients, *Salmonella enteritidis* in two patients, and methicillin-sensitive *Staphylococcus aureus* in one patient.

Cryptococcaemia was diagnosed in 34 patients (55%). Five patients had positive blood cultures for *Cryptococcus spp* at the referring institution, prior to transfer to our hospital. Cryptococcal meningoencephalitis was diagnosed in 55 patients (89%), respiratory involvement occurred in 14 (23%), and disseminated disease in 31 (50%).

All 61 patients diagnosed ante-mortem received antifungal treatment for cryptococcosis (Table 3). Delay in treatment was