

Title: Cryptococcal meningitis in overtly immunocompetent patients: Association with idiopathic CD4+ lymphopenia

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ABSTRACT

We present two cases of cryptococcal meningitis in patients subsequently diagnosed with idiopathic CD4+ lymphopenia. Both presented with new onset headaches without sinister features and were sent home on multiple occasions from emergency departments. Cryptococcal meningitis in HIV-negative patients poses major diagnostic and management problems and the associated mortality is 9-27%. We suggest blood and CSF cryptococcal antigen tests are performed for all patients with lymphocytic meningitis.

CASE 1

A 33-year-old Irish woman with a background of juvenile idiopathic arthritis in remission without treatment presented to the emergency department with headaches but no red flag features and was discharged. She re-presented several weeks later with vomiting and was again discharged. Five weeks after onset she presented for a third time. Neurological examination was unremarkable and she was afebrile. Initial investigations demonstrated a raised C-reactive protein of 68mg/L (<5mg/L) and a low lymphocyte count $0.2 \times 10^9/L$ ($1.1-4.0 \times 10^9/L$). Lumbar puncture revealed; opening pressure 28 cmH₂O, lymphocyte count $14 \times 10^6/L$, protein 0.83g/L and glucose 1.9mmol/L (serum 4.4mmol/L). *Cryptococcus neoformans* was grown from her blood culture and subsequently from the CSF. The cryptococcal antigen was also positive in blood at 1:160 and CSF at 1:1280.

HIV serology was negative but immunological investigations revealed a CD4+ lymphopenia with cell count $10 \times 10^6/L$ ($500-1500 \times 10^6/L$). There was also a mild CD8+ lymphopenia that resolved on repeat testing. She had normal serum immunoglobulins and IgG subclasses, except for a mildly elevated serum IgA. A serological screen for other viral causes of lymphopenia was negative. The

antinuclear antibody titre was 1:1280 and RNP antibody was positive raising the possibility of subclinical mixed connective tissue disease. T2-weighted MRI of the brain showed small patches of non-specific hyperintensity within the cerebral white matter, associated with mildly enlarged perivascular spaces and, after contrast, small focal enhancing lesions within the right internal capsule and left caudate nucleus (figure 1).

She was an inpatient for six weeks and was treated with intravenous amphotericin B and flucytosine, had three lumbar punctures for CSF pressure monitoring (table 1) and made a full recovery. She has remained well for 12 months on maintenance treatment with oral fluconazole and prophylactic co-trimoxazole. Blood tests have continued to show CD4+ counts below $50 \times 10^6/L$.

Day	Opening pressure (cmH ₂ O)	White cell count (x 10 ⁶ /L)	Protein (g/L)	Cryptococcal antigen titre
1	28	14	0.83	1:1280
2	27	32	0.88	-
4	30	20	0.71	1:80
10	22	12	0.50	1:128

Table 1 CSF opening pressure and analyses in case 1.

CASE 2

A 48-year-old man of Pakistani descent developed headaches and photophobia after a cataract operation, and was discharged from three different emergency departments over a two-week period. He was finally admitted when he was found to be profoundly confused at a routine outpatient appointment. Upon transfer to the emergency department he required sedation and intubation due to agitation, and to

enable further assessment. He had abused cocaine and heroin until four years earlier and had received treatment for tuberculosis two years previously after developing night sweats and cervical lymphadenopathy; a lymph node biopsy had shown granulomata without necrosis or acid-fast bacilli and culture was negative.

An initial CT head showed right maxillary antral mucosal thickening only. Lumbar puncture revealed an opening pressure of 44 cmH₂O, white cell count 212 x 10⁶/L (75% lymphocytes), protein 0.89g/L, glucose 1.1mmol/L and no growth after prolonged incubation. HIV serology was negative. Intravenous aciclovir and ceftriaxone was initiated and anti-tuberculous therapy subsequently added. An MRI brain showed gyral-sulcal restricted diffusion in keeping with meningoencephalitis. Repeat lumbar puncture revealed a positive CSF cryptococcal antigen and *Cryptococcus neoformans* was grown.

The patient was treated on the intensive care unit with intravenous amphotericin B and flucytosine for four weeks. During this time he had a persistently raised CSF pressure despite lumbar puncture and underwent a temporary lumbar drain insertion after a communicating hydrocephalus was identified on further imaging. Cervical and axillary lymphadenopathy was noted on examination and a repeat lymph node biopsy showed granulomatous inflammation with encapsulated yeasts in keeping with disseminated cryptococcal infection. A repeat MRI brain showed patchy areas of abnormal signal related to the frontal, parietal and temporal and insular cortices bilaterally (figure 2) without meningeal enhancement. Immunological investigations subsequently revealed a CD4+ lymphopenia. The CD56+ and CD19+ cell counts were also mildly reduced and increased, respectively. His lymphocyte subsets were as follows: CD3 absolute 911 x 10⁶/L (59%), CD4 absolute 115 x 10⁶/L (7%), CD8 absolute 840 x 10⁶/L (53%), CD19 absolute 526 x 10⁶/L (35%), CD56 absolute 80 x 10⁶/L (5%). He had normal serum immunoglobulins and IgG subclasses.

During his six-month admission he made a gradual recovery but still has major cognitive problems. He remains distractible and impulsive, cannot comply with formal cognitive testing, can follow simple commands but needs prompting to eat or wash. He continues to take maintenance oral fluconazole and prophylactic co-trimoxazole.

DISCUSSION

Diagnosis was delayed in both cases, and may have contributed to the adverse outcomes in the second case. There is a need for increased awareness of cryptococcal infection in HIV-negative patients with no overt iatrogenic immunocompromise.

Cryptococcal disease in the absence of HIV infection is rare, and is most commonly described in association with organ transplants, haematological malignancies, renal or liver failure and chronic steroid use. In the last 15 years there has been increasing recognition of a group of patients with previously unknown primary immunological disturbances that predispose to cryptococcal infection. These include idiopathic CD4+ lymphopenia, antibodies against granulocyte-macrophage colony stimulating factor or IFN- γ and monogenic disorders such as chronic granulomatous disease, hyperimmunoglobulin E syndromes and hyperimmunoglobulin IgM syndromes.[1] Idiopathic CD4+ lymphopenia (ICL) first described in 1992 is defined by a CD4+ count below $300 \times 10^6/L$ on at least two occasions in the absence of HIV infection or other known immunodeficiency or treatment associated with lymphopenia.[2] The aetiology remains unclear, but may be due to defects in T-cell receptor signalling, including CXCR4 expression, and UNC119 gene mutations.[1] Autoimmunity is reported to occur in around 25% of patients, most frequently systemic lupus erythematosus (SLE).[3]

These “pseudo” immunocompetent patients present major diagnostic problems. Zonios *et al* reviewed all 53 cases of cryptococcal meningitis with ICL in the literature to 2007.[4] Median age was 41 years with CD4+ count $82 \times 10^6/L$ (range $7-292 \times 10^6/L$) at presentation. Fever was present in only 42% and other opportunistic infections suggestive of immunodeficiency were only present in 4%. [4] Absence of fever introduced significant diagnostic delay as with our cases. Cryptococcal meningitis may be caused by *C. neoformans*, as in the cases described, or *C. gattii*. *C. neoformans* occurs worldwide, with infections typically occurring in immunocompromised individuals. *C. gattii*, endemic in tropical and subtropical regions, has recently emerged in the Pacific northwest of North America and causes infection in seemingly immunocompetent individuals.[1]

The investigation of HIV-negative cryptococcal meningitis is usually straightforward, but only if the diagnosis is considered. CSF white cell count and protein are higher and glucose is lower than in HIV patients.[5] Cryptococcal antigen tests are positive in the CSF of 97% and serum of 87% of all patients with cryptococcal meningitis. The sensitivity in serum may be lower in HIV-negative individuals and was 77% in a cohort of solid organ transplant recipients.[6] False negatives with the latex agglutination assay have been reported in patients with high antibody titres, due to the prozone effect, and in infection with *C. gattii*. A relatively recently developed lateral flow assay is a point of care antigen test with an improved sensitivity in blood and CSF, including for *C. gattii*, and is not subject to the prozone effect.[7] MRI findings are non-specific and include dilated Virchow-Robin spaces, pseudocysts, cryptococcomas and lacunar or cortical infarcts. Large cryptococcomas are associated with *C. gattii*. [1] Referral for immunological investigations should also be considered following a diagnosis of cryptococcal infection in an HIV-negative patient.

Treatment protocols derived from limited trials in HIV positive patients are covered in the 2010 guidelines from the Infectious Disease Society of America.[8] Treatment in HIV-positive patients is usually with amphotericin and flucytosine for at least two weeks. Liposomal amphotericin is preferred in HIV-negative patients because it is equally effective but better tolerated as treatment duration is four to six weeks.[1] Intravenous fluids, electrolyte replacement and a reduction in the dose of flucytosine may be needed in acute kidney injury to avoid bone marrow toxicity. Consolidation involves high dose oral fluconazole for eight weeks followed by maintenance therapy with lower doses usually for at least 6-12 months.[1] There is no clear evidence base to guide the duration of maintenance therapy, or secondary prophylaxis, in HIV-negative patients. Extrapolating from guidelines for HIV-infected patients, we recommend monitoring the CD4+ count and having a low threshold for continuing maintenance fluconazole (assuming no attributable side effects), and considering co-trimoxazole prophylaxis, if the CD4+ count remains below $100 \times 10^6/L$. [8]

Raised CSF pressure occurs in 19% of HIV-negative patients (median $23\text{cmH}_2\text{O}$), [9] and was encountered in both reported cases. This is caused by impaired CSF reabsorption and a communicating hydrocephalus can develop. Management of raised CSF pressure involves regular therapeutic lumbar punctures until the pressure is below $25\text{cmH}_2\text{O}$. [1] Occasionally lumbar drain insertion is carried out, as in case 2. Obstructive hydrocephalus can also occur in HIV-negative patients, particularly in those with large cryptococcomas secondary to *C. gattii* infection, and ventricular shunting may be required. Pappas *et al* reported that 11% of HIV-negative patients with Cryptococcal meningitis required a permanent ventricular shunt. [9] Steroids are not routinely recommended but may have a role in large cryptococcomas associated with oedema or in immunocompetent patients that develop a post-infectious inflammatory response syndrome. [8]

Mortality in cryptococcal meningitis is higher in HIV-negative patients and was reported as 27% by Pappas *et al* in 2013.[5] In the aforementioned series of ICL patients alone it was only 9%. The reasons behind these large differences in the two major series are unexplained and more recent data on mortality in ICL is lacking. Haematological malignancy, organ failure, absence of headache, male gender, altered mental status and possibly raised intracranial pressure were associated with increased mortality in HIV-negative patients.[1]

CONCLUSION

HIV-negative patients with cryptococcal meningitis pose diagnostic and management problems because these patients may have no prior history suggesting immunosuppression and if due to a primary immunodeficiency, immune reconstruction may be impossible. The high mortality in HIV-negative patients necessitates a low index of suspicion in patients with a new onset of headaches. We advocate blood and CSF cryptococcal antigen testing in all cases of lymphocytic meningitis.

KEY POINTS

- Cryptococcal meningitis rarely occurs in apparently immunocompetent patients
- Multiple novel non-HIV immunodeficiency syndromes have been identified of which idiopathic CD4+ lymphopenia is the best known
- Fever is absent in half of cases
- Diagnostic delay is common and may contribute to mortality rates of 9-27%
- We recommend Cryptococcal antigen testing in blood and CSF in all cases of lymphocytic meningitis

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LEGENDS

Figure 1 Axial T2-weighted MRI Brain image in case 1 showing multiple deep white matter nonspecific lesions.

Figure 2 Axial T2-weighted MRI Brain image in case 2 showing patchy areas of abnormal signal related to the frontal, parietal and temporal and insular cortices.