Original article

Successful treatment of Cryptococcus gattii neurocryptococcosis in a 5-year-old immunocompetent child from the French Guiana Amazon region

Anne Debourgogne a, b, Ferry Hagen c, d, Narcisse Elenga e, Laurence Long e, Denis Blanchet a, Vincent Veron a, Olivier Lortholary f, g, h, Bernard Carme a, Christine Aznar a, ∗

a Laboratoire Hospitalier et Universitaire de Parasitologie-Mycologie, CH André de Rosemon et et EA 3593, Faculté de Médecine, Université des Antilles et de la Guyane, Cayenne, Guiana
b Service de Parasitologie-Mycologie, CHU de Nancy, Vandoeuvre-lès-Nancy, France
c Department of Yeast and Basidiomycete Research, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
d Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands
e Service de Pédiatrie, CH André Rosemon, Cayenne, Guyana
f Institut Pasteur, Unité de Mycologie Moleculaire, Centre National de Référence Mycologie et Antifongiques, Paris, France
g CNRS URA3012, Paris, France
h Université Paris Descartes, Hôpital Necker-Enfants Malades, Service de Maladies Infectieuses et Tropicales, Centre d’Infectiologie Necker-Pasteur, Paris, France

A R T I C L E  I N F O

Article history:
Received 26 September 2011
Accepted 31 January 2012
Available online 22 February 2012

Keywords:
Neurocryptococcosis
Cryptococcus gattii
Children
French Guiana

A B S T R A C T

Compared to the incidence in adults, cryptococcosis is rare among children. We report a case of neurocryptococcosis due to Cryptococcus gattii in a five-year-old girl without identified risk factors living in French Guiana. Neurological surgery in combination with long-term antifungal treatment with amphotericin B and 5-flucytosine successfully resolved the cryptococcal infection. Subsequent molecular characterization of the Cryptococcus isolate revealed that the infection was caused by a C. gattii genotype AFLP6B/VGIIb strain.

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A R T I C L E  I N F O

Palabras clave:
Neurocryptococcosis
Cryptococcus gattii
Niños
Guyana francesa

R E S U M E N

Comparado con la incidencia en pacientes adultos, la criptococosis es una infección excepcional entre niños. Describimos un caso de neurocryptococosis debida a Cryptococcus gattii en una niña de 5 años de edad, sin factores de riesgo identificados, que vivía en la Guyana francesa. La cirugía neurológica combinada con un tratamiento antimicótico prolongado con anfotericina B y flucitosina (5-flucytosina) resolvieron satisfactoriamente la infección. La caracterización molecular posterior del aislamiento de Cryptococcus reveló que la infección se debió al genotipo AFLP6B/VGIIb de C. gattii.

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Cryptococcosis is a potential life threatening fungal infection that affects humans and animals. Cryptococcal infections are mainly caused by the pathogenic basidiomycetic yeast species Cryptococcus neoformans and Cryptococcus gattii. 2, 25 The former causes disease mainly among immunocompromised subjects, such as HIV-infected patients or those who underwent organ transplantation, while C. gattii has a predilection to infect apparently immunocompetent individuals. 2, 26 C. neoformans has a worldwide distribution pattern but the geographical distribution of C. gattii has changed in the last decade. 2, 26 Initially, this yeast occurred more frequently among humans and animals in tropical and subtropical regions, but an
ongoing outbreak emerged a decade ago on Vancouver Island, British Columbia (Canada), and has subsequently expanded to the mainland of Canada and the Pacific Northwest.\textsuperscript{1,5,19}

Detailed genotyping of \textit{C. gattii} outbreak strains revealed that a previously rare genotype, named AFLP6/VGII, was the cause of this unprecedented outbreak. This is one of the five genotypes within \textit{C. gattii} (AFLP4/VGI, AFLP5/VGIII, AFLP6/VGII, AFLP7/VGIV and AFLP10/VGIV) that can be discerned from each other using molecular biological techniques such as M13 PCR fingerprinting, PLB1 and URA5 Restriction Fragment Length Polymorphism fingerprinting (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Multi-Locus Sequence Typing (MLST).\textsuperscript{2,4,15,22}

Treatment options for cryptococcal infections are dependent on the severity and localization of the infection. Recently updated treatment guidelines from the Infectious Diseases Society of America (IDSA) recommend treatment of severe cases of cryptococcosis in immunocompetent and immunocompromised patients using induction therapy for two weeks with a combination of amphotericin B and 5-flucytosine, followed by ten weeks of consolidation therapy using fluconazole.\textsuperscript{24} These updated IDSA guidelines contain recommendations for several patient categories, such as those infected with \textit{C. gattii} or children, and describe therapeutic variations on the generally recommended treatment strategy.\textsuperscript{24} Compared to \textit{C. neoformans}, infections with \textit{C. gattii} might need a more aggressive antifungal therapy due to the higher probability of severe neurological complications and possible delayed response to used antifungal compounds.\textsuperscript{24} It has been observed that in vitro antifungal susceptibilities significantly differ between genotype AFLP4/VGI and AFLP6/VGII \textit{C. gattii} strains, which might affect the outcome of antifungal therapy, and which is an indication that in vivo differences may exist.\textsuperscript{15}

Cryptococcal infections in children are rarely reported and are outnumbered by the large number of adult patients. In most countries where \textit{C. gattii} is endemic, this disease rarely occurs in children except in North-Eastern and Northern regions of Brazil. A recent Brazilian study of paediatric cases shows that cryptococcal infections are more frequently observed in middle childhood, and that \textit{C. gattii} infections were all characterized by CNS infection.\textsuperscript{25}

The current case report describes a severe case of neurocryptococcosis due to \textit{C. gattii} in a five-year-old girl living in a remote village in French Guiana. Neurological surgery in combination with antifungal treatment with amphotericin B and 5-flucytosine, followed by long-term treatment using fluconazole, successfully resolved the cryptococcal infection. Subsequent molecular characterization of the \textit{Cryptococcus} isolate revealed that the infection was caused by a \textit{C. gattii} genotype AFLP6B/VGIIb strain.

Case report

A five-year-old girl, living in an Amerindian village (Wayampi and Emerillon tribe) located in the Amazon forest along the Oyapock river at the Eastern border of French Guiana and Brazil, presented at the local field hospital with acute gastro-enteritis, fever and weight loss. During the past 2-3 months, she showed signs of asthenia and irregular febrile seizures without aetiology. The patient was rehydrated and treated with ceftriaxone; however, there were no signs of improvement. Therefore, she was transferred to the paediatric unit of the Cayenne hospital two days later.

Diarrhoea and vomiting were cured by symptomatic treatment, but fever persisted. A few days later, she presented two seizures of vomiting with convulsions, behaviour disorders and left facial paralysis. Due to the deterioration, a cerebral CT scan and a lumbar puncture were performed. The CT-scan did not reveal any anomalies. However, the CSF had a slightly turbid appearance and direct examination of an Indian ink-stained sample showed abundant presence of encapsulated yeasts suggestive of a \textit{Cryptococcus} infection.

CSF showed an elevated glucose concentration of 1.6 mmol/L (reference value: 1.1–1.2 mmol/L), protein level of 0.63 g/L and a lactic acid concentration of 6.3 mmol/L. CSF was found to contain two WBCs per mm\(^3\) and no RBCs. Cryptococcal infection was confirmed by the detection of cryptococcal antigen in the CSF (Pastorex\textsuperscript{13}M CryptoPlus, Bio-Rad, Marne-la-Coquette, France), with a titre of 1:200. CSF cultures on Sabouraud-chloramphenicol-gentamicin agar (Bio-Rad, France) were positive after 48 h incubation at 30 °C and 37 °C. Cryptococcus colonies were observed as being round cream-colored mucoid colonies.

At the time of diagnosis of cryptococcal infection, the girl was somnolent and presented with an increasing frequency of respiratory pauses. She was directly admitted to the intensive care unit where she received antifungal treatment with amphotericin B deoxycholate (paediatric dose of 1 mg/kg QD) and 5-flucytosine (paediatric dose of 25 mg/kg QID) in parenteral form. Examinations included blood cultures, which were found to be negative, and serum cryptococcal antigen tests that yielded a titre of 1:2520. With an improved clinical state, she was transferred back to the paediatric care unit where her CSF was directly examined microscopically, as well as by culture and antigen agglutination, on days 1, 8, 15 and 30, post-diagnosis of the cryptococcal infection. On day 1, CSF was found to contain encapsulated spherical budding cells, and a cryptococcal antigen titre of 1:200 was observed. CSF cultures were found to be positive for \textit{Cryptococcus} species. Identical results were obtained on day 8, but the cryptococcal antigen titre decreased to 1:10 and a \textit{C. neoformans}/\textit{C. gattii} real time-PCR test was positive.\textsuperscript{27} Despite a negative CSF culture on day 15, the cryptococcal antigen titre was found to be 1:10. On day 30, culture and real-time PCR were both negative, and the antigen titre was found to be 1:5.

Despite the antifungal consolidation treatment using fluconazole (paediatric dosage of 12 mg/kg/day) initiated at day 30, the fever and neurological signs persisted after one month of hospitalization. The patient was transferred to the Necker-Enfants Malades hospital in Paris (France) for the management of her fungal infection and to further investigate the presence of any immunological abnormality or other predisposing factor. Upon arrival, the patient presented hydrocephalus and underwent external ventricular drainage. No immunological disorders were observed (HIV and HTLV negative, CD4 count and cytokine values were normal). The patient presented subsequently with a meningeal drain infection caused by \textit{Staphylococcus epidermidis}, which was successfully treated. Throughout this period of hospitalization the patient received fluconazole, and cryptococcosis was monitored by CSF and blood culture (always negative) and serum antigen titre (which decreased from 1:1429 to 1:72).

After one month of hospitalization in Cayenne (French Guiana) and five months in Paris (France), the patient returned to her village in the Amazonian forest without any neurological impairment at follow-up. A familial survey was carried out to trace a potential source of the \textit{C. gattii} infection. The patient’s parents, brothers and sisters were medically examined but pulmonary radiography presented no abnormalities and detection of cryptococcal antigen tests remained negative for the patient and all examined family members.

Identification of the \textit{C. gattii} strain

The \textit{Cryptococcus} strain obtained from the CSF of the patient was deposited in the CBS-KNAW publicly accessible yeast culture collection under accession number CBS11998. Species identification was performed using API32C (BioMérieux, Marcy l’Étoile, France).
followed by determination of the serotype and in vitro antifungal susceptibility profile using the EUCAST method. This revealed a C. gattii serotype B strain that was found susceptible to amphotericin B (MIC 0.25 μg/mL), 5-fluorocytosine (MIC 16 μg/mL), fluconazole (MIC 16 μg/mL), voriconazole (MIC 0.25 μg/mL) and posaconazole (MIC 0.5 μg/mL).

DNA sequencing of the ITS region identified the strain as C. gattii (Genbank accession number HQ386249).2,22

Further genotyping analysis using Amplified Fragment Length Polymorphism (AFLP) fingerprinting revealed that the C. gattii strain was genotype AFLP6B.3,19 Subsequently, the nuclear loci CAP59, GPD1, IGS1, LAC1, MPD1, PLB1 and TEF1a were sequenced to compare the strain with a large set of clinical, veterinary and environmental C. gattii strains.9 Additionally, the nuclear loci CAP10, SOD1 and URA5 were sequenced to complement the MLST dataset to that of the recently launched Cryptococcus MLST scheme, this makes future comparison of this strain possible.22 The sequences of these ten nuclear loci are available under Genbank accession numbers HQ606082–HQ606091. This detailed MLST analysis confirmed that the C. gattii strain CBS11998 belonged to genotype AFLP6B/VGI and that it was genetically identical to other C. gattii strains collected from French Guiana and Northern Brazil [F. Hagen, T. Boekhout and F. Dromer, unpublished data, 9].

Discussion

Cryptococcosis is mainly caused by the basidiomycetous encapsulated yeast species C. neoformans and C. gattii that differ in their natural habitat and geographical distribution.2,26 C. gattii, in contrast to its globally occurring sibling C. neoformans, has mostly been restricted to tropical and subtropical geographical regions until recently when outbreaks in Vancouver changed this geographical distribution.26

During the C. gattii outbreak described in Vancouver, cryptococcosis incubation periods ranged from 2 to 11 months.21 However, an early and effective treatment of infection is necessary because neurological symptoms appear rapidly and are relatively serious, and admission to the intensive care unit was required. An epidemiological survey to study the Vancouver Island C. gattii outbreak revealed that among the 176 cases, close to one-fifth of patients presented with central nervous system (CNS) disease, with or without concomitant pulmonary disease. Common signs and symptoms included headache, fever, night sweats and neck stiffness. The other patients presented with pulmonary disease.10 C. gattii is less likely to cause disseminated or CNS disease than C. neoformans, but more likely to form cryptococcomas in the lungs and brain. Cerebral disease is often associated with hydrocephalus, increased neurological deficits and a slower response to treatment.12,23

For this species, the major risk factor appears to be environmental exposure.2,23 C. gattii has been described in a biotope like the Brazilian Amazon rainforest16 including regions next to French Guiana.4 Indeed, Cryptococcus gattii molecular type VGI has been found on kassod trees (Senna siamea) in the city of Belém, Brazil.4

In the present clinical case, CSF results upon diagnosis were only moderately disturbed in spite of acute clinical manifestations. These data are consistent with cryptococcal disease contrary to other aetiological agents. In a large study of adult meningitis, among 514 cryptococcal meningitis cases, the CSF cell count ranged 0–3 neutrophils and 3–7 lymphocytes; the biochemical data ranged for glucose from 1.3 to 2.8 mmol/L and for protein from 0.5 to 1.7 g/L.17 These researchers present 81 (16%) cryptococcal meningitis cases with no abnormalities in the CSF, but these data are observed in HIV positive patients.

Biochemical identification with an API32C gallery cannot differentiate C. gattii from C. neoformans. Specific phenotyping methods with growth on specific media or antibody and genotyping methods are required. This identification and MICs determination were made by the National Reference Centre for Mycology and Antifungals at the Pasteur Institute, Paris. Antifungal susceptibility of this strain was in accordance with other Cryptococcus gattii strains tested at the National Reference Centre for Mycology and Antifungals or reported in the literature, in spite of high values for fluconazole and voriconazole.14,15 Given such profiles, drug resistance cannot be concluded, but clinical and biological surveillance are necessary.24

The patient underwent biological monitoring with weekly lumbar punctures. One week after diagnosis, direct examination, culture and PCR remained positive but cryptococcal antigen had decreased. One week later, the culture was negative despite a positive direct examination of CSF; this may be explained by the presence of non-viable yeast cells. Based on the favourable outcome, we consider that the induction regimen with the fungicidal combination amphoterin B and fluconazole, and consolidation treatment with fluconazole according to the updated guidelines, was successful.24

Another interesting point of this case is the age of the patient; Cryptococcus species rarely infect children. For example, during the Vancouver Island C. gattii outbreak from 1999 to 2002, no paediatric cases were observed among the 25 cases described by Hoang et al.16 and only four children among 218 cases were observed in a large scale epidemiological study.11

Another recent study by Joshi and co-workers revealed that cryptococcal infection in hospitalized children was observed during admission in 6.2 cases per million hospitalizations.18 In French Guiana, during a eleven-year period, only two cases of cryptococcosis were described among 43 cases reports.7 Humans develop antibodies against Cryptococcus sp. in early childhood,5,13 but children rarely develop symptomatic cryptococcal disease. Recently, Severo and colleagues have reviewed the Brazilian experience with paediatric cases of cryptococcosis.25 Epidemiological and clinical data are in agreement with our case. This study has also shown a relationship between geographical area and C. gattii or C. neoformans infection. Indeed, C. gattii seems more frequent in northeastern and northern Brazilian regions (10/11 C. gattii vs. 9/10 C. neoformans in southern region), and the disease is relatively frequent in children in this region.25

In conclusion, we report a case of neurocryptococcosis, with no specific symptoms at the early stage of the admission, in a five-year-old child living in an Amazonian village of French Guiana. The patient presented with no risk factors such as immune suppression. The aetiological agent of the disease was found to be C. gattii genotype AFLP6B which was successfully treated with the combination 5-flucytosine and amphoterin B followed by fluconazole long-term therapy.

Conflicts of interest

All authors declare no conflict of interest.

Acknowledgements

We are grateful to the National Reference Centre for Mycology and Antifungals at the Pasteur Institute, Paris, for identification and antifungal susceptibility testing.

We thank Dr Delphine Benoît, Dr Karine Lopez, and Camopi health center staff.

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