The incidence and description of Gynaecomastia in HIV positive adolescents on antiretroviral therapy

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DECLARATION

I, Khaukanani Neo Mungoni, declare that this dissertation report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics, at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signed: _____

On this: _____ day of: _____, 2019

DEDICATION

To my husband Rabelani and to my children Dakalo and Thendo, thank you for the amazing support throughout this journey.

PUBLICATION AND PRESENTATIONS ARISING FROM THE THESIS

None

ABSTRACT

Background: This study aimed to determine the incidence and clinical characteristics associated with gynaecomastia inHIV-infected adolescents attending Harriet Shezi Children's clinic (HSCC) in Johannesburg, South Africa.

Methods: This retrospective reviewwas conducted on 622 HIV-infected male adolescents between January 2004 and December 2014. Demographic andclinical characteristicswere assessed atantiretroviral therapy(ART) initiation,and start of person-time at risk (T_0) for developing gynaecomastia (either at age 10 years or first visit after 10 years). Gynaecomastia was diagnosed clinically by the attending clinician at clinic visits.Descriptive analysis and measures of association were conducted.

Results: While on ART44(7.1%)adolescents developed gynaecomastia at a median age of 14.6 years (IQR: 13.9-15.7).Overall incidence was 2.4/100person-years (95% CI: 1.8-3.3).Adolescents who initiated ART after 10 years of age were more likely to develop gynaecomastia than those initiated before 10 years (OR: 1.20, IQR 1.18-1.40).Adolescents who were wasted at ART initiation (18%) and adolescents on ART prior to T_0weresignificantly less likely to develop gynaecomastiacompared to those who were not (OR=0.22, IQR: 0.17-0.28 and OR=0.43, IQR: 0.23-0.79, respectively).In longitudinal regression analysis, adolescents on an EFV-basedregimen at T_0 were more likely to develop gynaecomastia than those who were not (OR=3.42, IQR: 1.12-10.42).

Conclusion: This study adds to the previously reported association between efavirenz and gynaecomastia in HIV-infected males. However, better designed studies are needed to clarify the relationship between gynaecomastia, HIV-infection and ART drugs, especially in adolescence where this relationship is confounded by physiological gynaecomastia.

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PROTOCOL

1. INTRODUCTION

The introduction of combination antiretroviral therapy (cART) in HIV infected children has led to a dramatic reduction in HIV-associated morbidity and mortality .[1] As a result of early cART initiation, HIV-infected children are ageing into adolescence and adulthood in South Africa and globally .[2] The World Health Organisation (WHO) defines an adolescent as an individual between 10 and 19 years. This coincides with the onset of puberty starting from 9 to 14 years. Puberty is associated with many physical, psychological and emotional changes.

Over the years combination ART treatment and prevention guidelines have changed due to new and ongoing research findings. cART has been associated with a number of adverse effects often specific to a particular antiretroviral drug, for example: gastrointestinal (anorexia, nausea, vomiting, diarrhea, abdominal pain, hepatitis), lactic acidosis, hypersensitivity reactions, haematological side effects, CNS related adverse effects, gynaecomastia etc. [3]

Table 1: National Department of Health ART treatment guidelines for children (2015).

Age group	Regimen
Children < 3yrs or older but <10kg.	ABC + 3TC + LPV/r 2nd line (expert consultation)
3-10yrs and < 10kg	ABC + 3TC + EFV
10-15years or < 40kg	ABC + 3TC + EFV Children started on ABC + 3TC + LPV/r before 3yrs, must remain on the same regimen Change all D4T to ABC if viral load (VL) suppressed. Change all DDI to ABC regardless of VL 2nd line: AZT+ 3TC + LPV/r OR AZT + ABC + LPV/r
>15yrs or >40kg, fulfilling eligibility criteria.	TDF + FTC +EFV (Fixed dose combination), Change D4T to Tenofovir (TDF) if virally suppressed

ABC =Abacavir; 3TC =lamivudine; LPV/r =lopinavir/ritonavir; EFV =efavirenz;
DDI =didanosine; AZT =zidovudine;TDF =tenofovir

Gynaecomastia is associated with puberty in many adolescent males. Gynaecomastia is the enlargement of the male breast secondary to stromal proliferation and ductal hyperplasia and is usually benign.[5,6] Most cases of gynaecomastia result from an imbalance between estrogenic (stimulatory) and androgenic (inhibitory) effects on the breast.[5] Gynaecomastia develops in about two thirds of boys during puberty. Some adolescents on long term cART have been reported to develop gynaecomastia which is difficult to manage in this vulnerable population.[2,5] Gynaecomastia occurs when the sex hormone balance favours estrogen, despite an increase in androgen production .[5] Drug induced gynaecomastia may account for 20 to 25% of all cases in menand has been reported as an emerging condition in HIV-infected patients on cART. [3,5] Meerkotteer presents two cases that demonstrate the association of cART and gyneacomastia.[6] Gynaecomastia associated with ART has been reported for several drugs such as efavirenz (EFV), didanosine (DDI), stavudine (D4T) and various protease inhibitors (PI). [7,8,9] A cross-sectional survey found that 2.9% of HIV infected patients between the ages of 12 and 58 years treated with ART developed gynaecomastia.[10] A study done in Uganda among patients aged 6 weeks to 18 years found that gynaecomastia contributed less than 1% of the total ART adverse events and there was no association between adverse events and patient baseline characteristics. [3]

Gynaecomastia in HIV-infected patients may be associated with HIV-associated lipodystrophy syndrome and dysmetabolism also known as metabolic syndrome.[10,11] A switch from PI containing ART to non-nucleoside reverse transcriptase inhibitors (NNRTI's) and abacavir (ABC) was effective for dysmetabolism but infrequently effective for lipodystrophy.[11]

A study done by Jose et al reported three cases of histopathologically confirmed gynaecomastia without lipodystrophy syndrome in HIV-infected male patients treated with EFV: the findings from this study suggested that EFV can produce gynaecomastia without the lipodystrophy syndrome by an unknown mechanism. [12] Another observational study of five patients diagnosed with gynaecomastia done by Jover et al suggested that cART induced gynaecomastia should be suspected in HIV patients receiving EFV- containing regimen, and in all cases gynaecomastia regressed after EFV withdrawal. Two possible mechanisms were implicated namely an immune restoration process and mediated via estradiol-like effects.[13]

Different hypothesis have been postulated regarding development of gynaecomastia and the pathophysiology of gynaecomastia may be multifactorial.[9]

Additional possible causes of gynaecomastia in HIV infected patients include hypogonadism secondary to Acquired immune deficiency syndrome (AIDS) or testicular tumor, other drugs used for treating comorbidities such as anti-tuberculous therapy, ketoconazole or metronidazole, cimetidine, enalapril, amiodarone, amphetamine and consumption of substances such as alcohol, heroin and marijuana.[1,9,14] A case control study done by Alejandra et al found that 1.8% of HIV-infected men had gynaecomastia, with this study suggesting that gynaecomastia among HIV-infected patients is related to hypogonadism, rather than to an adverse effect of antiretroviral drugs.[14] Overall the prevalence of gynaecomastia in HIV infected men on ART ranges from 2 to 3%.[9] One case of a prepubertal 7 year old girl who developed true gynaecomastia on cART has been reported.[15] Many of the case reports included small numbers of patients, and therefore it is difficult to make the assumption that cART causes gynaecomastia. However gynaecomastia causes much anxiety, psychosocial discomfort, and fear of breast cancer in patients and needs to be addressed sensitively.[5] Pseudogyneacomastia needs to be differentiated from true gynaecomastia.Pseudogyneacomastia is the accumulation of fat under and around the male breast nipple without ductal proliferation, common in obese males.

There is paucity of literature describing the incidence and outcomes of gynaecomastia in adolescents, with most cases described in adults. Therefore the aim of this study is to examine the incidence of gynaecomastia in HIV-infected adolescents on cART, to describe the clinical characteristics associated with gynaecomastia and to determine whether cART was associated with gynaecomastia in this cohort.

2. AIM AND OBJECTIVES

2.1 AIM

The aim of this study is to determine the incidence of gynaecomastia in HIV-infected adolescents attending Harriet Shezi Children's clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) and in those with gynaecomastia to describe baseline characteristics at gynaecomastia start and outcome.

2.2 OBJECTIVES

a) To estimate the incidence of gynaecomastia in adolescents at Harriet Shezi Children's clinic, CHBAH.

b) To compare the demographic and clinical characteristics and outcomes of male adolescents with gynaecomastia.

c) To estimate the association between gynaecomastia and demographic and clinical characteristics at cART initiation and in HIV-infected adolescent males on cART.

3. METHODS

3.1 STUDY DESIGN

This study is a retrospective longitudinal cohort study of HIV-infected male adolescents managed at Harriet Shezi Children's Clinic, CHBAH from 2004 to 2014.

3.2 SITE OF STUDY

This study will be conducted in the Harriet Shezi Children's Clinic in CHBAH in Soweto, Gauteng Province.

3.3 STUDY POPULATION

The study population will include all HIV-infected male adolescents between the ages of 10 to 19 years who attended at least two clinic visits between 1st April 2004 (when the South African Department of Health HIV rollout began) and 31 December 2014. According to the December 2014 Harriet Shezi Children's Clinic report, a total of 6964 patients were included in the database and an estimated ninety patients developed gynaecomastia.

3.3.1 INCLUSION CRITERIA

- All male adolescents in care at the age of 10 years will be included in the study.
- If patients transferred in between the ages of 10-19 years they have to be HIV naive

3.3.2 EXCLUSION CRITERIA

- Patients with chronic liver disease, endocrine disorder and kidney disorders that may result in gynaecomastia will be excluded.
- Patients receiving drugs apart from cART that have been associated with causing gynaecomastia
- Adolescent male patients transferred in already on cART

- Adolescent male transferred in already with gynaecomastia.

3.4 VARIABLES

The following variables will be collected at cART initiation and at all visits after the adolescent has turned 10.

- Age, cART regimen, co-morbid illness, CD4, VL, weight, Height, BMI, other adverse events that occurred at the same time as gynaecomastia.

3.5 PROCEDURES

Data will be collected from Harriet Shezi Children's Clinic Therapy edge from 2004-2014. All adolescents male patients will be evaluated, an estimated number of ninety adolescents developed gynaecomastia at the Harriet Shezi Children's Clinic. Missing information will be obtained from the paper- based patient records.

4. DATA ANALYSIS

Demographic and clinical characteristics at cART initiation and at the age of 10 will be compared between adolescents with gynaecomastia and those without. Continuous variables will be described using medians and interquartile ranges and compared between groups using the Wilcoxon rank sum test. Categorised variables will be described using percentages and compared between groups using the x^2 test. Incidence rates will be calculated by dividing the number of cases of gynaecomastia by the person-time at risk. Person-time at risk will start at the first visit after the age of 10 years until the end of follow-up or incidence of gynaecomastia with censoring due to death, transfer out or loss-to-follow-up.

The following advanced analyses will be undertaken with the assistance and guidance of a biostatistician. Kaplan-Meier survival curves will be used to determine the time to gynaecomastia in adolescence on cART, comparing different cART regimen and ages at cART initiation. The log rank test will be used to compare the survival curves. Cox proportional hazard models will be used to estimate the association between gynaecomastia and demographic, clinical and growth risk factors (such as age, CD4 count, VL, ART regimen, time on cART, Height-for-age Z-score, BMI, etc.).

Unadjusted and adjusted hazard ratios with 95% confidence intervals will be presented.

5. ETHICS

The protocol will be submitted for approval to the Human Research Ethics Committee (medical) of the University of the Witwatersrand. Permission to conduct the study will be requested from the department of paediatrics and the Chief Executive officer at CHBAH.

As this is a retrospective study, waiver for informed consent will be requested. No identifiable demographics such as name will be used as a means to maintain confidentiality. The study database will only be accessible to the student and supervisors. Each study participant will be assigned a patient identification number. The database will be password protected and only the student will have access to the file with linked name and specific identification number.

6. TIME LINES

	April 2015	May 2015	June 2015	July 2015	Aug 2015	Sept 2015	June 2017	Nov 2017	DEC 2017
Literature review									
Protocol preparation									
Protocol submission and assessment									
Ethical clearance									
Postgraduate committee clearance									
Data collection									
FC(PAED) SA Part 2 Exam preparation									
Data analysis and report									

write up									

7. FUNDING

There will be no funding required for this study, the costs that are incurred such as stationary, binding and printing will be borne by the investigator.

8. POTENTIAL PROBLEMS

Due to the retrospective nature of this study, records and data may be incomplete or missing, which may weaken the result. To limit this, initial data will be obtained from an electronic base and missing data will be collected from patient's files.

It is possible that some patients with gynaecomastia may be missed because of inadequate history or examination and inaccurate recording in the data base. Concomitant medication may not be recorded.

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BODY OF THE DISSERTATION

INTRODUCTION

Adolescence, defined as ages 10 -19 years,[1] coincides with the onset of puberty (9 – 14years[1]) and is associated with many physical, psychological and emotional changes. One of these changes is the development of gynaecomastia reported in about two thirds of boys during puberty, which may appear as early as 10 years of age, with peak onset between ages 13-14 years, followed by a decline in late teenage years.[2]Gynaecomastia is the enlargement of the male breast secondary to stromal proliferation and ductal hyperplasia and is usually benign.[2,3]In contrast, pseudogynaecomastia is the accumulation of fat under and around the male breast nipple without ductal proliferation and is common in obese males.

The introduction of antiretroviral therapy (ART) in HIV-infected children has led to a dramatic reduction in HIV-associated morbidity and mortality.[4] As a result of early ART initiation, HIV-infected children are ageing into adolescence and adulthood in South Africa and globally.[5]About 2.1 million adolescents worldwide were living with HIV in 2016, of whom 1.7 million (84%) residedin Sub-Saharan Africa.[6]

ART has been associated with a number of adverse effects often specific to a particular antiretroviral drug. Some adolescents and adults on long term ART have been reported to develop gynaecomastia whichis difficult to manage in an adolescent population already at risk of gynaecomastia, where determining causality is complicated by physiological norms.[2,5,7]Different classes of drugssuch as calcium channel blockers, cytotoxic agents and some antibiotics such as ketoconazole,metronidazole, and isoniazid induce gynaecomastia,accounting for 20 to 25% of all cases in men.[2,8]ART drugs most commonly associated with gynaecomastia are efavirenz (EFV) didanosine (DDI), stavudine (D4T) and various protease inhibitors (PI).[3,8-13] Gynaecomastia in HIV-infected males may be associated with HIV-associated lipodystrophy syndrome and dysmetabolism also known as metabolic syndrome.[8,14]Studies done in HIV-infected males aged 12-63 years, reportedgynaecomastia prevalence of 2.9-5%,, with a significant number of these patients receiving D4T and having lipodystrophy.[8,11,14]Published literature largely consists of case reports.A case series by Jose et al., of 3 adult males in Spain with histopathologically confirmed gynaecomastia without lipodystrophy syndrome in HIV-infected patients treated with EFV: in all 3 cases EFV was introduced as a simplification treatment instead of a protease inhibitor. Similarly in

2000Jover et al., reported 5 gynaecomastia cases in French males, ages 43-55years with EFV-associated gynaecomastia without lipodystrophy [12,13]syndrome: all of the cases had immunological and virological responses to ART and in all 5 cases gynaecomastia regressed after EFV withdrawal. These reports suggest that EFV may cause gynaecomastia by unknown mechanisms.[12,13]

There is a paucity of literature describing the incidence and outcomes of gynaecomastia in adolescents, with most cases described in adults. Therefore, the aim of this study is to estimatethe incidence of gynaecomastia in HIV-infected adolescents on ART, to describe the clinical characteristics associated with gynaecomastia and to determine whether specific antiretroviral drugs were associated with gynaecomastia in this cohort.

METHODS

This retrospective cohort study of HIV-infected male adolescents on cARTwas conducted at the Harriet SheziChildren's Clinic(HSCC), a public sector paediatric and adolescent HIV clinicatChris Hani Baragwanath Academic Hospital(CHBAH), Johannesburg. HSCC evolved over time from being one of few paediatric ART initiation sites in South Africafrom 2004-2010, to switching focus to an adolescent population from 2010 because of increasing numbers of children ageing into this age group, and since 2014 has been operating as a tertiary clinic, receiving referrals and managing complicated paediatric and adolescentsinfected with HIV.

The study population included all HIV-infected male adolescents between the ages of 10 to 19 years who attendedat least two clinic visits between1st April 2004 (when the South African Department of Health ART rollout began) and 31 December 2014. Patients transferred in on ART and already with gynaecomastia were excluded. Patients with chronic liver disease, endocrine disorders and kidney disease that may result in gynaecomastia were excluded.

At the time of the study, eligibility for adolescent ART initiation was determined using WHO clinical staging criteria and immunological staging criteria. Before 2010 children < 3years were initiated on lopinavir/ritonavir, lamivudine and stavudine (D4T) and in children over 3 years and ≥ 10kg efavirenz was initiated instead of lopinavir/ritonavir. From 2010, abacavir replaced D4T in first line regimens and all patients who were virally suppressed were switched. In September 2016, a universal test and treat approach was adopted for all HIV-infected South Africans.[15]

Routine longitudinal demographic and clinical datafor all eligible patients wasextractedfrom the HSCCTherapy Edge (ABL-SA, Version 19) database.Datawascollected at ART initiation and at all visits after the age of 10 years. Some patients only initiated ART after the age of 10 years.The following variables were collected at each visit:age, ART regimen, CD4 count and percentages, viral load (VL), weight, height, body-mass-index(BMI) or weight for height z-score(WHZ)and any other adverse events that occurred at the same time as gynaecomastia. Adverse events such as gynaecomastia were recorded by the attending clinicianwhen the adolescent complained of breast enlargement on history taking followed by clinical examination, or was noted on physical examination of the breast and followed up at subsequent visits.Therefore gynaecomastia wasa clinical definition, and the first visit when gynaecomastia was documented, was used as the time of onset of gynaecomastia.Where possible, missing data was obtained from paper-based patient records and inserted into the database.Data wasthen imported into Stata (StataCorp College Station, Texas, Version 14.0) for statistical analysis. Continuous variables were summarized using medians and interquartile ranges and compared between groups using the Wilcoxon rank sum test. Categorized variables weredescribed using percentages and compared between groups using the χ^2 test.

Immune suppression was classified into 3 groups based on absolute CD4 count if patient $\geq$5years and CD4 percentage if <5 years age: no immune suppression if CD4 absolute count $\geq$500 cells/µl or $\geq$ 25%; moderate immune suppression if CD4 absolute count $\geq$ 200 and <500 cells/µl or $\geq$15 <25%; severe immune suppression if CD4 absolute <200cells/µl or <15%.[16]The above immune suppression categorisation was applied to Table 1 and 2 only. For the purpose of regression analysis, table 3, 2 groups of categorisation were used: not immune suppressed ("none"if CD4 absolute count $\geq$500 cells/µl or $\geq$25% and immune suppressed if CD4 absolute count <500 cells/µl or <25%. Viral suppression was defined as a viral load (VL) value of <400 copies/ml.[17]WHO standard deviation scores (Z-scores) were used for height for age (HAZ),[18] categorized as stunted if $\leq$ -2, normal if $\geq$-2 and $\leq$ 2, and tall if >2.Wasted was defined as a body mass index (BMIZor weight for height z-score(WHZ) if <10years ,[18]stunted if$\leq$-2, normal if $\geq$-2 and $\leq$2and obese if >2.[18] Gynaecomastia was diagnosed clinically via a physical examination during routine clinic visits, and in some cases corroborated with a breast ultrasound.

The incidence rate of gynaecomastia was calculated by dividing the number of cases of gynaecomastia by the cumulative person-time at risk in the cohort. Person-time at risk started

at the first visit after the age of 10 years (T_0) until the end of follow-up or incidence of gynaecomastia with censoring due to death, transfer out, loss-to-follow-up or end of study period.

Cox proportional hazard models were used to estimate the association between gynaecomastia and socio-demographic, clinical and growth risk factors. Univariateanalyses included variables considered *a priori* to affect the probability of developing gynaecomastia such asage at ARTinitiation,ARTdrugs, no immune suppression and viral load suppression. Multivariable analyses were conducted using a backward stepwise process,starting with all variables with a p-value ≤0.2 in univariate analyses.

Kaplan-Meier survival curves showing the cumulative probability of developing gynaecomastia over time were stratified by age group at ART initiation, duration on ART, viral suppression, immune suppression and BMI/WHZ. Survival curves were compared using log rank tests. Statistical analyses were performed using STATA (Statacorp, LP), version 14.

Ethical clearance was granted by the University of the Witwatersrand Human Research Ethics Committee.

RESULTS

From January 2004 to December 2014 a total of 622 HIV-infected adolescent males were included in the study. Of these, 44(7.1%) were diagnosed with gynaecomastia. The incidence of developing gynaecomastia was 2.4 per 100 person-years (95% CI:1.8-3.3).

Demographics and clinical characteristics atART initiation

Majority (59%) of this cohort initiated ART prior to the age of 10 years at a median age of 9.2 years (IQR: 7.3-11.4)with a significantly higher median age at ART initiation seen among those who developed gynaecomastia (10.8 years, IQR: 8.6-12.3) compared to those who did not(9.1years, IQR: 7.2-11.4). Adolescent maleswho initiated ART after 10 years of age were more likely to develop gynaecomastia compared to thosewhoinitiated ART before 10 years of age (OR=1.2, IQR: 1.18-1.40) (Table 1).

Almost all adolescents (96%) were initiated on efavirenz-based regimens and 79% of these were on both efavirenz and D4T at ART initiation.However, ART regimens initiated (D4T-based, EFV-based or both EFV and D4T-based regimens) and the period of ART initiation, reflecting changing guidelines in South Africa (2004 to 2010 compared to 2010 to

2014),were not significantlydifferent between those who developed gynaecomastia and those who did not. Similarly, there were no significant differences in immune suppression, viral loads or stunting at ART initiation between male adolescents who developed gynaecomastia compared to those who did not. (Table 1)

Most adolescents (82%) in this cohort were not wasted at ART initiation.Median BMIZ at ART initiation overall was -0.68(-1.54 to 0.05). However, those who were wasted at ART initiation were significantly less likely to develop gynaecomastia compared to those who were not (OR: 0.22, IQR: 0.17-0.28) (Table 1).

Demographic and clinicalcharacteristicsat T_0

Just under 14% of the cohort initiated care at the clinic after the age of 13 years; reflecting transfer-in or late initiation on ART. A comparison of demographic and clinical characteristics at the start of person-time at risk (T_0) for gynaecomastia (Table 2), revealed no significant differences in age, immune suppression, stunting or wasting between adolescents who developed gynaecomastia and those whodid not.

At T_0, 60% of the cohort were already on ART for a median time of 28.6 months (IQR: 14.9-48.2).With 40% (18/44) of gynaecomastia cases and 62% (358/578) non-gynaecomastia cases on ART at T_0, we found thatHIV-infected adolescent males on ART prior to T_0 were significantly less likely to develop gynaecomastia compared to thosewho started ART after T_0 (OR=0.43, 95% CI:0.23-0.79).In addition, adolescents on an EFV-based regimen at T_0were more likely to develop gynaecomastia than those who were not ($p<0.05$) (Table 2). We found no significant differences in duration of ART, being on a D4T-based regimen and viral suppressionbetween male adolescents who developed gynaecomastia compared to those who did not ($p>0.05$).

Demographics and clinical characteristics of HIV-Infected Adolescentswho developed gynaecomastia

The median age at which the 44 adolescents developed gynaecomastia was 14.6 years (IQR: 13.9-15.7).The median HAZ when gynaecomastia developed was -2.13(IQR: -2.56 to -1.22) with 56% of adolescents stunted when gynaecomastia developed. At the time the adolescents developed gynaecomastia 88.6%(39/44) had normal BMIZ, median BMIZ was -0.56(IQR:-1.31 to-0.13) with only 5/44(11.56%) obese adolescents.None had severe immune suppression,69% (29/42)had a CD4 count above 500cells/µl and 78.57%(33/42) were virally

suppressed.The median time on ART whenthey developed gynaecomastia was 74.9months (IQR: 66.6-82.1).

Risk factors at ART initiation and $T_0$for gynaecomastia among HIV-infected males

Overall 602 adolescent males were included in the Cox proportional hazards regression analysis; the remaining 20 did not have sufficient follow updata to be included. A longitudinal analysis of the risk factors assessed for development of gynaecomastia are shown in Table 3. In both univariate and multivariable analyses, we found that adolescents who were wasted at ART initiation were significantly less likely to develop gynaecomastia (HR=0.19, 95% CI: 0.05-0.81 and aHR= 0.18, 95% CI: 0.05-0.76) and adolescents on an EFV-containing ART regimen (without D4T) at T_0 were three times more likely to develop gynaecomastia compared to those who were not (HR=3.42, 95% CI: 1.12-10.42 and aHR=3.42, 95% CI: 1.12-10.42) (Table 3).

Probability of developing gynaecomastia over time

Kaplan-Meier survival curves illustratethat adolescents who were wasted at ART initiation had a significantly lower probability of developing gynaecomastia over the follow-up period compared to those who were not wasted (log rank p-value 0.01); with marked differences noted from 2years after T_0(Figure 1).During this time, the incidence rate of gynaecomastia among males who were not wasted at ART initiation rose from 0.7 at $T_0$to 3.3/100 person-years 3 years later and increased further to 7.3/100 person-years by 6 years after T_0.

Adolescents on an EFV-based regimen with no D4T at T_0had a significantly higher probability of developing gynaecomastia compared to those on an EFV and D4T regimen (log rank p- value 0.02); with marked differences noted from 4 years after T_0(Figure 2). Over the first 3 years after T_0, the incidence of gynaecomastia rose from 0 to 11.30/100 person-years among adolescents on EFV withno D4T, compared to a much smaller increase from 0 to 2.83/100 person-years among those on EFV and D4T-regimen.By 6 years after T_0, the incidence of gynaecomastia rose to 27.47/100 person-yearsamong males on EFV (no D4T) ART regimens.

Adolescents with severe immune suppression at ART initiation had lower (but not significantly different) probabilities of developing gynaecomastia over time compared to adolescents who had non-severe immune suppression at ART initiation (log rank p-value 0.08) (Figure 3).The probability of developing gynaecomastia did not differ significantly

based on age at ART initiation, time on ART at $T_0$and viral suppression at T_0 (Figures 4-7) log rank p-value>0.05).

DISCUSSION

In this cohort of 622 adolescent males on ART, 44 (7%) developed gynaecomastia resulting in an incidenceof 2.4/100 person-years.This is similar to previous studies which have reported a gynaecomastia incidence between 0.8-2.4 cases/100 patient–years, albeit in the adult population.[8,9,14,19]This is lower than described in the literature, where two thirds of HIV-uninfected male adolescents may develop gynaecomastia in early puberty followed by a decline in late teenage years, likely reflecting delayed puberty in this HIV-infected population secondary to chronic HIV disease.[2]Additionally, Harriet Shezi Children's Clinic has a policy of transferring stable, clinically well adolescents to primary care facilities which may have lowered the incidence. Manfredi et al., reporteda prevalence of 2.9% in a study of HIV-infected adult males between 12-58 years of age[8,14]A study done in Barcelona in 2003 of 2275 HIV-infected adult men from two referral centres found a prevalence of 1.8%,and that D4T and EFV were independent factors associated with gynaecomastia.[20]Different hypothesis have been postulated regarding development of gynaecomastia and the pathophysiology of gynaecomastia may be multifactorial.[11]

Clinical factors associated with gynaecomastia

The hypothesis is that as part of immune restoration after ART initiation there is improvement in the T-helper cell cytokine response, specifically IL-2 and IL-6 which both increase breast cell proliferation. Additionally IL-6 has also been shown to increase aromatase activity, ultimately causing gynaecomastia.[21]Most of our patients initiated ART late(median age 9.20 years).This may have been due to late diagnosisor late introduction of measures to strengthen fast-tracking (acceleration of diagnosis and treatment) of HIV-infected patients requiring ART in the South African antiretroviral treatment guidelines in 2010.[22]Additionally, South African guidelines pre-2016 recommended ART initiation based on clinical and immunological criteria, not treat-all, as currently recommended.[15]In our cohort we found that most of the adolescents initiated ART at the time that they were at risk for gynaecomastia;after 10 years of age (59.01%) which coincides with pubertal age in boys. This could account for the higher prevalence in our cohort (7% versus 2-3%) compared to adult studies. In other studies of HIV-infected males gynaecomastia was confirmed by ultrasonography,[9,14,20] excluding patients with pseudogynaecomastia. In our study diagnosis

was clinical,based on examination by the attending clinician which may account for the high prevalence since pseudogynaecomastia was not excluded. Similar to other studies, there were no significant associationsbetween the occurrence and development of gynaecomastia with virological and immunological factors at ART initiation.[8,14]However, we did find that patients who were wasted at ART initiation were significantly less likely to develop gynaecomastia. It is likely that being clinically immunocompromised, measured in part by wasting, impacts hormonal changes around pubertyresulting in these patients being less likely to develop gynaecomastia.[23] Because of the clinical and immune status-based guidelines at the time, adolescents initiating ART later may have been well and in care for many years, not yet requiring ART, contrasting from more immunocompromised adolescents who initiated ART earlier. Three-quarters of patients were virally suppressed at the time gynaecomastia developed this may indicate good compliance to treatment and may very well suggest that ART may be associated with development ofthe gynaecomastia. D4T and EFV were independent factors associated with gynaecomastia in a study done by Alenjandra et al.[20] In this cohort HIV-infected adolescents who were on an EFV-based regimen were at three times greater risk for developing gynaecomastia, similar to other studies where EFV was associated with gynaecomastia.[12,13,20]

Gynaecomastia has a significant negative impact on the psychosocial well-being of affected adolescent males.[24] A Malawian study based on a standardised questionnairein men with gynaecomastia, reported 6.6% of men not adherent to ART as they believed ART was the cause, with 51.6% being embarrassed of the condition.[25]Adolescents are known to have difficulties with adherence compared to older adults and it is likely that this would be further impacted by gynaecomastia.[26,27] The management of gynaecomastia may include ART switches, hormonal therapy and surgery.[25]In a low middle incomecountry like South Africa with many other health challenges, access to treatment for gynaecomastia may be limited outside of specialised centres.

To our knowledge, this is one of the biggest cohorts of adolescents reporting the incidence of gynaecomastia in HIV-infected patients on ART. However, this study had a number of limitations. Because of the retrospective natureof the study incomplete data entry may have impacted data completeness and accuracy. In particular, the anthropometricmeasurements, and other clinical data such asCD4 count or percentages and VL especially at ART initiation were missing. However, where data was missing on the clinic database, a file review was conducted to minimize missing data as much as possible. There were no strict clinical criteria

or guidelines in the diagnosis of gynaecomastia; diagnosis was made solely by the attending clinician.Diagnosis may have been missed or not recorded which would have resulted in an underestimation of gynaecomastia in this cohort. Pseudogynaecomastia was not excluded by ultrasound scan.AsHSCC is also a referral center, patients who were transferred in from other health facilitiesmay have incomplete clinical details in the period prior to the referral and these missing data could not be retrieved. In this study there was no control group of HIV-uninfected adolescents from the same socio-economic background thus limiting the interpretation of the incidence of gynaecomastia within the general adolescent male population.The lack ofTanner staging anddata regarding the pubertal growth spurt that were not routinely recorded in the data base or patient records did not allow us to investigate the possibility of delayed puberty influencing the gynaecomastia incidence in this cohort.

CONCLUSION

This study adds to the previously reported association between efavirenz and gynaecomastia in HIV-infected males. However, better designed studies are needed to clarify the relationship between gynaecomastia, HIV-infection and ART drugs, especially in adolescence where this relationship is confounded by physiological gynaecomastia.Gynaecomastia causes much anxiety, psychosocial discomfort, and fear of breast cancer in patients, and may affect adherence and therefore needs to be managed sensitively.

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TABLES

Table 1Demographic and clinical characteristics of a cohort of HIV-infected male adolescents at the time of antiretroviral therapy initiation

	Overall (% or IQR)	**Gynaecomastia** (% or IQR)	**No Gynaecomastia** (% or IQR)	**P-value**
Total males (N)	622	44 (7.07)	578 (92.93)	
Age-group in years				
<10	367 (59.00)	18 (40.91)	349 (60.38)	
≥10	255 (41.00)	26 (59.09)	229 (39.62)	0.011
Age, median (IQR)	9.20(7.28-11.43)	10.77 (8.57-12.32)	9.09 (7.22-11.35)	<0.001
Immune suppression[1]	N=531	N=41	N=490	
None	90 (16.95)	5 (12.20)	85 (17.35)	
Moderate	212 (39.92)	21 (51.22)	191 (38.98)	
Severe	229 (43.13)	15 (36.59)	214 (43.67)	0.332
Log_{10} viral load	4.89(4.32-5.32)	4.87(4.37-5.30)	4.89(4.32-5.32)	0.913
Height-for-age z-score[2]	N=618	N=44	N=574	
Median	-2.18(-2.97 to -1.44)	-1.95(-2.99 to -1.51)	-2.18(-2.97 to -1.42)	0.603
Tall	0(0)	0(0)	0(0)	
Normal	270 (43.69)	22 (50.00)	248 (43.21)	
Stunted	348 (56.31)	22 (50.00)	326 (56.79)	0.381
BMIZ[3] **or Weight for-height z-score**[4]	N=613	N=42	N=571	
Median	-0.68(-1.54 to 0.05)	-0.54(-1.39 to 0.02)	-0.69(-1.55 to 0.06)	0.337
Obese	4(0.65)	0(0)	4(0.70)	
Normal	504 (82.22)	40 (95.24)	464 (81.26)	
Wasted	109 (17.13)	2 (4.76)	107 (18.74)	0.020
D4T-based regimen				
No	113 (18.16)	7 (15.91)	106 (18.34)	
Yes	509 (81.83)	37 (84.09)	472 (81.66)	0.630
EFV-based regimen				
No	24 (3.86)	1 (2.27)	23 (3.98)	
Yes	598 (96.14)	43 (97.73)	555 (96.02)	0.516
EFV- & D4T-based regimen				
No	131 (21.06)	7 (15.91)	124 (21.45)	
Yes	491 (78.94)	37 (84.09)	454 (78.55)	0.385
Period of ART initiation				
2004 to 2010	511 (82.15)	39 (88.64)	472 (81.66)	
2010 to 2014	111 (17.85)	5 (11.36)	106 (18.34)	0.244

[1] Immune suppression was classified into 3 groups based on absolute CD4 count if patient ≥5years and CD4 percentage if <5 years age: no immune suppression if CD4 absolute count ≥500 cells/µl or ≥ 25%; moderate immune suppression if CD4 absolute count ≥ 200 and <500 cells/µl or ≥15 <25%; severe immune suppression if CD4 absolute <200cells/µl or <15.

[2]Height-for-age z-score (HAZ) classified as stunted if HAZ ≤-2, normal if >-2 and ≤2 and tall if >2

[3]BMIZ: used if patient 10 years and older: body mass index z-score classified as wasted if ≤ -2, normal if >-2 and ≤2 and obese if >2.

[4]Weight-for height z-score (WHZ): used if patient less than 10 years old was classified as wasted if ≤-2, normal if >-2 and ≤2 and obese if >2.

N: Where N is specified per variable in the table, there is missing data and all percentages are calculated, excluding missing dataART[5] antiretroviral therapyEFV[6] Efavirenz, D4T[7] Stavudine

Table 2 Demographics and clinical characteristics of a cohort of HIV-infected male adolescents at start of person time at risk for developing gynaecomastia (T_0)

	Overall (% or IQR)	**Gynaecomastia**(% or IQR)	**No Gynaecomastia**(% or IQR)	**p-value**
Total males (N)	622	44	578	
Age, median (IQR)	10.17 (10.08-11.45)	10.77 (10.10-11.39)	10.17 (10.07-11.69)	0.060
Age in years				
<13 years	537 (86.33)	35 (79.55)	502 (86.85)	
≥13 years	85 (13.67)	9 (20.45)	76 (13.15)	0.174
Immune suppression[1]	N=556	N=44	N=512	
None	316 (56.83)	25 (56.82)	291 (56.84)	
Moderate	173 (31.12)	14 (31.82)	159 (31.05)	
Severe	67 (12.05)	5 (11.36)	62 (12.11)	0.987
HAZ[2]	n=604	n=44	n=560	
Median	-1.81(-2.52 to -1.15)	-1.74(-2.61 to -1.32)	-1.83(-2.52 to -1.12)	0.752
Tall	1(0.17)	0(0)	1(0.018)	
Normal	335 (55.47)	25 (56.82)	310 (55.18)	
Stunted	269 (44.54)	19 (43.18)	250 (44.64)	0.851
BMIZ[3]	n=599	n=43	n=556	
Median	-0.53(-1.22 to 0.19)	-0.18(-0.90 to 0.24)	-0.55(-1.23 to 0.17)	0.068
Obese	5(0.83)	1(2.33)	4(0.72)	
Not wasted	533 (88.35)	41 (95.34)	492 (88.49)	
Wasted	61 (10.18)	1 (2.33)	60 (10.79)	0.095
Adolescents on ART prior to T_0				
Adolescents on ART (N)	376/622 (60.45)	18/44 (40.91)	358/578 (61.94)	0.006
Time on ART, median (IQR)	28.55 (14.98-48.24)	22.71 (13.81-35.13)	28.55 (15.19-49.18)	0.192
Time on ART	N=376	N=18	N=358	
<12 months	73 (19.41)	4 (22.22)	69 (19.27)	
≥12 months	303 (80.59)	14 (77.78)	289 (80.73)	0.761
D4T-based regimen				
No	127 (33.78)	4 (22.22)	123 (34.36)	
Yes	249 (66.22)	14 (77.78)	235 (65.64)	0.444
EFV-based regimen				
No	74 (19.68)	0 (0)	74 (20.67)	
Yes	302 (80.32)	18 (100)	284 (79.33)	0.030
D4T & EFV based regimen	156 (41.49)	4 (22.22)	152 (42.46)	
No	220 (58.51)	14 (77.78)	206 (57.54)	0.139
Yes				
D4T no EFV				
No	347 (92.29)	18 (100.00)	329 (91.90)	
Yes	29 (7.71)	0 (0)	29 (8.10)	0.381
EFV no D4T				
No	294 (78.19)	14 (77.78)	280 (78.21)	
Yes	82 (21.81)	4 (22.22)	78 (21.79)	0.100
Viral load (copies/ml)	N=313	N=18	N=295	
<400	250 (79.87)	16 (88.89)	234 (79.32)	
>=400	63 (20.13)	2 (11.11)	61 (20.68)	0.544

[1]Immune suppression was classified into 3 groups based on absolute CD4 count if patient ≥5years and CD4 percentage if <5 years age: no immune suppression if CD4 absolute count ≥500 cells/µl or ≥ 25%; moderate immune suppression if CD4 absolute count ≥ 200 and <500 cells/µl or ≥15 <25%; severe immune suppression if CD4 absolute <200cells/µl or <15%.
[2]HAZ: height-for-age z-score classified as stunted if HAZ ≤-2, normalif >-2 and ≤2 and tall if >2.
[3]BMIZ: body mass index z-score classified as not wasted if wasted if ≤-2, normal if >-2 and ≤ 2, and obese if ≥2.
N: Where N is specified per variable in the table, there is missing data and all percentages are calculated, excluding missing data
ART: Antiretroviral therapy
D4T: Stavudine
EFV: Efavirenz

Table 3.Adjusted[ii] and unadjusted hazard ratios (HR) of potential risk factors for developing gynaecomastia in HIV-infected males, at ART initiation and T_0

Characteristics	Unadjusted HR (95% CI)	*P*-value	Adjusted HR (95%CI)	p-value
At ART initiation				
Age				
<10 years	1			
≥10 years	1.41 (0.77-2.57)	0.263		
HAZ[1]				
Not stunted	1			
Stunted	0.81 (0.44-1.47)	0.492		
BMI[2]& WHZ[3]				
Normal	1		1	
Wasted	0.19 (0.05-0.81)	0.024	0.18 (0.05-0.76)	0.019
Immune suppression				
None	1			
Immune suppressed	0.57 (0.30-1.08)	0.086		
EFV-based ART regimen				
No	1			
Yes	0.57 (0.08-4.17)	0.579		
D4T-based ART regimen				
No	1			
Yes	0.43 (0.19-1.01)	0.053		
EFV & D4T in ART regimen				
No	1			
Yes	0.51 (0.22-1.18)	0.115		
EFV, no D4T in ART regimen				
No	1			
Yes	1.95 (0.79-4.77)	0.146		
D4T, no EFV in ART regimen	N=0 in one cell			
At T_0[5]				
Time on ART for adolescents on ART				
<12 months	1			
≥12 months	1.23 (0.40-3.77)	0.712		
HAZ[1]				
Normal	1			
Stunted	0.87 (0.48-1.58)	0.639		
BMIZ[2]				
Normal	1			
Wasted	0.21 (0.03-1.53)	0.124		
Obese	4.26 (0.58-3.27)	0.154		
Immune suppression[4]				
None	1			
Immune suppressed	0.87 (0.34-2.21)	0.770		
Viral loadfor adolescents on ART				
Suppressed	1			
Not Suppressed	0.50 (0.11-2.22)	0.360		
EFV & D4T in ART regimen				
No	1			
Yes	0.92 (0.30-2.83)	0.897		
EFV-based ART Regimen	N=0 in one cell			

D4T-based ART regimen				
No	1			
Yes	0.60 (0.19-1.83)	0.372		
EFV (no D4T) ART regimen				
No	1		1	
Yes	3.42 (1.12-10.42)	0.031	3.42 (1.12-10.42)	0.031
On ART at T0				
Yes	1			
No	0.65 (0.36-1.19)	0.167		

HAZ[1]: height-for-age z-score, Not stunted if HAZ >-2, stunted if HAZ $\leq$-2
BMIZ[2] body mass index z-score classified as not wasted if >-2 and wasted if $\leq$-2 for those above 10 years age or WHZ[3] weight for-for height Z-score classified asnot wasted if >-2, wasted if $\leq$-2.
Immune suppression was classified into 2 groups based on absolute CD4 count if patient $\geq$5years and CD4 percentage if <5 years age: no immune suppression if CD4 absolute count $\geq$500 cells/µl or $\geq$ 25%; immune suppressed if CD4 absolute count <500 cells/µlor <25%T_0[5]: Time at risk for gynaecomastia at 10 years or first visit after 10 years age [µ]: Backward stepwise Cox proportional hazards regression analysis was performed by starting with all variables with a p-value<0.2, concluding with a model that included only variables with a p-value of <0.05.
ART: Antiretroviral therapy
D4T: Stavudine
EFV: Efavirenz

Table 4 Demographics and clinical characteristics of 44 HIV-infected adolescent males at the time gynaecomastia developed

Characteristic	N=44 (%, IQR)
Age,(median IQR)	14.60(13.85-15.69)
Immune suppression[1] Normal Moderate Severe	n=42 29(69.04) 13(30.95) 0(0.00)
Viral load (copies/ml[2)] <400 =>400	n=42 33(78.57) 9(21.43)
HAZ[2] Median Tall Not stunted Stunted	n=44 -2.13(-2.56 to-1.22) 0(0) 19(43.18) 25(56.82)
BMIZ[3] Median Obese Normal Wasted	n=44 -0.56(-1.31 to -0.13) 0 (0) 39(88.64) 5 (11.36)
ART regimen EFV only D4T only D4T & EFV Neither D4T nor EFV	n=43 18(41.86) 3(6.98) 9(30.93) 13(30.23)
Time on ART	74.9 months (IQR: 66.6-82.1).

[1]Immune suppression[1] classified into 3 categories: normal if CD4 absolute count ≥500 cells/µl; moderate immune suppression if CD4 absolute count ≥ 200 and <500 cells/µl: severe immune suppression if CD4 absolute <200cells/µl. Viral load

HAZ[2]: height-for-age z-score classified as stunted if HAZ ≤-2, normal if >-2 and ≤2 and tall if >2. .

BMIZ[3]: body mass index z-score classified as not wasted if ≤-2, normal if >-2 and ≤ 2 and obese if >2.

N: Where N is specified per variable in the table, there is missing data and all percentages are calculated, excluding missing data

1

FIGURES

Figure 1: Probability of developing gynaecomastia stratified by BMIZ-score at ART initiation

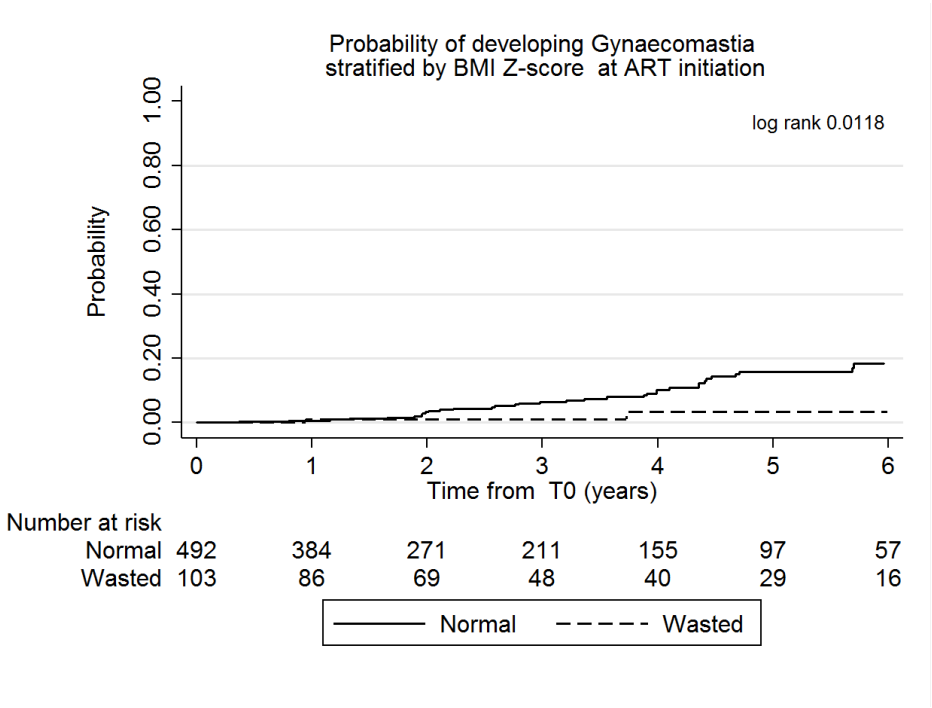


Figure 2: Probability of developing gynaecomastia stratified by EFV only at T_0

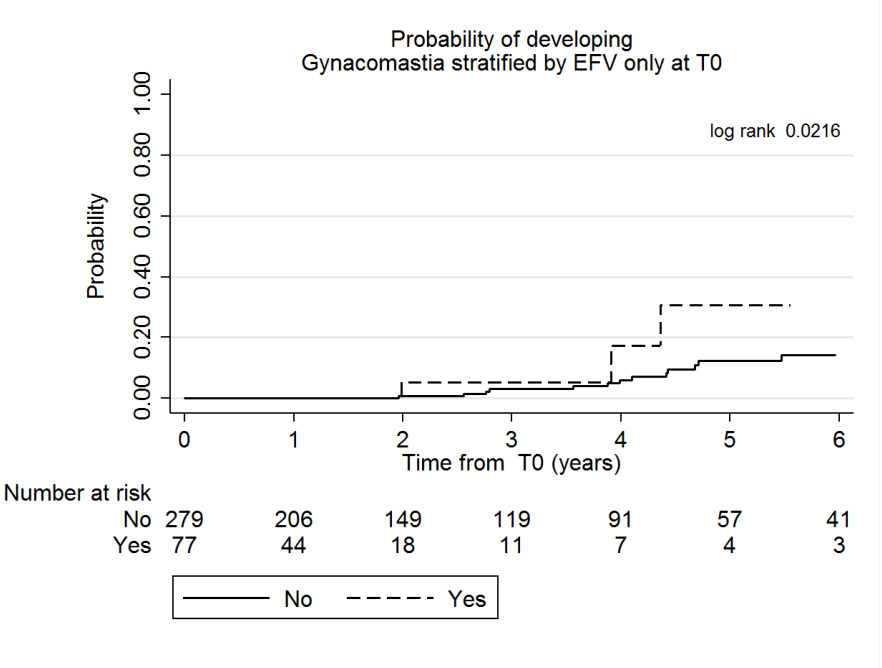


Figure 3: Probability of developing gynaecomastia stratified by immune suppression at ART initiation

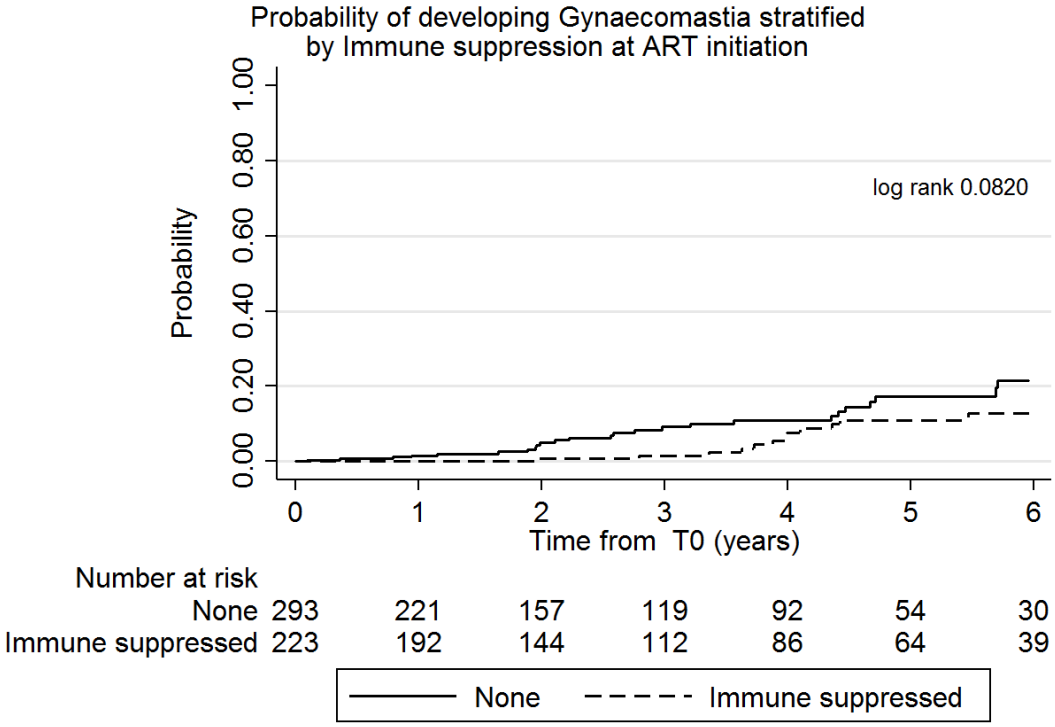


Figure 4: Probability of developing gynaecomastia stratified by age at ART initiation

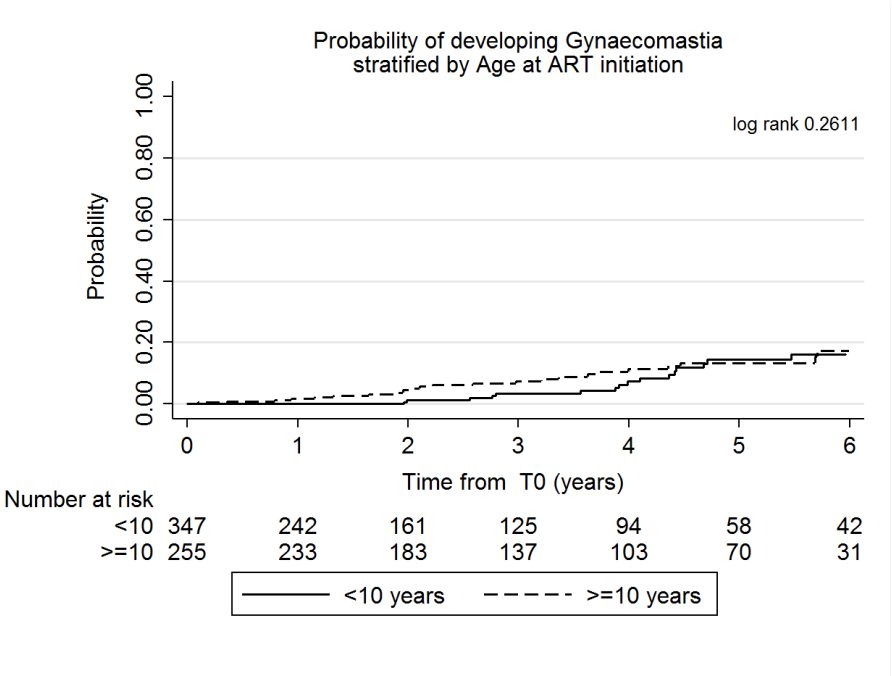


Figure 5: Probability of developing gynaecomastia stratified by time on ART

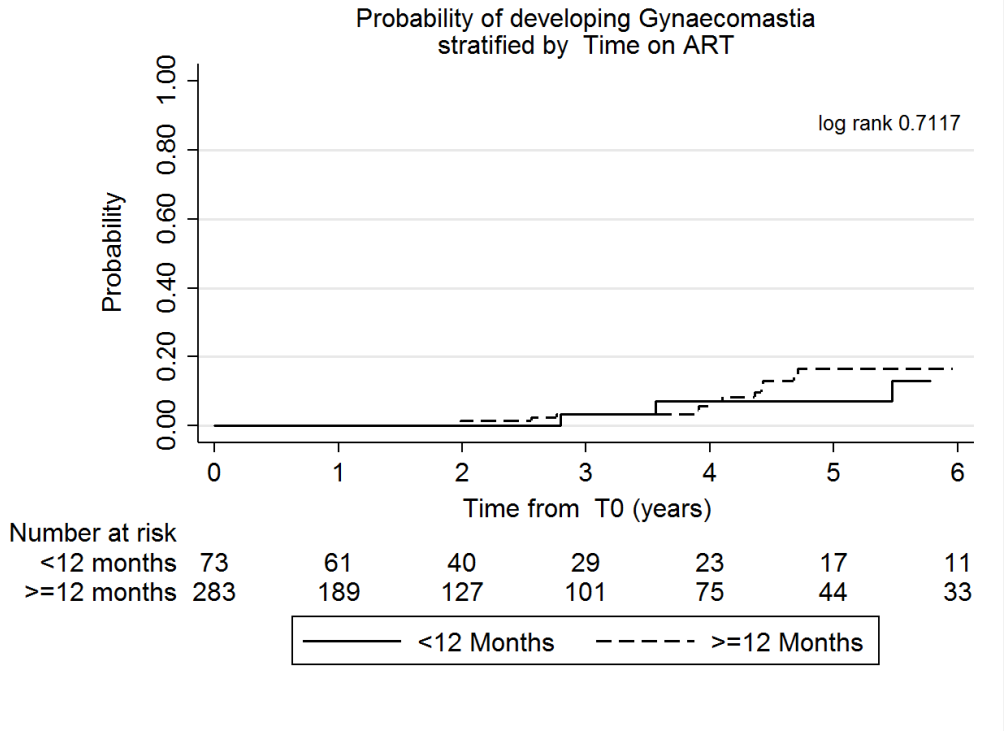


Figure 6: Probability of developing gynaecomastia stratified by EFV and D4T based regimens at ART initiation

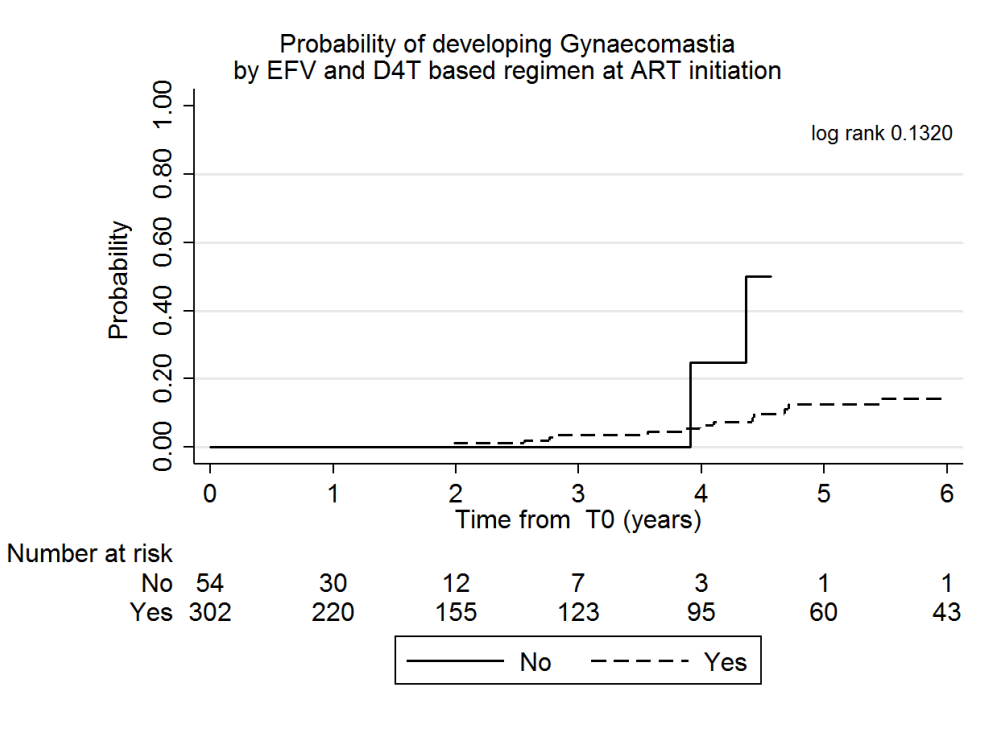
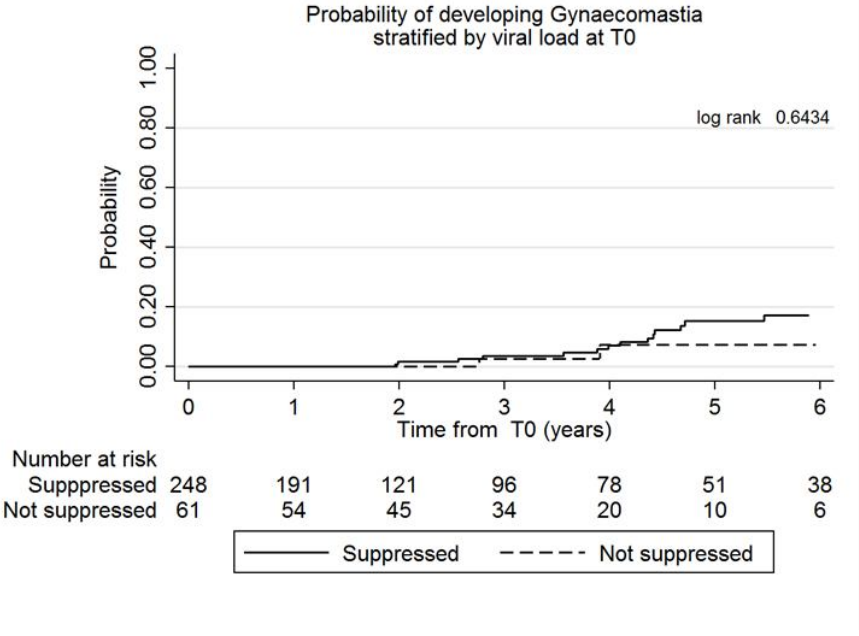


Figure 7: Probability of developing gynaecomastia stratified by viral load at T_0



APPENDIX A: TURNITIN REPORT

Turnitin Originality Report

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The incidence and description of Gynaecomastia in HIV positive adolescents on antiretroviral therapy Student: Dr Khaukanani Neo Mungoni 0304298T/A0003882 Supervisor: 1. Dr Lee Fairlie Co-supervisors: 2. Dr Nosisa Sipambo 3. Shobna Sawry A dissertation submitted to the faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Masters in Medicine. Johannesburg 2018 INTRODUCTION Adolescence, defined as ages 10 -19 years,1 coincides with the onset of puberty (9 – 14) years1 and is associated with many physical, psychological and emotional changes. One of these changes is the development of gynaecomastia, reported in about two thirds of boys during puberty.2 Gynaecomastia is the enlargement of the male breast secondary to stromal proliferation and ductal hyperplasia and is usually benign.2,3 In contrast, pseudogynaecomastia is the accumulation of fat under and around the male breast nipple without ductal proliferation and is common in obese males. The introduction of antiretroviral therapy (ART) in HIV-infected children has led to a dramatic reduction in HIV-associated morbidity and mortality. 4 As a result of early ART initiation, HIV-

infected children are ageing into adolescence and adulthood in South Africa and globally.5 About 2.1 million adolescents worldwide were living with HIV in 2016, of whom 1.7 million (84%) resided in Sub-Saharan Africa. 6 ART has been associated with a number of adverse effects often specific to a particular antiretroviral drug. Some adolescents and adults on long term ART have been reported to develop gynaecomastia which is difficult to manage in an adolescent population already at risk of gynaecomastia, where determining causality is complicated by physiological norms.2,5,7 Different classes of drugs such as calcium channel blockers, cytotoxic agents and some antibiotics such as ketoconazole, metronidazole, and isoniazid induce gynaecomastia, accounting for 20 to 25% of all cases in men.2,8 ART drugs most commonly associated with gynaecomastia are efavirenz (EFV) didanosine (DDI), stavudine (D4T) and various protease inhibitors (PI).3,8-13 Gynaecomastia in HIV-infected males may be associated with HIV-associated lipodystrophy syndrome and dysmetabolism also known as metabolic syndrome.8,14 Studies done in HIV-infected males report a prevalence range of 2.9-5%, ages between 12-63 years, with a significant number of these patients receiving D4T and had lipodystrophy.8,11,14 Published literature largely consists of case reports. A case series by Jose et al., of 3 adult males in Spain histopathologically confirmed gynaecomastia without lipodystrophy syndrome in HIV-infected patients treated with EFV: in all 3 cases EFV was introduced as a simplification treatment instead of a protease inhibitor. Similarly in 2000 Jover et al., reported 5 gynaecomastia cases in French males, ages 43-55 years with EFV-associated gynaecomastia without lipodystrophy 12,13syndrome: all of the cases had immunological and virological responses to ART and in all 5 cases gynaecomastia regressed after EFV withdrawal. These reports suggest that EFV may cause gynaecomastia by unknown mechanisms.12,13 There is a paucity of literature describing the incidence and outcomes of gynaecomastia in adolescents, with most cases described in adults. Therefore, the aim of this study is to estimate the incidence of gynaecomastia in HIV-infected adolescents on ART, to describe the clinical characteristics associated with gynaecomastia and to determine whether specific antiretroviral drugs were associated with gynaecomastia in this cohort. Methods This retrospective longitudinal cohort study of HIV-infected male adolescents on ART was conducted at the Harriet Shezi Children's Clinic (HSCC), a public sector paediatric and adolescent HIV clinic at Chris Hani Baragwanath Academic Hospital (CHBAH), Johannesburg. HSCC evolved over time from being one of few paediatric ART initiation sites in South Africa from 2004-2010, to switching focus to an adolescent population from 2010 because of increasing numbers of children ageing into this age group, and since 2014 has been operating as a tertiary clinic, receiving referrals and managing complicated paediatric and adolescents infected with HIV. The study population included all HIV-infected male adolescents between the ages of 10 to 19 years who attended at least two clinic visits between 1st April 2004 (when the South African Department of Health ART rollout began) and 31 December 2014. Patients transferred in on ART and already with gynaecomastia were excluded. Patients with chronic liver disease, endocrine disorders and kidney disease that may result in gynaecomastia were excluded. At the time of the study, eligibility for adolescent ART initiation was determined using WHO clinical staging criteria and immunological staging criteria. Before 2010 children < 3years were initiated on lopinavir/ritonavir, lamivudine and stavudine (D4T) and in children

over 3 years and ≥ 10kg efavirenz was initiated instead of lopinavir/ritonavir. From 2010, abacavir replaced D4T in first line regimens and all patients who were virally suppressed were switched. In September 2016, a universal test and treat approach was adopted for all HIV-infected South Africans. 15 Routine longitudinal demographic and clinical data for all eligible patients was extracted from the HSCC Therapy Edge (ABL-SA, Version 19) database. Data were collected at ART initiation and at all visits after the age of 10 years. Some patients only initiated ART after the age of 10 years. The following variables were collected at each visit: age, ART regimen, CD4 count and percentages, viral load (VL), weight, height, body-mass-index (BMI) or weight for height z-score (WHZ) and any other adverse events that occurred at the same time as gynaecomastia. Where possible, missing data was obtained from paper-based patient records and inserted into the database. Data was then imported into Stata (StataCorp College Station, Texas, Version 14.0) for statistical analysis. Continuous variables were summarized using medians and interquartile ranges and compared between groups using the Wilcoxon rank sum test. Categorized variables were described using percentages and compared between groups using the $\chi 2$ test. Immune suppression was classified into 3 groups based on absolute CD4 count if patient >5years and CD4 percentage if <5 years age: no immune suppression if CD4 absolute count >500 cells/µl or > 25%; moderate immune suppression if CD4 absolute count > 200 and <500 cells/µl or >15 <25%; severe immune suppression if CD4 absolute <200cells/µl or <15%.16 Viral suppression was defined as a viral load (VL) value of <400 copies/ml. 17 WHO standard deviation scores (Z-scores) were used for height for age (HAZ),18 categorized as stunted if < -2 and not stunted if > -2. Wasted was defined as a body mass index (BMIZ or weight for height z-score(WHZ) if <10years ,18 of < -2 or not wasted if > -2.18 Gynaecomastia was diagnosed clinically via a physical examination during routine clinic visits, and in some cases corroborated with a breast ultrasound. The incidence rate of gynaecomastia was calculated by dividing the number of cases of gynaecomastia by the cumulative person- time at risk in the cohort. Person-time at risk started at the first visit after the age of 10 years (T0) until the end of follow-up or incidence of gynaecomastia with censoring due to death, transfer out, loss-to-follow-up or end of study period. Cox proportional hazard models were used to estimate the association between gynaecomastia and socio-demographic, clinical and growth risk factors. Univariate analyses included variables considered a priori to affect the probability of developing gynaecomastia such as age at ART initiation, ART drugs, no immune suppression and viral load suppression. Multivariable analyses were conducted using a backward stepwise process, starting with all variables with a p- value ≤0. 2 in univariate analyses. Kaplan-Meier survival curves showing the cumulative probability of developing gynaecomastia over time were stratified by age group at ART initiation, duration on ART, viral suppression, immune suppression and BMI/WHZ. Survival curves were compared using log rank tests. Statistical analyses were performed using STATA (Statacorp, LP), version 14. Ethical clearance was granted by the University of the Witwatersrand Human Research Ethics Committee. Results From January 2004 to December 2014 a total of 622 HIV-infected adolescent males were included in the study. Of these, 44 (7.1%) were diagnosed with gynaecomastia. The incidence of developing gynaecomastia was 2. 4 per 100 person-years (95% CI: 1.8 -3.3).

Demographics and clinical characteristics at ART initiation Majority (59%) of

this cohort initiated ART prior to the age of 10 years at a median age of 9.2 years (IQR: 7.3-11.4) with a significantly higher median age at ART initiation seen among those who developed gynaecomastia (10.8 years, IQR: 8.6-12.3) compared to those who did not (9.1 years, IQR: 7.2-11.4). Adolescent males who initiated ART after 10 years of age were more likely to develop gynaecomastia compared to those who initiated ART before 10 years of age (OR=1.2, IQR: 1.18- 1.40) (Table 1). Almost all adolescents (96%) were initiated on efavirenz-based regimens and 79% of these were on both efavirenz and D4T at ART initiation. However, ART regimens initiated (D4T- based, EFV-based or both EFV and D4T-based regimens) and the period of ART initiation, reflecting changing guidelines in South Africa (2004 to 2010 compared to 2010 to 2014), were not significantly different between those who developed gynaecomastia and those who did not. Similarly, there were no significant differences in immune suppression, viral loads or stunting at ART initiation between male adolescents who developed gynaecomastia compared to those who did not. (Table 1) Most adolescents (82%) in this cohort were not wasted at ART initiation. However, those who were wasted at ART initiation were significantly less likely to develop gynaecomastia compared to those who were not (OR: 0.22, IQR: 0.17-0.28) (Table 1). Demographic and clinical characteristics at T0 Just under 14% of the cohort initiated care at the clinic after the age of 13 years; reflecting transfers-in or late initiation on ART. A comparison of demographic and clinical characteristics at the start of person-time at risk (T0) for gynaecomastia (Table 2), revealed no significant differences in age, immune suppression, stunting or wasting between adolescents who developed gynaecomastia and those who did not. At T0, 60% of the cohort were already on ART for a median time of 28.6 months (IQR: 14.9-48.2). With 40% (18/44) of gynaecomastia cases and 62% (358/578) non-gynaecomastia cases on ART at T0, we found that HIV-infected adolescent males on ART prior to T0 were significantly less likely to develop gynaecomastia compared to those who started ART after T0 (OR=0.43, 95% CI:0.23-0.79). In addition, adolescents on an EFV-based regimen at T0 were more likely to develop gynaecomastia than those who were not (p<0. 05) (Table 2). We found no significant differences in duration of ART, being on a D4T-based regimen and viral suppression between male adolescents who developed gynaecomastia compared to those who did not (p>0.05). Demographics and clinical characteristics of HIV-Infected Adolescents who developed gynaecomastia The median age at which the 44 adolescents developed gynaecomastia was 14.6 years (IQR: 13.9- 15.7). At the time the adolescents developed gynaecomastia, 88.6% (39/44) were not wasted; none had severe immune suppression, 65.9% (29/44) had a CD4 count above 500cells/µl and 75% (33/44) were virally suppressed. The median time on ART when they developed gynaecomastia was 74.9 months (IQR: 66.6-82.1). Risk factors at ART initiation and T0 for gynaecomastia among HIV-infected males Overall 602 adolescent males were included in the Cox proportional hazards regression analysis; the remaining 20 did not have sufficient follow up data to be included. A longitudinal analysis of the risk factors assessed fordevelopment of gynaecomastia are shown in Table 3. In both univariate and multivariable analyses, we found that adolescents who were wasted at ART initiation were significantly less likely to develop gynaecomastia (HR=0. 19, 95% CI: 0. 05 -0. 81 and aHR= 0. 18, 95% CI: 0. 05 -0.76) and adolescents on an EFV-containing ART regimen (without D4T) at T0 were three times more likely to

develop gynaecomastia compared to those who were not (HR=3.42, 95% CI: 1. 12-10.42 and aHR =3. 42, 95% CI: 1. 12 -10. 42) (Table 3). Probability of developing gynaecomastia over time Kaplan-Meier survival curves illustrate that adolescents who were wasted at ART initiation had a significantly lower probability of developing gynaecomastia over the follow-up period compared to those who were not wasted (log rank p-value 0.01); with marked differences noted from 2 years after T0 (Figure 1). During this time, the incidence rate of gynaecomastia among males who were not wasted at ART initiation rose from 0.7 at T0 to 3.3/100 person-years 3 years later and increased further to 7.3/100 person-years by 6 years after T0. Adolescents on an EFV-based regimen with no D4T at T0 had a significantly higher probability of developing gynaecomastia compared to those on an EFV and D4T regimen (log rank p- value 0.02); with marked differences noted from 4 years after T0(Figure 2). Over the first 3 years after T0, the incidence of gynaecomastia rose from 0 to 11.30/100 person-years among adolescents on EFV with no D4T, compared to a much smaller increase from 0 to 2.83/100 person-years among those on EFV and D4T-regimen. By 6 years after T0, the incidence of gynaecomastia rose to 27.47/100 person-years among males on EFV (no D4T) ART regimens. Adolescents with severe immune suppression at ART initiation had lower (but not significantly different) probabilities of developing gynaecomastia over time compared to adolescents who had non- severe immune suppression at ART initiation (log rank p-value 0.08) (Figure 3). The probability of developing gynaecomastia did not differ significantly based on age at ART initiation, time on ART at T0 and viral suppression at T0 (Figures 4-7) log rank p-value>0.05). Discussion In this cohort of 622 adolescent males on ART, 44 (7%) developed gynaecomastia resulting in an incidence of 2.4/100 person-years. This is similar to previous studies which have reported a gynaecomastia incidence between 0.8-2.4 cases/100 patient–years, albeit in the adult population.8,9,14,19 This is lower than described in the literature, where two thirds of HIV-uninfected male adolescents may develop gynaecomastia in puberty, likely reflecting delayed puberty in this population secondary to chronic HIV disease. 2. Manfredi et al., reported a prevalence of 2.9% in a study of HIV-infected adult males between 12-58 years of age8,14 A study done in Barcelona in 2003 of 2275 HIV-infected adult men from two referral centres found a prevalence of 1.8%, and that D4T and EFV were independent factors associated with gynaecomastia.20 Different hypothesis have been postulated regarding development of gynaecomastia and the pathophysiology of gynaecomastia may be multifactorial.11 Clinical factors associated with gynaecomastia The hypothesis is that as part of immune restoration after ART initiation there is improvement in the T-helper cell cytokine response, specifically IL-2 and 6 which both increase breast cell proliferation. Additionally IL-6 has also been shown to increase aromatase activity, ultimately causing gynaecomastia.21 Most of our patients initiated ART late (median age 9.20 years). This may have been due to late diagnosis or late introduction of measures to strengthen fast-tracking (acceleration of diagnosis and treatment) of HIV-infected patientsrequiring ART in the South African antiretroviral treatment guidelines in 2010. 22 Additionally, South African guidelines pre-2016 recommended ART initiation based on clinical and immunological criteria, not treat-all, as currently recommended.15 In our cohort we found that most of the adolescents initiated ART at the time that they were at risk for gynaecomastia; after 10 years of age (59.01%) which coincides with

pubertal age in boys. This also coincides with the time of physiological gynaecomastia, reported in about two-thirds adolescent males during puberty with a peak onset between 13 and 14 years.2 This could account for the higher prevalence in our cohort (7% versus 2-3%) compared to adult studies. Similar to other studies, there were no significant associations between the occurrence and development of gynaecomastia with virological and immunological factors at ART initiation.8,14 However, we did find that patients who were wasted at ART initiation were significantly less likely to develop gynaecomastia. It is likely that being clinically immunocompromised, measured in part by wasting, impacts hormonal changes around puberty resulting in these patients being less likely to develop gynaecomastia.23 Because of the clinical and immune status-based guidelines at the time, adolescents initiating ART later may have been well and in care for many years, not yet requiring ART, contrasting from more immunocompromised adolescents who initiated ART earlier. Three-quarters of patients were virally suppressed at the time gynaecomastia developed this may indicate good compliance to treatment and may very well suggest that ART may be associated with development of the gynaecomastia. D4T and EFV were independent factors associated with gynaecomastia in a study done by Biglia et al.20 In this cohort HIV-infected adolescents who were on an EFV-based regimen were at three times greater risk for developing gynaecomastia, similar to other studies where EFV was associated with gynaecomastia.12,13,20 Gynaecomastia has a significant negative impact on the psychosocial well-being of affected adolescent males.24 A Malawian study based on a standardised questionnaire in men with gynaecomastia, reported 6.6% of men not adherent to ART as they believed ART was the cause, with 51.6% being embarrassed of the condition.25 Adolescents are known to have difficulties with adherence compared to older adults and it is likely that this would be further impacted by gynaecomastia.26,27 The management of gynaecomastia may include ART switches, hormonal therapy and surgery.25 In a developing country like South Africa with many other health challenges, access to treatment for gynaecomastia may be limited outside of specialised centres. To our knowledge, this is one of the biggest cohorts of adolescents reporting on the incidence of gynaecomastia in HIV-infected patients on ART. However, this study had a number of limitations. Because of the retrospective nature of the study incomplete data entry may have impacted data completeness and accuracy. In particular, the anthropometric measurements, and other clinical data such as CD4 count or percentages and VL especially at ART initiation were missing. However, where data was missing on the clinic database, a file review was conducted to minimize missing data as much as possible. As HSCC is also a referral center, patients who were transferred in from other health facilities may have incomplete clinical details in the period prior to the referral and these missing data could not be retrieved. Tanner staging and details regarding further investigation and management were not routinely recorded in the data base or patient records. Conclusion This study adds to the previously reported association between efavirenz and gynaecomastia in HIV- infected males. However, better designed studies are needed to clarify the relationship betweengynaecomastia, HIV-infection and ART drugs, especially in adolescence where this relationship is confounded by physiological gynaecomastia. Gynaecomastia causes much anxiety, psychosocial discomfort, and fear of breast cancer in patients, and may affect adherence and therefore needs to be managed sensitively.

APPENDIX B: ETHICS CLEARANCE

R14/49 Dr Khaukanani N Mungoni

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150701

NAME: (Principal Investigator)	Dr Khaukanani N Mungoni
DEPARTMENT:	Paediatrics Chris Hani Baragwanath Academic Hospital Paediatrics Department Harriet Shezi Children's Clinic
PROJECT TITLE:	The Incidence and Description of Gynaecomastia in HIV Positive Adolescent Males on Antiretroviral Therapy
DATE CONSIDERED:	31/07/2015
DECISION:	Approved unconditionally
CONDITIONS:	Title Change (30/08/2017)
SUPERVISOR:	Dr Lee Fairlie
APPROVED BY:	Professor C. Feldman,Chairperson, HREC (Medical)
DATE OF APPROVAL:	12/08/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated,from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review in July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX C: PLAGIARISM DECLARATION

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS SENATE PLAGIARISM POLICY:

_____ (Student number: _____) am a student registered for the degree of _____ in the academic year _____. I hereby declare the following: - I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong. - I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise. - I have followed the required conventions in referencing the thoughts and ideas of others. - I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing. - I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: _____