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Cryptococcosis caused by *cryptococcus gattii*

2 case reports and literature review

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Abstract

Cryptococcosis caused by *Cryptococcus gattii*, is a life threatening fungal infection with recently increasing prevalence. *C. gattii* is a species complex comprising multiple independent species. However, many biological characteristics and clinical features of cryptococcosis due to *C. gattii* are relatively less well defined. In this paper, we identify 2 cases of *C. gattii* infection, and laboratory findings of genotype VGI and VGII in 2 groups of apparently immunocompetent Chinese individuals respectively. Upon detailed review of all 35 cases of *C. gattii* infections, it was observed that *C. gattii* can cause debilitating illness in both immunocompetent and immunocompromised individuals. Cryptococcosis due to *C. gattii* is a serious systemic fungal infection, with pulmonary central nervous system tropism. Epidemiologically, *C. gattii* infection is not only restricted in tropical and subtropical regions, but also in other geographical settings.

Abbreviations: CGBB agar = canavanine glycine bromothymol blue agar, CNS = central nervous system, CSF = cerebrospinal fluid, CT = computed tomography, MIC = minimal inhibitory concentration, MLST = multilocus sequence typing, MRI = magnetic resonance imaging.

Keywords: antifungal agents, *cryptococcus gattii*, epidemiologically, immunocompetence, infections

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1. Introduction

Cryptococcosis is a prevalent life-threatening fungal pathogen of global ramification with 2 notable etiologies mainly caused by 2 yeast species, *Cryptococcus neoformans* and *Cryptococcus gattii*.[1] Although both species usually cause pulmonary or central nervous system (CNS) infections, they differ in epidemiology, clinical features and pathophysiology.

C. gattii shares major virulence determinants with *C. neoformans*, and was previously thought to be a subtype of *C. neoformans*, but genomic and transcriptomic studies revealed their distinctions. *C. gattii* is now recognized as a unique species.[2] The recent proposal for naming them, based on multilocus sequence typing (MLST), is as follows: *C. neoformans* would be divided into *C. neoformans* (serotype A, VNI/AFLP1 and VNII/AFLP1A, AFLP1B,VNB, formerly *C. neoformans var. grubii*), *C. deneoformans* (serotype D, VNIV/AFLP2, formerly *C. neoformans var. neoformans*), and a *C. neoformans* × *C. deneoformans* hybrid (formerly VNIII/AFLP3 or AD hybrids). *C. gattii* would be recognized as 5 separate species, *C. gattii* (VGI/AFLP4), *C. deuterogattii* (VGII/AFLP6), *C. bacillisporus* (VGIII/AFLP5), *C. tetragattii* (VGIV/AFLP7), and *C. decagattii* (VGIV and VGIIIc/AFLP10), which differ in epidemiology and virulence.[3]

C. neoformans is the most common *Cryptococcus spp.* worldwide and mainly affects HIV/AIDS patients or other immunocompromised hosts. In contrast, *C. gattii* was initially isolated in tropical and subtropical regions, mainly affecting immunocompetent individuals.[4] Since 1999, an unprecedented outbreak of *C. gattii* in the temperate climate of the Pacific Northwest of North America has made people regard it as a fungal pathogen in other geographical settings.[5] Cryptococcosis caused by *C. gattii* has been a subject of increasing concern and become a subject of numerous recent research.

The fungal pathogen *C.gattii* can infect individuals with and without an identifiable immune defect.[6] However, recent studies

revealed that *C. gattii* is being becoming increasingly prevalent in immunocompromised hosts (including HIV/AIDS patients) and is isolated more frequently than previously expected.[7,8] Therefore, in this paper, we report 2 cases of cerebral and pulmonary cryptococcosis caused by *C. gattii* in Chinese individuals, and review 35 cases of *C. gattii* infections published in Pubmed after 2000, providing further details, to improve and contribute to a better understanding of this disease.

2. Case description

Case 1: This patient was a 40-years-old man, with a four-week history of headache and low-grade fever (highest 38.5°C). He was previously healthy and was not taking any medications prior to presentation at the hospital. The patient was a tobacco smoke and occasionally consumed alcohol. He is a resident of Shandong province (temperate zone), without previous travel history and exposure history to timber or animals. On physical examination, both neck stiffness and Kernig's sign were positive.

On laboratory examination, Cryptococcal antigen titer in serum was 1:1024. A lumbar puncture disclosed an elevated opening pressure (260 mm H_2O), and examination of cerebrospinal fluid (CSF) showed predominantly lymphocytic pleocytosis (lymphocytes 377×10^6/L) with low levels of glucose (0.5 mmol/L) and high protein concentration (1.57 g/L). The sediment of the centrifuged CSF, mounted in a drop of India ink revealed encapsulated *Cryptococci* as a halo against a black background. The titer of cryptococcal antigen by enzyme immunoassay of CSF was 1:1024. *Cryptococcus spp* was isolated in CSF standard fungal culture media, showing pure colony with morphology of round yeast cells. Cultures from the CSF grew yeast that we identified as *C. neoformans* by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Then *C. gattii* was initially identified by characteristic cobalt-blue color changes in canavanine glycine bromothymol blue (CGB) agar. Lastly, the cryptococcal strain isolated was identified as *C. gattii* genotype VGIIa using MLST, based on a DNA sequence analysis of a set of polymorphic loci. The brain magnetic resonance imaging (MRI) scan showed meningeal enhancement, and sheet hyperintense lesions in anterior angle of right ventricle.

During the diagnosis, the chest computed tomography (CT) scan revealed irregular nodule with spicules and lobulation in the periphery of the lungs (Fig. 1 A). The lesion was highly suspected to be primary lung cancer. The mass was subsequently resected completely and samples were sent for histology and microbiological studies (Fig. 1 B). Histology demonstrated numerous encapsulated yeasts with clear halos were scattered in the necrotic tissue and granuloma which differ from common *C. neoformans* encapsulated by multinucleated macrophages. The yeast was observed after HE-staining, Mucus carmine stains and Hexammonium silver staining (Fig. 1 C). We identified the strain as VGIIa by mass spectrometry analysis.

Based on these findings, liposomal amphotericin B (0.7–1.0 mg/kg/day) and 5-flucytosine (100 mg/kg/day) were intravenously administered immediately for 2 weeks, and changed to oral fluconazole with 400 mg/day for 1 month, subsequently, fluconazole with 200 mg/day for further maintenance therapy. After 84 days therapy, a repeat lumbar puncture showed normal opening pressure (13 cm H_2O), and the analysis of the CSF showed increased glucose (2.3 mmol/L) and protein (8.83 g/L), and less white blood cells (30×10^6/L). The titre of cryptococcal antigen of CSF decreased to 1:1. Final CSF cultures demonstrated no growth and India ink stain was negative. We followed up the patient and found that he was in good condition and had no recurrence.

Case 2: A 21-year-old man was admitted with chief complaints of severe headache, nausea, vomiting, and general seizures that appeared suddenly. During physical examination, the patient was febrile (39°C) and semi-unconscious. Signs of meningeal irritation (nuchal rigidity and Kernig's sign) and bilateral papilloedema were present. Examination of other systems revealed no obvious abnormality. He was previously healthy and did not have any history of seizure or respiratory manifestations; neither did he have any history of tuberculosis, malignancy, nor any other known medical history. He lived in Fujian province and had no history of travelling or close animal contact.

MRI scan of the head showed meningeal enhancement and multiple abnormal signals in basal nuclei. Lumbar puncture was performed and initial pressure was 33 cm H_2O. Analysis of CSF showed 0.35 g/L protein; 2.49 mmol/L glucose; leukocytes 720×10^6/L. Direct microscopic examination was negative for fungi

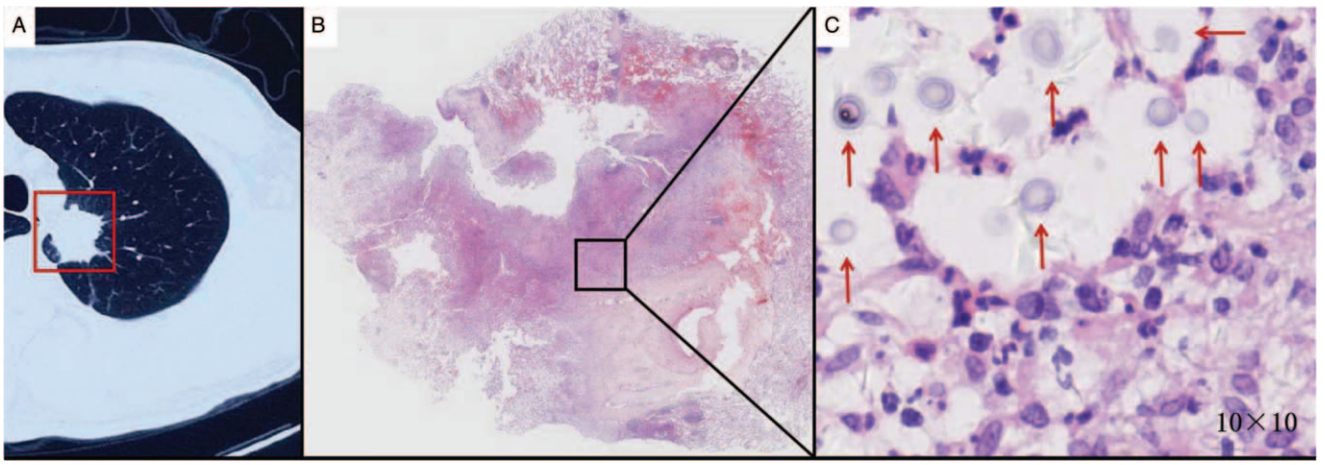

Figure 1. (A) Irregular nodule with spicules and lobulation in the periphery of the lungs in CT. (B) Pathological scanning of excised mass. (C) Histology demonstrated numerous encapsulated yeasts with clear halos were scattered in the necrotic tissue and granuloma.

and bacteria. However, India ink staining and culture of the patients CSF were positive. Phenotypic characterisation of the species was achieved by CGBB agar. Molecular typing was identified as *C. gattii* genotype VGI by mass spectrometry analysis.

Although the patient manifested severe CNS symptoms, he did not have any respiratory manifestations and CT scanning of the chest revealed no abnormalities. Then, antifungal therapy was administered with Liposomal amphotericin B (2 mg-4 mg-7 mg-10 mg/kg/day) and 5-flucytosine (1.5 mg/kg/tid-1 g/kg/q6 hour) intravenously for 2 weeks, then fluconazole (0.4–0.2 mg/kg/day) for 1 week. After 50 days continuous therapy, he regained consciousness. Repeat lumbar puncture showed a decreased opening pressure 20 cm H_2O. CSF analysis showed the following values: 0.24 g/L protein; 3.42 mmol/L glucose; leukocytes 81×10^6/L. But, India ink staining was still positive. Because of the relief of symptoms, he refused taking medications; eventually the infection recurred and led to death.

3. Review and discussion

In this paper, we describe 2 cases of *C. gattii* infection, with genotype VGI and VGIIa respectively, among Chinese immunocompetent individuals. As previously reported, VGII is the genotype most commonly associated with the outbreak in the United States and British Columbia, however, it is not common in other *C. gattii* endemic parts of the world.[9] A global molecular epidemiology analysis of *C. gattii* has shown that, in Asia, VGII genotype is in low percentages (1.7%), where VGI genotype is the most common pathogen (13.2%).[10] We also report of *C. gattii* VGIIa infection in a immunocompetent Chinese individual without a history of recent travel to an endemic area. Additionally, we reviewed 35 cases of *C. gattii* infections in Table 1 to present characteristics and clinical features of cryptococcosis due to *C. gattii*.

C. gattii is a basidiomycetous yeast known to widely exist in the environment, preferentially in soil around various kinds of trees and within animal hosts such as koalas.[10] The risk factors for *C. gattii* infection are not well defined, but several risk factors, such as a contact history of eucalyptus tree and pigeon, and host genetic factors have been reported.[2] In Table 1, among the immunocompetent patients (n = 27), there are 29.6% people having a travel history to an endemic area, 34.6% having a potential contact history, and 25.9% having a history of chronic disease. The data supports that these potential risk factors are very important in *C. gattii* infections. Avoiding these dangerous factors may help people effectively prevent infection.

C. gattii infection frequently presents as a lesion localized in the lung, CNS and rarely skin. Among the 2 Chinese patients we reported, *C. gattii* VGIIa infection has manifested both meningitis and respiratory symptoms, while VGI genotype has only affected CNS. In the 35 reviewed cases, 80.0% patients have shown neurological manifestations, 57.1% lung involvement, 20.0% skin lesions, and 1 seldom seen infection as an intra-abdominal mass.

Usually, *Cryptococcus* spores or yeast cells initially affects the lungs, where they encounter resident phagocytes, including macrophages, neutrophils and dendritic cells. However, *Cryptococcus* possesses several virulence factors including a polysaccharide capsule, melanin production and secretion of various enzymes that aid in evasion of the immune system or enhance its ability to thrive within the phagocyte. Recent studies suggest that *C. gattii* infection could dampen pulmonary neutrophil recruitment and inflammatory cytokine production in immunocompetent hosts.[11] We also have found that *C. gattii* might inhibit macrophages migration and phagocytosis in vitro (data not shown). Although *C. gattii* could be killed by dendritic cells, *C. gattii* capsule blocks surface recognition required for their maturation and dampens the DC-mediated effective Th1/Th17 immune responses.[12–14]*C. gattii* has developed numerous effective strategies to evade the immune system to survive and replicate in the hosts. Sometimes, these strategies also help the pathogen survive from the therapy, and cause recrudescence.

C. gattii can be transmitted by haematogenous dissemination and cross the blood-brain barrier. However, blood culture of the 2 Chinese patients in our study revealed no growth. Similarly, the 35 cases we reviewed have not reported positive infection of *C. gattii* in the patients blood. We speculate that the blood streaming movement is not fit for the growth of this pathogen. The titer is too low to detect in the blood. Moreover, *C. gattii* presents a predilection for the CNS, particularly the meanings. Maybe that is why so many cases only manifested CNS symptoms.

Cryptococcal meningoencephalitis is the most severe clinical manifestation caused by *C. gattii*. In some settings, *C. gattii* tend to produce more severe CNS manifestations compared with *C. neoformans*.[15,16] Headache, vomiting, and neck stiffness are the common neurological manifestations. However, intracranial hypertension, resulting from meningeal inflammation and cerebral oedema often produces irreversible damage of cranial nerve, even can be life threatening. To decrease a potentially harmful inflammatory response, in some cases, early use of dexamethasone is recommended.[17,18] And if necessary, serial lumbar punctures should be considered as an adjunct to antifungal therapy.[19,20] Moreover, *C. gattii* tends to be resistant to antifungal drugs, so it requires lengthy antifungal treatment, particularly in infections of the CNS, the aggressive management of increased intracranial pressure along with percutaneous lumbar drainage.[21]

Skin involvement has been described in disseminated forms and in primary cutaneous infections caused by *C. neoformans*. Cutaneous cryptococcosis caused by *C. gattii* is rare, and may be one of the first manifestations as well as the only manifestation of disseminated cryptococcosis. Most of the patients with cutaneous lesions reviewed in Table 1 have a history of injury or contact to animals. *C. gattii* commonly affects the face and neck with different morphologies including papules, pustules, plaques, ulcers, subcutaneous masses, and cellulitis or acneiform lesions. Diagnosis of it relied on skin histopathology and cultures for fungi. Majority of the patients with cutaneous involvement caused by *C. gattii* had a favorable outcome. Interestingly, a localized intra-abdominal cryptococcal mass due to *C. gattii* has been detected in a type 2 diabetic HIV-negative patient.[22] These prompt again that Cryptococcosis due to *C. gattii* is a serious systemic fungal infection.

Amphotericin B, 5-flucytosine and fluconazole are commonly used for antifungal therapy. Antifungal therapy courses during induction often should be extended for at least 6 weeks with close imaging monitoring to assure improvement and resolution. Majority of relapses are due to inadequate doses or duration during induction or maintenance.[55] VGII was more resistant to antifungal agents. In Bahia, *C. gattii* isolates that were significantly resistant to fluconazole.[56] Recent one research indicated that antifungal agents exhibited higher MICs against isolates of genotype VGII than genotype VGI.[57] It is suggested

Table 1

Overview of cryptococcosis due to *C. gattii* infection in Immunocompetent and Immunocompromised patients (n=35).

Year/ Reference	Age/ Gender	City/Country (Region)	Immunity	History(travel/contact/ history)	Infection position			Genotype	Treatment	Outcome
					CNS	Lung	Skin			
2000/[23]	47/M	Singaporean in China	Immunocompetent	pigeon contact history; travel history of Bangkok, Thailand, Kuala Lumpur, Malaysia	+	+	–	NR	AB (0.4 mg/kg/day) + flucytosine (150 mg/kg/day) iv for 3 weeks, Fl (400 mg/kg/day) for maintenance therapy	Improved slightly
2002/[24]	65/M	Brazil	Immunocompetent	No	–	-	+	serotype B	Fl (150 mg/3 capsules/day)	complete cure within 45 days
2003/[25]	60/M	Span	Immunocompetent	D2M for 2 years; parrot contact history	+	–	–	VGI, serotype B	AB (200 mg/day), AB (400 mg/day)+Fl (400 mg/12hour)+5F (2.5 g/6hour)	Improved
2005/[26]	46/M	Brazil	Immunocompetent	No	+	+	+	NR	AB (0.7 mg/kg/day)+5F (100 mg/kg/day) for 2 weeks, then oral Fl (400 mg/day)	Improved
2006/[27]	53/F	Switzerland	Immunocompetent	travel history of Vancouver Island	+	–	–	VGII, serotype B	AB (60mg/day) iv for 2 weeks, oral Fl (2x800 mg/day)	Improved
2006/[28]	36/M	Thailand	Immunocompetent	No	+	–	–	NR	AB (0.7 mg/kg/day)+flucytosine (100 mg/kg/day)	Improved
2006/[29]	45/F	Alberta, Canada	Immunocompetent	Travel history of Vancouver Island	+	+	-	serotype B	AB (1 mg/kg/day), flucytosine (25 mg/kg/6hourr–800 mg/day)+AB (0.7 mg/kg/day)	Improved
2007/[30]	44/M	Japan	Immunocompetent	hyperglycemia for 3 years	+	+	–	VGIIa, serotype B	Oral Fl (400 mg/day) for 1 year	Healed after one year therapy
2008/[31]	46/M	Southeastern United States	Immunocompetent	Regular exposure to birds; travel history of San Francisco and Western Europe	+	+	+	VGI, serotype B	AB+flucytosine, oral Fl (800 mg/day)	Improved
2009/[32]	37/ M	Southern Italy	Immunocompetent	travel history of Toronto and Montreal, Canada	+	+	–	VGI	AB+5F(3, 100 mg/kg/day)+Fl (800 mg/day) for 2 weeks, then Fl (400 mg) for maintenance	completely recovered
2010/[22]	51/M	Brazil	Immunocompetent	Controlled D2M	–	–	–	NR	AB (1 mg/kg/day)+Fl (800 mg/day)	Improved
2010/[33]	54/M	Peru	Immunocompetent	contact history of jungle, hens and guinea pigs	+	–	–	NR	AB (0.7 mg/kg/day) for 11 days, then Fl (450 mg/day)	Improved
2011/[34]	56/M	Villa Clara, Cuban	Immunocompetent	A moderate smoker; travel history of Honduras and Guatemala	+	+	–	VGIII	AB (0.7 mg/kg/day) iv+Fl (400 mg/bid) orally	Died
2011/[35]	37/M	Chinese in Singapore	Immunocompetent	traumatic history of plank	–	–	+	NR	—	NR
2011/[47]	18/F	Canada	Immunocompetent	Coinfection with tuberculosis	+	+	–	VGIIa	AB+Fl	Healed
2013/[36]	30/M	NR	Immunocompetent	No	+	–	–	NR	AB (1 mg/kg/day)+Fl (100 mg/kg/day) for 2 weeks, then Fl (800 mg/day) for 8 weeks	Improved
2014/[37]	62/M	Cuiaba, Brazil	Immunocompetent	a contact history of peridomicile and bat	+	–	–	VGII	AB (0.8 mg/kg/day) 2 days	Dead
2014/[38]	68/M	Brazil	Immunocompetent	Contact history of canaries and parakeets; chronic smoker and heavy beer drinker	–	–	+	VGI	—	Healed
2015/[39]	39/M	Japan	Immunocompetent	Smoke for 15 years; drink occasionally; a travel history of Hawaii, Dalian and Hong Kong	+	+	–	VGIIa	AB (5MG/KG/DAY)+Fl (6000 mg) for 8 weeks	Improved

(*continued*)

Table 1
(continued).

Year/ Reference	Age/ Gender	City/Country (Region)	Immunity	History(travel/contact/ history)	Infection position			Genotype	Treatment	Outcome
					CNS	Lung	Skin			
2015/[40]	33/M	NR	Immunocompetent	wood handling	+	+	+	NR	AB (50 mg/day)+Fl (450 mg/12hour) for 4 weeks, Fl (300 mg/week) for 8 weeks	Improved
2015/[49]	40/M	Illinois/ USA (temperate)	Immunocompetent	No	+	ND	–	VGI	EVD+AB+5F 2weeks iv Then Fl (800 mg) iv 8weeks FL (200 mg) po maintenance	Lost to follow-up
2016/[41]	Middle-aged/M	Southeastern United States	Immunocompetent	cocaine use	+	+	–	NR	AB+Fl	Improved
2016/[42]	65/M	Brazil	Immunocompetent	arterial hypertension, depression and thyroidectomy for nodular thyroid disease	+	+	–	VGII, serotype B	AB (60 mg/day)+Fl (800 mg/day)	Improved
2017/[51]	66/F	Thailand (NR)	Immunocompetent	Hypertension	+	–	–	VGI	EVD +AB (0.7 mg/kg/day)+Fl (800 mg) iv for 2 weeks, FL (200 mg) po for 6 months	Improved
2018/[52]	42/F	USA	Immunocompetent	Immigrated to the USA from Fiji 28 years prior; returned to Fiji for a 1 month in the last year.	+	+	–	VGIII	EVD+liposomal amphotericin B (5 mg/kg/day) and oral flucytosine (25 mg/kg po q6 hour) for 2 months, maintained on high-dose Fl for 2 months	Dead
2018/[53]	24/M	Quebec/Canada	Immunocompetent	Working in a factory producing frozen meals for the previous 3 years.	+	–	–	VGI	amphotericin B deoxycholate and 5-flucytosine (5-FC), then Fl 400 mg daily was continued for 18 months	recovered
2018[53]	20/F	Quebec/Canada	Immunocompetent	autosomal dominant osteopetrosis type 2	+	+	–	VGIIa	EVD + liposomal amphotericin B and 5-FC for 6 weeks, then 800 Fl for 6 months, then 200 mg for 10.5 months	complete resolution
1996[43]	31/M	Greece	Immunocompromised	HIV positive (diagnosed in 1992)	+	–	–	serotype B	AB (40 mg/day)+Fl (10 g/day)	Dead
1998/[43]	26/F	Greece	Immunocompromised	Drug use history of corticosteroid and cytotoxic for SLE	–	+	–	serotype B	AB (20 mg/day)+Fl (10 g/day)	Dead
1999-2000/[44]	59/M	Southern Brazil	Immunocompromised	lung transplantation in1998; smoker;	+	+	+	NR	AB (0.5 mg/kg/day)+Fl (37.5 mg/kg/day) for 1 month, Fl (400 mg/day)	Dead
2000/[45]	32/M	United Kingdom	Immunocompromised	HIV positive; 4-year stay in South Africa	+	–	–	NR	AB (0.7 mg/kg/day)+Fl for 10 days, Fl (400 mg/day)	Improved
2009/[46]	66/M	Southeastern Brazil	Immunocompromised	pigeon contact history; COPD for 20 years, chronic corticotherapy	+	+	–	NR	Fl (400 mg/day)	Healed after eight months therapy
2014/[48]	44/M	Formosa Province, Argentina	Immunocompromised	kidney transplant	+	+	–	VGI	Fl (800 mg/day), AB (1 mg/kg/day), Fl (800 mg/day)	Improved
2017[50]	76/F	Manchester /UK	Immunocompromised	10 years ago, to Cyprus and France; to 3 years ago to Spain (NHL, Ibrutinib+ chemotherapy)	_	+	_	NR	Lobectomy	Healed without therapy for 7 months
2018[54]	51/M	China	Immunocompromised	HIV 6 years+ chronic kidney disease	+	+	–	VGII	800 mg Fl for 2 months, 400 mg Fl for 20months	NR

5F = 5-Flucytosine, AB = amphotericin B, D2M = type 2 diabetes, EVD = external ventricular drain, Fl = fluconazole, ND = not done, NHL = non-Hodgkin lymphoma, NR = not represented,

that voriconazole, posaconazole and other azoles could be used for weeks to months (average 6 months) after the induction phase of treatment with amphotericin B-based formulations. The 2 Chinese patients infected with *C. gattii* have both improved after antifungal therapy with liposomal amphotericin B, 5-flucytosine, fluconazole, mannitol, and dexamethasone. However, the patient with genotype VGI got a worse outcome, and India ink staining of the CSF after therapy was still positive. Actually, the minimal inhibitory concentration (MIC) for all those drugs of the patient with genotype VGI was lower than that of the patient with genotype VGIIa. The different treatment outcomes may also depend on the host factors, strain of the pathogen and the timing of initiation treatment.

4. Conclusion

gattii infection can occur in not only tropical and subtropical regions, but also other geographical settings. *C. gattii* can affect both immunocompetent as well as immunocompromised individuals and result in a substantial systemic fungal infection, mainly in the lungs and the CNS. But we can not rule out other organ lesions, especially those only with cutaneous involvement and abdominal mass. Specimen culture and genomic analysis are considered as the gold standard for diagnosis. *C. gattii* infection requires lengthy anti-fungal treatment, particularly in infections of the CNS. Cerebrospinal fluid turning negative should be the ultimate treatment target. In this paper, the number of cases is small, only a basic descriptive analysis, no more statistical analysis. In the future, we will expand the sample size to explore the relationship between Cryptococcus genotyping and symptoms, as well as the mass spectrum characteristics of different types of Cryptococcus.

Author contributions

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