Case report: A fatal case of cryptococcosis in an immunocompetent patient due to Cryptococcus deuterogattii (AFLP6/VGII)

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Abstract

Introduction. Cryptococcosis in immunocompetent adults is a rare disease in Europe, mostly induced by members of the Cryptococcus gattii species complex. The diagnosis can be challenging due to its rarity, unspecific symptoms and long symptomless latency.

Case presentation. A 49-year-old woman with a three weeks history of headache was admitted to the hospital due to discrete ataxia and impaired vision. Cranial magnetic resonance imaging (MRI) showed a contrast-enhancing mass in the cerebellum. Further investigations detected a slight leukocytosis and a single subpleural nodule in the right inferior lung lobe. The cerebral lesion was surgically removed, and a direct frozen section only showed an unspecific inflammation. In the course of her admission she developed non-treatable cerebral edema and died ten days after surgical intervention. Histopathological examination of the surgical specimen and postmortem evaluation of the lung and the cerebrum demonstrated fungal elements. Molecular identification of the fungal elements in formalin-fixed paraffin-embedded tissue lead to the diagnosis of cryptococcosis induced by C. gattii sensu lato. Molecular genetic analysis identified the involved cryptococcal species as genotype AFLP6/VGII, recently described as Cryptococcus deuterogattii, which is known to be endemic to the west-coast of Canada and the USA. Additional heteroanamnestic information revealed that she had spent her holidays on Vancouver Island, Canada, two years before disease onset, indicating that infection during this stay seems to be plausible.

Conclusion. Cryptococcosis due to C. deuterogattii is a rarely encountered fungal disease in Europe, not particularly associated with immunodeficiency, and infection is likely to be contracted in endemic areas. Due to its rarity, long symptomless latency, unspecific symptoms and misleading radiological features the diagnosis can be challenging. Physicians need to be aware of this differential diagnosis in immunocompetent patients, as early adequate therapy can be lifesaving.

INTRODUCTION

In Western Europe Cryptococcus gattii infections have increased during the last two decades [1]. C. gattii can cause severe systemic disease in immunocompromised and immunocompetent hosts [2–4]. Cryptococcal yeasts usually enter the host via the respiratory tract [2]. Pulmonary symptoms as well as radiographic features and clinical parameters are mostly unspecific and often lead to misdiagnosis [5]. Both Cryptococcus neoformans and C. gattii species complexes show a high affinity for the central nervous system [6]. The median incubation time is six to seven
months [7, 8]. By using molecular techniques, such as restriction fragment length polymorphism (RFLP), PCR fingerprinting, multi-locus sequence typing (MLST) and amplified fragment length polymorphism (AFLP) fingerprinting it was observed that C. gattii species complex can be differentiated into five distinct genotypes [1]. Due to epidemiological, genetic and ecological differences the taxonomy of the C. neoformans/C. gattii species complexes have been recently thoroughly revised and the five C. gattii species complex genotypes were all raised to species as C. gattii sensu stricto (genotype AFLP4/VGI), Cryptococcus bacillisporus (genotype AFLP5/VGIII), Cryptococcus deuterogattii (genotype AFLP6/VGII), Cryptococcus tetragattii (genotype AFLP7/VGIV) and Cryptococcus decagattii (genotype AFLP10) [9]. The globally occurring species Cryptococcus gattii s.s. and C. deuterogattii are often involved in disease in apparently immunocompetent subjects, while the other three species are genetically restricted and have a prediction for immunocompromised hosts, mostly HIV-infected individuals [9]. An ongoing outbreak in British Columbia (Canada) that was first recognized in 1999 and started on Vancouver Island, was found to be caused by the rare genotype AFLP6/VGII (=C. deuterogattii). The outbreak expanded to the Pacific Northwest of the USA, although different subgenotypes were involved [10, 11] and has affected the health of hundreds of humans, and many more animals living in that area [12, 13]. In Germany, less than 25 hospitalizations for cryptococcosis are listed per year with 3% documented C. gattii s.l. infections. As molecular analysis identified that only half of the infections were acquired abroad [1], identification of environmental niches occupied by C. gattii s.l. in Germany is an important challenge to assess the associated risk of infection [14]. Here we report a fatal case of cryptococcosis due to C. deuterogattii infection that is likely to have been acquired on Vancouver Island two years before the onset of disease.

CASE REPORT

A 49-year-old woman was hospitalized in the University Hospital Halle/Saale, Germany, with an acute presentation of headache, discrete ataxia and impaired vision. No episodes of pyrexia were reported. Serum inflammatory markers were inconspicuous with only a slight leukocytosis (14.53 Gpt l⁻¹). Cranial magnetic resonance imaging (MRI) revealed a contrast-enhancing lesion with surrounding edema in the left cerebellum hemisphere next to the cerebellar pedunculus (Fig. 1a, b). In addition, computed tomography (CT) showed a small hyperintense subpialulderule node in the right lower lung lobe (Fig. 1e). To rule out malignancy the cerebellar lesion was surgically biopsied via a suboccipital craniotomy. A frozen section contained paucicellular glial tissue without signs of malignancy, purulence or specific infection (Fig. 1c, d). Postoperatively, the patient developed cerebral edema with displacement and compression of the fourth ventricle and the brainstem. Severe increase of intracranial pressure required suboccipital craniotomy and application of cerebrospinal fluid (CSF) drainage. A microbiological examination of the liquor was not carried out as there was no suspicion of an infection. Despite extensive supportive care the clinical condition did not improve and ten days after hospitalization the patient died. An autopsy demonstrated a purulent pneumonia with punctum maximum in the right inferior lung lobe. In addition, further processing of the cerebral biopsy was undertaken, including PAS and Grocott staining. These revealed the image of cerebral cryptococcosis with presentation of typical capsule, highlighted fungi next to a surrounding histiocytic-rich inflammation with abundance of foam cells and a rare lymphocytic infiltrate (Fig. 2a, b). The same pathogens were also seen in the inflamed pulmonary tissue (Fig. 2c, d). Cryptococcal DNA was amplified by a broad-range PCR assay targeting the ITS2 region of the ribosomal DNA. The amplicon was identified as C. gattii by LCD-Chip hybridization (LCD array fungi 2.1; Chipron). Natural reservoirs for C. gattii are found in Australia, Asia, Africa and some regions of America, including Vancouver Island which has been determined to be an endemic area [1]. In contrast, infection in Europe is rare, although C. gattii has been previously isolated in Greece, southern Italy and Spain [15]. When confronted with the autopsy results the relatives of the deceased mentioned that she spent her holidays on Vancouver Island two years prior her death. Tissue samples were transmitted to the Robert-Koch Institute (Berlin, Germany) where molecular pathogen analysis was confirmed by amplification of the ITS1 region of the ribosomal DNA with amplicon detection by LCD-Chip hybridization using a commercial system (Chipron) and ITS2 PCR with amplicon sequencing [16]. Subsequently in Nijmegen, the Netherlands, a standard multi-locus sequence typing analysis was performed [1], which did not yield any PCR product due to the fragmented formalin-fixed paraffin-embedded (FFPE)-DNA. Therefore a FFPE-MLST scheme was developed to cover the most variable parts of each of the seven standard MLST-loci (Table 1). PCRs and sequencing were performed as described previously [1]. Using the FFPE-MLST it was determined that the involved cryptococcal strain was C. deuterogattii, formerly known as C. gattii genotype AFLP6/VGII. By using phylogenetic analysis, it was determined that this strain clustered within the clade of Vancouver Island-outbreak-related C. deuterogattii strains (Fig. 3), which supports the earlier suggestion that the patient acquired the cryptococcal infection during her holiday to Vancouver Island.

DISCUSSION

Cryptococcus is a common yeast fungus with a natural reservoir on decaying wood and bird droppings [12–14]. Until recently, the genus Cryptococcus included over 100 species, but after a thorough taxonomic revision only ten remained [9]. Members of the C. gattii species complex, which is common in Sub-Saharan Africa, Asia, Australia, Europe, South and North America, can infect persons without immunodeficiency [2, 12, 13, 15–17]. As a diagnostic challenge, the patients do not present typical clinical or laboratory
findings. Furthermore, it has been reported that the infec-
tion first involves the pulmonary tract and spreads second-
arily to organs, especially the central nervous system [18].
Frequently, the patients present with a respiratory syn-
drome with cough, dyspnea and chest pain while neurologi-
cal symptoms, like headache and neck stiffness, as well as
night sweats, weight loss and anorexia may occur afterwards
[2]. Additionally, it has been reported that the clinical
symptoms and the progression of the disease differ between
species within the C. gattii species complex [12, 18–20],
even in vitro antifungal susceptibility varies [21]. Concern-
ing the case presented here, a woman with cough over a
period longer than one year suddenly developed acute head-
ache, discrete ataxia and impaired vision. Initially, the
blurred mass in the cerebellum together with the subpleural
nodule in the right lower lung lobe was misinterpreted as

Fig. 1. Preoperative and intraoperative diagnostic investigations. Cranial magnetic resonance imaging (a and b) showing a circum-
scribed mass in the left cerebellum hemisphere (arrows). Further investigations included a chest computed tomography (e) with pre-
sentation of a subpleural nodule in the right lower lobe (arrow). An intraoperative frozen section (c and d) revealed paucicellular glial
tissue with several infiltrating immune cells.
malignancy, a finding that is described in several other reports [5, 13, 18, 21–25]. The hypothesis of malignancy was further supported by clinical and laboratory findings, as the patient had no fever and only mild leukocytosis. Finally, the diagnosis of cryptococcosis was only made post mortem after histopathological and molecular-pathological evaluation. A main reason for the delayed diagnosis is the lack of experience due to the low incidence in an unexpected patient group. It should also be noted that the time between infection and the first clinical symptoms was uncommonly long, compared with a reported median incubation time of between six and seven months [7, 8]. According to data from the Robert Koch Institute in Berlin, Germany, only three percent of 155 cases of cryptococcosis were related to C. gattii s.l. between 2004 and 2013 [13, 14] and surveillance is challenging because fungal infections are not notifiable diseases. The available data indicate that there was no significant increase of cryptococcosis during that period [14]. However, in terms of health policy it is meaningful that C. gattii s.l. infections might also occur in Northern Europe, probably as a consequence of climate change [14, 26]. Therefore, nowadays this fungal pathogen is not only restricted to tropical and subtropical regions [14] and endemic areas for C. gattii s.l. within Europe have been described [1]. In 2012 the fungus was isolated from a tree in the Netherlands [27] and earlier C. gattii s.l. was detected in Italy, Greece, Portugal, and Spain [1, 15, 21, 28]. Furthermore, in Germany few cases of cryptococcosis due to C. gattii s.s. without a travel history to endemic regions have been documented [14]. Nonetheless at least half of the infections in Germany were acquired abroad [1, 14]. In summary, it is important that clinicians are aware of this challenging invasive mycosis that often infects immunocompetent subjects. Unspecific symptoms with cough in conjunction with acute cerebral symptoms are indicators for the diagnosis and it

Table 1. Newly designed Cryptococcus gattii sensu lato FFPE-MLST primers

| Locus | Primer name | Primer sequence* |
|-------|-------------|------------------|
| CAP59 | FFPE-CAP59Fwd | AGGGGAGGCAGCACAAGTA |
|       | FFPE-CAP59Rvd | TTGGCTTGTTGGAACC |
| GPD1  | FFPE-GPD1Fwd | AGGCTGTATGGTGAAGTTG |
|       | FFPE-GPD1Rvd | CCCATGTAAGTCAAGTCAAGA |
| IGS1  | FFPE-IGS1Fwd | TTGGCTAAGATGGGTATAGC |
|       | FFPE-IGS1Rvd | TTGCTTGACCGAGTTGACT |
| LAC1  | FFPE-LAC1Fwd | CATGGTATGGCAGAAG |
|       | FFPE-LAC1Rvd | ACGCTATGGTACSTCAGC |
| PLB1  | FFPE-PLB1Fwd | CGTGGAATTAAATGGCCACGT |
|       | FFPE-PLB1Rvd | TTGGGTGTTTCTCATGCA |
| SOD1  | FFPE-SOD1Fwd | ACTCTGAGGAGGACGGTGT |
|       | FFPE-SOD1Rvd | TGGAGTGAAGGAGGCAAG |
| URA5  | FFPE-URA5Fwd | AGGCGGTTAGGCCATATC |
|       | FFPE-URA5Rvd | CGTCCCTTCTTCTCTCTCT |

*All primers have a Tm of 60°C, PCRs were performed as described previously [1].

Fig. 2. Postmortem diagnostic investigations. Cerebellar tissue (a and b) showing single immune cells next to some poorly demarcating spherules in HE-staining. Assessment of lung tissue (c and d) revealed a purulent pneumonia with presentation of several foci with the same round and oval structures (Arrows). Further staining of the cerebellar tissue as well as of the lung tissue including PAS reaction and Grocott staining supported the assumption that this was due to Cryptococcus.
has to be pointed out that an early diagnosis allows cure of the majority of patients, albeit that despite effective treatment central nervous complications may persist [6].

**Conclusion**

In Western Europe, increased international travel leads to a rising number of infections with rare pathogens [29]. Additionally climate change creates new ecological niches in the environment. *C. gattii*, which has been formerly known to be a pathogen in tropical and subtropical regions [2, 3], has been recognized in temperate regions in Europe and North America [1, 10, 15]. In consequence of the lack of experience due to the low incidence in a particularly unexpected group of patients, determination of the diagnosis is often delayed and findings are misinterpreted [13, 14]. Early diagnosis is critical for a successful treatment of this otherwise lethal infection mostly due to central nervous complications [6].

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.
Ethical statement
This case-report is published with consent of the relatives.

References
1. Hagen F, Colom MF, Swinne D, Tintelnot K, Iatta R et al. Autochthonous and dormant Cryptococcus gattii infections in Europe. Emerg Infect Dis 2012;18:1618–1624.
2. Galanis E, MacDougall L, Kidd S, Morshed M. Epidemiology of Cryptococcus gattii, British Columbia, Canada, 1999–2007. Emerg Infect Dis 2010;16:251–257.
3. Bovers M, Hagen F, Kuramae EE, Diaz MR, Spanjaard L et al. Unique hybrids between the fungal pathogens Cryptococcus neoformans and C. gattii. FEMS Yeast Res 2006;5:599–607.
4. Chen SC, Slavin MA, Heath CH, Playford EG, Byth K et al. Clinical manifestations of Cryptococcus gattii infection: determinants of neurological sequelae and death. Clin Infect Dis 2012;55:789–798.
5. Yu JQ, Tang KJ, Xu BL, Xie CM, Light RW. Pulmonary cryptococcosis in non-AIDS patients. Braz J Infect Dis 2012;16:531–539.
6. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of America. Clin Infect Dis 2010;50:291–322.
7. Hagen F, van Assen S, Luijckx GJ, Boekhout T, Kampinga GA. Activated dormant Cryptococcus gattii infection in a Dutch tourist who visited Vancouver Island (Canada): a molecular epidemiological approach. Med Mycol 2010;48:528–531.
8. MacDougall L, Fyfe M. Emergence of Cryptococcus gattii in a novel environment provides clues to its incubation period. J Clin Microbiol 2006;44:1851–1852.
9. Hagen F, Khayhan K, Theelen B, Kolecak A, Polacheck I et al. Recognition of seven species in the Cryptococcus gattii/Cryptococcus neoformans species complex. Fungal Genet Biol 2015;78:16–48.
10. Byrnes EJ, Li W, Lewit Y, Ma H, Voelz K et al. Emergence and pathogenicity of highly virulent Cryptococcus gattii genotypes in the northwest United States. PLoS Pathog 2010;6:e1000850.
11. Hagen F, Ceresini PC, Polacheck I, Ma H, van Nieuwbergh F et al. Ancient dispersal of the human fungal pathogen Cryptococcus gattii from the Amazon rainforest. PLoS One 2013;8:e71148.
12. Hoang LM, Maguire JA, Doyle P, Fyfe M, Roscoe DL. Cryptococcosis in Europe: a review of recent epidemiological trends in Europe. Curr Med Res Opin 2013;29:1671–1679.
13. Kidd SE, Hagen F, Tscharke RL, Huynh M, Bartlett KH et al. A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc Natl Acad Sci USA 2004;101:17258–17263.
14. Smith IM, Stephani C, Hogardt M, Klawe C, Tintelnot K et al. Cryptococcosis due to Cryptococcus gattii in Germany from 2004–2013. Int J Med Microbiol 2015;305:719–723.
15. Cogliati M, D’Amicis R, Zani A, Montagna MT, Caggiano G et al. Environmental distribution of Cryptococcus neoformans and C. gattii around the Mediterranean basin. FEMS Yeast Res 2016;16:fov045.
16. Rickerts V, Khot PD, Myerson D, Ko DL, Lambrecht E et al. Comparison of quantitative real time PCR with sequencing and ribosomal RNA-FISH for the identification of fungi in formalin fixed, paraffin-embedded tissue specimens. BMC Infect Dis 2011;11:202.
17. Chen S, Sorrell T, Nimmo G, Speed B, Currie B et al. Epidemiology and host- and variety-dependent characteristics of infection due to Cryptococcus neoformans in Australia and New Zealand. Clin Infect Dis 2000;31:499–508.
18. Mischnik A, Klein S, Tintelnot K, Zimmermann S, Rickerts V. Kryptokokkose: kausalen, epidemiologischen und therapeutischen Strategien. DMW – Dtsch Med Wochenschr 2013;138:1533–1538.
19. Chen SC, Meyer W, Sorrell TC. Cryptococcus gattii infections. Clin Microbiol Rev 2014;27:980–1024.
20. Bartlett KH, Cheng PY, Duncan C, Galanis E, Hoang L et al. A decade of experience: Cryptococcus gattii in British Columbia. Mycosystema 2012;173:311–319.
21. Hagen F, Ilnait-Zaragozi MT, Bartlett KH, Swinne D, Geertsen E et al. In vitro antifungal susceptibilities and amplified fragment length polymorphism genotyping of a worldwide collection of 350 clinical, veterinary, and environmental Cryptococcus gattii isolates. Antimicrob Agents Chemother 2010;54:5139–5145.
22. Harris JR, Lockhart SR, Sondermeyer G, Vugia DJ, Crist MB et al. Cryptococcus gattii infections in multiple states outside the US Pacific Northwest. Emerg Infect Dis 2013;19:1621–1627.
23. Dora JM, Kelbert S, Deutschendorf C, Cunha VS, Aquino VR et al. Cutaneous cryptococcosis due to Cryptococcus gattii in immunocompetent hosts: case report and review. Mycopathologia 2006;161:235–238.
24. Ulett KB, Cockburn JW, Jeffree R, Woods ML. Cerebral cryptococcoma mimicking glioblastoma. BMJ Case Rep 2017;2017:brer2016218824.
25. Oliveira FM, Severo CB, Guazzelli LS, Severo LC. Cryptococcus gattii fungemia: report of a case with lung and brain lesions mimicking radiological features of malignancy. Rev Inst Med Trop Sao Paulo 2007;49:263–265.
26. Cogliati M, Puccianti E, Montagna MT, de Donno A, Susever S et al. Fundamental niche prediction of the pathogenic yeasts Cryptococcus neoformans and Cryptococcus gattii in Europe: Cryptococcus fundamental niche in Europe. Environ Microbiol 2017;19:4318–4325.
27. Chowdhary A, Randhawa HS, Boekhout T, Hagen F, Klaassen CH et al. Temperate climate niche for Cryptococcus gattii in Northern Europe. Emerg Infect Dis 2012;18:172–174.
28. Colom MF, Hagen F, Gonzalez A, Mellado A, Morera N et al. Cere- tonia siliqua (carob) trees as natural habitat and source of infection by Cryptococcus gattii in the Mediterranean environment. Med Mycol 2012;50:67–73.
29. Tintelnot K. Kryptokokkose: Cryptococcus gattii-infektionen können importiert werden und schwer verlaufen. Robert-Koch-Institut Epidemiologisches Bulletin 2008;20:159.