

## Fungal burden and raised intracranial pressure are independently associated with visual loss in HIV-associated cryptococcal meningitis

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**Author contributions:** SM and TB conceived of and designed the study. BR performed the data analyses, together with SM, with statistical input from DW and SJ. CK, SM, SL, RH, NK, JE, CK, DC, ET, SL, AKC, JvO, YM, PM, DGL, OL, MCH, AL and TSH led on and conducted the parent clinical trial, enrolling patients, performing visual acuity assessments and collecting the clinical data. AM advised on visual acuity assessments. SM and TB interpreted the data and wrote the paper, with input from all the authors.

**Abstract**

Amongst 472 patients with HIV-associated cryptococcal meningitis, 16% had severe visual loss at presentation, of whom 46% of 4-week survivors remained severely impaired. Baseline CSF opening pressure  $\geq 40$  cmH<sub>2</sub>O (aOR 2.56, 95% CI 1.36-4.83, p=0.02) and fungal burden  $>6.0$  log<sub>10</sub>Colonies/ml (aOR 3.01, 95%CI 1.58-5.7, p= 0.003) were independently associated with severe visual loss.

1 **Introduction**

2 HIV-associated cryptococcal meningitis (CM) is the commonest cause of adult meningitis in sub-  
3 Saharan Africa, and accounts for ~180,000 deaths/year globally[1]. In addition to headache, CM often  
4 presents with altered mental status, raised intracranial pressure (ICP) and hearing and visual loss[2],  
5 with survivors often experiencing neurological sequelae[3-5]. In CM, visual loss is usually bilateral,  
6 can be sudden or gradual in onset, occurs before or during antifungal treatment, and - especially  
7 without intervention - is frequently irreversible[3, 5, 6]. Evaluation of visual acuity in encephalopathic  
8 patients is challenging: the few CM cohorts (HIV and non-HIV) that systematically examined visual  
9 acuity report a prevalence of 33%-46% for any visual impairment (<6/6 Snellen chart), with profound  
10 visual loss (<6/60) occurring in 13% of patients[5-7].

11 Proposed mechanisms of visual loss include direct optic nerve infiltration by cryptococci  
12 (demonstrated on autopsy as well as tissue biopsy), inflammatory arachnoiditis, or optic nerve  
13 compression through raised ICP or a more localised optic nerve compartment syndrome[5], with both  
14 fungal burden and raised ICP implicated in its pathophysiology[3, 8]. Prior, small retrospective cohorts  
15 (<100 patients) suggested that high fungal burden (cerebrospinal fluid (CSF) cryptococcal antigen  
16 titre>1024) or raised ICP (CSF opening pressure(OP) >30cm H<sub>2</sub>O) are associated with visual loss in CM,  
17 but have been too small to undertake multivariable analyses[3, 6]. Here we report on the prevalence,  
18 risk factors and reversibility of visual loss in a large prospective CM patient multi-country cohort in  
19 sub-Saharan Africa[9].

20

21

## 22 **Methods**

23 Anonymised data from the ACTA (Advancing Cryptococcal Meningitis Treatment in Africa, ISCRTN  
24 45035509) trial formed the dataset for this pre-planned sub-study[9]. Between 2013 and 2016, 678  
25 adults with HIV CM were randomised to oral fluconazole plus flucytosine or amphotericin B-based  
26 therapy for one or two weeks. Patients had protocol-specified lumbar punctures (LPs) on day 1, 7 and  
27 14 with OP measurements and therapeutic CSF drainage according to guidelines[10].

28 Visual acuity (VA) was measured at baseline ( $\leq 3$  days from enrolment) and at 4 weeks using the  
29 standardised logMAR chart in surviving patients whose conscious level permitted assessment. The  
30 score, ranging from 0 (able to read all letters on the smallest line) to 1.375 (unable to read any letters),  
31 was recorded for each eye. Those unable to read any letters were assessed for finger counting, hand  
32 movement and light perception. Those without light perception were considered blind. Patients were  
33 classified into 6 categories: 1. Near-normal vision (score  $< 0.5$ ), 2. Moderate visual loss (score  $\geq 0.5$  but  
34  $< 1.0$ ), 3. Finger counting (score  $\geq 1.0$  and able to count fingers), 4. Hand motion perception, 5. Light  
35 perception, or 6. No light perception. When VA varied between the left and right eye (9.3%, 44/472),  
36 data for the worst eye was analysed. Patients did not routinely undergo CT brain or fundoscopy to  
37 assess for papilloedema or other ocular pathology.

38 Data were analyzed using Stata v15 (StataCorp, USA), using Kruskal-Wallis tests for continuous  
39 variables, chi-squared tests for categorical variables and quantile regression to test for trend across  
40 the 6 visual acuity categories. Based on proposed pathophysiological mechanisms, univariable and  
41 multivariable logistic regression was used to investigate the association between baseline visual loss  
42 and CSF white cell count (WCC), OP and fungal burden, adjusted *a priori* for age. Sex, CD4 count and  
43 ART status (naïve or exposed) were not associated with visual loss and no other measured variables  
44 were hypothesised to confound the relationship. For analysis of the association between VA, CSF OP  
45 and fungal burden, VA was dichotomised into near-normal/moderate vision (categories 1 and 2) and  
46 severe visual loss (categories 3-6), similar to definitions in [4].

47 To assess impact of antifungal therapy and therapeutic LPs on reversibility of visual impairment, rate  
48 of clearance of infection, number of LPs performed, total CSF volume removed and change in OP over  
49 2 weeks' treatment was compared between those with repeat measurements at 4 weeks in whom  
50 acuity deteriorated and those who improved; and in those that remained severe vs improved from  
51 severe.

52 Patient consent statement

53 Written informed consent was obtained from all participants in ACTA. Ethical approval was granted  
54 by the London School of Hygiene and Tropical Medicine Research Ethics Committee and by national  
55 research ethics committees and regulatory bodies in Malawi, Zambia, Cameroon and Tanzania.

56

## 57 Results

### 58 **Baseline data**

59 Of 678 ACTA participants, VA was measured in 472 (69.6%) at baseline. 75 patients (15.9%) had severe  
60 visual loss with 20 (4%) classed as blind. One fifth (15/75) of those with severe visual loss also reported  
61 hearing loss.

62

63 Overall, 206 (47.1%) patients had raised baseline ICP ( $OP \geq 25 \text{ cm H}_2\text{O}$ ) and median fungal burden was  
64  $4.9 \log_{10} \text{ colonies/ml}$  (IQR: 3.7-5.8) (Supplementary Table 1). *C neoformans* was the predominant  
65 species causing CM in this African cohort (337/382 sequenced isolates). Due to difficulties in  
66 measuring VA in unconscious patients, the VA cohort had less abnormal mental status ( $GCS < 15$ )  
67 (12.5% vs 50.5%,  $p < 0.001$ ) and lower median OP (19 vs  $25 \text{ cm H}_2\text{O}$ ,  $p < 0.001$ ), but no significant  
68 differences in median fungal burden ( $4.9$  vs  $5.1 \log_{10} \text{ colonies/ml}$ ,  $p = 0.74$ ), compared to the entire trial  
69 cohort.

70

### 71 **Association between severe baseline visual loss, opening pressure and fungal burden**

72 Across 6 categories from near-normal to blind, decreasing visual acuity was associated with both  
73 increased OP and higher fungal burden at baseline ( $p = 0.02$  and  $p < 0.008$ , respectively) (Fig 1a and b).  
74 High OP and fungal burden were also associated, with 55 (25.6%) patients with fungal burden above  
75 the median ( $\geq 4.9 \log_{10} \text{ colonies/ml}$ ) also experiencing high OP ( $> 40 \text{ cm H}_2\text{O}$ )- compared to 33 (15.4%)  
76 with lower fungal burden ( $p = 0.009$ ).

77 In multivariable analyses, age; baseline CSF pressure  $< 25 \text{ cm}$  vs  $\geq 40 \text{ cm H}_2\text{O}$ : aOR 2.56 (95% CI 1.36-  
78 4.83,  $p = 0.02$ ) and fungal burden ( $< 5$  vs  $> 6.0 \log_{10} \text{ Colonies/ml}$ : aOR 3.01 (95% CI 1.58-5.7,  $p = 0.003$ )  
79 were independently associated with severe visual loss (Table 1). There was no association between  
80 baseline CNS inflammation ( $\text{CSF WCC} > 10 \times 10^9/\text{L}$ ) and visual loss on either univariable or multivariable  
81 analyses.

82

83 ***Changes in visual acuity over time***

84 Paired visual acuity data were available for 336/472 (71.2%) patients at baseline and week 4. Of these  
85 171 (50.9%) had normal vision, 128 (38.1%) had moderate vision and 37 (11.0%) had severe visual loss  
86 at baseline. Death before follow-up was the main reason for missing data at 4 weeks (98/136, 72.1%).

87 VA remained unchanged over time for 210 (62.5%) patients. No significant differences were observed  
88 in rate of clearance of infection, number of LPs performed, total volume of CSF removed and change  
89 in OP over the first 2 weeks of treatment between the group remaining the same, those in whom VA  
90 deteriorated (n=51, 15.2%) and those that improved (n=75, 22.3%) (p>0.1, all comparisons  
91 Supplementary Table 2).

92 Of 37 (11%) patients with severe visual loss at baseline, 12 (32.4%) improved to near-normal, 8 (21.6%)  
93 improved to moderate and 17 (46.0%) remained severe. Again, there were no significant differences  
94 in the above parameters between patients remaining severely impaired and those improving  
95 (Supplementary Table 3), though numbers for comparison were low.

96

97 **Discussion**

98 This prospective study within a large African trial is the first to demonstrate an independent  
99 association of both fungal burden and raised ICP, in addition to age, with visual loss at presentation  
100 with HIV-CM. At presentation, 16% of patients had severe visual loss and 4% were blind: in those with  
101 repeat measurements at 4 weeks, following fungicidal treatment regimens and aggressive  
102 management of raised ICP, 54% of patients with severe baseline visual loss improved to near-normal  
103 or moderate vision, while 46% remained severely impaired.

104 In a case series comprising both HIV-infected and uninfected patients (n=49), the authors proposed  
105 dichotomous mechanisms of visual loss, optic neuritis due to fungal infiltration accounting for early  
106 rapid visual loss, and optic nerve compression due to raised ICP accounting for a slower onset.  
107 Interventions aimed at reducing ICP were the only ones associated with any success[3]. In a study of  
108 immunocompetent adults with *C gattii* meningitis, where ICP was not consistently measured or  
109 managed, 37% of 57 survivors remained blind, compared to 5% in our cohort[6]. The uniformity of our  
110 cohort, in terms of use of fungicidal regimens and pressure management, may not have permitted us  
111 to show a significant impact of rate of clearance or changes in pressure-related parameters on  
112 reversibility of visual loss.

113

114 The most comprehensive mechanistic study of visual loss in HIV-CM[5] observed frequent optic nerve  
115 conduction and visual field defects compatible with raised ICP, and no evidence of optic neuritis on  
116 MRI, presenting evidence for an optic nerve compartment syndrome, caused by cryptococcal plugging  
117 of channels between the intracranial and the peri-optic subarachnoid space, as an additional cause of  
118 optic nerve dysfunction in CM[8]. In practise, these mechanisms may well overlap[8]: our prior  
119 work[2] and findings from this study suggest a relationship between fungal burden and raised pressure  
120 at the highest extremes of each. These two factors likely converge in an individual patient, possibly  
121 more susceptible due to neuroanatomical variation, rendering them either temporarily or- in the  
122 absence of intervention- permanently visually impaired.



123

124 Limitations of our cohort include the lack of visual assessment at baseline or reversibility at 4 weeks  
125 in the sickest of patients who subsequently die, possibly underestimating the prevalence of visual loss.  
126 We did not capture self-reported visual impairment predating CM onset which may have impacted on  
127 baseline VA, nor were we able to exclude other conditions that can impair vision in this advanced HIV  
128 cohort (e.g. CMV retinitis). Nonetheless, our findings underscore the importance of interventions  
129 targeted at *both* fungal burden and raised ICP in mitigating visual loss, and underline the need for  
130 continued efforts towards earlier diagnosis and treatment of CM, given that, almost half of those with  
131 severe impairment failed to improve despite fungicidal regimens and aggressive management of  
132 raised CSF pressure.

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## **Conflict of Interests**

TB has received Speaking fees from Gilead Sciences and Pfizer and research funding from Gilead Sciences unrelated to the submitted work. TSH reports non-financial support from Immuno-Mycologics during the conduct of the study; grants and personal fees from Gilead Sciences, personal fees from Viamet, and personal fees from Pfizer outside the submitted work. OL reports grants and personal fees from Gilead Sciences, personal fees from Merck, personal fees from Astellas, personal fees from Pfizer, and personal fees from Novartis outside the submitted work. All other authors report no conflicts.

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**Figure 1: a.** Box plot (median, IQR, range) of baseline Cerebrospinal fluid (CSF) opening pressure by visual acuity group at presentation. **b.** Box plot of baseline fungal burden by visual acuity group at presentation. NN-Near normal; ML- moderate loss; CF- Counting fingers; LP- Light perception; NV- No vision (Blind)

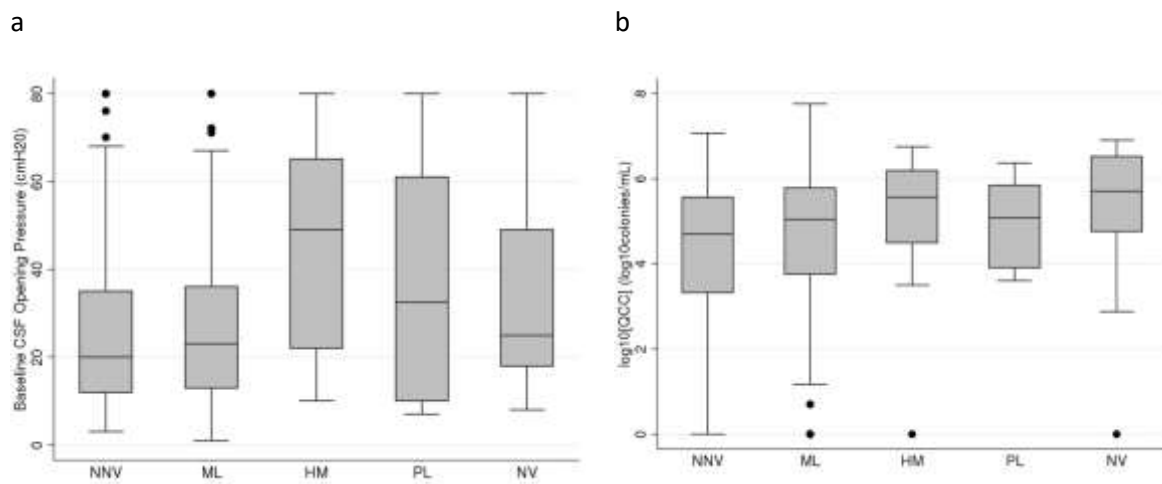


Table 1. Univariable and multivariable analysis for the association between age, baseline CSF opening pressure, baseline CSF fungal burden and CSF white cell count and severe visual loss at presentation.

Variable (baseline)	Visual Acuity at CM presentation (no. (%))		Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value*
	Normal or Moderate Loss	Severe Loss				
<b>Age</b>						
<50 year	356 (89.7)	41 (10.3)	1		1	
≥50 years	61 (81.3)	14 (18.7)	1.99 (1.03-3.87)	0.04	2.47 (1.22 - 5.05)	0.04
<b>CSF opening pressure (cmH<sub>2</sub>O)</b>						
<25	200 (86.6.0)	31 (13.4)	1		1	
25-39	98 (85.2)	17 (14.8)	1.12 (0.59-2.12)		1.28 (0.66-2.47)	
≥40	66 (72.5)	25 (27.5)	2.44 (1.35-4.43)	0.003	2.56 (1.36-4.83)	0.02
<b>Fungal Burden (log<sub>10</sub>colonies/ml)</b>						
<5.0	206 (88.4)	27 (11.6)	1		1	
5.0-5.9	113 (84.3)	21 (15.7)	1.42 (0.77- 2.62)		1.31 (0.69 - 2.47)	
>6.0	62 (70.5)	26 (29.5)	3.20 (1.74 -5.88)	0.001	3.01 (1.58 - 5.70)	0.003
<b>White cell count (x 10<sup>9</sup>/L)</b>						
<10	249 (82.2)	54 (17.8)	1		1	
≥10	126 (86.3)	20 (13.7)	0.73 (0.42 - 1.28)	0.27	0.70 (0.39 - 1.25)	0.22

\*Adjusted for each variable in the table OR: Odds ratio CI: Confidence Interval CSF: Cerebrospinal fluid