Central Nervous System Cryptococciosis due to Cryptococcus gattii in the Tropics

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Abstract
Purpose of Review Cryptococcosis of the central nervous system due to Cryptococcus gattii species complex is a serious mycosis with worldwide distribution but of great importance in the tropics. This article aims to review the progress made in these regions in the knowledge of this disease and its etiological agent.

Recent Findings They can be summarized in the presence in apparently immunocompetent patients of autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF), which is a hidden risk factor for acquiring C. gattii infection; this finding strengthens the concept that C. gattii is an opportunistic pathogen. A greater knowledge of the clinical and molecular epidemiology of C. gattii infection and of the different environmental niches of this fungus in the tropics. The discovery of a new lineage of C. gattii, VGV, in environmental samples from Africa. Until now, the COVID-19 pandemic has not meant an increase in cryptococcosis cases.

Summary Advances have been made in the identification of risk factors for cryptococcosis due to C. gattii as well as in the knowledge of its etiological agent and its relationship with the environment. Remarkably, there have been no significant achievements in diagnosis and treatment notwithstanding the documented importance.

Keywords Cryptococcus gattii species complex · Cryptococcosis · Meningitis · Encephalitis · Cryptococcoma · AIDS

Introduction
Cryptococcosis is a systemic mycosis caused by two fungal species complexes Cryptococcus neoformans and Cryptococcus gattii, belonging to the Basidiomycota phylum. They are easily distinguishable from other pathogenic yeasts due to the presence of a polysaccharide capsule, which constitutes one of their main studied virulence factors. The most serious and frequent clinical form of presentation is meningoencephalitic and is preceded by the inhalation of infective propagules that inhabit the environment, reach the lungs, and subsequently spread to the central nervous system (CNS) [1].

History
In 1894, Sanfelice isolated C. neoformans from peach juice for the first time, and in the same year, Busse and Buschke described the first case of cryptococcosis in a woman with a bone lesion. For decades, pathogenic isolates of Cryptococcus were treated as a single species: C. neoformans; however, the heterogeneity between the isolates led in the 1960s to the recognition of four serotypes (A, B, C, and D) based on polysaccharide epitopes of the capsule. The discovery of two different teleomorphs, Filobasidiella neoformans (anamorph: C. neoformans) and Filobasidiella bacillispora (anamorph: C. gattii) confirmed the species split, which has been subsequently verified by genome studies [1]. In 2002, isolates with serotypes B and C were classified as C. gattii and serotypes A, AD, and D as C. neoformans [2].

The first case of cryptococcosis due to C. gattii was described in 1970 in an African child with leukemia who developed meningitis [3, 4]; however, already at the end of the nineteenth century, Curtis described an isolate from a lumbar tumor mass of a young man. The isolate was recovered in cultures and inoculated in animals where the
subcutaneous lesion reproduced (Fig. 1). Curtis named it *Saccharomyces subcutaneous tumefaciens* that was later identified as *C. gattii* [5, 6].

In this century, analysis of the two species using molecular typing methods such as simple restriction fragment length polymorphism (RFLP) analysis to DNA and PCR fingerprinting, amplified fragment length polymorphism (AFLP), multilocus microsatellite typing (MLMT), multilocus sequence typing (MLST), and whole-genome sequence typing (WGST) has shown that both species *C. neoformans* and *C. gattii* contain genetically different lineages, leading to consider them as two species complexes. These in turn have been subdivided into numerous molecular types. Until now, four main lineages have been recognized for *C. gattii* called VGI/AFLP4, VGII/AFLP6, VGIII/AFLP5, and VGIV/AFLP7 [7]. Recently, a new lineage, VGV, has been identified in environmental isolates in Zambia, Africa [8]. Different names have been proposed for the 4 main lineages: *C. gattii* (VGI), *C. deuterogattii* (VGII), *C. bacillisporus* (VGII), and *C. tetragattii* (VGIV) [9]. This taxonomy is controversial because the known genotypes of *C. gattii* have revealed greater diversity than the 4 proposed species, which will lead to continued instability in the nomenclature. In the absence of biological differences between the clades and of consensus about which DNA sequence represents a species, a group of researchers has recommended continuing to use the term *C. gattii* species complex; this also applies to *C. neoformans* [10].

**Epidemiology**

Meningoencephalitic cryptococcosis is very frequently associated with advanced HIV infection, and an annual incidence of 223,000 cases and 180,000 deaths is estimated in this population, most of whom live in sub-Saharan Africa [11].
Globally, it is estimated that *C. neoformans* causes 95% of cases of cryptococcosis and *C. gattii* the remaining 5% [12]. For decades, it has been accepted that *C. neoformans* has a global distribution and that it especially affects immunosuppressed patients, the vast majority HIV infected and that *C. gattii* is distributed in tropical and subtropical areas and mainly affects individuals without HIV infection, with a significant percentage of apparently immunocompetent patients. In relation to *C. gattii*, this situation changed in 1999 with the appearance of the cryptococcosis outbreak in the Vancouver region, Canada, a temperate region, caused by the VGII lineage [13] and by the subsequent observation of cases of cryptococcosis due to *C. gattii* in the Pacific Northwest of the USA [14].

To date, it is known that the distribution of *C. gattii* is more global although minor compared to *C. neoformans*. Cases of *C. gattii* infections have been reported for all molecular lineages in a wide range of regions including Australia, Papua New Guinea, South America, Southeast Asia, Central and Southern Africa, the USA, and Canada. Sporadic cases have been described in Europe, most of them imported. However, there is evidence of the emergence of *C. gattii* in Europe in the last two decades, especially in Portugal, France, and Southern Italy [15].

*C. neoformans*, with a global distribution, causes disease in both immunocompetent and immunosuppressed hosts; however, most reported cases show compromise of the immune system, mainly due to infection by HIV but also due to the use of corticosteroids or other conditions that produce immunosuppression. For this reason, *C. neoformans* is known as an opportunistic pathogen, although there are countries such as China, Korea, and Japan where most of the *C. neoformans*-infected patients are not infected by HIV [1]. Until a few years ago, studies showed that *C. gattii* more frequently affected individuals without known risk factors, regardless of geographic region and was therefore considered a primary pathogen [1]. Significantly, HIV-infected patients rarely present cryptococcosis due to *C. gattii*, except for lineages VGIII and VGIV, a situation well known in Africa [16]. Infection of apparently immunocompetent individuals with *C. gattii* was thought to be the result of increased environmental exposure to the fungus and was based on the high prevalence of *C. gattii* infection in rural Aboriginal Australians, where they are daily exposed to the natural reservoir of this fungus, such as eucalyptus and other trees, organic debris, and other soil components. This concept began to change when it was shown that patients from British Columbia, Canada, in addition to their environmental exposure, had medical conditions that caused immunosuppression such as steroid use, chronic obstructive pulmonary disease and other noninfectious lung pathologies, and pneumonia [17]. Despite the high exposure to *C. gattii* in endemic regions such as South America, Southeast Asia and Vancouver, Canada, the number of cases of cryptococcosis is very low, and in a tropical country such as Colombia, the annual incidence is less than one case per million [18].

This has led to the belief that certain risk factors must exist in these individuals, which allow the appearance of the disease. New evidence has been obtained in recent years, and it has been detected that a significant percentage of apparently healthy patients have autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF) [19, 20••]. This factor is essential for the differentiation of monocytes into macrophages and in modulating the immune response [21]. Other subtle defects in immunity are likely to be risk factors for *C. gattii* cryptococcosis in apparently healthy individuals. In short, it is time to consider *C. gattii* as an opportunistic pathogen [21].

Regarding the clinical manifestations, the high percentage of patients in Papua New Guinea with meningitis due to *C. gattii* who lost their vision initially drew attention [22], a fact that we can attribute to the lack of aggressive management of intracranial hypertension, which is very common in these patients [23]. The first clinical studies from Australia and New Zealand on cryptococcosis due to *C. gattii* showed, in addition to the state of apparent immunocompetence, the higher frequency of pulmonary and cerebral cryptococcomas, lower mortality but greater neurological sequelae that required surgery, and prolonged therapy [24–26].

The appearance of the COVID-19 pandemic has generated an increase in the diagnosis of some systemic mycoses such as aspergillosis and mucormycosis [27]; the latter has occurred with an unusually high frequency in India [28]. The occurrence of these mycoses has been favored by different factors or comorbidities associated with COVID-19, such as hypoxemia, diabetes mellitus, steroid use, increased ferritin and free serum iron levels, mechanical ventilation, and CD4 lymphopenia (total cells, CD4+ and CD8+). However, only a handful of cases of cryptococcosis associated with COVID-19 have been described (two of them in the tropics), and no *C. gattii* infection has been reported [27].

Progress has been made in the knowledge of the molecular epidemiology of the etiological agents of cryptococcosis in Latin America [29, 30]. In this region, *C. neoformans* molecular-type VNI is the main cause of this mycosis (76%), followed by *C. gattii* molecular-type VGII. Africa, the continent with the highest number of cases of cryptococcosis in the world has the lowest proportion of isolates subjected to molecular typing [31]. Table 1 shows the distribution of molecular types of *C. gattii* isolates in Latin America and Africa. In India, *C. neoformans* VNI also predominates, and the representation of *C. gattii* is scarce (1.4% of the isolates) and with the molecular patterns VGI and VGIV [32].

A recent publication [33] of the study of strains obtained before 1975 showed that the HIV pandemic altered both the molecular epidemiology and the virulence of *C.
 neoformans/C. gattii species complexes. Before the pandemic, VNI predominated to a lesser extent (64%) and VGIII (7.5%). Overall, high genetic variability and recombination rates were found for the pre-HIV-pandemic era among strains of the C. neoformans/C. gattii species complexes.

Ecology

The natural habitat of C. gattii is trees. Since the end of the last century, the association of serotype B molecular pattern VGII with eucalyptus (Eucalyptus camaldulensis) in Australia [34] and serotype C molecular pattern VGIII with almond trees (Terminalia catappa) in Colombia [35] was established. Since then, an increasing number of trees have been identified as environmental reservoirs of C. gattii, many of these studies have been carried out in Latin America [29, 30] and in Africa [31]. Extensive environmental sampling in Zambia, Africa, allowed to recognize a new lineage of C. gattii, the molecular pattern VGV [8•].

The study of household dust in the Rio Negro microregion in the Brazilian Amazon suggests that humans may be exposed to the agents of cryptococcosis (C. neoformans VNI and C. gattii VGII) and that this continuous exposure could lead to the development of clinical or subclinical infections [36, 37].

Genomic analyzes have pointed to the origin of C. gattii VGII in the South American Amazon rainforest [38, 39] while the origin of C. neoformans is in Africa [40, 41]. It is presumed that these two species separated about 80–100 million years ago [41]. The study of environmental isolates of C. gattii and its relationship with the different regions of the world has been recently reviewed [42].

It has been postulated that global warming in recent years has significantly affected the distribution of C. gattii VGI in the European Mediterranean basin. Niche modeling using Maxent analysis showed a gradual expansion of the fundamental niche that has been more noticeable since 2010. This model predicts that the distribution of this pathogen will increase its distribution area towards more internal areas of the continent [43].

Diagnosis and Treatment

The diagnosis of meningeal cryptococcosis is made by identifying the fungus in the cerebrospinal fluid on direct examination using India ink exclusion, by determining the capsular antigen by lateral flow test, by detecting nucleic acid by PCR, and by cultivation in media for fungi and even in basic bacteriology media such as blood agar. Once the isolation is obtained, the differentiation of C. gattii from C. neoformans is easily done with the use of the canavanine glycine bromothymol blue (CGB) agar, a medium in which C. gattii grows, but C. neoformans not. Molecular methods allow a more precise identification indicating molecular lineages [44]. Also, proteomics with the MALDI-TOF-MS test allows differentiating species in a fast, sensitive, and less expensive way than molecular tests [45].

The treatment of meningeal cryptococcosis in patients with HIV, where the vast majority are caused by C. neoformans, has been established through controlled studies carried out in Africa, a continent where thousands of cases of this disease occur, which makes possible the conducting of these studies [46, 47]. In contrast, the treatment of C. gattii meningitis is based on extrapolation from studies of AIDS patients [48] and on retrospective case series studies [49] (Table 2) or expert recommendations [50]. Basically, the treatment of CNS cryptococcosis is based on 3 antifungals: amphotericin B, 5-fluorocytosine, and fluconazole. In many tropical countries, fluocytosine or amphotericin B is not available, which makes treatment difficult. In addition, amphotericin deoxycholate is used, which is more toxic and difficult to handle, due to its lower cost than liposomal presentations. Prospective studies are needed to determine the best antifungal treatment for CNS cryptococcosis due to C. gattii. Cerebral cryptococomas, which are more frequent in patients with C. gattii, require longer therapies [48].

| Region          | Source  | VGI | VGII | VGIII | VGIV | Total |
|-----------------|---------|-----|------|-------|------|-------|
| Latin America   | Clinical| 60  | 492  | 93    | 10   | 655   |
|                 | Environmental| 34  | 350  | 90    | 11   | 485   |
|                 | Veterinary | 1   | 14   | 1     | -    | 16    |
|                 | Total     | 95  | 856  | 184   | 21   | 1156  |
| Africa          | Clinical  | 17  | 11   | -     | 55   | 83    |
|                 | Environmental | 12  | -    | -     | -    | 12    |
|                 | Veterinary | -   | -    | -     | 7    | 16    |
|                 | Total     | 29  | 11   | 1     | 56   | 96    |

*Six environmental isolates of the molecular type VGV from Zambia, Africa.
Aggressive management of intracranial hypertension, which occurs in most patients, is necessary to improve patient survival. The recommendation of repeated lumbar punctures and in refractory cases of cerebrospinal fluid diversion also comes from observational studies [49]. In summary, no new advances have been made in the antifungal treatment of C. gattii CNS cryptococcosis. Recently, neurocritical and neurosurgical management of C. gattii cryptococcosis has been emphasized due to frequent intracranial hypertension [51]. These patients do not present the classic radiological signs of increased intracranial pressure such as ventriculomegaly, cerebral edema, or effacement of the basal cisterns. Therefore, the diagnosis is based on symptoms such as disabling headache, progressive vision loss, and papilledema and is confirmed by measuring the opening of the cerebrospinal fluid during lumbar puncture. The intraparenchymal deposition of the abundant capsular polysaccharide causes poor brain compliance, which leads to the so-called frozen brain state. If we want to reduce mortality and improve clinical outcomes, early diagnosis, antifungal therapy, and aggressive management of intracranial hypertension are necessary [51]. The benefit of steroids in these patients remains to be demonstrated. Traditionally, cryptococcosis management guidelines did not consider it necessary to establish the species of Cryptococcus in clinical practice [48, 52, 53]. The most recent guidelines recommend doing this speciation in the clinical laboratory due to the differences in the morphology, biology, and phylogenetics of the two species [54, 55]. Mortality and sequelae of CNS cryptococcosis due to C. gattii remain high (20.2%) [56]. The limitations in the diagnosis and treatment of this serious mycosis in many tropical countries mean that the poor outcomes are perpetuated.

However, there is renewed interest in the development of new antifungal drugs whose targets are essential genes or cellular functions, the blocking of virulence factors or the inhibition of the stress-response signaling, the use of drug combinations, and the development of therapeutics immunomodulatory and vaccines [57•]. We hope that in the near future the fruit of these investigations will be beneficial for patients affected by cryptococcosis.

### Conclusion

Progress has been made in the knowledge of C. gattii and in the epidemiological and ecological aspects of cryptococcosis. Unfortunately, no significant progress has been made in the management of patients with CNS cryptococcosis, a situation that is more evident in the tropics.

### Declarations

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

**Conflict of Interest** The authors declare no competing interests.

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- Of importance
- Of major importance

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