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# Cardiac abnormalities in HIV infected older children and adolescents in Zimbabwe

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## **Declaration of work**

I, Edith Delewe Majonga, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.



## **Role of the candidate**

The idea of investigating cardiac complications in HIV infected INHALE (Investigation of Heart and Lung Diseases in HIV among older children) belonged to Professor, Rashida Ferrand (my supervisor). I developed the idea, designed the studies and wrote the protocol. I led the field work and trained the research assistants and nurses and was responsible for all data collection and data management with the assistance of Tsitsi Bandason (our onsite Data manager). I have analysed all the data with statistical guidance from Dr Andrea Rehman and Victoria Simms. With regards to the papers for publication, I drafted all the papers and am first author.

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## **Abstract**

The global scale-up of antiretroviral therapy (ART) has resulted in a dramatic decline in mortality in HIV infected individuals. Longstanding HIV infection is associated with an increased risk of chronic co-morbidities. One of the most well-recognised co-morbidities is cardiac disease. An increased risk of cardiac disease in HIV infected children was reported in the pre-ART era, but the burden and natural history in the ART-era, particularly in SSA is not known. Assessment of cardiac abnormalities is further complicated by the lack of regional (African) echocardiographic reference ranges for healthy children. The main aims of this research were to establish echocardiographic reference ranges for older children in sub-Saharan Africa (SSA), and to determine the prevalence, incidence and progression of, and risk factors for cardiac abnormalities in children with HIV established on ART.

A total of 282 healthy HIV-uninfected children aged 6-16 years with no known history of cardiac disease were enrolled to derive echocardiographic reference ranges. Standard M-mode and two-dimensional echocardiography was performed following the American Society of Echocardiography guidelines and a gamma-weighted model was used to calculate the z-scores for ventricular and atrial dimensions. The first echocardiographic references (z-scores) were established for older children in SSA and were normalised to body surface area.

A total of 201 children with HIV aged between 6 and 16 years, taking ART for at least 6 months and clinically stable underwent transthoracic echocardiography, using a standard protocol at baseline and at 18 months.

A total of 197 had echocardiograms at baseline and abnormalities were found in 83 (42%); left ventricular (LV) diastolic dysfunction was commonest in 45 (23%) and LV hypertrophy (LVH) in 22 (11%). Right ventricular (RV) dilatation and systolic dysfunction were found in 13 (7%) and 4 (2%) respectively, of whom 60% had concurrent left heart abnormalities. Current use of nevirapine and hypertension were associated with LVH and LV diastolic dysfunction respectively. A total of 175 (89%) participants were followed up for 283.9 person-years (pys). Ten participants developed left heart and 16 developed right heart abnormalities constituting an incidence of left and right heart abnormalities was 3.52 and 5.64 per 100 pys respectively. The risk of RV dilatation was highest, 12/163 (7%) Stunting was associated with development of any new cardiac abnormality (aOR 2.59 (95% CI, 1.03-6.49; p=0.043). Cardiac abnormalities persisted at follow up in majority of participants. Cardiac abnormalities present at baseline reverted to normal at 18 months in 11(6%). There was an overall increase in mean z-scores for LV, left atrial, LVH, interventricular septal and LV posterior wall diameters at 18 months (p<0.001).

Despite ART, children with HIV have a high prevalence and incidence of cardiac abnormalities, with only a minority being transient. The increase in mean z-scores for LV, left atrial, LVH, interventricular septal and LV posterior wall diameters a short period of follow up suggests potential for progression of cardiac abnormalities. Longer follow up is recommended to understand the clinical implications of these abnormalities.

## Acronyms

2D	Two-dimensional
3D	Three-dimensional
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral drugs
AZT	Zidovudine
BSA	Body surface area
CDC	Centers for Disease Control
CW	Continuous wave Doppler
DCM	Dilated cardiomyopathy
ddI	Didanosine
EID	Early infant diagnosis
ECG	Electrocardiography
GALT	Gut-associated lymphatic tissue
HIV	Human immunodeficiency virus
HSV2	Herpes simplex virus-2
KS	Kaposi's sarcoma
LA	Left atrium
LV	Left ventricle
LVH	Left ventricular hypertrophy
MTCT	Mother to child transmission
NNRTI	Non-nucleoside reverse transcriptase
NRTI	Nucleoside reverse transcriptase
NVP	Nevirapine
PASP	Pulmonary arterial systolic pressure
PI	Protease inhibitor

PMTCT	Prevention of mother to child transmission
PW	Pulsed wave Doppler
RV	Right ventricle
SIV	Simian immunodeficiency virus
SSA	Sub-Saharan Africa
STE	Speckle tracking echocardiography
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler imaging
WHO	World Health Organization

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# 1. Introduction

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## 1.1 Background

The human immunodeficiency virus (HIV) pandemic has been established for the last three decades. An estimated 36.7 million people globally were living with HIV in 2016.<sup>1</sup> Sub-Saharan Africa (SSA) remains the global epicentre of the HIV pandemic, representing 12% of the global population while accounting for 70% of the global burden of HIV-infections.<sup>2-4</sup> The predominant mode of HIV transmission in SSA has been through heterosexual intercourse, resulting in severe generalised epidemics by the mid-1990s e.g. the HIV prevalence in Uganda and Zimbabwe peaked at 25% by 1995 and 29% by 1997 respectively. The risk of mother-to child transmission (MTCT) of HIV without intervention is 35-45%, occurring ante-, intra- or post-partum (through breastfeeding).<sup>5</sup> The adult HIV epidemic was followed by high rates of MTCT reflecting the high prevalence of HIV infection in women of childbearing age and unavailability of interventions for prevention of mother to child transmission (PMTCT).<sup>6</sup>

Untreated HIV infection is associated with a high risk of mortality in infants, with a median survival of two years observed in African cohorts in the pre-ART era.<sup>7</sup> However, a third of infants with HIV have slow-progressing disease, with a median survival of at least 16 years without treatment. HIV infection causes progressive immunosuppression, resulting in an increased risk of infections. ART dramatically reduces the risk of disease progression by suppressing viral replication and facilitating immune reconstitution.<sup>8</sup> In infants, immediate initiation of ART regardless of disease or immunological stage was shown to be associated with a 76% decrease in mortality and has been recommended by the

World Health Organisation (WHO) since 2010.<sup>9,10</sup> In older children, until 2010, the WHO recommended starting ART only in those with WHO Stage 3 or 4 HIV disease or until CD4 count thresholds were  $\leq 350$  cells/mm<sup>3</sup>, revised successively (<200 cells/ $\mu$ l in 2006; <350 in 2010; <500 in 2013) and finally 2015 all children diagnosed with HIV were being treated regardless of CD4 count. With the remarkable global scale-up of ART for both PMTCT and treatment, the incidence of HIV infection in children has declined, but growing numbers of children living with HIV globally are surviving to adolescence and young adulthood.<sup>11</sup> Notably, however, a recent systematic review showed that children in SSA start ART much later than children in high-income settings (median age of starting ART 7.9 (IQR 6.0-9.3) years in SSA vs 0.9 (0.4–2.6) years in high-income settings such as North America, where the vast majority of children are diagnosed in infancy.<sup>6</sup>

HIV in children is associated not only with an increased risk of infections, but also with chronic complications, for example growth failure, chronic lung disease, neurocognitive disease and cardiac disease. In the pre-ART era, cardiac complications were reported in approximately >31% and up to 100% in some studies of HIV-infected infants and younger children and cardiac disease was frequently clinically evident.<sup>12-14</sup> The cardiac complications were commonly congestive heart failure, left ventricular (LV) systolic dysfunction and sinus tachycardia.<sup>12,13</sup> The likely causes were HIV infection itself or a consequence of HIV-associated infection and ART, when it became available. Much of the data, however, was from high-income settings, and in younger children most of whom had advanced HIV infection.<sup>13,15,16</sup> The decline in paediatric infections and the improved survival as a result of ART scale-up means that the proportion of older

children and adolescents living with HIV is growing. In this ART era, there is limited data on cardiac disease in children and adolescents established on ART in SSA. Children <10 years have been mostly studied and had symptomatic HIV infection.<sup>17-19</sup> Available studies are also limited by their cross-sectional design, small sample sizes or different populations investigated i.e. children studied were either ART-naïve or include ART-exposed.<sup>17-20</sup>

## **1.2 Gaps in Knowledge of cardiac abnormalities in HIV-infected children**

The major gap in knowledge is the lack of data on the burden of cardiac disease among older children and adolescents living with HIV in the ART era in SSA. Most studies on paediatric cardiac complications in the context of HIV infection have been conducted in infants and younger children, and mostly in high-income settings.

Echocardiography is a non-invasive technique which allows assessment of cardiac structure and function. Cardiac evaluation of both adults and children largely depends on availability of reference ranges to define normality and abnormality. However, there are no regional African echocardiographic reference ranges for Black children, restricting the validity of available studies. Echocardiographic reference ranges particularly for children are complex as they are influenced by age, race, gender and growth.<sup>21</sup> Thus it is difficult to establish the true prevalence and incidence of cardiac abnormalities among HIV-infected children and adolescents in SSA.

### **1.3 Rationale of the study**

HIV-infected children were observed to be at an increased risk of developing cardiac complications in the pre-ART era. ART has been a remarkable achievement in terms of improving survival and enabling children, who would otherwise have died in early childhood, to reach adulthood. However, many of the children in SSA have either had delayed diagnosis of HIV and/or started ART in older childhood.<sup>6</sup> Infection with HIV during the perinatal period when the immune system is not developed, and immunosuppression and opportunistic infections before ART initiation may all have an impact on the risk of cardiac disease. Notably, in addition to immune suppression, HIV causes dysregulated systemic activation which leads to chronic inflammation, and is not completely suppressed by ART.<sup>22</sup> Cardiac dysfunction secondary to chronic inflammation has been reported in HIV-infected adults.<sup>23</sup> Furthermore, some antiretroviral drugs (ARV) have been associated with cardio-toxicity and cardiac complications.<sup>24</sup>

There has been a decline in numbers of infections in children due to the scale-up of PMTCT programmes and the roll-out of ART has resulted in improved survival and growing numbers of HIV-infected children are reaching older childhood and adolescence. However, these children face the prospect of taking life-long ART and the impact of HIV infection and its treatment is not known. Understanding the burden and manifestations of cardiac disease among children growing up with HIV will be important to ensure that children not only reach adulthood but do so healthy and without disability.

## **1.4 Research aims and objectives**

The aims of the research were firstly, to develop echocardiographic reference ranges for older children and adolescents for Zimbabwe and secondly, to investigate the features of cardiac abnormalities and their progression in HIV-infected older children and adolescents established on ART in Zimbabwe.

The main objectives were to:

1. Establish echocardiographic reference ranges for African children and adolescents.
2. Determine the prevalence and spectrum of cardiac abnormalities, in older children and adolescents on ART.
3. Investigate the risk factors for cardiac abnormalities in older children and adolescents on ART.
4. Investigate the incidence and progression of cardiac abnormalities in older children and adolescents on ART.

This research was conducted as part of a larger study called INHALE (Investigation of Heart and Lung Diseases in HIV among older children), aiming to investigate cardiorespiratory disease in children with HIV infection taking ART. A prospective cohort design was undertaken to answer the above objectives in a cohort of HIV-infected older children and adolescents aged between 6 and 16 years to reflect older childhood and adolescence, on ART for at least 6 months and clinically stable. A minimum of 6 months on ART was selected to allow sufficient time for viral suppression and for the risk of immune

reconstitution inflammatory syndrome to decline. A second group of healthy HIV-uninfected children of the same age group were also enrolled for determining normative data.

## **1.5 Thesis outline**

This thesis follows the format of the “research paper style” dissertation. The thesis is made up of manuscripts that have been published, are under review or ready for submission together with introductory, literature review and discussion chapters. Each paper establishes an independent chapter. The outline of the chapters is as follows:

**Chapter 1** (the current chapter) presents an overview of the research background, gaps in the knowledge, study rationale as well as the research aims and objectives and the outline of the thesis.

**Chapter 2** presents the review of literature undertaken for this thesis on the epidemiology of paediatric HIV, cardiac manifestations in the pre-ART and ART-era, risk factors for cardiac complications in HIV-infected children and echocardiographic measurements.

**Chapter 3** is a published systematic review and is an extension of **Chapter 2** describing the distribution of available echocardiographic reference ranges for the left-sided cardiac chamber dimensions. The paper is titled “**Racial variation in echocardiographic reference ranges for left chamber dimensions in children and adolescents: A Systematic Review**” published in the Pediatric



Cardiology. Reference: Majonga ED, Norrish G, Rehman AM, Kranzer K, Mujuru HA, Nathoo K, Odland JO, Kaski JP, Ferrand RA. Racial Variation in Echocardiographic Reference Ranges for Left Chamber Dimensions in Children and Adolescents: A Systematic Review. *Pediatric cardiology* 2018:1-10

**Chapter 4** is a published paper titled “**Echocardiographic reference ranges in older children and adolescents in sub-Saharan Africa**” published in the *International Journal of Cardiology*. Reference: Majonga ED, Rehman A, McHugh, G, Mujuru, HA, Nathoo, K, Patel, MS, Munyati, S, Odland, JO, Kranzer, K., Kaski, JP, Ferrand, RA. Echocardiographic reference ranges in older children and adolescents in sub-Saharan Africa. *International Journal of Cardiology*. 2017;248(Supplement C):409-13

**Chapter 5** consists of the manuscript which is under-review. The manuscript describes the prevalence and risk factors for left and right heart abnormalities in HIV-infected children on ART and is titled “**High prevalence of echocardiographic abnormalities in older HIV-infected children on antiretroviral therapy.**”

**Chapter 6** consists of a draft paper titled “**Incidence and progression of echocardiographic abnormalities in HIV-infected older children and adolescents taking antiretroviral therapy: A prospective cohort study.**” This paper highlights the incidence and clinical course of abnormalities present at baseline in children taking ART.

**Chapter 7** includes the main discussion of all study findings, conclusions and recommendations for future research.

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## 2. Literature review

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## **2.1 Human Immunodeficiency Virus Infection**

### **2.1.1 Origins of HIV infection**

HIV is a retrovirus belonging to the family of lentiviruses and it causes a fatal disease called acquired immunodeficiency syndrome (AIDS) which destroys the immune system.<sup>1</sup> The major targets of the HIV are the CD4<sup>+</sup> T-cells which are progressively depleted because of reduced production and increased damage.<sup>2</sup> It is believed that the HIV epidemic began after zoonotic transfer of simian immunodeficiency virus (SIV) from African primates.<sup>3</sup> Two distinct types of viruses, HIV-1 and HIV-2, whose genome organizations and phylogenetic (i.e. evolutionary) relationships are closely related with other primate lentiviruses, originated from the simian species.<sup>4</sup> Evidence suggests that HIV-1 came from a strain of SIV in chimpanzees (*Pan troglodytes*) referred to as SIVcpz while HIV-2 arose from a monkey species called sooty mangabey (*Cercocebus atys*) and the virus strain was SIVsm.<sup>4</sup>

HIV-1 comprises of four distinct virus groups (termed M, N, O and P) and they differ in their distribution within the human population: M group is the predominant one and is responsible for the global HIV epidemic.<sup>3</sup> Group N, O and P are less common with group O accounting for 5% of the infections in West and Central Africa, while group N and P have been rarely identified in a handful of people in Cameroon. Within the virus group M, are at least nine distinct subgroups A, B, C, D, F, G, H J and K. The different subgroups have combined their genetic material to form hybrid virus called circulating recombinant form.<sup>5</sup> Multiple independent zoonotic transmissions of SIV have caused the different

HIV lineages in the human population. HIV has massive genetic variability and rapid evolution mainly because of the high mutation and recombination rates of error-prone transcription which lacks proof-reading mechanism including high rates of replication.<sup>5,6</sup> There are frequent insertions and deletions in the viral genome giving rise to the fast generation of genetically diverse viral populations.<sup>5</sup>

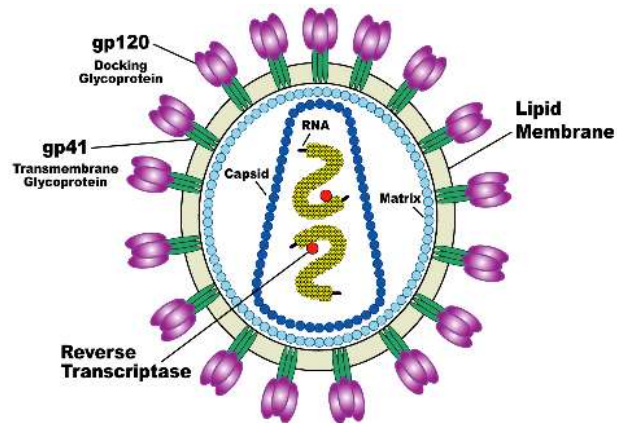
Molecular epidemiological studies have indicated that a high genetic diversification of HIV-1 group M occurred in Kinshasa, Democratic Republic of Congo, and is the epicentre of group M viruses.<sup>7</sup> Similarly, HIV-2 strains infecting humans have at least eight distinct phylogenetic lineages (A to H).<sup>3</sup> Group A and B are the endemic groups in the population while the remainder strains are largely single person infections.<sup>8</sup> Plasma viral loads in HIV-2 infection tend to be lower than in HIV-1 infection.<sup>9,10</sup> While HIV-1 has spread globally, HIV-2 is largely confined to West Africa. This is likely due to its lower transmissibility secondary to lower circulating viral loads. HIV-1 is responsible for the global HIV pandemic and is the virus group referred to in this thesis.

The first cases of AIDS were reported by the Centers for Disease Control (CDC) in 1981 in the United States of America, pneumocystis pneumonia (PCP) and a type of cancer called Kaposi's sarcoma (KS) in previously healthy young homosexual men.<sup>11-14</sup> The disease was associated with immunosuppression and CDC described AIDS as "a disease at least moderately predictive of a defect in cell mediated immunity, occurring in a person with no known cause for diminished resistance to that disease".<sup>15</sup> Further cases of AIDS, with individuals presenting with a variety of opportunistic infections, were later reported from other parts

of the world and in diverse population groups including, heterosexuals, recipients of blood transfusions including patients with haemophilia receiving regular Factor VIII concentrate, and intravenous drug abusers suggesting an infectious aetiology transmitted through sexual or parenteral transmission.<sup>13,16-19</sup> Isolated cases of children with immunosuppression and clinical characteristics similar to adults with AIDS born to intravenous drug abusers and recipients of blood products were also identified in 1982, suggesting that vertical transmission was also possible.<sup>20,21</sup> In 1985 CDC reported that 217 of the 15 172 AIDS cases were children under 13 years.<sup>22</sup> HIV was isolated and recognised as the cause of AIDS in 1983.

### **2.1.2 Pathogenesis of HIV Infection**

Following transmission, HIV targets CD4<sup>+</sup> T-cells.<sup>23</sup> A spherical-lipid bilayer surrounds the virus and has two major viral glycoproteins: gp120 and gp41 whose main function is to mediate attachment of the virus and entry into the CD4<sup>+</sup> T-cell.<sup>2</sup> Notably HIV is a retrovirus, holding duplicate single strands of viral RNA, which code for envelope proteins and enzymes (reverse transcriptase, protease and integrase), required for virus production (fig 2.1). Within the host cell, the viral RNA is transcribed into a single DNA strand using reverse transcriptase.<sup>24</sup> The single DNA strand is converted into a double-stranded DNA using host enzymes and integrated within the host genome using integrase. Proteins produced by translation of viral DNA that has been integrated within the host genome are cleaved by protease and packaged into a virion.



**Figure 2.1. Structure of the HIV**

HIV virions bud off through the cell membrane and infect other CD4<sup>+</sup> T-cells. Viral replication results in destruction of increasing numbers of CD4<sup>+</sup> T-cells, resulting in a gradual decline of CD4<sup>+</sup> T-cells and consequent immunosuppression (fig 2.2).<sup>2</sup> HIV primarily infects cells that have CD4 receptor and chemokine receptors to gain entry, including CD4 T-cells, monocytes and macrophages and dendritic cells and therefore viral replication following infections occurs largely in lymphatic tissue.<sup>23</sup> Notably, the earliest signs of infection (within a week of infection) is observed in the gut which is enriched with gut-associated lymphatic tissue (GALT).<sup>25</sup> During acute HIV-1 infection, the viral replication destroys the majority of the CD4 T cells in the gut. This induces inflammation and results endotoxins leaking out which activates the immune system.<sup>26</sup>

Humoral and cell-mediated immune responses are activated following infection but are unable to eradicate the infection.<sup>27</sup> The probability of transmission of HIV through unprotected sexual contact is in part reliant on the infectiousness of the infected partner when their viral load is higher.<sup>28</sup> Sexual transmission of HIV is also enhanced by presence of sexually transmitted diseases especially the ulcerative ones e.g. herpes simplex virus-2 (HSV2).<sup>28</sup>



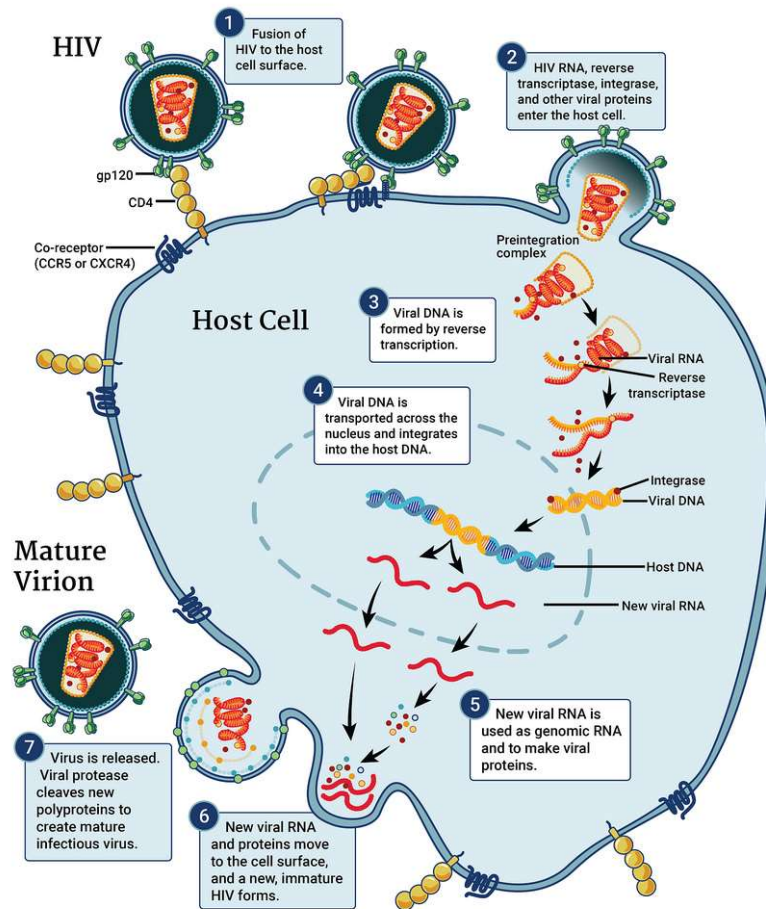
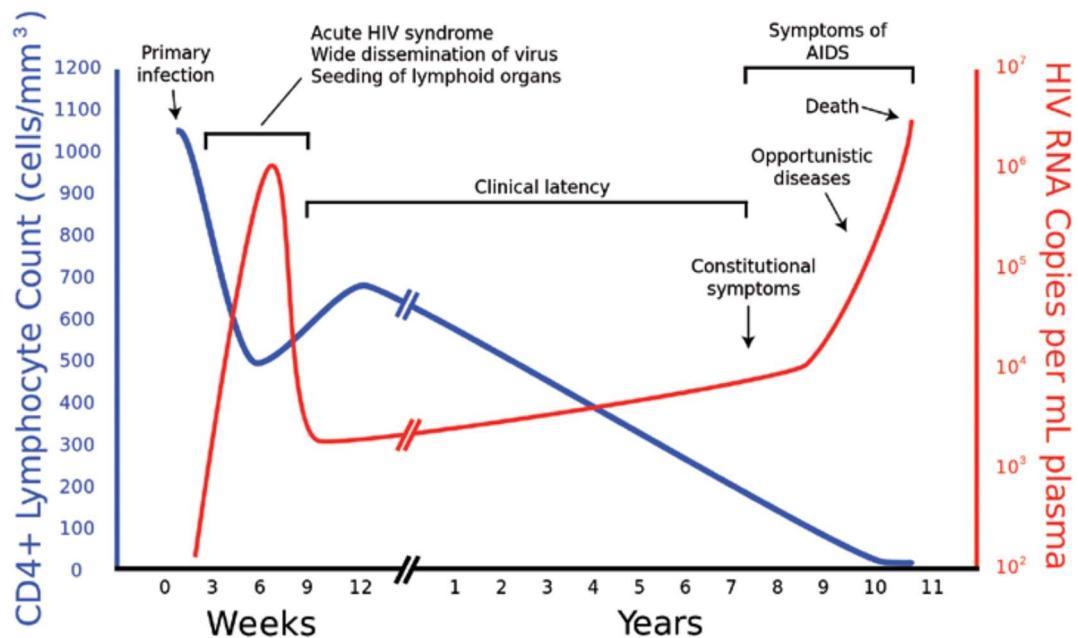


Figure 2.2. HIV replication cycle

### 2.1.3 Clinical course of untreated HIV infection

The first couple of weeks following HIV infection are referred to as acute phase or primary infection and are accompanied by a dramatic depletion of CD4<sup>+</sup> T-cells (blue line in fig 2.3). This results in marked HIV viraemia due to active viral replication (red line in fig 2.3).<sup>29</sup> The immune response within the infected individual is activated and there is recovery of almost near normal concentrations of CD4<sup>+</sup> T-cells although they will not reach pre-infection levels and there is eventual decline in HIV viraemia. This marks the period of clinical “latency” which is variable in length (median 10 years) when the infected individual is asymptomatic. However, there is continual low-level viral replication and the CD4<sup>+</sup> T-cells gradually decline.<sup>2</sup> Continued viral replication

and CD4+ T-cell destruction leads to increasing immunosuppression and development of infection, AIDS and subsequent death. (fig 2.3). The WHO Disease Staging is most widely used to clinically stage HIV infection.<sup>30</sup>



**Figure 2.3** Time course of untreated HIV infection. Progression of CD4 T-cell counts during course of the diseases are denoted by the blue line. Progression of viral loads is denoted by the red line.

## 2.2 Antiretroviral therapy

### 2.2.1 Regimens and mode of action

ART drugs target various replication stages of the virus and suppress viral replication: inhibition of virus entry (fusion /entry inhibitors); reverse transcription (reverse transcriptase inhibitors); integration (integrase inhibitors) and viral maturation (protease inhibitors).<sup>31</sup>

The first antiretroviral drug (ARV) to be introduced was a nucleoside reverse transcriptase (NRTI) called zidovudine (ZDV) in 1985. This drug was associated

with survival benefits after 24 weeks in HIV infected individuals and was approved for use in 1987 in patients with advanced HIV infection. Soon after, three other NRTIs were approved in succession for use including zalcitabine, didanosine (ddI) and stavudine.<sup>32</sup> There was rapid development of resistance to the drug when administered as a mono- or dual therapy.<sup>33</sup> The next advancement of HIV treatment was the development of drugs from different classes including nevirapine (NVP), a non-nucleoside reverse transcriptase (NNRTI), saquinavir and indinavir, protease inhibitors (PIs). To date, ART regimens typically consist of three or more drugs from at least two classes of drugs (combination ART) to prevent the emergence of drug resistance and enable more long-lasting viral suppression.<sup>31</sup>

ART suppresses viral replication, enabling immune restoration.<sup>34</sup> Treatment is monitored through measurement of viral load (copies/ml of the virus) and CD4+ T cell levels (CD4 count); an “undetectable” viral load (defined as a viral load below the limit of detection) is a marker of successful control of viral replication. A rise in CD4 count is a marker of immune restoration, although there may be qualitative defects in immunity persisting even if there is a quantitative increase in CD4 count.<sup>34</sup>

Following introduction of combination ART in the mid-1990s access was limited in resource poor settings because the drugs were highly expensive but there was rapid uptake in high-income countries.<sup>35</sup> With increasing availability of generic formulations in the following years, ART delivery extended into other resource poor countries including SSA.<sup>36</sup> The virological and immune response to ART is

equivalent in high- and low-income settings.<sup>37</sup> However, early mortality rates were observed to be much higher in low-income settings for both children and adults, with approximately 9-26% of the patients in SSA dying in the first year following ART initiation.<sup>37,38</sup> This is most likely because the majority of patients in SSA started ART when they had already developed advanced disease.<sup>37</sup>

HIV infection is incurable, and control of HIV infection requires sustained viral suppression, achieved with life-long treatment with ART.<sup>39</sup> Once an individual is infected, infection cannot be eradicated because viral DNA is incorporated into the host cell genome following transcription of viral RNA to DNA. ART drugs are only active against cells in which active viral replication is occurring. Resting memory cells which have archived the viral DNA will therefore act as reservoirs for virus and once activated will promote viral replication, which can persist in the host genome of cells including resting memory CD4<sup>+</sup> cells which prevents eradication of the virus by ART.<sup>39,40</sup>

### ***2.2.2 Timing of ART initiation***

The optimal time during the course of HIV infection has varied over time. Table 1 shows how the ART guidelines changed from 2006 up to 2015. The rationale for deferring ART until immunosuppression occurred i.e. drop in CD4 count or development of clinical disease (WHO Stage 3 or 4 HIV infection) was to reduce i) exposure time to drugs that have toxic effects which are often cumulative, ii) risk of drug resistance mediated by suboptimal adherence, and iii) cost for providing life-long treatment, particularly in low-income settings e.g. in SSA, the epicentre of the HIV epidemic. Some of the reported toxic effects of ART on the

heart may include mitochondrial dysfunction, which has been observed after the use of stavudine, dDI and AZT. Cardiotoxicity from abacavir and dDI increase the risk of myocardial infarction.<sup>41</sup> Lipodystrophy and other metabolic abnormalities have also been reported in patients taking PIs.

However, several randomised controlled trials have showed that early initiation of ART reduced morbidity and mortality, for both adults and children with HIV infection. The Strategies for Management of Antiretroviral Therapy (SMART) trial (2002-2006) was conducted in adults with HIV and CD4 count  $>350\text{cell}/\text{mm}^3$ .<sup>42</sup> Participants were randomly assigned to continuous ART or episodic use of ART when CD4 count dropped to  $\leq 250\text{ cell}/\text{mm}^3$  and use of therapy until CD4 count increase to  $>350\text{cell}/\text{mm}^3$  again. Significantly more opportunistic infections and death from any cause occurred in the group with episodic treatment than in the continuous ART group, with the trial stopped early. Importantly, there were more non-AIDS events including cardiovascular, renal, or hepatic disease in the group with interrupted ART than continuous ART, suggesting that ART is important in reducing the risk of non-infectious co-morbidities.<sup>42</sup>

The Strategic Timing for Antiretroviral Therapy (START) trial (2002-2006) evaluated the risks and benefits of the immediate initiation of ART in asymptomatic HIV-infected adults with CD4 count  $>500\text{ cells}/\mu\text{L}$  compared to deferral of ART until CD4 count dropped to  $350\text{ cells}/\mu\text{L}$ . There was a significant reduction in serious AIDS-related (72%) and serious non-AIDS-related events (39%) the early ART versus deferred ART group demonstrating the beneficial

effect of earlier ART initiation, even at high CD4 counts.<sup>43</sup> Similar findings were found in the TEMPRANO trial (2008-2012) conducted among HIV infected adults in West Africa.<sup>44</sup> The HIV Prevention Trials Network (HPTN 052) trial found a reduction transmission of HIV in sero-discordant couples. The infected partners were randomised to immediate ART and delayed ART groups and there was a reduction in the number of transmission in the immediate ART group (4 events vs 35 events).<sup>45</sup> This study was instrumental in the use of ART as a part of a public health strategy to reduce the spread of HIV infection.

These trials were instrumental in the revision of the 2015 WHO guidelines which recommended that all identified HIV infected persons, adults and children be treated (universal ART for all), both for individual benefit plus a public health benefit in terms of reduction of risk of transmission. In addition, contemporary ART regimens are comparably more potent, convenient, better tolerated and with fewer side-effects compared to earlier regimens.

The Children with HIV Early Antiretroviral Therapy (CHER) trial (2005-2008) was a landmark trial in infants reported a 75% reduction in early infant mortality in those randomised to immediate compared to deferred ART, and prompted change of WHO 2010 guidelines to recommend immediate HIV treatment in infants and children up to 2 years.<sup>46</sup>

The current WHO guidelines recommend immediate treatment for all individuals regardless of immunological or disease. However, there is no evidence of a mortality benefit of immediate ART in older children, and as described above,

trials have been conducted in infants and adults. The PREDICT trial is the only trial in older children aged 1-12 years which showed no mortality benefit of early vs deferred ART.<sup>47</sup> There were only 3 events in the early treatment group vs 2 events in the deferred ART group and the trial was therefore underpowered to detect differences in mortality or severe disease events.<sup>48</sup>

## **2.3 Epidemiology of HIV**

### ***2.3.1 Global HIV epidemiology***

Since the HIV pandemic was recognised 40 years ago, more than 70 million people have died and in 2017, there were 34.5 million adults and 2.1 children living with HIV.<sup>49,50</sup> The subsequent global scale-up of ART programmes has resulted in a dramatic decline in HIV-associated mortality globally, In 2005, there was an estimated 1.9 million global AIDS-related deaths, with a drop to 1 million in 2016.<sup>50</sup>

In addition, there has been a decline in overall new infections from 3 million in 2000 to 1.8 million in 2016. There are several reasons for this decline including scale-up of ART programmes resulting in reduced infectiousness of HIV-infected people on ART, and scale-up of HIV prevention programmes including PMTCT programmes.<sup>50</sup> Notably, the HIV epidemic is highly heterogeneous in terms of its epidemiology across the globe. In some regions, HIV is concentrated within key populations defined by WHO as vulnerable and most-at-risk populations.

Age-group	2006	2010	2013	2015
<1 year	Absolute CD4 count thresholds may be used (i.e. <1500 cells or WHO clinical stage 3 and 4)	Treat all	Treat all	Treat all
1-2 years	<750 cells/mm <sup>3</sup>	Treat all	Treat all	Treat all
2-5 years	<350 cells/mm <sup>3</sup>	CD4 count ≤750 cells/mm <sup>3</sup> or %CD4+ ≤25	Treat all	Treat all
>5 years	<200 cells/mm <sup>3</sup> (as in adults)	CD4 count ≤350 cells/mm <sup>3</sup> (as in adults)	Initiate ART if CD4 ≤500 cells/mm <sup>3</sup> (CD4 ≤350 cells/mm <sup>3</sup> as a priority) or WHO clinical stage 3 and 4 irrespective of CD4 count.	Treat all
Adolescents and adults	CD4 count ≤200 cells/mm <sup>3</sup>	CD4 counts of ≤350 cells/mm <sup>3</sup> or WHO clinical stage 3 and 4 irrespective of CD4 count.	Initiate ART if CD4 ≤500 cells/mm <sup>3</sup> (CD4 ≤350 cells/mm <sup>3</sup> as a priority) or WHO clinical stage 3 and 4 irrespective of CD4 count.	Adults (>19 years old): treat all Adolescents (10–19 years of age): treat all
Milestones in evolution of ART guidelines	↑ SMART Trial (2002-2006)	↑ CHER Trial (2005-2007)	↑ HPTN 052 Trial (2007-2010)	↑ PREDICT Trial (2012)
				↑ TEMPRANO Trial (2008-2012) START Trial (2009-2013)

**Table 2.1 A summary of WHO treatment guidelines by age in recent years and trials which have informed HIV treatment guideline**



Most-at-risk populations are disproportionately affected by HIV in most epidemic contexts and include men who have sex with men (MSM), transgender people, people who inject drugs and sex workers.<sup>51</sup> Vulnerable populations are groups of people who are particularly susceptible to HIV-infection; subject to societal circumstances and may vary depending on specific situations and contexts e.g. adolescents.<sup>51</sup> Some regions, notably SSA have experienced a generalised epidemic, defined as one that is self-sustaining in the general population through heterosexual transmission. Typically, HIV prevalence usually exceeds 1% among pregnant women attending antenatal clinics.<sup>52</sup>

Although there has been a global decline in incidence of HIV infection and AIDS-related deaths, prolonged survival due to ART has resulted in an increased number of people living with HIV to 36.7 million in 2016 from 27.7 million in 2000.<sup>50</sup>

### ***2.3.2 Epidemiology of HIV infection in Sub-Saharan Africa***

SSA has the highest HIV prevalence and globally and accounts for 70% of the world's HIV infected individuals.<sup>28</sup> There is considerable heterogeneity in the severity of the epidemic with Eastern and Southern Africa having the largest proportion of people currently living with HIV (19.4 million in 2016).<sup>53</sup> Western and Central Africa have lower HIV prevalence than Eastern and Southern Africa and the lowest prevalence is North Africa.<sup>50</sup> Women in the region are disproportionately infected (59%) compared to men and this contrasts with many high-income countries with concentrated epidemics where more men than women live with HIV infection.<sup>54,55</sup> The high HIV prevalence rates among

pregnant women in SSA resulted in a severe epidemic of HIV among children, predominantly infected by mother-to-child HIV transmission (MTCT).

## **2.4 Paediatric HIV infection**

The global epidemiology of HIV infection in children reflects the epidemiology of HIV in women.<sup>56</sup> The HIV epidemic in SSA has rapidly spread through heterosexual transmission. Large numbers of children were infected with HIV before interventions to prevent MTCT became available.<sup>57</sup> SSA currently accounts for 90% of new infections in children globally.<sup>58</sup>

### **2.4.1 Mother to child HIV transmission**

MTCT can occur during pregnancy, delivery or postnatally during breastfeeding.<sup>23</sup> Without intervention, the risk of mother-to-child transmission (MTCT) of HIV is 35-45%.<sup>59,60</sup> In addition, advanced maternal immunosuppression, high maternal viral load and prolonged rupture of membranes increases the likelihood of MTCT of HIV.<sup>60</sup> In utero transmission most often occurs through placental transfusion, and disruption of the placental barrier for example through abruption or tears increases risk of transmission in utero.<sup>61</sup> Intrapartum transmission occurs through direct contact of the foetus with infectious maternal blood and secretions during delivery.<sup>61</sup> In SSA, breastfeeding is common practice.<sup>62,63</sup> Both maternal viral load and subclinical mastitis have been associated with increased risk of transmission through breastfeeding.<sup>60</sup>

### **2.4.2 Prevention of mother-to-child transmission**

In the 1990s, interventions for PMTCT of HIV infection were introduced and these included, elective caesarean section delivery to minimise contact of the foetus with contaminated maternal secretions and avoidance of breastfeeding.<sup>64</sup> While breastfeeding poses the risk of HIV transmission, the risk of infant mortality outweighs the risk of malnutrition and infectious diseases (the major causes of death in SSA) through not breastfeeding.<sup>65</sup> Therefore, exclusive breastfeeding, rather than use of substitutes, has been recommended for women with HIV in low-income settings.<sup>66</sup> Similarly, elective caesarean section delivery is not safe and affordable for most people in settings where the prevalence of HIV is substantial, although they are successful methods of reducing HIV transmission.<sup>67</sup> ART reduces maternal viral load and thus the risk of transmission during pregnancy, delivery and postnatally, and opt-out HIV testing for pregnant women registering for antenatal care and combination ART has been recommended for all HIV-infected pregnant women, regardless of immunological stage, since 2013.<sup>59</sup>

The current recommendations for PMTCT can reduce the risk of MTCT to <1%, and the numbers of infections among children dropped from 460 000 in 2000 to 160 000 in 2016.<sup>50</sup> However, the coverage of PMTCT programmes across SSA remains suboptimal. In 2012, there was a coverage of 62, so that MTCT continues to occur.<sup>68</sup> Reasons include women not registering with health facilities for antenatal care, poor adherence and lost-to-follow up of pregnant women initiated on ART.<sup>69,70</sup> Early infant diagnosis (EID) among HIV-exposed infants is a critical component of PMTCT programs to enable timely initiation of ART in

infants who are infected.<sup>71</sup> Again, coverage of EID is variable, with about 39% of exposed infants received their HIV results and had timely initiation of ART in sub-Saharan Africa.<sup>72</sup> Challenges to EID include poor uptake, low retention at designated re-testing intervals, delayed test results, passive systems of communication of test results, and poor linkage to treatment.<sup>73</sup>

### ***2.4.3 Natural history of paediatric HIV infection***

Children with untreated HIV infection are at high risk of rapid disease progression with a median survival of 2 years observed among African cohorts in the pre-ART era, significantly lower compared to adults, and survival beyond early childhood was considered exceptional.<sup>59,74</sup> Typical manifestations included failure to thrive, rapid immunosuppression and multisystem and often multiple opportunistic infections, and progressive encephalopathy, usually manifest during the first months of life.<sup>74,75</sup>

### ***2.4.4 Long-term survival following untreated vertical HIV infection***

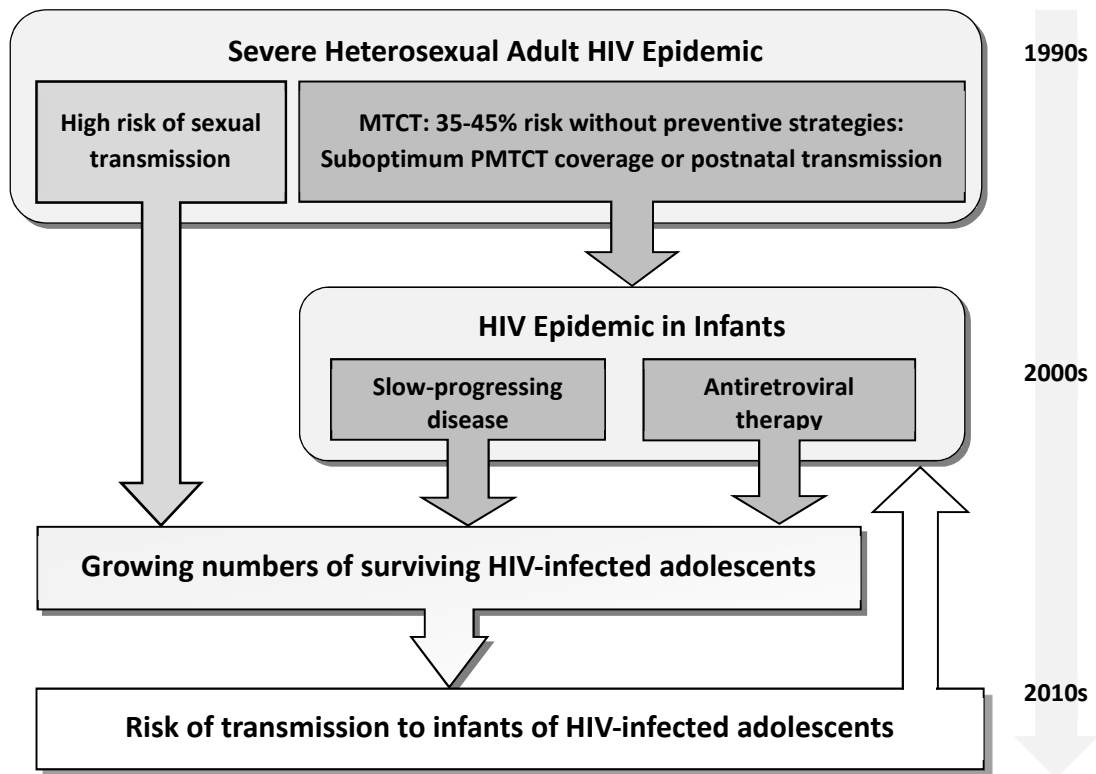
As HIV epidemics matured, it became apparent that about a third of children infected with HIV experience slow-progressing disease. It is estimated that this group has a median survival of at least 16 years, with no upward estimates available (as cohorts were not followed up for enough).<sup>76</sup> This group was recognised when increasing numbers of older children and adolescents started to present to health services with features suggestive of longstanding infection including stunting and history of chronic, minor illnesses across childhood in SSA.<sup>77</sup> The difference in natural history among infants with slow and fast progressing disease could in part be explained by timing of infection. Postnatal

HIV transmission is associated with slower progression of disease compared to in utero transmission.<sup>78</sup> The development of the foetal immune system begins in the first trimester around 4–6 weeks of gestation. The nonspecific innate and the adaptive immune systems develop throughout the gestation period and at birth the adaptive immune response is fairly immature due to lack of antigenic exposure in-utero.<sup>79,80</sup> Therefore, the immune response of the neonate against infection relies upon passively acquired maternal antibodies and components of the innate immune system.<sup>79</sup> In children who acquire HIV infection through breastfeeding, infection occurs when the immune system is more mature and therefore able to mount a more effective immune response and possibly influence disease progression.<sup>59,81</sup> Other postulated factors that may influence disease progression in the infant include maternal viral load and strain of infecting virus.<sup>82,83</sup>

#### ***2.4.5 “Ageing” of the paediatric HIV epidemic***

The scale-up of PMTCT programmes has resulted in a decline in the number of children being born with HIV globally. At the same time, the improved survival as a result of the scale-up of ART has resulted in increasing numbers of children, reaching adolescence and adulthood. In addition, as discussed above, children infected before PMTCT programmes were available, and did not die in early childhood (i.e. slow-progressors) have also been presenting to health care services with previously undiagnosed HIV in older childhood and adolescence.<sup>77</sup> This implies a long delay in diagnosis of HIV infection, and is due to the relative neglect until recently by paediatric HIV programmes of older children and adolescents.<sup>84</sup> Overall, therefore the paediatric epidemic is “ageing” with

paediatric HIV programmes seeing relatively larger numbers of older children and adolescents compared to infants (fig 2.4).



**Figure 2.4 Evolution of the paediatric HIV epidemic in sub-Saharan Africa (adapted from.<sup>84</sup>)**

It is important to note that global coverage of ART among children remains lower than in adults 43% vs 54%, respectively in 2016 and this is mainly due to delayed diagnosis of children particularly in low-income countries.<sup>50</sup> In a recent systematic review, the median age of ART initiation in high-income settings was 0.9 (0.4–2.6) years, compared to 7.9 (6.0–9.3) years in low-income countries.<sup>85</sup>

## 2.5 Chronic co-morbidities and HIV infection

The scale-up of ART has substantially increased life-expectancy of HIV-infected individuals. However, there is increased recognition that HIV infection is associated with a multi-system co-morbidities, disease, liver disease, osteopenia

and osteoporosis, neurocognitive disease and cardiovascular disease.<sup>86</sup> It is important to note that the background risk of these comorbidities increases with age and therefore this increase risk may be a cohort effect of individuals with HIV living longer, or confounding by established risk factors such as smoking, alcohol and drug use.<sup>87</sup>

However, HIV is now known to also have a pro-inflammatory effect, with HIV infection resulting in persistent chronic inflammation which is implicated in the pathogenesis of some co-morbidities, particularly cardiovascular disease.<sup>88</sup> ART reduces the risk of infections. And the SMART trial showed a beneficial effect of ART against development of non-AIDS defining events.<sup>42</sup> However, several studies have shown that that residual chronic immune activation persists despite ART.<sup>89,90</sup> The SMART trial showed higher levels of inflammatory and coagulation markers in patients with treated HIV disease than in uninfected control subjects.<sup>91</sup> Chronic immune activation causes release of cytotoxic cytokines which contribute to progressive and late tissue damage and.<sup>92</sup> Other possible causes of chronic co-morbidities may likely be long term sequelae of infections or effects of ART e.g. tenofovir has been associated with renal and bone disorders.<sup>93</sup> Nucleoside reverse transcriptase inhibitors (NRTIs), the backbone of ART including abacavir, and zidovudine have been associated with cardiotoxicity.<sup>94,95</sup>

### ***2.5.1 Chronic complications of HIV infection in children***

HIV in children is also associated with multisystem complications, including encephalopathy, growth failure, chronic respiratory and cardiac disease. These

can cause long-term disability. In a study conducted in Malawi among children with HIV, there was a high prevalence of self-reported disability in most domains including hearing, physical, learning/comprehension and speech.<sup>96</sup>

In infants, immediate ART is associated with a significant reduction in mortality and other benefits of early ART include improved growth and prevention of neurodevelopmental disorders.<sup>46,97</sup> Until relatively recently, ART in older children followed adult guidelines and was deferred until development of immunosuppression due to the lack of evidence of a mortality benefit and concern about the relatively longer exposure to ART (compared to adults) and its side-effects, particularly when physiological systems were immature, and poor adherence leading to development of drug resistance. There many children, particularly in low-income settings have initiated ART in later childhood. It is not clear how this will impact on the risk of co-morbidities. For example, catch-up growth is much poorer in children started on ART in older compared to younger childhood. Furthermore, the long-term effects of HIV infection acquired before the immune system is developed and the long-term adverse effects of ART may contribute to development of chronic comorbidities.

## **2.6 Cardiac disease in HIV infection**

### ***2.6.1 Cardiac disease in adults***

Cardiac disease is a well-recognised complication of HIV infection. The first case of HIV-associated cardiomyopathy was reported in adults in 1986.<sup>98</sup> A high prevalence and incidence of cardiac disease was reported in those with AIDS in



several studies in the pre-ART era (Table 2.2). The spectrum in adults included pericardial effusion and dilated cardiomyopathy.<sup>99-101</sup> The incidence of dilated cardiomyopathy in was 15.9 per 100-pys, with a consequent high mortality rate.<sup>102</sup> In patients with LV dysfunction, the median survival to death was 101 days compared to 472 days in those who had normal heart function at similar stage of HIV infection.<sup>103</sup>

In the ART era, subclinical cardiac abnormalities have been reported in HIV infected adults. In a study from USA, >50% HIV infected adults had abnormalities including LV systolic and diastolic dysfunction and pulmonary hypertension, 73% of whom were on ART and 91% were virally suppressed.<sup>104</sup> In a study from Cameroon among adults, 70% of whom were on ART, a higher prevalence of symptomatic cardiac disease was reported. However, the cohort was comparatively more immunosuppressed compared to those in the study from the USA, with two-thirds having a CD4 count  $\leq 200$  cell/mm<sup>3</sup>, suggesting that HIV-related immunodeficiency may be associated with development and possibly progression of cardiac disease.<sup>105</sup> Notably, in both studies, there was a high prevalence of traditional risk factors for cardiovascular disease.

### ***2.6.2 Cardiac disease in children in the pre-ART era***

In children in the pre-ART era, LV systolic dysfunction with dilated LV was the predominant abnormality observed and almost exclusively seen in patients with advanced HIV infection and AIDS (Table 2.3).<sup>106,107</sup> Cardiac morbidity and associated mortality were reportedly high in children with advanced HIV-infection.<sup>108,109</sup> There was a high incidence of HIV-associated cardiac dysfunction

with a five-year cumulative incidence in HIV-infected children ranging from 18-39%.<sup>110</sup> Luginbuhl *et al*, followed-up children from a median age at enrolment of 1.5 years up to a median age of 3.6 years and 10% of these children had congestive heart failure.<sup>108</sup>

Cardiac abnormalities were a predictor of all-cause mortality. Lipshultz *et al*, found that reduced LV systolic function and increased LV wall thickness predicted mortality in HIV-infected children.<sup>111</sup> More than half (51.6%) of children with HIV-associated deaths had a diagnosis of chronic cardiac disease prior to death and cardiac related mortality increased with age.<sup>112</sup> Dysrhythmias also occurred more frequently.<sup>108</sup>

Subclinical cardiac abnormalities were also reported and were progressive.<sup>113</sup> In the paediatric pulmonary and cardiac complications (P<sup>2</sup>C<sup>2</sup>) study, all the children (median age 2.1 years) had decreased LV systolic function, and this decreased further over the two-year period of follow-up. Overall, there was a notable increase in LV mass and after load.<sup>113</sup> Notably, most of these studies have been conducted in high-income settings and in younger children.

### **2.6.3 Current understanding about paediatric cardiac disease**

ART has transformed HIV-infection into a manageable chronic condition with longer life expectancy among children with perinatally-acquired HIV.<sup>84</sup> This has resulted in more questions about the long-term effects of HIV and ART on the incidence and course of cardiac disease.<sup>92</sup> Reports from high-income countries have confirmed a decline in the incidence of HIV-associated cardiomyopathy

suggesting a cardio-protective role of ART.<sup>94,114,115</sup> A study conducted by Patel et al in the USA in children taking (median age 10.5 years), a six-fold decrease in incidence of cardiomyopathy was reported compared to the pre-ART era from 25.6 per 1000 person-years to 3.9 per 1000 person-years.<sup>94</sup> This study relied on clinician report or initiating digoxin for meeting the diagnosis of cardiomyopathy, without echocardiographic scanning.<sup>94</sup> In a small cohort of Italian children (n=38 and median age 5.5 years) with HIV infection, all the children who had dilated cardiomyopathy (DCM) resolved after 6 months of ART initiation. Normal cardiac function persisted in these children during the 5-year follow-up.<sup>115</sup> However, a recent study conducted in the USA by Lipshultz et al has suggested that the cardio-protective effect of ART in children diminishes over time.<sup>116</sup> Lipshultz et al assessed the long-term effects of ART and HIV in children in two US cohorts.<sup>116</sup> The cohorts were HAART-Associated Cardiotoxicity in HIV-Infected Children (CHAART-2) study participants and the historic controls from P<sup>2</sup>C<sup>2</sup> study conducted in the pre-ART era. LV fractional shortening and LV contractility (measures for systolic function) were consistently and significantly higher in children exposed to ART. However, LV systolic function began to decrease with longer follow-up and at 11 years, the mean measures for systolic function were similar to the baseline values of the ART-unexposed children.<sup>116</sup> Whether this decrease in function will continue to worsen, is unknown.

#### ***2.6.4 Cardiac disease in African children***

These findings from a high-income setting cannot be generalised to children in Africa in whom a significant proportion of the children have had delayed

diagnosis and/or ART initiation, may have been more immunosuppressed when starting ART, and be at higher risk of infections.<sup>117</sup>

There is paucity of data from SSA on the long-term effects of ART in HIV-infected children (Table 2.3). Pepeta *et al*, retrospectively assessed the impact of ART on LV function in South African HIV-infected children (median age 94 months) pre- and post-ART and observed a significant improvement of myocardial function following ART initiation.<sup>118</sup> In addition to the improved myocardial function, there was also immunological improvement demonstrated by the rise in CD4 count levels and viral load suppression.<sup>118</sup> The sample size was very small (34 children) and due to the study design, there was potential selection bias in the selected patients.

Several cross-sectional studies have been conducted in African children which give a snapshot of the high burden of HIV-associated cardiac complications post-ART.<sup>119-124</sup> The prevalence of cardiac disease varies significantly between studies and range between 13.7% to greater than 76%. There is no consistency in definitions of cardiac abnormalities in the different studies. Chelo *et al*, used adult references to define right ventricular dilatation in their cohort which may have resulted in over-reporting of the abnormality.<sup>120</sup> The populations of HIV-infected children studied were either ART-exposed or ART-naïve or mixed.<sup>119,121,124</sup> Some children were very ill and hospitalised, and the others were outpatients and hence potentially less ill.<sup>121,124</sup> These variations limit the generalisability of the findings across the region.

In addition to echocardiographic findings in the studies, conduction abnormalities were also assessed. Namuyonga *et al*, found that the most common electrocardiographic (ECG) abnormality was nonspecific T wave changes among children on ART.<sup>119</sup> Ige *et al*, found that prolonged corrected QT (QTc) interval was more common in HIV-infected children compared to uninfected controls and this was not associated with use of ART.<sup>125</sup> Prolonged QTc interval may progress and result in fatal arrhythmias. Electrolyte imbalance related to malnutrition and/or chronic diarrhoea may induce ventricular arrhythmias by prolonging QTc interval.<sup>126</sup>

### **2.6.5 Risk factors for cardiac disease**

Low CD4 count and advanced stage of HIV infection were commonly reported risk factors in both adult and children (Tables 2.2 & 2.3).<sup>106,113,127,128</sup> Immunosuppression increases the risk of infection including cardiac infections or infections that also involve the heart, for example TB pericarditis.<sup>129</sup> Specific ART drugs were also associated with cardiotoxicity.<sup>94,104,130</sup> Domanski *et al*, in a retrospective study, found that use of zidovudine was associated with reduced LV performance.<sup>130</sup> Mitochondrial toxicity has been attributed to nucleoside reverse transcriptase inhibitors (NRTIs) exposure and clinical manifestations can include cardiomyopathy and myopathy.<sup>131</sup> Older age, smoking, hypertension, diabetes and higher BMI are well-recognised risk factors for cardiovascular disease and although most studies have tried to address confounding by these risk factors, residual confounding is hard to rule out. Nevertheless, it is widely accepted that HIV and/or its treatment is an independent risk factor for cardiac disease in adults.<sup>128,132</sup> Underlying systemic immune activation and chronic

inflammation, a consequence of HIV infection is thought to contribute to pathogenesis of cardiac disease.<sup>88</sup> Systemic immune activation is not completely suppressed even with ART.

Studies in children can provide insight into pathogenesis of HIV-associated cardiac disease because of the absence of traditional risk factors. In a study conducted in USA in children with HIV (median age 10.5 years), older age at ART initiation and lower nadir CD4 count increased the likelihood of cardiomyopathy.<sup>94</sup> This may have been due to a prolonged period of uncontrolled HIV infection. HIV encephalopathy was also reported as a risk factor for cardiac disease in children, likely a proxy for immunodeficiency.<sup>106,113</sup> Miller *et al*, found that stunting was associated with LV diastolic dysfunction, and longer duration of ART (>12 months of ART) had higher odds for RV dilatation among Zimbabwean adolescents with perinatally acquired HIV. However, a quarter of participants in this study were ART naïve.<sup>121</sup> Stunting in children is a marker of chronic inflammation and associated with childhood inflammatory disease such as inflammatory bowel disease.<sup>133</sup> It is possible that underlying systemic inflammation may have a role in pathogenesis.<sup>88,133,134</sup> There may be an interaction between the effects of ART and HIV, although, the magnitude and direction of the effects are unknown.<sup>92</sup> HIV-infected children may have been exposed to ART drugs in utero and/or in early childhood when physiological systems that metabolise drugs may not be mature.

Author	Year	N	Country	Age, y	% On ART	Cardiac abnormalities, %	Cardiac abnormalities	Risk factors
Currie et al	1994	296	UK	21-67y	None	15	DCM, isolated RV dilatation, borderline LV dysfunction, survival in patients with DCM was reduced compared to those without (102 days vs 472 days)	NR
Heidenreich et al	1995	231	USA	>18 y	None	11	11% incidence of PE/year. Survival of PE patients was shorter.	NR
Barbaro et al	1998	952	Italy	>18 y	None	8	Incidence of DCM was 15.9 per 1000pys	Low CD4 count (<200cells/mm <sup>3</sup> )
Silva Cardoro	1999	181	Portugal	>18 y	None	41	Acute pericarditis, PE and pericardial masses	NR
Twagirumukiza et al	2007	416	Rwanda	19-61	None	18	DCM	Low-socio economic status, Duration of HIV infection, Low CD4 count, Higher viral load, Stage of HIV, Low plasmatic selenium
Mondy et al	2011	656	USA	41	73	>50	LVDD, LVSD, PHT, dilated LA, increased LVM	Elevated hsCRP, Smoking, ART regimen, Diabetes, Hypertension, Non-white race, Female sex, ↑ BMI >25
Menanga et al	2015	44	Cameroon	>18 y	70.5	45	PE, DCM, PHT, sinus tachycardia	Hypertension, dyslipidaemia

NR= not reported; LA= left atrium; PE= pericardial effusion; RV= right ventricle; DCM= dilated cardiomyopathy; LVH= left ventricular hypertrophy; PHT= pulmonary hypertension; LVSD= left ventricular systolic dysfunction; LVDD= left ventricular diastolic dysfunction

**Table 2.2 Studies reporting on cardiac disease and risk factors in adults with HIV**

Author	Year	Type of Study	N	Country	Age	% On ART	Cardiac abnormalities, %	Cardiac abnormalities	Risk factors
Stewart et al	1989	Cross-sectional	8	USA	1	NR	100	LVSD, DCM, RV dilatation, PE	NR
Lipshultz et al	1998	Longitudinal	196	USA	1	63 (Zidovudine)	100	All abnormal at baseline LVH, DCM, depressed systolic function)	Lower CD4 (baseline) HIV encephalopathy
Al-Attar et al	2003	Retrospective	68	USA	15y & 1y	32 (Zidovudine or /DDI)	28	CHF, ECG abnormalities, PE	Recurrent bacterial infections, Wasting, Encephalopathy Low CD4 count
Plebani et al	2004	Longitudinal	38	Italy	5.5 y	100	13	13% DCM at baseline which resolved after 6 months on ART,	
Fisher et al	2005	Longitudinal	185	USA	2	56% (Zidovudine)		↑ heart rate, ↑ LVM, depressed FS, dilated cardiomyopathy	
Lubega et al	2005	Cross-sectional	230	Uganda	7	1 participant 2 wks.	51.3	RV dilatation, DCM, LVSD	Advanced HIV
Brown et al	2005	Cross-sectional	87	South Africa	<1	None	60	PE, LVSD, LV dilatations, sinus tachycardia	
Okoromah et al	2011	Cross-sectional	83	Nigeria	4	85	75.9	PE, ↑ LVM, CCF, LVSD	ART therapy Low CD4 count Older age
Ige et al	2012	Cross-sectional	150	Nigeria	6	56	50	LVSD, DCM	
Pepete et al	2012	Retrospective	34	South Africa	2 -12y	53	100	All had LV dysfunction which improved following ART	NR
Patel et al	2012	Longitudinal	3035	USA	10.5 y	100		5.6 per 1000pys incidence of cardiomyopathy	ART regimen, older age at ART initiation, Lower nadir CD4
Miller et al	2013	Cross-sectional	110	Zimbabwe	15	71	>67	LVH, PHT, RV dilatation, DD, LV dilatation, LVSD	Female sex, Stunting, Longer duration of ART
Ige et al	2014	Cross-sectional	150	Nigeria	6	56	44	↑LVM, DD	Younger age and advanced HIV
Chelo et al	2015	Cross-sectional	100	Cameroon	1 -15 y	91	89	RV dilatation, LV diastolic dysfunction, LVH, PE, LVSD	WHO clinical stage,
Namuyonga	2016	Cross-sectional	285	Uganda	1-18y	100	39	Pericardial disease, non-specific T-wave changes	None
Lipshultz et al	2017	Prospective	74 ART, 860 No ART	USA		74 (8%)	-	ART-exposed had better cardiac function than ART naïve. FS, LVM and septal thickening were normal in ART group but began to decrease after 11 yrs. of follow-up	-

NR= not reported; LA= left atrium; FS= fractional shortening; PE= pericardial effusion; RV= right ventricle; LVDD= left ventricular diastolic dysfunction DCM= dilated cardiomyopathy; LVH= left ventricular hypertrophy; PHT= pulmonary hypertension; CHF= congestive heart failure; LVM= left ventricular mass; LVSD= left ventricular systolic dysfunction

**Table 2.3 Studies reporting on cardiac disease and risk factors in children**



Notably, many of the studies investigating cardiac disease have included both ART naïve and ART- experienced children have had relatively small sample sizes, which may partly explain the variation in reported risk factors.

### **2.6.6 Hypertension in HIV**

People living with HIV are at an increased risk of developing hypertension and the prevalence of hypertension in HIV is reportedly higher than the general population (35% vs 30%).<sup>135</sup> Hypertension is a well-known risk factor for cardiovascular events in adults. Individuals on ART have a heavier burden of hypertension compared to those who are ART-naïve.<sup>135,136</sup> Older age, overweight and ART were associated with hypertension.<sup>135,136</sup> In a retrospective study, Chatterton-Kirchmeier and colleagues reported a high prevalence (18%) of high blood pressure in children, adolescents and young adults with HIV (aged 2-25 years).<sup>137</sup> The pathophysiology of hypertension in HIV is likely multifactorial. HIV-induced pro-inflammatory effect on vascular endothelium and ART altering the metabolic processes, leading to lipodystrophy, overweight and obesity may contribute to the increased risk of hypertension.<sup>135,138</sup> Fewer studies have investigated the pathophysiologic mechanisms leading to hypertension in people living with HIV.

## **2.7 Assessment of cardiac disease**

### **2.7.1 Methods of cardiac assessment**

Cardiac disease is assessed by various methods and techniques. Evaluation of heart disease includes assessment of cardiac electro-conductivity, structure and function. Each of the available methods of assessment has advantages and

limitations which may necessitate use of more than one investigative method on a patient.

Chest radiography allows the assessment of the cardiac size and of pulmonary oedema , and is relatively cheap and widely accessible in low-income settings.<sup>139</sup>

Electrocardiography (ECG) is non-invasive, and also relatively accessible and inexpensive. It assesses the electrical conduction system of the heart and the 12-lead ECG, along with chest radiography is widely used for initial assessment of individuals presenting with symptoms and signs suggestive of cardiac disease.<sup>140</sup>

Both modalities give limited information about cardiac structure and function and are insensitive diagnostic modalities.

Other methods of assessment that are more sensitive and give much more detailed information on cardiac structure and function include:

- Cardiac catheterization, an invasive procedure involving insertion of a catheter in the arteries and/or veins through to the heart to measure pressure in the various cardiac chambers. Right heart catheterisation is the gold standard for measuring right heart pressures in diagnosis of the pulmonary hypertension.<sup>141</sup>
- Computerized tomography (CT), is non-invasive and evaluates the heart using radiation similar to chest radiography and uses x-rays. One of its advantages is the excellent tissue characterization, high spatial resolution and short acquisition times.<sup>142</sup>

- Cardiac magnetic resonance (CMR) imaging is the gold-standard for cardiac assessment.<sup>142</sup> It uses magnetic fields and radiofrequency waves to produce detailed cardiac images.

These are however either invasive, require technical expertise, are expensive and not easily accessible in low-income settings.

### **2.7.2 Echocardiography**

Echocardiography (ultrasound of the heart) is the mainstay of cardiac assessment and can evaluate cardiac morphology, function and haemodynamics. It is widely used as one of first line diagnostic tools. It is safe, suitable to use in both adults and children, relatively inexpensive and relatively accessible in low-income settings. There are published guidelines and standards for performing echocardiography in different patient groups e.g. assessment of right heart in adults and for different cardiac evaluations e.g. evaluation of left ventricular diastolic function.<sup>143,144</sup> The guidelines define the echocardiographic windows to use for optimal evaluation, routine measurements required with their advantages and disadvantages. The most common guidelines available have been published by the American Society of Echocardiography (ASE) with endorsements from other societies including the European Society of Cardiology (ESC) and the Canadian Society of Echocardiography.<sup>145,146</sup> In this thesis guidelines from the ASE have been used.

### **2.7.3 Echocardiographic reference ranges**

Accurate measurement of cardiac dimensions and interpretation of imaging is a prerequisite for echocardiography.<sup>147</sup> This necessitates reliable reference ranges for defining normality or abnormality. This is particularly important in children who are undergoing linear and organ growth through childhood, and cardiac dimensions must be adjusted for body size. Body size surrogates include body surface area (BSA) or height or weight and these are typically used to normalise the cardiac dimensions to size.<sup>148</sup>

Available reference ranges have been derived predominantly from studies in Caucasians. It is important to note that anthropometric standards of a population are also influenced by genetics, environmental and economic factors.<sup>149</sup> Therefore, the reference ranges used to define normality of cardiac size will likely vary within different population groups. Echocardiographic reference ranges also depend on the parameters used to define anthropometric parameters used to normalising cardiac dimensions to body size, and on image acquisition and measurements techniques.<sup>150,151</sup> Racial differences in cardiac dimensions are reported between populations, for example the black children in a Brazilian study reportedly has larger cardiac dimensions compared to white children.<sup>149</sup> A systematic review in Chapter 3 considers racial differences in left chamber dimensions (LV and left atrium) in available echocardiographic references for children.

Notably, studies investigating cardiac complications in HIV-infected children are difficult to compare due to varying echocardiographic definitions, for example Chelo et al used adult reference ranges to define RV dilatation in children.<sup>120</sup>

## **2.8 Zimbabwe**

Zimbabwe is a Southern African land-locked country bordering Zambia, Mozambique, Botswana and South African to the, north, east, west and south, respectively. It comprise ten provinces and 63 districts , with an estimated population of 16.15 million in 2016.<sup>152</sup> Zimbabwe has experienced a decline it is economy over the past decade, with a near collapse of its health system in 2008 when the country had severe hyperinflation and an outbreak of cholera. .<sup>153</sup> The majority of the population (72%) live below the national poverty line, and 21% living on less than \$1.90 a day.<sup>152</sup>

### ***2.8.1 HIV epidemic in Zimbabwe***

The first AIDS case was reported in 1985 and this was followed by reports of adults and children presenting with immunodeficiency.<sup>154</sup> Zimbabwe has experienced an early-onset of severe and generalised HIV epidemic with HIV prevalence peaking at 34.2% among pregnant women in 1996, and resultant high rates of MTCT, given the lack of PMTCT programmes.<sup>155</sup> The prevalence of HIV subsequently declined (Zimbabwe being one of the first countries to observe the decline), likely due to the success of HIV control programmes leading to reduced incidence, maturation of the epidemic and HIV-associated mortality.<sup>156,157</sup> The adult HIV prevalence in Zimbabwe has declined from a high of 25.3% in 1997 to

14.5% in 2016.<sup>158</sup> It continues to have a severe epidemic (the 6<sup>th</sup> highest) prevalence country globally, with a health system weakened by the severe economic challenges and the heavy HIV epidemic. and shortage of public health workers.

The scale up of ART and PMTCT programmes in the mid-2000s have contributed to a decline in the prevalence among pregnant women as well as in number of infections in children.<sup>159</sup> However, as the epidemic matured, growing numbers of older children and adolescent survivors of MTCT were presenting to health care services with undiagnosed HIV. These children were infected at the peak of the HIV epidemic when PMTCT programmes were not available. The lack of recognition of the possibility of slow progression of disease in children led to many being diagnosed when they presented with advanced disease in adolescence or even in early adulthood. PMTCT coverage has improved with 85% coverage in 2015, leading to a decline in the number of babies born with HIV.<sup>160</sup> As in other parts of the region, the scale-up of ART has resulted in increased survival so that older children and adolescents constitute a proportionally larger (and growing) group being cared for by paediatricians.

### ***2.8.2 HIV care delivery***

Zimbabwe's health care system has four-tiers which include primary level including clinics (rural and urban) and rural health centres; district level, including district, rural, mission and municipals; provincial level comprising of provincial hospitals and finally central hospitals in the main cities are the third referral level.<sup>161</sup>

ART became available in the public health sector in 2004. A public health approach to HIV care, including guidelines for treatment, standardised treatment regimens, task-shifting from doctor to nurse-led HIV care (include ART initiation and follow-up treatment), and decentralisation of ART delivery from secondary to primary care level have been introduced to achieve universal coverage of HIV services. At the end of 2015 there were 1545 sites offering HIV services and of these 1475 initiated ART with the remainder providing follow up services.<sup>161</sup>

While this is a remarkable achievement, the focus of HIV programmes inevitably has been on HIV testing and on ART delivery to achieve universal coverage. The health system has to date not focused on screening and management of the long-term complications that accompany HIV infection. Also, while ART is provided free of charge, there is a user fee associated with each contact with health services. In addition, the cost of investigation and treatment of any infectious or non-infectious complications incurs a fee.

## **2.9 Summary**

SSA has experienced a severe HIV epidemic over the past 30 years, with the scale-up of ART reversing the tide of mortality in adults and children accompanying the HIV epidemic. Growing numbers of children, many of whom would have died in early childhood are reaching adolescence and adulthood because of ART. However, many were diagnosed and started ART in later childhood, rather than in infancy.

While that is undoubtedly a remarkable achievement, there is increasing recognition that longstanding HIV infection and possibly treatment may be associated with long-term comorbidities. In adults with HIV, cardiovascular disease is of major concern, but it is confounded by age and the traditional risk factors. A high incidence and prevalence of cardiac disease was also noted among children in the pre-ART era, many of whom had advanced immunosuppression and the majority were conducted in young children and in high-income studies settings.

There is sparse data on cardiac disease in children in SSA, where 90% of the world's HIV-infected children live. Findings from high-income settings cannot be generalised to children with HIV in SSA for several reasons including but not limited to level of immunosuppression before, and timing of, ART initiation, different genetic factors, the infectious milieu and the relative poorer access to health services. Furthermore, study of cardiac complications among HIV-infected children in SSA is complicated by lack of regional echocardiographic reference ranges in children. My research aims to address these knowledge gaps and to investigate the prevalence, incidence and progression of cardiac abnormalities using echocardiography.

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**3. Racial variation in echocardiographic reference ranges in children and adolescents: A systematic review**

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### **3.1 Introduction**

A systematic review on echocardiographic reference ranges of the right heart has been previously published by Lemmer and colleagues which identified the gaps in available reference ranges especially with respect to SSA.<sup>1</sup> Several areas of right heart dimensions lacked sufficient data including right atrial size, tricuspid valve area, RV dimensions and areas, RV percent fractional area change, pulmonary artery pressure gradients and right-sided haemodynamics, including the inferior vena cava dimensions. Importantly, they found no data from SSA. This implies that most African countries rely on reference ranges from other regions (mainly Western countries) which may not accurately represent the Black African children.

This chapter is a published systematic review of racial variations in echocardiographic reference ranges for left chamber dimensions in children and adolescents. The main aim of the review was to highlight the importance of race-specific reference ranges in children. Briefly, we found that most of the available echocardiographic references were conducted in North American and European Caucasian populations. There was only one study each from South America and Africa (Black children). The study from Africa was conducted as part of my PHD and is reported in Chapter 4. The comparison of selected reference ranges among Black African, Indian, German and US American children showed significant differences for interventricular septal thickness. The study further highlighted the non-standardisation of echocardiographic methods and normalisation parameters in deriving the reference ranges.

These findings demonstrate that available reference ranges cannot be generalised to all settings.

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### **3.2 References**

1. Lemmer CE, Stanfliet, Mayosi BM. Reference intervals for the echocardiographic measurements of the right heart in children and adolescents: a systematic review. *Cardiovascular ultrasound* 2014;12.

### 3.3 Research paper I

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### SECTION A – Student Details

Student	Edith Delewe Majonga
Principal Supervisor	Professor Rashida Ferrand
Thesis Title	Cardiac abnormalities in HIV-infected older children and adolescents in Zimbabwe

**If the Research Paper has previously been published please complete Section B, if not please move to Section C**

### SECTION B – Paper already published

Where was the work published?	Pediatric Cardiology		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I wrote the study protocol, led all field work and was responsible for echocardiographic and electrocardiographic data acquisition and data analysis. I wrote the first and subsequent drafts of the manuscript.
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Student Signature: \_\_\_\_\_

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# Racial Variation in Echocardiographic Reference Ranges for Left Chamber Dimensions in Children and Adolescents: A Systematic Review

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## Abstract

Echocardiography plays a critical role in the assessment of cardiac disease. Important differences in echocardiographically derived cardiac chamber dimensions have been previously highlighted in different population groups in adult studies, but this has not been systematically studied in children, whose body size changes throughout childhood. The aim of this study was to review the distribution of available reference ranges for the left cardiac chamber dimensions in older children and adolescents. The following electronic data bases were searched: Medline, Embase and Web of Science were searched to identify studies which have established echocardiographic reference ranges of left heart parameters in children and adolescents from 1975 to December 2017. There was no geographical limitation. All results were imported into Endnote. Retrieved articles were screened and data extracted by two independent reviewers. A total of 4398 studies were retrieved, with 36 studies finally included in this review. 29 (81%) references were from North America and European (Caucasians) populations, with only one study each from Africa and South America. Two-dimensional and M-mode techniques were the most commonly used echocardiography techniques. There were methodological variations in techniques and normalisation of references. Comparison of selected cardiac measures showed significant differences for interventricular septal thickness among Black African, Indian, German and US American children. Available echocardiographic references cannot be generalised to all settings and therefore, there is need for locally relevant reference ranges. Africa and South America are particularly under-represented. Future studies should focus on developing comprehensive echocardiographic reference ranges for children from different racial backgrounds and should use standardised techniques.

**Keywords** Echocardiography · Reference ranges · Left ventricle · Left atrium · Children

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## Introduction

Echocardiography plays a critical role in the assessment of cardiac structure and function. Due to changes in body size during childhood, the evaluation of cardiac chambers is highly reliant on the availability of reference ranges, the quality of which depends largely on the availability of a representative sample of healthy subjects and the methods employed to collect the data. The definition of what is “normal” varies widely according to age, body surface area (BSA), gender and race [1, 2]. Studies in adults have shown racial differences in echocardiographically derived cardiac chamber dimensions [1]. These differences may be more apparent in children whose body size changes throughout childhood, but this has not been investigated systematically.

The aim of this study was to systematically review racial distribution and methods used in available echocardiographic reference ranges for left ventricular (LV) and atrial (LA) chamber dimensions in children and adolescents. In addition, we compared values of selected chamber dimensions in different racial groups which have utilised the same methods for any differences.

## Materials and Methods

This review was registered with the international prospective register of systematic reviews (PROSPERO; registration number CRD42015026030).

## Type of Studies

All available studies that reported echocardiographic reference ranges for left cardiac chamber dimensions in healthy children and adolescents, regardless of echocardiographic technique, were considered for inclusion in this review.

## Inclusion Criteria and Exclusion Criteria

Studies including at least 50 healthy participants aged 5–21 years that reported echocardiographic measurements at rest were included. We considered studies written in English and published in peer-reviewed journals. Studies that only included neonates and infants, were conducted at high altitude (> 24,000 m above sea level); involved performing cardiac measures during or after exercise; or were based on autopsy specimens were excluded. Systematic reviews and meta-analyses were also excluded.

## Search Strategy

The following electronic databases were searched: Medline, Embase and Web of Science were searched. In addition, reference lists of selected studies and other systematic reviews were manually reviewed to identify other possible studies for inclusion. The search strategy included the following words: “echocardiography” AND “reference values” OR “normative” OR “reference standards” OR “reference intervals” AND “child” OR “children” OR “adolescent” OR “z score” (Table 1). Appropriate Boolean operators and truncation were used on synonyms. Both medical subjects and

**Table 1** Search strategy

Concepts	Set	Search terms
Echocardiography	1	echocardiography.mp or exp echocardiography/
References	2	exp reference values/or normative.mp
	3	Reference adj1 values
	4	Reference adj1 standards)
	5	Reference adj1 interval*
	6	Normal values/or reference value/or reference interval*.mp
Children	7	Child.mp or child/
	8	adolescent*.mp or exp adolescent/
	9	pediatric.mp or pediatrics/
	10	Paediatric.mp or paediatrics/
	11	Children*.mp
	12	Infant.mp or exp Infant/
Z-score	13	z-score.mp
	14	2 or 3 or 4 or 5 or 6 or 13
	15	7 or 8 or 9 or 10 or 11 or 12 or 13
	16	1 and 14
	17	15 and 16

keywords were used. The same search strategy was adapted for all the listed databases. All results were imported into Endnote X7 (Thomas Reuter).

Duplicate citations were removed. Titles and abstracts from the search results were screened independently by two reviewers (EDM) and (GN). The full texts of potentially eligible studies were obtained and assessed in duplicate using a standardised checklist. Any disagreements about inclusion of studies were resolved by consensus.

## Data Extraction and Analysis

The following data were extracted using a standard data extraction form: author; population studied; sample size; age range; echocardiography technique; parameters measured, and type of normalisation used.

A two-sample Kolmogorov–Smirnov non-parametric test was used to compare distributions of  $z$  score = 0 and 2 for selected cardiac measures of studies which used the same technique for performing echocardiography and the same method for calculation of BSA for normalisation. The selected  $z$  score represents the mean predicted value and the upper cut-off for the normal range for a cardiac measure. The null hypothesis was that the compared groups were sampled from populations with identical distributions. Due to multiple testing, the chance of obtaining a significant  $p$  value when in fact there is not a true difference between distributions was high. Therefore, we used the Bonferroni adjustment, with a  $p$  value < 0.005 considered significant.

## Quality of the Studies

We adapted the Newcastle-Ottawa Scale for assessing quality of non-randomised studies to suit cross-sectional studies in the systematic review [3]. In our tool, we assigned scores instead of stars (Table 2). The following criteria were used to determine the quality of the studies: representativeness of the sample; sample size; sample selection; standardisation of image acquisition and statistical methods used.

## Results

A total of 4398 citations were retrieved dating from 1975 to June 2017. Of these, 1193 duplicates were removed, and a further 3075 citations were excluded based on title and abstract (Fig. 1). Full texts of a total of 130 studies were reviewed and 36 studies were included. Characteristics of the included studies are in Table 3.

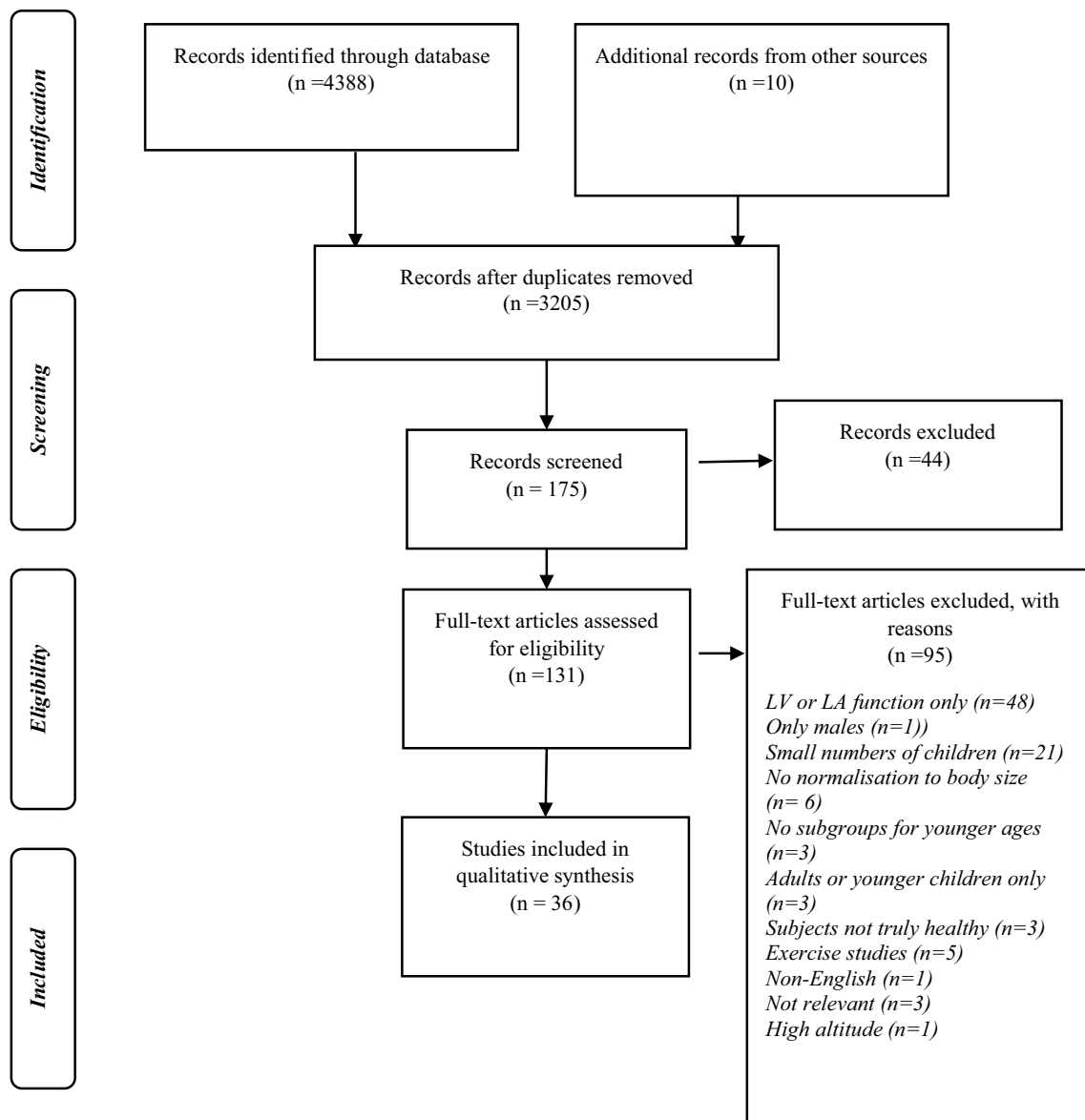
Sixteen (44%) studies were conducted in North America, followed by Europe ( $n = 13$ , 36%) and Asia ( $n = 5$ , 14%). Only one study each was conducted in South America (Brazil) and Africa (Zimbabwe). Nine studies reported the race

**Table 2** Criteria for assessment of quality of studies using the Newcastle-Ottawa Scale adapted for cross-sectional studies

Scale of coding
Representativeness of sample
Truly representative = 3
Somewhat representative = 2
No description of sampling strategy = 1
Sample size
Justified and acceptable = 2
Not justified = 1
Sample selection
Hospital/volunteer = 3
Databases = 2
Not reported = 1
Standardisation of images
American Society for Echocardiography guidelines/other = 2
Not reported = 1
Statistical methods
Rigorous with clear exclusion criteria of abnormal cases = 3
Acceptable = 2
Not appropriate or incompletely described = 1
Total score
11–13 = good quality
8–10 = acceptable
5–7 = poor

of children studied [6, 8, 9, 19, 23, 25, 33, 38, 39]. Sample sizes of the studies ranged from 95 to 9858. The quality of the studies was good ( $n = 16$ ) or acceptable ( $n = 20$ ) in all cases.

M-mode and/or two-dimensional (2D) echocardiographic techniques were used in 33 (92%) studies. One study used three-dimensional (3D) echocardiography in addition to M-mode and 2D, another utilised 3D only and a third study used a rarely practiced echocardiographic technique called acoustic quantification [18, 21, 22]. Anthropometric and non-anthropometric measures were used for normalising the results: body surface area (BSA) in 18 studies, height ( $n = 4$ ) [8, 11, 17, 28], weight ( $n = 3$ ) [10, 20, 35], age ( $n = 2$ ) [14, 15], and lean body mass ( $n = 1$ ) [7]. The remaining studies used age and heart rate, ( $n = 1$ ); [22] age and BSA, ( $n = 2$ ); [26, 37] age and height, ( $n = 1$ ); [27] height and BSA, ( $n = 1$ ); [33] weight and BSA, ( $n = 1$ ) [36] and one study used height, BSA and lean body mass [25]. Varying methods to calculate the BSA were used: 12 (33%) studies used the Dubois and Dubois method, and 4 (14%) studies used the Haycock. Daubeney et al. used the Boyd method; [24] Saito et al. calculated BSA using the West Nomogram [37] and five studies did not report the method used for calculating BSA [25, 29, 32, 39]. 23 (64%) studies standardised



**Fig. 1** PRISMA flow diagram for process of selecting included studies

images and performed measurements according to recommendations by the American Society of Echocardiography (ASE); one study used ASE and European Society of Cardiology recommendations and another study used ASE and Penn convention [23, 26]. The remaining studies used other methods of performing measurements, including inner edge to inner edge method in five studies [24, 28, 30, 31, 34]; trailing edge to leading edge in one study [32]; leading edge to leading edge in one study [35]; standard and Penn convention in one study [37]. Three studies described how they measured parameters without stating a specific convention [21, 38, 39].

### Left Ventricular Dimensions

The following LV dimensions were reported: diameter at end-diastole (LVEDD) and/or end-systole (LVESD), posterior wall and interventricular septal, area, length, volume, LV mass and index. Saito et al. developed references for LV muscle volume, which is a rarely used measure in clinical practice [37]. 13 studies were from North American children; 12 studies among European children; five studies from Asia and one study each from South American and African children (Table 3).

**Table 3** Characteristics of studies

Author	Population studied	Sample size	Age range (y)	Technique	Parameters measured	Standardisation of images	Normalisation	Quality of the study
Lopez et al. [4]	US American and Canadian	3215	0–18	2D	LVEDD, IVSd, LVPWd, LV length, LV area, LV volume, LVM	ASE	BSA (Haycock)	Good
Gokhroo et al. [5]	Indian	746	4–15	M mode/2D	LVEDD, LVESD, IVSd, IVSs, LVPWd, LVPWs, LA length	ASE	BSA (Haycock)	Good
Majonga et al. [6]	Zimbabwean (Black)	282	6–16	M mode	LVEDD, LVESD, IVSd, IVSs, LVPWd, LVPWs, LA	ASE	BSA (Dubois)	Good
Foster et al. [7]	US Americans and Canadian	1710	5–18	M mode/2D	LVM	ASE	Lean body mass	Good
Chinai et al. [8]	Italian and German (Caucasians)	400	0–18	M mode/2D	LVM	ASE	Height	Good
Cantinotti et al. [9]	Italian (Caucasians)	1091	0–17	M mode/2D	LVED area, LVES area, LVED length, LVES length, LVEDD, LVESD, LA length, LA area	ASE	BSA (Haycock)	Good
Oran et al. [10]	Turkish	1200	0–17	M mode/2D	LVEDD, LVESD, IVSd, LVPWd, LA	ASE	Weight	Good
Motz et al. [11]	German	9858	0–19	M mode/2D	LVEDD, LVESD, IVSd, LVPWd	ASE	Body length	Acceptable
Bhatia et al. [12]	US American	300	0–18	2D	LA volume	ASE	BSA (Haycock)	Acceptable
Kervancioglu et al. [13]	Turkish	208	0–14	M mode/2D	LVM	ASE	BSA (Dubois)	Acceptable
Taggart et al. [14]	US American	522	0–17	2D	LA volume, LA volume index	ASE	Age	Acceptable
Khoury et al. [15]	US American	2273	0–18	M mode/2D	LVM and LVMI	ASE	Age	Good
Pettersen et al. [16]	US American	782	0–18	2D/M-mode	IVSd, IVSs, LVEDD, LVESD, LVPWd, LVPWs, LA	ASE	BSA (Dubois)	Good
Foster et al. [17]	US American & Canadian	440	0–17	M mode	LVM	ASE	Height	Good
Poutanen et al. [18]	Finnish	169	2–27	M mode/2D/3D	LVM	ASE	BSA (Dubois)	Acceptable
Bonatto et al. [19]	Brazilian (Blacks and whites)	595	0–12	M mode	LVEDD, IVSd, LVPWd, LA, LVM, LVMI	ASE	BSA (Dubois)	Acceptable

Table 3 (continued)

Author	Population studied	Sample size	Age range (y)	Technique	Parameters measured	Standardisation of images	Normalisation	Quality of the study
Overbeek et al. [20]	Netherlands (Dutch)	587	0–18	M mode	LVEDD, LVESD, IVSd, LVPWd	ASE	Weight	Acceptable
Poutanen et al. [21]	Finnish	169	2–27	3D	LA volumes, LVEDV, LVESV	Other	BSA (Dubois)	Acceptable
Branzenberg et al. [22]	German	150	0–14.5	Acoustic quantification	LV volume and area	ASE	Age/HR	Good
Kampmann et al. [23]	German (Whites)	2036	0–18	M mode	IVSd, IVSs, LVEDD, LVESD, LVPWd, LVPWs, LA	ASE/ESC	BSA (Dubois)	Good
Daubeney et al. [24]	British + Australian	125	0–18	2D	LV inflow, LV area	Other (inner edge to inner edge)	BSA (Boyd)	Good
Daniels et al. [25]	US American (Blacks and Whites)	192	7–17	M mode	LVM	ASE	Height/lean body mass/BSA (NR)	Acceptable
Huwez et al. [26]	British	127	0–19	M mode	IVSd, LVEDD, LVESD, LVPWd, LA, LVM, LV volume	ASE & Penn convention	Age/BSA (Dubois)	Good
Malcolm et al. [27]	US American	904	6–16	M mode	LVM	ASE	Age/height	Good
Nidorf et al. [28]	US American	196	0–18	2D	LV diameter, LV length, LA diameter	Other (inner edge to inner edge)	Height	Acceptable
Vogel et al. [29]	German	95	1–17	2D	LVM, LV volume	ASE	BSA (NR)	Acceptable
Pearlman et al. [30]	US American	268	0–18	2D	LA diameters, LA volume	Other (inner edge to inner edge)	BSA (Dubois)	Acceptable
Pearlman et al. [31]	US American	268	0–18	2D	LV length, LV area	Other (inner edge to inner edge)	BSA (Dubois)	Acceptable
Hanseus et al. [32]	Swedish	120	0–16	2D	LA length, area and width, LV diameter, area and width	Other (trailing edge to leading edge)	BSA (NR)	Acceptable
Daniels et al. [33]	US American (Blacks and Whites)	334	6–23	M mode	LVM	ASE	Height/BSA (Dubois)	Acceptable
Akiba et al. [34]	Japanese	110	0–15	M mode	LVEDD, LVESD, LVPWd, LVPWs, LV volume	Other (inner to inner edge)	BSA (Haycock)	Acceptable
Voogd et al. [35]	Netherlands (Dutch)	432	4–17	M mode	IVSd, IVSs, LVEDD, LVESD, LVPWd, LVPWs, LA	Other (leading edge of echoes)	Weight	Good
Henry et al. [36]	US America	92	0–23	M mode	LVEDD, LVESD, IVSd, IVSs, LVPWd, LVPWs, LA	ASE	Weight/BSA (NR)	Acceptable

Table 3 (continued)

Author	Population studied	Sample size	Age range (y)	Technique	Parameters measured	Standardisation of images	Normalisation	Quality of the study
Saito et al. [37]	Japanese	301	6–15	M mode	LV muscle volume	Other (Standard & Penn convention)	Age/BSA (West Nomogram)	Acceptable
Henry et al. [38]	US American (Caucasians, Blacks and Oriental)	105	0–23	M mode	IVSd, IVSs, LVEDD, LVESD, LVPWd, LVPWs, LA, LVM	Other	BSA (Dubois)	Acceptable
Epstein et al. [39]	US American (Whites, Blacks, Oriental/East Indians)	205	0–18	M mode	IVSd, IVSs, LVEDD, LVESD, LVPWd, LVPWs, LA	Other	BSA (NR)	Acceptable

*M-mode* motion mode, *2D* two-dimensional, *3D* three-dimensional, *US* United States, *M-mode* motion mode, *BSA* body surface area, *LV* left ventricle, *LVEDD* left ventricular diameter at end-diastole, *LVESD* left ventricular diameter at end-systole, *LVEDV* left ventricular volume at end-diastole, *LVESV* left ventricular volume at end-systole, *IVSd* interventricular septum at end-diastole, *IVSs* interventricular septum at end-systole, *LVPWd* left ventricular posterior wall at end-diastole, *LVPWs* left ventricular posterior wall at end-systole, *LVM* left ventricular mass, *LVMi* left ventricular mass index, *LA* left atrium, *HR* heart rate, *NR* not reported

### Left Atrium Dimensions

LA diameter references were established in twelve studies, six of which were conducted in US American children [16, 28, 30, 38, 39]. LA length references were from Indian, Italian and Swedish children and of these, two studies also reported data on LA area [5, 9, 32]. LA volume was derived in four studies and three of these were in US American children (Table 3) [12, 14, 21, 30].

### Comparison of LV and LA Dimension Between Studies

Reference values of selected cardiac measures from different racial groups which used M-mode technique and normalised results with BSA were identified and compared (Tables 4, 5). The *p* values for the compared references are shown in Supplementary Tables 1 and 2 and these are for *z* score = 0 and +2 distributions. The graphical representations of the distributions for the selected cardiac measures are shown in Supplementary Figs. 1 and 2. Mean left ventricular diameter at end-diastole (LVEDD) among US American children and German children was similar (*p* = 0.906). On the other hand, Zimbabwean children had thicker mean interventricular septum at end-diastole (IVSd) than German children had (*p* < 0.001) while US American children and German children were similar (*p* = 0.281). Mean LA diameter was also similar between British and German children (*p* = 0.699).

Comparison using predicted values of *z* score = +2 (cut-off for upper limit of normal), significant differences were noted in the IVSd measures between Zimbabwean and German children (*p* = 0.001). IVSd measures between German and US American children were significantly different (*p* < 0.001). No significant differences were noted on the distributions of LA measures between the compared studies. The predicted values of *z* score = +2 by Kampmann et al. progressed in a step-wise fashion so for example, a value of IVSd > 10.4 mm had a *z* score = +2 for BSA between 1.7 and 1.9 m<sup>2</sup>. The shape of Kampmann’s distribution for *z* score = +2 was therefore strikingly different from the other references [23].

### Discussion

Accurate assessment of left cardiac chamber size in children relies on the availability of representative reference ranges. In this study, we have systematically reviewed differences between reference ranges for LV and LA chamber dimensions in children and adolescents. We found many studies which have established reference ranges for LV and LA chamber dimensions, reflecting the significant interest in, and importance of, establishing reference ranges

**Table 4** A comparison of published echocardiographic normal references for LV dimensions in studies using M-mode and normalised to BSA (for a child with 1 m<sup>2</sup> BSA)

Author	Population studied	Method for BSA	LVEDD(mm)	LVESD (mm)	IVSd (mm)	IVSs (mm)	LVPWd (mm)	LVPWs (mm)
Gokhroo et al. [5]	Indian	Haycock	35.02 (27.02–42.04)	21.32 (13.81–28.84)	7.4 (5.5–9.3)	11.0 (8.11–13.9)	7.2 (5.4–9.1)	10.8 (10.1–11.5)
Cantinotti et al. [9]	Italian	Haycock	37.86 (31.56–45.42)	22.97 (17.46–30.20)				
Majonga et al. [6]	Zimbabwean	Dubois	37.10 (32.43–41.76)	25.29 (20.84–29.74)	7.0 (5.0–9.1)	9.2 (6.7–11.6)	6.8 (5.2–8.5)	9.0 (6.5–11.4)
Pettersen et al. [16]	US American	Dubois	39.09 (32.06–48.79)	25.1 (19.6–32.1)	5.9 (3.9–9.0)	8.6 (6.0–12.3)	5.4 (3.7–7.9)	10.3 (7.7–13.9)
Kampmann et al. [23]	German	Dubois	38.50 (31.70–45.30)	24.4 (18.6–30.2)	5.8 (4.0–7.6)	8.4 (5.1–11.7)	5.9 (3.7–8.1)	9.5 (6.8–12.2)
Huwez et al. [26]	British	Dubois	38.27 (33.05–43.49)	24.28 (19.79–28.77)	7.1 (5.2–7.1)		6.4 (4.6–8.3)	

Dimensions are mean ( $\pm 2SD$ )

US United States, *M-mode* motion mode, *BSA* body surface area, *LV* left ventricle, *LVEDD* left ventricular diameter at end-diastole, *LVESD* left ventricular diameter at end-systole, *IVSd* interventricular septum at end-diastole, *IVSs* interventricular septum at end-systole, *LVPWd* left ventricular posterior wall at end-diastole, *LVPWs* left ventricular posterior wall at end-systole, *LVM* left ventricular mass

**Table 5** A comparison of published echocardiographic normal references for LA dimensions in studies using M-mode and normalised to BSA (for a child with 1 m<sup>2</sup> BSA)

Author	Population studied	Technique	Method for BSA	LA diameter (mm)
Majonga et al. [6]	Zimbabwean (Blacks)	M-mode	Dubois	24.05 (19.19–28.91)
Huwez et al. [26]	British	M-mode	Dubois	25.9 (20.3–31.6)
Kampmann et al. [23]	German (Caucasian)	M-mode	Dubois	25 (19.2–30.8)

Dimensions are mean ( $\pm 2SD$ )

US United States, *M-mode* motion mode, *BSA* body surface area, *2D* two-dimensional, *LA* left atrium, *A4C* apical four chamber view

for chamber dimensions in children. Most of the reference ranges in children were, however, developed in European and North American (US populations) and mainly in Caucasians. Notably, there was only one study each from South America (Brazil) and Africa (Zimbabwe) and the latter was published very recently, implying that most African countries rely on Western references in clinical practice which may not accurately represent black African children [6, 19].

Comparability of the available reference ranges was limited due to substantial methodological variations, including parameters of normalisation; technique of image acquisition (e.g. 2D or M-mode); measured chamber dimension and/or method used for performing the measurements (e.g. ASE guidelines or other). Cantinotti et al. highlighted that it is imperative to standardise methods of image acquisition and consistency in normalisation of the references [40].

However, we were able to compare a few studies which used similar methods from different races for selected cardiac measures. There were notable differences in some of the measures, particularly interventricular septal thickness among Zimbabwean, German and US American children.

Differences between Zimbabwean and German children were consistently demonstrated at both mean-level and upper cut-off for normal distributions. Although no interventricular septal thickness difference was found in the mean distribution of German and US American children, it was evident in the predicted values  $z$  score = +2. In practice, it is the upper cut-off for normal which is used to define abnormality rather than the mean. Our findings suggest that differences in reference ranges between different racial groups do exist but may be overlooked because of the scarcity of data e.g. African children. The use of inappropriate reference ranges may result in either under- or over-diagnosis of cardiac abnormalities or missing of early cardiac chamber remodelling due to cardiac disease [41]. This highlights the importance of using racial-specific reference ranges in clinical practice.

In a recently published study on effect of age, sex, race and ethnicity in echocardiographic  $z$  scores of children, significant effects by all the four parameters were observed on  $z$  scores. However, the authors concluded that these were not of clinical significance [4]. Given that this study was conducted mainly in US American and Canadian children, findings cannot be



generalisable to the rest of the world due to non-standardisation of methods in the available references. There are also other geographical confounders such as nutrition and altitude which may affect cardiovascular development [4].

Most studies used the M-mode or 2D echocardiography techniques. Advanced techniques such as 3D echocardiography, which may overcome some of the technical challenges of angle dependence and other geometric assumptions associated with conventional techniques, should be used to more accurately quantify chamber sizes and development of reference ranges [42].

This study is limited by the fact that many of the studies did not report the actual race of the children, and we therefore made assumptions based on the country the study was conducted in. We compared very broad racial groups due to scarcity of data. In addition, we were unable to compare references for other cardiac measures due to varying methods used in the studies. However, in the few selected references and cardiac measures where this was possible, we were able to demonstrate significant differences in different races. We also showed similarities in same racial groups. It is also highly likely that in addition to the varying methods used in other studies, racial differences are also present.

## Conclusion

This review underlines the importance of using race-specific reference ranges for children, as well as the need for standardising echocardiographic methods in deriving those reference ranges. Furthermore, these reference ranges need to be comprehensive, including a wide range of cardiac measures, as some studies only reported normal values for a single cardiac measure. Future studies should focus on including 3D parameters in addition to 2D and M-mode.

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## Compliance with Ethical Standards

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

**Ethical Approval** All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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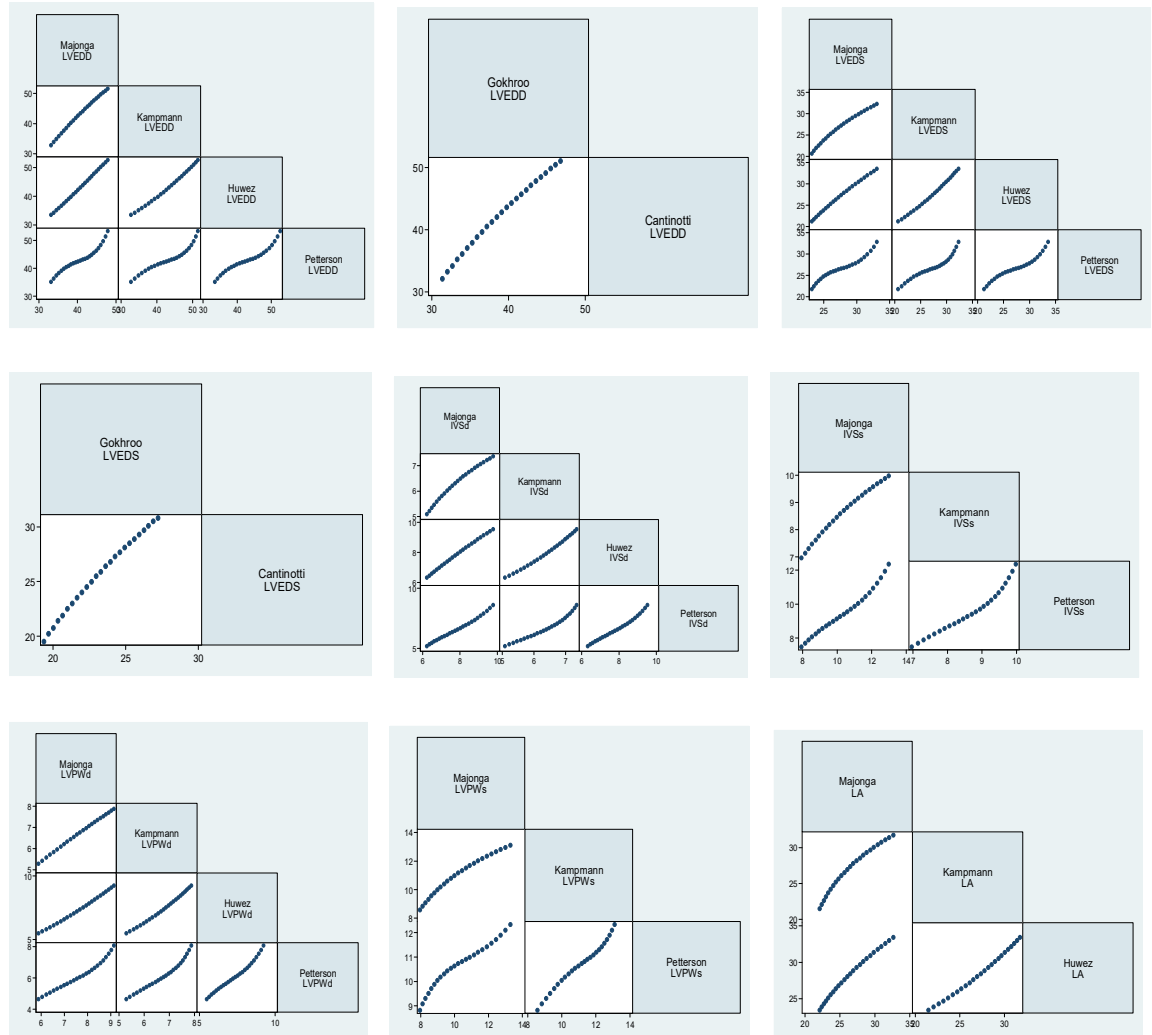
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### 3.4 Published Supplementary material

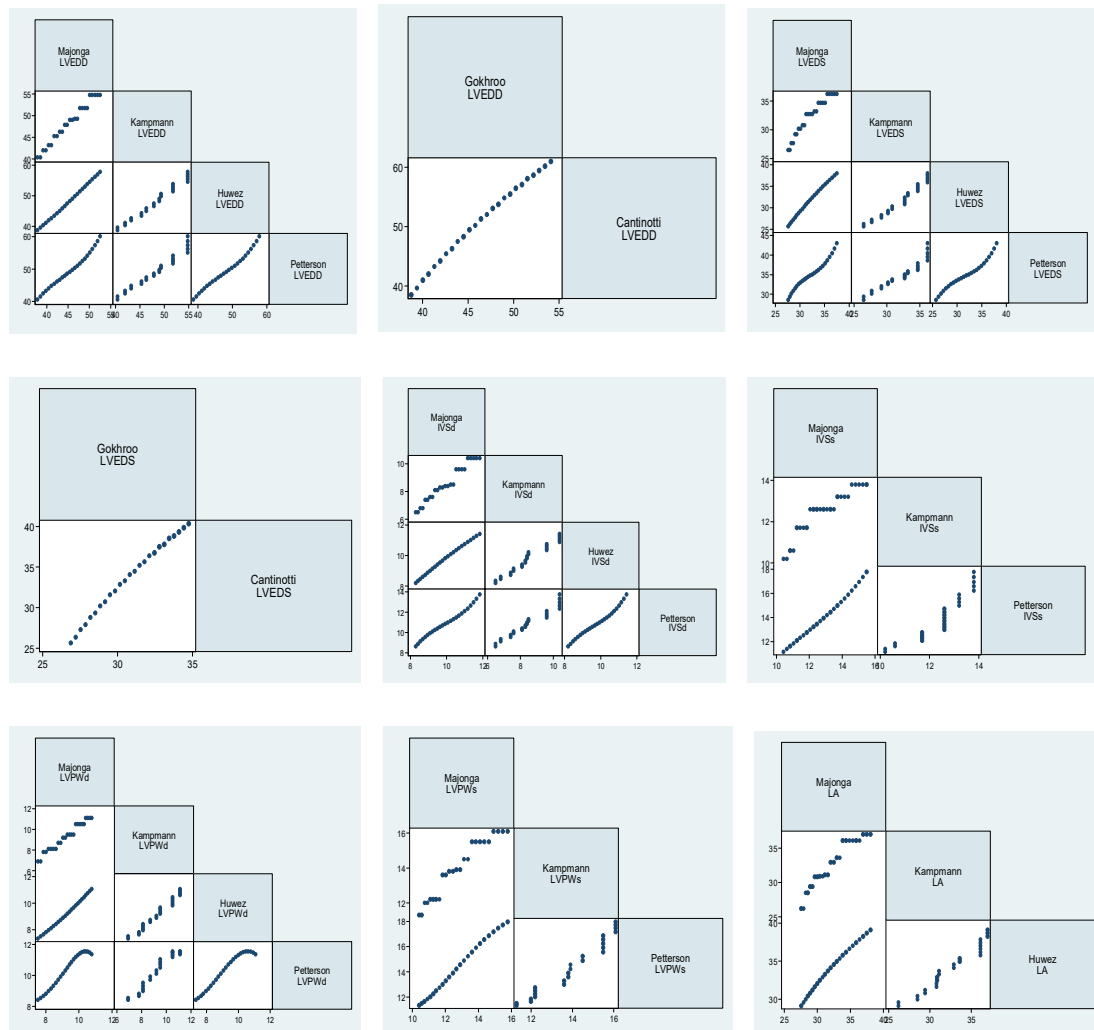
**Figure S1** A comparison of distributions (Z-score=0) of echocardiographic references for LV dimensions in studies using M-mode technique and BSA normalisation



Z-score=0 is representing the predicted mean value

*Abbreviations: LVEDD, left ventricular diameter at end diastole; LVESD, left ventricular diameter at end systole; IVSd, interventricular septum at end diastole; IVSs, interventricular septum at end systole; LVPWd, left ventricular posterior wall at end diastole; LVPWs, left ventricular posterior wall at end systole*

**Figure S2 A comparison of distributions (Z-score=2) of echocardiographic references for LV dimensions in studies using M-mode technique and BSA normalisation**



Z-score=0 is representing the predicted mean value

*Abbreviations: LVEDD, left ventricular diameter at end diastole; LVEDS, left ventricular diameter at end systole; IVSd, interventricular septum at end diastole; IVSs, interventricular septum at end systole; LVPWd, left ventricular posterior wall at end diastole; LVPWs, left ventricular posterior wall at end systole; LA, left atrium*

**Table S1** Kolmogorov Smirnov test of equality of distribution, p-values comparing references for each of the following variables for zscore=0

Reference 1	Reference 2	LVEDD (p-value)	LVEDS (p-value)	IVSD (p-value)	IVSS (p-value)	LVPWD (p-value)	LVPWs (p-value)	LA diameter (p-value)
Majonga [4]	Kampmann [8]	0.468	0.699	<b>0.000*</b>	<b>0.001*</b>	0.016	0.699	0.994
Majonga [4]	Huwez [25]	0.468	0.906	1.000		0.994		0.699
Majonga [4]	Petterson [35]	0.468	0.699	0.037	0.468	<b>0.002*</b>	0.468	
Kampmann [8]	Huwez [25]	1.000	0.994	<b>0.000*</b>		0.078		0.699
Kampmann [8]	Petterson [35]	0.906	0.906	0.281	0.078	0.281	0.468	
Huwez [25]	Petterson [35]	0.906	0.699	0.016		0.037		
Gokhroo [36]	Cantinotti [6]	0.281	0.037					

\*, significance at p <0.005

Compared references used the same M-mode technique and BSA normalisation (from Table 4 and 5).

Z-score=0 is representing the predicted mean value

*Abbreviations: LVEDD, left ventricular diameter at end diastole; LVEDS, left ventricular diameter at end systole; IVSd, interventricular septum at end diastole; IVSs, interventricular septum at end systole; LVPWd, left ventricular posterior wall at end diastole; LVPWs, left ventricular posterior wall at end systole*

**Table S2** Kolmogorov Smirnov test of equality of distribution, p-values comparing references for each of the following variables for Z-score= 2

Reference 1	Reference 2	LVEDD (p-value)	LVEDS (p-value)	IVSD (p-value)	IVSS (p-value)	LVPWD (p-value)	LVPWs (p-value)	LA diameter (p-value)
<b>Majonga [4]</b>	Kampmann [8]	0.281	0.906	<b>0.001*</b>	0.155	0.468	0.155	0.699
<b>Majonga [4]</b>	Huwez [25]	0.281	0.906	0.994		1.000		0.468
<b>Majonga [4]</b>	Petterson [35]	0.281	0.155	0.281	0.281	0.016	0.155	
<b>Kampmann [8]</b>	Huwez [25]	0.906	0.906	<b>0.002*</b>		0.699		0.281
<b>Kampmann [8]</b>	Petterson [35]	0.699	0.078	<b>0.000*</b>	<b>0.002*</b>	0.078	0.281	
<b>Huwez [25]</b>	Petterson [35]	0.994	0.155	0.078		0.078		
<b>Gokhroo [36]</b>	Cantinotti [6]	0.078	<b>0.016**</b>					

\*, significance at p <0.005

\*\* , significance at p <0.05

Compared references used the same M-mode technique and BSA normalisation (from Table 4 and 5)

Z-score=2 is representing the upper cut off for normal

*Abbreviations: LVEDD, left ventricular diameter at end diastole; LVEDS, left ventricular diameter at end systole; IVSd, interventricular septum at end diastole; IVSs, interventricular septum at end systole; LVPWd, left ventricular posterior wall at end diastole; LVPWs, left ventricular posterior wall at end systole*

#### **4. Echocardiographic reference ranges in older children and adolescents in sub-Saharan Africa**

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## **4.1 Introduction**

The systematic review in the previous chapter identified a lack of echocardiographic reference standards among Black African children. The aim of this study was to address this lack of reference ranges among African children aged 6 to 16 years. This chapter presents the published paper in its entirety on echocardiographic reference ranges among older children and adolescents in Zimbabwe. This is the first published study to establish echocardiographic reference ranges in Black African older children and adolescents in sub Saharan Africa.

These reference standards can be used for both clinical practice and research studies. These reference ranges were used to define cardiac abnormalities in HIV-infected children on ART described in the next chapters.

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## 4.2 Research paper II

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### SECTION A – Student Details

Student	Edith Delewe Majonga
Principal Supervisor	Professor Rashida Ferrand
Thesis Title	Cardiac abnormalities in HIV-infected older children and adolescents in Zimbabwe

**If the Research Paper has previously been published please complete Section B, if not please move to Section C**

### SECTION B – Paper already published

Where was the work published?	International Journal of Cardiology		
When was the work published?	June 2017		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I wrote the study protocol, led all field work and was responsible for echocardiographic and electrocardiographic data acquisition and data analysis. I wrote the first and subsequent drafts of the manuscript.
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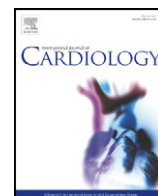
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## Echocardiographic reference ranges in older children and adolescents in sub-Saharan Africa



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### ABSTRACT

**Background:** Echocardiographic reference ranges are important to identify abnormalities of cardiac dimensions. Reference ranges for children in sub-Saharan Africa have not been established. The aim of this study was to establish echocardiographic z-score references for Black children in sub-Saharan Africa.

**Methods:** 282 healthy subjects aged 6–16 years (143 [51%] males) with no known history of cardiac disease were enrolled in the study in Harare, Zimbabwe between 2014 and 2016. Standard M-mode echocardiography was performed and nine cardiac chamber dimensions were obtained. Two non-linear statistical models (gamma weighted model and cubic polynomial model) were tested on the data and the best fitting model was used to calculate z-scores of these cardiac chamber measures. The reference ranges are presented on scatter plots against BSA.

**Results:** Normative data for the following cardiac measures were obtained and z-scores calculated: right ventricular diameter at end diastole (RVEDD); left ventricular diameter at end diastole (LVEDD) and systole (LVESD); interventricular septal wall thickness at end diastole (IVSd) and systole (IVSs); left ventricular posterior wall thickness at end diastole (LVPWd) and systole (LVPWs); left atrium diameter at end systole (LA) and tricuspid annular plane systolic excursion (TAPSE). Girls had higher values for BMI and heart rate than boys ( $p = 0.048$  and  $p = 0.001$ , respectively). Mean interventricular septal and left ventricular posterior walls thickness was higher than published normal values in predominantly Caucasian populations.

**Conclusion:** These are the first echocardiographic reference ranges for children from sub-Saharan Africa and will allow accurate assessment of cardiac dimensions in clinical practice.

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## 1. Introduction

Transthoracic echocardiography enables non-invasive assessment of cardiac size and function and is an essential tool for cardiac evaluation in children and adults. As cardiac chamber dimensions can change

with somatic growth in childhood and adolescence, it is important to normalise echocardiographic measurements to body size. Several echocardiographic references have been published for children and adolescents from various regions including Europe, Asia and North America [1–5]. However, no echocardiography references have been established for children and adolescents in sub-Saharan Africa, and echocardiographic studies from this region have utilised published references for normative data mostly derived from predominantly white populations [4,6]. Racial differences in cardiac dimensions have been reported from previously published nomograms with Black race children found to have significantly larger cardiac dimensions than White race children [7]. In addition, the standards of growth for a

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<sup>1</sup> This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

population may be influenced by environmental, social and economic factors of that region. This necessitates development of regional echocardiographic references. The aim of this study was to establish echocardiographic z-score references in Black African children.

## 2. Methods

The study was conducted at the Harare Children's Hospital, Zimbabwe, between August 2014 and December 2016. Children aged between 6 and 16 years who were HIV-uninfected were invited to participate in the study. Participants were recruited from seven primary care clinics in Harare that were offering HIV-testing to all attendees regardless of the reason for presentation as part of a project evaluating HIV testing services. The study was also advertised at the hospital to the general public. Those who tested HIV-negative were given a flyer with information about the study and asked for consent to be contacted for possible inclusion in the study. Participants were included if they had no known congenital and/or acquired cardiac disease, no cardiac symptoms, a normal electrocardiogram (ECG) and no evidence of structural or functional heart disease on echocardiography. The age, height and weight were obtained and body surface area (BSA) was calculated using Dubois and Dubois method [8].

Ethical approval was obtained from Harare Central Hospital Ethics Committee, Medical Research Council of Zimbabwe, London School of Hygiene and Tropical Medicine Ethics Committee and Biomedical Research and Training Institute Institutional Review Board. Written informed consent from guardians and assent from participants were obtained prior to enrolment in all cases.

### 2.1. Echocardiographic examination

Echocardiography was performed using a Mindray DC N6 multipurpose ultrasound machine (Mindray, Shenzhen, China) by a trained and experienced paediatric echocardiographer (EM). 2D guided M-mode echocardiography was performed on all children using a standard protocol, according to published guidelines [9]. No sedation was required prior to the examination. Participants were scanned in the left lateral or supine position to obtain an optimum image quality. Images were acquired using a transducer with frequencies ranging from 3.5 MHz to 7.0 MHz and simultaneous 3-lead ECG monitoring and were saved in DICOM format for subsequent off-line analysis.

The following cardiac measures were obtained over three cardiac cycles: right ventricular diameter at end diastole (RVEDD); left ventricular diameter at end diastole (LVEDD) and end systole (LVESD); interventricular septal wall diameter at end diastole (IVSd) and end systole (IVSs); left ventricular posterior wall thickness at end diastole (LVPWd) and end systole (LVPWs); left atrium diameter at end systole (LA); and tricuspid annular plane systolic excursion (TAPSE). End diastole was defined as the start of the QRS-wave on the ECG tracing, or preferably described as the frame in the cardiac cycle in which LV dimension is largest and systole as the frame prior to mitral valve opening in which LV dimension is smallest, as previously described [10]. Measurements were performed in the parasternal long axis (PLAX) or parasternal short axis (PSAX) views using the leading edge to leading edge method [11]. TAPSE was measured in apical 4-chamber view. Measurements were only made on technically adequate images, thus not all measurements were obtained in every patient (Table 1). Repeated measurements were performed on 20 randomly selected echocardiograms by the same rater (EM) and a further 28 (10%) of all the echocardiograms were randomly selected and re-measured by an independent rater (MSP).

### 2.2. Statistical analysis

Data were analyzed using STATA version 12 software (StataCorp, Texas, USA). Continuous variables are presented as mean  $\pm$  standard

**Table 1**  
Clinical and echocardiographic characteristics of participants.

Variable	Overall N = 282	Males N = 143	Females N = 139	P-value
Age (y)	10.7 (3.0)	10.7 (3.1)	10.7 (2.9)	0.922
6–9 years	107 (38)*	55 (38)*	52 (37)*	
10–13 years	107 (38)*	51 (36)*	56 (40)*	
14–16 years	68 (24)*	37 (26)*	31 (22)*	
Height (cm)	140.3 (16.2)	140.4 (17.5)	140.2 (14.8)	0.920
Weight (kg)	35.6 (13.2)	34.3 (12)	37 (14.3)	0.008
BSA (m <sup>2</sup> )	1.18 (0.3)	1.16 (0.3)	1.19 (0.3)	0.421
BMI (kg/m <sup>2</sup> )	17.4 (3.1)	16.8 (2.0)	18.1 (3.9)	<b>0.001**</b>
Systolic BP (mmHg)	112.7 (12.9)	112 (13.0)	113 (12.9)	0.571
Diastolic BP (mmHg)	74 (9.6)	73 (9.0)	74.7 (9.7)	0.205
Heart rate (bpm)	81.7 (13.0)	80.2 (12.0)	83.3 (13.7)	<b>0.048**</b>
RV diastole (mm)	14.9 (2.0)	14.9 (2.0)	14.9 (2.0)	0.969
LV diastole (mm)	39.2 (4.1)	39.7 (4.0)	38.7 (4.1)	<b>0.036**</b>
LV systole (mm)	26.8 (3.1)	27 (3.1)	26.5 (3.2)	0.170
IVS diastole (mm)	7.5 (1.3)	7.6 (1.3)	7.5 (1.3)	0.303
IVS systole (mm)	9.9 (1.7)	10 (1.8)	9.8 (1.6)	0.473
LVPW diastole (mm)	7.3 (1.1)	7.4 (1.1)	7.2 (1.1)	0.253
LVPW systole (mm)	9.8 (1.7)	9.8 (1.7)	9.7 (2.9)	0.511
Left atrium (mm)	25.6 (3.4)	25.7 (3.3)	25.5 (3.5)	0.579
***TAPSE (mm)	20.7 (2.3)	20.7 (2.1)	20.8 (2.4)	0.873

Results are mean  $\pm$  SD.

BSA, body surface area; BMI, body mass index; BP, blood pressure; RV, right ventricle; LV, left ventricle; IVS, interventricular septum; LVPW, left ventricular posterior wall; TAPSE, tricuspid annular plane systolic excursion.

\* N(%).

\*\* Significant at  $p \leq 0.05$ .

\*\*\* N = 237.

deviation (SD) for normally-distributed data or median and interquartile range (IQR) for non-normally-distributed data. To compute the z-scores, two regression models were tested on the data to optimise goodness of fit for the various cardiac measures and the selected independent variable, BSA. The two models were a gamma function weighted model [ $y = \alpha x^{\beta} \times e^{-\lambda x}$ ] proposed by Nevill et al. [12] and a cubic polynomial model [ $y = ax^3 + bx^2 + cx + d$ ] used by Petterson et al. [5] The final best model was selected based on several characteristics: first, by visual inspection of the goodness of fit of data plotted on a graph; secondly, the association between the residual and/or z-score plots against the BSA were assessed and no significant association should be observed if there is an adequate fit; thirdly, residual values were assessed for normal distribution; fourthly, the tails of distributions were assessed to ensure that individuals falling outside of the reference ranges (i.e. individuals with z-scores  $> +2$  or  $< -2$ ) were  $< 2.28\%$  [13] to ensure that no bias was introduced and finally the model with the smallest Akaike information criterion (AIC) value was selected. Intra-rater and inter-rater reliability was calculated using Intra-class correlation coefficient (ICC). A value of  $p < 0.05$  was considered statistically significant.

## 3. Results

A total of 282 children were enrolled in the study, of whom 143 (51%) were male. All participants were in sinus rhythm. The baseline demographic and clinical characteristics are shown in Table 1, stratified by gender. Girls had higher values for BMI and heart rate than boys ( $p = 0.048$  and  $p = 0.001$ , respectively) whereas boys had higher LVEDD dimensions ( $p = 0.036$ ).

Both the gamma function weighted and the cubic polynomial models had comparable visual goodness-of-fit of the data on plotted graphs and no significant association was observed between residuals and BSA. However, the gamma weighted model was selected because of the higher  $R^2$  values and smaller AIC values compared to the cubic polynomial model. In addition, the cubic polynomial did not have enough observations in the tail of the distribution. The regression analysis coefficients of the gamma weighted model are presented in Table 2. These include alpha, beta, lambda, root mean squared error (RMSE) and

**Table 2**  
Coefficients of the gamma weighted model for the echocardiographic measures.

Measurement	Alpha	Beta	Lambda	AIC	RMSE	R <sup>2</sup>
RV diastole	7.086	-0.320	-0.665	808.1	1.009	0.996
LV diastole	32.223	0.198	-0.141	1281.1	2.333	0.997
LV systole	18.623	-0.010	-0.306	1253.8	2.223	0.993
IVS diastole	5.119	0.071	-0.317	817.7	1.026	0.982
IVS systole	7.064	0.183	-0.259	927.1	1.246	0.985
LVPW diastole	6.508	0.381	-0.050	687.6	0.8145	0.988
LVPW systole	5.555	-0.063	-0.481	921.4	1.233	0.985
Left atrium	15.774	-0.116	-0.422	1303.7	2.428	0.991
TAPSE	16.287	-0.139	-0.222	1047.7	2.193	0.989

AIC, Akaike information criterion; RMSE, root mean squared error; R<sup>2</sup>, r-squared; RV, right ventricle; LV, left ventricle; IVS, interventricular septum; LVPW, left ventricular posterior wall; TAPSE, tricuspid annular plane systolic excursion.

the r-squared (R<sup>2</sup>) values. The scatter plots of the cardiac measures against BSA are shown in Figs. 1–2. The various lines in each graph represent the regression equations for z-scores ranging from -3 to +3,

with 0 as the predicted or mean value. The z-scores for the children can either be approximated using these graphs or may be calculated by substituting the coefficients in Table 2 directly into the gamma equation. The z-scores are calculated using the standard formula:  $(X - \mu) / \sigma$  where X is the observed value,  $\mu$  is the predicted mean and  $\sigma$  is the standard deviation. For example, a z-score for a 14-year-old male with a BSA of 1.3m<sup>2</sup> and measured LA of 30 mm, is calculated as follows:

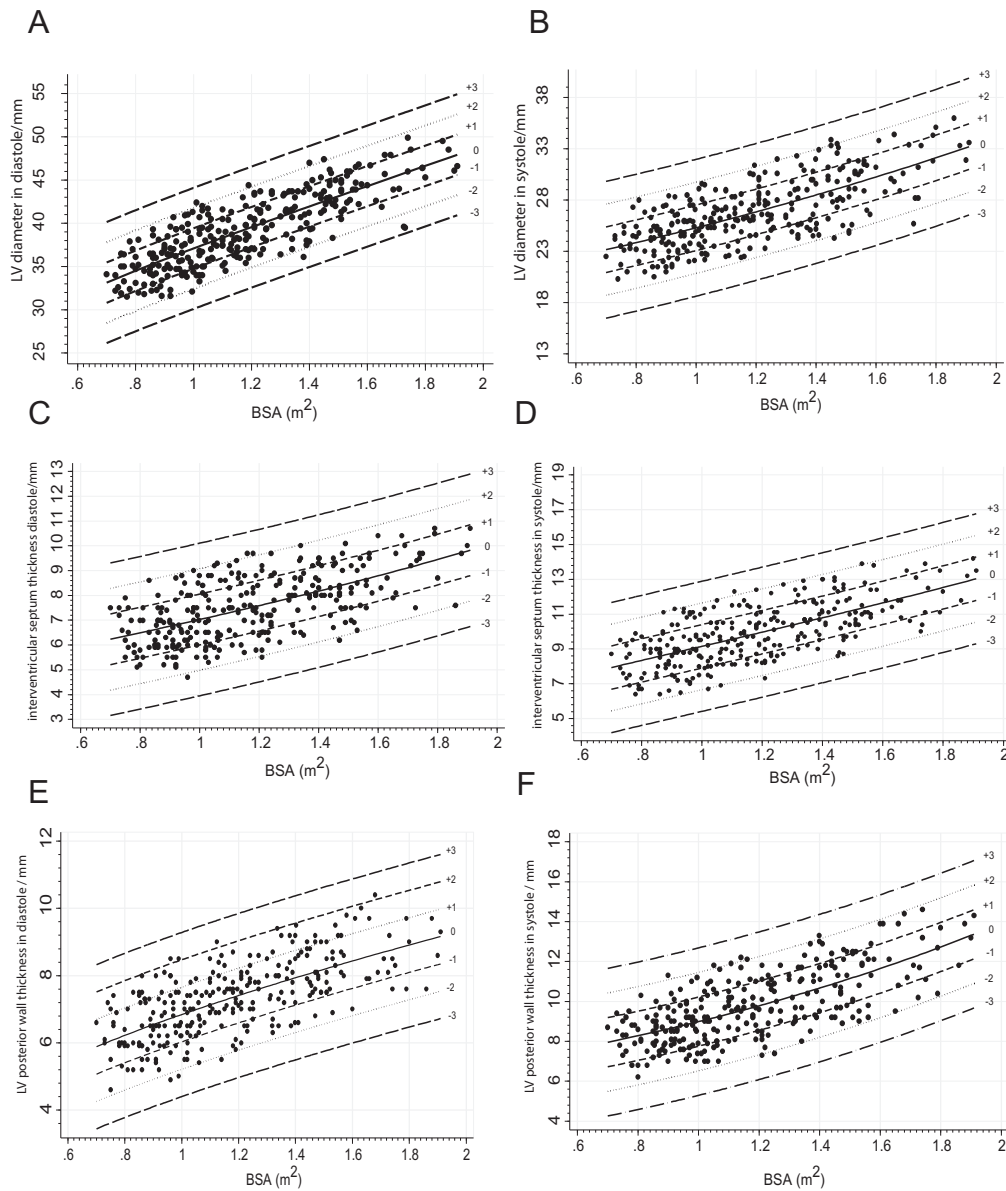
The mean LA is predicted using the coefficients in Table 2 in the equation. Y, is the value to be predicted and X is the BSA.

$$y = \alpha X^{\beta} \times e^{-\lambda x}$$

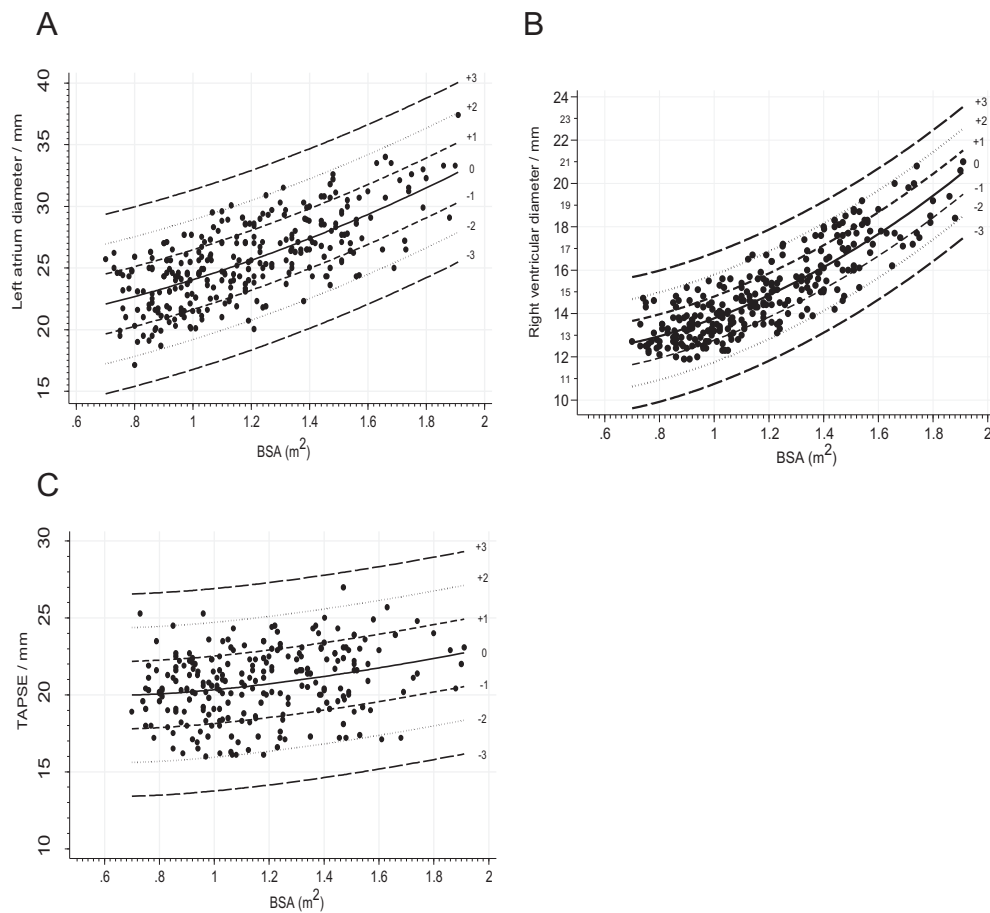
Therefore, the mean predicted LA is:  $Y = 15.774 \times 1.3^{(-0.116)} \times e^{-(-0.422 \times 1.3)} = 26.481$ . The z-score is calculated as:  $(30 - 26.481) / 2.428 = 1.449$ .

Thus, the boy has an approximate z-score of +1.45.

Inter-rater and intra-rater reliability was good for all cardiac measures, with an ICC  $\geq 0.88$  and  $\geq 0.87$ , respectively.



**Fig. 1.** Scatter plots for left ventricular dimensions against BSA. BSA, body surface area; LV, left ventricle The scatter plots show left ventricular related chamber dimensions. **A**, left ventricular diameter end diastole; **B**, left ventricular diameter end systole; **C**, interventricular septum thickness end diastole; **D**, interventricular septum thickness end systole; **E**, left ventricle posterior wall thickness end diastole; **F**, left ventricle posterior wall thickness end systole.



**Fig. 2.** Scatter plots for left atrium, right ventricle and TAPSE against BSA. BSA, body surface area; TAPSE, tricuspid annular plane systolic excursion; The scatter plots are showing A, left atrium diameter end systole; B, right ventricular diameter end diastole; C, TAPSE (N = 232).

#### 4. Discussion

This study is the first to establish normal reference ranges for M-mode echocardiography measures among older children and adolescents in sub-Saharan Africa. The results have important clinical implications, as they will allow more accurate assessment of cardiac dimensions in clinical practice and in future research studies.

Throughout childhood, cardiac size changes dramatically. This presents a challenge in the interpretation of echocardiographic measures in the paediatric population [5]. Unlike in adults, where reference ranges have been presented and applied as a single “normal range”, this approach is imprecise in children as body size needs to be considered [14]. Z-scores quantify the degree of normality or abnormality by the number of standard deviations above or below a size-specific population mean of a particular measure [14]. A normal range of z-scores for the cardiac measures is defined as +2 to −2 with 0 as the mean. To date, no consensus exists as to which growth parameter is most appropriate in standardizing cardiac measures for children [15]. Different parameters have been used in the published references, including height [16], weight [3], BSA [2,4], lean body mass [17,18] and age [19–21]. Roge et al. showed that height, weight and BSA were strongly correlated, resulting in similar regression equations using either of the parameters [22]. We chose to express our results in relation to BSA as this is the most widely used parameter in clinical practice. Age alone is an unreliable standardizing parameter because at any age of a child, height, weight and BSA are variable [23].

Several authors have published M-mode references in non-African children standardised to BSA [5,7,24,25]. Our references have a higher mean for interventricular septum and posterior wall thickness

compared to the published references. Left ventricular and atrial diameters are comparable to Kampmann et al. [24] and Huwez et al. [25]. Right ventricular diameter results for our study are higher than those reported by Kampmann et al., and Huwez et al., while being lower than those of Bonatto et al. [7] and Petterson et al. [5]. Our TAPSE values are comparable to Nunez-Gil et al. [26] among Spanish children and lower than Uysal et al. [27] among Turkish children. These variations may be explained by differences in race and environment. Only Caucasians were included in Kampmann's cohort, while other studies did not mention the race of the children studied [5,25]. Bonatto et al. studied Black and White children and found that Black children had overall larger cardiac dimensions than White children, consistent with the higher measurements reported in the present study and highlighting the importance of having specific reference ranges for sub-Saharan populations. To date, no previous studies have focused specifically on this population.

Several authors have reported gender differences in their cohorts, mainly due to variations of growth of boys and girls, particularly at puberty [6,28,29]. In our study, no significant gender differences were observed on the cardiac measures, except for LVEDD. The difference in LVEDD between boys and girls was small and a possible explanation for this difference could be differences in growth rates between boys and girls.

The strength of this study is the relatively large sample size and the fact that reference ranges have been prospectively derived from a homogenous group of Black African children from the same racial group. Our population was not completely unselected and there may be some selection bias. Nevertheless, only healthy children with no known heart disease were included. We have established an important

set of normal cardiac measurements for clinical practice. Lack of ethnic variation may be viewed as a limitation. However, as Black African children have not had representative references in the past, it allows comparison of these findings with data from other racial groups. We were not able to measure all parameters in all the children, particularly TAPSE (45 were missing) due to technically inadequate apical 4-chamber views. Our study is limited in that not all echocardiograms were interpreted by an independent reader; however, 10% of randomly selected echocardiograms were re-measured by the independent reader and the inter-rater reliability was good, suggesting that the results are valid. Furthermore, we followed a standard protocol to acquire images and perform measurements and, in clinical practice, echocardiograms are interpreted mostly by a single person. No two-dimensional (2D) measures, chamber volumes and functional parameters were measured and establishment of these normative values will be the focus of future studies in sub-Saharan populations. However, although M-mode is known to have reduced spatial resolution compared to 2D echocardiography [11], it has higher temporal resolution and remains the most commonly used method in clinical practice to assess cardiac chamber size and/or function due to its reproducibility and ease of performance. Given that our population is limited to only Zimbabwean children, the findings in this study may not be applicable to other African populations.

To our knowledge, this is the first study to establish echocardiography reference ranges in Black African children in sub-Saharan Africa. These reference standards can be used for both clinical practice and research studies. Addition of our references to existing literature will allow a more precise assessment of cardiac dimensions in children.

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## Conflict of interest

No conflict of interest.

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**5. High prevalence of echocardiographic abnormalities in older HIV-infected children taking antiretroviral therapy**

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## **5.1 Introduction**

The scale of ART has resulted in a dramatic decrease in mortality of individuals with HIV. Growing numbers of children with HIV, who would otherwise have died in infancy or early childhood are surviving to adolescence and beyond. However, longstanding HIV infection is associated with chronic co-morbidities that may be an effect of HIV infection itself, its treatment or a sequela of co-infections associated with HIV. This chapter reports the prevalence, spectrum and risk factors for cardiac abnormalities among children taking ART, investigated using echocardiography. Local echocardiographic reference ranges, derived as part of this project and reported in the previous chapter, were used to define cardiac abnormalities. We found a high prevalence of cardiac abnormalities in children on ART. Notably, the use of European echocardiographic reference ranges would have overestimated the prevalence of cardiac abnormalities, highlighting the importance of using race-specific echocardiographic reference ranges.

This chapter is a paper which has been submitted and is under review.



## 5.2 Research paper III

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Principal Supervisor	Professor Rashida Ferrand
Thesis Title	Cardiac abnormalities in HIV-infected older children and adolescents in Zimbabwe

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Student Signature: \_\_\_\_\_

Date: 14/09/2018

Supervisor Signature: \_\_\_\_\_

Date: 14/09/2018

# **High prevalence of echocardiographic abnormalities in older HIV-infected children taking antiretroviral therapy**

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## **Introduction**

Of the approximately 3.2 million children with HIV globally, 90% live in sub-Saharan Africa.[1] The global expansion of prevention of mother-to-child HIV transmission and scale up of antiretroviral therapy (ART) programs has resulted in a substantial decrease in the number of infants born with HIV and an increase in numbers of older children and adolescents living with HIV due to improved survival on ART.[2]

While ART has dramatically decreased the risk of opportunistic infections and mortality, there is mounting evidence that longstanding HIV infection and/or its treatment are associated with non-infectious complications, including cardiac disease.[3] Cardiac disease has been reported among treated and untreated HIV-infected adults, with prevalence ranging from 18-78%.[4, 5] Low CD4 cell count and high viral load have been identified as independent risk factors for cardiac disease, but residual confounding by well-recognised traditional risk factors such as smoking and hypertension remain an issue in these studies.[5] A small number of studies have suggested that children with HIV also are at risk of cardiac disease, despite the absence of traditional risk factors, with the most common reported abnormalities being left ventricular (LV) dilatation and systolic dysfunction.[6, 7] HIV infection can also lead to right heart abnormalities, either as a consequence of pulmonary arterial hypertension (PAH) or secondary to chronic lung disease. We have previously reported a high prevalence of chronic lung disease among HIV-infected older children on ART[8, 9], which may result in pulmonary hypertension and subsequent right ventricular (RV) dilatation and/or RV systolic dysfunction.[10] However, most studies were conducted in

children on zidovudine monotherapy or in mixed cohorts of ART-naive and experienced participants, and were mainly performed in younger children in high-resource settings.[7, 11]

In the ART era, there are contrasting reports on cardiac disease in HIV-infected children who are on ART from high and low-resource settings.[12-14] We therefore investigated the prevalence, spectrum and risk factors for cardiac abnormalities in children established on ART in Harare, Zimbabwe.

## **Methods**

A cross-sectional study was conducted at the paediatric HIV clinic at Harare Central Hospital, Zimbabwe, between August 2014 and June 2015. Harare Central Hospital is the largest public-sector hospital in Harare and provides HIV care to over 4000 children. This study was conducted within a larger cohort study called INHALE (Investigation of Heart and Lung Diseases in HIV among older children), aiming to investigate cardiorespiratory disease in children with HIV infection. Findings of the clinical and radiographic features of chronic lung disease have already been published.[8, 9] This report is limited to echocardiographic abnormalities in the same cohort of HIV-infected children.

HIV-infected children aged 6-16 years attending for HIV care, not acutely ill and taking ART for at least six months, were consecutively recruited on week days, restricted to the first five eligible participants per day for logistical ease. A minimum of 6 months on ART was selected to enable sufficient time for viral suppression and for the risk of immune reconstitution syndrome to have dropped.

### ***Study procedures***

An interviewer-administered questionnaire was used to collect socio-demographic data and clinical history, including previous illnesses, drug history and current symptoms. A standardized clinical assessment was performed, including anthropometric measurement (height and weight), heart rate, respiratory rate, blood pressure, New York Heart Association (NYHA) functional class, pulse oximetry and Medical Research Council (MRC) Dyspnoea scale. Fasted blood was collected for measurement of plasma glucose, and for HIV-1 viral load and CD4 count testing. The CD4 count was measured on site using an Alere PIMA analyser (Alere Technologies GmbH, Jena, German) and plasma VL was measured using the COBAS protocol Ampliprep/Taqman 48 Version 2.0 (Roche Molecular System, Branchburg, USA). Spirometry was performed according to American Thoracic Society guidelines to assess respiratory function.[15]

A transthoracic echocardiogram was performed using a Mindray DC N6 multipurpose ultrasound machine (Mindray, Shenzhen, China) by an echocardiographer trained in paediatric echocardiography (EM). A standard protocol consisting of 2-dimensional, M-mode, pulsed and continuous wave Doppler and colour flow mapping as recommended for transthoracic echocardiography was adopted [16]. Participants were scanned in the left lateral or supine position to obtain an optimum image quality and all measurements made over three cardiac cycles. Images were acquired using a 7MHz transducer and were saved in DICOM format for off-line analysis. Cardiac measurements were based on the American Society of Echocardiography (ASE) criteria [16].

Echocardiography scans were quality controlled by an experienced paediatric cardiologist (JPK) for adequacy of views. Furthermore, a random sample of 10% scans were re-measured by an independent rater (MSP) for interobserver agreement.

### ***Definitions***

Echocardiography measures for LV and right ventricular (RV) dimensions were normalized to body surface area calculated using Du Bois and Du Bois formula,[17] and converted to z-scores using local references[18] and further compared to European published references among Caucasian children.[19] Local reference ranges were used as the primary basis for defining cardiac abnormalities in the study. LV and RV dilatation were defined as a z-score  $>+2$  for LV and RV diameter in diastole respectively, left atrial dilatation was a z-score  $>+2$  for left atrial diameter in systole and LVH was defined as maximal wall thickness of interventricular septum and/or LV posterior wall greater than  $+2$  z-scores.[18] LV systolic function was assessed by Simpson's Biplane method and an ejection fraction  $\geq 55\%$  was considered normal.[16] LV diastolic dysfunction was assessed through transmitral Doppler (peak early (E) and late diastolic (A) filling velocities), E/A ratio, deceleration time and pulmonary venous flow velocities including peak systolic (S) and diastolic (D) waves, S/D ratio and atrial reversal (Ar) velocity and paediatric reference ranges were used to define abnormality.[20] Patients were classified as having diastolic dysfunction when at least four parameters were abnormal.[21] RV systolic dysfunction was defined as a tricuspid annular plane systolic excursion (TAPSE) z-score of  $<-2$ .[18] Pulmonary hypertension was defined as present if the tricuspid regurgitation

velocity was  $\geq 2.9$  m/s, estimated pulmonary arterial systolic pressure (PASP)  $\geq 37$  mmHg with/without additional echocardiographic variables suggestive of pulmonary hypertension (assuming right atrial pressure of 5mmHg).[22] PASP was indirectly calculated from the pressure gradient across the tricuspid valve (TV) by measuring the regurgitant jet and applying the simplified Bernoulli equation ( $4V^2$ ) and adding right atrial pressure (RAP) estimate to the tricuspid pressure gradient.[22] Hypoxia was defined as a resting oxygen saturation  $< 88\%$  or a  $\geq 5\%$  desaturation immediately following exercise; resting tachypnoea was defined as a respiratory rate  $> 25$ /min. Sinus tachycardia was defined as  $> 130$  beats per minute (bpm) for children aged 6-8 years and  $> 110$ bpm for those aged 9-16 years.[23] Stunting and wasting were defined as a z-score  $< -2$  for height-for-age and weight-for-age respectively, using British 1990 growth references.[24] Hypertension was defined as the systolic blood pressure and/or diastolic blood pressure  $\geq 95$ th percentile; prehypertension was systolic blood pressure and/or diastolic blood pressure between the 90th and 95th percentile for age, gender and height.[25] Chronic lung disease was defined as having at least one of the following criteria: chronic cough ( $\geq 3$  months) with tuberculosis excluded; hypoxia ( $SpO_2 < 90\%$  or desaturation  $\geq 5\%$  upon exertion); abnormal spirometry (defined as reduced ratio of the highest forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) (FEV1: FVC) or reduced FVC regardless of normal FEV1: FVC ratio) irreversible with salbutamol[9] and MRC Dyspnoea scale  $> 1$ .[26]



### ***Data management and statistical analysis***

Data were extracted from paper forms using optical character recognition software (Cardiff TELEFORM Intelligent Character, Version 10.7). Data were analysed using STATA version 12 software (StataCorp, Texas, USA).

A previous, retrospective, cross-sectional study in Zimbabwean adolescents found the prevalence of cardiac abnormalities to be between 24% and 67%.[27] We calculated a sample size of 200 was required to estimate an assumed prevalence of 65% with a precision of +/- 8% to 10%, for a 95% confidence interval. The association between HIV-related and clinical factors, determined a priori, and left and right cardiac abnormalities was investigated using multivariate logistic regression and odds ratios were calculated. Age and sex were included as *a priori* variables. HIV-related factors were categorized as follows: CD4 count (>200 cells/ $\mu$ L and  $\leq$ 200 cells/ $\mu$ L), HIV viral load ( $\leq$ 400 copies/ml and >400 copies/ml), age at ART initiation (0-5, 6-10 and 11-16 years), duration on ART ( $\leq$ 2 years and >2 years) and age was categorized as 6-10 years and 11-16 years. Antiretroviral drugs (ARVs), cardiac signs and symptoms, hypertension and chronic lung disease were dichotomized into yes and no. Zidovudine and nevirapine were selected because of evidence that zidovudine is associated with development of cardiomyopathy in children and nevirapine is associated with LVH in adults.[28, 29] Clinical factors evaluated include cardiac signs and symptoms, hypertension and chronic lung disease. Cardiac signs and symptoms included hypoxia, chest pains, tachypnoea and ankle swelling, and were grouped into one variable called cardiac symptoms. HIV-related variables that were significant at  $p \leq 0.1$  on univariate logistic regression analysis were

included in a multivariate logistic regression model. Clinical variables were added to the model and any clinical variable that was significant at  $p \leq 0.1$  was retained for inclusion in the final model. A value of  $p \leq 0.05$  was considered statistically significant in the final model. Intra- and interobserver agreement was assessed through Bland-Altman plots.[30] Variability was estimated by calculating the mean (95% CI) of the arithmetic differences between repeated cardiac measures of the same participant. Normally distributed differences would fall within a range of mean  $\pm 1.96SD$  and the range is referred to as limits of agreement (LoA).

Ethical approval was obtained from the Medical Research Council of Zimbabwe, the London School of Hygiene and Tropical Medicine Ethics Committee, the Biomedical Research and Training Institute Institutional Review Board and the Harare Central Hospital Ethics Committee. Written informed consent from guardians and assent from participants was obtained prior to enrolment. Any abnormal findings during the course of the study were recorded in the child's notes and the child was referred to a clinician on the same day for further management.

## **Results**

### ***Clinical characteristics***

Of the 921 attendees to the clinic aged 6-16 years over the study period, 397 were eligible and of these, 201 children were enrolled; the remainder were excluded due to the total number of enrolments being restricted to the first five eligible attendees per day (Figure 1). The median age was 11.1 years (IQR, 9-12) and 92

(46%) were female. Mother-to-child transmission was assessed as the likely mode of HIV acquisition in all but one participant, in whom we speculate that HIV had been acquired through sexual transmission because the mother had tested HIV negative when the child was diagnosed at the age of 6 years. Participants were taking ART for a median duration of 4.7 years (IQR, 2.6-6.4) and 154/197 (78%) had an HIV VL<400 copies/ml; the median CD4 count at enrolment was 727 cell/ $\mu$ l (IQR, 473-935) (n=198). No participant was taking any medication for cardiac disease. Eligible children who were not enrolled in the study were on average 5 months younger than those enrolled and had initiated ART at a year younger (Supplementary Table 1).

Thirty-four (17%) children had a history of chest pain, 36/201 (18%) were in NYHA functional class >1 and 24/198 (12%) had hypoxia. An abnormal blood pressure was observed in 106/198 (54%) children: 85/106 (80%) had hypertension and 21/106 (20%) had prehypertension. Chronic cough was reported in 30 (15%) and dyspnoea using the MRC dyspnoea scale in 30 (15%) and 42 (24%) had abnormal lung function on spirometry (Table 1).

#### *Echocardiographic findings*

Of the 201 enrolled participants, echocardiograms were performed on 197; the remaining four did not return for the examination. Echocardiographic measures are summarized in Table 2. Eighty-three (42%) participants had an echocardiographic abnormality of either the left and/or right heart. The most common abnormal finding was LV diastolic dysfunction in 45/197 (23%) children (Table 3). Isolated diastolic dysfunction, without associated LV

dilatation or LVH, was observed in 33/45 (73%) children. LVH was the second most common finding in 22/197 (11%) with the following patterns: interventricular septal hypertrophy 2/22 (9%) and posterior wall hypertrophy 20/22 (91%). Seven children (32%) with LVH had diastolic dysfunction and global systolic function was impaired in one child. LV dilatation was observed in 9/197 (5%) children, one of whom had impaired systolic function and pericardial effusion. Left atrial dilatation was found in 16/197 (8%) children and of these 4 (25%) had LVH; 4 (25%) had diastolic dysfunction; and 2 (12%) had LV dilatation.

RV dilatation was found in 13/197 (7%) and RV systolic dysfunction in 4/180 (2%) including two children with both RV dilatation and systolic dysfunction. Of the 15 participants who had RV abnormalities, nine (60%) had concurrent left heart abnormalities, including isolated LVH (n=2), isolated LV dilatation (n=1), isolated Left atrial dilatation (n=1), LV systolic dysfunction and LVH (n=1), isolated LV diastolic dysfunction (n=3) and one participant who had a dilated cardiomyopathy with LV and Left atrial dilatation and LV systolic dysfunction. Five (33%) of the participants with right heart abnormalities also met the case definition for chronic lung disease. None of the participants met the echocardiographic criteria for pulmonary hypertension. The prevalence of echocardiographic abnormalities was much higher when European reference ranges were used (Table 3).

Twenty (10%) echocardiograms were used to determine inter-observer agreement. Bland-Altman plots showed that there was good agreement for

repeated measures by the same observer two weeks apart and repeated measures between the two observers (Figure S1).

### ***Factors associated with echocardiographic abnormalities***

LV diastolic dysfunction was associated with hypertension (aOR 3.12 (1.48-6.57;  $p < 0.01$ ). Hypertension and LV diastolic dysfunction were present in 33/45 (73%) of the participants. LVH was associated with current use of nevirapine (aOR 3.14 (1.13-8.72;  $p = 0.03$ ) (Table 4). No HIV-related factors nor symptoms were associated with left atrial dilation. No associations were found between RV abnormalities and HIV-related factors including CD4 count, viral load, duration on ART, age at ART initiation and lung function (supplementary Table 2).

## **Discussion**

This study demonstrates a high prevalence of echocardiographic abnormalities among HIV-infected children established on ART, more than three-quarters of whom were virologically suppressed. Most of the participants with diastolic dysfunction had preserved systolic function. LV diastolic dysfunction is well described in adults with HIV but there are limited data on diastolic dysfunction in children. Diastolic parameters are difficult to measure in children and very slight alterations can result in significant changes in interpretation of the diastolic function. Diastolic dysfunction is difficult to measure in children because parameters such as transmitral inflow are influenced by heart rate, preload and sonographic techniques. Interestingly, in the pre-ART era, systolic dysfunction and LV dilatation rather than impaired LV diastolic function were the predominant abnormalities observed, suggesting that LV diastolic impairment

may be an earlier manifestation of cardiac disease.[31] It is not uncommon for diastolic dysfunction to present in isolation, typically before systolic impairment becomes apparent, for example in ischaemic cardiomyopathy.[32]

Among those with LVH, LV posterior wall thickening was predominant, in keeping with our previous report.[27] Idris and colleagues found that children with HIV and exposed to ART had thicker LV posterior walls compared to ART-naïve children and larger LV dimensions compared to uninfected children.[33] LV dilatation was less common than LVH in our study. We found that current use of nevirapine was associated with LVH, which may suggest possible treatment induced effect on the myocardium. Nevirapine has been associated with LVH among HIV infected adults in Spain, most of whom were on ART and virally suppressed.[28]

An important, previously unreported, abnormality found in our study was Left atrial dilatation, which is associated with adverse clinical outcomes such as atrial fibrillation and all-cause mortality in a range of cardiac disease, including ischaemic heart disease and hypertrophic cardiomyopathy.[34] Left atrial dilatation may occur because of increased LV filling pressures in the context of impaired LV diastolic function, for example among those with LVH, or because of LV dilatation.[34] Our data suggest that, in this population, Left atrial dilatation is related to the former. Further studies are required to investigate whether Left atrial dilatation also represents a marker of increased risk of mortality among individuals living with HIV.

More than half of the children had strikingly high blood pressure, but no association was found between LVH and abnormal blood pressure. However, we did find that LV diastolic dysfunction was associated with elevated blood pressure; hypertension is an established risk factor for LV diastolic dysfunction and a major contributor of heart disease.[35] However, not all patients with diastolic dysfunction had hypertension, suggesting a possible additional primary myocardial aetiology. Chatterton-Kirchmeier and colleagues also reported a high prevalence of elevated blood pressure among HIV-infected children and adolescents (age range 2-17 years), most of whom were on ART.[36] The reasons for this remain unclear, but this finding merits further investigation in this population. A high prevalence of elevated blood pressure in African children has been previously reported, in keeping with the present study.[37-40] There is a possibility of misclassification of participants as hypertensive; although we used the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, we did not perform repeated measures of BP for our participants on separate visits.[25] Furthermore, there may have been an element of “white coat hypertension”. Another explanation could be the possibility that the definition used for hypertension in this study, which is derived from reference ranges obtained from 70,000 children and adolescents from USA, is not suitable for the population under study.[25] Currently, there are no blood pressure references for African children. Miller *et al.* reported that stunting was associated with LV diastolic dysfunction in their retrospective study of HIV infected adolescents, 71% of whom were on ART.[27] Stunting is an indicator of chronic inflammation in childhood and chronic inflammation has been implicated in the pathogenesis of cardiac disease.[41-50]

We found RV abnormalities (dilatation and systolic dysfunction) in 8% of the children. RV dilatation was the most common right sided abnormality, although lower than the 29% previously reported among Zimbabwean perinatally HIV-infected adolescents.[27] The former study enrolled older children aged between 10-19 years and included both ART naïve and ART-experienced children. Chelo and colleagues reported an even higher prevalence of RV dilatation (76%) among Cameroonian children aged 1–15 years, 91% of whom were on ART. The Zimbabwean study used European children’s references by Kampmann *et al.*[19] and the Cameroonian study used adults’ references as recommended by the ASE[51], and it is possible that the prevalence of RV dilatation may have been overestimated in both studies. A comparison of African reference ranges, derived from Zimbabwean HIV-uninfected children and the European references by Kampmann *et al.*[19] and Huwez *et al.*[52] showed that RV diameters are higher among Black African children.[18] In this study we used locally developed reference ranges as a control population to define echocardiographic abnormalities.[18] European echocardiographic references derived from Caucasians[19] over-estimated the prevalence of some abnormalities in our cohort, underscoring the importance of using local reference ranges. Western reference ranges may not be appropriate and may explain the differences in prevalence of cardiac disease with the present study.

We did not find any association between RV abnormalities and chronic lung disease or other HIV related factors and this may be due to a lack of power to detect the associations. More than half of the children with RV abnormalities had left heart abnormalities too, suggesting that the abnormalities in the right heart



may be part of a global cardiomyopathic process. RV impairment may be secondary to left-sided cardiomyopathy and several mechanisms have been postulated, including the possibility that the same cardiomyopathic process may affect both ventricles. Alternatively, LV failure may result in reduced coronary perfusion for both LV and RV, or the dilated LV may cause RV diastolic dysfunction due to cumulative pericardial limitation.[53]

Most of the children in our study had normal RV systolic function despite having RV dilatation. Ventricular dilatation commonly occurs as an early structural change to maintain stroke volume when there is reduced wall motion.[54] It is possible that ventricular systolic dysfunction was subclinical and would clinically manifest over time.[55]

None of the children had elevated PASP in our study, using Doppler echocardiography. Although Doppler echocardiography is recognised as an important tool for screening and assessment of patients at risk of pulmonary hypertension[56], right heart catheterisation is the gold standard in diagnosis of pulmonary hypertension, and recent reports have shown that Doppler echocardiography may under- or over-estimate pulmonary pressures, especially in children with elevated right heart pressures.[57] It is possible, therefore, that right heart pressures may have been underestimated in our study. However, the concurrent right and left heart abnormalities in some of the children suggest that the observed RV abnormalities are not related to undiagnosed pulmonary hypertension but, rather, may reflect an underlying biventricular cardiomyopathic process. Further studies are required to investigate this.

Although one fifth of the children reported one or more cardiac symptoms, most children with cardiac abnormalities were asymptomatic. These findings highlight the importance of regular cardiac screening in this population, even in the absence of symptoms. Myocardial disease is often subclinical and may only become symptomatic once it progresses and leads to significant cardiac dysfunction. Relying on symptoms alone without echocardiography screening may therefore result in delayed diagnosis of cardiac disease.

Pathogenesis of cardiac disease in HIV infection is likely multifactorial. HIV causes dysregulated systemic activation which leads to chronic inflammation. These mechanisms contribute to organ damage. Furthermore, damage to the immune system before subsequent access to ART maybe responsible for this long-term effect of cardiac abnormalities. Although children in this study were stable on ART with high CD4 counts and virally suppressed at the time of the study, low nadir CD4 count and/or opportunistic infections may have occurred prior to ART initiation and contributed to cardiac damage. Cardiotropic viruses including cytomegalovirus, coxsackievirus and Epstein-Barr virus have been reported to cause cardiac dysfunction in HIV [58] although we did not investigate presence of these viruses in these children. Deficiency of trace elements such as selenium have also been associated with HIV-associated cardiomyopathy,[59] but were not measured in this study. Selenium deficiency related HIV-cardiomyopathy may include dilated cardiomyopathy [5, 60] .

A major strength of this study was the systematic cardiological assessment, with echocardiography performed prospectively by a paediatric echocardiographer.

Most importantly, we used local echocardiography reference ranges to define cardiac abnormalities.[18] Participants were not recruited selectively based on symptoms. Our study is limited by lack of data on global LV longitudinal strain, which may have been more sensitive to detecting subclinical LV systolic dysfunction, not apparent in the form of reduced LV ejection fraction. It is also limited by lack of a control group, but all cardiac abnormalities were defined using reference measures derived locally. Local reference ranges were however, not available for blood pressure. Hyperlipidaemia is a recognized risk factor for cardiac disease, but we were unable to assess lipid profiles due to resource constraints. The children in this study did not undergo routine blood haemoglobin testing, although there was no clinical suspicion of anaemia. The study is cross-sectional and may have been underpowered to detect any associations between risk factors and cardiac abnormalities. Due to the cross-sectional design, no causality can be attributed to the factors that were associated with cardiac abnormalities.

In conclusion, our study demonstrates that there is a high burden of echocardiographic abnormalities in children, despite good control of HIV infection with ART. Our findings also suggest that right heart abnormalities in HIV-infected children on ART appear to be associated with abnormalities of the left heart. The impact and clinical course of these abnormalities and potential for reversibility still need to be investigated in prospective longitudinal studies. Further study of the pathogenesis of cardiac abnormalities will inform development of appropriate screening and therapeutic strategies for an

increasing number of children growing up with HIV who face the prospect of taking lifelong ART.

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**Table 1: Baseline characteristics of participants (n=201)**

<b>Variable</b>	<b>N (%) unless otherwise stated</b>
Female	92 (46)
Age, years (median, IQR)	11.1 (9 – 12)
Age at HIV diagnosis, years (median, IQR)	5.1 (3-7)
CD4, cell/ $\mu$ l (median, IQR)	726 (473-935)
Viral load <400 copies/ml**	154 (78)
Duration on ART, y (median, IQR)	4.7 (2.6-6.4)
Age at ART initiation, y (median, IQR)	6 (3-8)
Treated for TB	74 (37)
<b>ART-Regimen</b>	
2NRTIs + PI n	40 (19.9)
2NRTIs + NNRTI n	154 (76.6)
Unknown n	7 (3.5)
<b>Antiretroviral drugs<sup>§</sup></b>	
Zidovudine	105 (52)
Nevirapine	103 (51)
<b>Symptoms and signs</b>	
Chest pains on exertion	34 (17)
Dizziness	19 (9)
Tachycardia at rest	13 (6.5)
Tachypnoea	14 (7.2)
Hypoxia*	24 (12)
Chronic cough	30 (15)
Abnormal spirometry	42 (24)
Ankle swelling	4 (2)
Wasting	44 (22)
Stunting	48 (24)
NYHA functional class >1	36 (18)
<b>Abnormal Blood Pressure<sup>†</sup></b>	106 (54)
Pre-hypertension	21 (20)
Hypertension	85 (80)
High fasting glucose (>7) <sup>‡</sup>	2 (1)

*Abbreviations: ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase; NNRTI, non-nucleoside reverse transcriptase; NYHA, New York heart association; IQR, interquartile range*

*\*missing data on n=3; †missing data on n=4; ‡missing data on n=7; § antiretroviral drugs evaluated in the logistic regression model*

**Table 2: Echocardiographic measures**

Measurement	N=197 Median (IQR)
Body surface area (m <sup>2</sup> )	1.03 (0.92- 1.15)
LV diameter z-score	0.68 (-0.22- 1.25)
IVS diameter z-score	0.09 (0.66- 0.80)
LV posterior wall z-score	0.28 (-0.51-1.07)
Left atrium diameter z-score	0.32 (-0.44- 1.20)
Fractional shortening (%)	31 (5.2) *
Ejection Fraction (%)	62 (6.6) *
E wave (m/s)	0.91 (0.81- 1.02)
A wave (m/s)	0.53 (0.47- 0.60)
E/A ratio	1.70 (1.50- 1.99)
Deceleration Time (ms)	173 (156- 190)
PV S wave (m/s)	0.49 (0.41-0.56)
PV D wave (m/s)	0.50 (0.46- 0.57)
PV A wave (m/s)	0.18 (0.16- 0.21)
PV S/D ratio	0.96 (0.79- 1.16)
RV diameter, z-score	0.37 (-0.52 -1.28)
PASP, mmHg	12.8 (8.7 -16.9)
TAPSE, z-score	-0.73 (-1.44 - -0.09)

*LV, left ventricle; IVS, interventricular septum, LA, left atrium; E/A ratio, mitral valve peak early to late left ventricular filling velocity; PV, pulmonary venous; S, systolic; D, diastolic; PASP, pulmonary arterial systolic pressure; TAPSE, tricuspid annular plane systolic excursion; \* mean (SD)*

**Table 3: Proportions of cardiac abnormalities**

Abnormalities	Local references*	European references
	[18]	[19]
	N=197	N=197
	N (%)	N (%)
LV dilatation	9 (5)	9 (5)
LVH	22 (11)	73 (37)
- <i>Interventricular septal hypertrophy</i>	2 (9)	52 (71)
- <i>Posterior wall hypertrophy</i>	20 (91)	4 (6)
- <i>Concentric hypertrophy</i>	-	17 (23)
Left atrial dilatation	16 (8)	12 (6)
LV systolic dysfunction	3 (2)	3 (2)
LV diastolic dysfunction	45 (23)	45 (23)
RV dilatation	13 (7)	57 (29)
RV systolic dysfunction	4 (2)	-
Any echocardiographic abnormality	83 (42)	
Any left heart abnormality	77 (39)	
Any right heart abnormality	15 (8)	

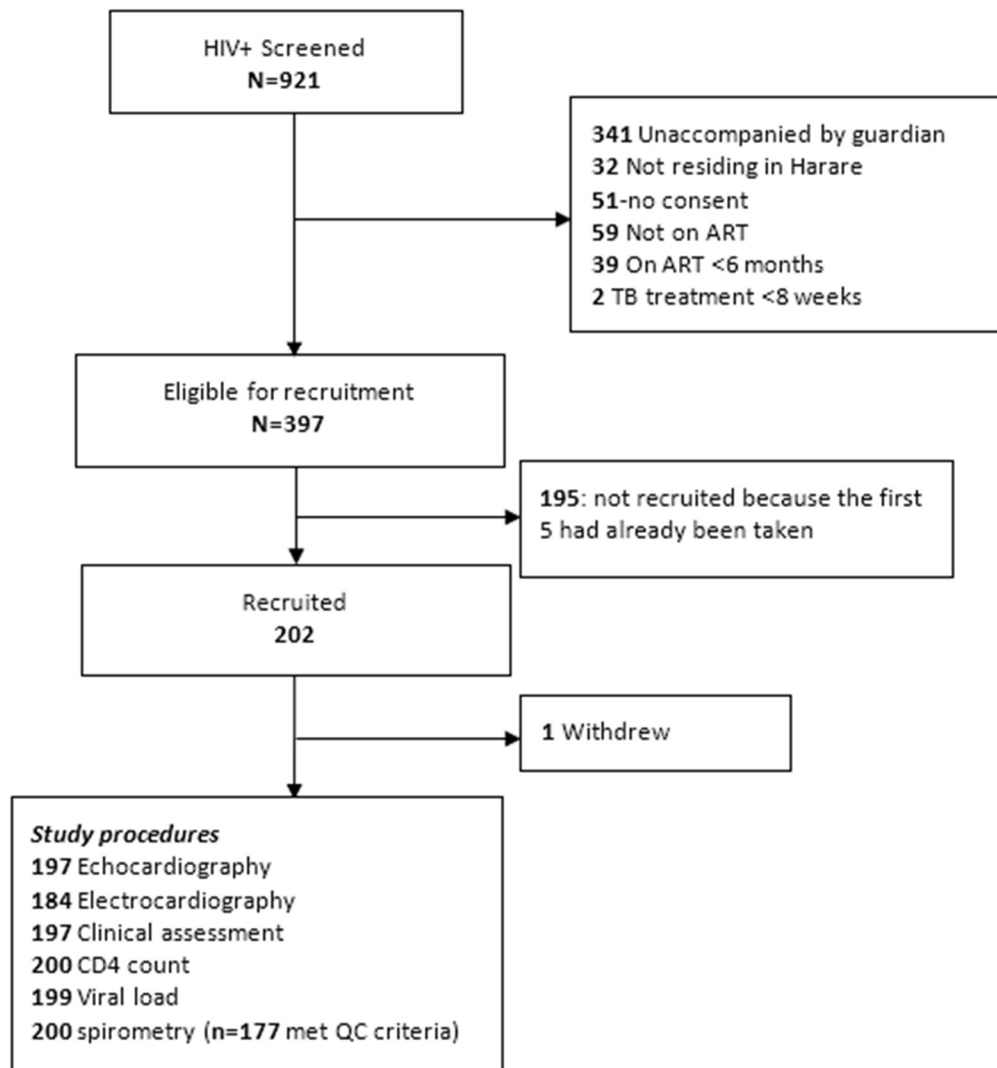
*\*, local reference ranges were used as the primary basis of defining cardiac abnormalities. Abbreviations: LA, left atrium; LV, left ventricle; LVH, left ventricular hypertrophy; RV, right ventricular missing data on n=4*

**Table 4: Factors associated with left and right heart abnormalities**

Variable	Prevalence n/N (%)	LV Diastolic Dysfunction				LVH				
		Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value	Prevalence n/N (%)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
<b>Sex</b>										
Female	20/94 (21)	1				14/94 (15)	1			
Male	25/103 (24)	0.19 (0.16-2.31)	0.61			8/103 (8)	0.48 (0.12-1.21)	0.12		
<b>Age</b>										
6-10 years	25/91 (27)	1				10/91 (11)	1			
11-16 years	20/106 (19)	0.61 (0.31-1.20)	0.15			12/106 (11)	1.03 (0.42-2.52)	0.94		
<b>Age at ART initiation</b>										
0-5 years	27/93 (29)	1.91 (0.93-3.90)	<b>0.08</b>	1.95 (0.93-4.07)	0.08	13/93 (14)	2.14 (0.77-5.91)	0.14		
6-10 years	15/85 (18)	1		1		6/85 (7)	1			
11-16 years	3/17 (18)	1.00 (0.26-3.92)	1	1.14 (0.28-4.64)		3/17 (18)	2.82 (0.63-12.6)	0.18		
<b>Duration on ART</b>										
≤ 2 years	10/55 (18)	1				6/55 (11)	1			
>2 years	35/142 (25)	1.47 (0.67-3.22)	0.33			16/142 (11)	1.04 (0.38-2.80)	0.94		
<b>CD4 count</b>										
>200 cell/ $\mu$ l	44/187 (24)	1				21/187 (11)	1			
≤200 cell/ $\mu$ l	1/9 (11)	0.41 (0.05-3.34)	0.40			1/9 (11)	0.99 (0.12-8.30)	0.99		
<b>Viral load</b>										
≤400 copies/ml	35/152 (23)	1				18/152 (12)	1			
>400 copies/ml	9/41 (22)	1.06 (0.46-2.44)	0.88			3/41 (7)	1.70 (0.48-6.08)	0.41		
<b>Nevirapine *</b>										
No	22/98 (22)	1				<b>6/98 (6)</b>	<b>1</b>			
Yes	23/99 (23)	1.05 (0.54-2.03)	0.90			<b>16/99 (16)</b>	<b>2.96 (1.10-7.91)</b>	<b>0.03</b>	<b>3.14 (1.13-8.72)</b>	<b>0.03</b>
<b>Zidovudine*</b>										
No	17/95 (18)	1				8/95 (8)	1			
Yes	28/102 (27)	1.74 (0.89-3.43)	0.11			14/102 (14)	1.73 (0.69-4.33)	0.242		
<b>Cardiac symptoms<sup>†</sup></b>										
No	27/119 (23)	1				13/119 (10)	1			
Yes	18/78 (23)	1.02 (0.52-2.02)	0.95			9/78 (12)	1.06 (0.43-2.62)	0.89		
<b>Hypertension</b>										
No	12/92 (13)	1				12/92 (13)	1			
Yes	<b>33/103 (32)</b>	<b>3.14 (1.51-6.55)</b>	<b>&lt;0.01</b>	<b>3.12 (1.48-6.57)</b>	<b>&lt;0.01</b>	10/103 (10)	0.72 (0.29-1.75)	0.46		

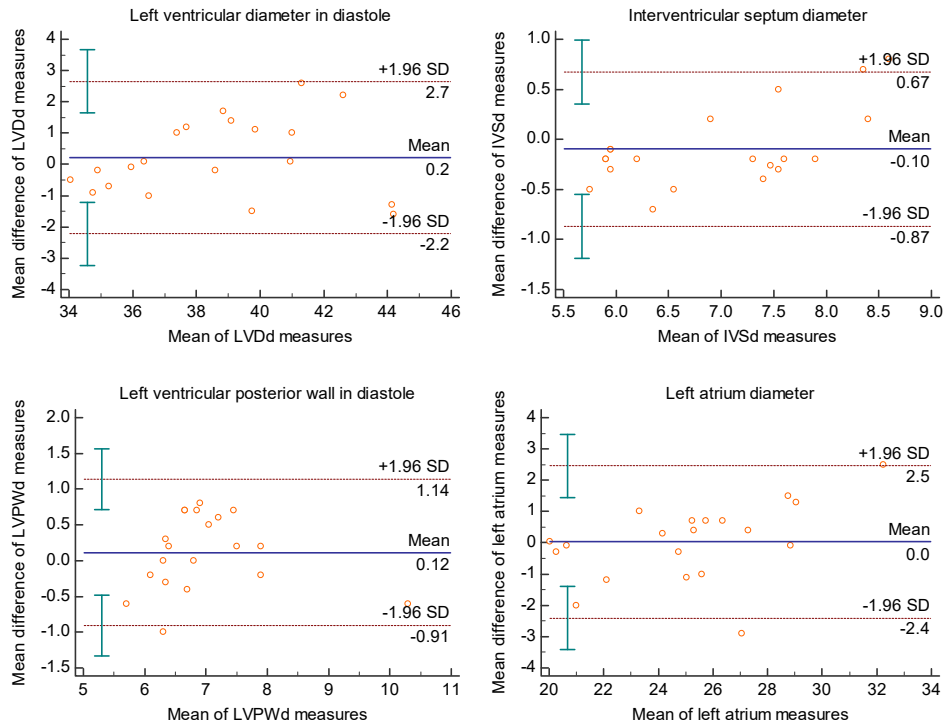
ART, antiretroviral therapy; LV, left ventricular; LVH, left ventricular hypertrophy \* Antiretroviral drugs; † cardiac signs and symptoms included hypoxia, chest pains, tachypnoea, and ankle swelling

**Figure 1: Participant Recruitment**

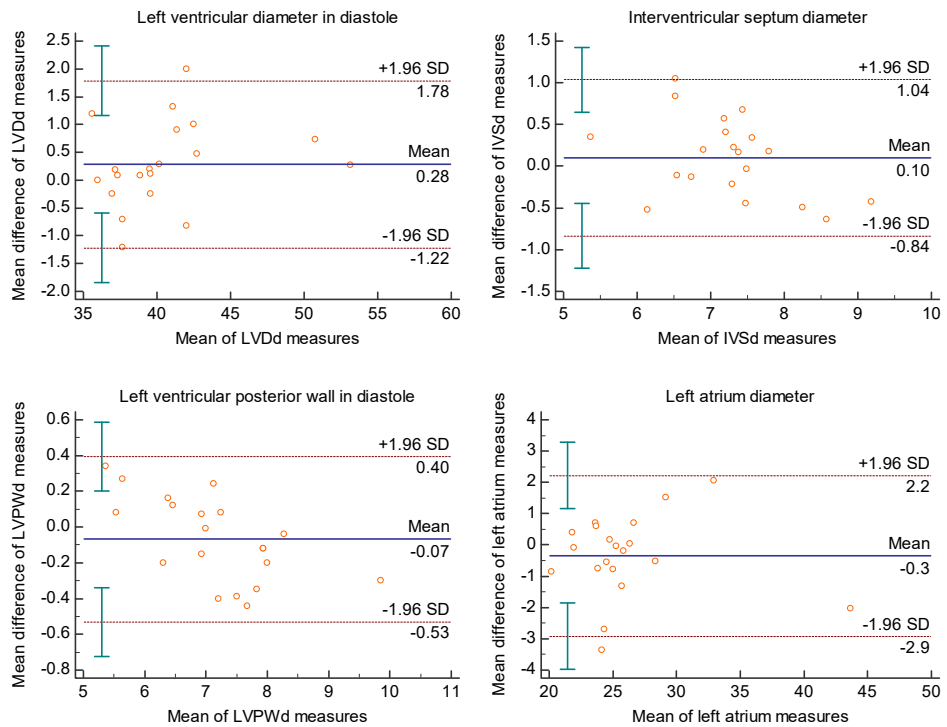


**Figure S1: Bland-Altman plots for intra-and interobserver agreement for cardiac measures**

**A: Intra-observer agreement Bland-Altman plots**



**B: Inter-observer agreement plots**



**Supplementary Table 1: Factors associated with enrolment in the study**

Variable	Enrolled N=200	Not enrolled N=197	Test coefficient, p-value
Age, y, mean (SD)	10.5 (2.5)	9.9 (2.7)	t=-2.20, p=0.03
Sex, n (%) female	96 (48.0)	86 (43.7)	Chi <sup>2</sup> =0.75, p=0.39
Taking TB treatment, n (%)	1 (0.5)	1 (0.5)	Chi <sup>2</sup> =0.00, p=0.99
Age at ART initiation, y, mean (SD)	6.1 (3.2)	5.1 (3.3)	t=-3.04, p=0.003

*SD, standard deviation; TB, tuberculosis; ART, antiretroviral therapy*



**Supplementary Table 2: Factors associated with RV abnormalities**

	RV abnormalities		
	<i>Prevalence</i>	<b>Unadjusted</b>	
<b>Variable</b>	<b>n/N (%)</b>	<b>OR (95% CI)</b>	<b>P-value</b>
<b>Sex</b>			
Female	9/94 (9)	1	
Male	6/103 (5)	0.58 (0.20- 1.71)	0.33
<b>Age</b>			
6-10 years	6/91 (7)	1	
11-16 years	9/106 (8)	1.31 (0.45- 3.84)	0.50
<b>Age at ART initiation</b>			
0-5 years	6/93 (6)	0.77 (0.25-2.38)	0.65
6-10 years	7/85 (8)	1	
11-16 years	2/17 (12)	1.49 (0.28-7.86)	0.64
<b>Duration on ART</b>			
≤ 2 years	5/55 (9)	1	
>2 years	10/142 (7)	0.80 (0.28- 2.30)	0.6
<b>CD4 count</b>			
>200 cell/ $\mu$ l	14/187 (7)	1	
≤200 cell/ $\mu$ l	0	-	-
<b>Viral load</b>			
≤400 copies/ml	15/152 (10)	1	
>400 copies/ml	0	-	-
<b>Nevirapine *</b>			
No	9/98 (9)	1	
Yes	6/99 (6)	0.64 (0.22-1.87)	0.41
<b>Zidovudine*</b>			
No	8/95 (8)	1	
Yes	7/102 (7)	0.80 (0.28-2.30)	0.68
<b>Cardiac symptoms<sup>†</sup></b>			
No	9/119 (8)	1	
Yes	6/78 (8)	1.02 (0.35-2.98)	0.97
<b>Hypertension</b>			
No	8/92 (9)	1	
Yes	7/103 (7)	0.77 (0.27-2.20)	0.62
<b>Chronic lung disease</b>			
No	10/121 (8)	1	
Yes	5/76 (7)	0.78 (0.26-2.38)	0.67

ART, antiretroviral therapy; RV, right ventricular; \* Antiretroviral drugs; † cardiac signs and symptoms included hypoxia, chest pains, tachypnoea, and ankle swelling

**6. Incidence and progression of echocardiographic abnormalities in HIV-infected older children and adolescents taking antiretroviral therapy: A prospective cohort study**

---

## **6.1 Introduction**

In the previous chapter we reported a high prevalence of cardiac abnormalities in HIV-infected children taking ART. Participants enrolled in the study were followed up for a median of 1.5 years. This chapter reports the incidence and progression of cardiac abnormalities, assessed by echocardiography. To our knowledge, this is the first study to report on incidence and clinical course of cardiac abnormalities in HIV-infected children in Sub-Saharan Africa.

This chapter has been prepared into a manuscript and is ready for submission.

## 6.2 Research paper IV

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### SECTION A – Student Details

Student	Edith Delewe Majonga
Principal Supervisor	Professor Rashida Ferrand
Thesis Title	Cardiac abnormalities in HIV-infected older children and adolescents in Zimbabwe

**If the Research Paper has previously been published please complete Section B, if not please move to Section C**

### SECTION B – Paper already published

Where was the work published?	
When was the work published?	
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	
Have you retained the copyright for the work?*	Was the work subject to academic peer review?

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### SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Pediatrics
Please list the paper's authors in the intended authorship order:	Edith D Majonga, Andrea M Rehman, Grace McHugh, Hilda A Mujuru, Kusum Nathoo, Jon O Odland, Rashida A Ferrand, Juan P. Kaski
Stage of publication	Not yet submitted

### SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I wrote the study protocol, led all field work and was responsible for echocardiographic and electrocardiographic data acquisition and data analysis. I wrote the first and subsequent drafts of the manuscript.
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Student Signature: \_\_\_\_\_

Date: 10/06/2018

Supervisor Signature: \_\_\_\_\_

Date: 10/06/2018

**Incidence and progression of echocardiographic abnormalities  
in HIV infected older children and adolescents taking  
antiretroviral therapy: A prospective cohort study**

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**Short title:** Incidence of cardiac abnormalities in children on ART

**Key words:** Cardiac, HIV, children, ART, Africa

**Word count:** 2736

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## **Abstract**

### ***Background***

A high prevalence of cardiac abnormalities has been reported in children with HIV taking ART in sub-Saharan Africa. We investigated the incidence and progression of cardiac abnormalities in children taking ART in Zimbabwe.

### ***Methods***

A prospective cohort study was conducted at a paediatric HIV clinic from 2014 to 2017. Children with HIV aged between 6 and 16 years and taking ART  $\geq 6$  months were enrolled. Transthoracic echocardiography was performed at baseline and at 18 months, with local reference ranges used to define cardiac abnormalities.

### ***Results***

Of 197 participants recruited at baseline, 175 [(89%), 48% female, median age 12 (IQR, 10-14) years] were followed up, constituting 283.9 person-years (pys) of follow up. The duration on ART was 6.5 (IQR, 4.3-8.1) years and CD4 count was 734 (IQR, 462-989) cells/ $\mu\text{l}$ . The incidence of left and right heart abnormalities was 3.52 and 5.64 per 100 pys, respectively. The risk of developing right ventricular (RV) dilatation was highest [12/163 (7%)]; left atrial (LA) dilatation and LV hypertrophy (LVH) were 1% each. Stunting was associated with the development of any cardiac abnormality] adjusted OR 2.59 (95% CI, 1.03-6.49);  $p=0.043$ ]. Among those with abnormalities at baseline, RV dilatation persisted at follow up in 11/12 (92%); LA dilatation in 14/14 (100%) and LV diastolic dysfunction in 35/40 (88%) participants. Cardiac abnormalities present at

baseline reverted to normal by follow up in 11(6%). There was an overall increase in mean z-scores for LV, LA, RV, interventricular septum and LV posterior wall diameters at 18 months ( $p<0.001$ ).

### ***Conclusions***

Despite ART, children with HIV have a high incidence of cardiac abnormalities, with only a minority being transient. Mean z-scores for LV, LA, RV, interventricular septum and LV posterior wall diameters increased over a relatively short follow up period, suggesting the potential for progression of cardiac abnormalities. However, longer follow up is required to understand the clinical implications of these abnormalities.

Words: 312



## Introduction

The global scale-up of antiretroviral therapy (ART) programmes has been followed by a dramatic decline in mortality among individuals living with HIV infection.<sup>1</sup> While ART facilitates immune reconstitution and reduces the risk of infections, there is increased recognition that longstanding HIV infection is associated with an increased risk of chronic co-morbidities. This may be a result of the HIV infection itself, its treatment or sequelae of infections.<sup>2</sup> One of the most well-recognised co-morbidities is cardiac disease, with several studies showing an increased risk of developing cardiac disease in adults despite ART.<sup>3, 4</sup> An important limitation of studies in adults is the confounding by well-established risk factors for cardiac disease such as age, smoking and hypertension.<sup>4</sup>

In the pre-ART era, infants and younger children with HIV were also reported to be at increased risk of developing cardiac disease, with a five-year cumulative incidence of cardiac dysfunction of 18-39%.<sup>5</sup> The most commonly reported abnormalities were left ventricular (LV) systolic dysfunction and LV dilatation; these were often progressive and were a predictor of all-cause mortality.<sup>6, 7</sup> Most of these studies were conducted in high-income settings, mainly in younger children and with some cohorts including children taking monotherapy for treatment of HIV infection.<sup>5, 7, 8</sup> In high-income countries, most children start ART in infancy, which not only decreases mortality but may prevent organ damage;<sup>9</sup> in these settings, the incidence of cardiac disease in children on ART has decreased.<sup>10</sup> These findings cannot be generalised to children growing up with HIV in Sub-Saharan Africa (SSA), where 90% of the world's children with HIV live,

most of whom have had delayed diagnosis of HIV and/or started ART in older childhood.<sup>11, 12</sup>

Nevertheless, increasing numbers of children in SSA, who would have died in early childhood, are now reaching adolescence and adulthood due to ART.<sup>13</sup> We and others have reported a high prevalence of cardiac abnormalities among African children despite treatment with ART and absence of the traditional risk factors for cardiac disease.<sup>14-17</sup> However, little is known about the incidence and clinical course of cardiac abnormalities in African children in the ART era. We investigated the incidence and progression of cardiac abnormalities in children taking ART in Harare, Zimbabwe.

## **Methods**

A prospective cohort study was conducted from August 2014 to December 2017 at the paediatric HIV clinic at Harare Central Hospital, Zimbabwe, a public-sector HIV clinic that provides care to over 4000 children. ART is provided free of charge according to national guidelines. This study was part of a larger study aiming to investigate cardiorespiratory disease in children with HIV infection taking ART [INHALE (Investigation of Heart and Lung Diseases in HIV among older children)]. Findings from this cohort pertaining to chronic lung disease have been published.<sup>18, 19</sup>

Children with HIV aged 6 to 16 years attending the outpatient HIV clinic, taking ART for at least six months and clinically stable (defined as not requiring hospital admission and not too ill to participate) were consecutively enrolled on week

days, limited to the first five eligible participants per day for logistical ease. Participants were followed up at 18 months. Baseline cardiac findings in this cohort of HIV infected children have been reported.<sup>14</sup>

### ***Study procedures***

An interviewer-administered questionnaire was used to collect socio-demographic data and clinical history, including previous illnesses, treatment history and symptoms. Clinical assessment was performed, including height and weight, heart and respiratory rates, pulse oximetry, blood pressure measurement and spirometry. Assessment of pubertal stage was based on Tanner staging.<sup>20, 21</sup> Transthoracic echocardiography was performed by an echocardiographer trained in pediatric echocardiography (EDM). A standard protocol consisting of 2-dimensional, M-mode, pulsed and continuous wave Doppler as recommended by American Society of Echocardiography (ASE) was adopted for image acquisition and cardiac measurements.<sup>22</sup> Images were acquired and saved in DICOM format for off-line analysis. Blood samples were collected for HIV viral load and CD4 count testing. All clinical assessments, including anthropometry, pulse oximetry, blood pressure, heart and respiratory rate, spirometry, transthoracic echocardiography and CD4 count and HIV viral load testing, were repeated at 18-month follow up.

### ***Definitions***

The echocardiographic cardiac measures were normalized to body surface area (BSA) (calculated using the Du Bois and Du Bois method)<sup>23</sup> and converted to z-scores using previously-published local references.<sup>24</sup> Echocardiographic

abnormalities were broadly categorized as right and left heart abnormalities. Right heart abnormalities refer to either right ventricular (RV) dilatation (defined as a z-score  $>+2$  for RV diameter end-diastole); and/or systolic dysfunction [defined as a tricuspid annular plane systolic excursion (TAPSE) z-score of  $< -2$ ]; and/or pulmonary hypertension [defined as present if the tricuspid regurgitation velocity was  $\geq 2.9$  m/s, pulmonary arterial systolic pressure (PASP)  $\geq 37$  mmHg with/without additional echocardiographic variables suggestive of pulmonary hypertension (assuming right atrial pressure of 5mmHg)<sup>25</sup>]. Left heart abnormalities included LV dilatation (defined as a z-score  $>+2$  for LV end-diastolic diameter) or hypertrophy (defined as maximal wall thickness of interventricular septum (IVS) and/or LV posterior wall (LVPW) greater than  $+2$  z-scores); and/or left atrial (LA) dilatation (defined as z-score  $>+2$  for LA end-systolic diameter); and/or systolic and/or diastolic dysfunction. LV systolic function was assessed using Simpson's Biplane method and an ejection fraction  $\geq 55\%$  was considered normal.<sup>22</sup> LV diastolic dysfunction was assessed using transmitral Doppler peak early (E) and late diastolic (A) filling velocities, E/A ratio, deceleration time and pulmonary venous flow velocities, including peak systolic (S) and diastolic (D) waves, S/D ratio and atrial reversal (Ar) velocity; paediatric reference ranges were used to define abnormality.<sup>26</sup> Participants were classified as having diastolic dysfunction when at least four parameters were abnormal.

Resting tachypnoea was defined as a respiratory rate  $>25$ /min. Stunting and wasting were defined as a z-score  $<-2$  for height-for-age and weight-for-age respectively, using British 1990 growth references.<sup>27</sup> Hypoxia was defined as a

resting oxygen saturation <88%. Abnormal spirometry was defined as a reduced ratio of the highest forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) (FEV1: FVC) or reduced FVC regardless of normal FEV1: FVC ratio irreversible with salbutamol.<sup>19</sup>

### ***Data management and statistical analysis***

Data were extracted from paper forms using optical character recognition software (Cardiff TELEFORM Intelligent Character, Version 10.7) and analysed using STATA version 12 software (StataCorp, Texas, USA). Continuous data were presented as mean  $\pm$  standard deviation (SD) if they were normally distributed or median (interquartile range, IQR) if not normally distributed. A paired t-test, Mann-Whitney and McNemar tests were used to compare clinical characteristics of participants and mean values of cardiac measures and z-scores at baseline and follow up. The mean change in z-scores for each cardiac measure was calculated as mean z-score (18 months) minus mean z-score (baseline) and adjusted for baseline z-scores using linear regression. The incidence rate and risk for right and left heart abnormalities, respectively, were calculated in those without abnormalities at baseline and risk is reported as a proportion. Logistic regression was used to assess for risk factors for incident cardiac abnormalities at follow up.

Ethical approval was obtained from the Medical Research Council of Zimbabwe, the London School of Hygiene and Tropical Medicine Ethics Committee, the Biomedical Research and Training Institute Institutional Review Board and the Harare Central Hospital Ethics Committee. Written informed consent from guardians and assent from participants was obtained prior to enrolment.

## **Results**

### ***Clinical characteristics***

A total of 197 participants were recruited of whom 175 (89%) were followed up at 18 months, giving 283.9 person-years (pys) of follow up (Figure 1). The median age of participants at 18 months follow up was 12 (IQR, 10-14) years and 84 (48 %) were female. The proportion of participants who were virally suppressed (<400 copies/ml) at 18 months was lower compared to that at baseline (40% vs 78%) (Table 1). Supplementary Figure 1 shows the flow diagram of viral suppression over time. Four participants died, all of whom had echocardiographic abnormalities at baseline. The causes of death were pulmonary tuberculosis; meningitis; cardiac failure due to dilated cardiomyopathy; and unknown. The 22 patients lost to follow up either relocated or were unreachable due to change of contact details. There were no significant differences in age, CD4 count, viral load, duration on ART, clinical characteristics, height-for-age and weight-for-age z-scores and cardiac dimensions z-scores at baseline between participants who were followed up and those lost to follow up (Table 2). Data on pubertal staging were available at follow up on all participants. Thirty (35%) participants had reached menarche. Of the female participants, 34 (40%) and 40 (48%) were in Tanner stage 1 of breast and pubic hair development, respectively, compared to 3 (3%) testicular volume and 66 (73%) pubic hair development of the male participants (data not shown).

### ***Echocardiographic findings***

Ten participants developed left heart abnormalities and 16 developed right heart abnormalities, giving an incidence of 3.52/100 pys and 5.64/100 pys,

respectively. Table 3 shows baseline, 18-month and change in mean (SD) z-scores for cardiac parameters. The risk of developing RV dilatation was 12/163 (7%). Mean (SD) z-score for RV diameter at baseline was +1.10 (0.7) and increased to +2.51 (0.5). Of the 23 (13%) participants at follow up who had RV dilatation, 10 (43%) had concurrent left heart abnormalities and 11 (48%) had had RV dilatation at baseline as well. The risk of developing RV systolic dysfunction was 4/158 (3%). Of the 6 participants with RV systolic dysfunction at 18 months, two also had systolic dysfunction at baseline. None of the patients met the criteria for pulmonary hypertension by echocardiography. The risk of LV diastolic dysfunction was 5/140 (4%), LV 1/168 (1%) and LA dilatation 1/161 (1%) and LVH 2/174 (1%). Of the 15 (9%) participants who had LVH at follow up, 13/15 (87%) also had LVH at baseline and 3 (20%), 5 (33%) had LA dilatation and LV diastolic dysfunction respectively at baseline. LV dilatation persisted from baseline in 7 (4%) participants, 2 of whom had concurrent LA dilatation at baseline and follow up. Regression of abnormalities was observed in 11(6%) participants; two had LV systolic dysfunction, five had LVH (one with concurrent LV systolic dysfunction), five had isolated LV diastolic dysfunction (one with concurrent LVH) and one had RV dilatation at baseline. Cardiorespiratory symptoms were present in 41/48 (85%) at baseline but were all asymptomatic at 18 months.

There was an overall increase in mean z-scores for LV, LA, RV, interventricular septum and LV posterior wall diameters and TAPSE for all participants at follow up ( $p < 0.001$ ) (Table 1). There was a negative correlation between change in z-score and baseline z-scores, e.g. participants with high z-scores at baseline had a

smaller change in z-score at follow up. Mean changes in z-scores adjusted for baseline z-score of the same parameter were 0.64 SD (95% CI: 0.52, 0.77) for RV diameter; 0.36 SD (95% CI: 0.25, 0.47) for LV diameter; 0.63 SD (95% CI: 0.52, 0.74) for IVS diameter; 0.74 SD (95% CI: 0.63, 0.85) for LVPW diameter; 0.42 SD (95% CI: 0.33, 0.51) for LA diameter and -0.06 SD (95% CI: -0.24, 0.13) for TAPSE. Supplementary figure 2 shows an increase in z-score for LVPW diameter in a participant at baseline and follow up.

On multivariate logistic regression, stunting was associated with development of any cardiac abnormality (adjusted OR 2.59 (95% CI, 1.03- 6.49; p=0.043). No HIV-related factors, including CD4 count, viral load, duration on ART, age at ART initiation and type of ART, were associated with incidence of any cardiac abnormalities. There was no association between incidence of right heart abnormalities and abnormal spirometry. Menarche or the different indices of pubertal growth were not associated with change in mean z-scores for cardiac dimensions (data not shown).

## **Discussion**

To our knowledge, this is the first cohort study from SSA reporting the incidence and progression of cardiac abnormalities in children taking ART. Despite ART, participants continue to develop cardiac abnormalities, with the highest risk being development of RV dilatation, which was not associated with abnormal lung function. Most of the children with RV dilatation had isolated dilatation without associated RV systolic dysfunction. Currie *et al*, reported that heart muscle disease among people with HIV manifests as global or borderline LV dysfunction and isolated RV dilatation<sup>28</sup> and we hypothesize that the isolated RV



enlargement seen in the present study may be part of the spectrum of HIV-related cardiomyopathy and that it is manifest before LV involvement becomes evident. Notably, none of the participants with RV dilatation met the criteria for pulmonary hypertension using Doppler echocardiography. Despite the limitations of Doppler echocardiography in assessment of right heart pressures in children, there was no association between right heart abnormalities and abnormal lung function, suggesting that the abnormalities are more likely due to primary heart muscle disease

Most cardiac abnormalities present at baseline persisted at follow up, and we observed an overall increase in mean z-scores for cardiac dimensions over this short period of follow up. However, the majority had no symptoms, suggesting that there may be a prolonged period of subclinical cardiac manifestation before overt cardiac disease develops. The results of our study suggest the potential for progression of cardiac abnormalities and highlight the importance of routine screening for cardiac disease in children with HIV, even in the absence of symptoms.

A minority of participants had transient cardiac abnormalities. LV systolic dysfunction and LVH may have been a consequence of an acute infectious myocarditis.<sup>29</sup> Transient ventricular wall thickening has previously been observed in patients with acute myocarditis.<sup>30</sup> The participant with RV dilatation at baseline which resolved by follow up had a respiratory tract infection near time of the initial echocardiogram, which then resolved. Lung infection may result in transient pulmonary hypertension and RV enlargement, which resolve

with effective treatment of the infection.<sup>31</sup> Reversible cardiac abnormalities have also been reported among HIV infected adults in the USA, more than two-thirds (71%) of whom had AIDS.<sup>32</sup>

The aetiology of myocardial disease in HIV infection is complex. Several pathogenic mechanisms have been hypothesised, including chronic systemic immune activation of cardiac myocytes that occurs in HIV infection and is not completely reversed by ART.<sup>33</sup> Infection of the heart by opportunistic pathogens, including cytomegalovirus, Epstein Barr virus, coxsackievirus and adenovirus, may also result in cardiac damage.<sup>34</sup> Cardiotoxicity from ART has also been suggested, particularly nucleoside reverse transcriptase inhibitors (NRTIs), the backbone of ART including abacavir, and zidovudine.<sup>35, 36</sup> This may be related to mitochondrial dysfunction, which has been observed following use of stavudine, didanosine and zidovudine, while abacavir reportedly increases risk of myocardial infarction in adults.<sup>37</sup> Abacavir and zidovudine were being taken by 4% and 52% of the participants, respectively, but no association between ART and cardiac abnormalities was identified in this study. This further supports the possibility of the abnormalities being primary cardiomyopathy.

Stunted children had a higher likelihood of developing cardiac abnormalities. Miller *et al.* also reported that stunting was associated with LV diastolic dysfunction in their retrospective study of HIV infected adolescents, 71% of whom were on ART.<sup>17</sup> Stunting is a marker of chronic inflammatory conditions in childhood and underlying systemic inflammation may also play a role in pathogenesis of cardiac disease.<sup>38-47</sup> Notably, the proportion of participants who

were virally non-suppressed had increased over the follow up period. Poor adherence and high rates of viral non-suppression among adolescents have been reported in multiple studies.<sup>48, 49</sup> This would mean ongoing viral replication which is in turn associated with dysregulated systemic immune activation. This may have possibly contributed to the increase in cardiac disease in our cohort. In adults with HIV, systemic immune activation is a risk factor for development of cardiac disease.<sup>39</sup> HIV persists in reservoir cells even after effective treatment with ART and may continue to release cytotoxic cytokines which subsequently contribute to progressive and late tissue damage (cardiac myocytes).<sup>50</sup> The median age of ART initiation in this cohort was 6 (IQR, 3-8) years and immunosuppression and opportunistic infections prior to ART initiation may cause cardiac damage.

### ***Strengths and limitations***

The main strengths of our study include the prospective, systematic evaluation for cardiac abnormalities and use of local reference ranges to define the abnormalities, rather than reference ranges derived from North American or European populations, which have been shown not to be appropriate for SSA.<sup>24, 51</sup> Furthermore, participants were consecutively enrolled and not selectively enrolled on the basis of symptoms. Limitations included lack of viral load measures in all participants. The use of advanced echocardiographic techniques such as speckle tracking would have been more sensitive for detecting subclinical ventricular function, which may not be identified by measurement of the LV ejection fraction.

## **Conclusion**

There is a high incidence of cardiac abnormalities in children with HIV and taking ART in SSA and the lack of association with lung and HIV factors suggests that these abnormalities are primary HIV-related heart muscle disease. There is some evidence of disease progression over a short follow up period. Longer follow up is needed to understand the clinical implications of these abnormalities, and the pathogenesis of these abnormalities needs further study.

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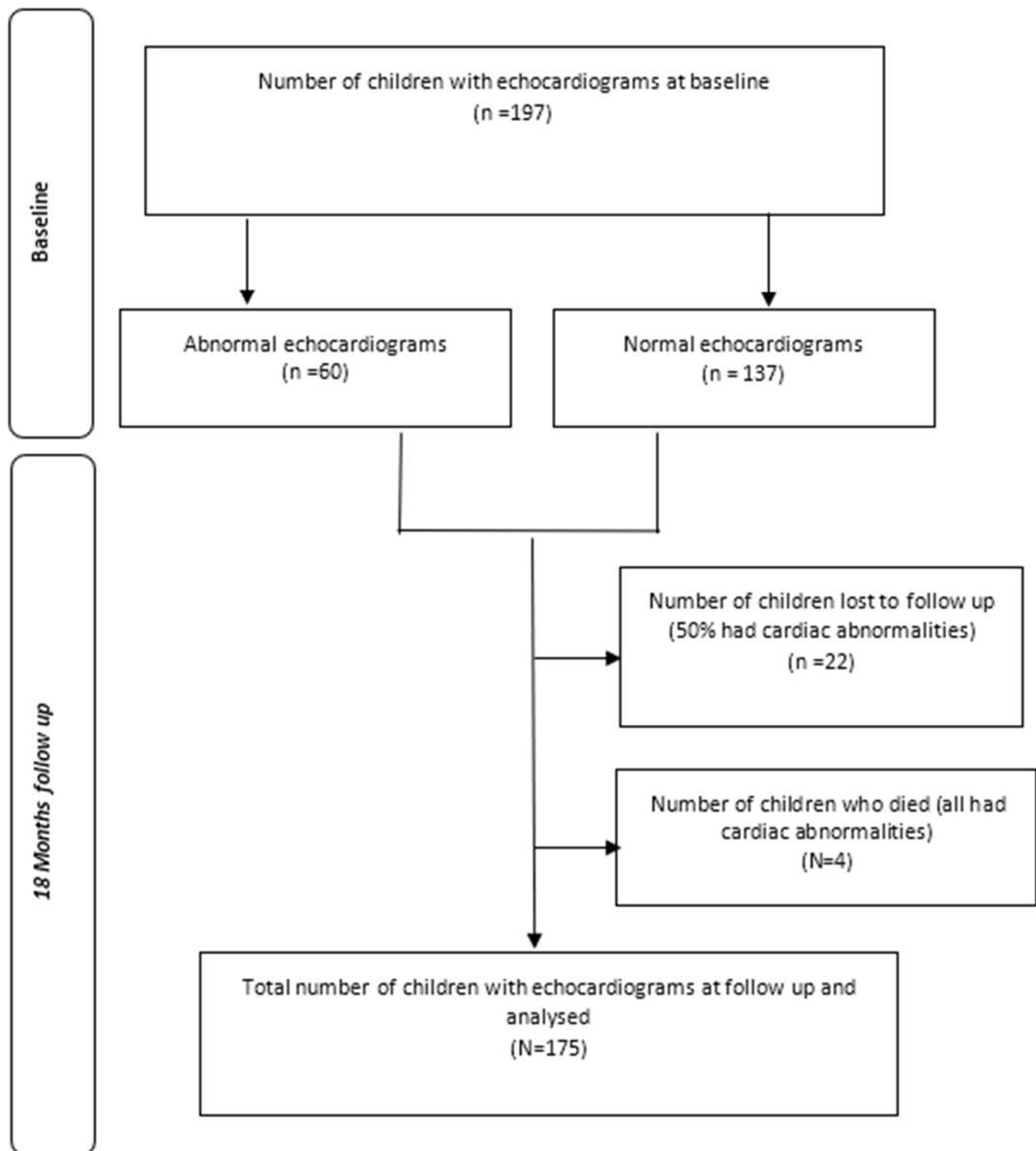
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**Figure 1. Flow chart for participant recruitment and follow up**



**Table 1. Clinical characteristics of participants**

Variable	N=175		P-value
	Baseline Mean (SD)	18 Months Mean (SD)	
Female, N (%)	84 (48)		
Age, y (median, IQR)	11 (9 – 13)	12 (10 – 14)	<b>&lt;0.001</b>
CD4, cell/ $\mu$ l (median, IQR)	726 (473 - 935)	734 (462 - 989) *	0.455
Viral load, copies/ml, (median, IQR)	19 (19 - 208)	456 (165 - 4080) **	<b>&lt;0.001</b>
Duration on ART, y (median, IQR)	4.8 (2.8 - 6.4)	6.5 (4.3 - 8.1)	<b>&lt;0.001</b>
Systolic Blood Pressure, mmHg	110 (11)	109 (10)	0.247
Diastolic Blood Pressure, mmHg	73 (9)	72 (9)	0.392
Respiratory rate, breaths per min	21.8 (4.5)	21.9 (2.3)	0.697
<b>Signs and Symptoms n (%)</b>			
Chest pains on exertion	20 (11)	5 (3)	<b>&lt;0.001</b>
Tachycardia	10 (6)	6 (3)	0.317
Tachypnoea	24 (14)	10 (6)	<b>0.013</b>
Hypoxia at rest	1 (1)	0	-
Abnormal spirometry***	37 (24)	23 (15)	0.144
Wasting	38 (22)	35 (20)	0.106
Stunting	40 (23)	39 (22)	0.117
<b>Cardiac Measures</b>			
RV diameter z-score	0.40 (1.3)	0.91 (1.1)	<b>&lt;0.001</b>
LV diameter z-score	0.49 (1.1)	0.72 (1.1)	<b>&lt;0.001</b>
IVS diameter z-score	0.06 (1.0)	0.65 (0.8)	<b>&lt;0.001</b>
LVPW diameter z-score	0.29 (1.2)	0.88 (0.9)	<b>&lt;0.001</b>
LA diameter z-score	0.36 (1.1)	0.66 (1.0)	<b>&lt;0.001</b>
TAPSE	-0.63 (0.9)	-0.26 (1.0)	<b>&lt;0.001</b>
Ejection fraction (%)	61.7 (6.2)	64.5 (6.6)	<b>&lt;0.001</b>
E wave (m/s)	0.91 (0.1)	0.93 (0.2)	0.142
A wave (m/s)	0.53 (0.1)	0.55 (0.1)	0.105
E/A ratio	1.76 (0.4)	1.74 (0.4)	0.656
Deceleration Time (ms)	174 (27.7)	169 (15.6)	<b>0.044</b>
PV S wave (m/s)	0.49 (0.1)	0.51 (0.1)	0.069
PV D wave (m/s)	0.55 (0.5)	0.51 (0.1)	0.388
PV A wave (m/s)	0.19 (0.2)	0.22 (0.5)	0.352
PV S/D ratio	0.98 (0.3)	1.02 (0.2)	0.062
<b>Cardiac abnormalities n (%)</b>			
LV dilatation	7 (4)	8 (5)	0.317
LVH	18 (10)	15 (9)	0.257
LA dilatation	14 (8)	15 (9)	0.317
LV systolic dysfunction	2 (1)	1 (1)	0.563
LV diastolic dysfunction	40 (23)	40 (23)	1.000
RV dilatation	12 (7)	23 (13)	<b>0.002</b>
RV systolic dysfunction	2 (1)	6 (3)	<b>0.046</b>

\*, n=159; \*\*, n=89; \*\*\*, n=150

LV, left ventricle; IVS, interventricular septum, LA, left atrium; RV, right ventricle; E/A ratio, mitral valve peak early to late left ventricular filling velocity; PV, pulmonary venous; S, systolic; D, diastolic; TAPSE, tricuspid annular plane systolic excursion

**Table 2. Baseline characteristics of the HIV infected children followed up and those lost to follow up**

	<b>N=175</b>	<b>N=22</b>
<b>Variable</b>	<b>Followed up mean (SD)</b>	<b>Lost-to-follow up mean (SD)</b>
Age, y (median, IQR)	11 (9 – 13)	11 (10 – 12)
CD4, cell/ $\mu$ l (median, IQR)	737 (473-935)	534 (416 – 807)
Viral load, copies/ml (median, IQR)	19 (19-208)	26 (165- 147)
Age at HIV diagnosis, y (median, IQR)	5 (3- 7)	6 (3- 8)
Duration on ART, y (median, IQR)	4 (2- 6)	4 (1- 5)
Heart rate, bpm	88.3 (15.5)	83.1 (12.3)
Height-for-age z-score	-1.24 (1.1)	-1.42 (1.1)
Weight-for-age z-score	-1.09 (1.2)	-1.19 (1.6)
Systolic Blood Pressure, mmHg	110 (10.8)	113 (15.7)
Diastolic Blood Pressure, mmHg	73 (8.7)	77 (8.2)
Respiratory rate, breaths per min	21.8 (4.5)	22.4 (5.8)
Oxygen Saturation, (%)	96.8 (2.2)	96.4 (2.7)
RV diameter z-score	0.40 (1.3)	0.10 (2.1)
LV diameter z-score	0.49 (1.1)	0.93 (1.6)
IVS diameter z-score	0.06 (1.0)	0.18 (1.0)
LVPW diameter z-score	0.29 (1.2)	0.40 (1.1)
LA diameter z-score	0.36 (1.1)	0.58 (2.1)
TAPSE	-0.63 (0.9)	-0.95 (1.3)
Ejection fraction, (%)	61.7 (6.2)	62.3 (6.6)

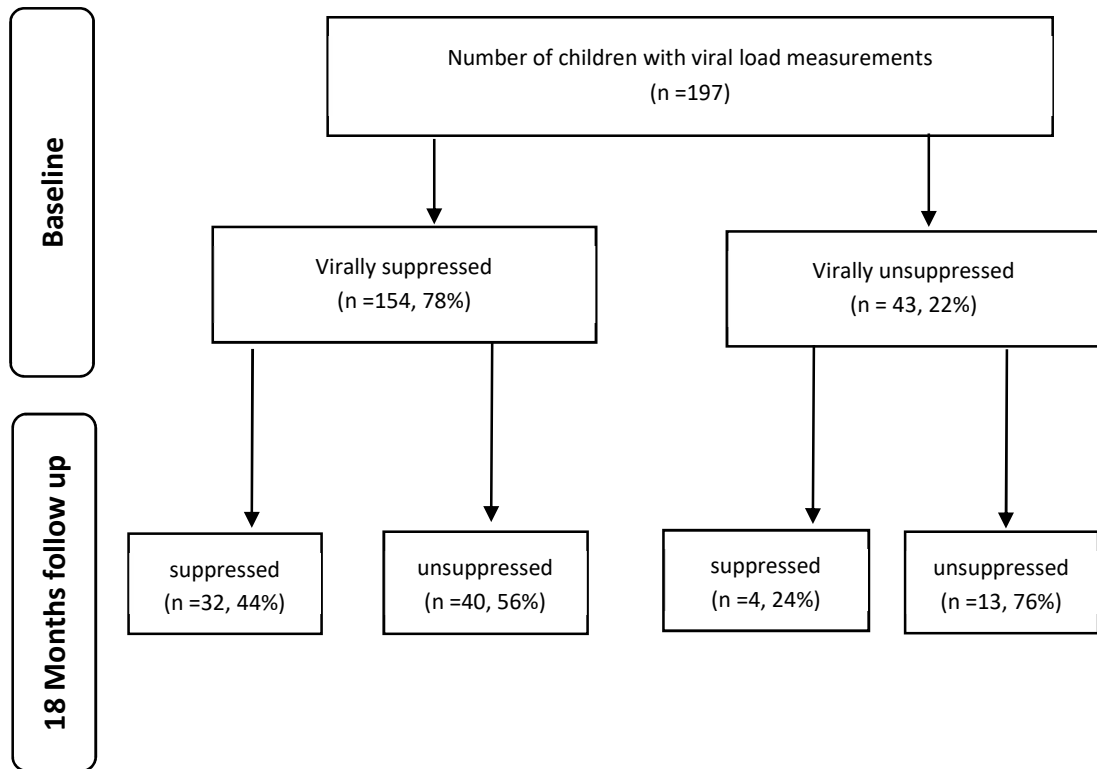
*RV, right ventricle; LV, left ventricle; IVS, interventricular septum, LVPW, left ventricular posterior wall; LA, left atrium, TAPSE, tricuspid annular plane systolic excursion; IQR, interquartile range; ART, antiretroviral therapy*

**Table 3. Z-scores at baseline and follow up for participants**

	Baseline	Follow up					
Cardiac variable			N (%)	Z-score baseline mean (SD)	Z-score 18 months mean (SD)	Adjusted change in z-score after 18 months, SD (95% CI)	Risk
RV diameter diastole	Normal	Normal	151 (86)	0.15 (1.1)	0.64 (0.9)	0.55 (0.43- 0.67)	
	Normal	RV dilatation	12 (7)	1.10 (0.7)	2.51 (0.5)	2.47 (1.90- 3.03)	12/163 (7%)
	RV dilatation	Normal	1 (1)	2.23 (0.0)	1.81 (0.0)	-0.79 <sup>a</sup>	
	RV dilatation	RV dilatation	11 (6)	2.91 (0.7)	2.85 (0.8)	0.59 (-1.21- 2.39)	
LV diameter diastole	Normal	Normal	167 (95)	0.37 (1.0)	0.62 (1.0)	0.35 (0.24- 0.47)	
	Normal	LV dilatation	1 (1)	1.04	2.10	1.06 <sup>a</sup>	1/168 (1%)
	LV dilatation	LV dilatation	7 (4)	3.27 (0.3)	2.94 (0.4)	-0.46 (-2.20- 1.27)	
IVS diameter diastole	Normal	Normal	172 (98)	0.02 (0.9)	0.62 (0.8)	0.61 (0.51- 0.71)	
	Normal	IVS hypertrophy	2 (1)	1.82 (0.2)	3.13 (1.1)	1.31 (1.3)	2/174 (1%)
	IVS hypertrophy	Normal	1 (1)	2.50	1.63	-0.87 <sup>a</sup>	
LVPW diameter diastole	Normal	Normal	158 (90)	0.05 (1.0)	0.72 (0.8)	0.70 (0.59- 0.81)	
	LVPW hypertrophy	Normal	4 (2)	2.43 (0.2)	1.63 (0.2)	-0.80 (0.4) <sup>a</sup>	
	LVPW hypertrophy	LVPW hypertrophy	13 (7)	2.61 (0.5)	2.61 (0.4)	1.00 (0.11- 1.89)	
LA diameter diastole	Normal	Normal	160 (91)	0.17 (1.0)	0.52 (0.9)	0.41 (0.31- 0.50)	
	Normal	LA dilatation	1 (1)	1.99	2.07	0.08 <sup>a</sup>	1/161 (1%)
	LA dilatation	LA dilatation	14 (8)	2.44 (0.3)	2.44 (0.2)	2.74 (1.77-3.70)	
TAPSE	Normal	Normal	154 (88)	-0.59 (0.9)	-0.16 (1.0)	-0.04 (0.22-0.14)	
	Normal	RV systolic dysfunction	4 (2)	-1.36 (0.6)	-2.32 (0.3)	-0.96 (0.8) <sup>a</sup>	4/158 (3%)
	RV systolic dysfunction	RV systolic dysfunction	2 (1)	-2.23 (0.1)	-2.20 (0.1)	-0.02 (0.1) <sup>a</sup>	

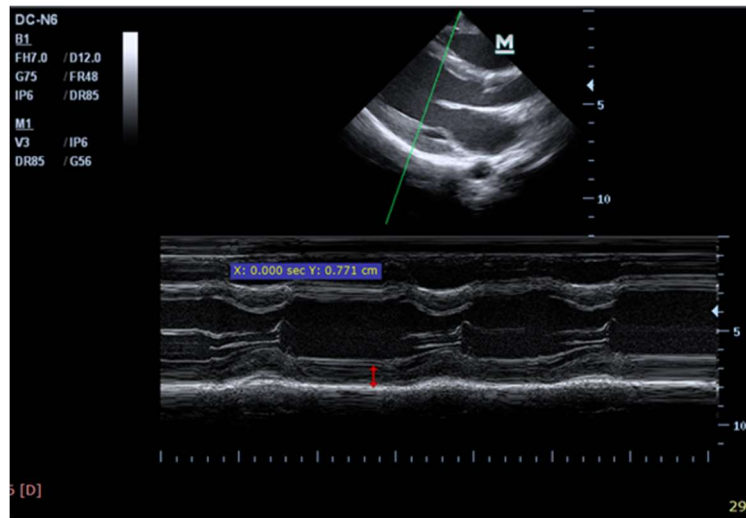
<sup>a</sup>, unadjusted change (if n=1), or adjusted mean and SD if (n>1) has been reported; RV, right ventricle; LV, left ventricle; IVS, interventricular septum, LVPW, left ventricular posterior wall; LA, left atrium, TAPSE, tricuspid annular plane systolic excursion

**Supplementary figure 1: Flow diagram of viral suppression over time**



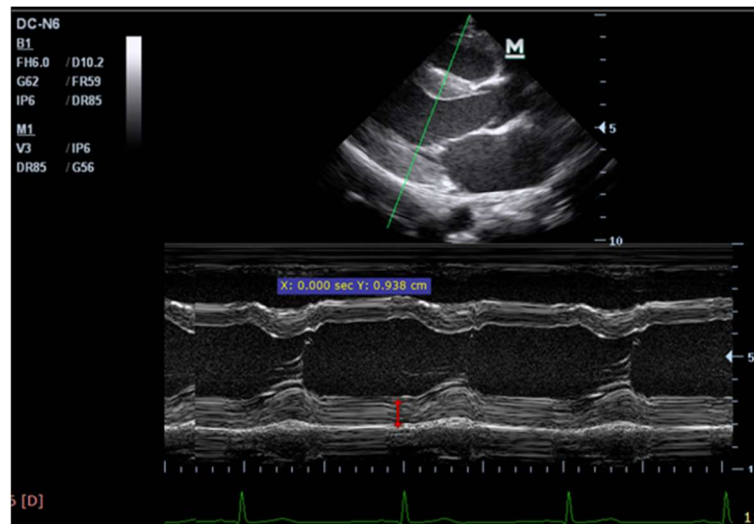
## Supplementary figure 2: Progression of cardiac abnormality over time.

a.



An M-Mode tracing for a 6-year-old boy with a body surface area of  $0.74\text{m}^2$ . The LV posterior diameter measured  $7.71\text{mm}$  at baseline which corresponded to a z-score of  $+2.10$ .

b.



At 18-month follow up the body surface area was  $1.02\text{m}^2$ , the LV posterior wall diameter for the same boy measured  $9.38\text{mm}$  which corresponded to a z-score of  $+2.94$ .

## 7. Discussion

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The main aims of this thesis were to develop echocardiographic reference ranges for older children and adolescents for Zimbabwe and to investigate the features of cardiac abnormalities and their progression in HIV-infected older children and adolescents taking antiretroviral therapy. The following objectives were met:

1. Established echocardiographic reference ranges for Zimbabwean children and adolescents.
2. Determined the prevalence and spectrum of cardiac abnormalities, in older children and adolescents on ART.
3. Assessed the risk factors for cardiac abnormalities in older children and adolescents on ART.
4. Examined the incidence and progression of cardiac abnormalities in older children and adolescents on ART.

This chapter summarises and discusses the key findings and links them together with implications of findings, strengths, limitations and focus for future studies outlined.

## **7.1 Echocardiographic reference ranges in children**

One of the key outputs were the first echocardiographic reference ranges for Black African older children and adolescents from Zimbabwe and SSA at large. Some cardiac dimensions including interventricular septal and right ventricular diameters were larger in this group when compared to available references from other races and regions. Lopez et al found a significant difference in measures of



echocardiographic references with respect to age, sex, race, and ethnicity, which they found to have no clinical significance.<sup>1</sup> Given that this study was conducted mainly in USA and Canadian children, findings cannot be generalisable to African children and elsewhere because of other methodological variations in the available references.<sup>2</sup> Furthermore, geographical confounders such as nutrition and altitude may affect cardiovascular development in different races.<sup>1</sup> However these were not evaluated in Lopez's study. The altitude of Harare is 1490m above sea level and it is categorized as low altitude according to altitude classification by Imray et al.<sup>3</sup> Increasing altitude results in a decrease in atmospheric and oxygen pressure, humidity and air temperature and physiological changes are caused to the cardiopulmonary system including hypoxia, myocardial work and increased pulmonary arterial pressure.<sup>4</sup> The exact altitude at which physiologic changes affecting cardiovascular performance occur are variable with significant changes typically occurring at altitudes >2,500m.<sup>4</sup> The altitude in Harare is relatively low and may not significantly influence cardiovascular system.

The systematic review conducted in this thesis revealed that there are other factors other than race which contribute to the variability of reference ranges including normalisation parameters.<sup>5,6</sup> The parameter of normalisation to growth such as BSA can be determined by more than one formula which also adds to the variability of echocardiographic reference ranges.<sup>7</sup> Currently, echocardiographic assessment of children in our setting is based on Western reference ranges,<sup>8</sup> and so, the references that have been established will fill a gap in knowledge. Abnormalities may potentially be over- or under-estimated if inappropriate references are used. The need for reference ranges appropriate to

the population under study has been demonstrated in this research. Furthermore, standardization of methodology and guidelines for developing reference ranges is required to minimise variation.

We found no children with bicuspid aortic valves, atrial septal defects, mild mitral regurgitation, or rheumatic heart disease in the healthy uninfected children possibly because they had been excluded at eligibility screening level. However, children excluded based on the above were very few (n=3) partly because of our recruitment strategy which was not completely unselective. There is a variable burden of rheumatic heart disease among African children with some areas reporting higher prevalence compared to others.<sup>9,10</sup> A cross-sectional survey among children (aged 1 to 12 years) presenting to two referral hospitals in Harare, Zimbabwe found that 50 children out of the 3 627 (1.37%) had acute rheumatic fever or rheumatic heart disease, suggesting that rheumatic heart disease prevalence in our setting is relatively low.<sup>11</sup>

Stunting has been associated with changes in cardiovascular function in African children.<sup>12</sup> Sub-Saharan Africa is one of the regions with a high prevalence of stunted children although it varies by country. The prevalence ranges from 16.6% to 57.7% with Zimbabwe having a rate of 32%.<sup>13</sup> Severe malnutrition may cause significantly reduced LV mass. Although we appreciate that malnutrition has an impact on cardiac growth, children were not excluded because of malnutrition or stunting in this study. We compared our z-scores with available references and found that LV and LA diameters were comparable to European references while RV diameter, interventricular septal and LV posterior wall

thickness were higher than published references supporting the need for local reference ranges for African children.<sup>5</sup>

## **7.2 Cardiac abnormalities in children taking ART**

A high prevalence of subclinical cardiac abnormalities was observed in our setting in children taking ART defined by the established reference ranges. Notably, an overestimation of LVH and RV dilatation would have occurred in this cohort if the Western reference ranges were applied. The pathogenesis of these abnormalities remains unclear but is most likely multifactorial.<sup>14-17</sup> Despite cardiac abnormalities being common, more than three-quarters of the children were virally suppressed at baseline which may suggest that ART or immune reconstitution may not be protective against development of cardiac abnormalities. An important risk factor, hypertension, was associated with LV diastolic dysfunction, although, not all patients with hypertension had LV diastolic dysfunction. Hypertension is a well-recognized traditional risk factor for cardiovascular disease.<sup>18</sup> Elevated high blood pressure has been previously reported in children taking ART and may potentially be an additional aetiology of myocardial disease in HIV infected children.<sup>19</sup> Further studies investigating hypertension in HIV infected children taking ART is required to confirm this.

At 18 months follow up, most of the cardiac abnormalities which were present at baseline persisted. Strikingly, they had not worsened nor caused further cardiac symptoms. ART is undoubtedly beneficial and may potentially slow progression of the abnormalities, which may potentially explain lack of progression. Children

in the USA (3-16 years) who were treated with ART in the CHAART-2 study had preserved cardiac function for up to 10 years, following which it began to decline.<sup>20</sup> CHAART-2 was conducted in a high-income country where the vast majority of children with HIV are started on treatment in infancy and they vary significantly from children in our setting for several reasons including: immune status at time of HIV diagnosis, timing of ART initiation, different genetic factors, the infectious background and the relative poorer access to health services. Also, other risk factors may be contributing to development of cardiac abnormalities including malnutrition and trace elements deficiency such as selenium.<sup>17,21,22</sup>

While most of the children taking ART in our study were virally suppressed, it is important to note that they had started ART in older childhood. Therefore, cardiac insults prior to ART may have occurred, including co-infections and chronic inflammation.<sup>15</sup> Early ART initiation may go a long way in delaying cardiac manifestation.

There was a high incidence of cardiac abnormalities over a relatively short period of follow up and an overall increase in mean z-scores of cardiac dimensions. This suggests potential for progression of cardiac abnormalities and may become overt cardiac disease. Cardiac abnormalities in HIV may be transient as observed in a minority of the children who had abnormalities which reverted to normal. This is likely associated with an episode of acute illness, which upon treatment may result in resolution of associated cardiac abnormalities. Transient cardiac abnormalities were also reported in the pre-ART era in adults with AIDS

and may certainly occur in the general population e.g. a period of myocarditis may be associated with myocardial thickening which may resolve.<sup>23,24</sup>

### **7.3 Strengths**

This was a prospective cohort study and incidence and progression of the cardiac abnormalities over time could be determined and assessed. The follow up rate was high which minimizes bias of findings. Locally derived reference ranges from children in the same setting were used to define echocardiographic abnormalities and has allowed a more accurate characterisation of cardiac abnormalities. Another particular strength of being part of a larger research, was the ability to compare lung function data and cardiac findings.

### **7.4 Limitations**

The study was embedded within a larger project (INHALE) whose primary aim was to determine the prevalence of chronic lung disease. The sample size was therefore determined based on the prevalence of chronic lung disease, it may have underpowered for detection of risk factors for cardiac abnormalities. The study did not include an HIV-uninfected comparison group. However, abnormalities were defined as standardized z-scores derived from a reference population from the same setting.

The current CD4 count was assessed as a risk factor for cardiac disease. However, it's likely the abnormalities developed in the past and a more suitable measure would have been a nadir CD4 count which is a measure of level of

immunosuppression a child would have reached. However, nadir CD4 counts were not available. Additionally, at follow up viral load measures were missing for a significant proportion of children. The follow up time was relatively short- this research is unable to establish the clinical significance of these abnormalities and whether they will progress to overt disease. Much longer periods of follow-up would be required.

The sample size for healthy HIV uninfected children for deriving reference ranges was constrained by time and resources and therefore, could not reach the intended target of 300 participants. The participants were not completely unselected and there may have been some selection bias. Participants were enrolled from seven primary care clinics in Harare that were offering HIV-testing to all attendees regardless of the reason for presentation as part of a project evaluating HIV testing services. We also approached stable patients in orthopaedic wards at Harare Central Hospital. Nevertheless, only healthy children with no known heart disease were included.

Pulmonary hypertension was assessed using echocardiography. Right heart catheterisation is the gold standard in diagnosis of pulmonary hypertension, and there is generally a poor correlation between echocardiographic measurements and pulmonary pressures derived through direct measurement.<sup>25</sup> Doppler echocardiography may under- or over-estimate pulmonary pressures, especially in children with elevated right heart pressures.<sup>26</sup>

The cohort was pauci- symptomatic and symptoms at follow up were significantly less frequent than at baseline. It is possible there may have been reporting bias and there is subjectivity associated with self-report. More importantly, the measure of hypertension was based on a single measurement and so misclassification of hypertension is a possibility due to diurnal variation and possibly due to “white coat” hypertension. Furthermore, the references we used we used from the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents were derived children in the USA and may have been unsuitable for this population.<sup>27</sup>

## **7.5 Implications of findings**

This study demonstrates a high prevalence of a variety of left-sided structural and functional cardiac abnormalities, determined by echocardiography. The natural history and clinical significance of these findings is not currently clear but there is certainly evidence from adult studies to show that HIV is an independent risk factor for cardiac disease. Notably, there was absence or low prevalence of some of the strong known risk factors for cardiac disease, namely smoking, elevated blood sugars and age. While a relatively high prevalence of hypertension was observed, a finding also noted in recent studies of African cohorts, misclassification as discussed above cannot be excluded.<sup>28-30</sup> Notwithstanding the limitations noted above, screening for and close monitoring of cardiac abnormalities in children with HIV may be warranted. This has resource implications in the health systems in resource-limited settings, where health systems are already overwhelmed by the large numbers of individuals

with HIV who will require life-long monitoring and treatment for HIV. Furthermore, HIV service delivery is largely decentralised to lower levels of health care and even to community settings, which makes implementation of screening for chronic co-morbidities such as cardiac disease challenging.

The generalisability of these findings is limited to this generation of children who have had delayed HIV diagnosis. With continued efforts of scaling up PMTCT and EID, new HIV infections are declining, and children likely starting ART earlier and cardiac outcomes may be different in children initiating ART in infancy. However, there is a generation of children who face lifelong treatment for an incurable infection. ART has been available for only a decade in SSA and this group of children with HIV is the first to survive childhood and reach adolescence. We therefore do not know what the long-term complications of HIV and/or treatment are likely to be. There is no precedent on how to screen for co-morbidities that accompany HIV infection as these children reach adulthood. Notably, children provide an optimum group for investigation of pathogenesis of HIV-related cardiac disease, due to the absence of confounding by established risk factors.

## **7.6 Future Work**

The future focus would be to extend follow of paediatric cohorts to understand natural history and the clinical implications of the observed cardiac abnormalities, and to evaluate screening algorithms for cardiac disease. Parallel standardized clinico-epidemiological studies of cardiac disease in different settings will provide insight into the likely risk factors and importantly whether



timely ART is sufficient to prevent development and progression of cardiac disease and other co-morbidities in children. In addition, investigation of the impact of periods of viraemia on the risk of complications will need studying given that adolescence is notably a period of poor adherence, and several studies have reported disproportionately poorer viral suppression rates in adolescence compared to other age-groups.<sup>31,32</sup>

As noted above, children are an optimum group to obtain insights into pathogenesis of HIV-associated cardiac disease. For example, it is postulated that the ongoing systemic immune activation and consequent chronic inflammation may lead to premature ageing of the immune system (“inflammaging”) and this is one postulated mechanism for development of chronic co-morbidities such as cardiac disease.<sup>33-35</sup> These studies will ultimately inform the need for and type of strategies to address long-term co-morbidities associated with HIV infection.

Undoubtedly ART is remarkable achievement and has meant that millions of children can now reach adulthood. However, HIV is a formidable foe and continued efforts to understand the virus will be required if we are to ensure that children who have now been given the chance to reach adulthood do so and achieve their full potential.

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