



Cryptococcal meningitis: Cases from Uganda

Dr. Edwin Nuwagira (MBCh.B, MMED)

Declaration

- Consultancy fees from Biomerieux East Africa
- No other conflicts of interest

Mbarara University of Science and Technology



Population 47 million
(75 % <30 years)
Area: 241, 037km²



Clinical Case 1

- 23/Female
- Presenting complaints:
 - Headache for 2 weeks
- Living with HIV infection for two years, defaulted from Antiretroviral treatment (ART, TLE) care for three years
- Recently on TLD x3 weeks
- He had headache for two weeks
 - One episode of vomiting and 2 seizures prior to admission
 - Blurring of vision
 - No abnormal behavior, altered mental state
- Other systems: cough, 1 month ago, no night sweats
- Separated, mother of 1, not sexually active

Physical Examination

- Lethargic and oral thrush
- Vitals: Blood Pressure= 113/78 mmHg, Pulse 98 beats per minute, temperature 36.0 °C, and respiratory rate 20 breaths per minute.
- GCS=15/15, with positive Kernig's sign
- Neck stiffness, right eye cranial nerve VI palsy, and bilateral dilated pupils (5-6mm) which were sluggishly reactive to light
- No other neurological findings and the rest of her systemic examination was normal

More tests

- Basic tests done
 - RBS= 8.5mmol/l (153 mg/dl)
 - Bedside Cryptococcal antigen (CrAg) lateral flow assay was **Positive**
- Patient agreed to have a lumbar puncture: Opening Pressure was 7 cmH₂O (Normal Range < 20 cmH₂O) and 10mls drained.
 - Bedside CSF CrAg was **Positive**
 - Microscopy with Indian Ink was also **Positive**
 - WCC was 05 cells, Glucose of 4.6mmol/l (82.8 mg/dl) and Protein of 10 mg/dl
- POC CD4 T cells = 06 cells/mm³ (normal is between 500 to 1500 cells/mm³)

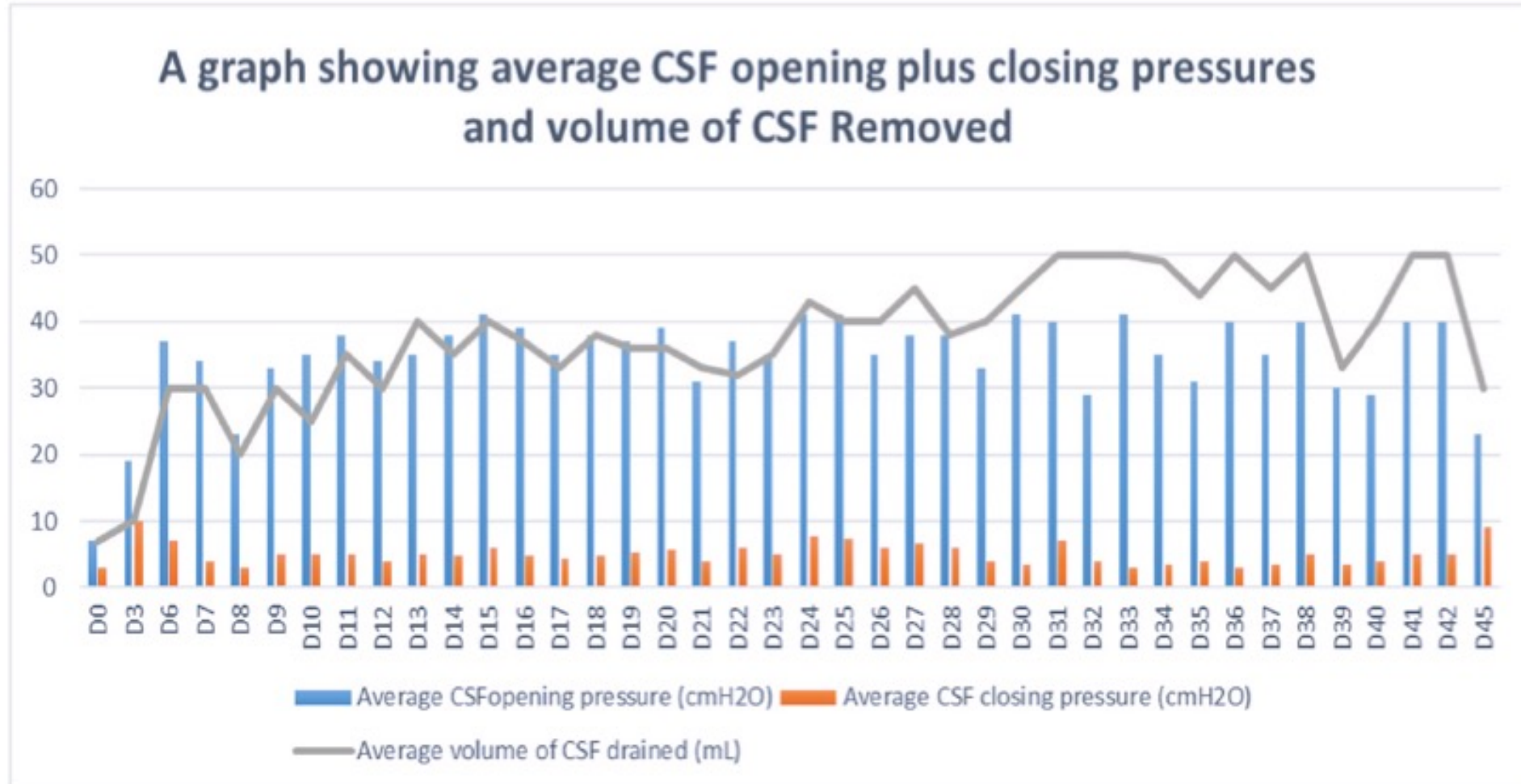
More tests

- CSF gram stain showed yeast cells
- Baseline blood chemistry and hematology were within normal limits.
- Sputum Xpert MTB/Rif and urine TB lipoarabinomannan (LAM) for *Mycobacterium tuberculosis* were negative.
- A chest radiograph was unremarkable
- HIV viral load was 3302 copies/mL.
- Quantitative Cryptococcal culture of 2,400,000 colony forming units per mL of CSF

Treatment

- I.V Amphotericin B deoxycholate 1mg/kg/day for 7 days plus oral flucytosine 100mg/ kg/day in four divided doses for 7 days followed by 7 days of fluconazole 1200 mg/day.
- The patient also received potassium and magnesium supplementation
- Oral Sodium valproate to control the seizures, and continued ART.
- **On day six:** she developed symptoms of raised ICP: vomiting, severe headache and new onset convulsions
- A therapeutic LP identified a raised opening pressure of 37 cmH₂O with 30mL of CSF being drained
- Serial therapeutic LPs were performed however the patient had recurrent headaches, and CSF opening pressures were persistently elevate

A graph representing trend in average daily CSF opening plus closing pressures and volume of CSF drained



Follow-up

- A total of 76 LPs were performed during the patient's entire admission,
- Frequency which of was guided by the signs and symptoms (headache, vomiting and convulsions)
- Follow up CSF cultures were sterile at both day 7 and 14
- Patient outcomes were good
- Elevated ICP normalized with resolution of signs and symptoms of raised ICP,
- She was discharged on day 46 of hospitalization
- She was actively followed through 16 weeks and later linked to a primary HIV clinic for follow up and ongoing care.

The pathogen: *Cryptococcal species*

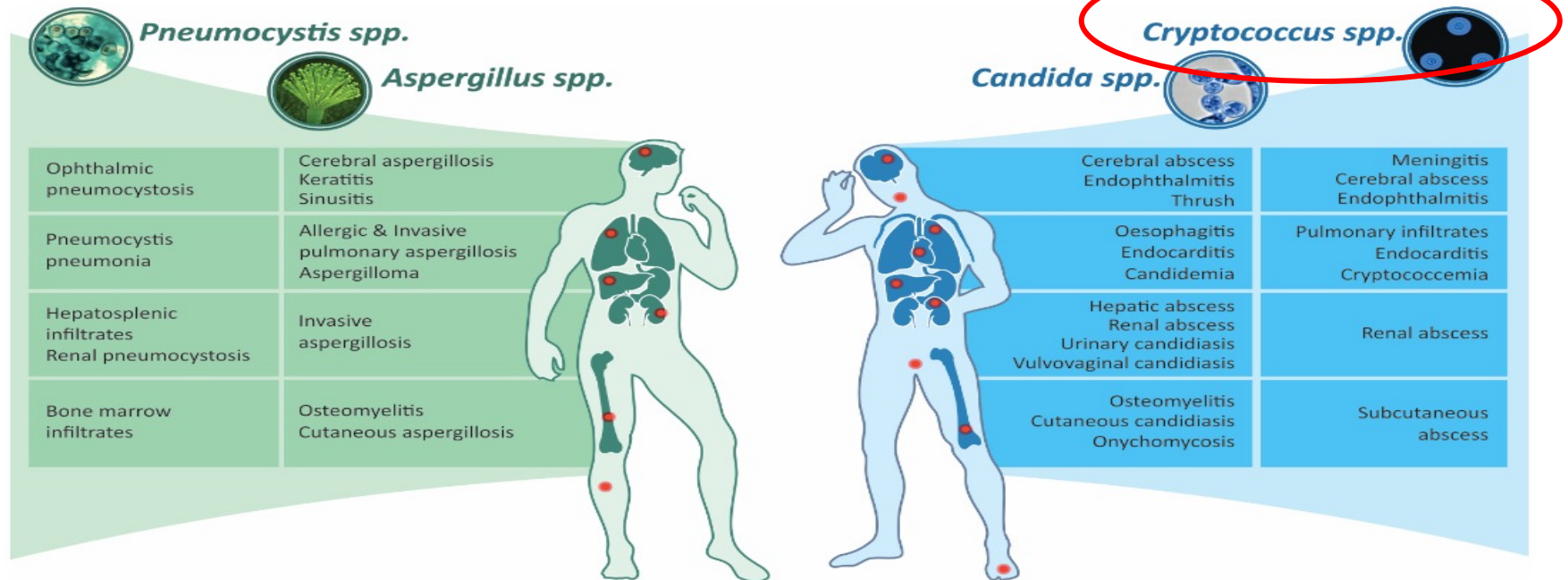
- Belongs to the WHO “critical” group of fungal pathogens.
- High priority due to
 - The high mortality, >25%.
 - Need for research into better diagnostic approaches and treatment regimen

REVIEW

MEDICAL MYCOLOGY

Hidden Killers: Human Fungal Infections

Gordon D. Brown,^{1*} David W. Denning,^{2*} Neil A. R. Gow,^{1*} Stuart M. Levitz,^{3*} Mihai G. Netea,^{4*} Theodore C. White^{5*}



90% of all reported fungal-related deaths result from species that belong to one of four genera

Epidemiology

Hospital	Country	Sample Size	HIV infected	Meningitis Prevalence			
				Bacterial / Pyogenic	Tuberculosis	Cryptococcal	Aseptic / Viral Meningitis
Mulago and Mbarara ¹	Uganda	416	98%	4%	8%	59%	29%
GF Jooste ²	South Africa	1,737	96%	19%	13%	30%	38%
Queen Elizabeth Central ³	Malawi	263	77%	20%	17%	43%	20%
Harare Central & Parirenyatwa ⁴	Zimbabwe	200	90%	16%	12%	45%	28%
Pooled Average		2616	93%	9.3% (8.2-10.5%)	12.7% (11-14%)	37% (35-39%)	41% (40-43%)

¹Durski K et al. *JAIDS* 2013; 63(3);e101-e108.

²Jarvis JN, et al. *BMC Infect Dis.* 2010; 10: 67.

³Cohen DB, et al. *Trop Med Int Health.* 2010; 15: 910-917.

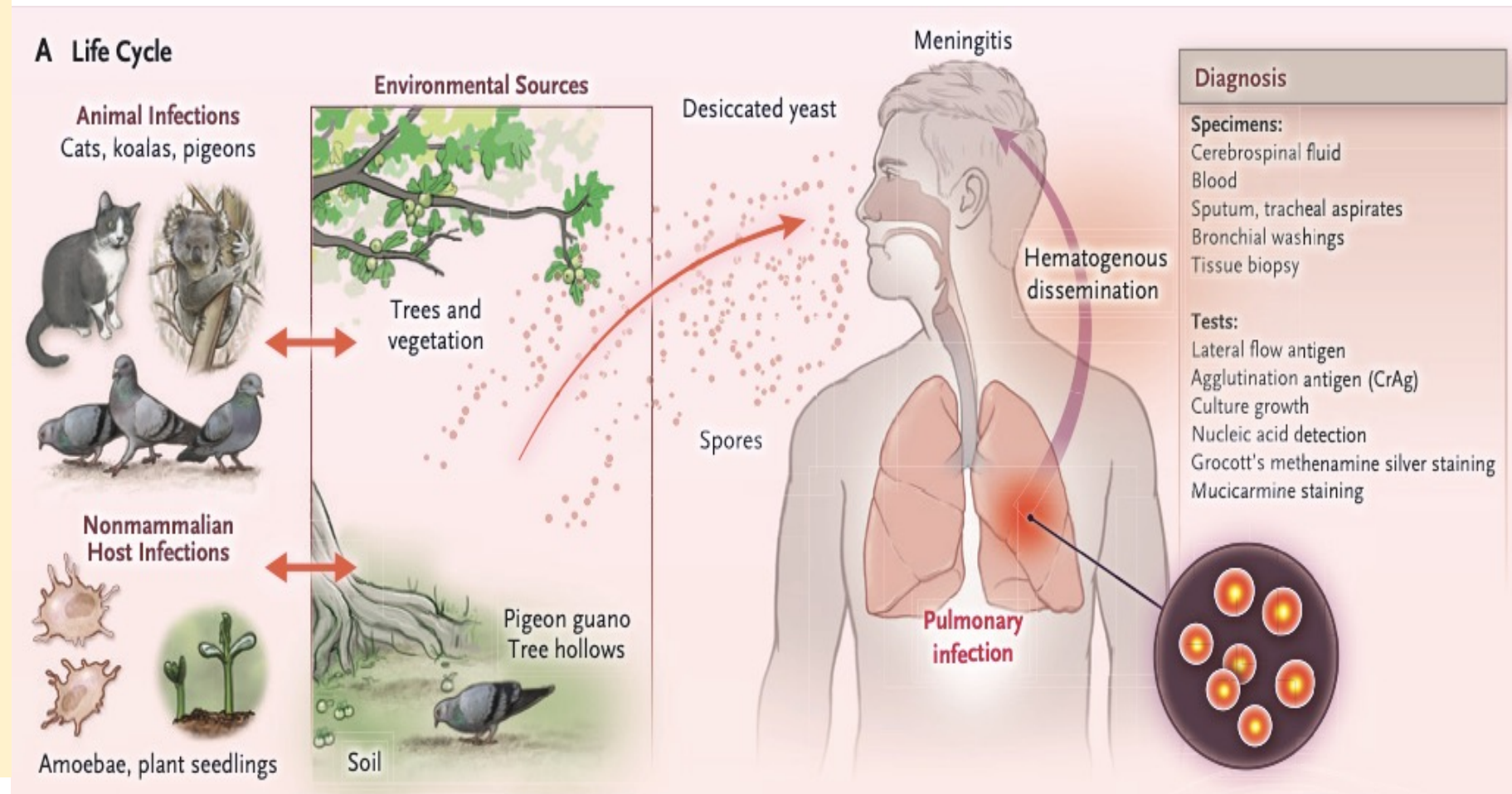
⁴Hakim JG et al. *AIDS.* 2000; 14: 1401-1407.

The pathogen: *Cryptococcal species*

- Cryptococcus is a basidiomycetous yeast.
- First discovered in 1894 by Busse and Buschke
- Resurgence was due to the use of immunosuppressive medication in the 1970s
- Two main virulence factors: polysaccharide capsule and melanin (cell wall laccase).
- These two make the organism neurotropic.
- The cryptococcus genus is generally considered to consist of two species complexes, *Cryptococcus neoformans* and *C. gattii*.

From the trees to the brain

- Leading cause of meningitis in Africa
- Cases are on the rise in the US.
- Pets like cats, birds/penguins can be asymptomatic
- Human hosts are usually immunocompromised.
- Up to 25% of patients die due to delays in diagnosis and treatment challenges



Spectrum of disease

Entity	Localised disease	Asymptomatic antigenaemia	Symptomatic antigenaemia	Subclinical CM	Symptomatic CM
Lumbar puncture	CSF-	CSF-	CSF-	CSF+	CSF+
Symptoms	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
Blood CrAg	Blood CrAg-	Blood CrAg+	Blood CrAg+	Blood CrAg+	Blood CrAg+
CrAg titre	-	Low CrAg titre	?	High CrAg titre	Very high CrAg titre
Blood culture	Fungaemia-	Fungaemia-	?	Fungaemia+/-	Fungaemia+/-

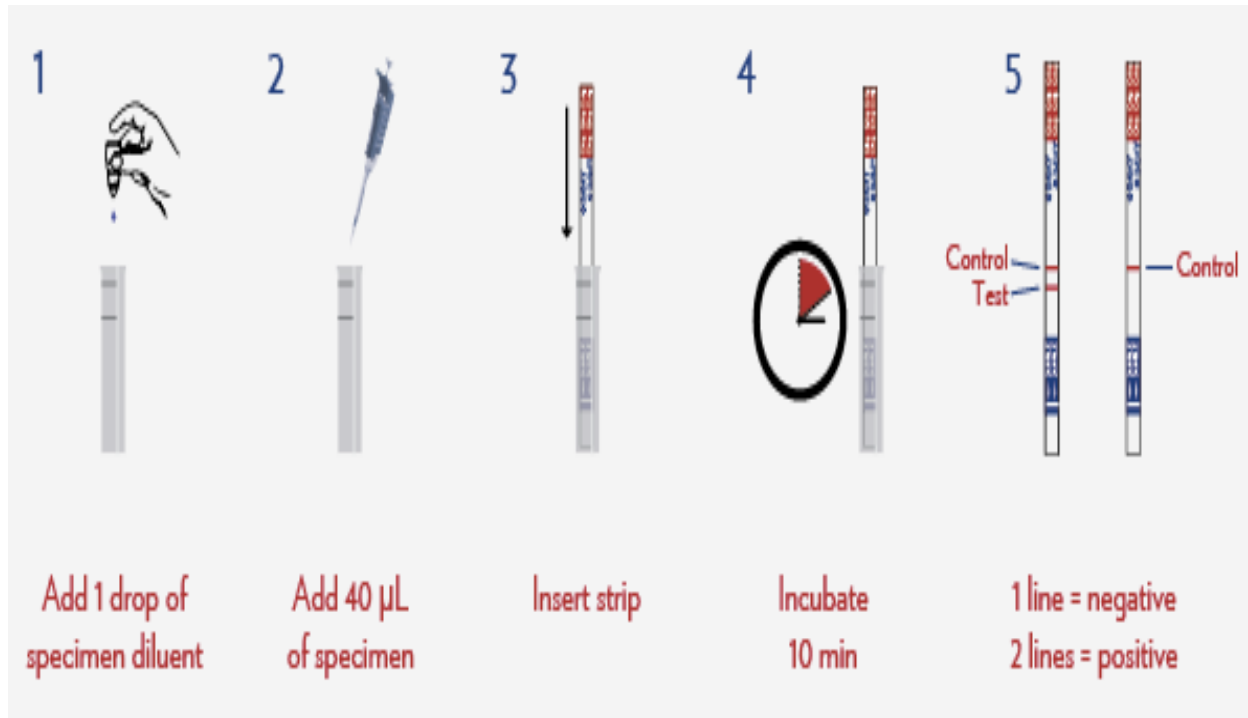
Diagnosis

- Serum CrAg is a screening test
- CSF CrAg is a diagnostic test
 - Validated globally
 - Both Sensitivity and specificity of 99%
- Indian ink microscopy in resource limited settings
- Cultures are the gold standard
- Multiplex PCR-Biofire can be used

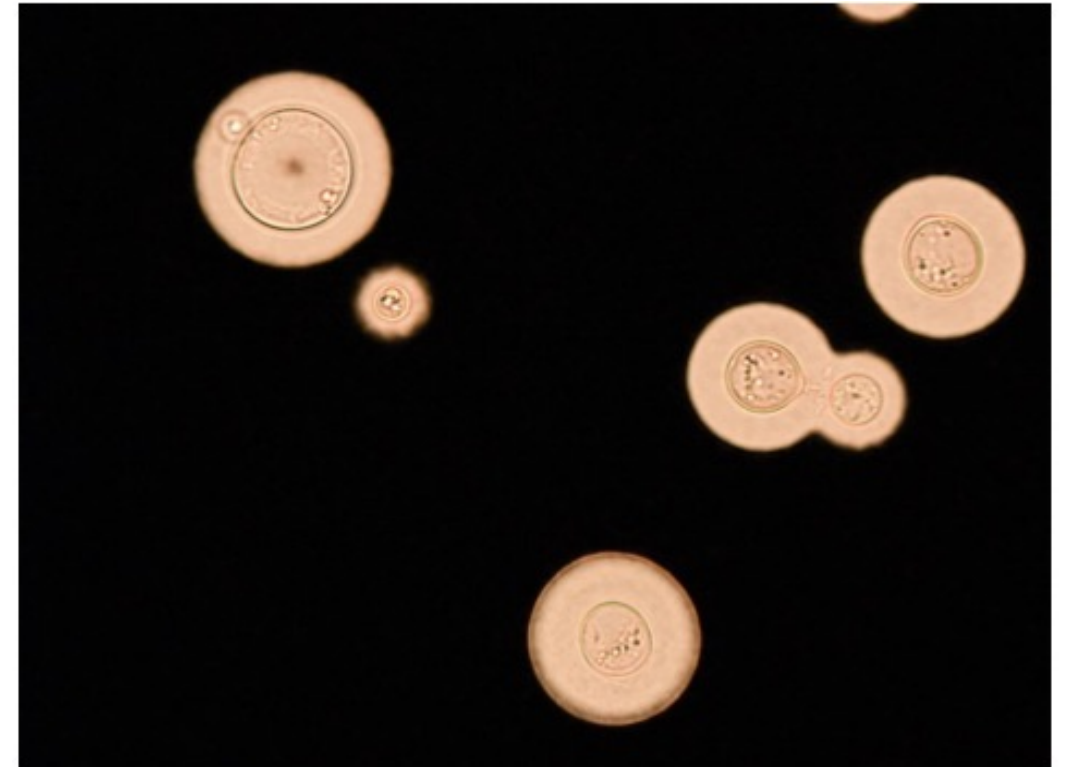
Table 1. Performance Characteristics of Cryptococcal Diagnostic Assays in Cerebrospinal Fluid (CSF) from Persons with Suspected Meningitis.*

Diagnostic Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
			<i>percent</i>	
Cryptococcal antigen lateral flow immunochromatographic assay	99.3	99.1	99.5	98.7
CSF culture†	90.0	100	100	85.3
100 mm ³	94.2	100	100	91.2
10 mm ³	82.4	100	100	75.8
India ink microscopy	86.1	97.3	98.2	80.2
Cryptococcal antigen latex agglutination assay				
Meridian	97.8	85.9	92.6	95.5
IMMY	97.0	100	100	95.3
Metagenomic next-generation sequencing	93.5	96.0	87.8	98.0
PCR assay‡	82.0	98.0	98.0	79.0

IMMY CrAg

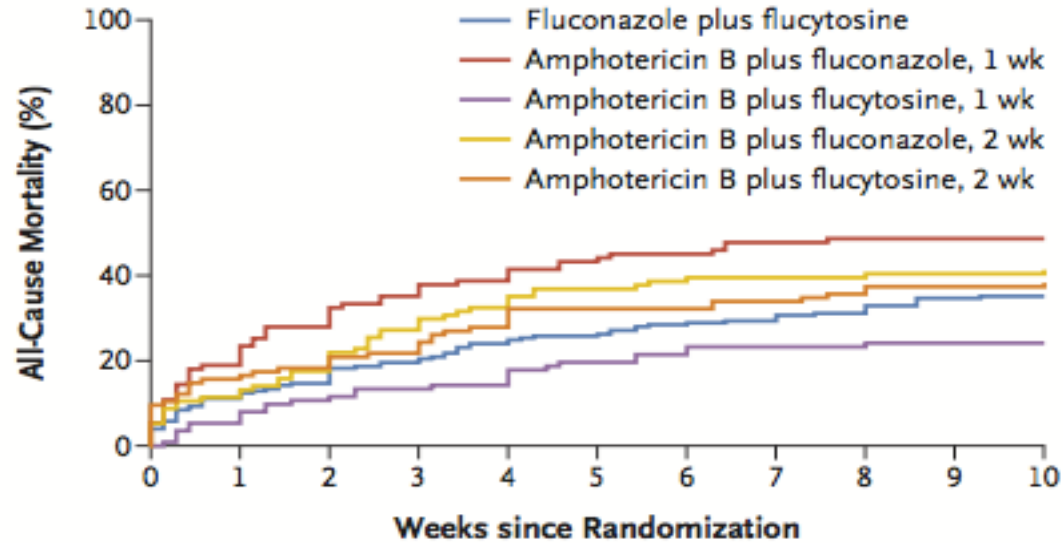


Indian Ink

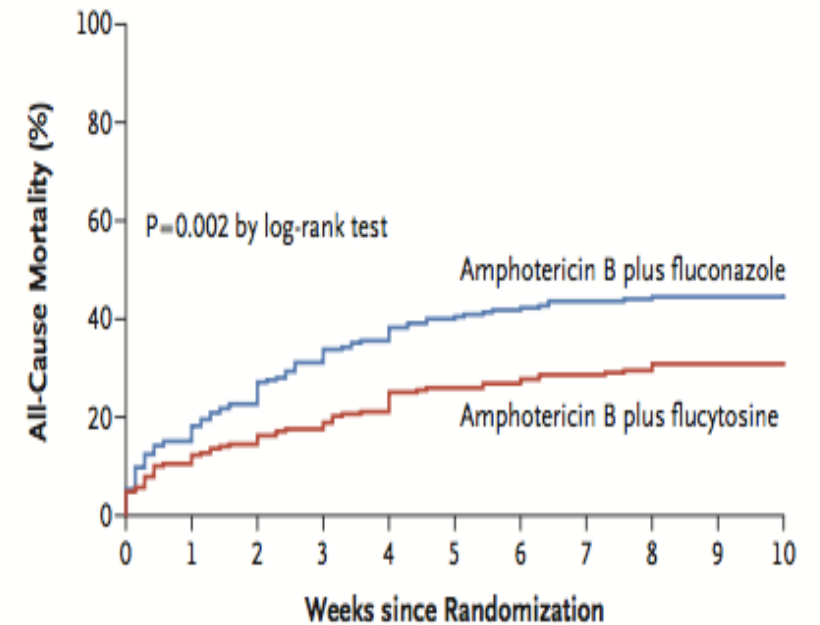


N.T.T. Ngan et al, 2022

Treatment



No. at Risk	0	1	2	3	4	5	6	7	8	9	10
Fluconazole plus flucytosine	225	200	192	181	171	167	161	159	155	147	144
Amphotericin B plus fluconazole, 1 wk	111	90	80	72	68	63	61	58	57	57	57
Amphotericin B plus flucytosine, 1 wk	113	106	100	97	96	89	87	85	85	84	82
Amphotericin B plus fluconazole, 2 wk	114	101	94	83	77	72	69	68	68	67	65
Amphotericin B plus flucytosine, 2 wk	115	97	94	90	83	78	78	76	74	72	71



No. at Risk	0	1	2	3	4	5	6	7	8	9	10
Amphotericin B plus fluconazole	225	191	174	155	145	135	130	126	125	124	122
Amphotericin B plus flucytosine	228	203	194	187	179	167	165	161	159	156	153

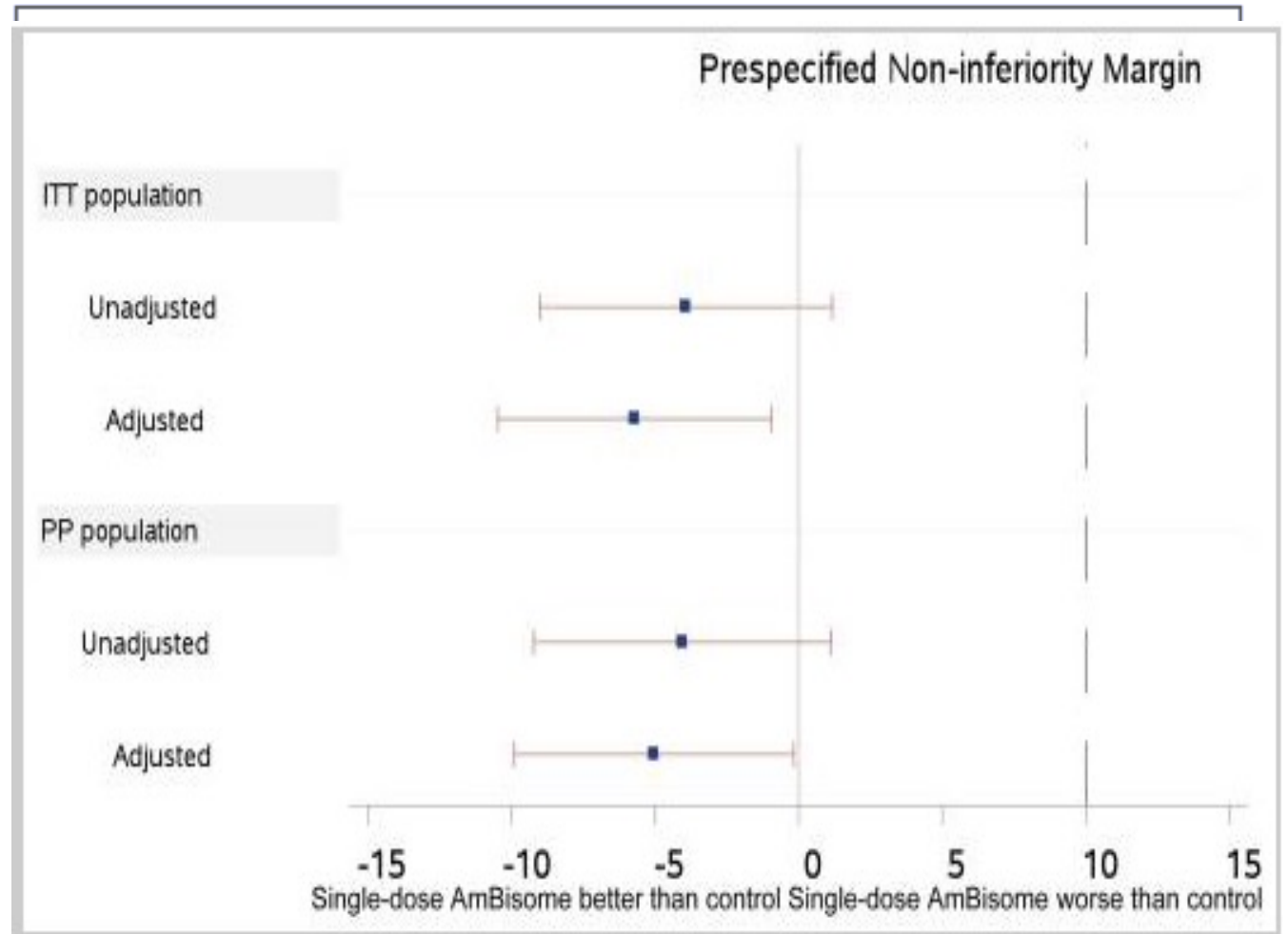
Treatment

Table 2. Unadjusted Analysis of Mortality and Rate of Fungal Clearance in CSF According to Treatment Strategy in the Intention-to-Treat Population.*

Outcome	Oral Regimen (N=225)	1-Wk Amphotericin B (N=224)	2-Wk Amphotericin B (N=229)	Difference (95% CI)†	
				Oral Regimen vs. 2-Wk Amphotericin B	1-Wk Amphotericin B vs. 2-Wk Amphotericin B
Mortality at 2 wk					
No. of deaths	41	49	49		
% (95% CI)	18.2 (13.2 to 23.3)	21.9 (16.5 to 27.4)	21.4 (16.1 to 26.7)	-3.18 (-10.50 to 4.15)	0.48 (-7.11 to 8.06)
Mortality at 4 wk					
No. of deaths	56	66	77		
% (95% CI)	24.9 (19.2 to 30.5)	29.5 (23.6 to 35.5)	33.6 (27.5 to 39.7)	-8.74 (-17.06 to -0.41)	-4.16 (-12.71 to 4.39)
Mortality at 10 wk					
No. of deaths	79	81	91		
% (95% CI)	35.1 (28.9 to 41.3)	36.2 (30.0 to 42.7)	39.7 (33.5 to 46.2)	-4.63 (-13.52 to 4.27)	-3.58 (-12.51 to 5.35)
Fungal clearance‡					
No. of patients	182	179	182		
Clearance rate — log ₁₀ CFU/ml/day	-0.26±0.18	-0.40±0.24	-0.42±0.25	0.10 (0.07 to 0.13)§	0.01 (-0.01 to 0.04)¶

Treatment: Ambition CM trial

- The 10-week mortality was 24.5% (95 of 388) in the liposomal amphotericin B group and 28.5% (113 of 396) in the control group
- A single 10-mg-per-kilogram dose of liposomal amphotericin B is effective in clearing cryptococcus from the cerebrospinal fluid.



Updated WHO treatment guidelines

Table 2. Current Approaches to Treatment According to Patient Group and Resource Availability.*

Treatment Phase and Patient Group	Duration
Induction therapy	
In HIV-coinfected patients in resource-rich settings: liposomal amphotericin B, 3–4 mg/kg daily, plus flucytosine, 25 mg/kg 4 times per day	2 wk
In HIV-coinfected patients in resource-limited settings	
Liposomal amphotericin B, 10 mg/kg as a single dose, plus flucytosine, 100 mg/kg/day, and fluconazole, 1200 mg/day	2 wk of flucytosine and fluconazole
Liposomal amphotericin B, 3–6 mg/kg/day, or amphotericin B deoxycholate, 0.7–1.0 mg/kg/day, plus flucytosine, 100 mg/kg/day (for both oral and intravenous formulations)	1 wk
Alternative induction therapy in resource-limited settings	
If flucytosine is not available: amphotericin B deoxycholate, 0.7–1 mg/kg/day given intravenously, plus fluconazole, 800–1200 mg/day	2 wk, although 1 wk of amphotericin B deoxycholate is better than none
If amphotericin B deoxycholate is not available: fluconazole, 1200 mg/day, plus flucytosine, 100 mg/kg/day given orally, if available	2 wk
In organ-transplant recipients: liposomal amphotericin B, 3 mg/kg daily, plus flucytosine, 100 mg/kg daily	2 wk
In previously healthy patients or those who have not received a transplant: liposomal amphotericin B, 3–5 mg/kg daily, or amphotericin B deoxycholate, 0.7–1.0 mg/kg daily, plus flucytosine, 100 mg/kg daily in 4 divided doses	4–6 wk or 2 wk after negative CSF, and flucytosine for first 2 wk
Consolidation therapy	
Fluconazole, 400–800 mg/day†	8 wk
Maintenance therapy	
Fluconazole, 200 mg/day; in HIV-infected patients, start ART at 4–6 wk, and consider discontinuing maintenance therapy after a minimum of 1 yr if CD4+ cell count is >100/mm ³ and HIV viral load is suppressed	12–18 mo

Lumbar punctures are part of the treatment

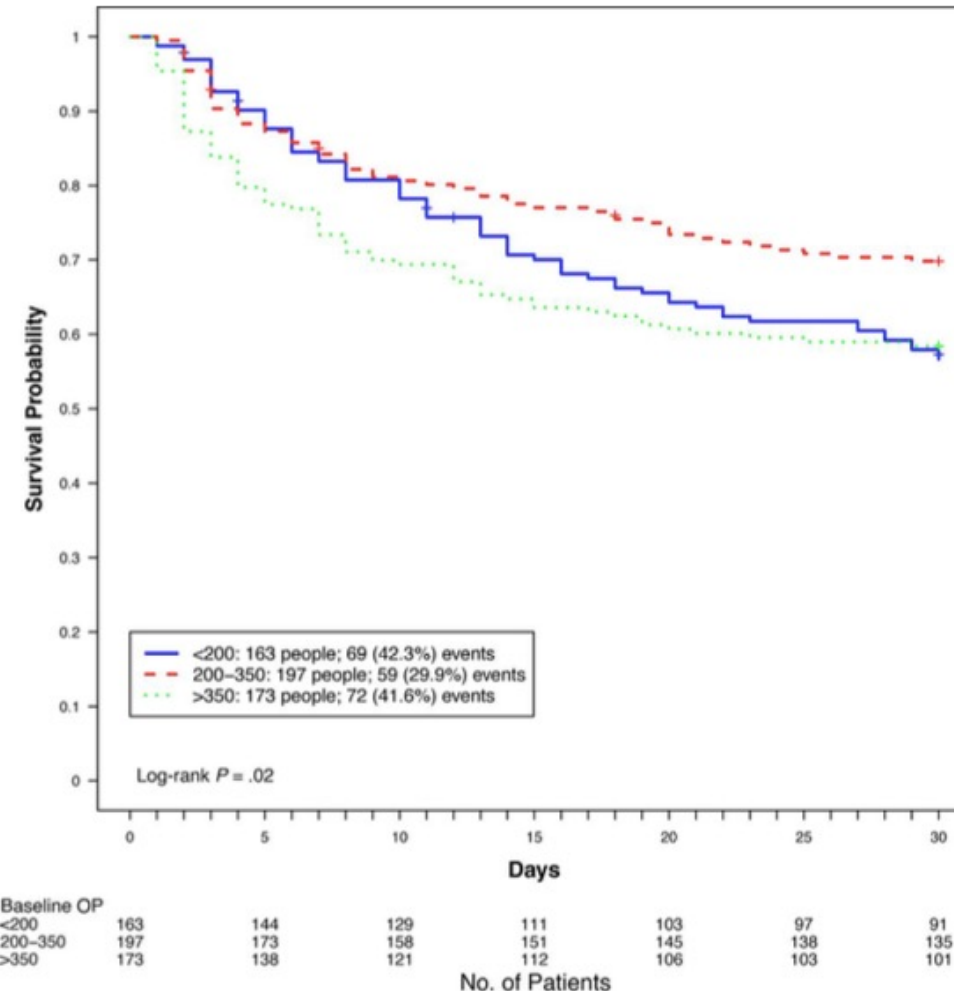
- Follow up quantitative cultures
- Help in prognosis



Lumbar punctures: Should Opening Pressure Direct Management?

1. Participants with very high baseline ICP (>350 mm H₂O) are likely to present with a GCS score <15, seizures, focal neurologic deficit, vision changes and high quantitative cryptococcal culture

2. More deaths at 30 days among those who had very high ICP.



<https://doi.org/10.1093/ofid/ofac416>

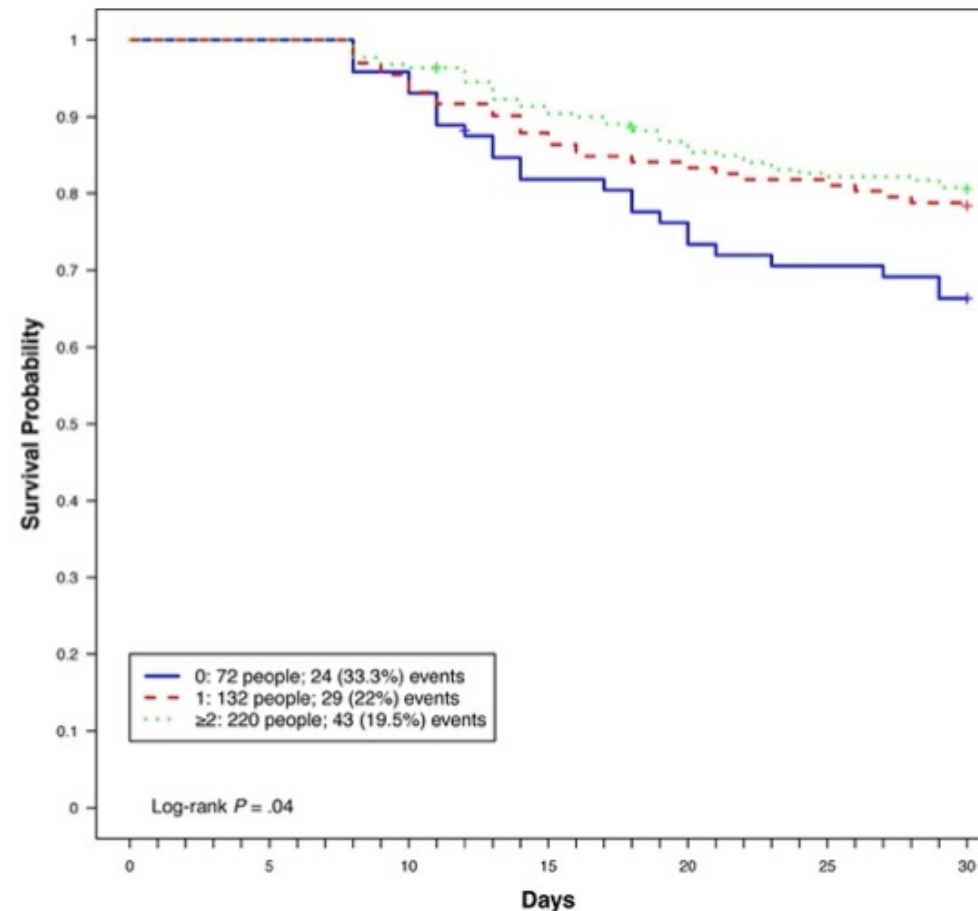
Kagimu et al, 2023

Do as many

Irrespective of the baseline ICP, at 7 days, participants who received no additional LPs had a higher mortality: $n = 24/72$ (33%) vs.

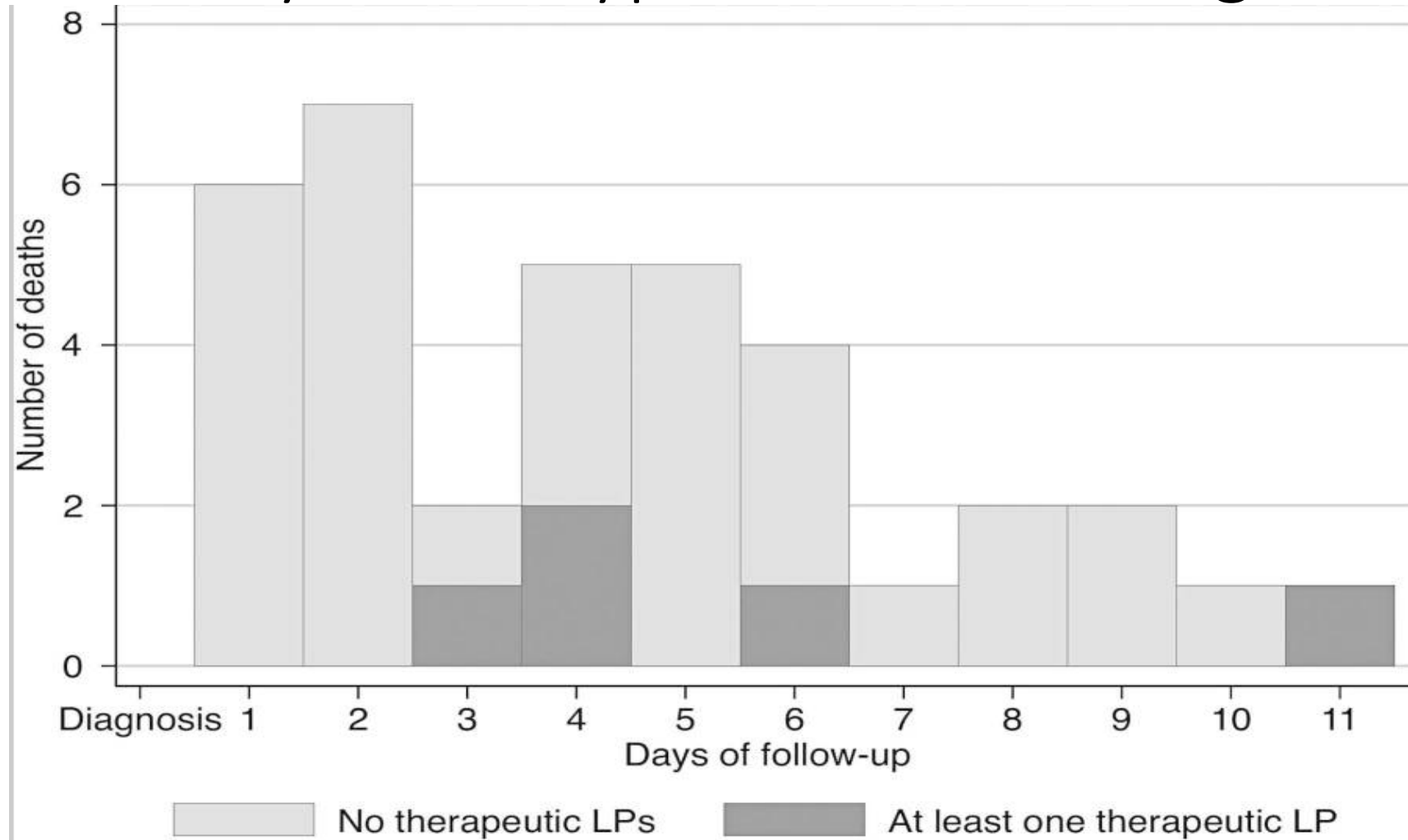
1 additional LP, $n = 29/132$ (22%) or

≥ 2 LPs, $n = 43/220$ (19.5%) ($P = .04$)



No. of follow-up LPs	72	69	58	54	50	47
0	72	69	58	54	50	47
1	132	126	116	111	108	104
≥ 2	220	213	200	189	180	176
No. of Patients						

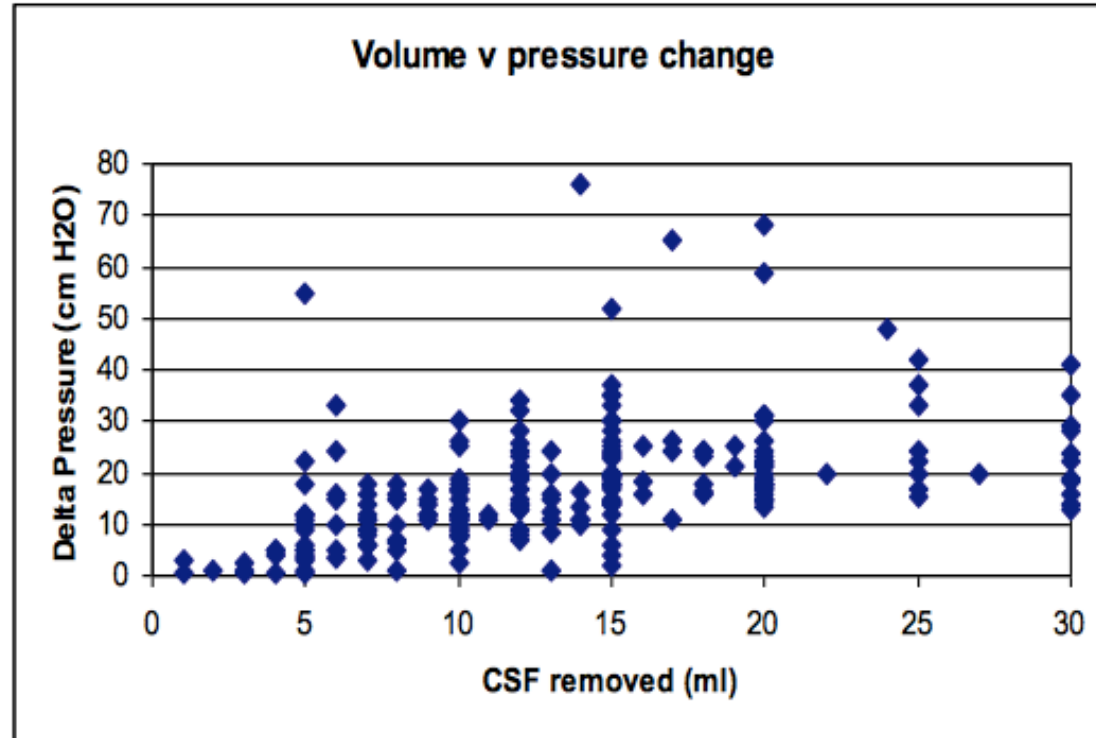
The effect of therapeutic lumbar punctures on mortality from cryptococcal-meningitis



Rx LPs were associated with a **69%** relative improvement in survival, regardless of initial ICP.

The relationship between volume of CSF drained and fall in CSF pressure

LP: Lumbar puncture
OP: Opening pressure
CP: Closing pressure



n=631 LP total
n=235 data on
OP/ CP/ volumes
drained

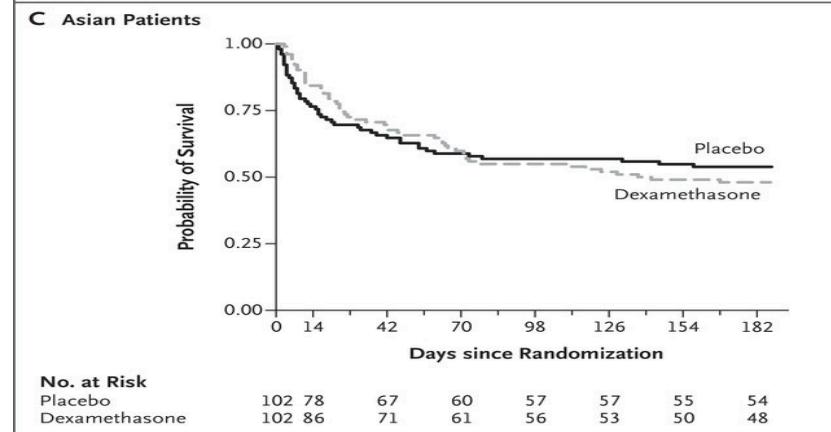
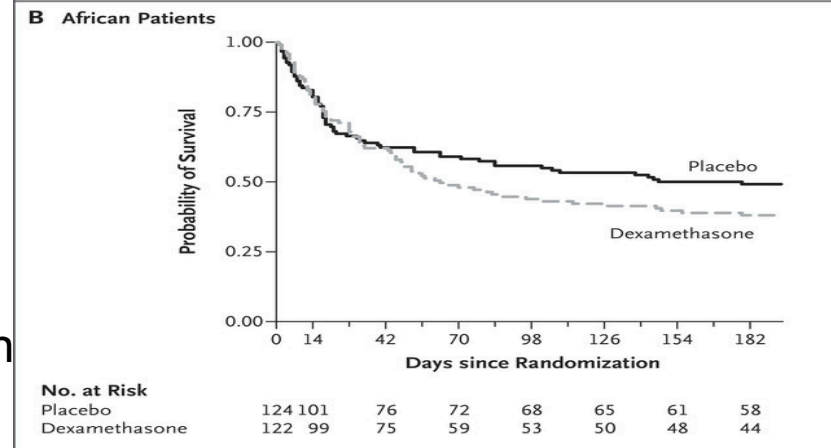
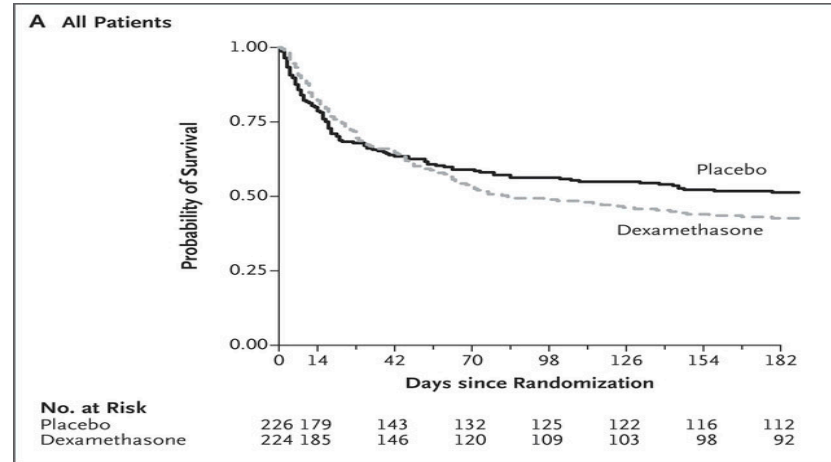
Mean OP= 31cm
Mean CP= 13cm
Mean volume
drained =14 mL

i.e. just over **1mL/cm** reduction in OP; Note extreme points
Therefore do NOT exceed 30mL and re-check pressure every 10mL

Adjunctive therapy: Dexamethasone in HIV-Associated

C.M

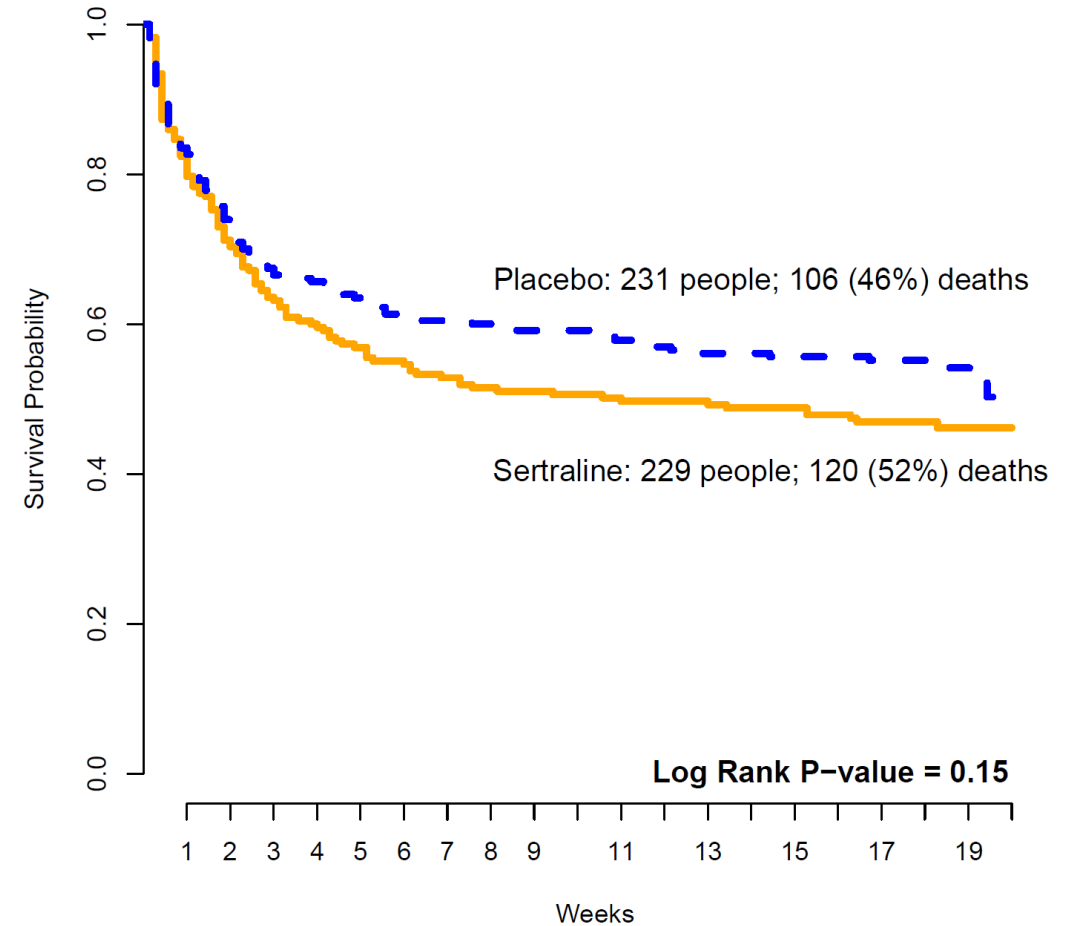
- Panel A: All patients
- Panel B: for those in Africa
- Panel C: Asia during the 6 months of follow-up.
- By 10 weeks 106 of 224 patients (47%) in the dexamethasone group and 93 of 226 (41%) in the placebo group had died.
- At 6 months, the estimated risks of death were 57% and 49%, respectively.



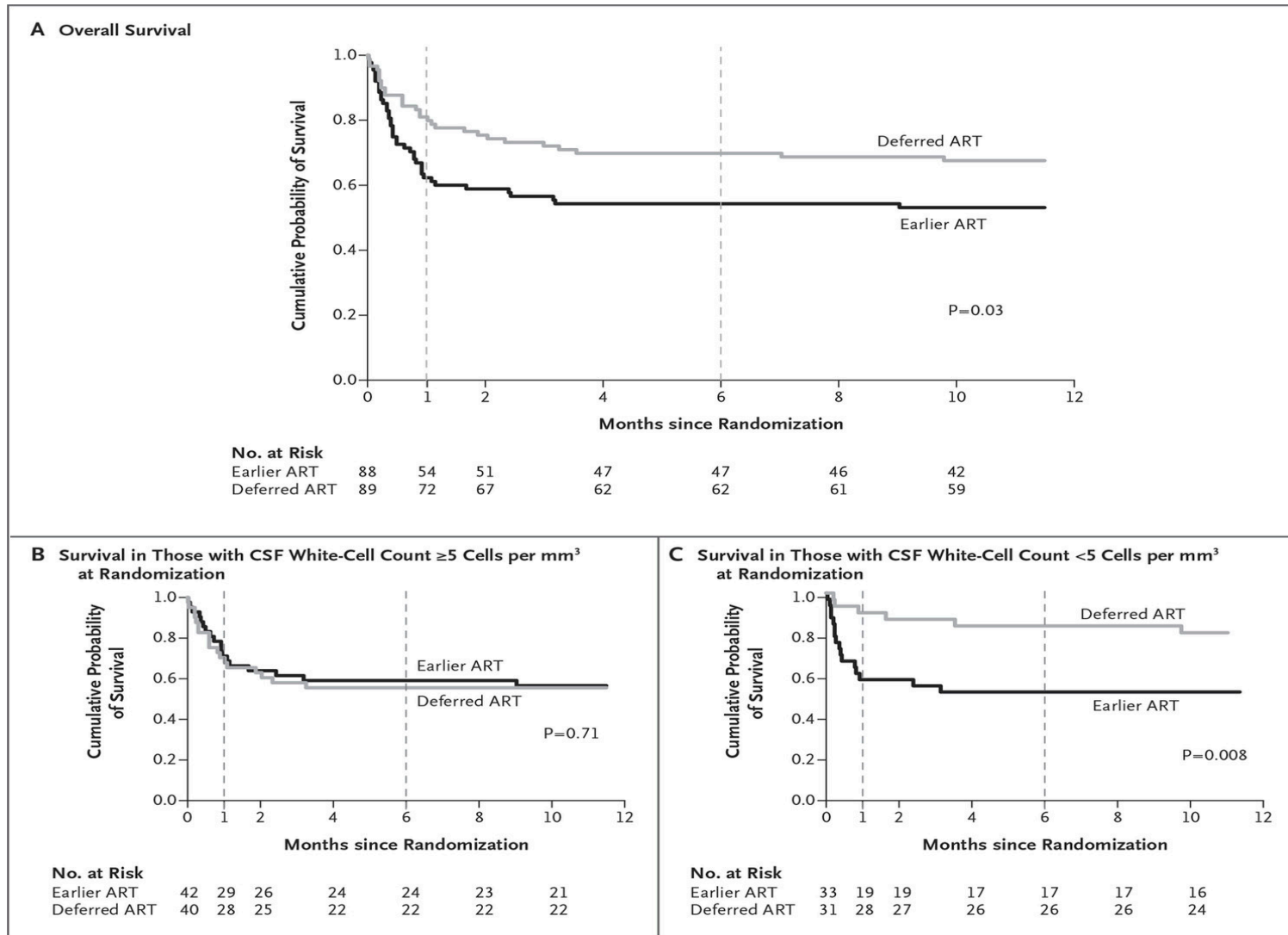
Beardsley J et al. N Engl J Med 2016;374:542-554.

Adjunctive therapy: Sertraline, 18 week Mortality

- Trial stopped for futility after enrolling 460 of planned 550 patients
- 18-week mortality
 - 52% in sertraline group vs.
 - 46% in the placebo group
 - Hazard ratio for sertraline: 1.21 (95% CI, 0.93-1.57; $p=0.15$)
- Mortality was similar among ART naïve and ART experienced patients

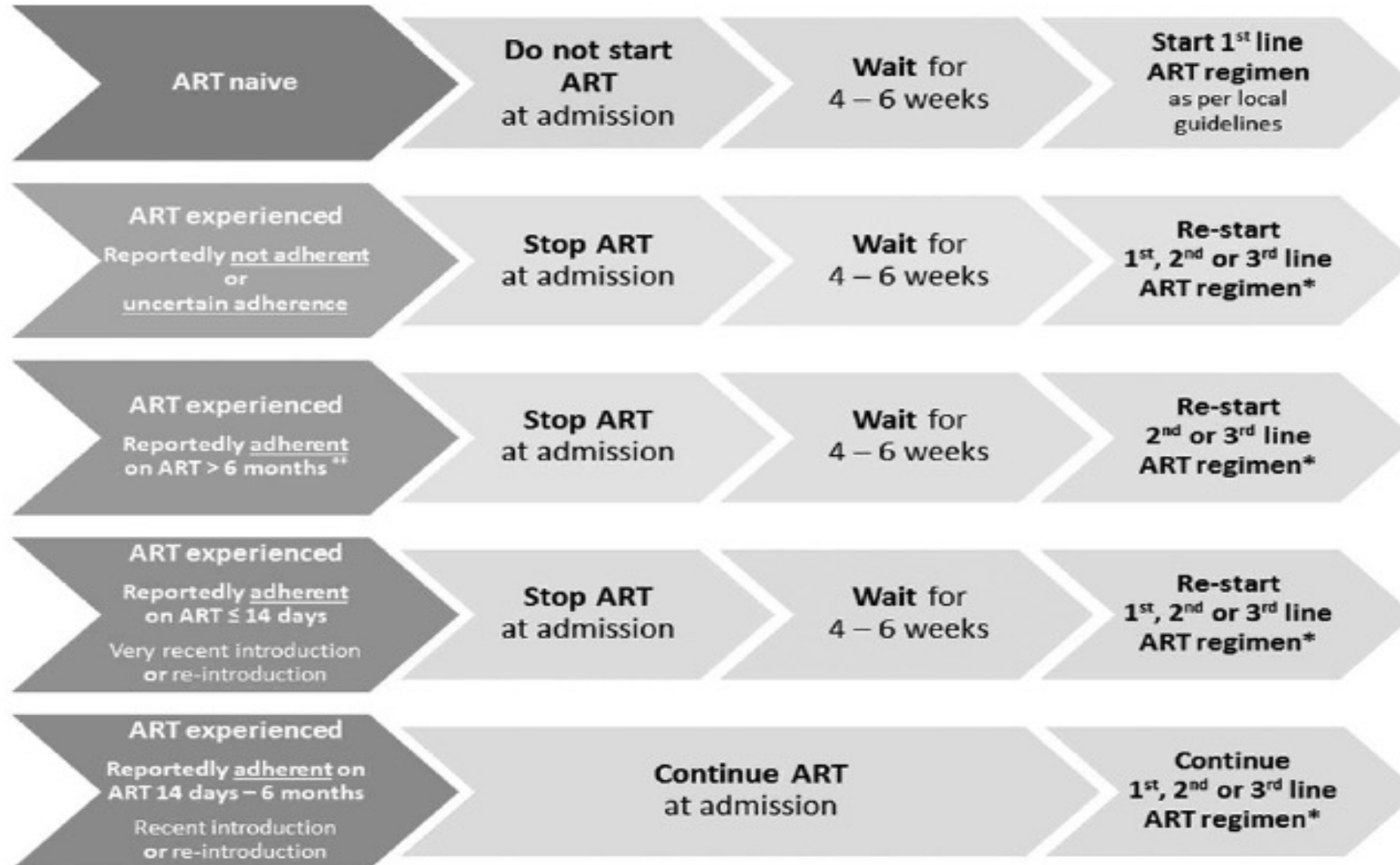


Timing of ART: Cumulative Probability of Survival According to ART timing



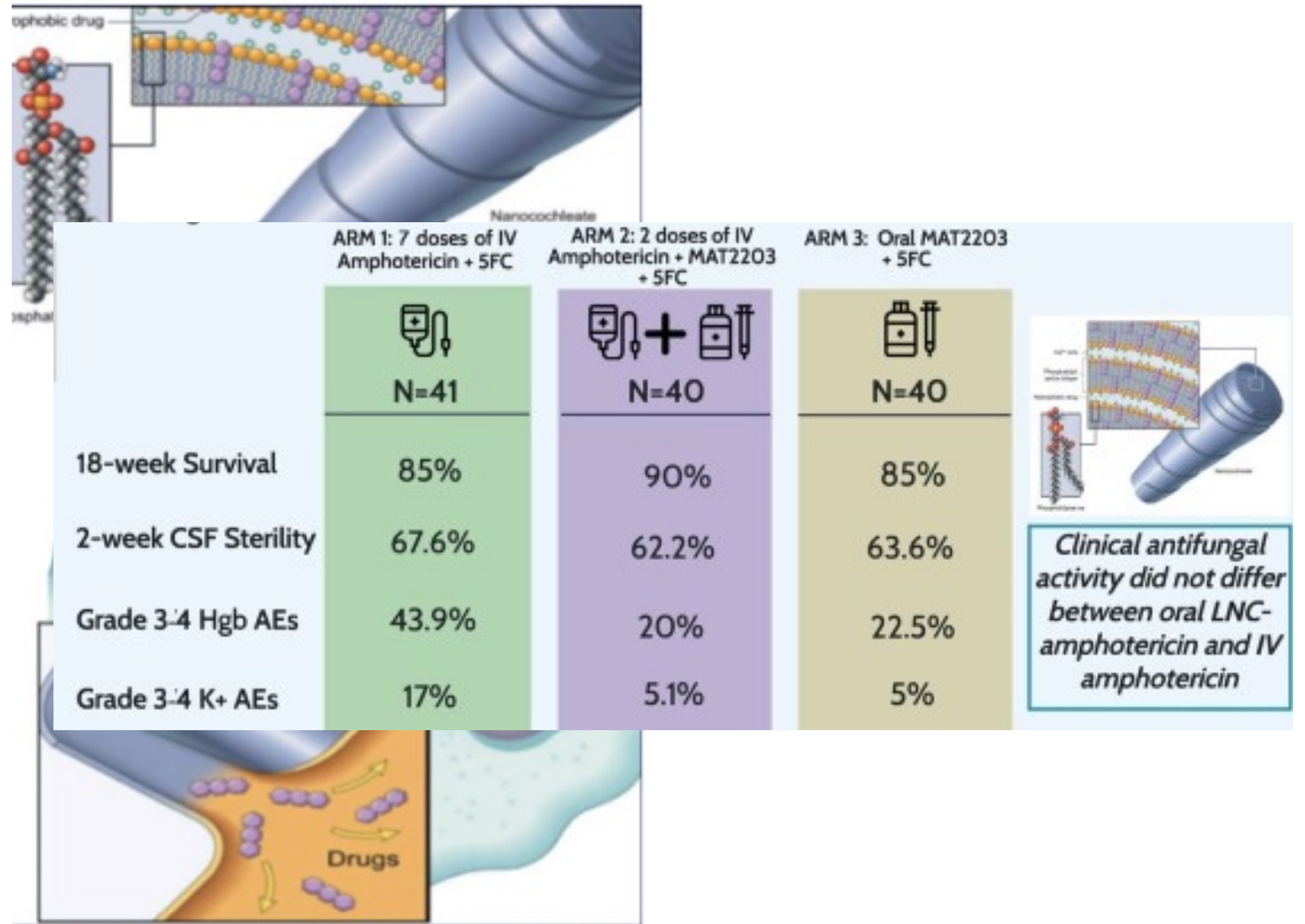
**Early ART:
70%
Mortality**

Timing of ART



The future: Oral Amphotericin B?

1. Encapsulation of Amphotericin B in a Lipid nanocrystal Particle
2. Lipid nanocrystal particle encapsulates amphotericin B molecule and delivers the drug to antigen presenting cells.



Summary!

- Increased awareness about Cryptococcus species
- High mortality
- Need for more affordable medicines, efficacious regimens
- Lumbar drains/punctures should be part of the treatment
- Secondary prophylaxis, ART timing and adherence counselling are key
- Post meningitis disability studies are ongoing
- No available vaccines, studies are on going

Thank you for listening

