Comparison of old and new treatment regimen for Cryptococcal Meningitis within the induction phase at Klerksdorp/Tshepong Hospital Complex, a retrospective audit.

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Declaration

I Dr Khutsisho Motlokwe Barnard Motubatse registered for Masters of Medicine degree student no 348 421 declare that the work submitted is entirely my own, except in areas where I have stated otherwise.

Signed 

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ABSTRACT

Background

Cryptococcal meningitis causes substantial morbidity and mortality in people living with HIV(LWH), especially in sub-Saharan Africa. Appropriate therapy with effective and safe regimens mitigates consequences of cryptococcal meningitis.

Objectives

This study compares mortality rates during and after the induction phase between the prior and current regimens for treating cryptococcal meningitis.

Methods.

In this retrospective review, we included adults LWH diagnosed with cryptococcal meningitis, who received either amphotericin B 1mg/kg/day with fluconazole (1200mg) for 14 days -old regimen - or amphotericin B (1mg/kg/day) with flucytosine(100mg/kg/day) in the first week followed by fluconazole (1200mg) in the second week-new regimen. We evaluated differences in mortality during and after the induction phase and compared characteristics and side effects between the groups using descriptive statistics.

Results

From a potential total group of 300 patients, we included the data of 91 patients between Jan 2015 to Dec 2022; 29 (31.87%) received the old regimen while 62 (68.13%) received the new regimen. All patients had laboratory-confirmed cerebrospinal fluid evidence of cryptococcal meningitis. After the start of induction , there were seven deaths among the patients treated with the old regimen ,compared to four deaths in the group treated with the new regimen.

Conclusion.

Both regimens demonstrated safety : however ,our findings indicate that the old regimen was less effective than the new regimen.. Moreover the new regimen's potential for reduced hospital stay suggests preference for its use compared to the old

regimen.

Key words : Cryptococcal meningitis, flucytosine, amphotericin B, HIV

BACKGROUND

Cryptococcus is a fungal yeast responsible for severe illness, particularly in immunocompromised individuals(1). The transmission of the fungus occurs through the inhalation of infected aerosols(1). Following acquisition, infection typically starts in the lungs and spreads to the bloodstream, eventually reaching the subarachnoid space by crossing the blood-brain barrier, leading to meningitis. While the infection may remain latent in individuals with healthy immune systems, it becomes more aggressive in people with immunosuppression(1). Cryptococcal infection, particularly meningitis, is prevalent in Sub-Saharan Africa, especially among adults living with Human Immunodeficiency Virus (HIV) (2) .and low and middle-income countries with high prevalence of HIV(3). Globally, 10 - 20% of HIV-related deaths in Africa are attributed to cryptococcal meningitis(4, 5).

Given the burden of cryptococcal meningitis, better treatment regimens with minimal adverse effects are required. Various therapeutic approaches, including monotherapy and combinations of fluconazole, amphotericin B, and flucytosine, have been explored to address the increasing challenges associated with cryptococcal meningitis. Three phase cryptococcal meningitis treatment includes: induction, consolidation, and maintenance. Induction typically lasts for two weeks and involves a combination of drugs. Flucytosine is recommended for use during the first week of induction(6). However, in regions where flucytosine is unavailable, amphotericin B and fluconazole are alternatives during the induction phase. During the consolidation phase, fluconazole is administered daily for eight weeks(6). For maintenance, oral fluconazole is prescribed at a lower dose for an extended period based on the cluster of differentiation 4 (CD4) cell count.

Flucytosine, although highly effective, may be limited in its availability in countries such as in South Africa, prompting the use of alternative medications(7). In the absence of flucytosine, the amphotericin B-fluconazole regimen has shown effectiveness in reducing mortality rates, reported in the Advancing Cryptococcal Meningitis Treatment for Africa (ACTA) trial(6). However, despite its efficacy, amphotericin B is associated with potentially life-threatening adverse effects, including hypokalaemia, anaemia, and renal dysfunction(8, 9). It is expensive(10), requires several preparatory steps before administration and can only be stored for 24hrs at 2-

18C before being discarded. Conversely, flucytosine's side effects mainly involve pancytopenia with neutropenic dominance(11, 12). It is cheaper than amphotericin B and taken as a capsule orally and can be stored at room temperature(10, 13). Other treatment approaches, such as monotherapy with high-dose fluconazole, amphotericin B, or different combinations, have demonstrated higher mortality rates and significant adverse events compared to the amphotericin B-flucytosine regimen(4, 14-18). The primary objective of this study is to assess mortality rates between the old and new regimens , with a hypothesis that mortality rates would be lower among patients treated with the new regimen(flucytosine based).

METHODS

Study design

A retrospective cohort study was conducted, abstracting data from the clinical records of patients who were admitted with cryptococcal meningitis. We evaluated treatment outcomes by the treatment received among patients with cryptococcal meningitis admitted from January 2015 through December 2022. The research site was Klerksdorp/Tshepong Hospital Complex in the Northwest Province of South Africa.

Study Population

We included adults living with HIV ≥ 18 years of age with a diagnosis of cryptococcal meningitis who received either amphotericin B 1mg/kg/day plus high dose fluconazole (1200mg) for 14 days (Old regimen) or amphotericin B 1 mg/kg/day with flucytosine 100mg/kg/day for 7 days followed by high dose fluconazole (1200mg) per day for the next 7 days(New regimen). Patients with

immunocompromised states caused by any other illness other than HIV including metastatic-malignant disease proven on staging computerized tomography(CT) and/ or patients on immunosuppressive drugs including but not limited to chronic glucocorticoid use, methotrexate, azathioprine, cyclosporine, mycophenolate mofetil, rituximab, and or any chemotherapeutic agents were excluded from this analysis.

In this study, patients were included if they had evidence of HIV infection confirmed

through the double rapid HIV test algorithm, an enzyme-linked immunoassay (ELISA) test result, or if they had initiated antiretroviral therapy. The diagnosis of cryptococcal meningitis was established based on positive results from various diagnostic tests, namely: 1. Cryptococcal Antigen Lateral Flow Assay(CrAg), (IMMY,Norman,USA) 2. India Ink 5% Nigrosin Stain, (Clinical Sciences Diagnostics, Germiston , Gauteng, South Africa) 3. Chocolate and 5% agar culture blood medium, (Thermo Fisher and Media Mage, Randburg and Stormill, Gauteng, South

Africa).

Data collection.

We identified potential study candidates through a variety of methods. Firstly, the Klerksdorp/Tshepong hospital pharmacy provided a list of all patients for which the old and/or new regimen were issued as therapy for cryptococcal meningitis, secondly, the public sector National Health Laboratory Service (NHLS) at Tshepong Hospital provided a list of all patients who had a positive cerebrospinal fluid India ink ,Crag or cryptococcal culture result. Lastly, we screened internal medicine ward admission logs in Klerksdorp Tshepong Hospital to identify patients admitted for cryptococcal meningitis between January 2015 and December 2022. A final combined list of 300 potential patients was used to identify and retrieve patient's files for screening.

Gender, age in years, CD4 cell count (cells/mm^3), method of diagnosis, Glasgow Coma Scale(GCS) on the date before commencement of therapy, regimen used during the induction phase, outcome at two weeks of starting treatment classified as alive or dead, whether patient was on antiretrovirals and the duration(in years) thereof at the time of diagnosis of cryptococcal meningitis was abstracted from those files that were eligible to be included. Additionally new or worsening renal failure and anaemia noticed during the induction phase of cryptococcal treatment was recorded. Neurological sequelae of cryptococcal meningitis pose significant concerns for long-term disease outcomes, therefore evaluating clinical parameters to gauge illness severity before initiating treatment

is crucial for optimizing patient care and predicting prognosis. We abstracted the Glasgow Coma Scale (GCS) at the time of presentation with illness. The duration of hospital admission in days was recorded..

Objectives and Research Hypothesis.

Our primary objective was to compare hospital mortality rates of HIV positive patients with cryptococcal meningitis at two weeks of induction therapy in two treatment groups.

1.1 Amphotericin/fluconazole group - old regimen

1st week fluconazole 1.2g daily and amphotericin B 1mg/kg/day

2nd week fluconazole 1.2g daily and amphotericin B 1mg/kg/day

1.2 Flucytosine/Amphotericin/fluconazole group - new regimen

1st-week amphotericin B 1mg/kg/day and flucytosine 100 mg/kg/day.

2nd week fluconazole 1.2g daily

Our secondary objectives included the following

1. To describe characteristics of patients with cryptococcal meningitis.
2. To compare the incidence of renal failure and anaemia in the two treatment groups at two weeks of induction phase of treatment. Based on the findings from previous reports such the ACTA(6) we hypothesized that mortality rates will be lower in the group that received the new regimen.

Data analysis

Continuous measures were assessed descriptively using medians (interquartile ranges) and frequencies (percentages) were determined for categorical measures. For continuous measures, the Kruskal-Wallis test was used to test the null hypothesis of no difference between groups whereas the Fishers exact test compared categorical variables. Mean values and their 95% confidence intervals were determined for continuous measures and

plotted over time. Statistical analysis was conducted in SAS Enterprise Guide 7.15 (SAS Institute Inc., NC, USA) assuming a 5% level of significance. We used a two-sample proportions Pearson's chi-squared test to compute power at different sample sizes and proportions of mortality for patients in the flucytosine group (p1) versus the Amphotericin group (p2), where the null hypothesis is HO: p1 = p2 and the alternative hypothesis is Ha: p2=! p1 and alpha= 0.05. Power calculations were conducted in Stata 15, using the Stata power command. A minimum sample size of 160 (80 in each group) will be powered at 79% to detect a difference of 20% between the groups. However we intended to use data from 180 patients (90 in each group) as a minimum.

Ethical considerations

The study received ethical approval from the University of the Witwatersrand Human Research Ethics Committee (Medical) (Reference Number: M210 867) as well as the North West Provincial and Hospital Research Committees.

Results

Of 300 patients ≥18 years of age diagnosed and treated for cryptococcal meningitis in Klerksdorp/Tshepong hospital from January 2015 through December 2022; 140/300 (46.7%) patient records were able to be retrieved. Of the 140 retrieved records, 91 met eligibility criteria and were included in this study. . The leading reasons for non-inclusion were treatment duration of less than 14 days, coexisting comorbidities contributing to immunosuppression other than HIV infection, and patient's age <18 years.

63.8% of all patients had a positive cerebrospinal fluid india ink test ,while 36.3% were diagnosed with a positive cryptococcal antigen(CrAg) test and 86% had a positive serum CrAg test. Twenty nine patients (31.87%) were treated with the old regimen and 62 (68.13%) with the new regimen.

TOTAL NO OF PATIENTS IDENTIFIED (n=300)
- Diagnosed with cryptococcal meningitis.

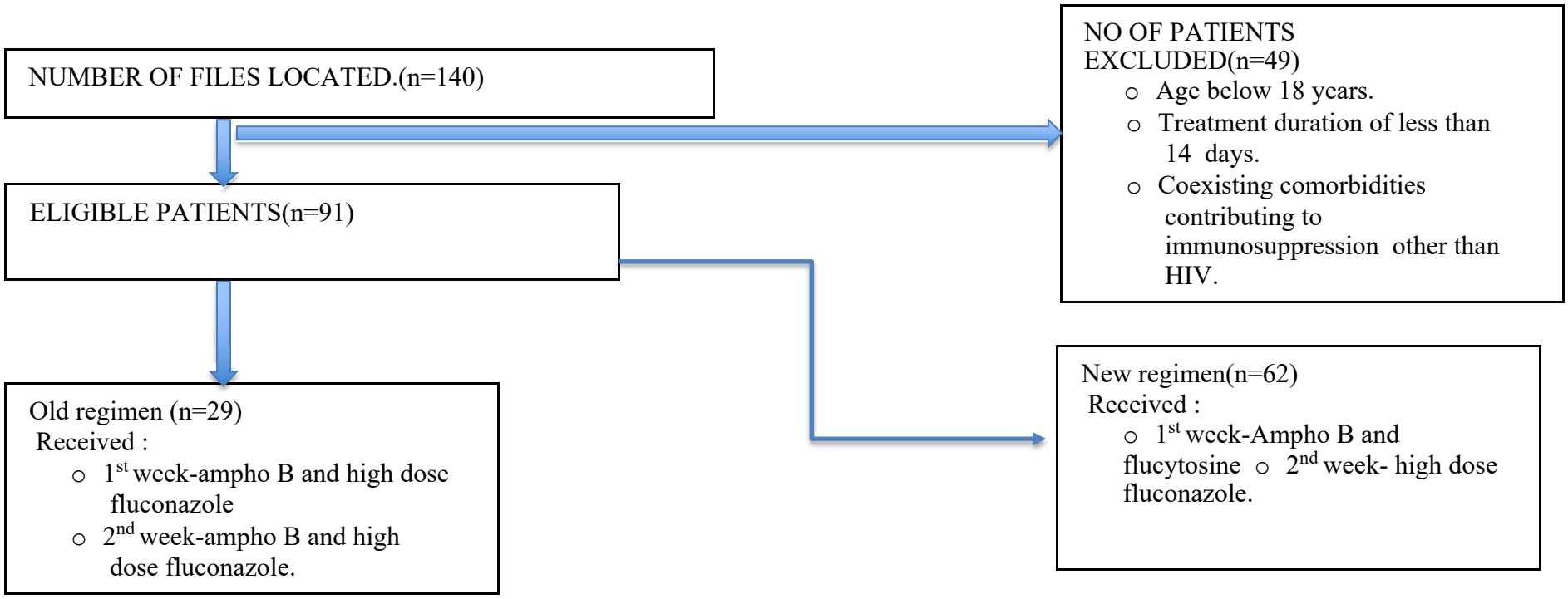

Figure 1 : STUDY AT VARIOUS PHASES

Characteristics of the two treatment groups

The median age for the entire sample was 37 years (IQR: 33-46). Those treated with the old regimen had a median age of 40 years (IQR: 33-46), while the median age in the new regimen group was 36 years (IQR: 33-44). 31.9% were female and 68.1% were male. The median CD4 cell count was 36 cells/mm^3(IQR15-66)); 54.0 cells/mm^3 (16-65) and 31.5 cells/mm^3 (12-66) in the old and new treatment groups (p-0.371).Among reviewed patients,79% were known to have HIV infection, while 21% were newly diagnosed at presentation with cryptococcal meningitis. Eighty four percent were virally unsuppressed, with 68% of those having defaulted on their antiretroviral treatment, and 13% were virally suppressed. The median duration of the antiretroviral therapy was 4 years..

The median GCS was 15.0 (IQR(14.0-15.0)) in both groups(p= 0.6106).

Duration of Hospital Stay and Mortality

The median duration of hospital stay was 16 days in the old treatment group, compared to 12 days in the new regimen. (p=0.0051). The study observed a total of eleven deaths : seven in the old regimen and four in the new regimen. Of these, ten occurred during the induction period-seven in the old regimen and 3 in the new regimen. After induction only one death was recorded in the new regimen.

Side effects and cell counts.

Table 1 : Interquartile range for the urea (mmol/L), creatinine (umol/L) ,haemoglobin (g/dL) and white cell count (10^9/L) over 14 days.

variable	total	Amphotericin	Flucytosine	P value	varr
Median (IQR) Urea (day 1)	n= 87 ; 3.7 (3.0-5.1)	n= 25 ; 3.6 (3.0-4.6)	n= 62 ; 3.9 (3.0-5.2)	0.5734	1
Median (IQR) Urea (day 4)	n= 79 ; 4.0 (3.0-7.0)	n= 25 ; 5.1 (3.0-6.9)	n= 54 ; 4.0 (3.0-7.0)	0.8002	1
Median (IQR) Urea (day 7)	n= 84 ; 4.7 (3.4-6.6)	n= 25 ; 4.8 (4.1-5.6)	n= 59 ; 4.3 (3.2-7.1)	0.5440	1
Median (IQR) Urea (day 14)	n= 60 ; 4.6 (3.2-6.0)	n= 25 ; 4.9 (3.6-5.5)	n= 35 ; 4.0 (3.1-6.3)	0.7189	1
Median (IQR) Creatinine (day 1)	n= 87 ; 67.0 (57.0-80.0)	n= 25 ; 70.0 (60.0-81.0)	n= 62 ; 64.0 (57.0-79.0)	0.3222	1
Median (IQR) Creatinine (day 4)	n= 79 ; 78.0 (65.0-96.0)	n= 25 ; 86.0 (70.0-113.0)	n= 54 ; 76.5 (62.0-94.0)	0.0989	1

Median (IQR) Creatinine (day 7)	n= 85 ; 88.0 (71.0-111.0)	n= 25 ; 91.0 (79.0-112.0)	n= 60 ; 84.0 (68.0-109.5)	0.1548	1
Median (IQR) Creatinine (day 14)	n= 60 ; 91.5 (72.0-127.5)	n= 25 ; 111.0 (87.0-135.0)	n= 35 ; 79.0 (64.0-120.0)	0.0039	1
Median (IQR) Haemoglobin (day 1)	n= 87 ; 12.4 (10.7-13.7)	n= 26 ; 12.3 (11.5-13.3)	n= 61 ; 12.4 (10.4-14.0)	0.9150	1
Median (IQR) Haemoglobin (day 4)	n= 57 ; 10.9 (9.6-12.1)	n= 15 ; 11.1 (9.8-12.0)	n= 42 ; 10.8 (9.5-12.2)	0.7373	1
Median (IQR) Haemoglobin (day 7)	n= 63 ; 9.9 (8.4-11.6)	n= 17 ; 9.9 (8.4-11.2)	n= 46 ; 9.9 (8.4-11.9)	0.6421	1
Median (IQR) Haemoglobin (day 14)	n= 51 ; 10.5 (8.6-12.2)	n= 17 ; 10.1 (8.3-11.3)	n= 34 ; 10.7 (8.9-12.3)	0.1773	1
Median (IQR) White Cell Count (day 1)	n= 87 ; 5.8 (4.4-9.0)	n= 26 ; 6.6 (4.1-9.0)	n= 61 ; 5.7 (4.5-9.0)	0.8059	1
Median (IQR) White Cell Count (day 4)	n= 54 ; 5.2 (3.5-7.1)	n= 15 ; 5.8 (3.6-9.5)	n= 39 ; 5.0 (3.4-6.9)	0.5239	1
Median (IQR) White Cell Count (day 7)	n= 62 ; 5.0 (4.1-7.0)	n= 17 ; 4.8 (4.1-5.7)	n= 45 ; 5.0 (4.1-7.0)	0.4488	1
Median (IQR) White Cell Count (day 14)	n= 51 ; 5.0 (3.7-6.8)	n= 18 ; 5.4 (3.8-6.8)	n= 33 ; 5.0 (3.7-7.1)	0.9059	1
Median (IQR) Protein	n= 79 ; 0.9 (0.6-2.0)	n= 22 ; 1.4 (0.8-2.8)	n= 57 ; 0.9 (0.5-1.8)	0.0213	1
Median (IQR) Glucose	n= 81 ; 2.2 (1.2-2.7)	n= 22 ; 2.0 (1.0-2.6)	n= 59 ; 2.2 (1.3-2.8)	0.2403	1
Median (IQR) PMM	n= 81 ; 0.0 (0.0-1.0)	n= 21 ; 0.0 (0.0-0.0)	n= 60 ; 0.0 (0.0-1.0)	0.3876	1

Median (IQR) Lymphocytes	n= 81 ; 5.0 (0.0-37.0)	n= 21 ; 3.0 (0.0-11.0)	n= 60 ; 5.5 (0.0-42.0)	0.3058	1
Median (IQR) Erythrocytes	n= 81 ; 0.0 (0.0-3.0)	n= 21 ; 0.0 (0.0-3.0)	n= 60 ; 0.0 (0.0-2.5)	0.7554	1

Figure 2 : Trend of urea (mmol/L) and creatinine (umol/L) during the 14 day period.

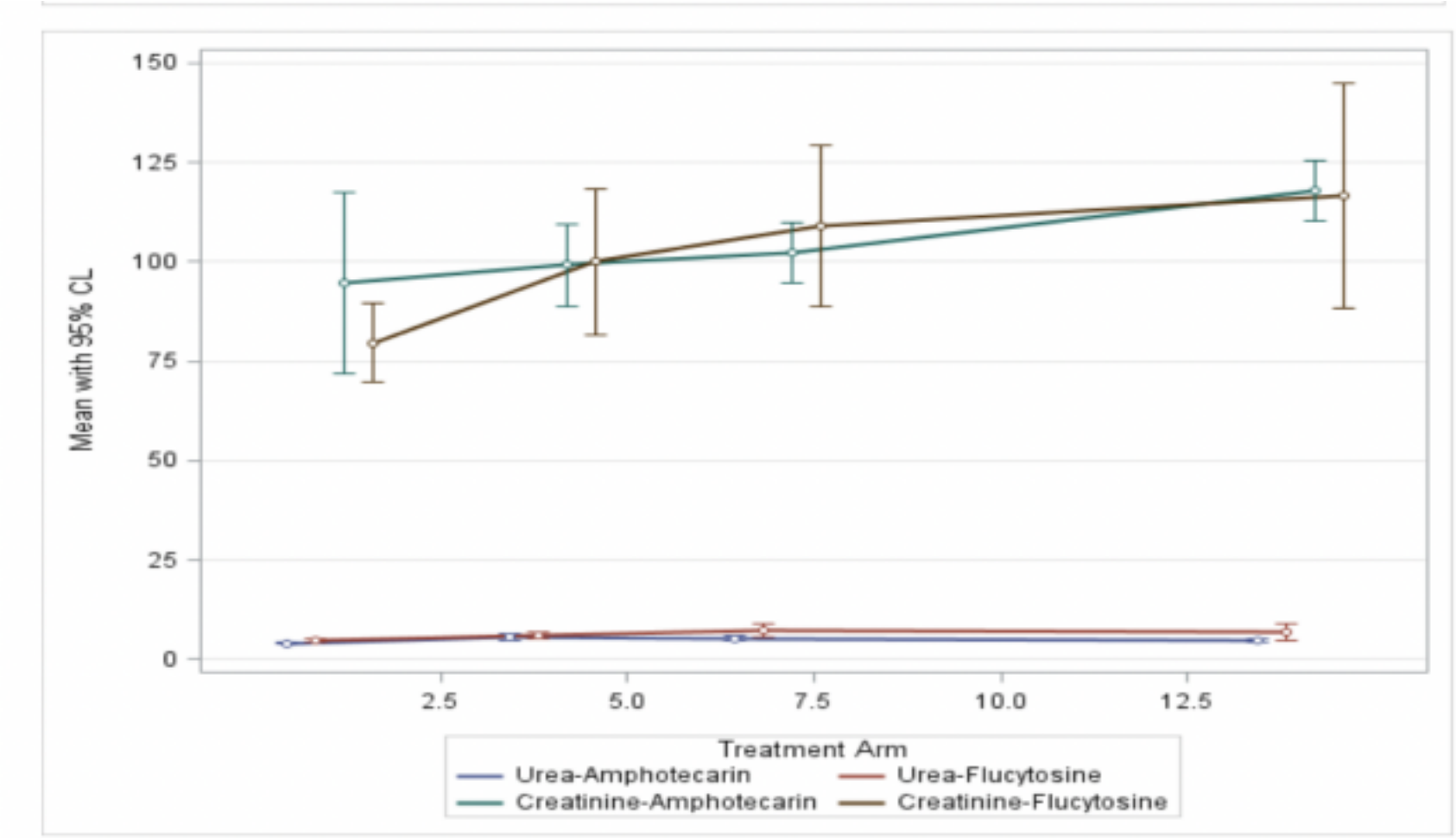

Figure 3 :Trend of haemoglobin (g/dL) and white cell count (10^9/L) over the 14 days period.

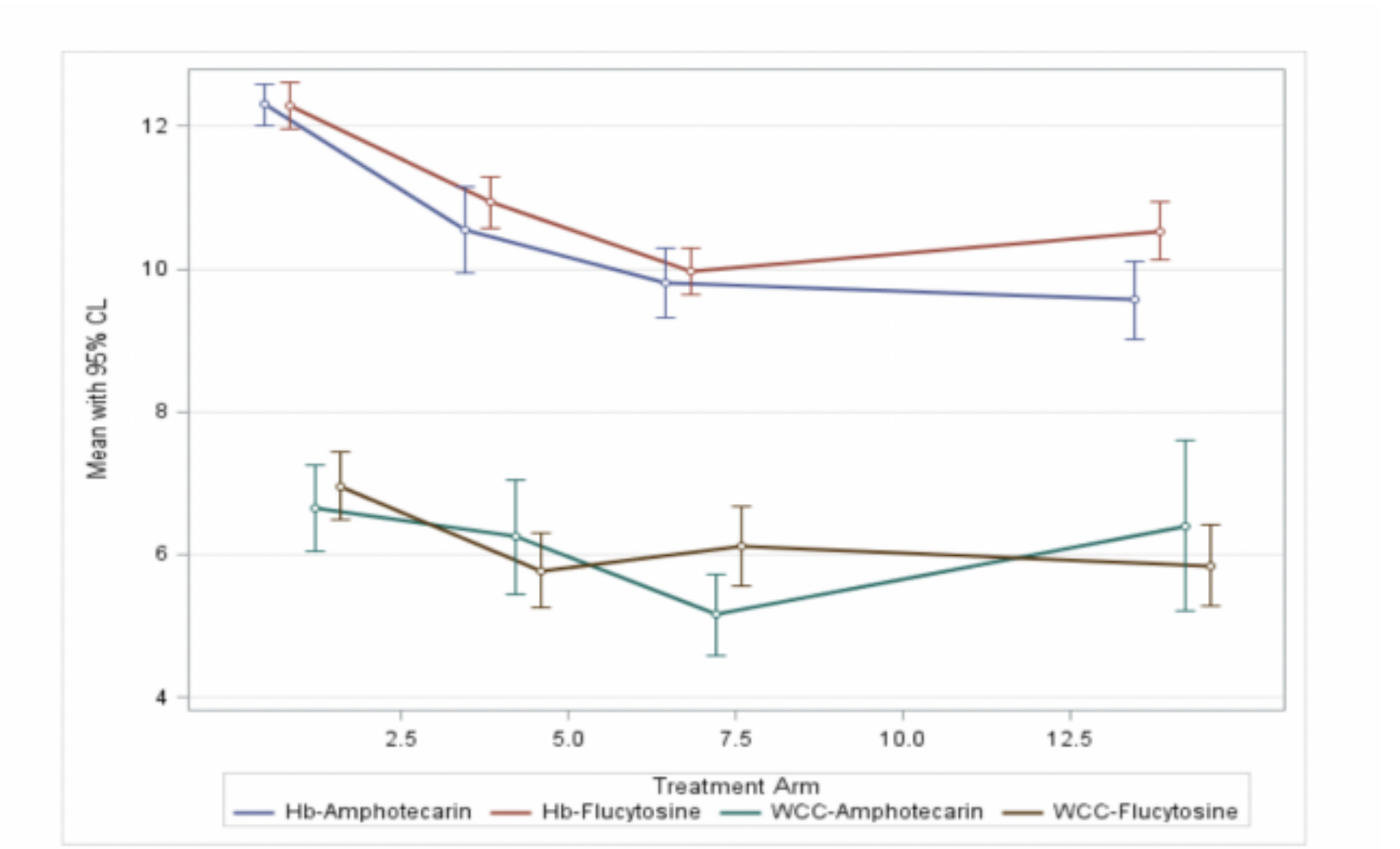

Table 2 : Characteristics between the two groups.

variable	total	Amphotericin	Flucytosine	P Value	varr
Median (IQR) age in years	n= 91 ; 37.0 (33.0-46.0)	n= 29 ; 40.0 (33.0-46.0)	n= 62 ; 36.0 (33.0-44.0)	0.3706	1
Gender					
Female	29 (31.9)	5 (17.2)	24 (38.7)	0.0537	
Male	62 (68.1)	24 (82.8)	38 (61.3)		
Method of diagnosis					
CSF CLAT	33 (36.3)	11 (37.9)	22 (35.5)	0.8195	
CSF India ink	58 (63.7)	18 (62.1)	40 (64.5)		
Outcome at 14 days after treatment					
Alive	90 (98.9)	29 (100)	61 (98.4)	1.0000	
Deceased	1 (1.1)	0 (0.0)	1 (1.6)		
HIV result					
Known positive	70 (79.5)	22 (84.6)	48 (77.4)	0.5684	
Newly diagnosed	18 (20.5)	4 (15.4)	14 (22.6)		
Defaulted treatment					
No	11 (16.4)	4 (18.2)	7 (15.6)	1.0000	

Yes	56 (83.6)	18 (81.8)	38 (84.4)		
Virally suppressed?					
No	68 (84.0)	21 (77.8)	47 (87.0)	0.3411	
Yes	13 (16.0)	6 (22.2)	7 (13.0)		
Median (IQR) CD4 Count (cells/mm3)	n= 91 ; 36.0 (15.0-66.0)	n= 29 ; 54.0 (16.0-65.0)	n= 62 ; 31.5 (12.0-66.0)	0.3710	1
Median (IQR) ARV duration in years	n= 77 ; 4.0 (0.0-8.0)	n= 22 ; 5.5 (1.0-7.0)	n= 55 ; 3.0 (0.0-8.0)	0.7102	1
Median (IQR) Glasgow coma scale	n= 81 ; 15.0 (14.0-15.0)	n= 24 ; 15.0 (14.0-15.0)	n= 57 ; 15.0 (14.0-15.0)	0.6106	1
Serum CRAG					
Negative	11 (14.3)	2 (9.5)	9 (16.1)	0.7172	
Positive	66 (85.7)	19 (90.5)	47 (83.9)		
Median (IQR) Length of hospital stay(days)	n= 82 ; 14.0 (10.0-18.0)	n= 22 ; 16.5 (14.0-18.0)	n= 60 ; 12.0 (9.0-18.0)	0.0051	1

Cerebrospinal fluid analysis

Of the 91 patients included in this study, 32 (35.16%) cultured *Cryptococcus neoformans in their cerebrospinal fluid*, while 17 (18.68%) grew non-Cryptococcus neoformans species, including laureti 12(13.19%) gatti 1 (1.10%) , albicans 1 (1.10%) and cryptococcal species unspecified 3 (3.29%). Regardless of culture results, every patient had at least one positive result from either the India ink or Crag test.

The diagnosis of cryptococcus was established based on a positive India ink test and Cryptococcal Antigen (Crag) in the remaining group of patients. The overall interquartile range for the cerebrospinal fluid protein found to be 0.9 g/L (0.6-2.0) with the highest value in the group that received the old regimen 1.4 g/L (0.8-2.8).The overall median glucose level was 2.2 mmol/L (1.2-2.7).With regards to cell count ,an overall interquartile range for the polymorphs, lymphocytes and erythrocytes was 0.00 /uL (0.0-1.0), 5.0 /uL(0.0-37.0) and 0.00 /uL (0.0-3.0).

DISCUSSION

In this retrospective study, we found no substantial differences in mortality at two weeks of the induction phase of treatment between patients with laboratory confirmed cryptococcal meningitis who received either the old or new treatment regimens for cryptococcal meningitis. But we did find that a markedly higher proportion of patients on the old regimen died during the induction phase (7/29 (24%) OLD versus NEW 3/62 (4.8%) regimen

Most patients (56%) in the study defaulted their antiretroviral medication, and had unsuppressed viral loads and very low CD4 cell counts. The average Glasgow Coma Scale (GCS) score of 15/15 on presentation suggests that patients exhibited normal levels of consciousness without any apparent acute neurological issues. Despite a high number of patients with unsuppressed HIV viral loads and low CD4 cell counts ,the presence of a good GCS with no reported acute neurological complications and normal cerebrospinal fluid glucose levels suggests the absence of poor prognostic factors(19, 20).

In our study, we found no significant differences in the prevalence of renal failure, anaemia and leukopenia between the two

treatment groups. However, there were notable differences in the length of hospital stay: patients receiving the old regimen had a longer hospital stay (16 days) compared to those receiving the new regimen (12 days). Our findings here corroborate what has been reported in other studies. Notably, Mashau et al. observed that the duration of hospital stay was 11 days for patients on the new regimen compared to 17 days for those receiving the old treatment regimen.

Most patients tested positive for serum cryptococcal antigen (86%), suggesting that in patients where the serum cryptococcal antigen result is negative but there is a strong suspicion of cryptococcal meningitis, cerebrospinal fluid analysis should still be performed. Microscopic detection of cryptococcal species using India ink stain was positive in the majority of patients (68.3%). India ink staining is considered the least sensitive test for diagnosing cryptococcal meningitis(21, 22).Given the high percentage of positive results with India ink staining in this cohort, we infer that the majority of patients had high fungal loads. A subset of the patient population cultured Cryptococcus laurentii, a rare pathogen not commonly known to cause disease in humans. This raises an interesting consideration, as this organism typically does not lead to human illnesses. Given its rare association with disease, it may be prudent to re-evaluate the accuracy, sensitivity, and specificity of the culture assay used.

Like other retrospective studies this one had multiple missing data points which primarily meant that although pharmacy records suggested that 300 people had received treatment during the study period, we were only able to recover and include in the analysis, less than one third of these. Moreover, there was an imbalance in numbers in the hospital record of patients who received either study regimen. . Instead of the planned 180 participants we analysed a smaller sample size of 91. The aforementioned limitations might have caused selection bias, which in turn likely lowered the statistical power to detect differences between the two groups, potentially obscuring the true differences in mortality rates. The mortality outcomes we found were inconsistent with those reported in other studies, including the ACTA(6) trial, likely largely due to the limitations mentioned.

CONCLUSION

In this retrospective cohort study, the old regimen appeared to be less effective than the new regimen . Our findings suggest shorter hospital stays associated with the use of the new regimen compared to the old regimen in the treatment of cryptococcal meningitis despite the lack of significant differences in adverse events between the two treatment groups. In view of the findings preference should be given to the new regimen. However, a clinical trial is required to show the evidence of benefit of the new regimen over the old regimen.

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