CORRELATION BETWEEN CD4 COUNT AND CEREBROSPINAL FLUID INFLAMMATORY RESPONSE IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PATIENTS WITH CRYPTOCOCCAL MENINGITIS

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i. DECLARATION

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FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION SIGNED BY ALL HIGHER DEGREE STUDENTS

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- I confirm that ALL the work submitted for assessment for the above course is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
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i. DEDICATION

To my loving husband for his unconditional support.

ii. PUBLICATIONS AND PRESENTATIONS ORIGINATING FROM THIS RESEARCH

iii. ETHICAL CONSIDERATIONS

The author obtained permission for this retrospective study from Professor G. Modi (Head of Neurology, Department of Neurosciences) and the Human Research Ethics Committee of the University of Witwatersrand (clearance number – M200318).

iv. ABSTRACT

Introduction: Cryptococcal meningitis is the most common central nervous system opportunistic infection in HIV seropositive individuals in sub – Saharan Africa, with South Africa having the highest prevalence. The cerebrospinal fluid findings indicate a lack of inflammatory response. Our study investigates if the cerebrospinal fluid (CSF) inflammatory response can be correlated to CD4+ counts level.

Methods: This was a retrospective cohort study of all patients with HIV associated cryptococcal meningitis at Charlotte Maxeke Johannesburg Academic Hospital. Demographics, CD4+counts and CSF findings were collected through all discharge summaries. Spearman's rank-order correlation statistic was used to assess the correlation between CD4+ counts and cerebrospinal fluid (CSF) inflammatory response. An R of +1 suggests a positive correlation, and a p-value < 0.05 was considered significant.

Results: One hundred and thirteen patients were included in the study. All were HIV seropositive, 44.25% (n = 50) were newly diagnosed and 44.25% (n = 50) were known. Thirty-four out of the fifty in the known HIV category were on antiretroviral treatment. Eighty – three percent (n = 94) of patients had CD4+ counts that were under 100 cells/ µL with the median CD4+ count of 21 cells/µL [Interquartile range (IQR): 9.00 – 56.00]. There was no statistically significant correlation between CSF polymorphonuclear cells and CD4+ count with a p-value of 0.068; however, there was a significant correlation with CSF protein with a p-value of 0.008.

Conclusion: There was a partial correlation between CD4+ counts and cerebrospinal fluid inflammatory response. Antiretroviral treatment did not significantly impact the CSF fluid inflammatory response, as suggested by other studies.

v. ACKNOWLEDGEMENTS

• I want to thank my supervisor, Professor G Modi, for his support, guidance and knowledge.

• I would like to also express gratitude to my colleagues for their extensive record keeping.

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ix. NOMENCLATURE

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral treatment/ therapy
CD	Cluster of Differentiation
CLAT	Cryptococcal Latex Agglutinin Test
CrAg	Cryptococcal Antigen
CSF	Cerebrospinal fluid
CT	Computerized Tomography
Glu	Glucose
GXM	Glucuronoxylomannan
HIV	Human Immunodeficiency Virus
IL	Interleukin
INF – y	Interferon-gamma
IQR	Interquartile Range
IRIS	Immune Reconstitution Inflammatory Response
LP	Lumbar Puncture
Lymphs	Lymphocytes
µL	Microliter
MRI	Magnetic Resonance Imaging
Polys	Polymorphonuclear
Prot	Protein

sTNFRII	soluble Tumor Necrosis Factor Receptor II
TNF	Tumour Necrosis Factor
UNAIDS	The Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization

1. PROTOCOL AND EXTENDED LITERATURE REVIEW

1.1. Introduction

Human Immunodeficiency Virus (HIV) is a devastating viral infection with a high global burden. The global incidence of infected people in 2018 was estimated at 37.9 million[(1)]. Based on data collected by UNAIDS in 2018, it is estimated that 1.7 million people are newly infected with HIV annually[(1)]. The largest affected population is in Africa. In sub – Saharan Africa, an estimated 25.7 million people are living with HIV[(1)]. The WHO/UNAIDS reported that South Africa harbours the highest burden of sub–Saharan countries with an estimated 270 000 new infections annually[(1)].

1.2. Opportunistic infections in HIV

HIV infected individuals are susceptible to opportunistic infections. HIV causes dysfunction of the CD4+ T-lymphocytes weakening the natural immune response[(2)]. A study from North India assessed opportunistic infections to CD4+ counts in HIV infected individuals[(3)]. This study demonstrated that the most common opportunistic infections included: Oral Candidiasis 40.8%; Cryptosporidiosis 23.68%; Tuberculosis 5.92%; and other diseases such as Cytomegalovirus (CMV), Pneumocystis pneumonia (PCP) and Cryptococcosis 2.95%. Opportunistic infections were more common in patients with CD4+ counts of less than 200 cells/microliter (µL)[(3)].

A more extensive study with a similar study design was conducted in Ethiopia. All patients in the study were on antiretroviral treatment[(4)]. This study demonstrated that most opportunistic

infections were in patients with CD4+ counts of less than 200 cells/mL (71.57%). Higher CD4+ counts were associated with the lowest opportunistic infections (8.6%)[4].

Bartlett et al. described common opportunistic infections concerning CD4+ counts in HIV infected population[5]. Tuberculosis infection was reported irrespective of CD4+ count. At CD4 + counts (≤) 250 cells/µL Coccidioidomycosis is common[5]. Pneumocystis occurs at ≤ 200 cells/µL [5]. Histoplasmosis is associated with CD4+ < 150 cells/µL [5]. Toxoplasmosis and Cryptococcus are prevalent in individuals with CD4+ counts of < 100cells/µL [5]. Mycobacterium avium complex is known to be prevalent at CD4+ ≤ 50 cells/µL[5].

1.3. Cryptococcosis and the immunocompromised

Cryptococcosis is a yeast contracted by inhalation of spores and subsequent colonisation of the lungs[6]. It infects the central nervous system (CNS) through the hematogenous spread manifesting as meningitis or meningoencephalitis[6]. It is a disease of the immunocompromised with a solid transplant, malignancies, long term immunosuppressive drugs, diabetes mellitus, rheumatological disorders, renal and liver failure are some of the risk factors. Cryptococcus neoformans is a common HIV infected population with a CD4+ ≤ 100 cells/µL [6]. Cryptococcus gattii typically affects the non - HIV population [6].

1.4. Prevalence of cryptococcal meningitis

Cryptococcal meningitis is the most common cause of morbidity and mortality in HIV infected populations in sub–Saharan Africa[7]. There is an estimated incidence of 278000 people globally per annum, of which 223000 were reported in sub – Saharan Africa[8]. A study conducted by

Govender et al. assessed the incidence of laboratory-confirmed cryptococcal meningitis by sampling 360000 CSF specimens with CD4+ counts of less than 100 cells/μL[7]. From 2002 to 2011, high rates of infection were noted ranging from 41.6% to 83.3%, with the highest incidence in 2006 (83.3%)[7]

1.5. Clinical presentation and diagnosis of cryptococcal meningitis

Individuals with cryptococcal meningitis present with headache, fever, nausea, vomiting and altered level of consciousness[9]. The median duration from symptom onset to presentation is two weeks in the HIV infected population [9]. The diagnosis is confirmed by analysing the cerebrospinal fluid (CSF)[9]. After excluding contraindications, a lumbar puncture (LP) is performed in patients with suspected new-onset cryptococcal meningitis [9]. Opening pressure is achieved by having a patient lie on their side[9]. The CSF sample is sent for laboratory confirmation of Cryptococcal Antigen (CrAg), India ink and Cryptococcal Latex Antigen Test (CLAT)[9].

The World Health Organization (WHO) recently published guidelines on the diagnosis of cryptococcal meningitis[9]. A rapid cryptococcal antigen assay is the preferred method if there is access to quick CSF results and no contraindications to a performance of an LP[9]. If the latter is not available, India ink is preferred to confirm the diagnosis[9]. If there are contraindications to an LP and no access to CSF rapid cryptococcal antigen (CrAg) or lumbar puncture, the WHO proposed that a serum cryptococcal antigen assay be performed, and the patient be referred without delay to a facility with the capacity to do an LP and CSF CrAg assay[9].

The sensitivity and specificity of Cryptococcal antigen in the CSF are 100% and 97.7%, respectively[10]. India ink sensitivity 50% and specificity 100%[11]. Cryptococcal Latex Agglutination Test sensitivity 91.1% and specificity 96%[12].

1.6. Pathophysiology of cryptococcosis

Cryptococcal meningoencephalitis can lead to fatal outcomes in HIV infected individuals [18]. Therefore, an effective immune response is vital in preventing the spread of cryptococcus neoformans to the central nervous system [18]. When an individual is infected with cryptococcus species, a cell-mediated immune response is activated, involving predominately mononuclear cells, including CD4+ T- cells [18]. It is suggested that leukocytes and CD4+ T-cells detected at the site (lungs) of infection provide a protective immune response [18].

Clearance of cryptococcus infection is dependent on the effectiveness of the host's immune system, and if it is intact, it will mount an immune response, thus clearing the infection [19]. Therefore, the key to controlling cryptococcosis lies in innate immune activation and sufficient inflammatory response [19].

The primary first-line response for host defence is initiated by the alveolar macrophages, which activate phagocytes through antibody and complement pathways [19]. Once internalised, the yeast is killed by interferon-y (IFN-y) produced by natural killer cells and CD4+ T-helper cells [19]. CD4+ T-helper cells are crucial in the host immune response; therefore, patients with cryptococcal meningitis who mount an immune response have a better outcome than those who don't [19]. However, this does not mean that patients with a higher CD4+ count automatically

mount an immune response as the CD4+ phenotype is more important than the number of cells one has [19].

1.7. HIV and Immunity

CD4+ T-cells are the primary mediators of immune response in human beings as they are essential for the production of both cellular and humoral immune responses against infections [22]. It is extensively researched that HIV harms the CD4+ T-cells [22]. HIV's main target is the CD4+ T-cells, leading to the reduced quantity of these cells and the quality, leading to a minimal and ineffective immune response to infections [22].

The level of immunity in HIV infected patients is quantified by measuring plasma CD4+ counts[13]. The typical values range from 500 to 1400 cells/mm^3. CD4+ counts are used to assess the progression and disability of the disease and the risk for opportunistic infections[14].

1.8. CSF inflammatory response

The CSF is used to assess the evidence of brain pathology caused by inflammation, infection and immunological processes [15]. The CSF is almost devoid of inflammatory cells such as macrophages, plasma cells, and eosinophils found in blood plasma [15]. The blood-brain barrier controls the communication between the CSF and systemic circulation, preventing these cells' leakage into the brain when the integrity of the blood-brain barrier is compromised, as, in meningitis, a CSF inflammatory response is activated [15].

The CSF is used to assess the evidence of brain pathology caused by inflammation, infection and immunological processes [15]. The CSF is almost devoid of inflammatory cells such as macrophages, plasma cells, and eosinophils found in blood plasma [15]. Cerebrospinal fluid (CSF)

inflammatory response refers to plasma cells evidenced by the presence of polymorphonuclear leukocytes, lymphocytes and elevated protein in the CSF sample analysis [(15)].

Cryptococcal meningitis is associated with minimal cerebrospinal fluid inflammation due to the inhibition of recruitment of mononuclear cells, namely, neutrophils by the yeast capsule glucuronoxylomannan (GXM) or binding of Cryptococcus neoformans to CD 18 [(20)]. Mononuclear cells produce both pro and anti-inflammatory cytokines [(20)]. Human Immunodeficiency Virus (HIV) potentially reduces the anti-cryptococcal activity of serum mononuclear cells or their ability to activate cytokines in the presence of cryptococcus neoformans (20). Glycoprotein 120 HIV envelop also changes the T cell response to the yeast [(20)].

1.9. Correlation between CD4+ and CSF inflammatory response in HIV associated cryptococcal meningitis

Lortholary et al., in partnership with The French Cryptococcus Study Group, conducted a study to test if there's any difference in levels of pro and anti-inflammatory mediators with the focus on the following cytokines: tumour necrosis factor – α (TNF- α), interleukins 6,8,10 (IL6, 8, 10) and soluble tumour necrosis factor receptor II (sTNFRII), in HIV associated cryptococcal meningitis and HIV seronegative extrameningeal cryptococcosis [(20)]. The study demonstrated that HIV related cryptococcal meningitis is associated with higher levels of several immune mediators compared to individuals with extra-meningeal cryptococcosis [(20)]. These findings were attributed to the fact that these immune mediators are produced by local cells, for example, microglial cells and not by mononuclear cells attracted to the infection site (lungs) [(20)].

Scriven et al. investigated if there's an association in the CSF immune response in HIV related cryptococcal meningitis characterised by macrophage activation to define if this affects the disease severity and use of antiretroviral therapy [21]. The study examined the CSF immune response measured by the inflammatory cytokines focused on the CD4+ and CD8+ T-helper cells [21]. The study found that persons with lower CSF CD4+ and CD8+ levels had a significantly higher CSF fungal burden and that serum and CSF CD4+ counts were closely correlated [21]. Further on, it also explored the relationship between CSF immune response and antiretroviral treatment [21]. The findings suggested that persons initiated on antiretroviral therapy (ART) 12 weeks before presentation had lower HIV viral loads and higher serum CD4+ counts [21]. However, there was no significant difference in the CSF fungal burden and opening pressure, white cell counts and outcome [21].

Cecchini et al. studied the variables that influence HIV CSF viral load in patients with cryptococcal meningitis [16]. He compared CSF cytology (leucocytes), biochemistry (protein and glucose) and CSF HIV viral loads in patients with cryptococcal meningitis to plasma HIV viral loads and CD4+ T lymphocytes[16]. The median plasma CD4+ count was 24 cells/mm3 with the median CSF leucocytes 10 cells/mm^3, glucose 39 milligram / deciliter (mg/dL) and protein 0.75gram/liter[16]. They found no correlation between CSF inflammatory response and plasma HIV viral loads; however, there was a correlation between CSF leucocytes and plasma CD4+ counts[16]. The study concluded that plasma CD4+ T- cells influence CSF cellular response in patients with cryptococcal meningitis[16].

1.10. Conclusion and reason for the study

Numerous studies assessed the CSF inflammatory response of HIV infected individuals who present with cryptococcal meningitis. These studies focused on interleukins, CD4+ and CD8+ levels in the CSF to evaluate the lack of a meningeal reaction.

The literature does not entirely understand why the CSF glucose is low and that protein and pressure are high in patients with cryptococcal meningitis. These CSF findings can be easily mistaken as mixed meningitis, for example, tuberculous meningitis. There is a lack of studies assessing the correlation between CD4+ counts and the CSF inflammatory response (i.e. cytology and biochemistry) in HIV infected populations with cryptococcal meningitis.

The study aims to test the hypothesis that lack of cerebrospinal fluid cellular response with low glucose and elevated protein in cryptococcal meningitis is due to the patient's immune state, which can be correlated with a CD4+ count.

1.11. Aim

To identify the correlation of the level of immunity as determined by the CD4+ count to cerebrospinal fluid (CSF) inflammatory response in HIV infected patients with cryptococcal meningitis

1.12. Study objective

To determine whether CD4+ count level has any effect on the CSF inflammatory response

1.13. Methods

1.13.1. Study design

This is a retrospective and descriptive study.

1.13.2. Population study

All the patients admitted with cryptococcal meningitis at Charlotte Maxeke Johannesburg Academic Hospital with an estimated sample size of 100 patients from 2015 to 2020.

1.13.3. Inclusion criteria

All patients diagnosed with cryptococcal meningitis

1.13.4. Exclusion criteria

- Cerebrospinal fluid erythrocytes, i.e. bloody or traumatic tap with erythrocyte count more than 50/cm^3

- Space occupying lesion as noted on brain imaging viz. Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) except those with cryptococcomas.
- Co-infection with another type of meningitis, e.g. Tuberculous, bacterial and viral meningitides.

1.13.5. Parameters

Immune response

1. Cerebrospinal fluid glucose and protein
2. Cytology: polymorphs, lymphocytes and erythrocytes

Immunity

1. CD 4+ count

1.14. Data collection and analysis

[APPENDIX II: data collection sheet]

- Data will be collected by myself
- I will go through the CMJAH neurology discharge summaries folder from the filing system and computer documents to extract information of all patients diagnosed with cryptococcal meningitis
- For the data from the discharge summaries folder, I will go through the folder and extract data of all patients diagnosed and treated for cryptococcal meningitis
- Demographics such as age and sex will be obtained from the discharge summary

- CD4+ counts will be tallied against the cerebrospinal fluid protein and glucose in conjunction with cytology
- Descriptive statistics will be used for data analysis using non-parametric Spearman Rank Order correlation statistics where CD4 counts are correlated with CSF findings
- STATA / STATISTICA will be used to aid data analysis in conjunction with a biostatistician.

1.15. Ethics

The protocol will be submitted for ethics approval to the Human Research Ethical Committee of the University of Witwatersrand.

1.16. Limitations

If there is missing data like CD4+ counts or viral load, I will not be able to access that information, as this is a retrospective study

1.17. Funding

No funding is required as it is standard procedure to do a lumbar puncture on each patient presenting with symptoms and signs suggestive of meningitis. Any printing or additional costs will be self-funded.

1.18. Timeline

Table 1 - GANT chart showing the timeline of the study

	Dec 2019 – Mar 2020	April 2020	May 2020	June 2020	July 2020	Aug 2020	Sep 2020	Oct 2020	Nov 2020	July 2021	Aug 2021	Sept 2021
Literature review												
Protocol preparation												
Protocol assessment												
Ethics application												
Data collection												
Data analysis												
Manuscript												

1.19. References (Extended Literature Review)

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2. MANUSCRIPT

CORRELATION BETWEEN CD4+ COUNT AND CEREBROSPINAL FLUID INFLAMMATORY RESPONSE IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PATIENTS WITH CRYPTOCOCCAL MENINGITIS.

2.1. Background

Human Immunodeficiency Virus (HIV) is a devastating viral infection with a high global burden, and the global incidence of infected people in 2018 was estimated at 37.9 million[1]. The largest affected population is in Africa. In sub – Saharan Africa, an estimated 25.7 million people are living with HIV[1]. The WHO/UNAIDS reported that South Africa harbours the highest-burden of sub–Saharan countries with an estimated 270 000 new infections annually [1, 3].

HIV infected individuals are susceptible to opportunistic infections. HIV causes dysfunction of the CD4+ T-lymphocytes weakening the natural immune response, thus leading to ineffective response to prevent opportunistic infections [7, 21]. CD4+ T – lymphocyte less than 100 cells/µL predisposes an individual to various opportunistic infections, including cryptococcosis, which can disseminate, resulting in cryptococcal meningitis [2, 12].

Our study aims to evaluate the correlation between CD4+ T- lymphocyte level and immune response in the cerebrospinal fluid defined by cell count and chemistry in HIV associated cryptococcal meningitis.

Cryptococcal meningitis is the most common cause of morbidity and mortality in HIV infected populations in sub–Saharan Africa[2, 7]. There is an estimated incidence of 278000 people globally per annum, of which 223000 were reported in sub – Saharan Africa[2, 3].

Clinical features in cryptococcal meningitis are headache, fever, nausea, vomiting and altered level of consciousness[4, 9]. The median duration from symptom onset to presentation is two weeks in the HIV infected population [4, 14]. The diagnosis is confirmed by analysing the cerebrospinal fluid (CSF)[4, 13].

The CSF is used to assess the evidence of brain pathology caused by inflammation, infection and immunological processes [5, 11]. The CSF is almost devoid of inflammatory cells such as macrophages, plasma cells, and eosinophils in blood plasma. The blood-brain barrier regulates the communication between the CSF and systemic circulation, preventing these cells' leakage into the brain [5]. When the integrity of the blood-brain barrier is compromised, as in meningitis, a CSF inflammatory response is activated [5, 14].

Cerebrospinal fluid (CSF) inflammatory response refers to plasma cells, evidenced by polymorphonuclear leukocytes, lymphocytes, and elevated protein in the CSF sample analysis [5, 11, 29]. Cryptococcal meningitis is associated with minimal cerebrospinal fluid inflammation due to the inhibition of recruitment of mononuclear cells, namely, neutrophils by the yeast capsule glucuronoxylomannan (GXM) or binding of Cryptococcus neoformans to CD 18 [8, 22, 27 - 28].

2.2. Methods

2.2.1. Design and setting

The study was a retrospective and descriptive cohort study conducted by the first author (SRM). The first author collected data from the Neurology ward discharge summary file at Charlotte Maxeke Johannesburg Academic Hospital, a tertiary level public hospital in South Africa's Gauteng Province, from January 2015 to March 2020. The study was approved by The University of Witwatersrand Human Research Ethics Committee (clearance number - M200318)

A total of one-hundred and thirty-five discharge summaries of patients admitted with cryptococcal meningitis were retrieved, of which twenty-two were excluded as per the protocols exclusion criteria. A total of one hundred and thirteen were then analysed. Of the excluded twenty-two: eleven had another type of meningitis, namely, tuberculous meningitis, two were HIV seronegative, and nine had high cerebrospinal fluid (CSF) erythrocyte count above 50/cm^3.

2.2.2. Measures

A thorough review of all included discharge summaries was conducted. Where there was any missing data, for example, CD4+ count levels, the National Health Laboratory Services website was used to retrieve the information using the patient's file number (which was not used on the final data sheet as per ethics requirements). A datasheet for each record was completed capturing demographic information. HIV status as either newly diagnosed or known, antiretroviral status (not on treatment, on treatment or defaulted treatment) was noted. Cerebrospinal fluid findings (glucose, protein, polymorphonuclear cells, lymphocytes, erythrocytes, India ink, Cryptococcal antigen, CLAT and fungal culture) were also recorded.

2.3. Statistical Analysis

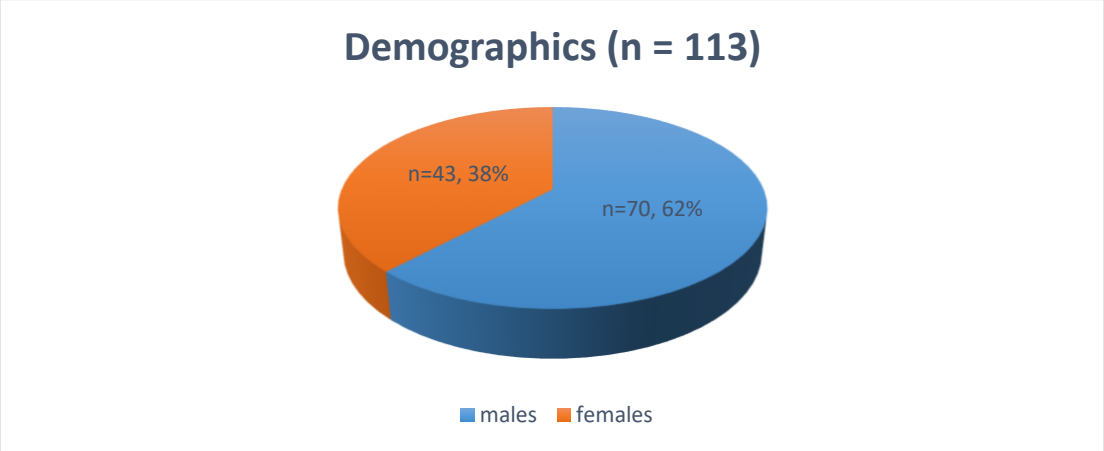
Statistical analysis was performed using Statistica Version 13.5.0.17. Patient demographics, immunological status and cerebrospinal fluid (CSF) parameters were described quantitatively using frequencies and percentages. Correlation between CD4+ and CSF parameters was calculated using non-parametric Spearman Rank Order Correlation statistic where: if R is -1 interprets negative correlation, and R +1 analyses positive correlation. A p-value of < 0.05 was considered statistically significant.

2.4. Results

2.4.1. Demographics and data summary

From January 2015 to March 2020, one-hundred and thirteen (n = 113) patient records with HIV associated cryptococcal meningitis were enrolled in the study. All the patients were of African descent and twenty-two were excluded as per exclusion criteria. The mean age was 37.7, of which 62% (n = 70) were males and 38% (n = 43) females.

Figure 1: Demographics (n = 113)



All enrolled patient records were HIV seropositive, of which 44.25% (n = 50) were newly diagnosed on presentation. Of those whose HIV seropositive status was known [44.25% (n = 50)], 30.1% (n = 34) were on antiretroviral treatment, and 9.7% (n = 11) had defaulted treatment. In the HIV and antiretroviral status groups, 11.50% (n = 13) and 14.2% (n = 16) were missing data, respectively.

Figure 2: HIV seropositive category (n = 113)

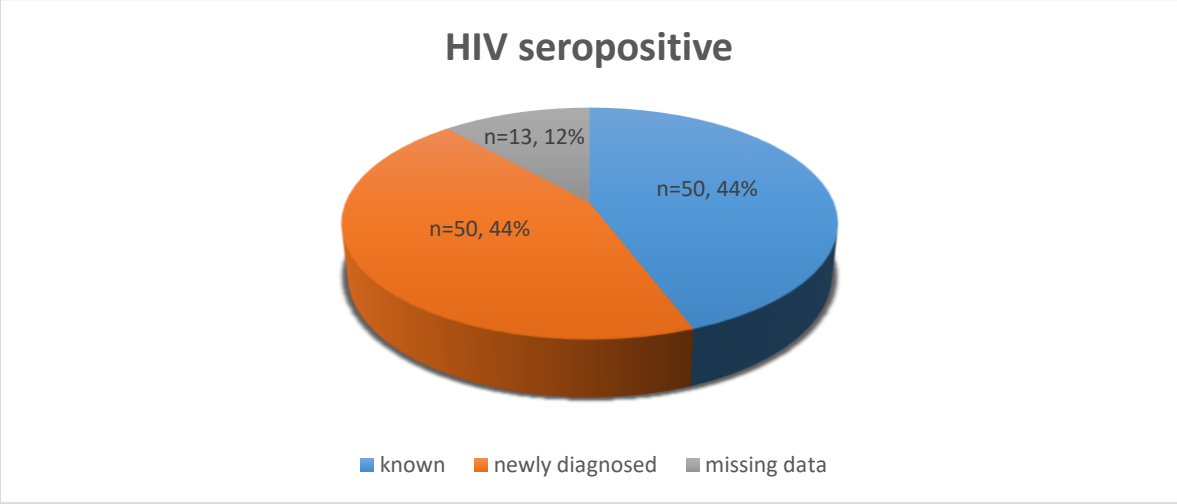
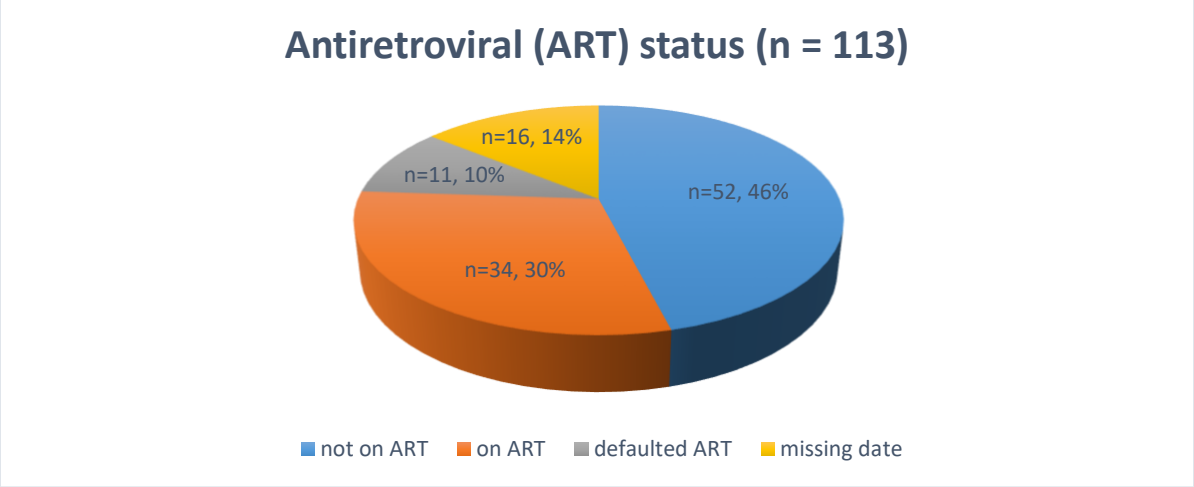
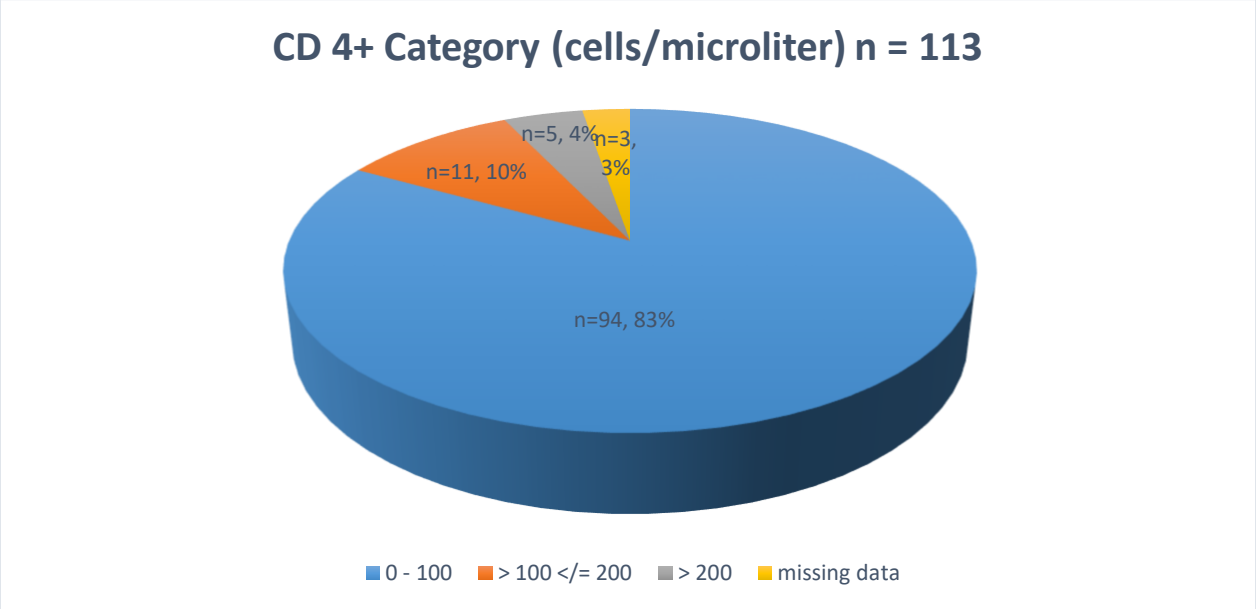


Figure 3: Antiretroviral category (n = 113)



CD 4+ counts were categorised into three groups: 0-100 cells/µL (first group), more than 100 cells/µL and less or equal 200 cells/µL (second group) and more than 200 cells/µL (third group). The first group accounted for 83.2% (n = 94) of the patients, followed by 9.7 % (n = 11) and 4.4% (n = 5) of the second and third groups, respectively, 2.7 % (3) was missing data. The median CD4+ count was 21 cells/µL (IQR: 9.00 to 56.00).

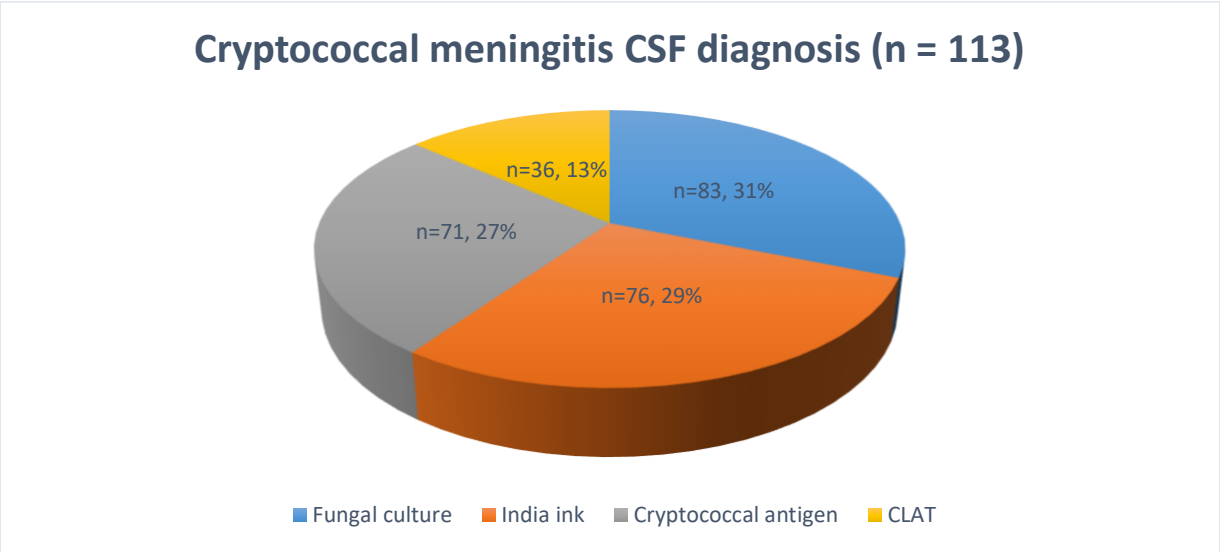
Figure 4: CD 4+ category (n = 113)



Thirty-eight of fifty newly diagnosed, including 86 % (n = 43) already known HIV seropositive [total n =81/100] patients CD4+ counts fell under ≤ 100 cells/µL group. Seven of the fifty newly diagnosed HIV patients had CD4+ counts of more than 100 but less than 200 cells/µL.

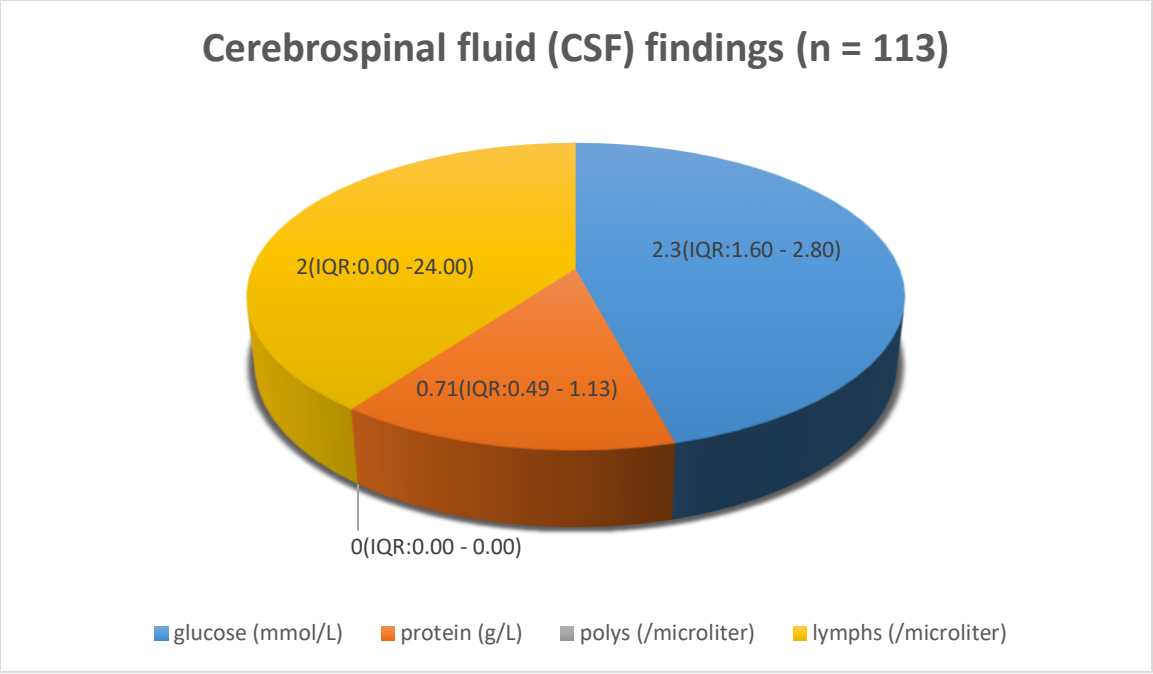
Cryptococcal meningitis was diagnosed through a positive fungal culture which grew Cryptococcal Neoformans in the CSF in 73.4 % (n = 83) of the patients, of which 67.3% (n = 76) had yeast observed on the India ink stain. Cryptococcal antigen assay was positive in 62.8 % (n = 71) of the patients, with CLAT detected in 31.9% (n = 36).

Figure 5: Cryptococcal meningitis CSF diagnosis (n = 113)



In assessing whether CD4+ counts had any influence on the CSF inflammatory response the median CD4+ 21 cells/microliter (µL) [Interquartile range (IQR): 9.00- 56.00]. The CSF findings were as follows: glucose median 2.3 mmol/L (IQR: 1.60 to 2.80), protein median 0.71 g/L (IQR: 0.49 -1.13), polymorphonuclear cells (polys) median 0.00/ µL (IQR: 0.00 – 0.00), lymphocytes (lymphs) median 2.00 /µL (IQR: 0.00 -24.00).

Figure 6: Cerebrospinal fluid findings (n = 113)



As summarised below, the study showed a weak positive correlation between CD4+ counts with the cerebrospinal fluid inflammatory response; however, only protein and lymphocytes were significant.

Table 2: Summary of correlation between CD4+ counts and CSF inflammatory response (n = 113)

Pair of variables	Number (n = 113)	Spearman R	p-value
CD4+ and Glucose	100	0.011	0.907
CD4+ and Protein	109	0.254	0.008
CD4+ and Polys	110	0.174	0.068
CD4+ and Lymphs	110	0.282	0.003

2.5. Discussion

The demographic profile of the patients presenting with cryptococcal meningitis were young patients with a mean age of 37.7 years, of which 62% (n = 70) were predominantly males. These findings are similar to a study conducted in Kwa – Zulu Natal Province by Ross et al., where their average age was 34.4 years, 64.5% being males [16]. The majority of the patients, 83.2% (n = 94/113) in our study, had CD4+ counts less than 100 cells/µL with the median CD4+ count of 21 cells/µL similar to a study done by Liu where the median CD4+ count was 21/mm^3 with CD4+ counts < 200/mm^3 [24]. The finding is in keeping with studies indicating that cryptococcal meningitis is an opportunistic infection in HIV infected patients with CD4+ counts less than 100 cells/µL [15, 19, 22].

However, HIV associated cryptococcal meningitis, though not expected, can occur in patients with higher CD4+ counts equal and more than 100 cells/µL as indicated by Tugume et al. [20]. The study showed that 9% of the patients with CD4 counts of ≥ 100 cells/µL had a median CD4 count of 166 cells/µL (IQR: 115 to 234 cells/µL) [20]. The finding compares to our study where 14.1% of our patients had CD4+ counts of more than 100 cells/µL; however, the difference is that they found that the CSF inflammatory response of these patients was higher than those who had CD4+ counts less than 100 cells/µL [20]. In our study, we found that there was no difference across all the CD4+ count groups

The diagnosis of cryptococcal meningitis was made through positive cryptococcal antigen assay 62.8% (n = 71) in the cerebrospinal fluid and about 73.4% (n = 83) Cryptococcus neoformans

was the offending pathogen in keeping with World Health Organization guidelines for diagnosis [13, 14, 22]. Qu et al. also used a positive Cryptococcal neoformans and cryptococcal antigen assay to diagnose cryptococcal meningitis [28]. Huang et al. used positive fungal culture (Cryptococcus neoformans) or the presence of yeasts on India ink staining to diagnose cryptococcal meningitis [11].

We also observed that antiretroviral treatment in our cohort had no significant impact on the CSF inflammatory response as 68.4% (n = 23/34) of patients who are on ART showed a lack of CSF inflammatory response. These findings are similar to Skripuletz et al., who found no difference in the baseline CSF findings between patients who were and were not on ART with HIV associated cryptococcal meningitis [13]. However, some studies found results contrary to ours, suggesting that patients on treatment mount better immune response possibly due to Immune Reconstitution Inflammatory Response (IRIS) depending on when treatment was started [9, 14, 23, 28]. Boulware et al. defined IRIS as the occurrence of opportunistic infections in HIV seropositive patients with baseline CD4+ count ≤ 200 cells/μL within six months of commencing antiretroviral therapy; in this case, Cryptococcal meningitis IRIS[10].

Our study aims to evaluate the correlation between CD4+ counts and immune response in the cerebrospinal fluid defined by cell count and chemistry in HIV related cryptococcal meningitis. In most studies, the CSF immune response was measured by various inflammatory cytokines [25-28].

Overall we found that the cerebrospinal fluid in HIV patients with cryptococcal meningitis had a lack of cellular response. The absence of polymorphonuclear cells, minimally raised protein, and

average glucose was observed as suggested by CSF findings [see Appendix II page 40] in previous studies, thus reflecting a lack of inflammatory response [17, 18, 22]

We also observed a weakly positive correlation between CD4+ counts and overall cerebrospinal fluid inflammatory response; however, only protein and lymphocytes were significant with p - values of 0.008 and 0.003, respectively. These findings are contrary to the study done by Qu et al., which found a positive correlation between CSF white cell counts (polymorphonuclear cells) with blood CD4+ T lymphocytes count [28]. Similar findings to Qu et al. were also observed by Cecchini et al.; the study concluded that plasma CD4+ T- cells influence CSF cellular response (CSF leukocytes) in patients with cryptococcal meningitis [6].

2.6. Limitations

This cohort was a small study limited to a single tertiary hospital with some missing data; therefore, it cannot reference the general population. It is also a retrospective study.

2.7. Conclusion

Despite readily available antiretroviral treatment, cryptococcal meningitis remains the most common central nervous system opportunistic infection in HIV seropositive patients. This observation is possibly due to the occurrence of IRIS, which is challenging to prevent. There was a partial correlation between CD4+ count and CSF inflammatory response. Antiretroviral treatment did not have any significant effect on the CSF inflammatory response. A more extensive prospective study will need to be conducted to be correlated with our findings.

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3. APPENDICES

APPENDIX I: DATA COLLECTION SHEET

Study number _____

HIV status

Unknown ☐

Known ☐

Newly diagnosed ☐

CD 4 count at presentation ☐

Viral load at presentation ☐

Age ☐

Sex ☐

ARV status

Not on treatment ☐

On treatment ☐

Defaulted treatment ☐

Cerebrospinal fluid findings

Appearance	
Protein	
Glucose	
Polymorphs	
Lymphocytes	
Erythrocytes	
India Ink	
Cryptococcal Antigen	
CLAT	
Fungal culture	
GeneXpert	
Gram stain	

IMAGING FINDINGS WHERE APPLICABLE (CT OR MRI)

APPENDIX II: CEREBROSPINAL FLUID FINDINGS IN CRYPTOCOCCAL MENINGITIS [17].

Parameter	Value/ result
Appearance	Clear
Pressure (cm H2O)	>25
Protein (mg/ dL)	40 – 150
Glucose	30 – 70
Polymorphs	Nil or not more than 5
Lymphocytes	>50%
Erythrocytes	Nil
Cryptococcal antigen	Positive
India ink	Yeasts observed
Cryptococcal latex antigen test	Positive

APPENDIX III. ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Sandra Refiloe Mashitela

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M200318

NAME: (Principal Investigator)	Dr Sandra Refiloe Mashitela
DEPARTMENT:	Neurosciences Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Correlation between CD4 count and cerebrospinal fluid inflammatory response in Human Immunodeficiency Virus infected patients with crytococcal meningitis
DATE CONSIDERED:	27/03/2020
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Girish Modi
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	09/06/2020

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **March** and will therefore be due in the month of **March** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

15/06/2020
Date

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