College of Medicine

Cardiac Disease in Children with HIV-Associated Chronic Lung Disease at Queen Elizabeth Central Hospital, Blantyre, Malawi

By

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(MBBS)

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DECLARATION

I, Gugulethu N. Mapurisa, hereby declare that this thesis is my original work and has not been presented for any other awards at the University of Malawi or any other University.

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ABSTRACT

Over the past decade, more perinatally-infected children have survived despite previous assertions that few would reach adolescence. More complications of the chronic human immunodeficiency virus (HIV) are surfacing with improving survival. These include HIV, chronic lung disease (HCLD), and cardiac disease (1-5). Such complications were previously associated with delayed diagnosis and poor HIV control. However, there is growing evidence that prolonged disease by itself predisposes to cardiac disease (6,7). Cardiac disease in HCLD has not been researched in children stable on ART.

The study aimed to describe the cardiac symptoms in HIV-infected children with chronic lung disease, who are stable on antiretroviral therapy (ART), and identify the prevalence of cardiac dysfunction.

The study was conducted at Queen Elizabeth Central Hospital, QECH, a large teaching hospital in Blantyre, Malawi. It was a nested study in a prospective randomised controlled trial that co-recruited consenting trial participants with HCLD who had been on ART for more than six months with virological suppression. Chronic lung disease was determined by spirometry of (FEV1 z-score < -1.0) with no reversibility (< 12%). Participant demographics were collected, and cardiac echocardiograms were done at baseline using a Sonosite M-turbo machine (8). Clinical data and demographic data were collected and analysed using STATA 14.

Fourty-nine (49) of the 180 participants were recruited. The median age was 14.5 years; the interquartile range [IQR] was 8.4 – 19.8 years; 51.1% female. The mean CD4 cell counts were 640 ± 439 (87 – 2969). The mean Medical Research Council (MRC) dyspnea score was 2.3 ±

1. Rheumatic heart disease was confirmed in 3 (6%) who were already on treatment at recruitment. 0 (0%) having pulmonary hypertension.

In conclusion, our findings demonstrate low cardiac dysfunction and pulmonary hypertension levels in this cohort of HCLD in children. However, there is significant co-morbidity with acquired heart disease in this group set of children. Longer-term follow-up of these children is essential to identify if further cardiac dysfunction does not emerge in children on ART for a longer duration.

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ABBREVIATIONS AND ACRONYMS

2D	Two-Dimensional
A4C	Apical 4 Chamber
ALT	Alanine Transferase
ART	Anti-Retroviral Therapy
BREATHE	Broncho-pulmonary function in REsponse to Azithromycin Treatment for chronic lung disease in HIV-infected children and adolescents
CLWH	Children Living With HIV
CXR	Chest X-Ray
DICOM	Digital Communication in Medicine
ECG	Electrocardiogram
FEV1	Forced Expiratory Volume in one second
HIV	Human Immuno-deficiency virus (HIV)
HCLD	Human Immunodeficiency Virus Chronic Lung Disease
HRCT	High-Resolution Computer Tomography
IPT	Intermittent Prophylactic Treatment
IQR	Inter-Quartile Range
LIP	Lymphoid Interstitial Pneumonitis

LV	Left Ventricular
MRC	Medical Research Council
NRTI	Nucleoside Reverse Transcriptase Inhibitor
QECH	Queen Elizabeth Central Hospital
PASP	Pulmonary Arterial Systolic Pressure
PH	Pulmonary Hypertension
RA	Right Atrial
SD	Standard deviation
SSA	Sub-Sahara Africa
TAPSE	Tricuspid Annular Plane Systolic Excursion
TB	Tuberculosis
VL	Viral Load

CHAPTER ONE: INTRODUCTION

Over the past decade, more children with perinatally-acquired Human Immunodeficiency Virus (HIV) infection have survived despite previous assertions that few would reach adolescence. With improving survival, complications of chronic HIV are surfacing. These include HIV-associated chronic lung disease (HCLD) and cardiac disease. Such difficulties were previously associated with delayed diagnosis and poor HIV control. However, there is growing evidence that even with early diagnosis, prolonged illness does predispose to cardiac disease and HCLD. Right heart abnormalities and pulmonary hypertension (PH) may occur secondary to chronic lung disease. No previous studies have explored the prevalence of cardiac disease in strictly screened HCLD children who are not failing ART. This study aims to determine the symptomatology and prevalence of echocardiogram confirmed cardiac disease in HCLD children managed at Queen Elizabeth central hospital, Blantyre, Malawi.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Over the past decade, there has been a global scale-up in antiretroviral therapy (ART) programs resulting in a dramatic decline in mortality among children living with human immunodeficiency virus (HIV) infection (9-10). As of 2019, 38 million people were reported to be living with HIV globally, with the majority being in sub-Saharan Africa (SSA). In Malawi in the same year, the ART coverage rate was 74%, with 1.1 million adults and an estimated 65,000 children aged 0-14 living with HIV (11).

Despite improved ART uptake and its benefits, evidence is increasingly reporting the burden of chronic comorbidities in those living longer with HIV (3,12-17). This is because, while ART facilitates immune reconstitution and reduces the risk of infections, a long-standing HIV infection is itself associated with an increased risk of chronic comorbidities (13,17-19). It may result from the HIV infection itself, its treatment, or sequelae of repeated chest infections (20). Complications can involve multiple systems and can be infectious and non-communicable (16).

2.2 HIV Chronic Lung Disease

Studies in sub-Sahara Africa (SSA), such as Zimbabwe and South Africa, have reported a high prevalence of chronic respiratory symptoms among older children and adolescents living with HIV (15,18,21-22). These findings are classified within HIV chronic lung disease (HCLD). The prevalence of HCLD can be as high as 25–37.5% amongst adolescent survivors of HIV disease. The typical clinical picture involves chronic cough with reduced exercise tolerance and an obstructive defect on spirometry with no bronchodilator response (21).

Patients with HCLD are typically hypoxic at rest with chest X-ray (CXR), finding inconsistent with Lymphoid Interstitial Pneumonitis (LIP) (14,21,23).

Multiple etiological factors may contribute to HCLD, including long-term sequelae of repeated bacterial and viral respiratory tract infections and possibly HIV-induced chronic inflammation and dysregulated immune activation (13,24). Treatment includes antibiotic therapy if there is clinical suspicion of a bacterial infection, pulmonary clearance techniques, and ART. Strategies to prevent HCLD include pneumococcal immunisation, chemoprophylaxis with azithromycin, and micronutrient supplementation (25-27).

In a study of 385 children in primary schools in Zimbabwe, 28% of ART-naïve aged 6 to 16 years had abnormal lung function with reduced forced vital capacity (FVC) (18,21). Upon ART initiation, there is evidence of initial improvement in lung growth and function seen in the first 2-years following ART initiation (19). Children developed chronic lung disease (HCLD) in the pre-ART era, most commonly due to lymphoid interstitial pneumonitis (LIP). This type of HCLD primarily affects the lung parenchyma and responds well to ART. In the post ART era, there has been a change in the kind of HCLD seen, in which the small airways are more commonly affected. The diagnosis of HCLD is challenging to make, partly because of the gold standard for investigation (high-resolution computer tomography imaging, cardiac imaging, and lung function testing) being out of reach of many affected. Findings on High-resolution computer tomography (HRCT) suggest that obliterative bronchiolitis may be the primary cause of HCLD (14).

2.3 Cardiac Disease in Children with HCLD

There is a high prevalence of cardiac disease in Children Living with HIV (CLWH), with dilated cardiomyopathy, inflammatory cardiomyopathy, left ventricular dysfunction, and pulmonary hypertension described in various studies in SSA (4,6,22,28-29). Despite theoretical toxicity risks, ART is generally reported to improve cardiac function, exercise tolerance, and lung function.

Despite this, children on ART had lower lung function (30), lower exercise tolerance (31), and lower cardiac function (2) compared to HIV-uninfected children in pediatric cohorts in South Africa, Malawi, and Zimbabwe, respectively. Miller *et al*. (4) reported a 29% prevalence of right ventricle dilatation in a Zimbabwe cohort of perinatally HIV-positive adolescents (71% on ART, median duration of ART 20 months). Nearly 50% of this population had chronic lung disease.

Right heart dysfunction may be secondary to chronic lung disease (4,28,32-33). Pulmonary hypertension (PH) may also develop as a complication of HCLD. PH may cause right ventricular (RV) re-modelling, hypertrophy, dilatation, and subsequent right heart failure (33). RV dysfunction, defined as abnormal RV structure and function, is associated with poor clinical outcomes regardless of underlying mechanisms (34). The pathogenesis of dilated and inflammatory cardiomyopathy in HIV is unclear, with several proposed mechanisms (35-37). The presence of HIV within the heart muscles initially suggests that cardiac damage resulted from direct infection of cardiac myocytes and nucleoside reverse transcriptase inhibitor (NRTI) induced mitochondrial toxicity (38-39). Many of these drugs are part of Malawi's pediatric national guidelines (40-41).

Patients with cardiac disease often benefit from early treatment with angiotensin-converting inhibitors and beta-blockers, which may halt the progression of the disease. Many children with diminished ejection fraction such as cardiomyopathy may be started on calcium channel blockers like digoxin. This, however, poses challenges in drug interaction (29).

2.4 Study Rationale

Challenges in diagnostic criteria for HCLD, and definitions of many cardiac dysfunction criteria like pulmonary hypertension, have made credibility and generalizability of research findings challenging. Few studies have looked at cardiac disease in children with CLD who are stable on ART in the post- ART era. The overall aim of this study was to identify the clinical symptoms and prevalence of cardiac dysfunction in children with vertically transmitted HIV who are established on ART with HCLD.

2.5 Aims and Objectives

a. To describe the cardiac symptoms in HIV-infected children with chronic lung disease aged 6 - 19 years, which are stable on antiretroviral therapy at QECH, Blantyre City, Malawi.

b. To determine the prevalence of cardiac dysfunction in HIV-infected children with chronic lung disease aged 6 - 19 years, which are stable on antiretroviral therapy at QECH, Blantyre City, Malawi using echocardiography.

CHAPTER THREE: METHODS

3.1 Introduction

The study was conducted as a nested sub-study to the randomised, double-blind, placebo-controlled trial of broncho-pulmonary function in response to azithromycin treatment for chronic lung disease in HIV-infected children and adolescents (BREATHE) (26). The overall trial was conducted at two sites, in Zimbabwe and Malawi. This study was conducted at Queen Elizabeth Central hospital, a large public teaching hospital, and other surrounding health centres with outpatient HIV clinics in Blantyre, Malawi. The study was conducted between June 2016 and September 2018. All participants recruited in the site trial were recruited prospectively.

3.2 Participants' Enrolment

The inclusion and exclusion criteria for the overall BREATHE trial was the same as this study. The inclusion and exclusion criteria are below:

3.2.1 Inclusion Criteria

a. Perinatally HIV-infected children and adolescents aged 6–19 years who have been receiving antiretroviral (ART) for at least six months.
b. The participant must have a firm home address and a stable guardian.
c. Must be able to obtain consent from the guardian and permission from the participant (for those aged < 18 years; those aged ≥ 18 years were asked to consent independently).
d. Participants met the definition of HCLD with a forced expiratory volume in one second (FEV1) z-score of -1 and lack of reversibility with salbutamol.
e. Disclosure of HIV status to the child aged 12 years and above.

f. Mother-to-child transmission was identified as the most likely mode of HIV acquisition.

3.2.2 Exclusion Criteria

a. All children with a condition that may prove fatal during the study period, such as malignancy.

b. Children with tuberculosis (TB) or acute respiratory tract infection at the time of screening were excluded, and so were participants who were

   i. pregnant or breastfeeding

   ii. abnormal creatinine clearance or elevated alanine transferase (ALT),

   iii. known macrolide hypersensitivity

   iv. Concomitant use of digoxin and/or fluconazole (or other drugs known to prolong the QTc interval).

   v. History of cardiac arrhythmia or a prolonged QTc interval (42).

Firstly, enrolment in the main trial and other trial procedures for screening and recruitment was conducted. Chronic lung disease was established by spirometry (forced expiratory volume in 1 second [FEV_1] z-score less than −1.0) with no reversibility (< 12% improvement in FEV_1 after salbutamol 200 μg inhaled using a spacer) (26). Spirometry was performed using the EasyOne™ spirometer. The participant made three attempts of forced expiration, and the best tracing was checked for acceptability (ndd Medical Technologies Inc., Andover, MA, USA) by trained research staff certified in performing spirometry and following the American Thoracic Society guidelines (43). A questionnaire asking for participant demographic information, their vital signs and cardio-respiratory symptoms, as well as their Medical Research Council (MRC) breathlessness score, were collected. A focussed cardiac

and respiratory clinical examination was conducted. Vital signs and demographic information were documented in the study form. See Appendix 1. A 12-lead electrocardiogram (ECG) was performed during screening and interpreted by a clinician for arrhythmias and QT prolongation, among other abnormalities using hospital standard ECG interpretation guidelines. A period of 6 months of training in echocardiography was conducted to equip the candidate in performing echocardiograms in Blantyre, Lilongwe, and Zimbabwe to ensure competence in scanning the appropriate views.

Secondly, a transthoracic echocardiogram was performed using a SonoSite™ M-Turbo echocardiography system (FUJIFILM SonoSite, Bothell, WA, USA). Only scans done in Malawi were included in the database for the study. Scans done at the other sites of the BREATHE trial were not included in the study. The echocardiogram was focused on assessing left and right-sided heart function and PH in the participants at baseline. Appendix 2 shows the data collection tools used for echocardiogram details. According to published guidelines, two-dimensional (2D), M-mode, and Doppler echocardiography were performed on all participants following a standard protocol. Participants were scanned in an either left lateral or supine position to obtain an optimum image quality using a transducer with frequencies from 3.5 MHz to 7.0 MHz and simultaneous 3-lead ECG monitoring. Acquired images were saved in Digital Communication in Medicine (DICOM) format for later offline analysis. A second experienced sonographer evaluated these images for adequacy of views, and errors were corrected. Where a need is, a second study was conducted to ensure the high quality of images and readings (44).

The following cardiac measures were obtained over three cardiac cycles: RV basal diameter in the apical 4-chamber (A4C) view at the basal level at end-diastole; tricuspid annular plane

systolic excursion (TAPSE) was measured using M-mode from the lateral tricuspid annulus; right atrial (RA) area in the A4C view; Tricuspid peak gradient was derived from the peak velocity of a tricuspid regurgitant jet using the Bernoulli Equation ($4V^2$), and pulmonary arterial systolic pressure (PASP) was calculated indirectly from the pressure gradient measured across the tricuspid valve (regurgitant jet) and adding right atrial pressure estimate to the tricuspid pressure gradient (45). Finally, left ventricular (LV) dimensions were measured using M-mode, and systolic function was assessed using Simpson's biplane method. To obtain an average, measurements were performed on technically adequate images only and over three cardiac cycles. Pulmonary hypertension was defined as present if the tricuspid regurgitation velocity was ≥2.9m/s with an estimated pulmonary arterial systolic pressure (PASP) ≥37mmHg (assuming right atrial pressure of 5mmHg) (45).

3.3 Ethical Considerations

Ethical approval was obtained at the same time as the broader trial from the Malawi College of Medicine Research Ethics Committee, Medical Research Council of Zimbabwe, Biomedical Research and Training Institute Institutional Review Board, Zimbabwe, London School of Hygiene and Tropical Medicine ethics committee, the University of Cape Town Research Ethics Committee and the Regional Ethics Committee for Medical and Health Research, Norway. Consent was obtained after full consenting. Participants were explicitly informed that they were not forced to be a part of the study and that this would not hinder their overall care in the ART clinic. Ascent was obtained from the primary caregiver for children, particularly those below 12 years old.

3.4 Statistical Analysis

Data collected was first cleaned and anonymised. Cardiac symptom frequency and other demographics were tabulated and presented in tabular form. Cardiac echocardiograms following validation for adequate quality had data variables entered into a database. Continuous data were presented as mean ± standard deviation (SD) if they were normally distributed or median (interquartile range, IQR) if not normally distributed. The number, total, and percentage in each category were reported for categorical variables. For continuous outcomes, mean values and standard deviations are reported. Analyses were performed using Stata. v14·0 software (Stata Corporation, College Station, TX, USA).

CHAPTER FOUR: RESULTS

4.1 Participant Characteristics

A total of 49 participants aged between 6 to 19 years and taking ART for at least six months were consecutively recruited into the study. This was 44.5% of the overall trial recruitments. Data to review the excluded participants if they differed from those recruited is unavailable when writing the manuscript. The primary trial had just ended, and unblinding of clinical data had not yet occurred (Figure 1).

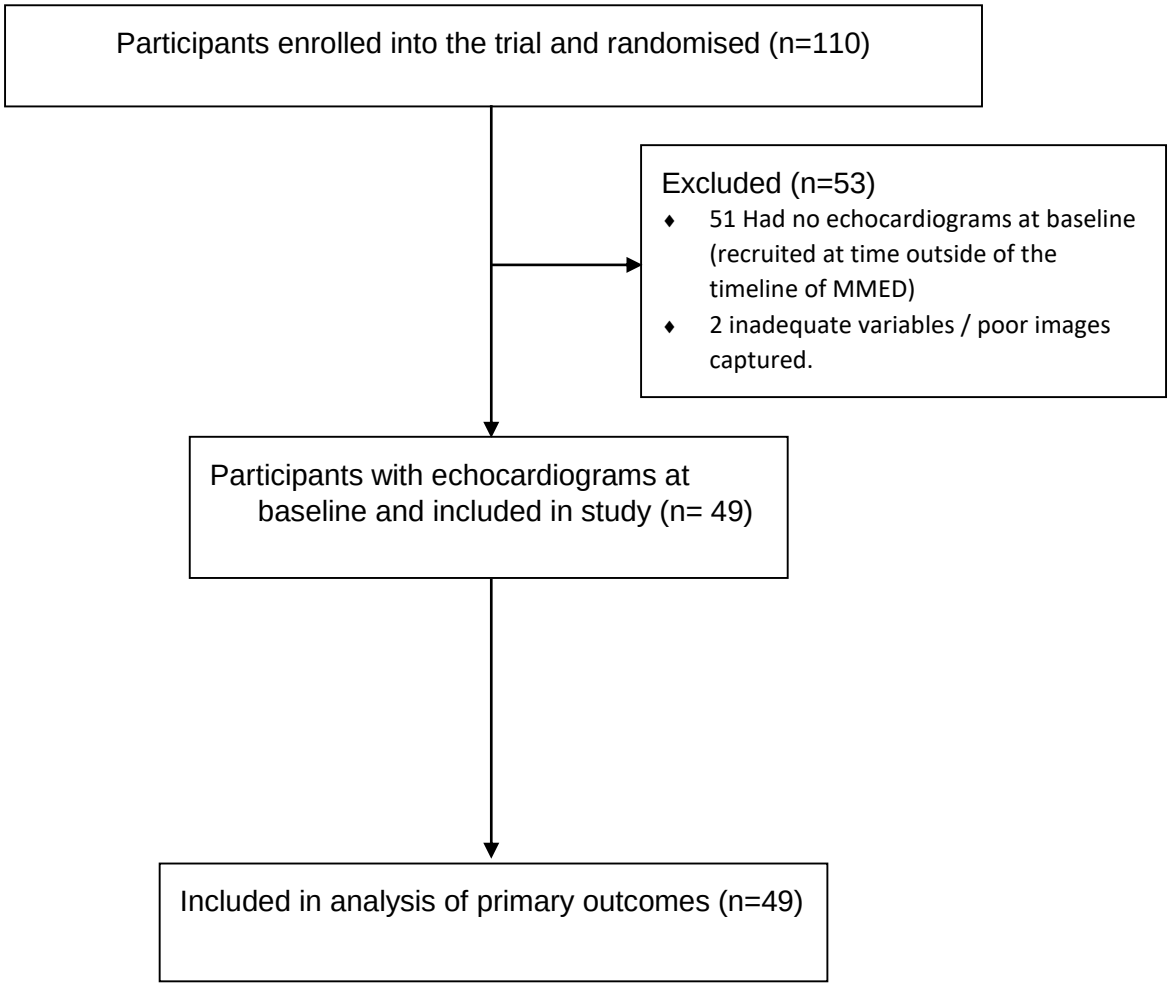

Figure 1: Participants' Characteristics

Participants had a median age of 14.5 (8.4- 19) years. There were 24 (51%) females. The median age at initiation of ART was seven years. The median CD4 was 640 (87 – 2969) cells/uL, with a median viral load (VL) was 1110 copies/mL. 45 % of participants were on cotrimoxazole prophylaxis. 22.9% of participants had been previously treated for pulmonary tuberculosis (Table 1).

Table 1: Participant Demographics (N=49)

Participant Variable	**n=49**
Mean Age, y	14.5 ± 3.2 (8.4 – 19.8)
Female sex	24 (51.1%)
Height – for age z score, median (IQR)	143cm ± 12 cm (111 - 167)
Weight – for age z score, median (IQR)	36.9 kg ±10.5 kg (17.5 – 60.6)
% Taking ART	100%
Age at HIV Diagnosis, y	7.00 ± 4.02
Age at Initiation of ART, y	7.96 ± 3.44
CD4 count, median (IQR), cells/Ul	640 ± 439 (87 – 2969)
Median viral load (VL)	1110
% on cotrimoxazole prophylaxis	45 (91.84)
Previous TB treatment (%)	11 (22.9)

The median MRC score was 2.3 ± 1.06. Up to 24 (49%) participants with CLD had an MRC score of 2.9 (18%) had tachycardia of >100bpm at rest. Hypoxia was identified in 2 (4.1%) participants with saturations at rest <88% and tachypnea of more than 30cpm (Table 2).

Table 2: Clinical Symptoms and MRC Scoring

MRC Dyspnea scale score	
-	n= Number of participants (% of cohort)
- 0	1 (2.04)
- I	8 (16.3)
- II	24 (49.0)
- III	9 (18.4)
- IV	5 (10.2)
- V	2 (4.08)
- Median score	2.3 ± 1.06 (0 – 5)
Tachycardia at rest (HR >100bpm)	9 (8.37)
SaO2 at rest <88%	2 (4.08)
RR > 30/min	2 (4.08)

4.2 Echocardiography Findings

No right heart abnormalities (RV systolic dysfunction or apparent RV dilatation or pulmonary hypertension) were observed in any participants.

The median volumetric EF was 59.6 ± 7.5 %. There were also no participants with septal flattening. 3(6.5%) of participants had features of rheumatic heart disease, with 1(2.1%) having a pericardial effusion. No participants had features of cardiac failure (Table 3).

Table 3: ECHO Findings

Measurement	Result, median (Range)
IVC diameter	10.36 ± 2.84 (4.6 – 18.9)
IVC collapsing on inspiration (>50%)	47 (95.9)
RVEDD, mm	28.6 ± 5.41 (13.9 – 41.5)
LVEDD, mm	40.4 ± 4.86 (29.7 – 54.3)
LVESD, mm	27.4 ± 4.22 (19.9 – 44.2)
IVS, mm	9.63 ± 3.05 (3.5 – 24.6)
LVPW, mm	9.54 ± 2.56 (4.4 – 16.2)
RA area	10.6 ± 3.52 (5.52 – 27.9)
FS, %	32.1 ± 4.89 (18.6 – 42.1) %
EF, % volumetric	59.6 ± 7.5 (31 – 73) %
ePASP, mmHg	10.42 ± 5.4 mmHg
PASP, mmHg > 37	0 (0%)
Tricuspid peak velocity	5.52 ± 5.39 (1.26 – 26.0)
Pericardial effusion	1 (2.1%)
Septal flattening	0 (0%)
Features of RHD	3 (6.5%)
TAPSE (average of 3)	20.9 ± 3.75 (10.6 – 33.0)
TV area*	3.12 ± 1.80 (0.15 – 8.50)

CHAPTER 5: DISCUSSION

5.1 Introduction

Despite effective ART, there is a high burden of HCLD in children with perinatally acquired HIV in sub-Saharan Africa (15-17,46). This is one of the few studies investigating cardiac symptoms and dysfunction among children with HCLD in Malawi and sub-Saharan Africa. In our study, twenty-three percent of children had some degree of shortness of breath on exertion, with 18% tachycardia at rest. This suggests a growing inability of the body to cope with daily activity demands and potentially impacts the quality of life in these growing children (12,21,30). Although the participants had HCLD, they were relatively well and were on ART for several years. This is similar to what other studies have shown previously (12,21,30). Further longitudinal studies would be needed to follow up this cohort for longer periods to identify the incidence of cardiac dysfunction prospectively.

5.2 Cardiac Disease

Interestingly, no participants had pulmonary hypertension (PH). This is similar to the low incidence of PH in HIV-infected child (0.5%) cohorts reported previously in the pre-ART era (47). Despite the knowledge that chronic lung disease is associated with PH and right heart dysfunction in adult studies (1,33). Doppler echocardiography used in this study is recommended as a screening tool for pulmonary hypertension but can frequently under- or overestimate pulmonary arterial pressures in patients (48). In the Zimbabwe cohort of this trial interesting, there was also a low PH prevalence rate of 0.6%. This was in a cohort that also used rigorous screening tools for CLD (27). This finding may highlight the complexity of abnormal lung and cardiac function interactions (1).

Traditionally, alterations in right-sided chambers were secondary to an increase in RV afterload. However, HIV *per se* may induce structural and functional changes in the LV in the absence of changes in loading conditions, suggesting that RV structure and function might be affected similarly. The CHAART-2 Study conducted among children established on ART found that cardiac function started declining after a decade of follow-up (6). It is also plausible that ART and viral suppression play a significant role in preventing clinical cardiomyopathy. However, long-term follow-up in children with HIV is still lacking to understand the lifetime risk of developing cardiovascular disease. RV dilatation was rare in this study, contrary to what has been previously reported in Zimbabwean children with HIV, which reported RV remodelling in the absence of elevated pulmonary pressures in patients with HIV in 29% of adolescents were identified (4). A more recent study among children established on ART found a much lower proportion of RV dilatation (7%). This was also not associated with elevated right heart pressure but rather was associated with left heart abnormalities (32). The differences in reported proportions in the two studies may be due to the different reference ranges used to define RV dilatation. The former used European, and the latter used local reference ranges (49-50).

Three (6%) participants had rheumatic heart disease. All had been previously diagnosed and were on intermittent prophylactic treatment (IPT). This was much higher than other cohorts, which reported a prevalence of 0.82% (51). IPT would have included monthly Intramuscular penicillin V (52-53). Further studies would be required to determine the prevalence of rheumatic heart disease in this pediatric population.

There were no identified cases of dilated cardiomyopathy or other left ventricular (LV) dysfunction besides the RHD cases mentioned above. One (2%) participant had a pericardial

effusion with no other endocarditis or pulmonary tuberculosis features. No congenital heart disease defects were identified. There were no cases of myocarditis. In contrast, a study in Nigeria has reported left ventricular dysfunction in 27% of children with HIV (54). This may also be due to LV dysfunction, which is not seen using traditional echocardiography as described by Sims et al. Their team has reported that HIV-infected participants demonstrated impaired strain and strain rate despite having a normal systolic function and ejection fractions (55).

5.3 Limitations

Right heart catheterisation was not conducted on the participants. In other studies, this modality has been used as the gold standard to measure pulmonary hypertension. Other potentially insightful echocardiography indices in RV assessment, including strain analysis, could not be performed in this study due to machine limitations. Quantification of the RV using 2D-echocardiography also posed some challenges due to the RV's complex geometry and retrosternal position and angle dependence of measurements such as TAPSE. However, a well-trained echocardiographer performed the scans. This has been reported in other studies (56).

Secondly, due to technical challenges such as the scanner malfunctioning and time constraints in my b timelines, it was not possible to consecutively scan all participants in the trial. Similar findings have been found in a study conducted in Zimbabwe at the other recruitment site for this trial, where 50% of participants were recruited in their trial site. In that recently published study, Majonga et al. also found a low prevalence of pulmonary hypertension of 0.6% (27).

Furthermore, due to the slow recruitment pace, it was not possible to recruit controls to form the basis and reference values for right heart measurements and normative data in Malawi. European reference values for the right cardiac disease have been used in other studies (57). References values have been described in Zimbabwe recently (49). Due to potential differences in malnutrition, body mass index (BMI), and other variables, it was thought cautious not to analyse some of the right heart disease variables data that required using these reference ranges. Using reference values mentioned above could potentially produce misleading results because of significant differences in population variables.

CHAPTER SIX: CONCLUSION

In conclusion, our findings demonstrate low levels of echocardiogram confirmed cardiac dysfunction and pulmonary hypertension in this cohort of HCLD in children. However, there is significant co-morbidity with acquired heart disease in this group set of children. Longer-term follow-up of these children is essential to identify if further cardiac dysfunction does not emerge in children on ART for a longer duration.

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APPENDICES

Appendix 1: Clinical Research Form Used to Collect Clinical Symptoms

F01	*STUDN*	Study number	□□□□	
F02	*DATE*	Date of interview *dd/mm/yyyy*	□□/□□/20□□	
HIV HISTORY				
F03	*HIVNO*	HIV clinic number	□□□□□□□□□□□□	
F05	*STATUS*	Is the participant aware of his/her HIV status?		Yes□ No□
F06	*DATEHIV*	Date of HIV diagnosis ***(if day unknown assign 15, if month unknown assign 06)***	□□/□□/20□□ Unknown □	
F07	*AGEHIV*	Age at HIV diagnosis	□□ years	
F08	*REASONHIV*	Was HIV diagnosis done because: ***(check ONE box only)***	Child was sick□ Routine screening□ Not known□	
F09	*DATEART*	Date of ART initiation ***(if day unknown assign 15, if month unknown assign 06)***	□□/□□/□□□□ Unknown □	
F10	*AGEART*	Age at ART initiation	□□	
F11	*COTRI*	Is the participant taking cotrimoxazole?	Yes□ No□	
F12	*DRUGS*	Please tick current ART drugs the child is taking: ***(tick all that apply)***		
		AZT (zidovudine)		Yes□ No□
		D4T (stavudine)		Yes□ No□
		TNF (tenofovir)		Yes□ No□
		Abacavir		Yes□ No□
		3TC/FTC		Yes□ No□
		DDI (didanosine)		Yes□ No□
		Nevirapine		Yes□ No□
		Efavirenz		Yes□ No□
		ATZ (atazanavir)/R (ritonavir)		Yes□ No□
		LPV (kaletra, alluvia)/R(ritonavir)		Yes□ No□

		Other ART Drug	Yes☐ No☐
		Specify Other ART Drug ……..	
CLINICAL HISTORY			
F13	*ADM*	Has the participant been admitted to hospital for chest problems in the last 12 months?	Yes☐ No☐
F14	*NADM*	If yes, how many times has the participant been admitted to hospital for chest problems in the last 12 months	☐☐
F15	*TBTREAT*	Has the participant ever been treated for TB?	Yes☐ No☐
F16	*NOTBTREAT*	If yes, how many times has the participant been treated for TB?	☐☐ NA ☐
F17	*BREATH*	Is the participant currently breathless?	Yes☐ No☐
F18	*MRC5*	Does the participant get breathless when dressing or too breathless to leave the house?	Yes☐ No☐
F19	*MRC4*	Does the participant have to stop for breath after walking 100 m?	Yes☐ No☐
F20	*MRC3*	Does the participant walk slower than most people or has to stop after 15 minutes walking?	Yes☐ No☐
F21	*MRC2*	Is the participant short of breath when hurrying on the level or walking uphill?	Yes☐ No☐
F22	*MRC1*	Does the participant get breathless on moderate exercise?	Yes☐ No☐
F23	*MRCSCORE*	What is the MRC Dyspnoea Scale score? ***(please check consistency with answers on questions F18 to F22)***	☐
F24	*COUGH*	Does the participant have a cough now?	Yes☐ No☐
F25	*COUGHT*	Has the cough changed over time? ***(tick what applies)***	Same ☐ Improving ☐ Getting worse ☐ Do not know ☐ Not coughing ☐
F26	*SPUTUM*	Does the participant cough up sputum?	Yes☐ No☐

F27	*SPUTUM Q*	How much sputum does the participant cough up each day? ***(tick what applies)***	Less than a table spoon □ A few table spoons □ A cup □ Do not know □ Not coughing □
F28	*LYWH*	Does the participant get wheezing or whistling in the chest?	Yes□ No□
F29	*INH*	Does the participant use an inhaler?	Yes□ No□
F30	*FINH*	How often does the participant use an inhaler? ***(tick what applies)***	Once a week □ Daily □ More than once a week □ Don´t know □ Not applicable □
F31	*SALBT*	Does the participant use salbutamol tablets?	Yes□ No□
F32	*ASTHMA*	Has the participant ever been told by a doctor or nurse that he or she has asthma?	Yes□ No□
EXAMINATION			
F33	*WEIGHT*	Weight	□□.□ Kg
F34	*HEIGHT*	Height	□□□.□ cm
F35	*RR*	Respiratory rate	□□ breaths per minute
F36	*HR*	Heart rate	□□□ per minute
F37	*SAT*	Oxygen saturation	□□□ %
SPIROMETRY			
F38	*SPIRD*	Was spirometry done?	Yes□ No□
F39	*RSPIR*	If not done, specify reason	Participant unable to follow instructions □ Participant acutely unwell □ Logistic problems □ Not applicable □
ELIGIBILITY FOR SHUTTLE WALK TEST			
F40	*SHUTY*	Is the participant capable of doing the shuttle walk test	Yes□ No□

F41	*SHUTN*	If not capable of doing a shuttle walk test, give reason why not (check all reasons that apply):	SpO_2 <88% □ RR>24/min□ Resting heart rate >120/min□ Feels too ill to exercise □ Unable to stand/ walk□ Other□ If other, specify _____ _____
FOR THOSE ELIGIBLE FOR SHUTTLE WALK TEST			
F42	*SHUTM*	Time participant walked	□□minutes □□seconds
F43	*SHUTO2*	O2 saturation **immediately after SWT**	□□□%
F44	*SHUTHR*	Heart rate **immediately after SWT**	□□□/min
F45	*SHUTRR*	Respiratory rate **immediately after SWT**	□□/min
F46	*SHUTDNC*	When the participant had to stop, what was the reason?	Chest pain□ Breathlessness□ Leg tiredness□ Staggering □ Excessive sweating (diaphoresis) □ Other□ If other, Specify _____
FORMS AND TESTS			
F47	*TESTS*	Which tests were collected? ***(tick all that applies)***	Sputum storage Yes□ No□ (BO.26) Stool storage Yes□ No□ (BO.27) NPA Yes□ No□ (BO.28) Blood sample immunology Yes□ No□ (BO.25) Cardiac Echo Yes□ No□ (BO.15)
F48	*LOGD*	Drug recording diary given to designated caregiver?	Yes□ No□
F49	*FORM*	Form BO.04 (SCHOOLING FORM) Completed	Yes□ No□

NEXT VISIT			
F50	*ECHOD*	Cardiac echo appointment date ***(if not possible to be done on the baseline visit date)***	□□/□□/20□□ NA □
F51	*NVISIT*	Follow up appointment date	□□/□□/20□□

Appendix 2: Echocardiogram Form

C01	*STUDYN*	Study number	□□□□
C02	*ECHO*	Echo date	□□/□□□/□□□□
C03	*HEIGHT*	Height	□□□.□ cm
C04	*WEIGHT*	Weight	□□.□ Kg
C05	*BODYS*	Body surface	□□.□□ m^2
APICAL VIEW			
C06	*TAPSEd*	Tricuspid annular plane diastole	□□□.□ mm
C07	*TAPSEs*	Tricuspid annular plane systole	□□□.□ mm
C08	*TVDa*	Tricuspid annular diameter apical	□□□.□ mm
C10	*TPV*	TRUCUSPID PEAK VELOCITY	□.□□ m/s
C11	*TPG*	TRICUSPID PEAK GRADIENT	□□.□□ mmHg
C12	*PASP*	Estimated Pulmonary Arterial Systolic Pressure	□□.□□ mmHg
C13	*A4Cd*	Left ventricular area in diastole, apical 4 chamber view	□□.□□ cm^2
C14	*A4Cs*	Left ventricular area in systole, apical 4 chamber view	□□.□□ cm^2
C15	*A2Cd*	Left ventricular area in diastole, apical 2 chamber view	□□.□□ cm^2
C16	*A2Cd*	Left ventricular area in diastole, apical 2 chamber view	□□.□□ cm^2
C17	*EFv*	EJECTION FRACTION (volumetric method)	□□.□ %
Parasternal long axis			
C18	*LVDd*	Left ventricular diameter in diastole (M Mode)	□□□.□ mm
C19	*LVDs*	Left ventricular diameter in systole (M Mode)	□□□.□ mm
C20	*FS*	Fractional shortening	□□.□ %
C21	*EFlin*	Ejection Fraction (linear method)	□□.□ %
C22	*TVDpsla*	Tricuspid valve diameter, parasternal long axis view	□□□.□ mm