Demographic and hematological factors associated with discordant immunologic response to antiretroviral therapy in an African cohort.

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DECLARATION

I declare that this research report is my own, unaided work. It is being submitted in partial fulfilment of the requirements for the Degree of Master of Science in the field of Epidemiology and Biostatistics, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Batanayi Prinsloo Muzah 07 October 2011

Dedication

I dedicate this work to my parents

And

My wife, Yvonne

for always encouraging and supporting me to achieve more in life.

Abstract

Background

The therapeutic goal of HAART is sustained immune recovery and viral suppression. However some patients still have poor CD4 count responses despite achieving viral suppression. Such discordance immune response has been associated with poor clinical outcomes. We describe the prevalence of discordant immune response during the first 6-months of HAART and determine risk factors associated with this discordance at two large public sector clinics in South Africa.

Methods

We analysed data from 6 460 HIV-infected adults initiated onto first-line HAART at Goba and Phola Park clinics, in Johannesburg, South Africa between November 2008 and December 2009. Multivariable logistic regression models were used to estimate adjusted odds ratios (AOR) for associations between discordant immune response and clinical and demographic factors. Models were adjusted for WHO clinical stage, baseline CD4 count, education level and HAART regimen.

Results

At initiation of HAART, most patients were female 592(64.6%) and 803(87.6%) were initiated on 3TC-d4T-EFV/NVP. The mean CD4 count was 155 cells/mm3 (±118.4 sd), mean age was 38.5 years (±8.7 sd) and most patients had haemoglobin >11g/dL (n=645, 71.2%). By 6-months after initiation of HAART, 24% (n=220) of patients had a discordant immune response, 7% (n=67) discordant virologic

response and 21% (n=1359) had been lost to follow-up. In multivariable analysis, higher baseline CD4 cell count (CD4≥200cells/mm3): AOR=3.02; 95%CI 2.08-4.38; p<0.001) and moderate anemia (8-9.4 g/dL) at baseline (AOR=2.30; 95%CI 1.25-4.59; p=0.007) were the strongest predictive factors for development of discordant immune response.

Conclusions

We found a significant proportion of patients with discordant immune response 6-months after initiating HAART. Simple algorithms utilizing baseline characteristics can be developed for use in clinics in order to identify those patients at risk of development of discordant immune responses. Intensive monitoring of individuals at risk may improve clinical outcomes.

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Nomenclature

AIDS: Acquired Immune-Deficiency Virus

ALT: Alanine Aminotransferase

CCMT: Comprehensive Care Management and Treatment

CD4: Cluster of Differentiation 4

D4T: Stavudine

EFV: Efavirenz

HTLV-1: Human T-Lymphotrophic Virus-1

HIV: human immunodeficiency virus

HAART: Highly Active Antiretroviral Therapy

MDR: Multi-Drug Resistance Transporter Gene.

NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor

NRTI: Nucleoside Reverse Transcriptase Inhibitor

NVP: Nevirapine

0I: Opportunistic Infection

WHO: World Health Organization

VL: Viral Load

3TC: Lamuvudine

CHAPTER ONE

General Introduction

This chapter gives an overview of the burden of discordant immunologic and virologic responses to antiretroviral therapy. The chapter also contains the background on discordant immunologic and virologic responses, a statement of the problem, justification of the study, objectives of the study and a review of relevant literature.

1.1 Background information

Significant progress has been made in the treatment of the acquired immune-deficiency syndrome (AIDS) caused by the Human Immuno-deficiency Virus (1). The introduction of antiretroviral therapy has seen a significant fall in morbidity and mortality associated with HIV infection (2). Antiretroviral drug treatment has evolved from the initial monotherapy with Zidovudine in 1987 to the current triple therapy known as highly active antiretroviral therapy (HAART). This reduction in morbidity and mortality has been achieved due to the ability of HAART to reduce HIV viremia to undetectable levels and in turn increase numbers of circulating peripheral CD4 lymphocytes(3). However studies have shown that in clinical practice only 40-60% of the patients receiving HAART achieve this concordant response , 12-23% have neither of the above responses and the remaining patients exhibit what is termed a discordant response (3). There are two types of discordant responses: an adequate immunologic response in the absence of viral suppression and good viral suppression in the absence of immunologic recovery(4).The latter response, poor

immunologic response despite viral suppression($CD4^-/VL^+$) has received greater attention because of concerns over poor prognosis associated with lack of immune reconstitution despite virological suppression(4).The long term reduction in the risk of developing opportunistic infection and tumors in patients receiving antiretroviral therapy is related to immunologic recovery(5). Studies on discordant immune responders have mainly been in developed countries where treatment is protease inhibitor (PI) based and involved treatment experienced patients (3).

1.2 Statement of problem

One of the biggest public health problems facing the world to date is the HIV/AIDS pandemic and its implications on global health, international development and security (6). Because of the high burden of HIV/AIDS, it is important that discordant responses to HAART are better understood because studies continue to show their association with increased risk of mortality and morbidity. Though there has been extensive research on discordant responses to HAART, most of these studies have been conducted in resource-rich settings and have also been focused on patient cohorts exhibiting all 4 types of discordance. Applicability/generalizability of these findings is a major issue as antiretroviral drug regimens in resource-rich settings are predominantly protease inhibitor based and the cohorts consists predominantly of treatment experienced patients. Findings on this topic in limited-resource settings are still limited and also very few if any have vigorously examined the sub-group of patients failing to achieve CD4 responses despite virologic suppression. The financial burden associated with the development of AIDS defining illnesses and their clinical management which often requires frequent physician consultation and hospitalizations is also a cause for concern.

1.3 Justification for the study.

Despite the relative frequency, information on the prevalence, risk factors and clinical significance of discordant immune response to HAART is still limited (4). The lack of knowledge on this sub-group of patients may contribute to inadequate clinical management as current HIV treatment guidelines do not provide guidance on this scenario. Studies on the prevalence and predictors of this discordant response from resource-limited settings (LRS) where the treatment is primarily nucleoside reverse transcriptase inhibitor (NRTI) based are still limited. Also in this setting, patients often initiate treatment at advanced stages of immunosuppression and often have co-morbidities that may compromise their response to treatment (7).

1.4 Literature review

1.4.1 Introduction

HIV infection is typically associated with progressive CD4 cell depletion and consequent immunodeficiency (8-10). However, the introduction of HAART has seen a fall in morbidity and mortality associated with HIV infection (2). This is through the ability of HAART to suppress HIV viremia to undetectable levels and concomitantly resulting in an increased number of CD4 cells (11).However in clinical practice not all patients receiving HAART achieve this desired concordant response as 20-40% respond in a discordant manner (12).

Some patients on HAART show a trend of sustained CD4 cell response despite constant viremia or do not show a significant increase in CD4 cell count despite viral suppression. These two scenarios are referred to as discordant response and are associated with an intermediate risk of developing an AIDS event or death (3, 12-14). Immunologic only response without viral response has been attributed to lower

baseline CD4 count, presence of non synctium- inducing viruses, viruses with multiple resistance mutations and low level viral replication with viruses with reduced fitness(15, 16). Virologic only response without immune recovery may arise due to either failed immune reconstitution or excessive destruction of CD4 cells (17). The reconstitution of peripheral CD4 cells is a biphasic process with an initial rapid increase of memory CD4 cells succeeded by a slow increase of naïve CD4 cells (18, 19). The second rise in CD4 cells may be due to expansion or sustenance of cell survival in the periphery and also by the central regeneration of cells by the thymus (17). Paradoxically, a few studies have shown contrasting results linking higher baseline CD4 cell count with development of discordant immune response (13, 20). This could be attributed to the different study settings as these studies were mainly conducted in resource- limited settings.

Previous studies conducted in resource rich settings show that discordant response occurs in 20-30% of patients six months to twenty-four months after initiating HAART(2). There is however paucity of data on discordant responders from developing countries. The Antiretroviral Therapy in Lower Income Countries Collaboration (ART-LINC) has written on the frequency of discordant responses in HIV treatment programs in Africa, Asia and South America. Information from 15 countries showed the frequency of virologic only response to be 19.07% and immunologic only response to be 14.8% after 6 months of treatment (21). Another study by Tan et al. from the University of Alabama outpatient HIV clinic showed frequency of immunologic only response to be 15.8% and that of virologic only response to be 8.7%(22). Moore at el. from Vancouver, Canada had similar findings with frequency of virologic only responders being 15.7% and that of immunologic only responders being 11.7% (11). This variation in frequency of discordant

responses could be due to variation in sample sizes, definition of discordant response and study settings. Viral suppression has been defined differently within these studies with some studies using a cut-off value of 400copies/ml and others using 1000copies/ml.

Despite the relative frequency of discordant immune response shown in resource rich settings, no study has been done in South Africa to ascertain its burden and associated hematologic and demographic factors. Our study will help quantify the burden of the condition and describe factors associated with the development of discordant response.

1.4.2 Risk factors for developing discordant immune response

The pathogenesis of discordant immune response is not well understood. It is thought to result from the interaction of viral, host and treatment related factors.

The host factors associated with a discordant immune response include old age which has been associated with diminished immune response as a result of decreasing thymic activity with increasing age (2). A lower pre-treatment nadir CD4 count which results from depletion of CD4 cells in the gut associated lymphoid tissue during initial primary HIV infection has also been associated with blunted immune recovery. This may lead to a slow or refractory reconstitution of immune function with antiretroviral therapy (23). Paradoxically, a few studies have shown contrasting results linking higher baseline CD4 cell count with development of discordant immune response (13, 20). This could be due to patients initiating treatment with a high baseline CD4 cell count resulting in minimal increase in CD4 cell count on follow up.

The patient gender and co- infection with opportunistic infections like Tuberculosis, Human T-Lymphotrophic Virus-1 (HTVL-1), HIV-2 and GB Virus C (GBV) has been also associated with discordant immune response (24-26).

Host genetics have been shown to play a part in the immune recovery. The multidrug resistance transporter gene MDR1 codes for P-glycoprotein which is essential for transportation of many different substrates including antiretroviral drugs. An over expression of P-glycoprotein lowers intracellular concentration of protease inhibitors and may affect reconstitution of CD4 cell production (2). Other studies have shown an association with genes responsible for T-lymphocyte turnover, activation and apoptosis (27). A combination of genes controlling T lymphocyte homeostasis and an allele that encodes IL-6 production have been shown to change CD4 cell recovery (26).

Treatment factors associated with discordant immune response include the combination of Tenofovir/Didanosine which is known to be Didanosine dose dependant (28). The hypothesized mechanism being the synergistic effect of both drugs' metabolites in producing an imbalance in the purine pool of CD4 cells which induces cytolysis (29). The use of myelotoxic drugs such as Zidovudine and Cotrimoxazole has also been linked to poor immune response (2). Also poor adherence to antiretroviral therapy has been associated with a discordant immune response (30).

1.4.3 Prognosis of discordant responders

Studies in resource rich settings have shown a stronger correlation of long-term prognosis with viro- immunological responses after 6 months on HAART than with baseline values(2). Discordant response at 3-9 months after HAART initiation play a

crucial role in predicting long-term clinical outcomes in treatment naïve patients(31).The majority of studies have shown that discordant immune responders are associated with an intermediate risk of death and clinical progression when compared to complete responders(2). The UK CHIC study showed incidence rate ratio (IRR) of death of 3.35 with 95% CI (1.73-6.47) in both types of discordant responders. Studies by Moore et al (11) and Tan et al.(22) showed an average relative hazard (RH) of death 3.33 95% CI (1.64-7.08) among virologic only responders and 2.64 CI (1.89-3.96) among immunologic responders.

Furthermore few studies have been done to assess the prognostic impact of discordant immune responders in HAART naïve patients and patients receiving non-nucleoside reverse transcriptase inhibitors (NNRTI) based regimen which are common in resource limited settings.

1.5 Study objectives

1.5.1 General study objective

The main objective of this study was to describe the prevalence discordant immune responders during the first 6-months of antiretroviral therapy and also to examine risk factors associated with this clinical scenario at two large public sector clinics in South Africa

1.5.2 Specific objectives

- To describe the prevalence of discordant immunologic and virological responders among patients receiving HAART at public sector clinics in South Africa.
- To identify the factors associated with discordant immune response to antiretroviral therapy.

CHAPTER TWO: METHODOLOGY

Introduction

Chapter two describes the study design and methodology. In this chapter the study population is described, selection of the study sample is illustrated and data sources and management are discussed. Details of study variables and definition of outcomes of interest is defined in this chapter. The chapter concludes with a synopsis of the data analysis plan and ethical considerations of the study.

2.1 Study design

We analysed prospectively collected cohort data for HIV-infected patients initiating HAART at two Ekurhuleni district Comprehensive Care Management and Treatment (CCMT) centres (Phola Park and Goba Clinics) in Johannesburg, South Africa.

2.2 Study setting

Ekurhuleni is the largest of the six districts of Gauteng province in South Africa. It has a population of 2 480 260 people and an HIV prevalence of 31.5% (32). Phola Park and Goba clinics were initially down referral clinics which started to initiate antiretroviral treatment in January 2009. Down referral clinics are primary care clinics designated to maintain care of patients on antiretroviral therapy who are deemed stable by central HAART initiation centres. Since inception of the clinics, over 6000 HIV infected people have been enrolled into care with more than 3000 currently receiving free antiretroviral treatment in accordance to the current South African National Department of Health Guidelines of 2010. However during the study period, the 2004 HIV treatment guidelines were in use (see Table 2). Tables 1 shows the patient follow-up schedule at these 2 clinics in accordance with these

guidelines. The 2004 guidelines which were employed during the study period had a two tier treatment readiness assessment process (33).

Table 1: The two-tier treatment readiness assessment process

Initial screening visit*(2-4 weeks before starting treatment)*	**Second visit***(often accompanied the HAART commencement visit)*	**HAART commencement visit**.
• Confirm the selection criteria: clinical and laboratory (make sure TB and pregnancy have been excluded). • Treat any opportunistic infection. • Patient's information records need to be completed. • Patient must meet with the multi-disciplinary team for group and individual information sessions. • Treatment counsellor/patient advocate will discuss treatment with the patient. • 28-day supply of Cotrimoxazole is given to patient. • Patient is given a date of return.	• Clinical assessment • Information and education session • Pill count (Cotrimoxazole) • Adherence counselling for patient and treatment counsellor if available.	• Re-assess patient's readiness. • Do Cotrimoxazole pill count. • Provide detailed description of the drugs. • Discuss further information and adherence issues with the patient and his/her counsellor or advocate. • Re-enforce drug-dosing details before the patient leaves the clinic. • Ensure that instructions are clearly written on the container with a permanent marker.

Table 2: HAART monitoring schedule

Regimen	Drugs	Monitoring test	Frequency
1a	**D4T/3TC/EFV**	• **CD4** • **VL** • **ALT**	• Staging,6 monthly • 6 monthly • symptomatic
1b	**D4T/3TC/NVP**	• **CD4** • **VL** • **ALT**	• Staging,6 monthly • 6 monthly • Baseline, week 2,4 and 8.thereafter 6 monthly

National antiretroviral therapy guidelines. South Africa, 2004.

Key: ***Staging:*** *initial testing for all patients when referred for ART*

Baseline*: testing for ART eligible at initiation of ART*

1a*:Stavudine/Lamuvudine/Efavirenz*

1b*: Stavudine/Lamuvudine/Nevirapine*

Above is a table showing first line ART regimens in use during the study period and the recommended monitoring schedule.

These clinics were selected because clinical data of the patients is captured electronically and such available for secondary analysis.

2.3 Study population

The study population consisted of 6 460 HIV infected people enrolled at Phola Park and Goba clinics CCMT centres in Ekurhuleni district from inception to 31 December 2009.

2.4 Study sample

The study sample consisted of 917 adults patients initiated on to HAART at Phola Park and Goba clinics since between 01 November 2008 and 31 December 2009. This also included individuals who had been down referred from larger HIV-treatment units.

Inclusion criteria

Patients had to fulfil the following criteria in order to be included in the study:

- patients older than 16 years at initiation of HAART.
- patients who received standard first-line HAART for at least 3 months.
- patients who had baseline CD4 count and the next scheduled CD4 count within a 6 month period in their records.
- patients who had viral load taken on the same day as the next scheduled CD4 count.
- patients who were ART naïve at treatment initiation.

Exclusion criteria

- HIV infected patients not on treatment
- patients with both incomplete CD4 count and viral load results.

2.5 Data sources

Patient clinical and demographic data at the treatment sites is captured and stored on an electronic patient management and decision support making system called TherapyEdge$^{T.M.}$. This system incorporates a data warehouse for aggregation of data from on-site installations of TherapyEdge$^{T.M}$- HIV. The system has a data capture module and rapid capture forms. Phola Park and Goba clinics record both demographic and clinical patient information using TherapyEdge$^{T.M}$. Trained data

capturers capture data from the patient files after each clinic visit. At the commencement visit the patient's demographic and contact details are recorded on the HAART initiation form. At ensuing visits the patient's vitals including weight as well as any symptoms and new diagnosis are recorded on the follow-up visit form. Blood tests results for CD4 cell count, HIV viral load, full blood count and liver function tests are tested and recorded onto the database as scheduled (Table 1). Data was extracted from Therapy-EdgeTM and exported to STATA version 11 (STATA Corp., College Station, TX) for analysis.

2.6 Study variables

Main outcome

The outcome of interest was the development of a discordant immune response. We defined viral suppression as a plasma HIV viral load less than 400 copies/mL at 6 months after initiating antiretroviral therapy. Immune response was defined as an increase of 50 in the absolute CD4 count value at 6 months from the baseline value. Discordant immune response was defined as viral suppression with immunological failure. Measurements within 3-9 months were used in this analysis as these patients are representative of the 6 month scheduled follow-up.

N.B: Those with virologic failure accompanied with an immune response were not analyzed because their risk factors are different.

Exposure variables.

- Age (years) and Age groups categorized in years as:(16-24)(25-44) (45-64) (>65) [34]
- Gender (male/female)
- Alcohol intake(yes/no)

- Smoking (yes/no)
- Employment status as : (unemployed/ employed)
- Level of education as: Illiterate/ Primary school / secondary school / beyond secondary school
- BMI: Body Mass Index categorized as: (Underweight< 18.5)(Normal:18.5-24.99)(Overweight25.0- 29.99)(Obese >=30)
- Baseline CD4 count categorized as: (CD4<200cells/ µL)(CD4≥200cells/ µL)
- Baseline Hemoglobin categorized as: (normal ≥ 11g/dl)(mild anaemia:9.5-10.9g/dl)(moderate:8.0-9.4g/dl)(severe:≤7.9)
- AST level categorized as:(Normal/Abovel normal)
- ALT level categorized as:(Normal/Abovel normal)
- HAART regimen
- WHO clinical stage categorized as: stages 1, 2, 3 and 4.
- History of previous Tuberculosis(yes/no)

2.7 Data Quality Control.

The data of patients receiving antiretroviral therapy at Goba and Phola park clinics is captured from their medical records onto TherapyEdge-HIV $^{T.M}$, a medical management software . This electronic database is managed and maintained by a Data management and Quality assurance team from Right To Care (34).

In addition to these quality assurance measures observations which were deemed implausible were set to missing before analysis since verification was impossible. This included weight of above 200kgs.

2.8 Data processing methods and Data Analysis.

All data was analysed in STATA version 11 (STATA Corp., College Station, TX). The prevalence of discordant immune response was computed. The Chi-square test was used to compare differences in characteristics between categorical variables (e.g. demographic and hematological characteristics of those with discordant immune response compared to those with a concordant response). The Student ttest was used to compare means of the continuous variables such as age, CD4 cell count and viral load which were noted to be normally distributed.

Preliminary analysis was conducted to compare patients who had missing data at six months and those who were included in the analysis. This comparison was done to determine whether significant differences existed between the two groups which may affect the generalizabity of findings and bias the results.

Factors that were significant at $p \leq 0.2$ in univariate analysis were considered for inclusion in multivariable logistic regression models to determine factors associated discordant immune response when controlling for other factors. Age was reported in 10 year increments to provide sensible interpretation. WHO stage was retained in all models as it had been included priori. This is because of the intimate link between baseline CD4 cell count and WHO stage at ART initiation.

We arrived at our final models using both forward selection and backward elimination techniques. The models were tested for goodness of fit using the Hosmer-Lemeshow goodness of fit test. Interaction between all the significant variables in the model was

also investigated. Collinearity was tested in all the regression models. In our final model, we adjusted for WHO clinical stage, baseline CD4 count, education level and HAART regimen. The latter three variables had been significant in the univariate analysis. Precision of estimate for odds ratios (OR) in the logistic regression model was at the 5 percent significance level (p=0.05). We also conducted a sensitivity analysis to demonstrate robustness of our study findings to variation in definition of CD4 response. Categorized continuous variables such hemoglobin level was tested for trend in the final model.

2.9 Ethical consideration.

Patient confidentiality was maintained through allocation of unique identifiers to individual participants and removal of any personal identifiers before the data was given to the researcher. The researcher was unable to obtain informed consent because secondary data analysis was conducted and as such study participants could not be reached to consent to the study. Ethical approval was obtained from the University of The Witwatersrand Human Research Ethics Committee (Medical),ethics number M10944 (see appendix 1) and permission to conduct the study was obtained from the Ekhuruleni Ethical Panel (see appendix 2).

CHAPTER THREE: RESULTS

INTRODUCTION

This chapter begins with a flow diagram illustrating how study sample was obtained. It then shows a comparison between those with complete data and those with missing data at 6 months (hence not eligible for analysis) in terms of exposure variables. Baseline characteristics of the study sample are then followed by a comparison of those who developed discordant immune response and those who had concordant response. The factors associated with development of discordant immune responses are then investigated using logistic regression analysis. The chapter concludes with sensitivity analysis.

3.1 Cohort demographics

The study population consisted of 6 460 adults enrolled on the antiretroviral program at Goba and Phola park clinics from the clinic inception to the 31st of December 2009. Out of this total, 962 were excluded because besides appearing in the ART program register no additional information was recorded (e.g no HAART regimen and baseline haematological measurements), a further 4 581 were excluded because there had either missing initial or 6 month CD4 count/ Viral load or both. These included those with mistaken HAART regimens.

Of the 917 eligible for analysis 810 made the study sample (590: concordant response ($CD4^+/VL^+$), 220: discordant immune response ($CD4^-/VL^+$). The remaining 107 had responses either a discordant virologic response ($CD4^+/VL^-$) or were non-responders ($CD4^-/VL^-$). These latter responses were not the subject of our study. Therefore the prevalence of discordant immune response which was the subject of

this analysis was 24%.All the above information is illustrated below in the flow diagram below.

Fig 1: Flow chart of study participants

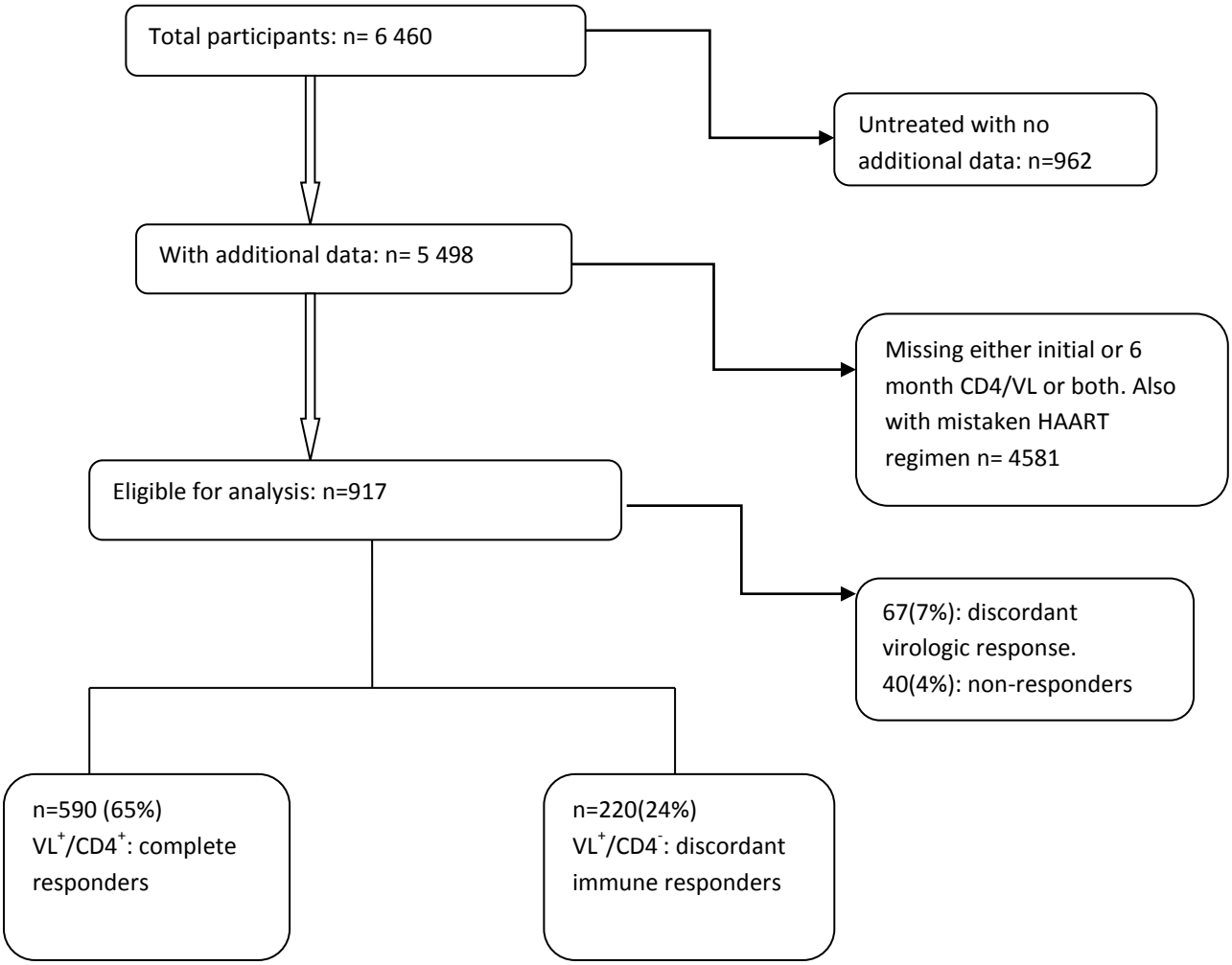

Table 3: Comparison of participants included in the analysis versus those with missing data at 6 months by baseline characteristics

characteristics	With missing data at 6 months	Eligible for analysis	P-value
Age(years)	(37.3)9.6	(38.5)8.9	0.009
Age groups(years)			
16-24	109(7.69)	31(3.39)	
25-44	961(71.03)	689(74.54)	
45-64	281(20.77)	196(21.42)	
≥65	7(0.52)	6(0.66)	0.001
Gender: female	883(64.97)	592(64.56)	
male	476(35.03)	325(35.44)	0.839
HAART D4T-3TC-EFV			
D4T-3TC-NVP	644(51.44)	803(87.57)	
AZT-3TC-EFV			
AZT-3TC-NVP	137(10.54)	81(8.83)	
3TC-TDF-EFV			
3TC-TDF-NVP	476(38.02)	33(3.60)	0.001
Baseline CD4 count(cells/ µL)	(179.9)148.8	(155.7)118.4	0.003
Baseline CD4 count			
<200 cells/ µL	901(66.35)	660(72.85)	
>200 cells/ µL	457(33.65)	256(27.95)	0.004
WHO clinical stage			
1	1 042(76.67)	672(73.28)	
2	21(1.55)	9(0.98)	
3	296(21.78)	236(25.74)	0.054
Hemoglobin(g/dl)			
Normal: ≥11	897(70.02)	645(71.19)	

Mild anemia:9.5-10.9	226(17.64)	154(17.0)	
Mod anemia:8-9.4	110(8.54)	76(8.39)	
Severe anemia:≤7.9	48(3.75)	31(3.42)	0.938
History of TB			
No	1 286(94.63)	814(88.77)	
Yes	73(5.37)	103(11.23)	0.001
Smoking			
No	868(78.55)	605(79.4)	
Yes	237(21.45)	157(20.6)	0.660
Alcohol no	814(84.79)	589(78.64)	
yes	146(15.21)	160(21.36)	0.0001
Body Mass Index			
Normal:18.5-24.99	509(49.8)	372(49.01)	
Underweight:<18.5	116(11.38)	64(8.43)	
Overweight:25-29.99	239(23.39)	198(26.09)	
Obese: ≥30	158(15.46)	125(16.46)	0.151
Occupation status			
Unemployed	825(66.86)	560(67.96)	
employed	409(33.14)	264(32.04)	0.600
Education level			
Illiterate	34(2.88)	27(3.33)	
1^0 school	228(19.34)	136(16.75)	
2^{nd} school	888(75.32)	630(77.59)	
Tertiary school	25(2.12)	14(1.72)	0.464
Aspatate transaminase level normal	800(69.14)	591(66.03)	
Above normal	357(30.86)	304(33.97)	0.135
Alanine aminotransferase normal	1 193(93.35)	858(94.29)	
Above normal	85(6.65)	52(5.71)	0.373

Key: *n(%) for categorical variables and (mean)standard deviation for continuous variable*

3.3 Comparison of participants included in the analysis versus those with missing data at 6 months by baseline characteristics

There is a significant difference in the mean ages of the two groups with those analyzed being a year older, though there was no significant difference in gender between the two groups. There was also a significant difference in the HAART regimen between the two groups with a higher proportion of those eligible for analysis (87.6%) initiating treatment on Stavudine based regimen compared to those with missing data at 6 months (51.4%) ($p<0.001$). A slightly higher proportion of those with missing data at 6 months (10.5%) were on a Zidovudine based regimen compared to (8.8%) who were analyzed. In addition an even higher proportion of those with missing data at 6 months (38%) were on Tenofovir based regimen compared to those analyzed (3.6%, p-value 0.001).

A higher proportion of those analyzed (155 cells/ µL) started HAART with a significantly lower mean baseline CD4 count than those with missing data at 6 months(179 cells/ µL) though there was marginally a significant difference (p-value 0.003) in the WHO clinical stage at initiation of treatment in the two groups.

There was also a significant difference in those with a history of Tb and alcohol use at commencement of HAART as a higher proportion of those analyzed (11%) were more likely (p-value 0.001) to have a history of Tb compared to those with missing data at 6 months (5.4%). Also a participant was more likely (p-value0.001) to use alcohol if there were analyzed (21.4%) compared to those who had missing data at 6 months (15%)

However there was no significant difference in those analyzed and those with missing data at 6 months in the following variables, smoking, body mass index, hemoglobin level education level, occupational status, and hepatic enzymes (ALT, AST) (p>0.05).

Table 4: Baseline characteristics of study sample

characteristics	**Study sample**
Age(years)	(38.4)8.7
Age groups(years)	
16-24	24(2.97)
25-44	610(75.4)
45-64	170(21.01)
≥65	5(0.61)
Gender: female	530(84.13)
male	100(15.87)
HAART D4T-3TC-EFV	
D4T-3TC-NVP	720(88.89)
AZT-3TC-EFV	
AZT-3TC-NVP	67(8.27)
3TC-TDF-EFV	
3TC-TDF-NVP	23(2.84)
Baseline CD4 count(cells/ µL)	(159)118
Baseline CD4 count	
<200 cells/ µL	584(72.10)
>200 cells/ µL	226(27.90)
WHO clinical stage	
1	598(73.83)
2	9(1.11)
3	203(25.06)

Hemoglobin(g/dl)	
Normal: ≥11	577(72.13)
Mild anemia:9.5-10.9	135(16.88)
Mod anemia:8-9.4	61(7.63)
Severe anemia:≤7.9	27(3.38)
History of TB	
No	721(89.01)
Yes	89(10.89)
Smoking	
No	540(79.30)
Yes	141(20.70)
Alcohol no	529(78.49)
yes	145(21.51)
Body Mass Index	
Normal:18.5-24.99	327(48.16)
Underweight:<18.5	55(8.10)
Overweight:25-29.99	181(26.66)
Obese: ≥30	116(17.08)
Occupation status	
Unemployed	492(67.86)
employed	233(32.14)
Education level	
Illiterate	21(2.95)
1^0 school	125(17.53)
2^{nd} school	553(77.56)
Tertiary school	14(1.96)
Aspatate transaminase level	
normal	523(66.29)
Above normal	266(33.71)
Alanine aminotransferase normal	761(94.77)

Above normal	42(5.23)

Key: *n(%) for categorical variables and (mean)standard deviation for continuous variable*.

3.3: Baseline characteristics of study sample

The mean age of the study sample was 38 (std 8.7) years and mean CD4 cell count was 159 (std 118). The majority of study participants were female 530(84%), were on Stavudine based regimen 720 (88%), had normal body mass index 327(48%), normal hemoglobin level 577(72%) and in WHO clinical stage 1 598(74%). It should be noted that no participant was classified as WHO clinical stage 4 at the time of initiation of treatment.

Table 5:

Comparison of discordant and concordant responders defined at 6-9 months

characteristics	**(VL^+/ CD4+) responders**	**(VL^+/ $CD4^-$) responders**	**p-value ψ**
Age(years)	(38.0)8.6	(39.5)8.9	0.0276
Age groups(years)			
16-24	18(3.05)	6(2.74)	
25-44	454(76.95)	156(71.23)	
45-64	114(19.32)	56(25.57)	0.279
≥65	4(0.68)	1(0.46)	
Gender: female	392(66.44)	138(62.73)	
male	18(33.56)	82(37.27)	0.323
HAART D4T-3TC-EFV			
D4T-3TC-NVP	532(90.17)	188(85.45)	
AZT-3TC-EFV			
AZT-3TC-NVP	40(6.78)	27(12.27)	
3TC-TDF-EFV			0.037
3TC-TDF-NVP	18(3.05)	5(2.27)	

Baseline CD4 count(cells/ µL)	(137.1)84.6	(218.7)167.9	0.0001
Baseline CD4 count			
<200 cells/ µL	462(78.56)	122(55.45)	
>200 cells/ µL	128(21.69)	98(44.55)	0.0001
WHO clinical stage			
1	434(73.56)	164(74.55)	
2	5(0.85)	4(1.82)	
3	151(25.59)	52(23.64)	0.444
Hemoglobin(g/dl)			
Normal: ≥11	415(71.31)	162(74.31)	
Mild anemia:9.5-10.9	104(17.87)	31(14.22)	
Mod anemia:8-9.4	38(6.53)	23(10.55)	
Severe anemia:≤7.9	25(4.30)	2(0.92)*2	0.017
History of TB			
No	529(89.66)	192(87.27)	
Yes	61(10.34)	28(12.73)	0.33
Smoking			
No	393(79.23)	147(79.46)	
Yes	103(20.77)*94	38(20.54)*35	0.948
Alcohol no	390(78.95)	139(77.22)	
yes	104(21.05)*96	41(22.78)*40	0.630
Body Mass Index			
Normal:18.5-24.99	234(46.15)	93(54.07)	
Underweight:<18.5	40(7.89)	15(8.72)	
Overweight:25-29.99	147(28.99)	34(19.77)	
Obese: ≥30	86(16.96)*83	30(17.44)*48	0.117
Occupation status			
Unemployed	364(69.20)	128(64.32)	
employed	162(30.80)*64	71(35.68)*21	0.209
Education level			

Illiterate		12(2.30)	9(4.59)	
1^0 school		92(17.66)	33(17.19)	
2^{nd} school		404(77.54)	149(77.60)	
Tertiary school		13(2.50)*69	1(0.52)*28	0.138
Aspatate transaminase level	normal	387(67.19)	136(63.85)	
Above normal		189(32.81)*14	77(36.15)*7	0.379
Alanine aminotransferase	normal	552(94.52)	209(95.43)	
Above normal		32(5.48)*6	10(4.57)*1	0.605

Key: n(%) for categorical variables and (mean)standard deviation for continuous variable.

*Missing values:**

***Ψ-** p-values were obtained using chi ^{2} test and student ttest*

Discordant immune responders where more likely to be older when compared to concordant responders. In addition concordant responders were more likely to be on a Stavudine based HAART regimen when compared to discordant immune responders. However discordant immune responders were more likely to be on a Zidovudine and Tenofovir based regimens than concordant responders.

Virologic only responders were more likely to initiate HAART with a higher baseline CD4 cell count than concordant responders.

Moreover there was a significant difference in the hemoglobin level at initiation of HAART. Discordant immune responders were more likely to initiate HAART with moderate anemia and concordant responders were more likely to have initiated treatment with a normal hemoglobin level.

However there was no difference among the groups with respect to the following variables:

Gender, history of Tuberculosis, smoking, WHO clinical stage, alcohol use, occupation status, level of education, body mass index and liver enzymes (AST, ALT).

3.4: Risk factors associated with poor CD4 response

Table 6:

Univariate and Multivariate analysis of factors associated with discordant immune response.

Variable	Univariate model			Multivariate model		
	Unadjusted Odds ratio	(CI)	P-value	adjusted OR	(CI)	P-value
Age(years)	1.02	(1.00-1.04)	0.028	1.02	(1.00-1.04)	0.031
Gender: female	1					
male	1.18	(0.85-1.66)	0.323			
HAART D4T-3TC-EFV						
D4T-3TC-NVP	1					
AZT-3TC-EFV						
AZT-3TC-NVP	1.91	(1.14-3.20)	0.014			
3TC-TDF-EFV						
3TC-TDF-NVP	0.78	(0.29-2.15)	0.639			
Baseline CD4 count						
<200 cells/ µL	1			1		
>200 cells/ µL	2.90	(2.08-4.03)	0.0001	3.02	(2.08-4.38)	0.001
WHO clinical stage						
1	1					
2	2.12	(0.56-7.98)	0.268			
3	0.91	(0.63-1.31)	0.616			
Hemoglobin(g/dl)						
Normal: ≥11	1			1		
Mild anemia:9.5-10.9	0.76	(0.49-1.19)	0.230	0.88	(0.54-1.45)	0.629

Mod anemia:8-9.4		1.55(0.90-2.68) 0.117	2.30(1.26-4.19)0.007
Severe anemia:≤7.9		0.20(0.05-0.88)0.032	0.36(0.08-1.63)0.188
History of TB			
No		1	
Yes		1.26(0.78-2.04)0.335	
Smoking			
No		1	
Yes		0.99(0.65-1.50) 0.948	
Alcohol no		1	
yes		1.11(0.73-1.67)0.630	
Body Mass Index			
Normal:18.5-24.99		1	
Underweight:<18.5		0.94(0.50-1.79)0.854	
Overweight:25-29.99		0.58(0.37-0.91)0.017	
Obese: ≥30		0.88(0.54-1.41)0.594	
Occupation status			
Unemployed		1	
employed		1.25(0.88-1.76)0.210	
Education level			
Illiterate		1	
1^0 school		0.48(0.18-1.24) 0.124	
2^{nd} school		0.49(0.20-1.19)0.116	
Tertiary school		0.10(0.01-0.93) 0.043	
Aspatate transaminase level	normal	1	
Above normal		1.16(0.83-1.61)0.379	
Alanine aminotransferase normal		1	
Above normal		0.83(0.40-1.71)0.605	

3.4.1: Univariate analysis

In the univariate analysis a ten year increase in age was significantly associated with 2 percent greater chance of developing discordant immune response (unAOR=1.02,p=0.028). Also, patients initiating treatment on Zidovudine based regimen had 1.9 greater odds of developing a discordant immune response compared to those initiating on Stavudine based regimen (p=0.014). Being on a Tenofovir based regimen was shown to be protective against developing a discordant immune response though this was not statistically significant (p=0.639). Initiating treatment with a baseline CD4 cell count greater than 200 was shown to be associated with a 2.9 greater odds of developing discordant immune response when compared to those with initial CD4 cell count less than 200(p<0.0001).

In the univariate analysis initiating treatment with severe anemia was significantly associated with an 80% reduced odds of developing a discordant immune response (p=0.032) compared to those who had normal hemoglobin. A similar trend was noted in those who initiated treatment with mild anemia as it was associated with a 25 % reduced odds of developing discordant immune response though this was not statistically significant (p=0.230). A contrasting trend was noted in those who initiated treatment with moderate anemia as there were associated with just over 50% greater odds of discordant immune response compared to those who had a normal hemoglobin, though this was not significant (p=0.117).

In addition, the univariate analysis showed a significant protective association of developing discordant immune response in patients who had an initial BMI class of overweight when compared to those who had normal BMI (OR=0.58, p=0.017), however the other BMI classes did not show a significant association.

Moreover, the univariate analysis showed a 90% reduced odds of developing discordant immune response in patients with tertiary level of education compared to those with were illiterate (p=0.043). However there was no significant association between developing immunologic discordant response and the other levels of education. No significant relationship was demonstrated with regards to the other variables in univariate analysis.

3.4.2: Multivariate analysis

In our final model, we adjusted for WHO clinical stage, baseline CD4 count, education level and HAART regimen. The latter three variables had been significant in the univariate analysis. Precision of estimate for odds ratios (OR) in the logistic regression model was at the 5 percent significance level (p=0.05)

Age was significantly associated with the development of discordant immune response. A ten year increase in age was associated with a 2 percent greater chance of developing discordant immune response (AOR=1.02,p=0.031).

Also accounting for the other factors in the model, initiating treatment with a baseline CD4 count above 200 cells/ µL was significantly associated with a three times greater odds of developing discordant immune response when compared to the odds of those who initiated treatment with a CD4 count of less than 200 cells/ µL (p<0.0001).

Initiating treatment with baseline moderate anemia was significantly associated with 1.6 greater odds of developing discordant immune response accounting for the other variables in the model when compared to the odds those who initiated treatment with normal baseline hemoglobin. However there was no significant association with the other classes of anemia after controlling for confounding.

3.5: Interaction

Interaction was investigated between all significant variables in the final model but none of the interaction terms were significant.

3.6: Model assessment

the final model was assessed by the Hosmer Lemeshow goodness of fit test and were noted to be adequate. In addition a test for trend in baseline hemoglobin was investigated and no trend was established.

3.7: Sensitivity analysis

Table 7

Univariate and Multivariate sensitivity analysis of factors associated with discordant immune responders

Variable	Univariate model		Multivariate model	
	Odds ratio (CI)	P-value	AOR (CI)	P-value
Age(years)	1.02 (1.00-1.04)	0.059	1.02(1.00-1.04)	0.030
Age groups(years)				
16-24	1			
25-44	1.18 (0.43-3.23)	0.740		
45-64	1.72 (0.61-4.86)	0.305		
≥65	0.95(0.09-10.50)	0.967		
Gender: female	1			
male	1.09 (0.79-1.52)	0.594		
HAART D4T-3TC-EFV				
D4T-3TC-NVP	1			
AZT-3TC-EFV				
AZT-3TC-NVP	2.00(1.19-3.37)	0.009		
3TC-TDF-EFV				
3TC-TDF-NVP	1.11(0.43-2.87)	0.820		
Baseline CD4 count(cells/ µL)	1.01(1.01-1.02)	0.0001		
Baseline CD4 count				
<200 cells/ µL	1		1	
≥200 cells/ µL	5.51(3.91-7.77)	0.0001	5.31(3.71-7.61)	0.0001

WHO clinical stage	
1	1
2	1.33(0.33-7.78)0.686
3	0.89(0.61-1.30) 0.023
Hemoglobin(g/dl)	
Normal: ≥11	1
Mild anemia:9.5-10.9	0.52(0.32-0.85) 0.009
Mod anemia:8-9.4	0.91(0.50-1.66) 0.011
Severe anemia:≤7.9	0.09(0.01-0.73)0.024
History of TB	
No	1
Yes	0.90(0.54-1.51)0.694
Smoking	
No	1
Yes	1.01(0.66-1.55) 0.962
Alcohol no	1
yes	1.07(0.70-1.64)0.743
Body Mass Index	
Normal:18.5-24.99	1
Underweight:<18.5	0.70(0.35-1.39)0.311
Overweight:25-29.99	0.52(0.33-0.82)0.005
Obese: ≥30	0.80(0.49-1.31)0.372
Occupation status	
Unemployed	1
employed	1.04(0.73-1.49)0.832
Education level	
Illiterate	1
1^0 school	0.35(0.13-0.90) 0.029
2^{nd} school	0.35(0.14-0.84)0.019
Tertiary school	0.18(0.03-1.03) 0.054
Aspatate transaminase level	
normal	1
Above normal	0.86(0.61-1.22)0.409
Alanine aminotransferase	
normal	1
Above normal	0.79(0.37-1.68)0.544

We conducted sensitivity analysis by varying immune response and considering a 30 percent increase from baseline CD4 as the desired response. The univariate analysis produced similar findings to those in the original analysis. However, only baseline hemoglobin level failed to be a significant risk factor for development of discordant response as in the original analysis.

Chapter four: Discussion

4.1 Introduction

The main objective of this study was to describe the burden of discordant immune response and identify the risk factors associated with discordant immune response to antiretroviral therapy at public sector clinics in South Africa.

The results of our study showed the prevalence of discordant immune response to be 24% and concordant response to be 65%. These findings are similar to those obtained by several studies (2, 12, 14, 35, 36). Discordant immune response was shown to be associated with age, higher baseline CD4 cell count and moderate anemia.

4.2 Representativeness of study sample

4.2.1 Baseline characteristics of those with missing data at 6 months and those eligible for analysis.

This study found that individuals who had missing data at 6 months were more likely to be younger on average of 37.3 years. This finding was similar to the one described by Dahab et al in South African cohort receiving HAART from public sector clinics were the average age was 37.5 years(std=37). However our study did not show that level of education as being associated with those lost to follow-up as Dahab et al showed.

Also those with missing data at 6 months were more likely to have a higher baseline CD4 cell count than those eligible for analysis. In addition those with missing data at 6 months were less likely to have had a history of Tuberculosis or use of alcohol than those eligible for analysis. These findings show that those with missing data at 6

months were more likely to have been of relatively better health than those eligible for analysis which is consistent with literature showing that individuals who view themselves as well are less likely to adhere to treatment than those who start treatment at more advanced state of poor health(37). This could have resulted in the study sample consisting mainly of older individuals of poorer health biasing our results.

The above could have resulted in selection bias as those who were likely to develop the outcome of interest were eligible for analysis.

4.2.2 Baseline characteristics of the study sample

Discordant responders were more likely to be older than concordant responders and this could have biased the results though it was adjusted for in the multivariate model.

There was a significant difference regarding HAART regimen among the groups of responders. Discordant immune responders were more likely to be on Zidovudine and Tenofovir based HAART regimens than concordant responders. In addition, discordant immune responders were more likely to have initiated treatment with moderate anemia than concordant responders.

Discordant immune responders were more likely to have a higher baseline CD cell count when compared to concordant responders.

All the above is congruent with literature were these factors have been associated with discordant response, except for moderate anemia. This will be explained further below.

4.3 Systematic overview of study findings

4.3.1 HAART regimen as a risk factor for discordant immune response

Our study showed that individuals on Zidovudine based HAART regimen were independently associated with1.9 greater odds of developing discordant immune response. This is in line with literature which shows that use of myelotoxic drugs like Zidovudine, Didanosine and Cotrimaxazole is associated with discordant immune response (2). However this was not significant after adjusting for other confounders.

4.3.2 Age as a risk factor for discordant immune response

After adjusting for potential confounders, a ten year increase in age was significantly associated with development of discordant immune response. This is similar to the findings of other studies were older age was associated with this outcome (5, 11, 14, 36). This has been linked to the immune restoration which is largely dependent on a decreasing thymus activity with aging.

4.3.3 Baseline CD4 cell count as a risk factor for discordant immune response

Our study also showed that initiating treatment with a high baseline CD4 cell count was associated with development of discordant immunologic response. This finding is marred by conflicting findings in literature. In a study on the largest cohort of patients receiving HAART in resource poor settings (ART-LINC study) similar findings to our study were found (21). In addition another study from resource-rich setting, the UK-CHIC study also showed similar results to ours though discordant response was assessed at 8 months after initiating treatment (31). However most studies in resource-rich settings show contrary findings as low baseline CD4 cell count is associated with discordant immune response (2). The latter finding has gained biological plausibility as a low nadir pre-treatment CD4 cell count is

suggestive of more extensive depletion of CD4 cells in the gut-associated lymphoid tissue during acute HIV infection, which may be delayed or refractory to reconstitution with antiretroviral therapy (23). In addition genetic variability has also been investigated as a possible modulator of immunological recovery. However, the association between initiating treatment with a high baseline CD4 cell count and development of discordant immune response can be explained by the nonlinear nature of CD4 cell count increases after HAART initiation across the different baseline CD4 count strata (2). Another reason cited is that starting treatment at higher CD4 cell counts limits the scope for further rise (31).

4.3.4 Baseline hemoglobin level as a risk factor for discordant immune response

To our knowledge our study is the first to describe of an association between baseline hemoglobin level and discordant immune response. In our study moderate anemia classified as hemoglobin of between 8-9.4g/dl was associated with 1.6 greater odds of developing a discordant immune response. The aetiology of anemia in HIV infection is multifactorial but is commonly due to underproduction of erythrocytes by the bone marrow stem cells (38). These stem cells are also responsible for production CD4 cells through the myeloid precursor cell (39). In addition erythrocytes of HIV infected individuals experience membrane changes which result in decreased fluidity of the cells leading to their premature destruction (40). This mechanism of membrane changes is similar to the one experienced by CD4 cells which also result in their destruction. These two mechanisms of poor production and membrane changes could explain the discordant immune response experienced by those with anemia when starting HAART.

Anemia is common in HIV infection, mainly in advanced disease states (38). The fact that patients who were eligible for our analysis were of relatively advanced stage of

immunosuppression compared to those not analyzed (missing data at 6 months) could have biased our study to significance. In addition our analysis group had an older population compared to those with missing data at 6 months.

However this finding should be viewed with caution as it failed to remain significant in the sensitivity analysis when definition of immune response was altered.

4.4 Study limitations

4.4.1 Technical limitations

Our study in particular had limitations which are inherent to secondary data analysis. These include errors in the data entry which because of the nature of the study could not be rectified. These errors in data entry could have resulted in non-differential misclassification of patients as outcomes could be inaccurately measured. This could have bias the results towards the null. However such errors could have been evenly distributed among the groups and their influence on the results would have been minimal.

Another problem plaguing secondary data analysis is that of missing data. However since this was at random, the influence on the results would have been minimal (see Table 4).

4.4.2 Epidemiological limitations

Selection bias could have influenced the findings in our study as only those presenting for follow up visits were available for analysis. There was also systematic difference between those eligible for analysis and those with missing data at 6 months as suggested by the greater proportion of those analyzed being of more advanced immune suppression than those with

missing data. This could have biased the results as those analyzed were more likely to develop the outcome of interest.

The outcomes in this study were measured at between 3-6 months after initiation of treatment. It is possible that factors associated with discordant immune response at 3 months could be different to those at 6 months. It is unfortunate that due to the secondary nature of the data, we were unable to explore this possibility which could potentially influence our results. Similarly, we were unable to explore factors associated with discordant immune response at later times which also affects the generalizability of our findings.

4.4.3 Statistical limitations

We had a study sample of 810 participants. This small sample could have influenced our study findings. However we feel our sample was comparable to most studies done elsewhere.

4.5 Generalizability

The major limitations in interpreting and generalizing our study results are the following:

4.5.1 Variation in the study designs

Study designs have been mainly secondary data analysis of prospectively collected data, prospective cohort studies and observational studies. All these studies are all plagued with their own flaws.

4.5.2 Variations in study population

Studies have also included treatment experienced (41), treatment naïve and at times mixed patients. In addition different studies have used patients who

have either used NRTI, NNRTI or have switched from a PI based regimen (42).

4.5.3 Variation in the sample size

Sample sizes have varied from as little as 162 by Piketty et al(43) to as much 2 584 in the UK-CHIC study (31).

4.5.4 Variation in the definition of outcomes

The definition of good virologic response varied from 400- 1000copies/ml(41, 42, 44) or 1 $\log_{10}$ copies/ml(41) decrease from baseline was used. The definition for CD4 count response also varied from 50-500cells/ul increase in CD4 cell count in some studies (11).

4.5.5 Variation in the timing after HAART Initiation

The timing after the initiation of HAART has ranged from 6 months – three year (45), hence the optimal time for assessing discordance is still unknown.

4.6 Residual confounding.

All factors of interest were initially examined in a univariate model to determine their association with development of discordant immune response. Significant factors at P≤0.2 were included in the multivariate logistic regression model where each factor of interest controlled for the confounding effect of the other. This method adjusted for confounding effect of several factors at the same time. Factors significant at the 5% level were then considered significant risk factors for development of discordant immune response.

However, despite controlling for confounding, the residual confounding effect in the association between the risk factors identified and discordant immune response remains a possibility. This is because we did not examine or measure all factors associated with discordant immune response such as co-infection

with HTLV-1, GB virus C and genetic factors. This information was lacking in our database.

4.7 Study strengths

- Our sample size was large enough to have detected any significant findings.
- Our study population was from two clinics from a densely populated area in the biggest district of Gauteng province making our study findings generalizable to many populations.
- Our study used routine clinical data from Resource Limited Settings which make our findings applicable to adults receiving antiretroviral therapy at urban clinics in South Africa.

4.8 Suggestion for further studies

Our study showed a clinically significant association between baseline CD4 cell count and discordant immune response. Moreover, it showed an association with baseline anemia which had not been described before to our knowledge. This finding will need further studies to confirm it as a risk factor which can be of use in resource-poor settings where it can be used for predicting patients who may develop the discordant immune response.

Chapter five: Conclusion and Recommendations

5.1 Conclusions

In conclusion, our study showed that the prevalence of early discordant immune response was 24 % and that of concordant response to be 65%. Our study also showed that age, baseline CD4 count and baseline hemoglobin level were significantly associated with development of discordant immune response after 6 months of initiating treatment.

However no association was observed between gender, body mass index and HAART regimen which have been observed in other studies.

5.2 Key points

- Age is significant risk factor for developing discordant immune response after six months of HAART.
- Baseline CD4 cell count is significant risk factor for developing discordant immune response after six months of HAART.
- Initiating HAART with baseline moderate anemia is significantly associated with development of discordant immune response six months on.

5.3 Summary

Some studies have shown an association between discordant immune response and gender or body mass index. It should be noted that these studies had small sample sizes and did not measure other known confounders. Even though our study failed to demonstrate a significant association between HAART regimen and discordant

immune response in the multivariable model, its significance has been shown in several studies. This could be due few patients initiating treatment on Zidovudine based regimen. However Zidovudine based regimen was independently associated with discordant immune response.

Regardless of the above, we feel our study findings outlined above are valid and unlikely to be due to chance, sampling bias or residual confounding.

5.4 Recommendations

5.4.1 Clinical management

It is currently recommended that patients with discordant immune response on a new HAART regimen maintain their current regimens. Changing or intensifying the regimen has not been shown to have an effect on the CD4 cell count. Benefit has however been observed in changing those on leucopenic antiviral drugs like Zidovudine or Didanosine. Administration of human recombinant growth hormone has also been shown to be of benefit in chronically infected patients. In addition use of concomitant medications which are myelosuppressive such as Cotrimoxazole can be interrupted or substituted to confer some benefit to these patients.

In view of the findings of our study we recommend that clinicians use hemoglobin which is available in resource-poor settings to predict patients who are likely to develop discordant immune response. These patients are at increased risk of developing an AIDS event compared to those with a concordant response hence require frequent clinical visits to detect opportunistic infections early.

Moreover we recommend for the development of a standardized definitions of discordant responses to permit for meaningful comparisons between studies to be made.

REFERENCES

1. Hsu DC, Quin JW. Clinical audit: virological and immunological response to combination antiretroviral therapy in HIV patients at a Sydney sexual health clinic. Intern Med J. 2010 Apr;40(4):26574.

2. Schechter M, Tuboi SH. Discordant immunological and virological responses to antiretroviral therapy. J Antimicrob Chemother. 2006 Sep;58(3):506-10.

3. Moore DM, Hogg RS, Yip B, Wood E, Tyndall M, Braitstein P, et al. Discordant immunologic and virologic responses to highly active antiretroviral therapy are associated with increased mortality and poor adherence to therapy. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2005;40(3):288-93.

4. Collazos JA, V .Antonio Carto,J. CD4 Responses in the Setting or Suboptimal Virological Responses to Antiretroviral Therapy:Features, Outcomes, and Associated Factors. AIDS RESEARCH AND HUMAN RETROVIRUSES [serial on the Internet]. 2009 [cited 2010 20 0ctober]; 25: Available from: http://www.ncbi.nlm.nih.gov/pubmed/19621987.

5. Piketty C, Weiss L, Thomas F, Mohamed AS, Belec L, Kazatchkine MD. Long-term clinical outcome of human immunodeficiency virus-infected patients with discordant immunologic and virologic responses to a protease inhibitor-containing regimen. J Infect Dis2001;183(9):1328-35.

6. Montaner JS, Hogg R, Wood E, Kerr T, Tyndall M, Levy AR, et al. The case for expanding access to highly active antiretroviral therapy to curb the growth of the HIV epidemic. Lancet. 2006 Aug 5;368(9534):531-6.

7. Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, Freedberg KA. Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. Clin Infect Dis. 2003 Mar 1;36(5):652-62.

8. Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science. 1996 May 24;272(5265):1167-70.

9. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med. 1997 Jun 15;126(12):946-54.

10. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995 Jan 12;373(6510):123-6.

11. Moore DM, Hogg RS, Yip B, Wood E, Tyndall M, Braitstein P, et al. Discordant immunologic and virologic responses to highly active antiretroviral therapy are associated with increased mortality and poor adherence to therapy. J Acquir Immune Defic Syndr. 2005 Nov 1;40(3):288-93.

12. Grabar S, Le Moing V, Goujard C, Leport C, Kazatchkine MD, Costagliola D, et al. Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy. Ann Intern Med. 2000 Sep 19;133(6):401-10.

13. Nicastri E, Chiesi A, Angeletti C, Sarmati L, Palmisano L, Geraci A, et al. Clinical outcome after 4 years follow-up of HIV-seropositive subjects with incomplete virologic or immunologic response to HAART. J Med Virol. 2005 Jun;76(2):153-60.

14. Marimoutou C, Chene G, Mercie P, Neau D, Farbos S, Morlat P, et al. Prognostic factors of combined viral load and CD4+ cell count responses under triple antiretroviral therapy, Aquitaine cohort, 1996-1998. J Acquir Immune Defic Syndr. 2001 Jun 1;27(2):161-7.

15. Sufka SA, Ferrari G, Gryszowka VE, Wrin T, Fiscus SA, Tomaras GD, et al. Prolonged CD4+ cell/virus load discordance during treatment with protease inhibitor-based highly active antiretroviral therapy: immune response and viral control. J Infect Dis. 2003 Apr 1;187(7):1027-37.

16. Garcia F, Vidal C, Plana M. Residual low-level viral replication could explain discrepancies between viral load and CD4+ cell response in human immunodeficiency virus-infected patients receiving antiretroviral therapy. Clin Infect Dis. 2000;30:392–4.

17. Benveniste, Flahault A, Rollot F, Elbim C, Estaquuier J, Pedron B, et al. mechanisms involved in the low-level regeneration of cd4 cells in HIV 1 infected patients recieving highly active antiretroviral

therapy who have prolonged undetecable plasma viral loads. Journal of Infectious Diseases. [MAJOR]. 2005;191:1670-9.

18. Mezzaroma I, Carlesimo M, Pinter E, Alario C, Sacco G, Muratori DS, et al. Long-term evaluation of T-cell subsets and T-cell function after HAART in advanced stage HIV-1 disease. AIDS. 1999 Jul 9;13(10):1187-93.

19. Fleury S, Pantaleo G. Tcell regeneration in HIV-infected subjects under highly active antiretroviral therapy. Int J Mol Med. 1999;4:91-7.

20. Anthropometric and immunlogical success of antiretroviral therapy and prediction of virological success in west african adults [database on the Internet]. world health organization. 2008 [cited 28/12/2010]. Available from: http://www.who.int/bulletin/volumes/86/6/07-042911/en/.

21. Tuboi SH, Brinkhof MW, Egger M, Stone RA, Braitstein P, Nash D, et al. Discordant responses to potent antiretroviral treatment in previously naive HIV-1-infected adults initiating treatment in resource-constrained countries: the antiretroviral therapy in low-income countries (ART-LINC) collaboration. J Acquir Immune Defic Syndr. 2007 May 1;45(1):52-9.

22. Tan R, Westfall AO, Willig JH, Mugavero MJ, Saag MS, Kaslow RA, et al. Clinical outcome of HIV-infected antiretroviral-naive patients with discordant immunologic and virologic responses to highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2008 Apr 15;47(5):553-8.

23. Brenchly JM, Schaker TW, Ruff LE. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med. 2004;200:749-59.

24. Torti C, Quiros-Roldan E, .Scudeller L. Characterization of viro-immunological responses in a closely followed cohort of heavily pretreated patients:evidence from the GenPheRex Study. HIV Med. 2003;4: 263–70.

25. Montaner JS, Reiss P, Cooper D, Vella S, Harris M, Conway B, et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients:

the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA. 1998 Mar 25;279(12):930-7.

26. Fernandez S, Rosenow AA, James IR. Recovery of CD4 T cells in HIV patients with a stable virologic response to antiretroviral therapy is associated with polymorphisms of interleukin 6 and central major histocompatibility complex genes. J Acquir Immune Defic Syndr. 2006;41:1-5.

27. Solomon A, Cameron PU, Bailey M. Immunological and virological failure after antiretroviral therapy is associated with enhanced peripheral and thymic pathogenicity. J Infect Dis 2003;187 1915-23. .

28. Negredo E, Molto J, Burger D, Viciana P, Ribera E, Paredes R, et al. Unexpected CD4 cell count decline in patients receiving didanosine and tenofovir-based regimens despite undetectable viral load. AIDS. 2004 Feb 20;18(3):459-63.

29. Barrios A, Rendon A, Negredo E. Paradoxical CD4+ T-celldecline in HIV-infected patients with complete virus suppression taking tenofovir and didanosine. AIDS. 2005; 19: 569–75.

30. Mehta S, Lucas G, Astemborski J. Discordant responses to HAART and clinical outcomes among injection drugs users in Baltimore, Maryland.Programs the Thirteenth Conference on Retroviruses and Opportunistic Infections; Denver: Foundation for Retrovirology and Human Health, Alexandra, VA, USA; 2006.

31. Gilson RJ, Man SL, Copas A, Rider A, Forsyth S, Hill T, et al. Discordant responses on starting highly active antiretroviral therapy: suboptimal CD4 increases despite early viral suppression in the UK Collaborative HIV Cohort (UK CHIC) Study. HIV Med. 2010 Feb;11(2):152-60.

32. Africa NDoHS. 2008 national antenatal sentinel HIV & Syphilis prevalence survey2008: Available from: http://www.doh.gov.za/docs/reports/2009/nassps/foreword.pdf.

33. Africa. NDoHS. National antiretroviral therapy guidelines.South Africa2004.

Nations, U. (1982). provisional guidelines on standard international age classification. New York**:** 6.

34. Maskew M. gender differences in clinical and immunological outcomes in South african HIV infected patients on HAART [masters]. johannesburg: University of The Witwatersrand; 2008.

35. Mehta SH, Lucas G, Astemborski J, Kirk GD, Vlahov D, Galai N. Early immunologic and virologic responses to highly active antiretroviral therapy and subsequent disease progression among HIV-infected injection drug users. Aids Care-Psychological and Socio-Medical Aspects of Aids/Hiv. 2007;19(5):637-45.

36. Nicastri E, Chiesi A, Angeletti C, Sarmati L, Palmisano L, Geraci A, et al. Clinical outcome after 4 years follow-up of HIV-seropositive subjects with incomplete virologic or immunologic response to HAART. Journal of Medical Virology. 2005;76(2):153-60.

37. Dahab M, Charalambous S, Karstaedt AS. Contrasting predictors of poor antiretroviral therapy outcomes in two South African HIV programmes: a cohort study. BMC Public Health. 2010;10:430.

38. Moore RD, Forney D. Anemia in HIV-infected patients receiving Highly Active Antiretroviral Therapy. J Acquir Immune Defic Syndr. 2002;29:54-7.

39. Calles NR, Evans D, Terlounge D. Pathophysiology of the human immunodeficiency virus. HIV curriculum for health professionals2009. p. 13.

40. Martins-Silva J, Nuno C, Santos M. Changes in blood cells membrane properties in HIV-Type 1infected patients. AIDS Res Hum Retroviruses. 2006;22:849-53.

41. Florence E, Lundgren J, Dreezen C, Fisher M, Kirk O, Blaxhult A, et al. Factors associated with a reduced CD4 lymphocyte count response to HAART despite full viral suppression in the EuroSIDA study. HIV Med. 2003 Jul;4(3):255-62.

42. Kaufmann GR, Perrin L, Pantaleo G, Opravil M, Furrer H, Telenti A, et al. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. Arch Intern Med. 2003 Oct 13;163(18):2187-95.

43. Piketty C, Weiss L, Thomas F, Mohamed AS, Belec L, Kazatchkine MD. Long-term clinical outcome of human immunodeficiency virus-infected patients with discordant immunologic and virologic responses to a protease inhibitor-containing regimen. J Infect Dis. 2001 May 1;183(9):1328-35.

44. Kaufmann GR, Furrer, H. Ledergerber B, et al. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/mL in HIV type 1-infected individuals receiving potent antiretroviral therapy. Clin Infect Dis. 2005;41: 361–72.

45. Piketty C, Castiel P, Belec L, Batisse D, Si Mohamed A, Gilquin J, et al. Discrepant responses to triple combination antiretroviral therapy in advanced HIV disease. AIDS. 1998 May 7;12(7):745-50.

Appendices