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Survival after cryptococcosis in Germany: A retrospective multicenter cohort study of patients diagnosed between 2004 and 2021

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ABSTRACT

Cryptococcosis is the most prevalent fungal infection of the central nervous system worldwide. We performed a retrospective multicenter cohort study to gain insights into the epidemiology of cryptococcosis in Germany. We describe the use of diagnostic tests, clinical management and patient outcome. We included 64 patients with underlying HIV infection (55%) or other predispositions. Molecular typing by MLST documented 20 individual sequence types among 42 typed isolates. A fatal outcome was documented in 14% of patients in the first two months after diagnosis.

1. Introduction

Cryptococcosis is the most prevalent fungal infection of the central nervous system (CNS) worldwide. Most infections occur in patients with HIV infection and are diagnosed in low- and middle-income countries (LMIC) (Rajasingham et al. 2022). In high income countries, HIV infection is responsible for 40–80% of cryptococcosis cases. Other predisposing conditions, such as solid organ transplantation and immuno-suppressive therapy are increasingly gaining attention (Dromer et al. 2007; Bratton et al. 2012).

Management of disseminated cryptococcosis, currently relies on amphotericin B based antifungal combination therapy. However,

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adverse outcomes including persistent disabilities and mortality remain substantial. Cohort studies identified risk factors for adverse outcomes including delayed diagnosis, CNS involvement, high serum cryptococcus antigen (CRAG) level and lack of flucytosine as antifungal combination partner[2]. In addition, microbiological studies using isolates from HIV infected patients with CNS infection suggest, that prognosis of infection might be influenced by cryptococcus sequence type (ST). STs may differ by phenotypic traits including uptake of cryptococci into host cells and intracellular fungal replication (Altamirano et al. 2020).

Cryptococcosis in Germany is a non-reportable infection, and therefore, exact numbers of cases are not available. Previously, a median of 57 (range: 49–60) hospital discharges per year, reflecting 0.07 discharges per 100.000 inhabitants, has been attributed to cryptococcosis (Smith et al. 2015). Prior clinical studies on cryptococcosis in Germany consisted of small retrospective single center reports. They focused on the use of diagnostic tests that potentially impact patient management (Katchanov et al. 2015; Skripuletz et al. 2014).

We conducted a retrospective multicenter cohort study of cryptococcosis diagnosed in Germany between 2004 and 2021 to gain insights into the epidemiology of the infection, diagnostic practice, mycological findings, therapeutic measures, and survival after diagnosis of cryptococcosis in this cohort. We compare findings with open accessible data on hospitalization for cryptococcosis in Germany to assess the representativeness of our study.

2. Methods

2.1. Case finding and documentation

Physicians involved in the clinical care of patients with HIV infection were invited via the AIDS working party of the *Deutsche Gesellschaft für Infektiologie* (German Society for Infectious Diseases) to participate in this study. Patients were eligible if a first episode of cryptococcosis had been diagnosed between 2004 and 2021 in Germany according to EORTC/MSG criteria (Donnelly et al. 2020). In short, proven cryptococcosis requires the cultivation of *Cryptococcus* from sterile clinical samples showing signs of an infectious process, the cultivation or antigen detection from blood or cerebrospinal fluid (CSF) or histopathologic evidence for cryptococcosis. Probable cryptococcosis refers to recovery of *Cryptococcus* from a non-sterile sample, such as bronchoalveolar lavage (BAL) together with radiologic evidence for infection in a patient with an underlying condition predisposing to cryptococcosis.

Due to protracted recruitment, additional treatment centers were invited to document infections. In addition, selected patients diagnosed at the German reference laboratory for cryptococcosis were included if data on clinical follow up were available.

Patient characteristics consisting of underlying disease, antiretroviral therapy, immigration and travel history, clinical presentation, mycological findings and antifungal therapy were extracted from patient records by participating physicians using a case report form (CRF). In addition to baseline data, follow up information such as response to therapy and survival were documented for week 4 and 16 and in case immune reconstitution syndrome (IRIS) or relapse was suspected, and at the last clinical follow up. Data from CRFs were transferred into an Excel database for descriptive data analysis. Variables are reported as median with the range. Selected information such as the date of diagnosis, disease localization, dissemination, last follow up and outcome were transferred into Graphpad prism 9 for survival plots.

Ethics approval was obtained from the hospital of the Goethe-University Frankfurt/Main (198/18), which waived the need for informed consent for this retrospective analysis.

2.2. Definitions

Baseline (day 1) of infection was assumed as the date when the first positive sample (CSF, blood, BAL, histology) confirming the diagnosis of cryptococcosis was taken. Disseminated infection was defined as cultivation of cryptococcus from blood or CSF. IRIS was suspected in HIV infected patients starting antiretroviral therapy according to local definitions, used by the treatment center, including worsening clinical symptoms of cryptococcosis and the absence of evidence for a relapse, i.e., cultivation of cryptococcus.

Table 1
Demographic characteristics, disease, and etiology in 64 patients diagnosed with cryptococcosis between 2004 and 2021 in Germany.

sex		
male	50	(78%)
female	14	(22%)
age (years) (Median and range)	44.5	(25–95)
geographic origin		
German	39	(61%)
other European	3	(5%)
African	9	(14%)
other	5	(17%)
unknown	8	(13%)
underlying conditions		
HIV Infection	35	(55%)
hem. malignancy	6	(9%)
ESLD	4	(6%)
other*	5	(8%)
unknown	7	(11%)
transplantation	2	(3%)
rheumatic conditions	3	(5%)
sarcoidosis	2	(3%)
travel history**		
Africa	9	(14%)
Europe	11	(17%)
Asia	5	(8%)
Americas	5	(8%)
disease		
disseminated	59	(92%)
localized***	5	(8%)
organism		
C. neoformans var. grubii	42	(66%)
C. neoformans var. neoformans	9	(14%)
AD-hybrid	1	(2%)
C. gattii	2	(3%)
no organism identified	10	(16%)

Abbreviations: HIV, human immunodeficiency virus; ESLD, end stage liver disease; AD-hybrid, hybrid between serotypes A and D. *Other underlying diseases: Diabetes mellitus (n=1), chronic pulmonary disease (n=1), idiopathic CD4 lymphopenia (n=1), renal insufficiency (n=1), inflammatory bowel disease (n=1); ** one or more per patient, *** localized skin infection (n=1), localized pulmonary infection (n=4).

2.3. Laboratory investigations

Cryptococcus isolates were available for identification and molecular testing in a subgroup of patients. In case of previous deposition of the strain at the German reference laboratory, patient history and isolate were linked by identical patient identifier (sex, year of birth, treatment center) and date of infection.

Isolates were identified to species level by phenotypic tests and genotyping. Multilocus sequence typing (MLST) was performed according to the typing scheme of the international society of human and animal mycology as described previously (Selb et al. 2019; Meyer et al. 2009).

2.4. Data on hospitalization for cryptococcosis in Germany

In order to assess for the representativeness and completeness of this cohort, publicly available administrative data on hospitalizations for cryptococcosis in Germany were extracted from an online database. The number of hospital discharges for cryptococcosis in Germany and a summary of selected patient characteristics (age, sex, duration of hospitalization, lethal outcome during hospitalization) can be assessed

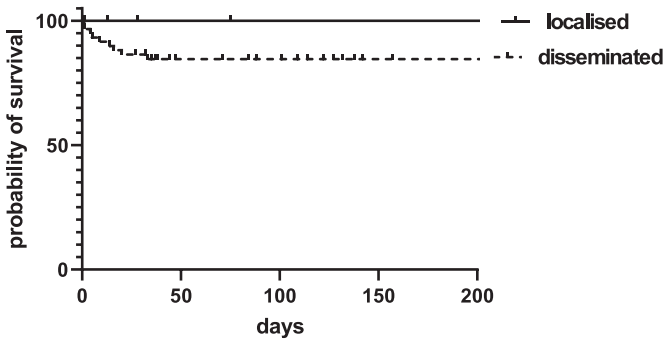

Fig. 1. Survival by extent of disease. Disseminated infection (n=59) was assumed when fungi were cultivated from blood or cerebrospinal fluid. Localized infections consisted of lung infection (n=4) or soft tissue infection (n=1).

online (www.gbe-bund.de). Data on patients hospitalized with cryptococcosis between 2004 and 2021 were retrieved June 01, 2023 using "Kryptokokkose" as query term.

3. Results

Case report forms were provided by 28 clinical sites. CRFs were provided from sites recruited by the AIDS working party (n=39), additional clinical sites (n=20) and the reference laboratory for cryptococcosis (n=5). Per site, a median of one (median) infection (range: 1–15) was documented. Cryptococcosis was proven according to EORTC/MSG criteria in 61 cases and probable in three patients with respiratory tract infection. Median patient follow up was 140 days (range 1 – 5008) days.

Patient characteristics are summarized in Table 1. HIV infection was the most frequent underlying condition documented in 35 of 59 (59%) patients with disseminated infection. Other underlying conditions included hematologic malignancy such as chronic lymphocytic leukemia (CLL) (n= 3) including one patient in whom ibrutinib was documented for CLL treatment, one patient with chronic myelogenous leukemia (CML) and autologous stem cell transplantation as well as two patients with multiple myeloma. Localized infection of the lung (n=2) or disseminated infection (n=4) were diagnosed in these patients. End stage liver disease, i.e. liver cirrhosis due to chronic hepatitis C (n=2), autoimmune and alcohol abuse was present in four patients with disseminated cryptococcosis. Information on the geographic origin of the patient were available 56 patients, 17 (30%) of whom had immigrated to Germany. In addition, a travel history was reported in 12 non-migrants. Taken together, 29 (45%) patients may have been exposed to cryptococcus outside of Germany.

Patients with localized infection (n=5) presented as pulmonary disease (pneumonia n=3; lung nodule n=1) or subcutaneous infection (n=1). Localized infection was diagnosed in patients with cancer (n=2), idiopathic CD4 lymphopenia or without underlying disease (n=2) by cultivation of *Cryptococcus* from pulmonary secretions (n=3), the skin (n=1) or histopathology from a lung biopsy (with PCR confirming *C. gattii*), and by positive CRAG in the absence of culture confirmation. Patients were treated according to guidelines with oral fluconazole (n=2) for localized pneumonia, with an amphotericin B formulation and flucytosine in a patient with pneumonia with high antigen titer and without antifungals in a patient with skin infection. One patient with localized pulmonary infection was treated with posaconazole. Fungi cultivated were *C. neoformans var. grubii* (n=2) and *C. neoformans var. neoformans* (n=2). Outcome of the infection was excellent with survival in all patients (Fig. 1).

HIV infection was the most common underlying condition in patients with disseminated crypto-coccosis. Infection was diagnosed mostly in patients with advanced immune deficiency as reflected by a median 38 CD4 cells/µl (range: 0–290) at time of cryptococcosis diagnosis. Clinical manifestations at diagnosis of disseminated infection included CNS disease (n=45), pneumonia (n=18) and skin infection (n=5). Lung CT was reported in 22 patients and showed nodular lesions (n=3), localized (n=5) and diffuse infiltrates (n=2), pulmonary edema (n=4) or intrathoracic lymphadenopathy (n=1). Results of brain imaging (CT or MRI) were reported in 42 patients and demonstrated meningeal enhancement (n=8), brain edema (n=6) or ischemia (n=4). CSF opening pressure was rarely documented (n=9) and was 35 cm H2O (median; range 19–50). The initial positive clinical sample confirming cryptococcosis was reported in 57 patients with disseminated infection and included CSF (microscopy, CRAG, culture) in 37 (65%), blood culture in 14 (25%), serum CRAG in 3 (5%), BAL, bone, and skin biopsy (one each). Subsequently, serum CRAG was reported in 23 cases with a median of 512 (range: 5–65,000). Antifungal therapy was specified in 47 cases with disseminated infection, consisting of amphotericin B (liposomal: 38; conventional:7) based combination therapy including triple therapy with fluconazole and flucytosine (n=29), double therapy with flucytosine (n=9) or fluconazole (n=9). Two patients initially received fluconazole monotherapy including one patient who died on day 1. Four of 20 patients (20%) with information on HIV treatment history received antiretroviral combination therapy 3, 18, 18, 1567 before diagnosis of cryptococcosis. Sixteen patients received antiretroviral therapy 17 days

Table 2
Patients with cryptococcosis and fatal outcome.

sex, age	underlying disease	clinical presentation	isolation site	species (seq. type)	serum CRAGscreening	antifungals	outcome (day)	comment
m, 38	ESLD	ARDS, sepsis	blood	CNN (unk)	ND	caspofungin	4	culture positive after death
m, 29	AIDS*	CNS	blood, CSF, BAL	CNG (23)	ND	fluconazole	1	culture positive after death
m, 35	AIDS*	unk	blood	CNG (93)	unk	unk	16	
m, 32	AIDS*	CNS	blood	CNG (2)	ND	unk	1	
m, 76	COPD**	CNS	blood	CNG (290)	unk	fluconazole	5	
f, 46	ESLD, DM	CNS	CSF	CNG (58)	ND	amphotericin B, flucytosine, fluconazole	9	
m, 52	AIDS	CNS	CSF	CNG (77)	ND	amphotericin B, flucytosine, fluconazole	14	HAART for 60 days
m, 48	AIDS	CNS	CSF	CNG (2)	unk	amphotericin B,fluconazole	20	HAART for 18 days
m, 68	sarcoidosis**	CNS	CSF	no isolate	ND	amphotericin Bfluconazole	33	

Abbreviations: ESLD, End stage liver disease; AIDS, Acquired immuno deficiency syndrome; DM, Diabetes mellitus; ND, not done; unk, unknown; CNS, central nervous system; BAL, bronchoalveolar lavage; ARDS, acute respiratory distress syndrome; COPD, Chronic obstructive pulmonary disease; HAART, highly active antiretroviral therapy. CNG, *C. neoformans var. grubii*; CNN, *C. neoformans var. neoformans*.

* HIV infection untreated

** treatment with glucocorticosteroids

(range: 0–50) after diagnosis of cryptococcosis. IRIS was suspected in 12 of 35 (34%) patients with AIDS and disseminated infection 188 days (range: 40–465) after diagnosis of cryptococcosis.

Patients with fatal outcome (n=9; 14%) had underlying HIV infection (n=5) with 20 CD4 cells/µl (range: 20–31), end stage liver disease (n=2) or chronic lung disease (COPD, sarcoidosis) (Table 2). All suffered from disseminated infection with cryptococci cultivated from blood (n=4), CSF (n=4) or both (n=1). CNS involvement was documented by clinical signs and pathologic cranial CT scan (n=7) and was absent in one patient with diffuse pulmonary infiltrates. Data on clinical presentation was missing in one. An absence of CRAG screening results from samples before cultivation of cryptococci was documented in six patients, whereas in three this information was missing. In two patients CRAG was determined after cultivation and revealed high CRAG titers (1:1096 and 1:4096). Four patients were treated with amphotericin B based combination therapy, while two received fluconazole or an echinocandin empirically and antifungal treatment was not documented in two.

Cultivation of the causative agent was achieved in 49 (77%) cases. Infections were caused by *C. neoformans var. grubii* (n=40), *C. neoformans var. neoformans* (n=7), an AD hybrid (n=1) and *C. gattii* (n=1). Molecular typing by MLST was available for 42 (86%) haploid *C. neoformans* isolates. The most prevalent genotypes of *C. neoformans var. grubii* consisted of ST 58 (n=7), ST 23 (n=7), ST 2 (n=3) ST 63 (n=3), ST 69 (n=3), ST 77 (n=2), ST 40 (n=2), ST 5 (n=2), ST 4, ST 6, ST 93, ST 288, ST 290 (one each). For *C. neoformans var. neoformans*, ST 116 (n=2), ST 122, ST 270, ST 273, ST 514, ST 597 and ST 661 (one each) were identified.

Administrative data documented 1055 hospitalizations (44–80 per year) for cryptococcosis during the study period in male (73%) and female (27%) patients in Germany. Patients were discharged from hospitals after a mean 24.7 days (range: 21.6–32 days). In 113 hospitalizations (10.7%) a lethal outcome was documented (Figure S1). Therefore, patients included in this cohort study (n=64) may represent 6% of cryptococcosis cases hospitalized in Germany during the study period with lethal outcomes being slightly overrepresented (9 of 64 (14%).

4. Discussion

We describe the first retrospective multicenter cohort study of patients with cryptococcosis diagnosed in Germany to gain insights into the epidemiology including molecular epidemiology of this infection, clinical management and patient outcome. Comparison with systematically collected data on hospital discharges suggest that we could only include a small portion that might represent selection toward more severe cases as reflected by comparison of mortality. However, some insights into the epidemiology and management of cryptococcosis in Germany are provided by this survey. First, in accordance with previous cohort studies in high-income countries, AIDS remains the predominant risk factor accounting for 42–77% of cryptococcosis cases (Dromer et al. 2007; Bratton et al. 2012; Pyrgos et al. 2013). However, as we have primarily included cases via physicians associated with the AIDS working party, this study may suffer from selection bias, suggesting that the prevalence of HIV infection in disseminated cryptococcosis may be less than 55% in Germany. Also, in line with previous cohort studies and recent case series, an increasing array of underlying conditions appears to predispose for cryptococcosis. First, hematologic malignancies including chronic lymphocytic leukemia may predispose for pulmonary and disseminated cryptococcosis. Recently, cryptococcosis in patients treated with kinase inhibitors including ibrutinib have been reported. These infections are predominantly localized in the lung, occur in the first 6 months of treatment, and are associated with a 30-day mortality of 18% (Schmalzle et al. 2016). Additional therapeutics under discussion as risk factors for cryptococcosis include fingolimod, used for treatment of multiple sclerosis (Del Poeta et al. 2022). Second, end stage liver disease with or without liver transplantation has been described as a predictor for cryptococcosis, manifesting as pulmonary, peritoneal and disseminated infections. These infections have been associated with a 90-day mortality of 57% (Singh et al. 2015). Not reflected in our cohort, is SARS-COV-2, as our survey period was mostly before the pandemic and SARS-COV-2 was not present in these patients. Case series described localized pulmonary (23%) or disseminated infection (76%). These infections were associated with a mortality of 59% (Walker et al. 2023). In contrast to these emerging risk groups, cryptococcosis in additional underlying groups has been documented for a longer time and some insights from these groups may also relate to emerging risk groups. Sarcoidosis has been well known to predispose for cryptococcosis. A retrospective study of patients with cryptococcosis in France found that about 3% of non-AIDS associated cryptococcosis cases were due to sarcoidosis (Bernard et al. 2013). The authors described significant diagnostic delays until diagnosis of cryptococcosis ranging from months to years. This is potentially due to infrequent use of CRAG detection as a rapid screening test in these patients. Of note, in our patients, serum CRAG was reported as the first diagnostic test in only 5% of patients. Diagnosis predominantly relied on cultivation from blood or CSF suggesting infrequent use of CRAG testing in patients with symptoms in line with cryptococcosis. As CRAG tests are widely available in Germany, awareness for cryptococcosis may be limiting in early diagnosis. However, we cannot exclude a lack documentation of antigen detection in our patients due to the retrospective character of this survey. However, a study analyzing patterns of CRAG testing in a large US clinical laboratory found that underlying conditions other than AIDS were listed in less than 10% of the cases, suggesting that awareness for cryptococcosis might be limited (Benedict et al. 2022). In line with this, a study analyzing healthcare utilization data from adult patients hospitalized with cryptococcosis in the US demonstrated, that patients hospitalized with cryptococcosis had previous admissions potentially related to subsequent cryptococcosis during 90 days before cryptococcosis diagnosis. Missed diagnosis were associated with increased mortality both in HIV and non-HIV associated infections (Salazar et al., 2020).

A metanalyses of cohort studies and randomized controlled trials documented a 20% mortality in AIDS patients with cryptococcosis in high income countries with available antiretroviral therapies, mostly occurring in the first 2–6 months after diagnosis (Pasquier et al., 2018). Altered neurologic status, low CD4 cell counts, high CSF cryptococcal burden and older age at diagnosis were independently associated with long term mortality. As fatal outcomes in our study were documented during the first two months, we assume that cryptococcosis was the ultimate cause of death in these patients. Early death in AIDS has been associated with high CSF fungal burden, altered mental state at presentation and rate of fungal clearance. It has been proposed that earlier diagnosis and rapid institution of fungicidal amphotericin B therapies may improve outcome (Jarvis et al., 2014). Case report forms in our study suggest that diagnosis in our patients was based primarily on cultivation from CSF and blood. In contrast, CRAG screening was rarely documented, suggesting that increasing use of CRAG testing in clinical care of HIV patients with low CD4 cells before start of antiretrovirals might improve the detection of early phases of the infection before severe neurologic disease manifests. In addition, early inclusion of infectious diseases physicians familiar with complex management decisions necessary for successful treatment of cryptococcosis improved survival in a retrospective cohort study (Spec et al., 2018). Lack of documentation of opening pressure as a marker for disease severity in our patients and fluconazole monotherapy in two patients with fatal disseminated infection and the early initiation of antiretrovirals in some patients suggest, that early inclusion of experienced physicians may improve care of patients with severe disseminated cryptococcosis in AIDS patients in Germany. Improvement in clinical care has been previously documented in a single center study demonstrating that involvement of infectious disease consultation is significantly associated with a reduced 90 day mortality associated by a higher rate of lumbar punctures when

indicated, and improved antifungal induction therapy (Spec et al., 2017). In addition, the recently introduced EQUAL scoring system may be used to foster best evidence practice of care of patients with cryptococcosis in hospitals caring for AIDS patients if applied in a stewardship audit and feedback context. The scoring system provides a checklist for the audit of performance of health care institutions in the management of cryptococcosis. This includes the use of diagnostic tests, antifungal therapy and other treatment measures. If this score is be used for feedback on the performance, hurdles to optimal patient management might be mitigated (Spec et al., 2018).

As previously reported in a large Australian multicenter study, outcome in AIDS associated cryptococcosis was not different than in non-AIDS associated cases (Coussement et al., 2023). A metanalysis established independent risk factors for mortality in non-AIDS patients, including delayed diagnosis, age above 60 years, altered neurologic status at presentation, high cryptococcal burden and induction therapy lacking amphotericin B (Pasquier et al., 2018). It is been increasingly documented that disseminated cryptococcosis in non-AIDS patients is associated with substantial long-term neurological sequelae despite aggressive antifungal therapies (Marr et al., 2020). Main impairments, documented in 10–20% of patients include vertigo, visual loss, hearing impairment and motor deficit (Pasquier et al., 2018). In addition to early diagnosis and amphotericin B based combination therapy, individualization of management might be needed to improve survival and prevent neurologic sequelae in patients with disseminated cryptococcosis, as damage caused by cryptococcus varies by the infecting fungus and also immune damage by the host. A framework has been proposed to guide clinicians by fungal clearance after two weeks of antifungal therapy, improvement of clinical signs and management options for the so called post infectious inflammatory syndrome (PIIRS) that manifests weeks after diagnosis with neurologic symptoms ranging from auditory and visual impairment up to altered mental status (Anjum and Williamson, 2019).

Molecular typing using MLST has been widely used for molecular typing of the agents of cryptococcosis. In some regions infections are predominately caused by a small number of sequence types (ST) of *C. neoformans var. grubii* such as ST5 in Asia or ST93 in some studies in Africa. This has facilitated to study a potential impact of sequence types with survival of AIDS patients with disseminated infections (Mukaremera et al., 2019; Ashton et al., 2019). Such associations could not be identified in our study due to a high number of STs documented. This might be caused by frequent infections in patients with travel or migration history. Of note, incubation period of cryptococcosis might vary between 6 weeks and 2 years (van der Torre et al., 2022). and may also reflect the diversity of genotypes found in environmental samples in Europe. While most sequence types identified for *C. neoformans var. grubii* have been previously found in environmental and clinical samples in Europe and represent closely related widely distributed groups, more genetic diversity is found in *C. neoformans var. neoformans* as previously described (Cogliati et al., 2019).

In order to identify fungal determinants of disease manifestation and prognosis, with a potential for individualization of patient management, documentation of phenotypic characteristics of isolates and genome-based typing may generate more actionable results (Fernandes et al., 2018; Gerstein et al., 2019).

In conclusion, this report confirms the low prevalence of cryptococcosis in Germany. The retrospective multicenter cohort study documents the broad range of underlying conditions besides AIDS and diversity of fungal genotypes. Early mortality associated with disseminated infections may be reduced by increased awareness for cryptococcosis especially in emerging risk groups including end stage liver disease, immune suppressive treatments and increased use of cryptococcus antigen testing which is possible in most microbiologic laboratories and may even be performed in clinical laboratories by a lateral flow assay to facilitate early diagnosis and prompt institution of established antifungal combination therapies to prevent mortality and persistent neurologic deficits (Fig. 2).

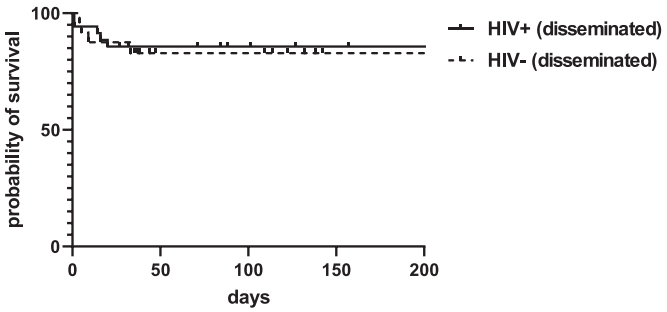

Fig. 2. Survival of patients with disseminated cryptococcosis in patients with HIV infection (n=35) and without (n=29).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijmm.2024.151614.

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