Assessment of CD4, viral load and prevalence of opportunistic infections during Antiretroviral Therapy initiation preceding and during the Test and Treat era in patients visiting a tertiary hospital in KwaZulu-Natal

By

Dr Zanele Ruth Moya

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As the candidate's supervisor, I have/have not approved this thesis for submission.

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I, Zanele Ruth Moya, declare:

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DEDICATION

To my love: my late mother, Abelina Mbatha, ngiyaziqhenya ngawe.

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OVERVIEW OF THE STUDY

Background

The cluster of differentiation 4 (CD4) cell count is used as a marker of immune status and, in the past, was used as a guide for initiating antiretroviral therapy (ART) in people living with HIV (PLHIV) to reduce the risk of opportunistic infections (OIs) associated with immunodeficiency. The literature suggests that higher CD4 counts are correlated with a lower risk of OIs. The province of Kwa-Zulu Natal (KZN) has one of the highest burdens of OIs, particularly tuberculosis (TB), in South Africa. Despite changes in guidelines, many patients present to healthcare facilities already suffering from acquired immunodeficiency syndrome (AIDS)-defining illnesses. This study aimed to determine whether the changing policy to increase the level of CD4 count for initiating ART was aligned with higher levels at which PLHIV were initiated on ART. The study examined whether ART was initiated before a decrease in CD4 cell count and/or the onset of OIs in PLHIV attending King Edward VIII Hospital in Durban, South Africa.

Aim

The study aimed to assess the alignment of the index CD4 count with the criteria for initiating ART at two different time points and the presence or absence of OIs on patient outcomes at twelve months.

Method

This study employed a retrospective cohort design to investigate the index CD4 cell count, viral load and the presence or absence of OIs in patients who initiated ART at King Edward VIII Hospital. Convenience sampling was utilised. Cohort 1 was comprised of 112 patients and cohort 2 of 129 patients. Patients presenting during two distinct periods were selected and designated as Cohort 1 and Cohort 2. Cohort 1 included patients that presented from January 1, 2014, to December 31, 2014, characterised by ART initiation recommendations for patients with CD4 cell counts $\leq$ 350 cells/mm^3. Cohort 2 included patients that presented from January 1, 2019, to

December 31, 2019, reflecting the current Test and treat era, where ART initiation was recommended regardless of CD4 cell count.

Results

The total study population was (n=241) with 112 patients in cohort 1 and 129 in cohort 2. The majority of patients in both cohort 1 & 2 (36.6% and 51.9%) were aged between 26 and 35 years, respectively with a higher proportion of females than males (58.9% in cohort 1 & 64.1% in Cohorts 2). At. ART initiation, the median CD4 cell was 170 cells/mm3 (IQR: 85.5 – 287) in cohort 1 and 243 cells/mm^3 (IQR: 120 – 411) in cohort 2. Tuberculosis was observed as the predominant OI with highest prevalence of TB infections in the group with CD4 cell count <200mm^3 in both cohort 1 (30 cases 26.8%) and cohort 2 (36 cases 27.9%), p=0.039. At one year follow-up, virological suppression (viral load <400copies/ml) was achieved in only 77.7% and 84.7% of Cohort 1 & 2 patients respectively.

Conclusion

This study provided compelling evidence that a significant proportion of patients were initiated on ART when their CD4 count falls below critical threshold. The median CD4 cell was 170 cells/mm^3 (IQR: 85.5 – 287) in cohort 1 when threshold for initiation was set at CD4 cell ≤ 350 cells/mm^3. In cohort 2 when treatment was initiated regardless of CD4, the median CD4 cell was 243 cells/mm^3 (IQR: 120 – 411). This was associated with a prevalence of Tuberculosis as the dominant OI, in cohort 1 (26.8%) and cohort 2 (27.9%). Viral suppression of 77.7% and 84.7% in Cohort 1 & 2 at one year, respectively. Which were below 90% target in the UNAIDS 90-90-90 criteria by 2020 set to end HIV as a public health threat by the year 2030.

Contributions

This study identified a delay in ART uptake, and a thorough reassessment is required to identify contributing factors and develop tailored interventions.

Keywords: HIV infection, antiretroviral therapy, test and treat era, opportunistic infections, tuberculosis, cluster of differentiation 4

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PART 1: LITERATURE REVIEW

Introduction

This section provides a comprehensive examination of scholarly works on the frequency of human immunodeficiency virus (HIV) infection and concurrent infections, both at local and global levels. Additionally, it explores the research that has played a pivotal role in shaping the creation of protocols for effectively managing HIV infection. The principal aim of this investigation was to evaluate the cluster of differentiation 4 (CD4) count, viral load and occurrence of OIs in two distinct cohorts across different time intervals.

Background

Acquired immunodeficiency syndrome (AIDS) emerged in the United States (US) in 1981 when a group of individuals experienced increased mortality rates attributed to uncommon OIs and rare malignancies (1). Subsequently, it was determined that the syndrome was caused by HIV. South Africa reported its first AIDS-related death in late December 1981 (1, 2). According to the 2021 Global HIV and AIDS statistics, the HIV epidemic has claimed the lives of over 40 million people due to related illnesses. Annually, there are 1.5 million new HIV infections. A total of 28.7 million individuals are on antiretroviral therapy (ART) (3). In South Africa, the prevalence of HIV among adults aged 15 and above is 18.3%, affecting 7.3 million individuals, with a higher prevalence among women (24.5%) compared to men (12.1%) (3). At present, 75% of HIV-infected individuals in South Africa are receiving treatment, and 67% of these individuals have achieved virological suppression (3).

CD4 cell count, viral load and OIs

Various markers have been devised to evaluate immune function in HIV-infected individuals, including CD 4 count, CD4 percentage, quantitative HIV-1 RNA (viral load) and p-24 antigenaemia (4). Among these markers, viral load is considered a more dependable indicator of disease progression to AIDS and mortality compared to CD4 cell count. However, during the acute phase of illness, viral load may be elevated. Therefore, the CD4 count remains the most valuable test for assessing the risk of opportunistic infections (OIs) (4).

An immune response to antiretroviral therapy is associated with an increase in CD4 T lymphocyte count by approximately 100 to 250 cells/mm^3, leading to a reduction in the risk of OIs, particularly at higher CD4 counts (5). Jung and Paauw conducted a review of studies examining the correlation between CD4 count and specific diseases and found that different disease profiles were observed at different CD4 count levels (4). Respiratory symptoms, such as cough and chest pain, were found to be common among HIV-infected patients. When CD4 counts exceeded 500 cells/mm^3, patients commonly experienced community-acquired pneumonia, viral infections and sinusitis. However, once CD4 counts declined below 500 cells/mm^3, infections such as tuberculosis arose (4).

Table 1 below indicates the CD4 counts below which selected diseases commonly occur.

Table 1: CD4 thresholds for commonly associated diseases

Disease Stage	CD4 Count	Respiratory Disease	Dermatologic Disease	CNS Disease	Systemic Disease
Early	>500	Bacterial pneumonia & sinusitis*	Oral hairy leukoplakia Vaginal candidiasis Kaposi's sarcoma	HIV meningitis	
Intermediate	<500	Tuberculosis	Thrush*		
	<400		Herpes zoster*		
	<300		Herpes simplex* Eosinophilic folliculitis		
Advanced	<250	PCP			
	<200			HIV dementia	Non-Hodgkin's lymphoma
	<150	Coccidioidomycosis			
	<100		Bacillary angiomatosis Molluscum contagiosum Esophageal candidiasis	Cryptococcosis Progressive multifocal leukoencephalopathy Toxoplasma encephalitis	
	<75				Disseminated *Mycobacterium avium* complex
Very advanced	<50	Pseudomonas pneumonia	Large, nonhealing HSV perirectal ulcers	CMV retinitis	
		Histoplasmosis Aspergillosis		CNS lymphoma	
	<10		Giant mollusca		

**Diseases increase in incidence and severity as CD4 counts decline.*

Retrieved from Geginat J, Paroni M, Maglie S, Alfen JS, Kastirr I, Gruarin P, De Simone M, Pagani M, Abrignani S. Plasticity of human CD4 T cell subsets. Frontiers in immunology. 2014 Dec 16; 5:630.

Table 1 above demonstrates that as CD4 cell counts decrease below 200 cells/mm^3, there is an elevated risk of developing conditions such as pneumocystis jiroveci pneumonia (PJP). Additionally, the prevalence of other OIs, such as candida, increases as the CD4 cell count falls below 100 cells/mm^3. Progressive multifocal leukoencephalopathy (PML), toxoplasmosis,

cryptococcal meningitis, cytomegalovirus (CMV) retinitis and central nervous system lymphoma all exhibit a higher incidence with CD4 counts below 50 cells/mm^3 (4).

Antiretroviral therapy background

The initial treatment approaches for HIV/AIDS proved to be ineffective. In 1987, clinical trials commenced with a focus on monotherapy, using zidovudine as the first drug (6), 7). These medications were administered weekly or on alternate days to mitigate toxicity. However, the response was short-lived and did not demonstrate any survival benefits (8), 9).

The Italy, Netherlands, Canada and Australia (INCA) study was the first to show that triple combination therapy had a more substantial and sustained impact on reducing HIV viral load (10). These new therapeutic options transformed the clinical landscape of HIV infection from a subacute disease to a chronic one (11). These drugs were categorised into distinct classes based on their molecular mechanisms and resistance profiles, including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, protease inhibitors (PIs), fusion inhibitors (FIs) and co-receptor antagonists (CRAs) (11).

The side effects of NRTI therapy are primarily due to the drugs' inhibition of host mitochondrial DNA polymerase, leading to lactic acidosis (13). Other side effects include subcutaneous lipoatrophy, peripheral neuropathy and pancreatitis, which are predominantly associated with stavudine. The main risks associated with the use of NNRTIs are skin reactions and hepatitis, which often occur early during therapy. Progressive accumulation of visceral fat and metabolic complications are the major complications of PI therapy (14, 15).

Despite significant progress in treatment development, the optimal timing for initiating ART remains in question. The potential benefits of early therapy include immune system preservation, reduced risk of HIV transmission and an earlier suppression of viral replication (12). However, the risks of early therapy include adverse effects of drugs on quality of life, leading to decreased adherence (12).

ART guidelines

The HIV guidelines of the South African Department of Health (DoH) align with the recommendations of the World Health Organisation (WHO). The initial DoH guidelines, established in 2004, advocated for initiating treatment when a patient's CD4 cell count was below 200 cells/mm^3 (16, 17). Clinical benefits were observed when initiating therapy before the CD4 cell count declined below 200 cells/mm^3. However, a specific CD4 cell count threshold above 200 cells/mm^3 for treatment initiation and its associated survival benefits had not yet been determined (18). It was believed that drug exposure beyond this target could lead to negative side effects (19), which led to the adoption of the threshold of 200 cells/mm^3 in the first guideline.

The randomised Comprehensive International Programme of Research on AIDS (CIPRA) HT 001 study demonstrated that delaying treatment in patients with a CD4 cell count between 200 and 350 cells/mm^3 resulted in long-term immune dysfunction and a persistent increase in tuberculosis (TB) infection (20). As a result, a policy change occurred, shifting the threshold for initiation of therapy to a CD4 cell count of 350 cells/mm^3 or less (21). In 2015, the WHO revised its ART initiation recommendations, advising the initiation of ART for all adults living with HIV regardless of CD4 cell count (22). This update was supported by evidence from several trials:

- The trial of early antiretroviral and isoniazid prevention therapy in Africa (TEMPRANO) demonstrated a lower risk of death or HIV-related illness with earlier ART initiation compared to deferred treatment (23).
- The International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) strategic timing of antiretroviral treatment (START) study showed a significant reduction in mortality and morbidity in patients initiated on ART with a CD4 cell count greater than 500 cells/mm^3, with benefits outweighing the risks of drug toxicity (24).
- The HIV prevention trials network (HPTN 052) revealed that early initiation of ART reduced sexual transmission of HIV in serodiscordant couples, highlighting the substantial benefit of test and treat (T&T) strategies in preventing transmission (25, 26).

In 2016, the United States National Institute of Health (NHI) and the WHO implemented universal T&T policies aimed at early detection of HIV infection, leading to lower HIV transmission rates

at the community level (27, 28). Against the backdrop of hospital admissions associated with HIVrelated complications in the high-burden setting of KwaZulu-Natal (KZN), South Africa, this study aimed to determine if patients were indeed receiving treatment based on the recommended CD4 cell count threshold.

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PART 2: SUBMISSION-READY MANUSCRIPT

Assessment of CD4, viral load and prevalence of opportunistic infections during Antiretroviral Therapy initiation preceding and during the Test and Treat era in patients visiting a tertiary hospital in KwaZulu-Natal

Moya ZR[1], Pillay S[2], Magula NP[3]

[1] Moya ZR*, MBChB, FCP (SA), Department of Internal Medicine, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal

[2] Pillay S, Senior PhD, PhD, FCP (SA), MMed, MBChB), Department of Internal Medicine, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal. King Edward VIII Hospital, Durban

[3] Magula NP, BSc, MBChB, MSc, FCP (SA), PhD, Department of Internal Medicine, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal

Corresponding author: zaneleruthmoya@gmail.com*

Background

The cluster of differentiation 4 (CD4) cell count is used as a marker of immune status and, in the past, was used as a guide for initiating antiretroviral therapy (ART) in people living with HIV (PLHIV) to reduce the risk of opportunistic infections (OIs) associated with immunodeficiency. The literature suggests that higher CD4 counts are correlated with a lower risk of OIs. The province of Kwa-Zulu Natal (KZN) has one of the highest burdens of OIs, particularly tuberculosis (TB), in South Africa. Despite changes in guidelines, many patients present to healthcare facilities already suffering from acquired immunodeficiency syndrome (AIDS)-defining illnesses. This study aimed to determine whether the changing policy to increase the level of CD4 count for initiating ART was aligned with higher levels at which PLHIV were initiated on ART. To examine whether ART was initiated before a decrease in CD4 cell count and/or the onset of OIs in PLHIV attending King Edward VIII Hospital in Durban, South Africa.

Aim

The study aimed to assess the alignment of the index CD4 count with the criteria for initiating ART at two different time points and the presence or absence of OIs on patient outcomes at twelve months.

Methods

This study employed a retrospective cohort design to investigate the index CD4 cell count, viral load and the presence or absence of OIs in patients who initiated ART at King Edward VIII Hospital. Convenience sampling was utilised. Cohort 1 was comprised of 112 patients and cohort 2 was comprised of 129 patients. Patients presenting during two distinct periods were selected and designated as Cohort 1 and Cohort 2. Cohort 1 included patients that presented from January 1, 2014, to December 31, 2014, characterised by ART initiation recommendations for patients with CD4 cell counts ≤ 350 cells/mm^3. Cohort 2 included patients that presented from January 1, 2019, to December 31, 2019, reflecting the current Test and treat era, where ART initiation was recommended regardless of CD4 cell count.

Results

The total study population was (n=241) with 112 patients in cohort 1 and 129 in cohort 2. The majority of patients in both cohort 1 & 2 (36.6% and 51.9%) were aged between 26 and 35 years. respectively with a higher proportion of females than males (58.9% & 64.1% in Cohorts 1 & 2 At ART initiation, the median CD4 cell was 170 cells/mm3 (IQR: 85.5 – 287) in cohort 1 and 243 cells/mm^3 (IQR: 120 – 411) in cohort 2. Tuberculosis was observed as the predominant OI with highest prevalence of TB infections in the group with CD4 cell count <200mm^3 in both cohort 1 (30 cases 26.8%) and cohort 2 (36 cases 27.9%), p=0.039. At one year follow-up, virological suppression (viral load <400copies/ml) was achieved in only 77.7% and 84.7% of Cohort 1 & 2 patients respectively.

Conclusion

This study provided compelling evidence that a significant proportion of patients were initiated on ART when their CD4 count falls below critical threshold. The median CD4 cell was 170 cells/mm^3 (IQR: 85.5 – 287) in cohort 1 when threshold for initiation was set at CD4 cell ≤ 350 cells/mm^3.

In cohort 2 when treatment was initiated regardless of CD4, the median CD4 cell was 243 cells/mm^3 (IQR: 120 – 411). This was associated with a prevalence of tuberculosis as the dominant OI, in cohort 1 (26.8%) and cohort 2 (27.9%). Viral suppression of 77.7% and 84.7% in Cohort 1 & 2 at one year, respectively, which were below 90% target in the UNAIDS 90-90-90 criteria by 2020 set to end HIV as a public health threat by the year 2030.

Contributions

This study identified a delay in ART uptake, and a thorough reassessment is required to identify contributing factors and develop tailored interventions.

Keywords: HIV infection, antiretroviral therapy, test and treat era, opportunistic infections, tuberculosis, cluster of differentiation 4

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Moya ZR[1], Pillay S[2], Magula NP[3]

[1] Moya ZR*, MBChB, FCP (SA), Department of Internal Medicine, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal

[2] Pillay S, Senior PhD, PhD, FCP (SA), MMed, MBChB), Department of Internal Medicine, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal. King Edward VIII Hospital, Durban

[3] Magula NP, BSc, MBChB, MSc, FCP (SA), PhD, Department of Internal Medicine, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal

Corresponding author*: *zaneleruthmoya@gmail.com

Introduction

AIDS was first identified in the United States (US) in 1981 as a syndrome characterised by increased mortality from unusual opportunistic infections and rare malignancies (1, 2). It was later discovered that the human immunodeficiency virus (HIV) is responsible for causing the acquired immunodeficiency syndrome (AIDS). South Africa documented its first case and AIDS-related death within the same year (2). According to the Global HIV and AIDS statistics of 2021, there are currently 38.4 million people living with HIV, and 28.7 million have been initiated on antiretroviral treatment (ART) (3). In South Africa, 7.3 million adults aged 15 years and older are living with HIV, with a prevalence of 18.3%. There is a gender difference in HIV prevalence, with a higher prevalence among women than men (24.5% vs 12.1%). Three-quarters (75%) of HIVinfected individuals in South Africa are on treatment, and 67% of these patients are virologically suppressed (3).

The cluster of differentiation 4 (CD4) count, CD4 percentage, quantitative human immunodeficiency virus type 1 ribonucleic acid (HIV-1 RNA) (viral load) and HIV p-24 antigenaemia are all tests that have been developed to quantify immune function in people living with HIV. While the viral load is a reliable marker for determining disease progression and risk of death, it can vary during acute illness. The CD4 count, however, is the most valuable test for assessing the risk of developing opportunistic infections (OIs), particularly as the count declines below 200 cells/mm^3. Multiple studies have shown that as the CD4 count decreases, the risk of developing opportunistic infections (OIs) increases (4).

With the emergence of the HIV epidemic, studies were conducted to explore drug treatment options. Early clinical trials examined the use of zidovudine monotherapy to reduce toxicity (5, 6). However, the success of this approach was short-lived and did not yield any survival benefit (7, 8). Subsequently, the Italy, Netherlands, Canada and Australia (INCA) study demonstrated greater effectiveness of triple combination therapy in continuously reducing HIV viral load (9). These new therapeutic options transformed the clinical profile of HIV from a subacute to a chronic disease (10). An immunological response to ART is linked with an increased CD4 T lymphocyte count of 100 to 250 cells/mm^3, which in turn lowers the risk of developing OIs at higher CD4 levels (11).

The Food and Drug Administration (FDA) approved drugs available for the treatment of HIV infections. They were categorised into distinct classes based on their molecular mechanism and resistance profiles and included nucleoside reverse transcriptase inhibitors (NRTIs), non–nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors (INSTIs), protease inhibitors (PIs), fusion inhibitors (FIS) and co-receptor antagonists (CRAs) (10).

The side effect profile of NRTI therapy is a consequence of the capacity of these drugs to inhibit host mitochondrial DNA polymerase, causing lactic acidosis, subcutaneous lipoatrophy, peripheral neuropathy and pancreatitis. These side effects are frequently seen with the use of stavudine (12). The risks associated with the use of NNRTIs predominantly include skin reactions and hepatitis, usually occurring early during therapy.

The major complications of PI therapy are a progressive accumulation of visceral fat and metabolic disturbances (13, 14). The timing of ART initiation remains a point of contention in HIV management guidelines. Early initiation has been associated with the preservation of the immune

system, decreased risk of HIV transmission and an earlier suppression of viral replication. However, early initiation also carries risks, such as the adverse effects of drugs on quality of life, leading to decreased adherence (15).

The South African Department of Health (DoH) guidelines follow the recommendations of the World Health Organisation (WHO). The first DoH guidelines of 2004 adopted treatment initiation at a CD4 count of less than 200 cells/mm^3 (16, 17). Initiating therapy before the CD4 cell count declined below 200 cells/mm^3 was shown to provide clinical benefit; however, the precise threshold above 200 cells/mm^3 at which treatment should be initiated had not been definitively identified. Furthermore, the survival outcomes for patients who initiated treatment at higher CD4 cell counts had not been firmly established (18), and it was believed that exceeding this target could lead to potential negative side effects associated with drug exposure (19).

As a result, the initial guidelines recommended adhering to the specified CD4 cell count threshold of 200 cells/mm^3. The Comprehensive Program for Research in AIDS (CIPRA HT 001) randomised study showed that delaying treatment in patients with CD4 cell counts between 200 and 350 cells/mm^3 resulted in long-term immune dysfunction and a persistent increase in tuberculosis (TB) infection (20). This led to a policy change to shift the threshold for initiation of therapy to a CD4 cell count of 350 cells/mm^3 or below (21).

In 2015, the WHO revised ART initiation recommendations to the initiation of all adults living with HIV at any CD4 cell count, based on supporting evidence from multiple trials (22). The Trial of Early Antiretroviral and Isoniazid Prevention Therapy in Africa (TEMPRANO) showed a lower risk of death or HIV-related illness with earlier ART initiation compared to deferred treatment (23). The International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) Strategic Timing of Antiretroviral Treatment (START) study showed a significant reduction in mortality and morbidity in patients initiated on ART when their CD4 cell counts were greater than 500 cells/mm^3, with benefits outweighing the risks of drug toxicity (24). The HIV Prevention Trial Network 052 (HPTN 052) demonstrated that early initiation of ART reduced sexual transmission of HIV in serodiscordant couples, revealing that the largest benefit in testing and treating was in the prevention of transmission (25, 26).

The United States National Institute of Health (NIH) and the WHO implemented the universal test and treat (T&T) strategy in 2016, with the aim of early detection of HIV infection to lower HIV transmission at the community level (27, 28). In September 2016, South Africa adopted the T&T strategy.

The primary objective of the study was to examine whether patients received treatment per the recommended CD4 cell count threshold. This investigation was prompted by the observation of increased hospital admissions related to complications associated with HIV in KwaZulu-Natal (KZN), South Africa, a region of particularly high HIV burden.

Methods

The study planned to assess the demographic characteristics of two distinct cohorts and their CD4 cell count at treatment initiation based on the recommended guidelines, as well as the incidence of OIs and therapeutic outcomes after twelve months of antiretroviral therapy.

Study design

A retrospective quantitative cohort study design was utilised. Patient records were the primary tool from which data were collected.

Study setting and population

The King Edward VIII Hospital HIV (KEH HIV) clinic is based in the eThekwini District of the province of KZN. All patients who met the inclusion criteria were included in the study.

Sampling strategy

The KEH HIV clinic serves approximately two thousand eight hundred and ten (2810) patients monthly, of which there are around ten (10) new patients per month. Sample size estimates were calculated for an independent t-test with alpha errors at 0.05 and powers of 0.80 and 0.90. It was calculated using Gpower®, which suggested an overall sample size of 260. Therefore, approximately 130 files were required to be reviewed from each of the periods mentioned below. This was based on a convenience sampling technique. Clinical charts from January to December of the selected years that met the inclusion criteria were reviewed.

Inclusion criteria

All adult patients 18 years and older initiating ART at the KEH HIV clinic during two periods:

1. 1st January 2014 - 31st December 2014: A period when the South African national HIV guidelines indicated that ART should only be initiated when the patient CD4 cell count was ≤ 350 cells/$mm^{3.}$
2. 1st January 2019 - 31st December 2019: A period when the South African national HIV guidelines indicated that ART should be initiated once an HIV test was positive irrespective of CD4 count. This period fell within the so-called T&T era

Exclusion criteria

1. Patients previously or currently on ART
2. Patients less than 18 years of age
3. Patients that were initiated on ART outside these study periods

Clinical outcomes were measured by viral load suppression and CD4 cell count at twelve months. Virological suppression was defined as a viral load of fewer than 400 copies/ml, measured 16 weeks after initiation (29).

Statistical analysis

The data collected were analysed with Statistical Package for the Social Sciences (SPSS) version 28.0 (IBM Corp, Armonk, NY, USA) and Stata version 16.0 (StataCorp, College Station, TX, USA). Categorical data were presented as frequencies and percentages and compared utilising chisquare tests (goodness-of-fit for single variables and test-of-independence for bivariate data). Descriptive statistics (mean and standard deviation) were used to describe the continuous data that were collected. Continuous variable group means were compared using the Kruskal-Wallis test. A p-value of less than 0.05 was regarded as statistically significant. All p-values were chi-square values unless otherwise specified (30).

Results

The total study cohort comprised 241 patients (112 vs 129 in Cohort 1 and 2 respectively). Most patients were between the ages of 26 and 35 years (36.6% in cohort 1 vs 51.9% in cohort 2), $p<0.001$). The studied patients were predominantly female, with the racial distribution being primarily black African as shown in Table 2 below, which presents the sociodemographic profile of the study population.

Table 2: Socio-demographic details of the study population (n = 241)

Demographic Variable		Cohort 1 n=112		p-value	Cohort 2 n=129		p-value	p-value for both cohorts
		Count	%		Count	%		
Age (years)	18 - 25	4	3.6%	< 0.001	19	14.7%	< 0.001	< 0.001
	26 – 35	41	36.6%		67	51.9%		
	36 – 45	34	30.4%		23	17.8%		
	46 – 55	23	20.5%		12	9.3%		
	> 55	10	8.9%		8	6.2%		
Sex	Male	46	41.1%	0.059	46	35.9%	0.001	0.428
	Female	66	58.9%		82	64.1%		
Race	Black	110	98.2%	< 0.00	122	94.6%	< 0.001	0.463
	Indian	1	0.9%		4	3.1%		
	Coloured	1	0.9%		1	0.8%		
	White	0	0.0%		2	1.6%		

Table 3 below delineates the differences in median CD4 cell counts between the two cohorts at two time points: at the baseline and after one year. At baseline, Cohort 1 had a lower median CD4 cell count of 170 cells/mm^3 (interquartile range [IQR]: 85.5 – 287) compared to Cohort 2 with a median count of 243 cells/mm^3 (IQR: 120 – 411). This difference was statistically significant with a p-value less than 0.001 within both cohorts. After one year, CD4 cell counts increased to 332.50 cells/mm^3 (IQR: 228 – 517.5) and 372 cells/mm^3 (IQR: 222 – 579) in Cohort 1 and Cohort 2 respectively, but the difference between the two cohorts was not statistically significant (p=0.680), as shown in Table 3 below.

3: The median CD4 count at baseline and one-year in each cohort

Variable	Cohort 1: n=112	p-value	Cohort 2: n=129	p-value	p-value for both cohorts
	Median (IQR)		Median (IQR)		
CD4 (cells/mm^3) at baseline	170 (85.5 - 287. 0)		243.0 (120-411)		0.001

Table

CD4 (cells/mm^3) at 1 year	332.50 (228.0-517)	<0.001	372.0 (222.0-579.0)	<0.001	0.680

Table 4 below presents the frequency distribution of CD4 cell counts at the baseline and the oneyear follow-up for Cohort 1 and Cohort 2, along with the corresponding p values for both cohorts. In Cohort 1, at the baseline, 57.1% of patients had a CD4 cell count less than 200 cells/mm^3, which was significantly higher than the proportion of patients with CD4 cell counts between 200-350 cells/mm^3 (36.6%) and those with CD4 cell counts above 350 cells/mm^3 (6.3%) ($p<0.001$). After one year of treatment, there was a notable improvement, with 45.5% of patients achieving CD4 cell counts above 350 cells/mm^3, followed by those with CD4 cell counts between 200-350 cells/mm^3 (33.0%). The proportion of patients with CD4 cell counts less than 200 cells/mm^3 decreased to 21.4% ($p=0.008$). The differences observed between the CD4 categories in Cohort 1 were statistically significant ($p<0.001$). In Cohort 2, at baseline, 41.9% of patients had a CD4 cell count less than 200 cells/mm^3, which was higher than the proportion of patients with CD4 cell counts between 200-350 cells/mm^3 (23.3%) and those with CD4 cell counts above 350 cells/mm^3 (34.9%) ($p=0.033$). After one year of treatment, most patients achieved CD4 cell counts above 350 cells/mm^3 (55.8%), followed by those less than 200 cells/mm^3 (24.8%). A smaller proportion of patients had CD4 cell counts between 200-350 cells/mm^3 (19.4%) ($p<0.001$).

The observed differences between the CD4 categories in Cohort 2 were statistically significant ($p=0.055$). These findings indicated that both cohorts showed significant improvements in CD4 cell counts after one year of treatment, with a higher proportion of patients achieving optimal CD4 levels above 350 cells/mm^3. However, as shown in Table 4 below, the distribution patterns and proportions of CD4 categories at baseline and the one-year follow-up differed between the two cohorts, suggesting a change in CD4 cell count distribution and response to treatment over time.

4: Frequency table describing CD4 counts at baseline and 1 year

CD4 category cells/mm^3	**CD4 at Index presentation**		**p-value for both cohorts**	**CD4 at 1 year**		**p-value for both cohorts**
	Cohort 1 n=112	**Cohort 2 n=129**		**Cohort 1 n=112**	**Cohort 2 n=129**	

Table

	Count (%)	p-value	Count (%)	p-value		Count (%)	p-value	Count (%)	p-value	
<200	64 57.1%	<0.001	54 41.9%	0.033	<0.001	24 21.4%	0.008	32 24.8%	<0.001	0.055
200-350	41 36.6%		30 23.3%			37 33.0%		25 19.4%		
>350	7 6.3%		45 34.9%			51 45.5%		72 55.8%		

OIs that were present at diagnosis and during the period under review were recorded. TB (both pulmonary and disseminated) was found to be the predominant OI, with the highest prevalence of TB infections in the group with CD4 cell counts <200mm^3 in both Cohort 1 (29 cases) and Cohort 2 (36 cases) (p=0.039). Other OIs occurred at a lesser frequency (recurrent severe bacterial pneumonia [3 cases], candidiasis [4 cases], HIV-associated malignancy such as lymphoma and cervical cancer [4 cases]), as indicated in Table 5 below.

5: The frequency of opportunistic infections and HIV-associated diseases in each cohort

Variables	**Cohort 1**			**Cohort 2**			**p-value**
	CD4 cells/mm^3			**CD4 cells/mm^3**			
	<200	**200-350**	**>350**	**<200**	**200-350**	**>350**	
Tuberculosis	23	7	0	26	4	6	0.039
Recurrent severe bacterial pneumonia	1	0	0	2	0	0	-
Candidiasis	2	0	1	1	0	0	-

Table

HIV-associated malignancy***	1	1	1	1	0	0	0.513
Pneumocystis jiroveci pneumonia	1	0	0	0	0	0	-
Herpes zoster	1	1	0	1	1	2	0.472
Other**	6	1	1	5	0	2	0.506
None	29	32	4	21	25	35	< 0.001

**Syphilis, cryptococcal meningitis

***Cervical cancer, Kaposi sarcoma, non-Hodgkin's lymphoma

Table 6 below demonstrates that 77.7% of patients in Cohort 1 and 81.4% of patients in Cohort 2 achieved virological suppression at one year post-ART initiation. This was statistically significant within each cohort ($p<0.001$); however, there was no significant difference between the two cohorts ($p=0.475$).

6: Outcomes in viral load suppression at 1 year within each cohort

Viral Load at 12 months	**Cohort 1 n = 112**	**P value**	**Cohort 2 n= 129**	**P value**	**Accumulative Total n=241**	**P value**
Suppressed	87 (77.7%)	p <0.001	105 (81.4%)	p<0.001	192 (79.7%)	p = 0.475

Table

Non suppressed	25 (22.3%)		24 (18.6%)		49 (20.3%)	
Suppressed viral load: <400copies/ml at 6 months Not suppressed viral load: >400 copies/ml at 6 months						

Discussion

This study presented an examination of individuals who commenced ART for HIV infection at distinct target CD4 levels, as suggested by the prevailing HIV guidelines during that period. The study scrutinised the initial CD4 cell counts of these patients upon their presentation and subsequently assessed their CD4 levels again after one year. Additionally, the study assessed the prevalence of virological suppression among these patients at the one-year mark. Furthermore, the investigation characterised the profile of OIs observed in this cohort of HIV-infected individuals.

South Africa continues to grapple with a substantial HIV epidemic, with KZN emerging as one of the provinces facing a disproportionate burden of disease, particularly among the younger population (31). This study revealed that most patients in both Cohort 1 and Cohort 2 who initiated therapy were between 26 and 35 years old, constituting 36.6% and 51.9% of the respective cohorts. This observed prevalence aligns with the findings of the household-based HIV serosurvey, which documented a higher incidence of infections among individuals aged 25 to 34, with the highest infection rates observed among 26-year-old females (32). The gender distribution in both cohorts primarily comprised females, reflecting the gender distribution patterns previously observed in

South Africa (33). Extensive research indicates that factors, such as increased female participation in rural-to-urban migration and socio-economic challenges including unemployment, may contribute to elevated infection rates among women (33). Regarding racial demographics, the study population predominantly consisted of black individuals, accounting for 98.2% in Cohort 1 and 94.6% in Cohort 2, aligning with both national epidemiological data and the specific geographical context of the study (34). Also, the public hospital where data was collected from, predominantly attracts black individuals.

Over more than two decades, the measurement of CD4 cell count has emerged as a pivotal tool in comprehending the progression of HIV disease and assessing the susceptibility to OIs (35). The development of guidelines concerning the initiation of ART at higher CD4 cell counts has been influenced by accumulating evidence suggesting that deferring treatment until CD4 cell counts decline below 200 cells/mm^3 is linked to increased mortality rates (36). These guidelines are formulated with consideration for a population that undergoes annual HIV testing, to commence treatment before the onset of immunodeficiency (37).

In Cohort 1, the median CD4 cell count at the initiation of antiretroviral therapy was measured at 170 (IQR: 85.5 – 287), while in Cohort 2, it was recorded as 243 (IQR: 120– 411). Despite the implementation of higher CD4 cell count targets and the era of universal T&T, the findings of the current study revealed that most patients in both cohorts (57.1% in Cohort 1 and 41.9% in Cohort 2) had CD4 cell counts below 200 cells/mm^3 at the time of treatment initiation. A meta-analysis conducted in sub-Saharan Africa, focusing on CD4 cell counts at treatment initiation, reported that there was no discernible change in the trend of CD4 cell counts over the past decade, irrespective of the specified threshold (38). This persistent lack of change may be attributable to factors such as reduced uptake of HIV testing due to prevailing HIV-related stigma, as well as geographic and transportation-related barriers (39, 40).

It is noteworthy that Cohort 2 exhibited a substantially greater proportion of patients with CD4 cell counts exceeding 350 cells/mm^3 (34.9%) in comparison with Cohort 1 (6.3%). This discrepancy was anticipated, as the patients of Cohort 2 were initiated on ART during the era of universal T&T, wherein treatment initiation was not solely based on CD4 cell count thresholds. In

contrast, patients in Cohort 1 were initiated on treatment due to alternative indications, such as coinfection with TB or other factors.

OIs pose a significant threat to the health and survival of individuals living with HIV. Moreover, the study revealed a higher prevalence of OIs among patients with CD4 cell counts below 200 cells/mm^3 (31.25% in Cohort 1 and 25.58% in Cohort 2). Among the observed OIs, TB infection occurred the most commonly, with higher numbers observed in the more immunodeficient group (30 cases in Cohort 1 vs 36 cases in Cohort 2). This finding aligns with previous retrospective studies that identified TB and oral candidiasis as the two most common OIs (41, 42). A prospective cohort study conducted by Murphy et al. (2010) at McCord Hospital in KZN also reported TB as the predominant OI, accounting for 76% of cases (43). Murphy et al.'s (2010) study additionally identified other OIs, including herpes zoster (2.9%), bacterial pneumonia (1.2%), candidiasis (2.1%), pneumocystis jiroveci pneumonia (0.4%) and HIV-associated malignancies such as nonHodgkin's lymphoma, cervical cancer and Kaposi's sarcoma (1.7%). Cases of syphilis and cryptococcal meningitis (7.1%) were also documented among the studied patients. These findings underscored the diverse range of OIs that can occur in HIV-infected individuals with compromised immune function and emphasised the importance of screening for such infections.

In the present study, at the one-year mark, the median CD4 cell count was 332.5 cells/mm^3 (IQR: 228 – 517.5) in Cohort 1 and 372cells/mm^3 (IQR: 222 – 579) in Cohort 2. These results aligned with those of a study conducted by Mocroft et al. (2007), who reported a median CD4 count of 204 cells/mm^3 (IQR: 85–330) at the initiation of ART (44). Mocroft et al. (2007) also observed an average increase of 100 cells/mm^3 in CD4 count after one year of initiating ART (44). Notably, they reported that patients with lower baseline CD4 counts exhibited a greater increase in CD4 count compared to those initiating treatment at CD4 counts above 500 cells/mm^3 (44). This highlighted the significance of initiating treatment at higher CD4 counts to preserve immunological function in patients.

In the current study, in Cohort 1, a total of 87 of 112 patients (77.67%) achieved virological suppression, compared to 105 of 124 patients (84.67%) in Cohort 2. One of the UNAIDS 90-9090 targets aimed to achieve 90% viral suppression in individuals receiving ART by the year 2020, with the overall goal of curbing the HIV epidemic by 2030. However, the present study found no

significant difference in the rates of virological suppression between the cohorts (p=0.475), irrespective of initial CD4 cell counts. However, within each cohort, there was a significant variation in the number of patients who achieved suppressed viral loads (p<0.001). It is important to acknowledge that during the study period, rates of viral suppression did not meet the UNAID's 90% target. Nevertheless, a notable proportion of patients achieved virological suppression. Factors contributing to virological failure included poor adherence to treatment and the development of OIs within the cohorts.

Limitations of the study

This study was limited by its design, which was retrospective. The sample size was small and examined for only twelve months. The cohort included patients from a tertiary hospital, which might not have reflected local and rural areas of KZN.

Conclusion

The study showed that, despite a change in the guidelines with the removal of CD4 count cut-off levels of 350 cells/mm^3 before initiating ART, a significant number of patients still presented with low CD4 counts at diagnosis in the T&T era. The median CD4 cell was 170 cells/mm^3 (IQR: 85.5 – 287) in cohort 1 when threshold for initiation was set at CD4 cell ≤ 350 cells/mm^3. In cohort 2 when treatment was initiated regardless of CD4, the median CD4 cell was 243 cells/mm^3 (IQR: 120 – 411). These findings suggested delays in diagnosis and/or treatment initiation. However, the reasons for the delay are not known and beyond the scope of the study, and further studies are required to investigate barriers to early diagnosis and/or treatment initiation to reduce the burden of HIV disease. TB was the most common OI in both cohorts (cohort 1 (26.8%) and cohort 2 (27.9%) which may be explained in part by a comparable proportion of those with low CD4 cell counts in both cohorts at baseline. Furthermore, viral suppression of 77.7% and 84.7% in Cohort 1 & 2 respectively at one year. This was below 90% target in the UNAIDS 90-90-90 criteria by 2020 set to end HIV as a public health threat by the year 2030 thus highlights the need to reinforce treatment adherence, investigate modifiable contributing factors and take necessary actions.

Competing interests

I declare that I have no financial gain or personal relationships that may have inappropriately influenced me in writing this paper.

Author contributions

ZM: The study concept and design, including data collection, data analysis, interpretation and preparation of the manuscript

NM and SP: Study concept and design; analysis and interpretation of data; and preparation of the manuscript

Ethical consideration

The Biomedical Research Ethics Committee of the University of KwaZulu-Natal granted ethical approval for the study (BREC/00001701/2020). Approval was obtained from the hospital and KZN provincial Department of Health.

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Data availability

Upon request, data are available from the corresponding author, ZM.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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PART 3: SUMMARY AND RECOMMENDATIONS

Summary and key findings

The study aimed to assess the CD4 cell count, viral load and the occurrence of OIs in patients who initiated treatment at a CD4 cell count $\leq 350mm^3$, versus those who started ART during the T&T era. The objective was to determine if the goals outlined in the evolving guidelines, such as

initiating therapy before the onset of immunodeficiency and OIs and achieving viral suppression in line with the UNAIDS 90-90-90 targets, were being met.

Findings revealed that most patients were younger than 36 years, with a female preponderance. Regardless of the suggested higher CD4 cell count threshold for treatment initiation, most patients were only initiated on ART at CD4 cell counts below 200mm^3. This indicates a delay in HIV testing and access to ART within the community, potentially influenced by factors such as HIVrelated stigma and socio-economic challenges.

TB emerged as the most prevalent OI, with a higher incidence observed in patients with CD4 cell counts $\leq$ 200mm^3 in both cohorts. This underscores the increased vulnerability to OI during immunodeficiency, which significantly contributes to morbidity and mortality.

It was observed that virological suppression rates fell below the UNAIDS 90-90-90 target, with an overall rate of 79.9% in the total cohort. However, both cohorts exhibited a significant increase in the proportion of patients achieving virological suppression at one year, with no significant difference between the two groups. This suggests that once treatment is initiated, viral suppression is attainable, irrespective of the baseline CD4 cell count.

Despite changes in guidelines and an increased CD4 cell count threshold, the outcomes at one year in terms of CD4 cell count and viral load were comparable. The delayed uptake of therapy necessitates further investigation to identify and address barriers to the timely initiation of ART.

Recommendations

The current South African HIV guidelines advocate for T&T. This study has shown similar outcomes in the increase in CD4 cell count and viral load suppression at twelve months in both cohorts. However, results indicated that OIs such as TB are more common in patients with a CD4 count of less than 200 cells/mm^3. Challenges faced in clinical practice with the Tt&T strategy include patients presenting with the unmasking of OIs after ART initiation or with AIDS-defining illnesses at the time of diagnosis of HIV.

Recommendations include the following:

- **Primary prevention:** A primary goal should be to reduce the number of new infections and HIV transmission, hence ongoing community education is required regarding barrier methods and the use of pre-exposure prophylaxis by high-risk groups through communitybased programs or local clinics.
- **Vulnerable population:** The burden of HIV appears to affect young women more than men in the studied community. Therefore, programmes should be developed to empower women on their reproductive rights, such as the use of female barrier methods and the right to know the HIV status of their partners. Community projects should be established to increase their skills, improve their income and alleviate economic dependence.
- **Community health:** Despite industrialisation, South Africans in rural areas face the struggle of reaching health care facilities due to distance and infrastructure challenges. Mobile clinics could be utilised, not only as means to provide chronic treatment to the community but also as a point of HIV testing. This could be undertaken in co-ordination with home-based care programs to reach communities at large. In addition, annual HIV testing should be encouraged. This may facilitate early HIV diagnosis, increasing HIV treatment coverage and curbing the incidence of OI in this population. Finally, the stigma around HIV persists. HIV awareness programmes should be in place throughout the community, including in high schools, making use of current technological platforms such as social media.
- **Service centres:** Health facilities should offer HIV counselling and testing to all patients that enter, at every visit. Patients should be encouraged to test annually for HIV. At these facilities, people living with HIV should be afforded the same respect and dignity as other patients with chronic comorbidities.
- **Patient care:** The urine lipoarabinomannan (LAM) assay should be used to screen for TB. In patients with symptoms suggestive of active TB. Adequate time should be provided to retrieve sputum and serum cryptococcus antigen latex test results before ART initiation.
- **Research and policies**: Government and policymakers should invest in further research to identify local factors that result in delayed HIV testing and poor compliance, to achieve UNAIDS 90-90-90 targets.

APPENDICES

Appendix 1: Final Study Protocol Research Protocol – MMed (Internal Medicine)

Title: Evaluation of the cluster of differentiation 4, viral load and presence of opportunistic infections at antiretroviral therapy initiation before and during the Test and Treat era in patients attending a tertiary hospital in KwaZulu-Natal

Principal Investigator: Dr Zanele Moya

Student number: 209501693

Address: King Edward VIII Hospital

Telephone: 031 360 3111

Cell: [redacted]

Email: [redacted]

Supervisor: Prof Nombulelo Magula

Email: magulan@ukzn.ac.za

Co-supervisor: Prof Somasundram Pillay

Email: drpillay@iafrica.com

June 2020

Table of Contents

Acronyms and Abbreviations

AIDS	acquired immunodeficiency syndrome.
ART	antiretroviral therapy
CD4	cluster of differentiation 4
D4T	stavudine
FDC	fixed-dose combination
HIV	human immunodeficiency virus
HCT	HIV counselling and testing
KEH	King Edward VIII Hospital
NRTI	nucleoside/nucleotide reverse transcriptase inhibitors
NNRTI	non-nucleoside reverse transcriptase inhibitors
OI	opportunistic infections
PJP	*pneumocystis jiroveci* pneumonia

PI	protease inhibitors
SA	South Africa
TB	tuberculosis
UTT	universal test and treat
WHO	World Health Organisation
VL	viral load

Executive Summary

The purpose of this retrospective study is to evaluate the index cluster of differentiation 4 (CD4) count, viral load and the presence or absence of prior or current opportunistic infections (OIs), in patients initiated on antiretroviral therapy (ART) at King Edward VIII Hospital (KEH). The periods assessed will be:

* 1st January 2014 to 31st December 2014, a period when ART was initiated at CD4 cell count ≤ 350 cells/mm^3

* 1st January 2019 to 31 December 2019 during the current test and treat era.

Data of all patients meeting the inclusion criteria who were initiated on ART during these periods will be evaluated.

The CD4 cell count has traditionally been used as a marker of immunity status and a guide for the initiation of ART. Previous studies have shown that patients with higher CD4 cell counts show preserved immunity and a reduced risk of opportunistic infections (OIs). This has influenced the evolution of the human immunodeficiency virus (HIV) management guidelines, from initiation at a targeted CD4 cell count to the current guideline of universal testing and treating (UTT). These measures are intended to initiate ART before the immune system is compromised, reducing the risk of OIs and onward HIV transmission. KwaZulu-Natal (KZN) is a province of South Africa (SA) with a heavy burden of OIs such as tuberculosis (TB). In clinical practice, despite these

changing guidelines, many patients' index presentation to health care facilities is with an acquired immunodeficiency syndrome (AIDS)-defining illness.

This study aims to evaluate whether, in the era of widespread access to ART, goals intended by policy change are being achieved. This will help to demonstrate if ART is being initiated before a decline in CD4 cell count and the appearance of opportunistic infections. This aim will be achieved through a retrospective review of clinical charts.

1 Background and Literature Review

Globally, 37.9 million people were living with HIV in 2018. (1) This figure includes the approximately 8 million South Africans living with HIV, with KZN being regarded as the epicentre of this pandemic in SA (2). HIV and TB co-infections are responsible for the majority of AIDSrelated deaths in SA. (3)

The Adult and Adolescent Spectrum of HIV Disease (ASD) study reported that 25 to 50% of patients with a CD4 count cell of less than or equal to 200 cells/mm^3 had an associated OI at presentation. (4) The decision to treat at a CD4 cell count less than or equal to 200 cells/mm^3 was based on the unknown benefits and known risks of treating at higher threshold CD4 cell count. (5) Drug toxicity is a major reason for delaying treatment, particularly with the use of stavudine (D4T), which is associated with metabolic complications. (6) The randomised Comprehensive Program for Research on AIDS (CIPRA HT 001) showed that the effects of delaying treatment in patients with CD4 cell counts between 200 and 350 cells/mm^3 resulted in long term immune dysfunction and a persistent increase in TB infection. (7) This resulted in a policy change which shifted the threshold for initiation of therapy to a CD4 cell count of 350 cells/mm^3 or below. (8)

In 2015, the World Health Organisation (WHO) revised ART initiation guidelines. (9) This was based on supporting evidence from multiple trials:

- The Trial of Early Antiretroviral and Isoniazid Prevention Therapy in Africa (TEMPRANO) demonstrated a lower risk of death or HIV-related illness with earlier ART initiation compared to deferred treatment. (10)
- The International Network for Strategic Initiatives in Global HIV (INSIGHT) Strategic Timing of Antiretroviral Treatment (START) study reported a significant reduction in mortality and morbidity in patients initiated on ART with a CD4 cell count greater than 500 cells/mm^3, with benefits outweighing the risks of drug toxicity. (11)
- The HIV Prevention Trial Network (HPTN 052) reported that early initiation of ART reduced sexual transmission of HIV in serodiscordant couples, suggesting that the greatest benefit of UTT was in preventing transmission. (12, 13) It has been shown that the UTT strategy led to a 14.7% reduction in transmissible HIV RNA levels as well as a prolonged life expectancy. (14) As new data emerged, the International Antiviral Society based in the United States (US)) concluded that the use of ART was beneficial in all individuals with established infection. (15) Using a mathematical model, it has been concluded that the impact of UTT i.e., depends on the epidemiological context, i.e. the testing of high-risk groups. (13)

In a local cluster randomised study conducted in KZN, SA, utilisation of UTT was linked to high levels of viral suppression; however, the overall access to health care remained poor. (16)

In light of observed hospital admissions linked to HIV-associated complications in the high-burden setting of KZN, SA, this study aims to establish whether patients are being initiated on treatment according to the recommended CD4 cell count threshold.

2. Problem Statement

HIV management guidelines have moved away from a model of initiating ART at a targeted CD4 cell count to one where ART is initiated at diagnosis, thereby increasing the threshold for treatment initiation from a potentially low CD4 cell c unit to that of a higher CD4 cell count.

3 Research Question

Are patients presenting with higher CD4 cell counts at ART initiation during the UTT era, as intended by the current HIV management guidelines, in comparison to when patients were initiated on ART at CD4 counts below 350 cells/mm^3?

4 Aims and Objectives

4.1 Overall Aim

To assess whether patients presenting during the UTT era have higher CD4 cell counts at ART initiation, achieve virological suppression and experience fewer OIs compared to those who were initiated at CD4 cell counts less than 350cells/mm^3 and whether patients are initiated on ART following HIV guidelines.

4.2 Objectives

1. Describe the demographic profile of each sample group (One group initiated at CD4 cell count $\leq$350cells/mm^3 and the other group initiated by UTT)
2. Evaluate clinical charts regarding:
    - CD4 cell count, viral load and OIs at the index consultation
    - CD4 cell count, viral load and OIs at twelve months.

5 Methodology

5.1 Study Design:

This will be a retrospective, quantitative study.

5.2 Study Setting

The KEH HIV clinic is situated in ward 33 in the eThekwini District of KZN. This hospital has a catchment area of approximately three million people. Approximately twenty-two thousand outpatients are consulted monthly at this tertiary hospital, of which two thousand are seen in the HIV clinic.

5.3 Study Population

This study will include all patients initiated on ART at the KEH HIV clinic within the two time periods described below.

5.3.1 Inclusion and Exclusion Criteria

Inclusion Criteria:

All adult patients 18 years and older initiating ART at the KEH HIV clinic during the following two time periods:

1. 1st January 2014 to 31st December 2014: Initiated at CD4 cell count less than or equal to 350 cells/mm^3
2. 1st January 2019 to 31st December 2019: Initiated treatment during UTT

Exclusion Criteria:

1. Patients previously or currently on ART.
2. Patients less than 18 years of age.
3. Patients initiated outside the abovementioned time frames.

5.6 Sampling

Approximately two thousand eight hundred and ten patients are seen at the KEH HIV clinic each month, about ten of which are new patients. These sample size estimates were calculated for an independent t-test with alpha errors at 0.05 and powers of 0.80 and 0.90. These estimates were calculated using G power, which recommended an overall sample size of 260. (17) Approximately 130 files will be reviewed from each period.

5.7 Data Source

Data will be collected from clinical records of patients attending the KEH HIV clinic and extracted by the primary investigator using a validated data collection tool.

5.8 Variables

The collected study variables will include:

- Demographics ○ Age, Sex, Race
- Clinical ○ Weight, ART history, OIs and comorbidities
- Laboratory ○ CD4 count, HIV Viral load

5.9 Data Analysis

After capturing the data into an Excel spreadsheet, the data will be imported and analysed using SPSS version 26.0 and STATA 16 with the help of a statistician. Percentages, means and standard deviations of numeric data will be determined and percentages of categorical data will be determined. Appropriate logistic regression and correlation analysis will be used to determine relationships between covariates such as age, sex, race, OIs, comorbidities, CD4 count and HIV viral load. The confidence interval for all statistical tests will be set at 95% and statistical tests will be declared significant if the p-value is found to be less than 0.05. If the data is non-parametric, the Wilcoxon rank sum test will be used to compare the differences.

5.10 Reliability and Validity

The study will be consistent and scientifically valid, as the same data collection tool will be used to collect the information, ensuring standardisation. Data will be reviewed with a qualified statistician.

The same standardised and established methods were used to record data in all patients, and this standardisation of measurement tools and their set accuracy level will increase validity of the results. (18)

6 Limitations

Data collection from clinical charts may have the following limitations:

- Lost files

- Duplicated files
- Illegible documentation of information
- Omitted information in clinical charts
- Staff shortages
- Lack of infrastructure to keep records on an online system ● Transfers out or deaths

7 Ethical Considerations

7.1 Risks to Participants

A medical chart review will be conducted, posing neither physical or emotional risk to patients.

7.2 Benefits

To identify if current HIV guidelines are aligned with clinical practice and identify gaps in their implementation.

7.3 Confidentiality

Patients' identities, either personal or hospital numbers, will not be disclosed.

7.4 Consent

There will be no interaction with patients, thus consent is not required.

7.5 Permissions

Required permissions will include the following:

- KEH HIV clinic and Department of Internal Medicine.
- Biomedical Research and Ethics Committee.
- KZN Department of Health.

8 Time Frame

Objectives	**Period**
Research Protocol	January 2020 –June 2020
Request Ethics Approval	July 2020 –September 2020

Data Collection (Chart Review)	October 2020-December 2020
Analysis of Data and Write-Up	January 2021 – June 2021
Submission	July 2021 –December 2021

9 Budget

Budget item	**Year 1**	**Year 2**
Stakeholder meetings (attending conferences and meetings)	R0	R0
Data collection		R20000
Publication	R0	R5000
Total direct costs		
Local conference: registration fee, transport, accommodation		R5000
Total annual costs	R0	
Total project budget	R0	30000

10 Data Collection Tool

Section 1: Patient Demographics

1.1 Age:

18-25years	
26-35years	
36-45years	
46-55years	
>55years	

1.2 Sex

Male	
Female	

1.3 Race

Black	
Indian	
Coloured	

White	

1.4 Weight

<45 kg	
46-65kg	
66-75kg	
>76kg	

Section 2: Clinical Background

2.1 Opportunistic infections (OIs)

Type of OI	Tick relevant	Date of Diagnosis	Mode of Diagnosis	Duration of treatment	Treatment completed
herpes zoster					Y or N
tuberculosis					Y or N
recurrent severe bacterial pneumonia					Y or N
candidiasis					Y or N
HIV-associated malignancy					Y or N
• Kaposi sarcoma					
• cervical cancer					
• lymphoma					
PJP					Y or N
Other					Y or N

2.2 Comorbidities

diabetes mellitus	
hypertension	
arthritis	
epilepsy	
Other	

2.3 ART History

Date of index initiation	
Date of re-initiation	

Section 3: Laboratory Results at Initiation

CD4 cell count	
HIV Viral load	

Section 4: Outcomes at Six Months

4.1 Immunological Outcomes

Latest CD4 cell count	

4.2 Virological Outcomes

Latest Viral Load	

4.3 Clinical Outcomes

Follow Up

a) Lost to follow up
b) Transferred to another clinic
c) Enrolled into CCMDD
d) KEH HIV clinic follow up

Treatment Adherence

(a) Good (no treatment interruption on his/her own)

(b) Poor (history of treatment interruption on his/her own)

Change in ART Regimen

a) Yes

b) No

If Yes (tick the appropriate box)

Drug Adverse Event	
Virological failure	
Treatment failure	
Other:	

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Appendix 2: The Guidelines for Authorship for the Journal Selected for Submission of a Manuscript: Southern African Journal of HIV Medicine

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an Original Research article should consist of six paragraphs labelled Background, Objectives, Method, Results, Conclusion and What this study adds.

- **Background:** Why do we care about the problem? State the context and purpose of the study. (What practical, scientific or theoretical gap is your research filling?)
- **Objectives:** What problem are you trying to solve? What is the scope of your work (e.g. is it a generalised approach or for a specific situation)? Be careful not to use too much jargon.
- **Method:** How did you go about solving or making progress on the problem? State how the study was performed and which statistical tests were used. (What did you do to get the results?) Clearly express the basic design of the study; name or briefly describe the basic methodology used without going into excessive detail. Be sure to indicate the key techniques used.

- **Results:** What is the answer? Present the main findings (that is, as a result of completing the procedure or study, state what you have learnt, invented or created). Identify trends, relative changes or differences on answers to questions.
- **Conclusion:** What are the implications of your answer? Briefly summarise any potential implications. (What are the larger implications of your findings, especially for the problem or gap identified in your motivation?)
- **What this study adds:** What key insights into the research results and its future function are revealed? How do these insights link to the focus and scope of the journal? It should be a concise statement of the primary contribution of the manuscript; and how it fits within the scope of the journal.

Do not cite references and do not use abbreviations excessively in the abstract.

Introduction: The introduction must contain your argument for the social and scientific value of the study, as well as the aim and objectives:

- Social value: The first part of the introduction should make a clear and logical argument for the importance or relevance of the study. Your argument should be supported by the use of evidence from the literature.
- Scientific value: The second part of the introduction should make a clear and logical argument for the originality of the study. This should include a summary of what is already known about the research question or specific topic and should clarify the knowledge gap that this study will address. Your argument should be supported by the use of evidence from the literature.
- Conceptual framework: In some research articles it will also be important to describe the underlying theoretical basis for the research and how these theories are linked together in a conceptual framework. The theoretical evidence used to construct the conceptual framework should be referenced from the literature.
- Aim and objectives: The introduction should conclude with a clear summary of the aim and objectives of this study.

Research methods and design: This must address the following:

- Study design: An outline of the type of study design.
- Setting: A description of the setting for the study; for example, the type of community from which the participants came or the nature of the health system and services in which the study is conducted.
- Study population and sampling strategy: Describe the study population and any inclusion or exclusion criteria. Describe the intended sample size and your sample size calculation or justification. Describe the sampling strategy used. Describe in practical terms how this was implemented.
- Intervention (if appropriate): If there were intervention and comparison groups, describe the intervention in detail and what happened to the comparison groups.
- Data collection: Define the data collection tools that were used and their validity. Describe in practical terms how data were collected and any key issues involved, e.g. language barriers.
- Data analysis: Describe how data were captured, checked and cleaned. Describe the analysis process, for example, the statistical tests used or steps followed in qualitative data analysis.
- Ethical considerations: Approval must have been obtained for all studies from the author's institution or other relevant ethics committee and the institution's name and permit numbers should be stated here.

Results: Present the results of your study in a logical sequence that addresses the aim and objectives of your study. Use tables and figures as required to present your findings. Use quotations as required to establish your interpretation of qualitative data. All units should conform to the **SI convention** and be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion: The discussion section should address the following four elements:

- Key findings: Summarise the key findings without reiterating details of the results.
- Discussion of key findings: Explain how the key findings relate to previous research or to existing knowledge, practice or policy.

- Strengths and limitations: Describe the strengths and limitations of your methods and what the reader should take into account when interpreting your results.
- Implications or recommendations: State the implications of your study or recommendations for future research (questions that remain unanswered), policy or practice. Make sure that the recommendations flow directly from your findings.

Conclusion: Provide a brief conclusion that summarises the results and their meaning or significance in relation to each objective of the study.

Acknowledgements: Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution. Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named. Refer to the acknowledgement structure guide on our Formatting Requirements page.

Also provide the following, each under their own heading:

Competing interests: This section should list specific competing interests associated with any of the authors. If authors declare that no competing interests exist, the article will include a statement to this effect: The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article. Read our policy on competing interests.

Author contributions: All authors must meet the criteria for authorship as outlined in the authorship policy and author contribution statement policies.

Funding: Provide information on funding if relevant

Data availability: All research articles are encouraged to have a data availability statement.

Disclaimer: A statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

References: Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote selfinterests. Refer to the journal referencing style downloadable on our Formatting Requirements page.

Appendix 3: Ethical approvals

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII HOSPITAL

Private Bag X02, CONGELLA, 4013
Corner of Rick Turner (Francois Road) & Sydney Road
Tel: 031-3603853; Fax:031-2061457; Email:
www.kznhealth.gov.za

Ref.: KE 2/7/1/(08/2020)
Enq.: Mrs. L. Mpanza
Research Programming

11 August 2020

Ms. Zanele Ruth Moya
School of Clinical Medicine
Medical School

UNIVERSITY OF KWAZULU-NATAL

Dear Ms. Moya

Protocol: "Evaluation of the CD4, Viral load and presence of opportunistic infections at antiretroviral therapy initiation before and during the Test and Treat era in patients attending a tertiary hospital in KwaZulu -Natal" Degree: Master of Medicine

BREC REF NO: 00001701/2020

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.

Kindly note the following:-

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgment in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

[redacted]

SUPPORTED / NOT SUPPORTED

11/8/2020

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 March 2023

Miss Zanele Ruth Moya (209501693)
School of Clinical Medicine
Medical School

Dear Miss Moya,

Protocol reference number: BREC/00001701/2020
Project title: Evaluation of the CD4, viral load and presence of opportunistic infections at antiretroviral therapy initiation before and during the Test and Treat era in patients attending a tertiary hospital in KwaZulu-Natal
Degree: Master of Medicine

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	**21 October 2022**
Expiration of Ethical Approval:	**20 October 2023**

I wish to advise you that your application for recertification received on 02 December 2022 for the above study has been noted and approved by a subcommittee of the Biomedical Research Ethics Committee (BREC). The start and end dates of this period are indicated above. Your response to BREC query dated 19 January 2023 has been noted by a subcommittee of the BREC.

Notes to PI:
Technically BREC approval should be uninterrupted/continuous until the PI chooses to close the approval after data analysis.
In this case the lapse is condoned as this is a minimal risk study.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 May 2023.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:
Health Research & Knowledge Management Unit

Postal Address: Private Bag X9050
Physical Address: 330 Langalibalele Str, PM Burg, 3201
Tel: 0333963189/3123/2805 Fax: 033-3943782
Email address: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

NHRD Ref: KZ_202010_002

Dear Dr Z Moya
(UKZN)

Approval of research

1. The research proposal titled **'Evaluation of the CD4, viral load and presence of opportunistic infections at antiretroviral therapy initiation before and during the Test and Treat era in patients attending a tertiary hospital in KwaZulu-Natal'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at King Edward VIII Hospital.

2. You are requested to take note of the following:
   a. *All research conducted in KwaZulu-Natal must comply with government regulations relating to Covid-19. These include but are not limited to: regulations concerning social distancing, the wearing of personal protective equipment, and limitations on meetings and social gatherings.*
   b. *Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.*
   c. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   d. *Provide an interim progress report and final report (electronic and hard copies) when your research is complete to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za*
   e. *Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.*

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

[redacted]

Dr E Lutge

Chairperson, Health Research Committee

Date: 14/10/2020

Appendix 4: Example data collection tool

Excel number_____

Initial_____

_

File Number_____

Data Collection Tool Section 1: Patient Demographics

1.1 Age:

18-25years	0
26-35years	1
36-45years	2
46-55years	3
>55years	4

1.2 Sex

Male	0
Female	1

1.3 Race

Black	0
Indian	1
Coloured	2
White	3

1.4 Weight

<45 kg	0
46-65kg	1
66-75kg	2
>76kg	3

Section 2: Clinical Background

2.1 Opportunistic Infections

N: No 0 Y:Yes 1 ND: Not documented 2

*Number collates with subsequent infection or disease

Type of OI	Tick relevant	Date of Diagnosis	Mode of Diagnosis	Duration of treatment	Treatment completed	

Herpes Zoster 1*					N 0 Y1 Not documented 2	1
Tuberculosis 2*					N 0 Y1 ND2	2
Recurrent severe bacterial pneumonia 3*					N 0 Y1 ND2	3
Candidiasis 4*					N 0 Y1 ND2	4
HIV- associated malignancy 5*					N 0 Y1 ND2	5
• Kaposi sarcoma						
• Cervical Cancer						
• Lymphoma						
PJP 6*					N 0 Y 1 ND 2	6
Other 7*					N 0 Y1 ND 2	7

2.2 Co-morbidities (Tick)

Diabetes Mellitus	

Hypertension	
Arthritis	
Epilepsy	
Other	

2.3 ART history

Date of Index Initiation	
Date of Re-Initiation	

Section 3: Laboratory Results at Initiation

CD4 cell count/mm^3	<200 200-350 >350
HIV Viral load	

Section 4: Outcomes: 6months

4.1 Immunological Outcomes

Latest CD4 cells/mm^3 :	<200 200-350 >350

4.2 Virological Outcomes

Latest Viral Load :	Below 1000 copies/ml : Y or N

4.3 Clinical Outcomes

Follow Up

a) Lost to follow up
b) Transferred to another clinic
c) Enrolled into CCMDD
d) KEH HIV clinic follow up

Treatment Adherence (circle A or B)

(a) Good (no treatment interruption on his/her own)
(b) Poor (history of treatment interruption on his/her own)

Change in ART Regimen

a) Yes
b) No

If Yes (tick the appropriate box)

Drug Adverse Event	
Virological failure	
Treatment failure	
Other:	