

## **A Comparison of Haematological Parameters in a Botswana HIV Cohort in which Cardiovascular Events were Common.**

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### **Abstract**

*The study was a comparison between the haematological profile parameters of Human Immunodeficiency Virus (HIV) positive patients who developed a cardiac event and those who did not. The aim was to assess the possibility of using haematological parameters as predictors of the risk of developing a cardiovascular event in a Botswana HIV positive cohort. Elevated white blood cell (WBC) count, neutrophil (NEUT), CD8 T cell count (CD8) and decreased lymphocyte (LYMPH), CD4 T cell count (CD4) and CD4/CD8 ratio have been associated with cardiovascular risk. Whether this association is present in an HIV positive cohort in Botswana is uncertain. Differences in the haematological parameters of HIV positive patients who developed a cardiac event and of those who did not were compared at 3 different time points using the Mann Whitney U Test. A total of 9 cases and 34 controls were studied. At baseline, cases had higher WBC counts with a mean rank of 25.86 compared to controls (16.04) with  $p$  value of 0.012. NEUT mean rank of 24.58 for cases was higher than 13.23 for controls ( $p=0.002$ ). At 6 months into study and at occurrence of event, the differences were not statistically significant. Study shows that at baseline, WBC and NEUT are higher for patients likely to develop a cardiac event hence can be a potential risk predictor.*

### **Abbreviations**

AIDS	Acquired Immuno-deficiency Syndrome
AMI	Acute Myocardial Infarction
ARV	Antiretroviral
ART	Antiretroviral Treatment
CAD	Coronary Artery Disease
CD4	CD4+ T-cell
CD8	CD8+ T-cell
CHD	Coronary Heart Disease
CVD	Cardiovascular Diseases
FBC	Full Blood Count
HAART	Highly Active Antiretroviral Treatment
HIV	Human Immunodeficiency Virus
LYMPH%	Lymphocyte percentage
MI	Myocardial Infarction
NADEs	Non-AIDS defining events
NCDs	Non Communicable Diseases
PAD	Pulmonary Artery Disease
WBC	White Blood Cell

### **Introduction**

The many advances in the treatment of HIV/AIDS by HAART has seen it transition from being a death sentence to a manageable chronic condition (Teague, n.d.). HIV medications and treatments have significantly changed the natural progression of HIV infection (AIDS.gov, n.d.). Highly Active Anti-retroviral Treatment (HAART) has proven beyond doubt in

successfully preventing HIV infected individuals from progressing to AIDS (Montaner et al., 2010). In Botswana, HIV is now a chronic condition with rare cases of progression to AIDS.

However, like many of the countries in sub-Saharan Africa, non-communicable diseases are on the increase posing a threat of having triple disease burden on the already exhausted primary healthcare systems. HIV infected individuals have been shown to have a higher risk of Acute Myocardial Infarction (AMI) compared to HIV uninfected individuals (Oluwatosin A. Badejo, Chung-Chou Chang, 2015). The rates of Non-AIDS complications have been predicted to increase in the African region thus necessitating enhanced laboratory and diagnostic capacity (Wester et al., 2009).

In a research conducted in Botswana, cardiovascular events were the most common non-AIDS defining events (Wester et al., 2011). Cardiovascular diseases (CVDs) are preventable. Screening and high-quality care in the community can prevent complications due to NCDs and also prevent hospitalizations, strokes and other cardiac events, thus lengthening and improving the quality of life of HIV infected patients. Health care systems thus need to be actively engaged in secondary prevention. In light of this view, there is great need to find an easy, inexpensive readily available diagnostic tool to predict those patients at risk of developing a cardiovascular event.

Inflammation is associated with endothelial dysfunction in treated and untreated HIV infected patients (Cerrato et al., 2015). Inflammation has been demonstrated to be an important risk factor for the development of cardiovascular events with leukocytes playing a major role in these inflammatory processes (Hoffman, Blum, Baruch, Kaplan, & Benjamin, 2004). Monocytes and T lymphocytes have been said to be prevalent and pathogenic within unstable coronary artery plaques (P Libby, 1995; Peter Libby, 2003; Ross, 1999). In patients with HIV, immune reconstitution after antiretroviral therapy (ART) is often incomplete increasing their risk of developing non-AIDS related events (Mussini et al., 2015). Possible mechanisms increasing risk to AMI in HIV positive patients include inflammation and CD4+ cell count depletion. Research has shown that high CD8+ T cell count among HIV infected people was associated with increased acute myocardial infarction risk compared to uninfected people (Oluwatosin A. Badejo, Chung-Chou Chang, 2015).

This study was focused on investigating if components of the Full blood count (FBC), CD4 and CD8 counts, which are widely available tests, could be used to predict the occurrence of a cardiovascular event in a cohort of HAART patients in Botswana. High white blood cell count has been shown to be a strong and independent predictor of coronary risk in patients with or without coronary heart disease (CHD) (Madjid & Fatemi, 2013). Other components of the complete blood count such as platelet count, haemoglobin, red cell count and hematocrit have also been associated with CHD and together with white blood cell count, can be used to predict coronary risk (Madjid & Fatemi, 2013). Low CD4 T cell count and high CD8 T cell count were also significantly associated with an increased risk of MI (Lang et al., 2012). This research investigated if these associations are present in a cohort of HAART patients in Botswana. The aim being to preserve life expectancy of HIV positive patients whose life span is being threatened by NADEs.

## **Literature review**

Botswana was the first African country to establish a national HIV/AIDS treatment programme in 2002, which saw its mortality rate reduce to levels similar to other low and middle income countries (Farahani et al., 2014). An excellent response to HAART was observed in a public pilot ARV treatment program conducted in Botswana (Wester et al., 2005). HAART has successfully reduced HIV infection associated mortality and morbidity among HIV positive patients (Panos et al., 2008) (Ledergerber et al., 1999). However, despite HAART initiation increasing life expectancy, long-term exposure to ARVs can also reduce expected life and quality of life due to toxicities (Gebo, 2006). HIV is now a chronic infection due to ART success but health systems particularly in sub-Saharan region have been designed

to provide acute care and are ill-equipped to provide chronic care (Deeks, Lewin, & Havlir, 2013).

Adverse metabolic complications and negative health outcomes classified as NADEs have emerged as a major health concern among long-term cART-treated adults (Deeks & Phillips, 2009)(Palella Jr. et al., 2006)(Martinez et al., 2007). NADEs include cancer, hepatic disorders, osteoporosis/osteopenia, neurocognitive disease, cardiovascular diseases, hypertension, diabetes and renal disorders. A study which compared cohorts from Botswana to the U.S found cardiovascular events to be the most common NADEs in both settings (Wester et al., 2011). Cardiovascular diseases are one of the most common causes of death worldwide and there is need to continually expand their diagnosis, screening and effective management in HIV infected patients in the sub-Saharan region (Wester et al., 2009). This study focused on cardiovascular events with the aim to investigate several options of improving laboratory diagnosis and prevention of cardiovascular events among HAART patients in Botswana.

Cardiovascular events in the context of this study referred to cerebrovascular accident (stroke), cerebral/subarachnoid haemorrhage, myocardial infarction (MI), coronary artery disease (CAD) and congestive heart failure (Wester et al., 2009). Cardiovascular disease is a term used to refer to different heart or blood vessel problems related to the heart. Atherosclerosis is the most common cause of cardiovascular diseases (Frostegard, 2013) and is a chronic inflammatory disease (Anogeianaki et al., 2011) hence making it slow in progression and cumulative. Nearly all of the cellular elements in the blood, including WBCs, red blood cells (RBCs), and platelets, are involved in the underlying pathogenesis of atherosclerosis (Peter Libby, 2002). It has also been shown that in the presence of atherosclerosis, activated monocytes and neutrophils may release products that promote plaque disruption and subsequent thrombus formation, which may eventually lead to a coronary event (Ernst, 1987; Fuster, 1994). Clearly components of the haematological profile are involved in inflammation and have been associated with coronary heart disease thus making them potential predictors of cardiovascular events. The haematological profile could be a potential predictor of cardiovascular events in a resource poor setting.

### **Relationship of blood vessels and atherosclerotic lesions**

Atherosclerosis involves hardening of the arteries, cholesterol deposits in the arteries and arterial blockages. Cells of artery wall, including endothelial cells, smooth muscle cells, and inflammatory cells are involved in the formation of an atherosclerotic lesion (atheroma). Blood borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being vascular endothelial and smooth muscle cells (Hansson, 2005). T-cells, macrophages and mast cells infiltrate the lesion and are particularly abundant in the shoulder region where the atheroma grows (Jonasson, Holm, Skalli, Bondjers, & Hansson, 1986)(Kovanen, Kaartinen, & Paavonen, 1995)(Stary et al., 1995).

Myocardial infarction (MI) occurs when atheromatous process prevents blood flow through the coronary artery (Hansson, 2005). Plaque rupture can be a reason for an occluding thrombus during an infarction (Hansson, 2005). Plaque rupture exposes pro-thrombotic material from the core of the plaque, phospholipids, tissue-factor and platelet adhesive matrix molecule to the blood. Platelets are the first blood cells to arrive at the scene of endothelial activation (Massberg et al., 2002). Activated endothelial cells express several types of leukocyte adhesion molecules which cause blood cells (lymphocytes and monocytes) rolling along the vascular surface to adhere at the site of activation (Eriksson, Xie, Werr, Thoren, & Lindbom, 2001). A cytokine in the inflamed intima, macrophage colony-stimulating factor, induces monocytes entering the plaque to differentiate into macrophages.

### **Full blood count parameters associated with atherosclerosis**

A full blood count is a haematological parameter used to measure the cellular components of blood and comprises of white cell count and white cell differential count which are neutrophils, monocytes, lymphocytes, eosinophil and basophils. It also measures red cell count, haemoglobin, haematocrit, red cell indices and platelet count. These blood cells have been shown to participate in atherosclerosis hence making them possible predictors of cardiac events. Research has shown association of components of the complete blood count with cardiovascular diseases (S J Turner, Ketch, Gandhi, & Sane, 2008)(Madjid & Fatemi, 2013).

#### **White blood cell count (WBC)**

Total WBC count has been the most investigated prognostic marker of cardiac events (Samuel J Turner, Ketch, Gandhi, & Sane, 2008). Benjamin Horne et al confirmed total WBC count to be an independent predictor of death or MI in patients with or at high risk for Coronary Artery Disease (CAD)(Horne et al., 2005). Elevated WBC was found to be predictive of increased risk of inhospitable mortality (Grzybowski et al., 2004). WBC count has been shown to be able to predict long-term mortality, defined as death occurring greater than 30 days after Acute Coronary Syndrome (ACS) (Cannon, McCabe, Wilcox, Bentley, & Braunwald, 2001)(Bhatt et al., 2003)(Sabatine et al., 2002). Development of cardiogenic shock was associated with elevated WBC counts (Menon et al., 2003). Elevated WBC counts were also a prognostic marker for worse angiographic outcomes (Sabatine et al., 2002). Multiple studies have shown that a high WBC count is associated with increased mortality ratios in patients who present with unstable angina pectoris (Zouridakis, Garcia-Moll, & Kaski, 2000)(Cannon et al., 2001)and acute myocardial infarction (AMI) (Menon et al., 2003)(Mueller, Neumann, Perruchoud, & Buettner, 2003).

#### **Other FBC parameters**

A good predictive power was provided by high neutrophil or low lymphocyte counts, but the greatest risk prediction was achieved by using the neutrophil-to-lymphocyte ratio (Horne et al., 2005). Elevated red blood cell (RBC) count within the upper limits of normal have been reported to have only weak or no association with cardiovascular risk (Puddu et al., 2002). Low haemoglobin and haematocrit values during ACS were associated with increased long-term mortality (Langston, Presley, Flanders, & McClellan, 2003). However, other studies did not show a significant relationship between haematocrit and CHD risk (Carter, McGee, Reed, Yano, & Stemmermann, 1983)(Abu-Zeid & Chapman, 1977)(Lowe, Lee, Rumley, Price, & Fowkes, 1997). No prognostic association was found between platelet count and mortality in patients with a recent MI (Burr, Holliday, Fehily, & Whitehead, 1992). In a cross-sectional study of patients undergoing coronary angiography, the mean platelet volume was higher in MI patients than in control patients, and stable angina pectoris correlated with the extent of CHD.

#### **T Cell profile in cardiovascular events**

The excess risk for AMI in HIV infected persons as opposed to those who are uninfected is in part predicted by immune status in those with HIV infection (Freiberg et al., 2013; Triant, Lee, Hadigan, & Grinspoon, 2007). The CD4/CD8 T- cell ratio provides information on the immune status beyond CD4 count alone(Oluwatosin A. Badejo, Chung-Chou Chang, 2015). In a nested case-control study, Lang and colleagues found out that a high current CD8+ T-cell count is associated with increased AMI risk, independent of cardiovascular risk factors and antiretroviral therapy (Lang et al., 2012). Independently of cardiovascular risk factors and antiretroviral therapy, HIV replication, a low CD4 T-cell nadir and a high current CD8 T-cell count were shown to be associated with an increased risk of MI in HIV-infected individuals (Lang et al., 2012).

By assessing haematological parameters which are independent predictors of cardiac events in other studies, this study aimed at determining their feasibility of predicting the risk of a cardiac event in a Botswana cohort of HIV infected patients.

## Methods

This study compared total WBC count, Lymphocyte percentage (LYMPH%), Neutrophil count (NEUT), CD4 T- cell count, CD8 T-cell count and CD4/CD8 ratio of patients who developed a cardiac event with those who did not. This was an HIV positive Botswana cohort in which the most common NADEs were cardiovascular diseases. The aim of the study was to determine if there is a difference in the haematological parameters of HIV positive patients who developed a cardiac event from those who did not in the duration of the study thus determining possibility of haematological parameters being used to predict the risk of a cardiovascular event.

The study was a retrospective (Hyde, 2004) analysis of a clinical trial cohort. It involved collecting FBC parameters, CD4 count and CD8 count data of patients who developed a cardiac event and comparing with patients who did not develop any cardiac event, in-order to establish an association between these haematological parameters and the occurrence of a cardiac event.

## Study population

This was a nested case-control study within a Botswana cohort of patients enrolled in the completed Adult Antiretroviral Treatment and Drug Resistance study ('*Tshepo*'), which was a clinical trial cohort, conducted between December 2002 and December 2007 (Wester et al., 2010). Study participants were HIV-1 positive adult men and women living within a 20 km radius of the Princess Marina Hospital in Gaborone, Botswana. They were qualified for cART based on the antiretroviral treatment guidelines for Botswana (Health, 2002, 2005). The study was an open-label, randomised combination ARV comparing three main factors in a 3x2x2 factorial design, these factors being: the choice between three NRTI combinations, the choice between two NNRTIs, and the choice between two adherence strategies.

Inclusion criteria was a CD4 cell count of  $\leq 200$  CD4 cells/mm<sup>3</sup> ( $\leq 90$  days prior to randomisation), OR a CD4 cell count between 201-350 CD4 cells/mm<sup>3</sup>, AND a plasma HIV-1 viral load of greater than 55,000 copies/ml, an absolute neutrophil count  $\geq 1.0$  /mm<sup>3</sup>  $\leq 31$  days prior to randomisation. Exclusion criteria was an active AIDS-defining illness or HIV/AIDS-related infection diagnosed within four weeks prior to enrolment for which the participant was not yet on appropriate treatment.

The study was carried out in such a way as to ensure adherence to good clinical practice described in the following documents: 1. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Clinicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996). Ethical review board approval was obtained from the Ministry of Health, Botswana (Health Research Development Committee), the Harvard School of Public Health (Human Subjects Committee), and the Vanderbilt University Institutional Review Board (Wester et al., 2011).

Cases (n=9) were patients who had a first prospectively reported cardiac event between January 2002 and December 2007. NADEs (Cardiac events included) were noted as grade 3 or higher clinical diagnosis using established Division of AIDS (DAIDS) tables for Grading Severity of Adult Adverse Experiences. All NADEs onset and resolution dates were confirmed by study data management staff and were reviewed by coordinating study physicians as needed (Wester et al., 2011). Controls (n=34) were HIV positive patients in the *Tshepo* study who did not develop any cardiac event between January 2002 and December 2007. Controls were matched for age and sex among patients who did not develop a cardiac event during the study. With the aim of having 3 controls per case patient, up to 5 controls per case from the list of patients meeting the matching criteria were randomly selected from the

remaining 641 patients using incidence-density sampling(Lang et al., 2012). Case patients were not eligible as controls up to the onset of a cardiac event.

### **Data collection**

Patients were seen on routine schedule and this included clinic visits and routine laboratory assessments to monitor toxicity, measure study treatment response and provide standard care (Wester et al., 2011). Haematology specimens were collected every month for the first 6 months and every 4 months thereafter. Haematology tests included haemoglobin, haematocrit, mean corpuscular volume (MCV), absolute neutrophil count, lymphocytes and platelets. CD4 specimens were collected at enrolment and every two months after for the duration of the Study.

Results were retrieved from The Botswana Harvard Partnership Data Management Centre database, which provides efficient collection of data, strict quality assurance routines, online reporting facilities, scheduled report distribution, tight security, and confidentiality.

### **Study variables**

The following biological parameters were retrospectively collected for all cases and controls; White blood cell count (WBC), Neutrophil count (NEUT), Lymphocyte % (LYMPH1), CD4 absolute count, CD8 absolute count and CD4/CD8 ratio. The CD4/ CD8 ratio was derived from dividing the CD4 by CD8. The FBC results were analysed using the Sysmex XT-2000i and Sysmex EX-1800 and the CD4 and CD8 counts were analyzed by the Beckman Coulter FACsCalibur.

Results for cases were collected for each parameter at baseline, after 6 months into study and at the time of cardiac event. Results for controls were collected for each parameter at baseline, after 6 months into study and at the time of cardiac event of the corresponding case. The biological parameters at the time of event were collected within 3 months of the case developing a cardiac event.

### **Other variables**

Study variables other than the biological parameters included age, gender and body mass index (BMI). The cases had BMI at baseline and at time of event while for the controls; only BMI at baseline was available.

### **Statistical analysis**

Patient variables are presented as mean ranks for continuous variables. It was of interest in this study to check if the cases differed from the controls in the haematological parameters selected.

Haematological parameters for cases and controls were collected at baseline, after 6 months into study and at time of event. Data was analysed using Microsoft Excel 2003 Data Analysis package. The median of each variable for the cases was compared with the controls. Observations were not normally distributed and definite outliers were present.

Transformation of the variables was not possible because the sample size was too small for analysis by parametric methods. Non parametric methods were the most ideal. Comparison of the median between cases and controls was thus done using the Mann-Whitney U test to determine the U statistic, and p-value determined by testing hypothesis at an  $\alpha$  level of 0.05 using the z test. Comparison of case variables at baseline and at the time of event was done using the Sign test for paired data. Using the w statistic of the Sign test, p values were determined at  $\alpha$  level of 0.05 using binomial distribution.

## **Results**

### **Characteristics of patients**

Of the 650 patients, the study population consisted of 9 case patients, and 34 controls, 3 cases had 5 controls, 4 cases had 4 controls, 1 case had 3 controls and 1 case had no available

data thus was excluded from the analysis. The summary statistics for the cases is shown in Table 1. The average time from baseline to time of cardiac event was 69.56 weeks with the earliest event at 11 weeks and the latest event 156 weeks into the study. Mean CD4 count at baseline for the cases was 203.11 with minimum of 29 and maximum of 262. Generally, there was an increase in the CD4 count in the cases at occurrence of event with an average of 383.37, minimum being 151.58 and maximum 566.14.

The average age of the case patients was 38.94 years, of which 6 were female and 3 were male. Of the all the cases, 5 had a Myocardial Infarction, 3 had a stroke and 1 had heart failure (Table 2). Only 3 of the cases survived to the end of the study. All the males died including 3 of the females leaving 3 female survivors. The mean age of the controls was 32.27 at baseline, with 22 being female and 12 being male.

**Table 1:** Summary Statistic of Cases (n=9.)

	Mean	Std. dev	Min	Max
Time to event (weeks)	69.56	51.48	11	156
Baseline CD4	203.11	69.83	29	262
CD4 at event	383.37	139.36	151.58	566.14
Baseline BMI	26.94	6.03	17.99	35.43
BMI at event	28.27	6.68	18.41	35.61

**Table 2:** Details of Case Outcomes

Event	Frequency	Detail
MI	5	4 died
Stroke	3	1 died
Heart failure	1	1 died

This study was a retrospective study which involved collecting data of events that have already occurred. The advantage was that the patients who experienced a cardiac event were already known making the study inexpensive since the outcome and exposure had already occurred, and so resources were mainly be directed at data collection (Hyde, 2004). However, the sample size was small since only 9 of the 650 participants in the study had a cardiac event. In order to increase the sample size, each case was matched to at most, 5 controls (Lang et al., 2012) by age and sex making the controls 34 in total.

The variables of interest in the study were not normally distributed in the two groups; cases and controls. Obvious outliers were also present thus data could not be analysed using parametric tests. The Mann Whitney U test was thus the non parametric method of choice. The Mann-Whitney U Test compares the ranks of one sample group to the average ranks of both sample groups to determine if ranks of each of the two populations are significantly different (Harmon, 2014a).

Using Mann Whitney U test to establish if the cases and the controls were different in the variables of interest, the variables were compared at 3 time points; at baseline, 6 months into the study and around the time the cardiac event occurred in the cases.

### Baseline characteristics

At baseline, the cases had higher WBC counts as shown by having a higher mean rank (25.86) than the controls (16.04), and this was statistically significant ( $p=0.012$ ) as shown in Table 3. Neutrophil count of cases was higher with mean rank 24.58 compared to 13.23 in the controls ( $p=0.002$ ). The BMI of the cases were significantly higher than the controls with mean rank of the cases BMI being 28.13 while that for controls was 19.94 ( $p=0.043$ ). The CD4 and CD8 counts for the cases was higher than that for the controls as shown by case mean rank of 23.27 and 24.06 respectively as compared to 20.33 and 20.26 in the controls respectively. For the Lymph% and CD4/CD8 ratio, the controls had higher values with higher mean rank of 17.65 in Lymph% and 21.68 in the CD4/CD8 ratio as compared to 14.08 in LYMPH % and 18.19 in CD4/CD8 ratio in the cases. However, the differences in the

Lymph%, CD4, CD8 and CD4/CD8 ratio were all not statistically significant at  $\alpha$  level of 0.05 as shown by p values  $> 0.05$  (See Table 3 below).

**Table 3:** Baseline Characteristics of Cases and Controls

Variable	Cases Mean Rank	Controls Mean Rank	Sum of Rank s	Mann Whitney U	z value	p value
WBC	25.86	16.04	630	43	-2.268	0.012
NEUT	24.58	13.23	465	17.5	-2.826	0.002
LYMPH (%)	14.08	17.65	561	63.5	-0.817	0.207
CD4	23.75	20.33	861	110	-0.724	0.235
CD8	24.06	20.26	861	107.5	-0.806	0.21
CD4/CD8	18.19	21.68	861	109.5	-0.74	0.23
BMI	28.13	19.94	903	83	-1.718	0.043

### Characteristics at 6 months

The same characteristics were compared after 6 months into the study for both cases and controls except for BMI which was not available. Higher mean rank values were observed in the cases in WBC 13.83 compared to 12.74 in controls ( $p=0.378$ ). CD4 mean rank for cases was 20.43 which was higher than 15.4 for controls, but not significantly different with p value of 0.105. Case mean rank for CD8 (19.43) and for CD4/CD8 ratio (17.57) were not significantly higher than that of controls CD8 (15.68) and CD4/CD8 ratio (16.2) with p values of 0.175 and 0.366 respectively. NEUT whose mean rank for controls was 13.11, yet for cases, it was 12.67 ( $p=0.45$ ) showed higher values in the controls but this was not statistically significant. The LYMPH % remained higher in the controls (15.11) as compared to cases (14.58) but this was not statistically significant ( $p=0.446$ ). All differences at this time point were not statistically significant (Table 4).

**Table 4:** Characteristics of Cases and Controls 6 Months into the Study

Variable	Cases Mean Rank	Controls Mean Rank	Sum of Ranks	Mann Whitney U	z value	p value
WBC	13.83	12.74	325	52	-0.312	0.378
NEUT	12.67	13.11	325	55	-0.127	0.45
LYMPH (%)	14.58	15.11	435	66.5	-0.135	0.446
CD4	20.43	15.4	528	60	-1.254	0.105
CD8	19.43	15.68	528	67	-0.933	0.175
CD4/CD8	17.57	16.2	528	80	-0.342	0.366

### Characteristics at event

The characteristics at the time of event were collected within 3 months of the cardiac event (Lang et al., 2012). The cases had higher values for LYMPH% as shown by a higher mean rank value of 15.17 compared to controls with mean rank value of 11.61. However p value was 0.143 thus difference was not statistically significant. The CD4/CD8 ratio was higher in the controls with mean rank of 20 while the cases had a mean rank of 17.44 ( $p=0.322$ ) but difference was not statistically significant. The other variables, CD4, CD8, WBC and NEUT were higher in the cases with mean ranks of 20.31, 22.44, 14.25 and 12.92 (respectively) than controls with mean ranks of 18.64, 18.05, 11.21 and 12.36 (respectively) but just as at 6 months, the differences at time of event were all not statistically significant (Table 5).

Further statistical comparison on the characteristics of the cases at baseline and at time of event was done using the Sign rank test. The Sign Test is a nonparametric alternative to the one-sample t-Test when normality of the sample or population cannot be verified and the



sample size is small(Harmon, 2014b). There was a significant increase between baseline CD4 and CD4 at event ( $p= 0.008$ ). There was however no significant difference in the CD4/CD8 ratio at baseline and at event ( $p=0.727$ ). CD8 also showed no statistically significant difference between baseline and at event ( $p=0.289$ ). Performing Sign rank test to compare baseline values and at event for the controls showed a statistically significant increase in CD4 ( $p=0.000$ ) and CD8 ( $p=0.013$ ). The CD4/CD8 ratio was not significantly different with  $p$  value 0.345.

**Table 5:** Characteristics of Cases and Controls at Time of Event

Variable	Cases Mean Rank	Controls Mean Rank	Sum of Ranks	Mann Whitney U	z value	p value
WBC	14.25	11.21	276	37.5	-0.945	0.172
NEUT	12.92	12.36	300	51.5	-0.167	0.434
LYMPH (%)	15.17	11.61	300	38	-1.067	0.143
CD4	20.31	18.64	703	105.5	-0.387	0.349
CD8	22.44	18.05	703	88.5	-1.015	0.156
CD4/CD8	17.44	20	703	103.5	-0.461	0.322

## Discussion

This was a nested case –control study in the Tshepo study on Adult Antiretroviral Treatment and Drug Resistance which was conducted 2002 and completed 2007. The study findings showed that cardiovascular events were the most common non-AIDS defining events among the participants (Wester et al., 2011). Several studies have shown that the Full blood count parameters, a routine laboratory test, could be used to predict the risk and outcomes of cardiac events such as MI (Burr et al., 1992; Cannon et al., 2001; Horne et al., 2005; S J Turner et al., 2008; Zouridakis et al., 2000). This research aimed at assessing if FBC parameters could predict the occurrence of a cardiac event in patients in the Tshepo study. The patients involved were HIV positive thus the study went on to also assess the CD4 and CD8 counts since increased risk of cardiac events in HIV positive patients is associated with immune status (Oluwatosin A. Badejo, Chung-Chou Chang, 2015).

The results at baseline showed statistically significant differences in the WBC, NEUT and BMI in which cases had higher rank means compared to controls (Table 3). These are consistent with findings from several studies. A high WBC count was shown to be predictive of future cardiovascular events in individuals who were disease free at baseline (Zalokar, Richard, & Claude, 1981). In a large, disease-free patient cohort from the Adult Health Study (AHS) of Hiroshima and Nagasaki, results showed a relationship between the total WBC count, including the eosinophil, neutrophil, and monocyte counts, and the incidence of CHD (Prentice et al., 1982). In another study of patients with PAD, only an elevated neutrophil count was predictive of an increased risk of major adverse cardiovascular events or death within the study's 20-month follow-up period (Haumer et al., 2005). Obesity is a known risk factor for MI in the general population (Hotchkiss & Leyland, 2011; Whitlock et al., 2009) hence the higher BMI in the cases was not unusual. The average BMI in the cases was 27kg/m<sup>2</sup> while that for controls was 22kg/m<sup>2</sup> showing that on average the cases were overweight while the controls were normal.

After 6 months into the study there was no statistical difference between the cases and controls in the WBC and NEUT. At this point, these results suggest that the WBC and NEUT cannot predict the risk of a cardiac event in the cohort as there was no difference between cases and controls. Cases had higher WBC while controls had higher NEUT although the differences were not statistically significant (Table 4). At event, higher mean ranks were observed in the cases for NEUT and WBC although; again this was not statistically significant. These results show that these parameters did not differ between the cases and controls making them insignificant in predicting a cardiac event.

The LYMPH% was not statistically significant at all time points and had lower mean ranks in the cases except at occurrence of event where the cases had higher mean rank value. It thus cannot be used to predict a cardiac event in the cohort as there was no definite difference between cases and controls. However, looking at the trend despite the statistical insignificance, cases had lower mean ranks for LYMPH% except at event occurrence. A prospective study of 1,037 patients who experienced an acute MI showed that low lymphocyte counts were independent predictors of all-cause death (Dragu et al., 2008). Elevated levels of almost all subtypes of WBCs, including lymphocytes (an inverse relationship), have been associated with increased risk of CHD (Olivares, Ducimetiere, & Claude, 1993; Ommen, Gibbons, Hodge, & Thomson, 1997). This shows that lower lymphocytes has been associated with CHD.

The absolute CD4, absolute CD8 count and CD4/CD8 ratio were not statistically significant making them insufficient for use in predicting the risk of developing a cardiac event in the cohort. The trend however shows higher mean ranks in the CD4 and CD8 at baseline, at 6 months into study and at occurrence of event in the cases. High CD8+ T-cell count among HIV-infected people has been associated with increased acute myocardial infarction risk (Oluwatosin A. Badejo, Chung-Chou Chang, 2015). A low CD4 T-cell nadir and a high current CD8 T-cell count are associated with an increased risk of MI in HIV-infected individuals (Lang et al., 2012). Looking at the CD4/CD8 ratio the controls showed higher mean rank at baseline and at occurrence of a cardiac event except at 6 months into study. This could suggest that the controls had better immune status at baseline and after treatment; at occurrence of event in the cases, the controls probably had better immune recovery. Research has shown that low ratios were associated with increased risk of serious events and deaths and suggested the CD4/CD8 ratio could be used by clinicians to identify patients at risk of non-AIDS-related events (Mussini et al., 2015).

The results of the sign test showed that for both cases and controls the CD4 count significantly increased from baseline to the time event occurred in the cases. Both controls and cases showed poor immune recovery from baseline to event date. However for the controls the CD8 count increased significantly from baseline to event date whereas for the cases the difference was insignificant. This suggests that the CD8 count could have a significant role in development of cardiac events in the cases. This is an inverse relationship to that suggested by Oluwatosin et al who found high CD8 T cells to be associated with occurrence of MI in HIV positive patients (Oluwatosin A. Badejo, Chung-Chou Chang, 2015).

## **Conclusion**

This study showed statistically significant differences in the WBC, NEUT and BMI between cases and controls at baseline. This is consistent with literature thus suggesting WBC and NEUT as potential predictors of a cardiac event occurring in HIV positive patients who are treatment naive. However, at other time points, the results were not statistically significant showing that haematological parameters were not different between cases and controls. This could be attributed to ARV treatment. This research shows that haematological parameters cannot be used as predictors of cardiac events except for WBC and NEUT at baseline. Previous studies were mostly done in HIV uninfected patients and this could explain the difference in the findings. The situation might be different in an HIV infected population. Research done on HIV infected patients was in different populations where clinical management of patients and ethnicity differed hence outcomes were not similar.

The trends however were consistent with other studies despite being statistically insignificant, with cases showing higher mean ranks in WBC, NEUT, and CD8 count at all time points except at 6 months into the study when the NEUT for controls was higher. LYMPH% and CD4/CD8 ratio were lower, again consistent with literature (Dragu et al., 2008; Mussini et al., 2015).

The research had potential limitations that need to be mentioned. The research had a small sample size with only 9 cases. The data was not normally distributed with outliers and cases outnumbered the controls thus non-parametric statistical methods of analysis were the most ideal method of analysis. The results were centred more on the median rather than on the mean in parametric tests. The disadvantage of non-parametric data is that they are wasteful of data as they sacrifice value of variable for a rank number or sign. Non-parametric tests generally have less power (ability to detect a difference) than their parametric equivalents (Harmon, 2014a).

In conclusion our study showed significant difference in the WBC and NEUT between cases and controls at baseline, thus making them potential risk markers for the occurrence of a cardiac event in HIV positive patients. The similar trends with literature shown in this study strongly support further research on haematological parameters in HIV infected patients in Botswana in a larger prospective study using parametric methods of analysis so as to reach more firm conclusions.

### Recommendations

Consideration should be given for closer follow-up, as well as more intensive screening and modifications of haematological parameters as potential risk factors in HIV positive patients. These can be a less expensive and readily available screening tool in management of HIV positive patients in resource poor settings. Future studies can look at traditional CVD risk factors, HIV related parameters and exposure to ARV treatment together with Haematological parameters to get a firm conclusion of their use in predicting the likelihood of developing a cardiac event. There is potential use of haematological parameters in developing clinical guidelines and promotion of their use in the most appropriate manner.

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