THE PREVALENCE OF CORONARY HEART DISEASE IN HUMAN IMMUNODEFICIENCY VIRUS POSITIVE INDIVIDUALS IN HARARE, ZIMBABWE

By

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Abstract

Coronary heart disease is complex with several well established as well as postulated risk factors and pathogenetic mechanisms. This public health problem is a major cause of morbidity and mortality in the industrialised world. HIV is now well recognised as a risk factor for coronary and ischaemic heart disease in the industrialised world but has been hitherto not studied in the black African population. In this study a case control autopsy series of HIV-positive and negative deceased black Zimbabweans was done with the objective of estimating the coronary heart disease rates in these individuals and establishing an association, if any, between HIV-infection and CHD. CHD was present with prevalence rates of 18.1% and 9.5% in HIV-positive and negative individuals respectively, although these prevalence rates were not as high as those in industrialised countries where CHD has reached epidemic proportions. There was however no statistically significant association between HIV infection and CHD. It has therefore become necessary for clinicians treating HIV-positive black African patients to actively investigate for and manage coronary heart disease and its risk factors. There is potential to reduce previously unrecognised morbidity and mortality in HIV. In addition coronary heart disease is now prevalent in the general black Zimbabwean population and may be a source of clinically apparent disease.
Acknowledgements

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This work is dedicated to my loving wife and inspiration, Siffie.
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<td>NRTI</td>
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Introduction and background

In 2011 Kalyani et al\(^1\) reported a case of a 36 year old HIV-positive and treatment naive Indian male who died as a result of injuries sustained in a road traffic accident. At autopsy he was noted to have extensive accelerated atherosclerosis of the aorta, coronary vessels and the carotid arteries. He was a smoker but did not take alcohol, was not hypertensive neither did he have diabetes mellitus or dyslipidaemia. The authors concluded that this “accelerated” atherosclerosis could not be explained away on the basis of his tobacco smoking alone and was likely to have been related to the HIV-positive status. The authors went on to further assert that cardiovascular disease and in particular coronary artery disease was poorly recognised by clinicians in HIV and perhaps undertreated.

The HIV /AIDS pandemic is a truly global menace that has established a foot print across continents in almost every nation; an estimated 35.3 million people are infected with HIV worldwide\(^2\). There is however a wide disparity in geographical prevalence and incidence with the burden of disease weighing heavily on developing countries particularly sub-Saharan Africa states.\(^2\)

The geographical and socioeconomic variation has resulted in striking differences in the pandemic. The industrialised countries have had their HIV-infected population on disease modifying therapeutic agents for at least twenty years resulting in a maturing population that has lived with the disease for a long time\(^2\). This population has shown susceptibility to the same age related and lifestyle modulated morbidity such as is seen in the ageing HIV-negative population which includes hypertension and its side effects as well as metabolic syndrome associated with obesity and (mal)nutrition.\(^3,4\)
Conversely in the developing world, HIV/AIDS has decimated and continues to wreak havoc in the reproductive and economically active population with devastation of the economies and social fabric of these nations. There are far reaching consequences that have resulted from these scenarios and unfortunately, in a typical negative feedback loop fashion, the ability of an affected nation to respond to this public health disaster is severely curtailed by the devastation caused by the disease itself.

Zimbabwe is still regarded as an epicentre of the HIV / AIDS pandemic with the prevalence having been estimated as high as 25% in the recent past and currently hovering around 15%. There are 1.4 million people infected with 61,000 deaths resulting every year and almost a million children under the age of 18 years orphaned yearly.

The public health sector in Zimbabwe is struggling to marshal a response to the burden; only 76.9% of eligible adults and 46.12% of eligible children are on the Anti-Retroviral Therapy accessible for free in the public health sector which is mainly funded by the government AIDS levy and the Global Fund donor pool.

In the industrialised countries illness and death in HIV – infected individuals is largely due to conditions considered to be unrelated to HIV infection and in fact arise as a result of genetic, environmental and age related factors, the morbidity and mortality in African HIV-infected patients is still largely attributed to opportunistic infection resulting from the acquired immuno - suppression of HIV / AIDS. A variety of systems have been described as being affected, with mortality having being described as occurring mostly as a result of infection in respiratory, gastrointestinal and central nervous systems.

Cardiac disease in HIV/ AIDS infection is well recognised and has been described by several workers in both industrialised and developing country settings. Whilst the spectrum of disease is wide, the majority of publications around this area have commonly reported
pericardial disease, endocarditis, myocarditis, and cardiomyopathy particularly in African studies.\textsuperscript{3, 10, 11} Much like the other systems affected by the HIV / AIDS disease, the aetiology of the bulk of these cardiac disorders is thought to be mainly related to opportunistic infection in these cardiac compartments. This is notwithstanding the fact that a growing body of research has been focused at the direct effect of the HIV virus itself on the heart, particularly in the cardiac myocyte.\textsuperscript{13}

In the ageing populations of the developed countries; ischaemic heart disease due to coronary vessel atherosclerosis is prevalent both in the HIV negative and HIV positive patients with aetiology being mostly attributed to the traditional risk factors of cardiac disease which include hypertension, smoking, alcohol, diabetes mellitus, obesity and dyslipidaemia.\textsuperscript{14,15} The situation in the developing countries, particularly in Africa, is unclear in as far as coronary heart disease (CHD) is concerned.\textsuperscript{16} Whilst traditionally CHD has not been a significant public health problem due to the relatively low prevalence of the aforementioned key risk factors, the impact of the HIV /AIDS on ischaemic heart disease and CHD has not been investigated in the African population where a large proportion of the infected patients have failed to access the live-saving Highly Active Anti - Retroviral Therapy (HAART) or the access is inconsistent and frequently interrupted. However anecdotal evidence from practising clinicians in Africa seems to point towards an increase in ischaemic heart disease and events subscribed to coronary heart disease in the HIV – positive patients.

This anecdotal evidence is not accompanied by scientific evidence largely due to inaccessibility of specialised investigations for this phenomenon in the African setting. This is further compounded by the fact that in the majority of deceased HIV – positive patients and any other deceased individuals for that matter, medical interest autopsy is not always performed in cases where it would be useful. The reasons for this anomalous situation are
multiple and include the resistance by the local population to post-mortem examination of their deceased relative due to religious belief systems, customs and traditions. In the current Zimbabwean economic climate, fears that the autopsy procedure will lengthen funeral proceedings and lead to a significant increase in funeral costs are also valid reasons for the relatives’ reluctance to consent for autopsy.

The acute shortage of competent personnel in the public sector available to perform the large number of autopsies required does not help matters. This has led to the tragic situation where the majority of autopsies are performed only to answer pertinent medico-legal and forensic questions whilst medical interest autopsy and the valuable scientific information it can provide has largely fallen by the wayside.\textsuperscript{17,18}
Review of literature

It is clear that cardiac disease in one form or another is a well-recognised complication of HIV infection and has been tackled by several authors. There is however very little work that has been reported, both in Africa and beyond to investigate this cardiac disease by autopsy, long regarded the gold standard of investigating and characterising disease scientifically.\(^\text{18}\)

In 2010 Garcia-Jardon et al carried out a combined retrospective and prospective autopsy study of HIV-positive deaths and found heart conditions in 10% of cases although they did not specify the nature of the heart pathology.\(^\text{19}\) Several other workers (Chintu et al, Rennert et al, Ruffini et al) also reported autopsy series performed on HIV-positive and HIV-negative African individuals but did not report any cardiac findings. The preponderance of reported pathological findings was of infectious conditions in the respiratory and central nervous systems.\(^\text{20, 21, and 22}\)

Coronary heart disease has been a recognised phenomenon in HIV /AIDS since very early on in the pandemic. As early as 1984 reports of various cardiac manifestations in HIV and in particular coronary heart disease were published initially based mainly on autopsy work and later on from clinical studies.\(^\text{23}\)

Coronary Heart Disease and HIV

In a 2013 review of coronary artery disease in HIV Malvestutto and Aberg found that although the traditional risk factors of cardiovascular disease appear to be the main contributors of coronary artery disease in this population, HIV infection is associated with an independent increased cardiovascular risk.\(^\text{24}\) This was corroborated by Saves et al who looked at data from a French study group and found that there were in fact significantly
increased rates of smoking and CHD amongst the HIV-positive population compared to the HIV negative cases. The observed increase in CHD rates could not however be attributed sorely to the differences in smoking between the two groups. Data from the Kaiser-Permanente database showed the same trend as reported by Klein, Hurley, Quesenberry and Sidney.

Pathogenetic mechanisms

A variety of Pathogenetic mechanisms are thought to be involved in the causation of atherosclerotic coronary heart disease in HIV infection and include direct toxic effect of the HIV itself, infection by cardiotrophic viruses, autoimmunity, nutritional deficiencies, and drug related toxicity and side effects.

A study published in 2009 in the journal AIDS by Hsue PY and co-workers reported an association between persistent chronic inflammation and the development of atherosclerosis compared to the HIV-negative controls. This was thought to be key in the pathogenesis of CHD. They also noted that interrupting HAART may increase coronary artery disease risk however the precise pathogenetic mechanism is not clear.

Coronary Artery Disease association with CD4+ cell count

There is emerging data of an association between increased rates of coronary atherosclerosis and HIV-infection in patients with low CD4+ cell counts. A low count is defined as a single value less than 200 cells/uL. Kaplan, Kingsley, Gange, et al studied data from two longitudinal clinical studies of HIV-positive and HIV-negative adults and found an association between advanced HIV disease characterised by low CD4+ cell counts and increased frequency of subclinical atherosclerotic lesions. Similar findings had been
reported by earlier workers (Hsue, Lo, et al; Riddler, Smit, Cole et al and Baker, Peng, et al) who had however worked with limited data sets.\textsuperscript{30,31}

*Heart Disease in Africa*

Cardiac disease in its various clinicopathologic subtypes has been studied fairly extensively in the African HIV-positive population. There are varying aetiologies encompassing a wide spectrum that includes but is not limited to congenital cardiac disease, infectious disease and its sequelae like RHD, hypertensive heart disease, and cardiomyopathy. Coronary heart disease has been noted to have a low prevalence.\textsuperscript{32,33, and 34}

Since the 1990s cardiac disease in African HIV – positive individuals has been studied via clinically based research such as the *Heart of Soweto Study*.\textsuperscript{35} The spectrum of disease published as being related to HIV infection is somewhat narrower than in the HIV – negative population but is skewed towards disease related to opportunistic infection such as pericarditis.\textsuperscript{36}

*African studies on Coronary Artery disease in HIV*

Rana, Hawken, Mwacheri et al\textsuperscript{37} conducted an autopsy study in Kenya and described heart disease as major pathology in only one of 75 autopsies (1.3%) performed on HIV-positive adults and in 3 of 47 (6.4%) of autopsies performed on HIV-negative adults. One of these cases with heart pathology was said to have a primary cardiac sarcoma. In this study the HIV-positive deceased had severely depressed CD4+ cell counts and the authors discussed this as one of the possible reasons to explain the presence of infectious disease as the predominant pathology.

Cardiac disease in African HIV-positive patients was also reviewed by Ntsekhe and Mayosi in 2008 in a review of papers published from 1998 to 2008.\textsuperscript{38} They found that in sub-
Saharan Africa pericarditis of the tuberculous type and dilated cardiomyopathy were the major forms of cardiac pathology in HIV infection in sharp contrast to the prevailing scenario in industrialised countries where coronary artery disease is a major cause of morbidity and mortality in HIV infection. In addition Ntsekhe and Mayosi reported that in their review they did not find any reports or studies of coronary artery disease in the pre-HAART era or afterwards. Ntsekhe and Hakim after reviewing data from eight African countries similarly reported that, although heart disease has emerged as an important cause of morbidity in HIV-infected Africans, coronary artery disease is not a prominent cause of morbidity.

There are varied reasons for this paucity of published data on CHD despite the improved availability of HAART in sub-Saharan Africa. These reasons include the fact that a few researchers are interested in the subject matter, the HAART regimes currently in use in the donor driven public health sectors do not include protease inhibitors which are implicated more than any other drug in causing dyslipidaemia and that the baseline dyslipidaemia (and hypertriglyceridaemia) is comparatively low at the first instance in these black African patients.

Anti-Retroviral Therapy and Coronary Heart Disease

Anti-retroviral drugs, particularly the protease inhibitors class, have been shown to cause hyperlipidaemias and predispose to coronary atherosclerosis. The mechanisms appear varied and the complex interplay between the possible pathogenetic mechanisms has not been fully elucidated.

The prevalence of coronary heart disease in HIV – positive black African adults in black African adults has not been established whilst several studies have put the estimated the
prevalence of CHD in the general black African population at anything between 0 to 8% depending on whether it is a rural or urban setting amongst other factors.\textsuperscript{35,41,42,43}

There are several criteria that have been described for grading coronary heart disease.\textsuperscript{44, 45} African researchers\textsuperscript{46} who have worked on coronary heart disease have mainly employed a grading system based on the degree of luminal narrowing and the presence or absence of complications; thus

\textit{Grade I: less than 50\% narrowing of the lumen}

\textit{Grade II: 50-75\% narrowing of the lumen}

\textit{Grade III: greater than 75\% narrowing of the lumen}

\textit{A complicated atheroma lesion is characterised by the presence of calcification, plaque rupture and/or thrombosis.}

This simplified grading system has been found to be easily reproducible and correlates well with the complex and technically more difficult classification/criteria described by the American Heart Association (AHA).\textsuperscript{45}
Statement of the problem

There is a wide body of research and convincing evidence that HIV infection and drugs (HAART) used as therapy of the same, result in increased rates of atherosclerotic coronary artery disease and increased risk of myocardial infarction in these patients.\(^{40}\)

The majority of the research done in this subject area was done in the industrialised countries where background rates of dyslipidaemia, atheromatous coronary artery disease and ischaemic heart disease are already relatively high. Sub-Saharan Africa is however the epicentre of the pandemic with the hardest hit populations but this has not resulted in corresponding scientific investigation of how HIV-related atherosclerosis has affected the black African HIV-positive patients. It remains largely unknown therefore how prevalent atherosclerotic coronary artery disease is in the HIV-infected black African population. This in turn leads to under-investigation and inadequate treatment of coronary heart disease in the black African HIV-positive population.
Research Question

What is the impact of HIV- infection on the prevalence of Coronary Heart Disease in black Zimbabwean adults?

Null Hypothesis

Coronary heart disease rates are the same in deceased HIV-positive and deceased HIV-negative black Africans adults in Harare, Zimbabwe.

Alternate hypothesis

HIV-positive deceased black African adults in Harare have higher rates of coronary heart disease compared to HIV-negative deceased black African adults in Harare, Zimbabwe.
Objectives

1. To determine the prevalence of coronary heart disease in black HIV – positive patients by performing an autopsy examination in HIV – positive deceased adults at Parirenyatwa Group of hospitals, Harare, Zimbabwe.

2. To determine the prevalence of coronary heart disease in black HIV – negative patients by performing an autopsy examination in HIV – negative individuals at Parirenyatwa Group of Hospitals, Harare, Zimbabwe.

3. To compare the prevalence of coronary heart disease in HIV – positive deceased black Zimbabwean adults and HIV – negative deceased black Zimbabwean adults.

4. To correlate the prevalence of coronary heart disease in HIV – positive deceased black Zimbabwean adults with peripheral CD4+ cell count.

5. To correlate the prevalence of coronary heart disease in HIV – positive deceased black Zimbabwean adults with treatment status and HAART regimen.
Materials and Methods
Study Design

A case control post-mortem based study was selected as the most appropriate design for the research question. The research was directed primarily at determining the impact of HIV – infection on coronary heart disease in black Zimbabwean adults; it was thus appropriate to select as cases individuals in whom HIV infection and treatment was previously documented and compare with a control group of HIV – negative black Zimbabwean selected from the general population whose HIV-negative status would be confirmed by testing. An additional factor which made the case-control study design attractive was the fact that it allows for study of outcomes like CHD which develop slowly over time and have a relatively low prevalence in the study population.

Sample size

The sample size for the study group was calculated by Dobson’s formula (see Appendix C) with precision set at 0.05 and a proportion of 5%; The number of HIV-positive cases was a minimum of eighty (80) and the size of the control group of HIV-negative deceased individuals was set at a minimum of 80 HIV – negative adult black Zimbabweans.

Inclusion Criteria

The autopsy study was conducted only on forensic autopsy cases at Parirenyatwa hospital being conducted as requested by the Zimbabwe Republic Police at Parirenyatwa Group of Hospitals police post. Forensic autopsies are requested by the state represented by the Zimbabwe Republic Police to answer medico – legal questions (See appendix A). The relatives of the study participants were however required to provide informed consent for
inclusion into the study of the deceased individual and to further provide the deceased’s clinical history and medical records.

The study was performed on black male and female adults between the ages of 18 years and 75 years inclusive.

The HIV – positive cases were required to have had a documented HIV screening test conducted by a competent healthcare provider and also required to have had a record of medical illness and treatment.

HIV – positive cases confirmed by next of kin to be positive but without accompanying documentation were excluded from the study as were HIV – positive cases where signed informed consent for study participation could not be obtained from the next of kin.

The HIV – negative group consisted of a minimum 80 consecutive cases of black Zimbabwean adults dying of accidental (unnatural) causes and availed for forensic autopsy at Parirenyatwa hospital mortuary.

The HIV – negative group had no record of a positive HIV screen and a confirmatory negative HIV screen performed by the researcher using rapid test kits (Determine and SD Bioline) was required for inclusion into the study. Cases that had a positive HIV screen after testing by the researcher were excluded from the study.

Cases without informed consent were excluded from the study.

Cases in the control group with an equivocal HIV screen result were excluded from the study.
Methodology

A complete (as far as was possible) medical history and clinical record of the deceased individual was obtained from the next of kin who gave consent for the deceased to participate in the study. This information was recorded on the data collection tool (See Appendix E).

A complete autopsy was performed in every case. A complete external examination was performed which was followed by the internal examination. The internal examination of the thoracic and abdominal compartment commenced with a standard Y shaped incision. This was followed by an in – situ examination of the pulmonary trunk for thromboembolism. Evisceration of the thoracic and abdominal viscera was then performed using the en-mass technique (Rokitansky’s technique) and every organ (system) examined grossly. Representative tissue blocks were then obtained from every organ for histological analysis.

Special attention was directed towards the heart in this study. The pericardial sac and cavity were inspected for inflammation, effusion or haemorrhage. The coronary vessels were then inspected, palpated for calcification and serially sectioned at 5mm intervals. The lumen was examined for atherosclerosis, calcification and /or thrombosis along the whole length of the vessel. Examination of the rest of the heart followed. This was achieved by serially sectioning the heart at 10mm intervals and examining the myocardium for pallor, necrosis or fibrosis. Inspection of the endocardial surface and valves was then performed for inflammation, luminal thrombus, vegetations or tumour.

Tissue blocks were obtained from the coronary vessels (left anterior descending, right posterior and circumflex coronaries) for histological examination and fixed in formalin solution (10% buffered formalin). The tissue blocks were then processed and embedded as paraffin wax blocks. The formalin-fixed paraffin-embedded (FFPE) blocks were then
sectioned and stained with Haematoxylin and Eosin [H & E] (See Appendix D). Special stains (Periodic acid Schiff with Diastase digestion [PAS/D], Ziehl – Nielsen, Masson Trichrome and Congo Red) were performed where indicated after examination of the H & E blocks.

All histological sections were examined by the researcher and reviewed by the consultant pathologist. Coronary artery disease was classified into grades I, II and III based on the amount of luminal narrowing as is set out below;

Less than (<) 50% luminal narrowing = Grade I

50% - 75% luminal narrowing = Grade II

Greater than (>) 75% luminal narrowing = Grade III.

Atherosclerosis was classified into uncomplicated or complicated based on the presence or absence of calcification, rupture and/or thrombosis.

HIV testing

Two millilitres (2mL) of blood were collected during the autopsy for testing by HIV Rapid testing. Determine rapid test kits were used for the screening and SD Bioline test kits were used as a confirmatory test in every case.

Storage of tissues

The sampled tissues were labelled and stored according to the Department of Pathology guidelines and procedures.
Results

83 consecutive autopsies were performed over a 6 month period on HIV – positive black African adults who were eligible for participation in the study and 84 consecutive autopsies were performed on individuals having forensic autopsies performed for accidental deaths who were confirmed to be HIV – negative by the rapid HIV screen. Four cases were excluded from the HIV-negative control group after a rapid HIV test confirmed them to be positive for HIV.

Demographics

The participants in the HIV- positive group were almost evenly matched with 42 males versus 41 females. The ages were distributed in the range 18 years to 75 years with the majority of the HIV – positive cases in the 30 years – 49 years band. The median age band was the 35 – 39 years group.

Figure 1: Bar-graph showing the age distribution of participants in the HIV-positive group
The HIV – negative control group consisted of 84 cases the majority of which were female (43 female cases = 51.2%). These cases fell into the 20 years – 74 years age range.

![Figure 2: Bar-graph showing the age distribution of participants in the HIV-negative controls](image)

**Cardiovascular Disease Risk Factors**

A clinical history was obtained in all 167 cases to elucidate the presence or absence of cardiovascular disease risk factors.

**Smoking**

In the HIV – positive age group there were 22 cases of smoking (26.5%) whilst smoking was present in 13 (15%) of the HIV-negative group.
Alcohol

29 males and 1 female consumed alcohol in the HIV-positive group and 29 had a history of alcohol use in the HIV-negative controls.
**Hypertension**

In the HIV – positive study group (15 cases = 19.3%) had been previously diagnosed of or were on treatment for hypertension. The majority of individuals with a positive history for hypertension were in the 35 – 55 age group (11 cases = 23%). Hypertension was identified in 30 (35%) of the HIV-negative controls.

![Graph showing the distribution of hypertension in the study subjects](image)

**Diabetes Mellitus**

Comparatively fewer cases had been diagnosed and treated for Diabetes Mellitus. 4 cases evenly split between male and female were identified in the HIV – positive group. There were 14 cases with diabetes mellitus in the negative controls.
Figure 6: Chart showing the distribution of diabetes mellitus cases in the study participants

**Dyslipidaemia and Hypercholesterolaemia**

There were no cases of hypercholesterolaemia identified by examination of clinical notes or by interviewing the next-of-kin of the deceased.

In the HIV – positive group the majority (48%) no risk factors, 38 (45.8%) cases had only one or two risk factors present. Four risk factors were identified in only one case which was a 55 year female.

Conversely in HIV-negative group 55 cases had at least one risk factor and none had four of the traditional risk factors.
Figure 7: Distribution of traditional risk factors in study participants

Data on the length of HIV disease was obtained in 81 of the 83 cases. The length of disease was within the range 1 month to 136 months (11.33 years) which corresponded to the period between actual diagnosis and death.

**CD4+ cell counts**

43 cases had a recent CD4+ cell count and the values fell within the range 13 to 601 cells/µL. The mean CD4+ count value was 234. In 40 cases no recent CD4+ cell count was available.
Anti-Retroviral Therapy

43 HIV-positive deceased were on Anti-Retroviral Therapy (ART). The regimens included various NNRTI and NRTI combinations. Of note was that none of the treatment regimens included protease inhibitors which are known to independently increase the risk of CHD by causing dyslipidaemia. The duration of treatment ranged from 1 month to 9 years.
Coronary Heart Disease

Coronary heart disease in varying degrees of severity was identified in 15 (18.1%) of the HIV – positive cases and in 8 (9.5%) of the HIV – negative controls.

In the HIV – positive study cases 6 individuals had mild disease (grade 1 disease), 7 had grade 2 disease and 2 had severe disease (grade 3). There was multiple vessel disease in 9 cases and 4 cases had complicated atherosclerosis with calcification. A single case had extensive involvement of the coronary vessel walls by amyloidosis which was a potential diagnostic pitfall. In another interesting case coronary vessels with atheroma were seen surrounded by adjacent tuberculous granulomata which did not directly involve the wall.
Gross findings at autopsy: coronary arteries

Figure 10: Photograph of coronary atherosclerosis of the left anterior descending (LAD) vessels

Figure 11: Photograph of coronary artery involved by circumferential atheromatous lesion with calcification

Figure 12: Coronary vessel with marked narrowing of the lumen (60%) due to atherosclerosis
Histological Findings

Figure 13: Coronary artery with eccentric atheroma and associated perivascular inflammation (x4, H&E)

Figure 14: Grade 1 atherosclerosis of coronary artery (x10, H&E)
Figure 15: Well developed coronary atherosclerotic lesion with a lipid core and fibrous cap (x4, H&E)

Figure 16: Eccentric coronary atheroma with 50% lumen narrowing (x4, H&E)
Histological evaluation showed well developed atheromatous lesions which were easily recognised at light microscopy on H&E stained slides. The atherosclerotic lesions identified were in various stages of development although the majority were grade I and grade II lesions. Isolated cases showed grade III disease (greater than 75% luminal narrowing). The only examples of complicated coronary atherosclerosis identified were ones with calcification of the atheromatous lesion. There was no rupture of atheroma plaque in any of the identified cases nor were there any associated thrombi.
Figure 19: Photomicrograph of complicated grade 3 atherosclerosis with calcification (Low, medium & high power views, H&E)
Figure 20: Photomicrograph showing coronary artery involved by amyloidosis in a 51 year old HIV-positive woman (x10, H&E)

Figure 21: Photomicrograph showing a high power view of coronary vessel amyloidosis in the same patient as in figure 20 (x40, H&E)
Figure 22: Photomicrograph of a coronary artery showing atherosclerosis with an adjacent caseous tuberculous granuloma (x4, H&E)
In the HIV – negative controls there were a total of eight (9.5%) cases of coronary artery atherosclerosis; one (1) case had grade 1 disease, seven (7) cases were of grade 2 disease and there were no examples of grade 3 disease. There was evidence of calcification in 3 of the eight cases.
Figure 24: Chart showing number of coronary vessels affected in each HIV-positive patient

Figure 25: Comparison of CD4+ cell counts in individuals with coronary atherosclerosis and those without.
Figure 26: Graph showing ART regimes in HIV-positive cases with coronary heart disease

Figure 27: Graph showing distribution of risk factors and CHD in HIV-positive cases
Figure 28: Graph showing distribution of risk factors and CHD in HIV-negative cases.
Statistical Analysis

A total of 167 participants were recruited into the study. There was no significant difference in the proportion of males (49.7%) and females (50.3%), \( p=0.913 \). The mean age of the participants was 34.4 years (sd=12.8 years). There was no significant difference in age distribution between males (mean =42.9, sd=10.8 years) and females (mean=44.0, sd=14.5 years), \( p=0.589 \). Table 1 shows the demographic profile of the participants. The proportion of patients who were CHD positive was 23/167(13.8%).

<table>
<thead>
<tr>
<th>Demographic characteristic</th>
<th>Frequency, n(%)</th>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>83(49.7)</td>
</tr>
<tr>
<td>female</td>
<td>84(50.3)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>131(78.4)</td>
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<td>Yes</td>
<td>35(21.0)</td>
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<td>109(65.3)</td>
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<tr>
<td>Yes</td>
<td>58(34.7)</td>
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Table 1: Demographic profile of study participants

Medical history

<table>
<thead>
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<td>Yes</td>
<td>44(26.3)</td>
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<td>Diabetes mellitus</td>
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<td>149(89.2)</td>
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<tr>
<td>Yes</td>
<td>18(10.8)</td>
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<tr>
<td>Dyslipidaemia</td>
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<td>No</td>
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<tr>
<td>Yes</td>
<td>1(0.6)</td>
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<tr>
<td>HIV status</td>
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<tr>
<td>Negative</td>
<td>84(50.3)</td>
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<tr>
<td>Positive</td>
<td>83(49.7)</td>
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Table 2: Frequency of risk factors in study participants
<table>
<thead>
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<th>ART Regime</th>
<th>Freq.</th>
<th>Percent</th>
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<td>125</td>
<td>74.9</td>
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<tr>
<td>D4T/3TC/NVP</td>
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<td>TDF/3TC/EFV</td>
<td>5</td>
<td>3.0</td>
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<tr>
<td>Total</td>
<td>167</td>
<td>100</td>
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</table>

Table 3: HAART regimes prescribed to study participants

There were 42 (50.6% of HIV-positive cases) individuals on ART and the median ART duration was 24 months (IQR: Q₁=12; Q₃=50).

**CHD and HIV**

<table>
<thead>
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<th>CHD</th>
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<tr>
<td></td>
<td>Positive</td>
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<tr>
<td>Positive (n=83)</td>
<td>15(18.1)</td>
</tr>
<tr>
<td>Negative (n=84)</td>
<td>8(9.5)</td>
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</table>

Table 4: Frequency of atherosclerotic lesions in study participants

There was no significant association between HIV status and CHD.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Frequency (N=167), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7(4.2)</td>
</tr>
<tr>
<td>II</td>
<td>14(8.4)</td>
</tr>
<tr>
<td>III</td>
<td>2(1.2)</td>
</tr>
</tbody>
</table>

Table 5: Frequency of atherosclerotic lesions by grade

In absolute terms smoking was more prevalent in the HIV-positive cases compared to the HIV-negative cases. Univariate and multivariate analysis however did not reveal a significant association between CHD and the individual traditional risk factors in both the HIV-negative and HIV-positive cases.
Discussion

18.1% of the HIV-positive subjects and 9.5% of the HIV-negative controls in this study had histologically demonstrable coronary heart disease with varying degrees of severity which would have very likely caused clinical symptoms and signs with the passage of time.

It is clear from these findings that coronary heart disease in black African Adults is not as rare as some authors maintain but is in fact present in varying degrees of severity within the Zimbabwean population. This is in contrast with much of the literature on the subject which has not considered coronary heart disease to be a significant cause of morbidity in the black African population.

Although there were more cases of coronary atherosclerotic heart disease found in the HIV-positive group compared to the HIV-negative control group there was no statistically significant association demonstrated between HIV-positive status and CHD. The null hypothesis was therefore not rejected. It was therefore not possible to confirm the positive association between HIV-infection and CHD that has been unequivocally demonstrated in patients being treated in industrialised countries. It is however likely with increased study subject numbers, the same positive association would have been demonstrable in the black Zimbabwean adult population.

The HIV-positive patients had higher rates of the traditional risk factors compared to the HIV-negative controls. This is similar to what has been reported by other researchers. In particular smoking was present with a frequency of 26.5% in HIV-positive individuals compared to only 15% in the HIV-negative group. This significant difference did not however translate to a significant statistical association of smoking with CHD in either group. Interestingly though a good proportion of the HIV-positive cases with coronary artery disease did not fall into the subset with the traditional risk factors and in fact 4
(26.7%) of 15 cases with CHD and HIV-positive status did not have any of these traditional risk factors at all. This may be tell-tale sign of what further research with larger study groups may reveal regarding the relationship of CHD and HIV in the black African patient.

When the corresponding data from the HIV-negative control group is examined it is also clear that the same traditional risk factors are prevalent in the general population with a significant baseline of atherosclerotic coronary heart disease present (9.5% in the current study).

Analysis of the CD4+ count data was inconclusive. 8 (53.3%) of the 15 HIV-positive patients with CHD had a CD4+ cell count which was classified as low (<200 cells/μL). There was no association statistically between these two variables. This can be attributed to the relatively low numbers of patients in this study who had a documented CD4+ cell count which is reflective of the prevailing situation in the Zimbabwean public health sector where resources are frequently unavailable for routine CD4+ cell testing. The scatter plot of CHD occurring in HIV-positive cases against CD4+ cell counts appeared to betray a skew towards the lower CD4+ cell counts (<200 cells/μL); a significant association between low CD4+ cell counts and CHD such as has been found by workers in industrialised countries would likely be established by further studies with higher subject numbers.

Limitations of the study

The study was performed on HIV-positive patients dying of complications of the disease. It is therefore possible that the majority of the HIV-positive cases would have had a low CD4+ and the attendant increase in opportunistic infections of other systems. This might have led to a selection bias of cases with predominantly low CD4+ counts qualifying for entry into the study. The majority of the study subjects however had a moderately decreased
CD4+ cell count (in the range 201-350 cells/u/L) which was higher than the range obtaining in the majority of HIV-positive cases with coronary artery disease.

All of the HIV-positive study subjects on HAART were taking or had taken regimes containing NRTIs (Nucleoside reverse transcriptase inhibitors) and NNRTIs (Non-Nucleoside Reverse Transcriptase Inhibitors). None of the patients were on or had been on a protease inhibitor containing regime. This is likely due to the fact that protease inhibitors are not widely available in the public health sector where they are used only as second-line and third line medications. It was therefore not possible to look at the association of HAART and CHD in black Zimbabwean patients because the regimes currently implicated in causing significant dyslipidaemias are the protease inhibitor containing HAART regimes.

The study was severely constrained by time and budgetary limitations; it was therefore not possible to design the study to have several controls per case or to at least match the controls with the cases exactly. This would have increased the power of the study. However the results in the present study are valid and can be extrapolated as a reasonable estimate for the situation prevailing in the black Zimbabwean population.

For future study

Several studies can be designed and carried out to build on the findings of the present one;

A similar case-control study with greater study cases and matched controls or multiple controls per case will determine prevalence rates with a higher degree of statistical significance.

A long term cohort study which will include clinical investigation and eventual autopsy will allow investigation of the interplay of the various pathogenetic factors of CHD and correlate with the pathological findings at autopsy.
In particular a cohort with black Zimbabwean patients on regimes containing protease inhibitors should be investigated in light of the current study which revealed the presence of coronary heart disease in patients who were not taking these drugs.
Conclusion

Coronary heart disease is complex with several well established as well as postulated risk factors and pathogenetic mechanisms. This public health problem is a major cause of morbidity and mortality in the industrialised world.

In the African setting coronary heart disease is present, although the prevalence rates are not as high as those in industrialised countries where CHD has reached epidemic proportions. A large proportion of this CHD in the black African population is related to the traditional cardiovascular risk factors, however more research is required to establish how much CHD in this population is related to HIV infection and treatment.

It has therefore become necessary for clinicians treating HIV-positive black African patients to actively investigate for and manage coronary heart disease and its risk factors. There is potential to reduce previously unrecognised morbidity and mortality in HIV. In addition coronary heart disease is now prevalent in the general black Zimbabwean population and may be a source of clinical disease. In addition more research into this phenomenon in the black African population is an urgent imperative which will impact on the treatment of affected individuals as well as the public health management of the HIV/AIDS pandemic.
References


23. Rakhlin N, Hsue P, Melvin D. Cardiac Manifestations of HIV. *HIV Insite Knowledge Base Chapter University of California San Francisco October 2005*. [http://hivinsite.ucsf.edu/InSite?page=kb-00&doc=kb-04-01-06](http://hivinsite.ucsf.edu/InSite?page=kb-00&doc=kb-04-01-06)


33. Mbewu, A. The burden of cardiovascular disease in sub-Saharan Africa. *SA Heart* 2009; 6:4-10


Appendix A

Forensic Autopsy: Criteria

Autopsy must be performed in all deaths falling within the following categories:

1. All accidental deaths including but not limited to;
   a) Road traffic accidents
   b) Domestic accidents
   c) Occupational accidents
   d) Accidents related to sporting activities
   e) All drowning deaths and boating accidents

2. Suicide

3. Murder or homicide

4. All deaths due to “Acts of God” including lightning strikes and natural disasters

5. Sudden unexplained deaths

6. All deaths related to illicit drug use

7. Maternal deaths (Death while pregnant or within six (6) weeks of termination of pregnancy or delivery)

8. Death during whilst under anaesthesia, during a surgical operation, or within 24 hours of surgical operation and/or administration of anaesthesia

9. Death within 24 hours of admission into a medical facility

10. All cases of death where a medical practitioner is unable to complete a death certificate
Appendix B

American Heart Association criteria for grading atherosclerotic lesions

• Grade 1 - isolated intimal foamy cells (minimal change)

• Grade 2 - numerous intimal foamy cells often in layers (fatty streaks)

• Grade 3 - pools of extra cellular lipid without a well-defined core (intermediate lesion or pre-atheroma)

• Grade 4 - well defined lipid core with luminal surface covered by normal intima (atheroma or fibro plaque)

• Grade 5 - lipid core with a fibrous cap with or without calcification (fibro-atheroma)

• Grade 6 - fibro-atheroma with cap defect such as haemorrhage and thrombosis

• Grade 7 - calcification prominent

• Grade 8 - fibrous tissue change prominent
Appendix C

Dobson’s formula for Sample size calculation

To estimate a sample size for a proportion, three numbers are needed:

1. Estimate of the expected proportion ($p$)
2. Desired level of absolute precision ($d$)
3. Estimated design effect ($DEFF$)

The sample size formula is:

$$n = \frac{1.96^2 \cdot p(1-p) \cdot DEFF}{d^2}$$

The level of absolute precision $d$ specifies the width of the confidence interval 30% and 50%) be acceptable? The selection of a value for $d$ (the desired absolute precision) may depend on the expected proportion and the purpose of the survey. Common values for $d$ are usually around ±5% for estimated proportions in the range of 20%-80%, and around ±3% for less common or very common events (<20% or >80%).

The sample size required for a cluster survey is almost always larger than that required for surveys using simple random sampling because of the design effect ($DEFF$). If the prevalence of a particular indicator is similar in each cluster, the $DEFF$ will be around one, which means the variability is the same as would have been with simple random sampling methods. The greater the clusters differ from one another, the larger the $DEFF$. As the $DEFF$ increases the sample size must be increased to maintain a desired level of precision.

1. (Gorstein J, Sullivan KM, Parvanta I, Begin F. Indicators and methods for cross-sectional surveys of vitamin and mineral status of populations. Micronutrient Initiative (Ottawa) and Centers for Disease Control and Prevention (Atlanta), May 2007, pg 29).
Appendix D

Histological Stain Protocols

Based on University of Utah protocols

1. **Hematoxylin and Eosin Stain**
   1. Deparaffinise in Xylene I and II and III (5 minutes)
   2. Rehydrate
      a. EtOH 100% (3 minutes)
      b. EtOH 100% (3 minutes)
      c. EtOH 95% (3 minutes)
      d. EtOH 95% (3 minutes)
      e. EtOH 70% (3 minutes)
   3. Rinse in distilled water (5 minutes)
   4. Stain in haematoxylin (6 minutes) *Filter before each use to remove oxidized particles*
   5. Rinse in running tap water (20 minutes)
   6. Decolorize in acid alcohol (1 second) *Can go up to 3 seconds. Longer = Lighter Discard after each use*
   7. Rinse well in tap water (5 minutes)
   8. Immerse in Lithium Carbonate (3 Seconds) *Longer time = floating tissue*
   9. Rinse in tap water (5 minutes)
   10. Counterstain in Eosin (15 seconds)
   11. Dehydrate
      a. EtOH 95% (3 minutes) *Discard after each use*
      b. EtOH 95% (3 minutes)
      c. EtOH 100% (3 minutes)
      d. EtOH 100% (3 minutes)
   12. Clear in Xylene I and II (5 minutes)

2. **PAS/Diastase stain**

   **PURPOSE:** To determine glycogen by digesting out and staining with PAS stain.
   **PRINCIPLE:** The diastase (or a-amylase) acts on glycogen to de polymerize it into smaller sugar units, maltose and glucose, that are washed out of the section.
   **CONTROL:** Identical sections are obtained on two separate slides. One is digested the other is not, both are stained with the PAS stain. Liver is a good control.
   **FIXATIVE:** Any well fixed tissue.

   **TECHNIQUE:** Cut paraffin sections at 4m to 5m
   **REAGENTS:**
Digestion Solution:
Diastase (with a-amylase) 0.05 gm
Distilled water 50.0 ml (Made fresh and used within 15 minutes, rest is discarded)
0.5% Periodic Acid, Schiff's Reagent and haematoxylin.

SAFETY: Hydrochloric acid is caustic
Schiff's reagent: Basic fuchsin (pararosaniline) is a known carcinogen

PROCEDURE:
1. Deparaffinise and hydrate to distilled water.
2. Warm the diastase solution in the microwave for 20 seconds.
3. Place the slide labelled "PAS/D" in the warm diastase solution and into the water bath for 15 minutes, do not over digest.
4. Rinse in running tap water, rinse in distilled.
5. Stain both slides simultaneously, following the PAS procedure.

RESULTS:
Glycogen will be stained magenta on the PAS stained slide and will be absent on the PAS/D stained slide.
Note: If slide is over digested, the tissue must be recut. Over digestion has the appearance of lace; there is no tissue left.

NOTES:
1. Do not celloidin the slides prior to digestion, it coats the tissue and digestion cannot occur.
2. Saliva can be substituted, rinse out mouth, lay slide horizontally in staining dish, in the heat for 30 minutes.

3. Congo Red stain

PURPOSE: To demonstrate amyloid deposits in tissue sections.
PRINCIPLE: Amyloid is homogeneous and eosinophilic; the deposits are extracellular and may become sufficiently large enough to cause damage to surrounding tissues when stained with the Congo Red Stain. Amyloid, with the aid of polarizing lenses, will birefringe an apple green colour under the microscope.

CONTROL: Known amyloidosis containing tissue
FIXATION: Carnoy's and absolute alcohol are recommended, 10% NBF or Bouin's.
TECHNIQUE: Cut paraffin sections at 10 microns.
EQUIPMENT: Rinse glassware in DI water. Coplin jars, funnel and glasswool, graduated cylinder, 1mm pipette, and microwave.

REAGENTS:
Mayer's Hematoxylin, 1% Sodium Hydroxide, Sodium Chloride, Congo Red
Sodium Hydroxide: is a severe irritant to skin and eyes, is corrosive to skin and eyes and can cause burns.
Congo Red: Developmental abnormalities were produced in mouse foetuses after chronic feeding studies and causes vascular changes in humans after ingestion.

PROCEDURE:
1. Deparaffinise and hydrate to water.
2. Mayer's Hematoxylin for 10 minutes.
3. Wash in tap water until blue.
4. Working sodium chloride solution, room temperature, 20 minutes.
5. Place directly into Working Congo red solution for 1 hour.
6. Dehydrate rapidly in absolute alcohol, 10 dips, 3 changes.
7. Clear in xylene, coverslip with DXT.

RESULTS:
Congo Red stains amyloid red to pink and nuclei blue

NOTES:
1. Sections must be cut at 8 to 10 microns for birefringence.
2. Solution must be filtered through glass wool, not paper filters for birefringence to occur.
3. Tissue fixed in solutions other than formalin may display false positive birefringence.

4. Masson’s Trichrome Stain

PROCEDURE:
1. *Mordant in Bouin's solution, microwave 1 minute and allow to stand for 15 minutes.
2. Wash in running tap water to remove the picric acid, 5 minutes.
3. Weigert's working haematoxylin, 10 minutes.
4. Blue in running tap water for 5 minutes, rinse in distilled water.
5. Biebrich scarlet for 5 minutes.
6. Rinse in distilled water.
7. Phosphotungstic/phosphomolybdic acid for 10 minutes, discard solution.
8. Transfer directly into Aniline blue for 5 minutes.
9. Rinse in distilled water.
10. 1% Acetic acid for 1 minute, discard solution, rinse in distilled water.
11. Dehydrate, clear, and coverslip.
*Conventional method: Mordant in Bouin's solution, 60°C for 1 hour.

RESULTS:
Masson Trichrome stains nuclei black; cytoplasm, muscle, and erythrocytes red. Collagen is stained blue

NOTES:
1. Light green may be substituted for Aniline blue.
2. 5% phosphotungstic acid for 5 minutes, must be substituted when using Light green.
3. When staining liver biopsies, the collagen is better light blue than dark blue.
5. **Ziehl-Nielsen stain**

**PURPOSE:** Used in the demonstration of acid-fast bacteria belonging to the genus 'mycobacterium', which include the causative agent for tuberculosis.

**PRINCIPLE:** The lipoid capsule of the acid-fast organism takes up carbolfuchsin and resists decolourization with a dilute acid rinse. The lipoid capsule of the mycobacteria is of such high molecular weight that it is waxy at room temperature and successful penetration by the aqueous based staining solutions (such as Gram's) is prevented.

**CONTROL:** Any tissue containing acid-fast organisms.

**FIXATIVE:** 10% formalin

**TECHNIQUE:** Cut paraffin sections at 4-5 microns.

**REAGENTS:**

- Carbol-Fuchsin Solution, 1% Acid Alcohol:
  - **CAUTION:** Corrosive, Carcinogen, toxin.

- Methylene Blue
  - **CAUTION:** Avoid contact and inhalation.

- Phenol: toxic by ingestion, inhalation and skin absorption; is readily absorbed by foetal systems.

- Methylene blue: produced deleterious effects on fertility in rats.

**PROCEDURE:**

1. Deparaffinise and hydrate to distilled water.
2. *Carbol-fuchsin solution, microwave 80 power, 45 seconds, allow slides to stand in hot solution for 5 minutes. Filter solution once a week.
3. Wash in running tap water.
4. 1% Acid alcohol until light pink and color stops running.
5. Wash in running tap water for 5 minutes.
6. Rinse in distilled water.
7. Working methylene blue for 30 seconds.
8. Rinse in water.
9. Dehydrate, clear, and coverslip.
   *Conventional Method: 60°C oven for 1 hour

**RESULTS:**

- Acid-fast bacilli bright red
- Background blue
## Appendix E

### Data Collection Tool

**Study:** The Prevalence of Coronary Vascular Disease in HIV Positive patients in Zimbabwe

**Researcher:** Dr Blessing Zambuko, MB.ChB. Department of Histopathology

### CLINICAL DATA

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### RISK FACTORS FOR HEART DISEASE

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<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### SMOKING

<table>
<thead>
<tr>
<th>ALCOHOL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

### HYPERTENSION

<table>
<thead>
<tr>
<th>DIABETES MELLITUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

### HIGH CHOLESTEROL

<table>
<thead>
<tr>
<th>HIV STATUS AND SEVERITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>DURATION OF DISEASE (DATE TESTED)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>CD4 COUNT/DATE</th>
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<tbody>
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<td></td>
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### ANTI-RETROVIRAL THERAPY

<table>
<thead>
<tr>
<th>DATE ART COMMENCED</th>
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<tbody>
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<td></td>
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### ART REGIMEN

<table>
<thead>
<tr>
<th>AIDS RELATED ILLNESSES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
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</table>

### WHO CLINICAL STAGE

<table>
<thead>
<tr>
<th>AUTOPSY FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

### DIAGNOSIS

<table>
<thead>
<tr>
<th>CARDIAC FINDINGS</th>
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<tr>
<td></td>
</tr>
</tbody>
</table>

### WEIGHT (g)

<table>
<thead>
<tr>
<th>LEFT VENTRICULAR THICKNESS (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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### TRICUSPID

<table>
<thead>
<tr>
<th>PULMONARY</th>
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</table>

### MITRAL

<table>
<thead>
<tr>
<th>AORTIC</th>
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<tbody>
<tr>
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### PERICARDIAL EFFUSION (mLs)

<table>
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<tr>
<th>PERICARDITIS</th>
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<tbody>
<tr>
<td></td>
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</table>

### GROSS CARDIAC FINDINGS

<table>
<thead>
<tr>
<th>MYOCARDIUM</th>
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<tbody>
<tr>
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### HISTOLOGY

<table>
<thead>
<tr>
<th>CORONARY VESSEL DISEASE</th>
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<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LEFT gross</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

### histology

<table>
<thead>
<tr>
<th>RIGHT gross</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

### histology

<table>
<thead>
<tr>
<th>NO. OF VESSELS AFFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III or more</td>
</tr>
</tbody>
</table>

### SCORING

<table>
<thead>
<tr>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = 0 – 50% reduction in diameter of lumen</td>
</tr>
<tr>
<td>2 = &gt;50 – 75% reduction in diameter of lumen</td>
</tr>
<tr>
<td>3 = &gt;75% reduction in diameter of lumen</td>
</tr>
</tbody>
</table>

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Appendix F

Consent Forms

SUBJECT INFORMED CONSENT TO PARTICIPATE IN RESEARCH

Study title: The Prevalence of Coronary Vessel Disease in HIV – positive individuals in Zimbabwe

Site: Parirenyatwa Group of Hospitals

Researcher: Dr Blessing Zambuko, MB.ChB.
Junior Registrar (M.Med.) in Histopathology
P.O. Box A178 Avondale, HARARE
Email: blessing.pvt@gmail.com
Mobile: +263 (0) 773 786 707

Study Description

You are invited to take part in this study. Postmortems (examination after death to determine the cause of death by a pathologist) will be conducted on HIV negative and positive individuals at PGH to determine the presence and severity of coronary vessel disease (heart vessel disease) present in these individuals.

Consent Given by

Informed consent shall be obtained from you, relatives of the deceased.

What is the consent being given for?

- You are being requested to consent to a postmortem being performed on your deceased relative, the results of which will be used in a study on heart disease occurring in HIV – positive individuals.
- You are consenting to removal of tissue from the body of your deceased relatives and storage of the same for the purposes of the aforementioned study.
- You are being asked to consent to the usage of the autopsy (postmortem) results in a study which will be published in journals and presented at medical forums.

Rights of the deceased and the consenter

1. Before consenting to a postmortem to be conducted on your relative for the purposes of the study, you must clearly understand as part of the process of informed consent;
   a. Its purpose
   b. The process and what is expected of you
   c. Any involved risk, costs and compensation due
   d. The benefit to the participant and your family
2. You will be allowed to know the results of the autopsy
3. Right to confidentiality
Your deceased relative’s personal details including name and HIV status will not be revealed. The autopsy result may be used as part of the study and will be published in scientific publications and will be presented at medical and academic meetings.

**Purpose of the study**

- In this we want to determine the prevalence of coronary vessel disease in HIV-positive individuals in Zimbabwe by performing autopsies and collecting tissue for microscopic study.
- To help the relatives gain closure by accurately determining the cause of death using postmortem techniques.
- To improve the diagnosis and treatment of heart disease in HIV–infected individuals in Zimbabwe.
- To compare the clinical and autopsy diagnoses.

**Procedures involved during the study**

1. A full autopsy will be performed on your relative and samples may be removed for further examination.
2. The tissue samples removed from your relative will be processed in the Histology laboratory and examined microscopically (using a machine which allows one to see very small things) to establish the pathology (disease).

**Risk and discomfort**

- There will be no physical discomfort or emotional discomfort to your relative or to yourself.
- The results of the autopsy will remain confidential.
- The results and findings of the autopsy will be discussed with you.

**Potential benefits**

- You as the relative of the disease will be able to know, as far as is possible, the cause of death in the deceased and any other afflictions he/she might have had whether previously diagnosed or not.

**Study withdrawal**

- You may choose not to enter the study or withdraw from the study at any time without loss of benefits entitled to you.

**Confidentiality of Records**

- The details of the deceased and the consenter are confidential and will not be revealed.
- The collected data will be kept by the researcher and in the ZRP Parirenyatwa post as part of forensic autopsy records.

**Questions**

You are free to ask the researcher questions about the research or consent now, at any point during the consenting process and/or at any point in the future.
CONSENT TO PARTICIPATE IN THE STUDY/AUTHORIZATION

By signing my name below, I confirm the following:

- I have read (or had read to me) this entire consent document. All of my questions have been answered to my satisfaction.
- The study’s purpose, procedures, risks and possible benefits have been explained to me.
- I agree to let the researcher/the pathologist perform an autopsy (postmortem) on my deceased relative …………………………………………………………………………………for the purposes of this study.
- I agree to let the study team use and share the health information and other information gathered for this study.
- I voluntarily agree to participate in this research study. I agree to follow the study procedures as directed. I have been told that I can stop at any time with no prejudice or risk to the deceased or myself.

IMPORTANT: You will receive a signed and dated copy of this consent form. Please initial all the pages of the consent form and keep it where you can find it easily. It will help you remember what we discussed today.

Signed

<table>
<thead>
<tr>
<th>Consenter’s (Relative) Name please print</th>
<th>Consenter’s Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Researcher’s Name please print</th>
<th>Researcher’s Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Name of Witness please print</th>
<th>Signature of Witness</th>
<th>Date</th>
</tr>
</thead>
</table>
CHIBVUMIRANO

Study title: The Prevalence of Coronary Vessel Disease in HIV – positive individuals in Zimbabwe

Site: Parirenyatwa Group of Hospitals

Researcher: Dr Blessing Zambuko, MB.ChB.
Junior Registrar (M.Med.) in Histopathology
P.O. Box A178 Avondale, HARARE
Email: blessing pivt@gmail.com
Mobile: +263 (0) 773 786 707

Zvichaitwa pachirongwa

Murikukumbirwa kuti mupinde muchirongwa ichi. Mutumbi we hama yenyu unenge uchitariswa kuti tsinga dze pamoyo dzanga dzichichifambifasa ropa zvakanaka here. Vanopinda muchirongwa vanhu vane hutachiwonawe HIV ne vasina vakashayikira pa Parirenyatwa Group of Hospitals.

Ndian anopa mvumo

Mvumo ine ruzivo hwa kakwana inopihwa nehama dzemufi dzepedyo.

Mvumo yamuri kupa iyi ndeyeyi?

- Murikukumbirwa kupinda muchirongwa chekuti hama yenyu ivhenekwe papostmortem patinenge tichitarisa chirwere chetsinga dzinofambisa ropa pamoyo.
- Murikukumbirwa kuti mupe mvumo yetsinga dzeropa idzi dziviso muchidimbu dzonotariswa nemuchina unoona zvidikidiki kuti paonekwe kuti dziafambisa ropa zvakanaka here.
- Murikukumbirwawo futi mvumo kuti zvichawanikwa zvishandiswe mumabhuku nemapepa avana chiremba, asi mazita enyu neemufu haabude.

Kodzero dzemufi nehama inopa mvumo

4. Musaiti masaina chibvumirano chine ruzivo rwakawana kuti hama yenyu ipinde pachirongwa makafanira kunzwisisa
   a. Chinangwa chechirongwa nekuvhenekwiko kwemufi
   b. Chii chichaitwa ne zvamunotarisirwa kuita
   c. Njodzi kana kusagadzikana kungakonzereswa ne kuvhenekwa kwe hama yenyu
   d. Zvamungangowana kubva muchirongwa.
5. Ikodzero yenyu kuziva zvichabuda pakuvhenekwa kwehama yenyu (postmortem)
6. Zvichawanikwa papostmortem hazivbiritswe kune vanhu vasina kodzero
   Zita remufi neronu hazvizionanga nezvichawanikwa pachirongwa uye zvichawongororwa.
   Zviwanikwa zvikuvenekwa kwemufi zvinogona kushandiswa pakunyora mabhuku nemapepa
   avanachiremba kuti vashandise ruzivo uhu pakurapa pane vamwe varwere.
Zvinanga zvechirongwa

- Muchirongwa ichi tinoda kuona huhwanda wechirwere chetsinga dzinofungisa ropa pamoyo mune vanhu vane utachiwana we HIV.
- Vanorapa varwere vane utachiwana weHIV vanenge vane ruzivo rwakawedzere nezvechirwere chemoyo chinokonerwa nekusafamba zvakakanaka kwe ropa, zvichaita kuti vakwanise kukurumidza kubata izvi pane varwere vawo nekuvabatsira paine nguva.
- Zvinowanikwa pakuvhenekwa kwemufi zvichabatsirawo imi hama yake kunzwisisa zvakansetsa pamufi.

Zvichaitwa pachirongwa chekuvhenekwa kwemufi

3. Pachaitwa ongororo yakazara yemufi pachitarisa nhengo dzese dzemuuriri kuti paonekwe zvirwere zvaakaita.
4. Tsinga dzinofungisa ropa dzepamoyo dzake dzichanotarwisa ku laboratory nemuchina unowona zvinhu zvidikidiki kuti paonekwe kuti dzaidzifambisa ropa zvakakanaka here.

Njodzi ne/kana kusagadzikana

- Hapana njodzi dzinotarisirwa kuvepo kwamuri imi hama yemufi kana kumufi zvakare.
- Zvinowanikwa pachirongwa zvin sorambwa zvakawanda.

Zvamungangowana kubva muchirongwa

- Muchataurirwa zvinenge zvawanikwa pakuvhenekwa (postmortem) kwehama yenyu kuti mukwanise kuziva zvakakonzeresa kushaika kwemufi

Kubuda muchirongwa

- Tapota zivai kuti kupinda kwenyu muchirongwa ichi kuda kwenyu kwakazara uye mungangosarudza kusapinda kana kubuda chero ipi nguva musingararikirwe nezvamunowana.

Zvakavanzika

- Mazita emufi neenyu haazi kuzoburitswa pachena
- Ruzivo rwese runowanikwa ruchachengetedzwa nemukuru wechirongwa nezvimwe zvinochengetedza nemapurisa apaParirenyatwa post.

Mibvunzo

Munokumbirwa kubvunza mubvunzo pese pamafungire, iye zvino kana nguva ipi zvayo pamberi.
CHIBVUMIRANO/PEJI YEKUSAINA

Nekusaina pasi apa, ndinobvuma kuti

- Ndaverenga kana kuti ndaverengerwa gwaro rese iri. Ndagutsikana nemhundiro dzandapihwa kumibvunzo yangu.
- Chinangwa chechirongwa, njodzi dzingangowanikwa uyezve zvandingangowana zvakurukurwa neni zvizere.
- Ndiovobvuma kuti vechirongwa vavheneke hama yangu anonzi........................................................................................................................................................................... vachiita chirongwa chekuvheneka tsinga dzinofambisa ropa.
- Ndinobvuma kuti vechirongwa vashandise ruzivo runenge rawanikwa.

ZVEKUBATISISA: Muchawana gwaro iri ramunenge masaina. Makafanira kunyora zita renyu pasi pepeji yese nekurichengetedza pamunozoriwana. Richakubatsirai kurangarira zvatakurukura nhasi.

Peji yekusaina

<table>
<thead>
<tr>
<th>Zita remunhu azvipira kupinda uchirongwa</th>
<th>Sainecha yemunhu azvipira kupinda muchirongwa</th>
<th>Zuva</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Sainecha yemukuru wechirongwa</td>
<td>Zuva</td>
</tr>
<tr>
<td>Zita remunhu achapa uchapupu</td>
<td>Sainecha wemunhu achapa uchapupu</td>
<td>Zuva</td>
</tr>
</tbody>
</table>

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Appendix G

Data sheet
Appendix H

JREC Approval

Joint Parirenyatwa Hospital
And College of Health Sciences
Research Ethics Committee

Sth Floor College of Health Sciences Building
Telephone: +263 4 708140 Email: medrural@medsch.uw.zw

APPROVAL LETTER

Date: 16th December 2013

JREC Ref: 195/13

Names of Researcher: Dr Blessing Zambuko

Address: University of Zimbabwe, Department of Histopathology

Re: The Prevalence of Coronary Vessel Disease in HIV-Positive Individuals in Zimbabwe.

Thank you for your application for ethical review of the above mentioned research to the Joint Research Ethics Committee. Please be advised that the Joint Research Ethics Committee has reviewed and approved your application to conduct the above named study.

- APPROVAL NUMBER: JREC/195/13
- APPROVAL DATE: 16th December 2013
- EXPIRATION DATE: 15th December 2014

This approval is based on the review and approval of the following documents that were submitted to the Joint Ethics Committee:

- a) Completed application form
- b) Full Study Protocol
- c) Informed Consent in English and/or appropriate local language
- d) Data collection tool version:

After this date the study may only continue upon renewal. For purposes of renewal please submit a completed renewal form (obtainable from the JREC office) and the following documents before the expiry date:

- a. A Progress report
- b. A Summary of adverse events.
- c. A DSMB report

- MODIFICATIONS:
  Prior approval is required before implementing any changes in the protocol including changes in the informed consent.
• TERMINATION OF STUDY:

On termination of the study you are required to submit a completed request for termination form and a summary of the research findings/results.

Yours faithfully,

[Signature]

Professor M.M Chidzonga
JREC Chairman