MAJOR ARTICLE

# Clinical Manifestations of *Cryptococcus gattii* Infection: Determinants of Neurological Sequelae and Death

**Sharon C-A. Chen,[1,2,3] Monica A. Slavin,[6] Christopher H. Heath,[8] E. Geoffrey Playford,[11] Karen Byth,[3] Deborah Marriott,[4] Sarah E. Kidd,[12] Narin Bak,[13] Bart Currie,[14] Krispin Hajkowicz,[14] Tony M. Korman,[7] William J. H. McBride,[15] Wieland Meyer,[1,2,5] Ronan Murray,[9,10] and Tania C. Sorrell[1,2,3]; on behalf of the Australia and New Zealand Mycoses Interest Group (ANZMIG)-Cryptococcus Study[a]**

[1]Centre for Infectious Diseases and Microbiology, Westmead Millennium Institute, Sydney, [2]The Sydney Institute for Emerging Infections and Biosecurity, University of Sydney, [3]Westmead Hospital, Western Sydney Local Health District, [4]Department of Microbiology and Infectious Diseases, St. Vincent's Hospital, Sydney, [5]Molecular Mycology Research Laboratory, Westmead Hospital, University of Sydney; [6]Department of Infectious Diseases, Peter MacCallum Cancer Centre, [7]Monash Infectious Diseases and Monash University, Melbourne; [8]Department of Microbiology and Infectious Diseases, Royal Perth Hospital, [9]Department of Microbiology, PathWest Laboratory Medicine WA, [10]Department of Infectious Diseases, Sir Charles Gairdner Hospital, Perth; [11]Infection Management Services, Princess Alexandra Hospital, Brisbane; [12]Mycology Unit, SA Pathology at Women's and Children's Hospital, Adelaide, [13]Department of Infectious Diseases, Royal Adelaide Hospital; [14]Infectious Diseases Department and Menzies School of Health Research, Royal Darwin Hospital, Casuarina; and [15]School of Medicine and Dentistry, James Cook University, Cairns, Australia

***Background.*** Longer-term morbidity and outcomes of *Cryptococcus gattii* infection are not described. We analyzed clinical, microbiological, and outcome data in Australian patients followed for 12 months, to identify prognostic determinants.

***Methods.*** Culture-confirmed *C. gattii* cases from 2000 to 2007 were retrospectively evaluated. Clinical, microbiological, radiological, and outcome data were recorded at diagnosis and at 6 weeks, 6 months, and 12 months. Clinical and laboratory variables associated with mortality and with death and/or neurological sequelae were determined.

***Results.*** Annual *C. gattii* infection incidence was 0.61 per $10^6$ population. Sixty-two of 86 (72%) patients had no immunocompromise; 6 of 24 immunocompromised hosts had idiopathic CD4 lymphopenia, and 1 had human immunodeficiency virus/AIDS. Clinical and microbiological characteristics of infection were similar in immunocompromised and healthy hosts. Isolated lung, combined lung and central nervous system (CNS), and CNS only disease was reported in 12%, 51% and 34% of the cases, respectively. Complications in CNS disease included raised intracranial pressure (42%), hydrocephalus (30%), neurological deficits (27%; 6% developed during therapy) and immune reconstitutionlike syndrome (11%). Geometric mean serum cryptococcal antigen (CRAG) titers in CNS disease were 563.9 (vs 149.3 in isolated lung infection). Patient immunocompromise was associated with increased mortality risk. An initial cerebrospinal fluid CRAG titer of ≥256 predicted death and/or neurological sequelae ($P = .05$).

***Conclusions.*** Neurological *C. gattii* disease predominates in the Australian endemic setting. Lumbar puncture and cerebral imaging, especially if serum CRAG titers are ≥512, are essential. Long-term follow up is required to detect late neurological complications. Immune system evaluation is important because host immunocompromise is associated with reduced survival.

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Correspondence: Sharon Chen, MD, PhD, Centre for Infectious Diseases and Microbiology, 3rd level, ICPMR Building, Westmead Hospital, Darcy Road, Westmead, NSW 2145, Australia (sharon.chen@swahs.health.nsw.gov.au).

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*Cryptococcus gattii* has historically been considered a pathogen of tropical and subtropical regions, especially in persons with apparently normal immune systems [1–5]. However, *C. gattii* has caused recent outbreaks in new climatic zones in British Columbia, Canada, and the US Pacific Northwest [6–8]. In these settings, new risk factors for infection have been identified including human immunodeficiency virus (HIV)/AIDS, cancer, and smoking [9, 10]. In the US Pacific Northwest, oral corticosteroid use within the preceding year increased risk of death in *C. gattii* infection caused by "outbreak" strains [10]. There is debate as to whether these results are generalizable to nonoutbreak settings or other regions [11]. In Australia, *C. gattii* infection is endemic and has predominantly affected nonimmunocompromised hosts, especially Aboriginal people [3–5]. Although the demographics, clinical presentation, and associated comorbidities of *C. gattii* infection have been described [4, 12, 13], longer-term outcomes and predictors of morbidity or mortality have not.

We took advantage of the sporadic occurrence of *C. gattii* infection in Australia to identify factors that influence patient outcomes by analyzing clinical, microbiological, radiological, treatment, and outcome data in patients followed for at least 12 months.

## METHODS

### Study Design

A nationwide retrospective study of *C. gattii* infection in adults diagnosed January 2000–December 2007 was conducted. Fourteen of 17 referral university institutions participated. Cases were identified by searching hospital and laboratory databases. Respective Human Ethics Review Committees approved the study. Data collected included demographics; comorbidities [14]; predisposing factors (eg, corticosteroids/immunosuppressive therapies within the last 12 months); clinical findings; complications (raised intracranial pressure [ICP], immune reconstitution inflammatory syndrome [IRIS]); and microbiological, radiological, treatment, and outcome data. Data were collected at four times: (1) diagnosis to 14 days after initiation of antifungal therapy, (2) approximately 6 weeks after initiation of therapy, (3) approximately 6 months after initiation of therapy, and (4) approximately 12 months after initiation of therapy.

### Definitions

A case was defined as culture-confirmed *C. gattii* infection during the study period. Cases with only positive serum/cerebrospinal fluid (CSF) cryptococcal antigen (CRAG) or compatible histopathology were excluded. Cryptococcal meningoencephalitis was defined as isolation of *C. gattii* from CSF. A brain infection was defined as a radiologist report of mass lesions (≥1 cm diameter) or other parenchymal abnormalities (eg, vasculitic lesions without an alternative diagnosis). Abnormal neurology was defined as seizures, abnormal mental status, or neurological deficits [15]. Raised ICP was defined as an opening pressure ≥25 cm water [16]. Induction therapy was defined as the antifungal regimen used in the initial 14 days of treatment. Immune reconstitution inflammatory syndrome was defined as the worsening and/or onset of new symptoms or radiological features consistent with inflammation in patients with a clinical and/or microbiological response to anticryptococcal therapy and negative cultures [17, 18]; diagnosis required agreement between 2 investigators (S. C-A. C., T. C. S.). Idiopathic CD4 lymphopenia (ICL) was defined as defined previously [19]. Clinical outcome was defined as death (all-cause mortality); progressive disease or failure (worsening clinical symptoms/signs); stable infection (no improvement in symptoms/signs); partial response (≤50% resolution of symptoms and signs); or complete response (resolution of clinical symptoms and signs). Mycological outcome was evaluated at 2 and 6 weeks after the initiation of therapy.

### Microbiology

*Cryptococcus gattii* was identified at source laboratories using standard phenotypic methods [20] and confirmed at 2 reference laboratories (Table 1).

### Data Analysis

The 2006 Australian census was used to calculate population incidences [21, 22]. Data were analyzed using SPSS software version 17. Continuous variables were compared using 2-sample *t* tests (or Mann–Whitney test for time to normalization of CSF parameters), and categorical variables were analyzed using $\chi^2$ or Fisher exact tests.

For the total patient cohort, log-rank tests were used to assess univariate associations between clinical and laboratory variables and all-cause mortality. Kaplan–Meier survival distributions (date of diagnosis to death) were plotted for all patients and by presence or absence of immunocompromise. For patients with central nervous system (CNS) infection, associations were assessed between the above variables and each of 2 outcomes: (1) all-cause mortality, and (2) neurological sequelae and/or deaths at 12 months. Multiple logistic regression analysis with backward stepwise variable selection was used to identify independent predictors of neurological sequelae and/or death in CNS infection. Candidate variables for inclusion in the multivariate model were those variables with a univariable $P \leq .1$ and previously reported outcome determinants of *Cryptococcus neoformans* infection [15, 23].

## RESULTS

Clinical, mycological, and outcome data on day 14 of antifungal therapy were available for 86 of 96 incident infections, and all-cause mortality data were available for 85 patients.

**Table 1. Characteristics of Patients With *Cryptococcus gattii*[a] Infection, 2000–2007**

| Patient Characteristic | No. (%) | |
|---|---|---|
| Male | 51 (59) | |
| Female | 35 (41) | |
| Age | | |
| 15–30 | 14 | |
| 31–49 | 40 | |
| 50–64 | 17 | |
| 65–80 | 15 (17) | |
| Ethnicity[b] | | |
| Caucasian | 50 (58) | |
| Australian Aborigine | 23 (27) | |
| Asian | 7 (8) | |
| Pacific Islander | 4 (5) | |
| Underlying conditions | | |
| None | 62 (72) | |
| Leukemia[c] | 4 (5) | |
| Solid organ cancer | 3 (3) | |
| Kidney transplantation | 3 (3) | |
| Collagen vascular disorders | 2 (2) | |
| Idiopathic CD4 lymphopaenia | 6[d] | |
| Corticosteroid/ immunosuppressive therapy | 12 (14) | |
| Diabetes mellitus | 5 (6) | |
| Pregnancy | 3[e] | |
| Induction antifungal therapy (initial) | | Dose range |
| c-AMB and 5FC | 64 (74) | 0.7 mg/kg/day and 100 mg/kg/day |
| c-AMB only | 7 (8) | 0.7–1.0 mg/kg/day |
| c-AMB and Fluconazole | 2 (2) | 400 mg daily |
| Fluconazole only | 13 (10) | 400–1600 mg daily[f] |

Abbreviations: c-AMB, conventional amphotericin B; 5FC, 5-flucytosine.

[a] Confirmed at Women's and Children's Hospital, Adelaide, and/or Westmead Hospital, Sydney

[b] Ethnicity unknown for 2 patients.

[c] Acute myeloid leukemia (n = 1), acute lymphoblastic leukemia (n = 1), chronic lymphocytic leukemia (n = 2)

[d] Of 44 patients who had T-cell subset studies performed.

[e] Of 21 women of child-bearing age.

[f] Includes 4 patients with isolated lung disease and 9 with central nervous system ± lung infection

The estimated mean annual population-based incidence of *C. gattii* infection was 0.61 per $10^6$ population (n = 96 cases). Annual incidence in Aboriginal Australians (2.3% of population) [21] was 6.3 per $10^6$ population (26.7% cases). Although 48% of cases occurred in the most populous states of New South Wales and Victoria, incidence was highest in the Northern Territory (6.5 per $10^6$ population) (Figure 1). Forty-five (52.3%) patients resided in urban areas, and 41 (47.7%) resided in semirural and/

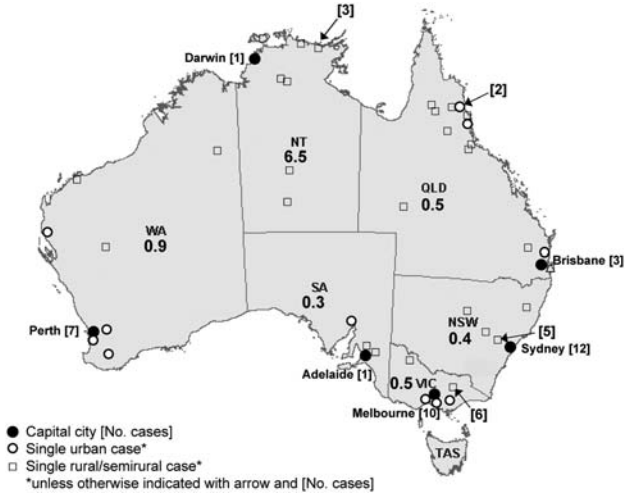


**Figure 1.** Map of Australia showing distribution of capital city (•), other urban (○), and rural and/or semirural (□) cases of *Cryptococcus gattii*, 2000–2007. Numbers within brackets indicate number of cases. If no number is given, a single incident is represented. Incidence per $10^6$ population is indicated within each jurisdiction. Abbreviations: NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

or remote regions. Table 1 summarizes patient characteristics. The proportion of males (59%) was not different from that in the Australian population (49.2%; *P* = not significant), but there was a trend for males to be overrepresented in immunocompetent hosts (*P* = .06) (Supplementary Table 1). Mean age was 46.8 years (standard deviation, 15.1; range, 17–80).

### Host Factors

Sixty-two (72%) patients had no immunocompromise. Of 24 (28%) immunocompromised hosts (Table 1), only 1 of 79 patients tested for HIV had AIDS. All 6 patients with ICL (44 patients had T-cell subset studies) had CD4 counts of $12 \times 10^6$/L to $179 \times 10^6$/L, which persisted for at least 12 months; 4 had concurrent nocardiosis. Comorbidities in 8 additional patients were pregnancy (3 of 21 women of child-bearing age, similar to the general population [approximately 260,000 viable pregnancies annually in women aged 15–49 years] [24]) and diabetes mellitus (5.8% patients vs 7.5% [25]; *P* > .05); 36% of patients were cigarette smokers (vs 21% in the general population [22]; *P* = ·001).

Of 37 (43%) patients with putative environmental exposure to *C. gattii*, 14, including 7 Aboriginals from remote communities, lived or consistently undertook occupational and/or recreational activities that exposed them to *Eucalyptus* trees or their by-products.

### Clinical Presentation

The median time from symptom onset to diagnosis was 45 days (interquartile range [IQR], 24–120) and was similar for

patients in rural and/or semirural areas and urban areas (data not shown). Eighteen (21%) patients required intensive care unit (ICU) admission: 15 due to severe *C. gattii* disease (8 with impaired consciousness and/or seizures and 7 post-CSF shunt placement) and 2 following excision biopsy of cerebral lesions. Mean length of ICU stay was 9.1 days (range, 1–29).

Most patients (85%) had CNS disease. In 44 (60%), lung disease was present concurrently, and all presented with neurological symptoms. There were 10 episodes of cryptoccocemia (Table 2). Demographics, clinical presentation, proportion of patients with serum CRAG titers of ≥512, and proportion of patients with CSF titers of ≥256 in hosts with and without immunocompromise were similar (Table 2). Although fewer immunocompromised patients had brain or brain and meningeal infection, this was not statistically significant.

Of 73 patients with CNS infection, meningoencephalitis (89%) and brain involvement (62%) were common (Table 2). Median time to diagnosis was 45 days (IQR, 21–120) and was ≥8 weeks in 27% of cases. Forty-three patients presented with neurological manifestations: impaired consciousness (n = 18, Glasgow coma scale range, 6–14), cerebellar deficits (n = 10), limb weakness (n = 6), seizures (n = 5), cranial nerve deficits (n = 13; 1 VIII nerve palsy, 7 VI nerve palsies, 5 optic atrophy), and papilledema (n = 9).

At diagnosis, cerebral computerized tomography scan with and/or without MR imaging was performed in 81 (94%) cases. Abnormalities consistent with cerebral cryptococcocosis were identified in 45 cases, including cryptococcomas in 30 cases (17 had multiple lesions, and 13 had a single lesion). There was no association between abnormal neurology at presentation

**Table 2. Clinical and Laboratory Characteristics and Site of Infection According to Host Immune Status in *Cryptococcus gattii* Patients**

| Characteristic | Immunocompetent Hosts, No./Total No. (%) | Immune-Deficient Hosts, No./Total No. (%) | All Patients, No./Total No. (%) | *P* Value |
|---|---|---|---|---|
| Age ≥65 years | 13/62 (21) | 2/24 (8) | 15/86 (17) | .22 |
| Aboriginal ethnicity | 14/62 (23) | 9/24 (38) | 23/86 (27) | .25 |
| ICU admission | 12/62 (19) | 6/24 (25) | 18/86 (21) | .78 |
| Smoking | 23/62 (37) | 8/24 (33) | 31/86 (36) | .94 |
| Site of infection | | | | |
| CNS | 54/62 (87) | 19/24 (79) | 73/86 (85) | |
| Meningitis[a] | 49/62 (79) | 16/24 (67) | 65/86 (76) | .36 |
| Brain[b] | 36 /62(58) | 9/24 (38) | 45/86 (52) | .14 |
| Brain and meningitis | 30/62 (48) | 7/24 (29) | 37/86 (43) | .17 |
| CNS without lung | 21/62 (34) | 8/24 (33) | 29/86 (34) | .84 |
| Lung[c] | 42/62 (68) | 12/24 (50) | 54/86 (63) | |
| Lung and CNS | 35/62 (56) | 9/24 (38) | 44/86 (52) | .18 |
| Lung only | 6/62 (10) | 4/24 (17) | 10/86 (12) | .46 |
| Blood[d] | 5 | 5 | 10 | |
| Skin/subcutaneous tissue | … | 1/24 (4) | 1/86 (1) | … |
| Bone/joint | 1/62 (2) | … | 1 /86 (1) | … |
| Clinical features (CNS infection) | | | | |
| Headache | 36/54 (67) | 11/19 (58) | 47/73 (67) | .68 |
| Abnormal neurology at presentation | 32/54 (59) | 11/19 (58) | 43/73 (59) | .31 |
| CRAG result | | | | |
| Serum titer ≥512 | 30/50 (60) | 11/23 (48) | 41/73 (56) | .47 |
| CSF titer ≥256 | 32/46 (70) | 10/19 (52) | 42/65 (65) | .36 |
| Complications | | | | |
| Raised intracranial pressure | 24/37 (65) | 7/10 (70) | 31/47 (66) | 1.0 |
| Hydrocephalus | 19/54 (35) | 3/19 (16) | 22/73 (30) | .19 |
| Neurological sequelae at death or 12 months | 14/54 (26) | 6/19 (32) | 20/73 (27) | .86 |

Abbreviations: CNS, central nervous system; CRAG, cryptococcal antigen; CSF, cerebrospinal fluid; ICU, intensive care unit.

[a] Seventy-five patients underwent lumbar puncture.

[b] At diagnosis, 81 patients had cerebral computerized tomography scans.

[c] Abnormalities on chest x-rays in 85 patients included cryptococcomas ≥1 cm (single, n = 36; multiple, n = 4) and consolidation or interstitial infiltrates (n = 23).

[d] Of 39 instances where blood cultures were drawn.

**Table 3. Cerebrospinal Fluid Parameters in *Cryptococcus gattii* Meningoencephalitis[a]**

| Parameter | No. Positive/No. Tests at Diagnosis | At 14 Days of Antifungal Therapy (No. Positive/No. Tests) | Total No. Tests |
|---|---|---|---|
| CSF culture | 65/65 | 12/50 | … |
| CSF India Ink | 62/65 | 41/47 | … |
| Raised CSF pressure | 23/39 | 20/32 | 47 |
| | Mean (range) | Mean (range) | |
| CSF protein (g/L) | 0.88 (0.29–2.3) | 1.1 (0..27–4.4) | 47 |
| CSF glucose (mmoL/L) | 2.2 (0–3.2) | 2.2 (0–4.1) | 46 |
| CSF mononuclear cells ($\times 10^6$/L) | 105.6 (2–599) | 72.1 (0–540) | 46 |

Abbreviation: CSF, cerebrospinal fluid.

[a]Sixty-five and 52 patients underwent lumbar puncture at diagnosis and at 14 days after initiation of antifungal therapy, respectively. Overall, 31 of 47 patients had raised CSF pressure.

and specific radiographic lesions, including single large cryptococcomas (23% of patients compared with 7% of patients without abnormal neurology; $P = .11$).

Fifty-four patients (63%) had abnormal chest imaging (Table 2). Median time from symptom onset to diagnosis in 10 patients with isolated lung infection was 56.8 days (IQR, 1–180 days); 9 presented with cough, haemoptysis, and dyspnea.

### Laboratory Studies

Baseline serum CRAG was positive in 71 of 73 cases, including 62 of 63 (98%) CNS infections and 9 of 10 (90%) isolated lung infections. Geometric mean (GM) titers were 563.9 (range, 16–>16 484) in CNS infection, and 149.3 (range negative result-8192) in isolated lung cryptococcosis.

The GM CSF CRAG titer in patients with meningitis (sensitivity, 100%) was 282.8; titers were ≥256 in 65% of cases. Table 3 summarizes CSF parameters at baseline and at 14 days of therapy. The ICP was raised in 23 of 65 (35%) patients at diagnosis; a further 8 developed it within 14 days, and none developed it thereafter. Elevated ICP did not correlate with CSF CRAG titers or presence of cerebral mass lesions; 15 of 31 patients had cryptococcomas (vs 15 of 32 patients without raised ICP; $P = .89$). Two-week CSF cultures were negative in 38 of 50 (76%) patients and in all but 1 patient (with abnormal neurology) at 6 weeks. The median time to an 8-fold drop in CRAG titer (approximately 6 months) was similar in patients with and without abnormal baseline neurology, but India ink, protein, and leukocyte abnormalities persisted significantly longer in the former (Table 4).

### Induction Antifungal Therapy

Sixty-four patients (74%) received induction therapy with amphotericin B (AMB) and 5-flucytosine (median, 6 weeks [range, 2–12]) (Table 1). Eighteen (24%) patients with CNS infection received fluconazole only (n = 9), AMB (n = 7), or AMB plus fluconazole (n = 2). Six patients with isolated lung infection received AMB plus 5-flucytosine and 4-fluconazole.

### Patient Outcomes

Eleven (13%) patients died within 4 months of diagnosis, 10 from *C. gattii*. At 12 months, a complete response was noted in 33% of patients, and a partial response was noted in 48% of patients. Mortality in CNS infection was 13.6% (10 of 73 patients); mortality was 11% in CNS plus lung infection and 17% in CNS infection only ($P = .51$). Only 1 of 9 patients with isolated lung infection died. There were significantly more *C. gattii*–related deaths in immunocompromised patients (7 of 24 patients) than in healthy hosts (3 of 62; $P = .002$).

Hydrocephalus due to CSF outflow obstruction was identified within 2 months in 18 patients with CNS infection but developed between 2 and 12 months in an additional 4 (22; 30%); 14 (64%) required placement of CSF shunts and/or drains a median of 2 weeks (range, 3 days to 18 months) after diagnosis. Of 31 patients with raised ICP, 17 (55%) had shunts and/or drains placed (median, 4 weeks; range, 2 days to 8 months); 5 patients had both raised ICP and hydrocephalus.

Twenty patients had neurological sequelae at 12 months (8 patients who died and 12 survivors). Deficits included visual impairment (8 patients; 3 became blind), deafness (n = 3), limb weakness (n = 2) and dysphasia (n = 2). Proportions of patients with CNS plus lung infection and with CNS infection only with neurological sequelae (24% vs 29%) were similar, as were the proportions with raised ICP (51% vs 36%; data not shown). In 4 survivors, sequelae developed during therapy. Immune reconstitution inflammatory syndrome occurred in 7 (11%) patients (5 were immunocompetent) a median of 16 weeks (range, 6–52) after starting antifungals.

#### *Determinants of Mortality and Neurological Sequelae*

Overall, ICU admission ($P < .001$) and immunocompromise ($P = .007$) increased the risk of death by univariate analysis

**Table 4. Resolution of Cerebrospinal Fluid Abnormalities With Time by Neurological Status at Diagnosis in 39 Patients With Meningitis**

| CSF Parameter (No. Tests Performed) | Abnormal Neurology at Diagnosis (n = 20 Patients) No. Patients With Normal Test/No. Patients | No Abnormal Neurology at Diagnosis (n = 19 Patients) No. Patients With Normal Test/Total No. Patients | *P* Value |
|---|---|---|---|
| India ink negative result (n = 37) | | | |
| Within 6 weeks | 8/18 | 12/19 | .03 |
| 6 weeks–6 months | 4/18 | 7/19 | |
| 6–12 months | 6/18 | …[a] | |
| Protein (n = 34) | | | |
| Within 6 weeks | 2/18 | 7/16 | .004 |
| 6 weeks–6 months | 5/18 | 6/16 | |
| 6–12 months | 5/18 | 3/16 | |
| >12 months | 6/18 | …[a] | |
| Glucose (n = 38) | | | |
| Within 6 weeks | 0/20 | 0/18 | .21 |
| 6 weeks–6 months | 4/20 | 5/18 | |
| 6–12 months | 7/20 | 9/18 | |
| >12 months | 9/20 | 4/18 | |
| Mononuclear cells (n = 38) | | | |
| Within 6 weeks | 2/19 | 7/19 | <.001 |
| 6 weeks–6 months | 2/19 | 8/19 | |
| 6–12 months | 9/19 | 4/19 | |
| >12 months | 6/19 | …[a] | |

Abbreviation: CSF, cerebrospinal fluid.

[a] No patient had an abnormal test at this approximate time point.

**Table 5. Univariable Analyses of Potential Risk Factors for Overall Survival at 12 Months: All Patients**

| Variable (No. Evaluable) | Deaths, % (No./Total No.) | Nondeaths, % (No./Total No.) | *P* Value |
|---|---|---|---|
| Overall survival[a] | | | |
| Male sex (n = 51) | 54.5 (6/11) | 59.2 (45/76) | .76 |
| Australian Aborigine (n = 23) | 54.5 (6/11) | 27.4 (17/62) | .051 |
| ICU (n = 18) | 63.6 (7/11) | 14.9 (11/74) | ≤.001 |
| Underlying immunocompromise (n = 24) | 63.6 (7/11) | 19.9 (14/74) | .007 |
| Time to diagnosis ≥4 weeks (n = 44) | 36.4 (4/11) | 54.1 (40/74) | .24 |
| Time to diagnosis ≥8 weeks (n = 24) | 27.3 (3/11) | 28.2 (21/74) | .85 |
| Abnormal neurology at presentation (n = 43)[b] | 72.7 (8/11) | 47.3 (35/74) | .09 |
| CNS disease (all) (n = 73) | 90.9 (10/11) | 85.1 (63/74) | 1.0 |
| CNS plus lung (n = 44) | 54.5 (6/11) | 51.3 (38/74) | 0.90 |
| CNS only (n = 29) | 36.4 (4/11) | 33.8 (25/74) | 1.0 |
| Serum antigen ≥512 (n = 41) | 60 (6/10) | 60.7 (37/61) | .82 |
| CSF antigen ≥256 (n = 42) | 72.7 (8/11) | 54 (34/63) | .22 |
| Raised intracranial pressure (≥25 cm) (n = 23) | 83.3 (5/6) | 47.3 (18/38) | .09 |
| CSF culture positivity at 2 weeks (n = 12) | 25 (2/18) | 21.3 (10/47) | .75 |
| Hydrocephalus (n = 22) | 36.3 (4/11) | 24.3 (18/74) | .25 |

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; ICU, intensive care unit.

[a] Log-rank test.

[b] Includes abnormal mental status (n = 18), cerebellar deficits (n = 10), pyramidal tract signs (n = 6), seizures (n = 5), cranial nerve deficits (n = 13; 1 VIII nerve palsy, 7 VI nerve palsies associated with raised intracranial pressure), papilloedema (n = 9), and optic atrophy (n = 5).

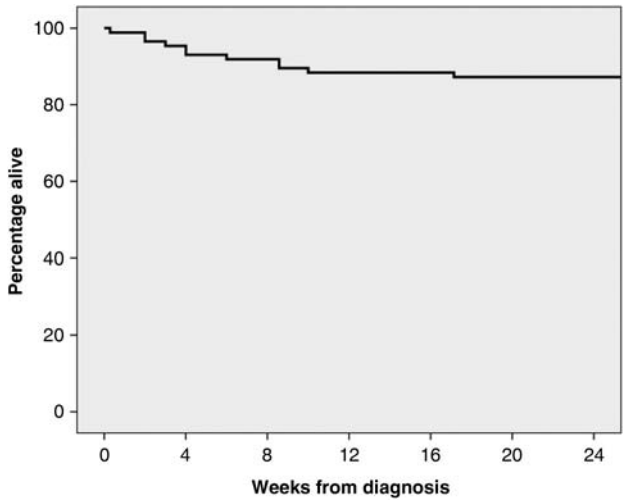


**Figure 2.** Kaplan–Meier survival curve for all patients (n = 86) with *Cryptococcus gattii* infection: percentage of patients alive by weeks from diagnosis.

(Table 5). Kaplan–Meier survival curves for the total patient cohort and by underlying immunocompromise are shown in Figures 2 and 3, respectively. Risk of death was not associated with late presentation (≥8 weeks) or baseline serum CRAG titers ≥512, but there was a trend toward significance for patients with initial abnormal neurological features and those with raised ICP (Table 5).

In patients with CNS infection, immunocompromise was again associated with increased mortality (Table 6). Although baseline abnormal neurology ($P = .03$) and CSF CRAG titers of ≥256 ($P = .02$) were associated with death and/or neurological sequelae at 12 months by univariable analysis, multiple logistic regression identified only CSF titers ≥256 as an independent predictor (odds ratio, 1.8; 95% confidence interval [CI], 1–26; $P = .05$). After adjusting for CSF titers of ≥256, the odds ratio for abnormal neurology as a risk variable was 3.9 (95% CI: .94–16.8; $P = .06$). Two-week CSF culture positivity was not a risk factor for adverse outcomes (Table 6).

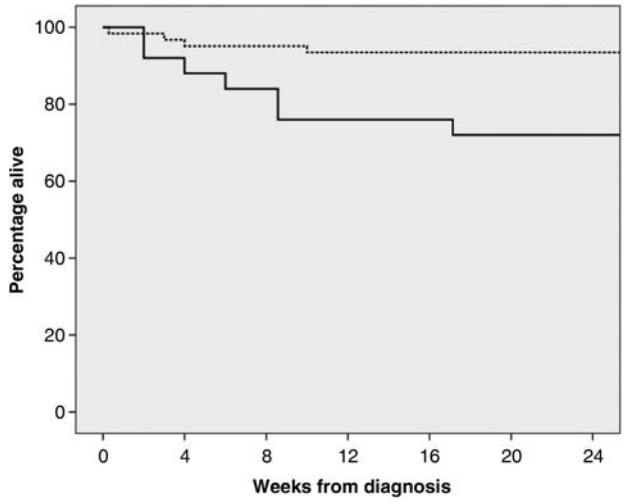


**Figure 3.** Survival of *Cryptococcus gattii* patients according to underlying immunocompromise: percentage of patients alive by weeks from diagnosis. Immunocompromised, solid line; nonimmunocompromised, dashed line.

## DISCUSSION

This contemporary, population-based Australian study confirmed that Aborigines and apparently healthy hosts are at highest risk of *C. gattii* infection and that underlying HIV/AIDS is rare [4, 26]. Unlike previous *C. gattii* studies, the 12-month patient follow-up enabled us to determine longer-term complications of *C. gattii* infection and predictors of outcomes.

The annual incidence and distribution of *C. gattii* infection in Australia remains unchanged at approximately 0.61 per $10^6$ population with the highest per capita case load in the Northern Territory. Exposure to *Eucalyptus* trees, the only known ecological niche of *C. gattii* in Australia [27] was common (43%). Although exposure may be the dominant risk factor for infection in Aborigines, the contribution of genetic determinants warrants study. The proportions of diabetic or pregnant patients were similar to the general population, but significantly more patients were smokers ($P = .001$). In the British Columbia outbreak in which lung infection was more common, cigarette smoking was also a risk factor [9]. Harris et al correlated "outbreak" strains of *C. gattii* with preexisting diseases in the United States, but the categorization of outbreak and nonoutbreak strains was based on genotype rather than epidemiological linkage [10]. Case–control studies are warranted to validate these findings.

The most striking change in epidemiology was the increase in HIV-negative immunocompromised patients (approximately 28% vs 8.5% in a previous Australian survey) [4] due to cancer (8%), ICL (at least 14%) and long-term immunosuppressive therapies (14%). Risk factors in British Columbia included steroid use, HIV infection, and cancer [9]. Although ICL is a risk factor for *C. neoformans* infection [28], its association with *C. gattii* is new. Possible explanations include failure to investigate for ICL previously or changes in host- or pathogen-related risk factors. Diagnosis of ICL is important because of increased risk of opportunistic coinfection; the 4 patients coinfected with *Nocardia* herein add to the experience of 2 previous cases, including 1 in a patient with impaired lymphocyte function [29, 30].

**Table 6. Univariable Analyses of Potential Risk Factors for Overall Survival, and for Death and/or Neurological Sequelae at 12 months: Central Nervous System Infection**

| Variable (No. Evaluable) | | | *P* Value |
|---|---|---|---|
| Overall survival[a] | | | |
| | Deaths, % (No./Total No.) | Nondeaths, % (No./Total No.) | |
| Male sex (n = 44) | 66.7 (6/9) | 59.4 (38/64) | .66 |
| Australian Aboriginal (n = 16) | 44.4 (4/9) | 19.4 (12/62) | .1 |
| ICU stay (n = 17) | 66.7 (6/9) | 17.2 (11/64) | <.001 |
| Underlying immunocompromise (n = 19) | 55.6 (5/9) | 21.9 (14/64) | .03 |
| Time to diagnosis ≥4 weeks (n = 37) | 33.5 (3/9) | 53.1 (34/64) | .25 |
| Time to diagnosis ≥8 weeks (n = 20) | 22.2 (2/9) | 28.1 (18/64) | .68 |
| Abnormal neurology at presentation[b] (n = 42) | 88.9 (8/9) | 53.1 (34/64) | .05 |
| Serum antigen ≥512 (n = 37) | 66.7 (6/9) | 59.6 (31/52) | .41 |
| CSF antigen ≥256 (n = 42) | 88.9 (8/9) | 61.8 (34/55) | .12 |
| Raised intracranial pressure (≥25 cm; N = 22) | 80 (4/5) | 54.3 (19/35) | .23 |
| CSF culture positivity at 2 weeks (n = 12) | 28.6 (2/7) | 21.3 (10/47) | .62 |
| Hydrocephalus (n = 22) | 44.4 (4/9) | 28.2 (18/64) | .21 |
| Neurological sequelae[b] and/or death at 12 months (n = 20)[c] | | | |
| | Death/Sequelae, % (No./Total No.) | Alive/No Sequelae, % (No./Total No.) | |
| Underlying immune compromise (n = 19) | 36.8 (7/19) | 20.5 (9/44) | .21 |
| Abnormal neurology at presentation[b] (n = 40) | 80 (16/20) | 54.5 (24/44) | .03 |
| Serum antigen ≥512 (n = 35) | 73. 3 (11/15) | 61.1 (22/36) | .41 |
| CSF antigen ≥256 (n = 38) | 88.9 (16/18) | 57.9 (22/38) | .02 |
| Raised intracranial pressure (≥25 cm) (n = 20) | 75 (6/8) | 52 (13/25) | .42 |
| CSF culture positivity at 2 weeks (n = 10) | 37.5 (6/16) | 13.3 (4/30) | .07 |

Abbreviations: CSF, cerebrospinal fluid; ICU, intensive care unit.

[a] Log-rank test.

[b] Includes abnormal mental status (n = 18), cerebellar deficits (n = 10), pyramidal tract signs (n = 6), seizures (n = 5), cranial nerve deficits (n = 13; 1 VIII nerve palsy, 7 VI nerve palsies associated with raised intracranial pressure), papilloedema (n = 9), and optic atrophy (n = 5).

[c] $\chi^2$or Fisher exact test.

As in a previous Australian study, most patients (85%) presented with CNS cryptococcosis, especially meningitis (76% vs 64%) [4], but the ratio of meningitis to brain infection decreased (1.5:1 vs 2.5:1), presumably because computed tomography scanning, which is less sensitive than MR imaging, was used in the first study. Of note, <10% of Canadian patients and 37% of US patients had neurological disease [7, 10]. Concurrent lung and CNS infection was common (52%), and blood cultures, although infrequently collected, were positive in 26% of cases. Clinical and microbiological characteristics in healthy and immunocompromised patients were similar.

Key findings in CNS infection included raised ICP (42%), baseline neurological abnormalities (50%), and severe illness as indicated by the number of *C. gattii*–related ICU admissions (17%). Papilledema and optic atrophy were noted initially in 15.8% and 8.8% of cases, respectively. Overall mortality was low (13%), compared with 8.7% in British Columbia and 22.4%–36.5% in Papua New Guinea, northern Australia, and the United States [10, 13, 31]. The low mortality is likely due partly to aggressive treatment of raised ICP and/or hydrocephalus; approximately 60% of cases were managed with a ventricular CSF shunt and/or drain, which is critical to prevent neurological sequelae [32]. Complications during therapy included raised ICP in the first 2 weeks, late (up to 12 months) hydrocephalus, and IRIS-like syndrome (5 of 7 cases were in nonimmunocompromised hosts). Visual impairment including blindness was the most common of the persistent and/or new sequelae (40%). *Cryptococcus gattii* may have a predilection for the visual apparatus because optic neuritis and/or papilledema and optic atrophy were noted in earlier studies [3, 31, 32].

That outcomes including *C. gattii*–attributable deaths were worse in immunocompromised patients is noteworthy. In a recent US report [10], immunocompromise was not associated with mortality, but oral corticosteroid therapy was. Because we included corticosteroid therapy as a cause of immunocompromise, these data are not directly comparable. In predominantly HIV-infected patients with *C. neoformans* meningitis, baseline

abnormal neurology and hematological malignancy were associated with reduced 3-month survival [15]. In another study, age >60 years, hematological cancer, and organ failure predicted death in largely HIV-negative *C. neoformans* patients [23]. In the present study, by univariate analysis, baseline abnormal neurology, high serum CRAG titers (≥512), and positive 2-week CSF cultures were not associated with mortality. Notably, however, the only independent predictor of neurological sequelae and/or death was a baseline CSF titer of ≥256.

Time from symptom onset to diagnosis did not influence outcome and was comparable to that previously reported in Australia (median, 45 days vs 6 weeks in CNS infection) [3]. Median time was longer in isolated lung infection than in CNS infection, presumably because neural tissue is less accommodating of growing cryptococcal lesions than pulmonary tissue and patients develop and present earlier with neurological symptoms. Time to presentation and/or diagnosis may also reflect differences in host factors, exposure dose, and patient recall of symptom onset.

Laboratory investigations are central to diagnosis. Even in isolated lung infection, the sensitivity of serum CRAG testing was 90%. Because GM serum CRAG titers in isolated lung infection were 4-fold lower than in CNS disease, titers of ≥512 should alert physicians to the likelihood of meningoencephalitis. That 76% of 2-week CSF cultures were negative represents a much higher rate of clearance than in HIV-infected patients [33]; culture positivity at 2 weeks did not correlate with mortality, although a correlation with death and/or neurological sequelae ($P = .07$) may become significant with a larger sample size. Delayed clearance of cryptococci from CSF is a poor prognostic marker in HIV-infected and other immunocompromised patients with *C. neoformans* infection [15, 34]. This may be due to higher CSF fungal loads in immunosuppressed patients or as yet undefined factors. Positive 2-week cultures were not associated with baseline neurological abnormalities; however, elevated 6-week CSF protein and mononuclear cell counts were 2–3-fold more common and remained abnormal in approximately 70% cases for ≥12 months.

The study limitations include its retrospective design, which introduces the possibility of unrecognized biases, incomplete data collection including serum/CSF CRAG and CSF pressure measurements, and the small number of deaths precluding multivariate analysis of factors influencing mortality.

In summary, the predominance of CNS involvement likely reflects differences in *C. gattii* infection between the outbreak and endemic setting. Raised ICP, hydrocephalus, and neurological sequelae were common and may be delayed. Host immunocompromise was associated with increased risk of death, and CSF CRAG titers of ≥256 predicted mortality and/or neurological sequelae. Key clinical practice points highlighted by this study include the need for lumbar puncture and cerebral imaging in all patients, especially if serum CRAG titers are ≥512; investigation for host immunocompromise, including ICL; determination of CSF CRAG as a prognostic indicator; and early measurement of CSF pressure and prompt management if raised.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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