A DESCRIPTION OF FULL BLOOD COUNT ABNORMALITIES IN HIV-INFECTED CHILDREN LESS THAN THREE YEARS OF AGE BEFORE AND AFTER COMBINED ANTIRETROVIRAL THERAPY INITIATION

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DECLARATION

I, Theodore Mlungisi Mabaso, declare that this is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics, in 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: _____, 2016

DEDICATION

To my beloved daughter, Nyeleti.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THE THESIS

This research has not been published but has been presented at the WITS Paediatric Research day on the 6th of June 2015, in Johannesburg.

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Abstract

***Background*:** Early cART reduces mortality and morbidity in HIV-infected children. Cytopaenias may develop in HIV-infected individuals, contributing to poor outcomes and few descriptions of cytopenias are available in children.

Objectives: Describe the prevalence and outcomes of cytopaenias at baseline (cART initiation) and 6 and 12 months in HIV-infected children at Chris Hani Baragwanath Academic Hospital.

Methods: Electronic and paper-based records of HIV-infected children under 3 years of age and initiating cART between the 1st January 2010 and 31st December 2012 were reviewed. Anthropometric measurements, clinical characteristics and laboratory findings at baseline, 6 and 12 months of cART were collected for analysis and compared at each outcome.

***Results*:** At baseline, the 185 HIV-infected children eligible for this study had a median age of 13 months (range 1 – 36), with advanced disease: 44% were underweight and stunted, 31% were wasted; 92% had WHO stage III/IV, CD4 count <15% in 36% and 85.5 % had a viral load ≥100 000 copies/ml. The median haemoglobin was 9.9 g/dl, white cell count 11.8 x 10^9/L and platelet count 354 x 10^9. Anaemia, thrombocytopaenia and leukopaenia were present in 80%, 6.9% and 1.2% of children respectively. At 6 months the proportion of children with anaemia decreased to 46% ($p<0.001$) and 32% ($p<0.001$) at 12 months after cART; and thrombocytopaenia decreased to 1.9% ($p<0.320$) and 0.86% ($p=0.017$).

***Conclusions*:** The majority of HIV-infected patients are anaemic at baseline. This study shows that at 6 and 12 months, there is significant reduction in anaemia and thrombocytopaenia, likely related to cART.

Study clearance certificate: Human research ethics committee (Medical) number M131042

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List of abbreviations

AIDS - acquired immunodeficiency disease syndrome
cART - combined antiretroviral treatment
CHBAH - Chris Hani Baragwanath academic hospital
DAIDS - division of AIDS
DNA - deoxyribonucleic acid
ELISA - enzyme linked immunosorbent assay
FBC - full blood count
HAZ- height for age Z-score
Hb - haemoglobin concentration
Hct - hematocrit
HIV - human immunodeficiency virus
HSCC - Harriet Shezi Children's Clinic
MANOVA - multivariate analysis of variance
MCH - mean corpuscular haemoglobin
MCHC - mean corpuscular haemoglobin concentration
MCV - mean corpuscular volume
N DoH - National department of health
NHLS - National health laboratory services
PCR - polymerase chain reaction
Plt - platelet count
PMTCT - Prevention Mother to Child Transmission
RBC - red blood cell count
RDW - red cell distribution width
SAPMTCTE- South African Prevention Mother to Child Transmission Effectiveness
SD - standard deviation
SSA - sub-Saharan Africa
T test - any statistical hypothesis test in which the test statistic follows a student's t
UNAIDS - Joint United Nations programme on HIV and AIDS
WAZ - weight for age Z-score
WBC - white blood cell count
WHO - World Health Organization
WLZ - weight for Length Z-score
Wits RHI -Wits Reproductive Health and HIV Institute

Introduction

1.1 Background

The human immunodeficiency virus (HIV) belongs to a large family of related retroviruses, known to cause immunodeficiency. In Africa 23 million people are HIV-infected constituting about 70% of the world's burden.[1] South Africa is one of the most severely affected countries, with more than 6 800 000 [6 500 000 - 7 500 000] infected persons.[2] With current Prevention Mother To Child Transmission (PMTCT) Programmes, transmission rates at the 6 week PCR test are around 2.4 % according South African Prevention Mother To Child Transmission effectiveness (SAPMTCTE).[3] Recently NHLS, through the District Health Barometer reported data showing that the early vertical transmission rate in South Africa for 2013/14 is was 2.2%, slightly lower. Over the past few years (2011-2014), the national average for Antenatal HIV prevalence was around 29.5%, with KwaZulu-Natal with highest and Northern Cape with lowest prevalence,[4] approximately 60 000 infants were born with HIV infection, and total of 360 000 children less than 15 years of age were living with HIV infection.[5]

Early combination antiretroviral therapy (cART) initiation in children, particularly those under 12 months has been included in local and international guidelines since 2010, based on findings from the CHER study which showed a 76% reduction in mortality in children initiating cART before they became symptomatic.[6] Despite this, less than half of children in South Africa who need cART, receive it. Treatment coverage for children remains a priority in South Africa and sub-Saharan Africa. In addition, the region needs to scale up programmes to diagnose, treat, and provide care and support to children living with HIV who have survived through childhood into their teens.[7] Without early cART, disease progression may be rapid in young children and over 50% of children perinatally infected will die before their second birthday. (Marston) Immune deterioration and Acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) and is characterized by progressive damage to immune system, which results in a number of opportunistic infections, immunological and haematological complications. The main immunological complication and hallmark of HIV infection is cellular CD4 T-lymphocyte depletion for which various mechanisms: HIV induced cytolysis; dysregulation of cytokines; cytotoxic T-lymphocyte responses and HIV- induced autoimmune reactions, all of which may co-exist. Haematological complications are the second most common cause of morbidity and mortality in HIV-infected individuals and are characterised by cytopaenias such as anaemia, lymphopaenia, neutropenia, and thrombocytopaenia.[8]

The full blood count (FBC) measures these abnormalities and a panel of tests examining whole blood includes the following: total white blood cell count (WBC) and differential count, red blood cell count (RBC), haemoglobin concentration (Hb), hematocrit (Hct), platelet count (Plt), red cell indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW).[9] Abnormalities of the FBC may result in clinical symptoms and signs such as weakness, fatigue, fever or bruising. The FBC may also diagnose conditions/ diseases such as anaemia, infection, and other disorders and additionally may be used to determine the stages of a particular disease. The FBC may provide evidence of infection and may assist with monitoring the response to treatment, usually a leukocytosis in children with an initial neutrophilic response to both bacterial and viral infections. If significantly abnormal FBC values are obtained, a peripheral blood smear should be prepared and examined (e.g., red cell morphology, WBC differential, platelet count estimation, identification of immature cells).[5]

Haematological abnormalities have been reported in HIV infected children and adults pre- and post-introduction of CART. These haematological manifestations of HIV are common and diverse, occurring in all stages of infection. Cytopaenias are one of the most common complications of HIV and may be broadly classified as being due either to a bone marrow production defect associated with chronic illness or to increased peripheral loss or destruction of blood cells by the HIV virus itself, adverse effect of medications such as (zidovudine) or medications to treat opportunistic infections (ganciclovir) and nutritional status.[10]

Anaemia is the most common cytopaenia and occurs in up to 95% of HIV-infected patients during their disease course, which may be due to haemolysis or bone marrow suppression. The main mechanism of anaemia of chronic disease is a disturbed bone marrow cytokine homeostasis. HIV is cytotoxic to T-helper lymphocytes, which in turn leads to dysregulation of B cells and altered release of cytokines. HIV-infected T cells directly suppress growth of bone marrow progenitors, thus suppressing haemopoeisis. CD4, the cell-surface receptor target of HIV, is carried by T-helper lymphocytes, monocytes and microvascular endothelial cells, which are prevalent in marrow.[11]

Combination ART is an important intervention in cytopaenic HIV patients, as it reduces the cytokine disturbances. However, if patients develop a new cytopaenia while taking cART, then a drug-induced cytopaenia must be considered and a change of cART regimen may be required.[12] There is a risk of anaemia among HIV-infected children on antiretroviral therapy

containing zidovudine (AZT) a drug associated with bone marrow suppression and is recommended in first-line regimens in the WHO guidelines.[13] Thus current SA guidelines recommend a baseline FBC and repeats only if receiving AZT on the 1st, 2nd and 3rd month.[14]

In the literature the haematological abnormalities of HIV are commonly described in adults and they are a significant cause of morbidity and mortality. Early intervention with cART is vital in correcting the haematological abnormalities. Descriptions of haematological abnormalities in HIV infected children are less frequent, particularly in the current era of earlier cART initiation. A study of children from Rural Uganda in November 2007 to June 2009 described a high prevalence of anaemia at ART initiation occurring in 57.6% of participants. Anaemia is associated with low CD4 count, young age and advanced WHO clinical disease.[15] Anemia is the most common hematological abnormalities in HIV patients and it is a wide spread public health problem. The World Health Organization estimates that over 2 billion people are anemic worldwide with more than 100 million of these anemic children living in Africa.[16]

It has been suggested that the impact of age at infection may be due to background mortality patterns.[17] Anaemia is associated with increased mortality in infants hence starting ART very early reduces disease progression and early mortality. Children with low haemoglobin level and delayed development shall get proper diagnosis and care such as nutritional interventions and cotrimoxazole prophylaxis should be initiated as early as possible to all eligible infants and children to reduce early mortality to reduce the risk of death.[18]

Given the paucity of data in young HIV-infected children regarding cytopaenias in the era of early cART initiatuion, this study sought to describe the prevalence and outcomes of cytopaenias at baseline (cART initiation) and 6 and 12 months in HIV-infected children at Chris Hani Baragwanath Academic Hospital.

1.2 Study approval

The study was approved by the Human Research Ethics Committee (Medical) of the University of Witwatersrand, clearance certificate number M1313042. Permission to conduct research was approved by the medical advisory committee of Chris Hani Baragwanath Hospital.

2.0 METHODS

2.1 Study design: This report was a retrospective descriptive study

2.2 Study setting, population and period: Harriet Shezi HIV Children's Clinic **(**HSCC) is a secondary/tertiary level HIV clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) that cares for HIV-infected paediatric patients that are referred from inpatient wards and surrounding clinics as result of HIV related comorbidities. Medical care, drugs and laboratory testing (including viral load testing) are provided at no cost to patients. Routine medical care includes a clinical assessment and HIV staging according to WHO at the initial visit. In this study HIV infected children under 3 years of age attending HSCC, CHBAH, Soweto from 1st January 2010 and 31st December 2012 with confirmed HIV infection were included. Testing was according to the guidelines at the time of diagnosis.[19] Children were eligible if they had a full blood count (FBC) done within 3 months of each study period including baseline (cART initiation), 6 months and 12 months. Combination ART regimens were selected according to guidelines at the time. All children received abacavir, lamivudine and lopinavir/ritonavir (LPV/r) unless there was any contra-indication to this regimen. Children co-treated with rifampicin-based tuberculosis treatment received additional ritonavir or double dose LPV/r.

2.3 Study definitions

Full blood count abnormalities in children were defined as follows: white cell count (< 4.0 or > 10) X 10^9 cells/microliter, haemoglobin < 11g/dl (-2 SD for age) or > 20g/dl and platelet count < 150 000 or > 450 000 X10^9 cells/microliter. Baseline defines FBC values of HIV infected in the study population prior initiation of cART.

2.4 Data collection and analysis

Data was collected from the HSCC database through Therapy Edge Medical Data System and file reviews were captured in MS Excel. Data was collected at baseline, 6 months and 12 months after cART initiation. Data collected included demographic information (age, sex, race), anthropometrical parameters including weight and height for age, clinical including WHO staging, significant clinical findings or diagnosis, immunological including CD4 absolute count and percentage, viral load as well as haematological test results during the study period. The captured data from microsoft excel spreadsheet was imported to statistical software where medians, means and proportions were calculated using Statistica. ANOVA was used to compare means at various time points. The haematological findings and the key

outcomes are reported in percentages along the 95% confidence intervals and significance using p-values comparing baseline, 6 months and or 12 months.

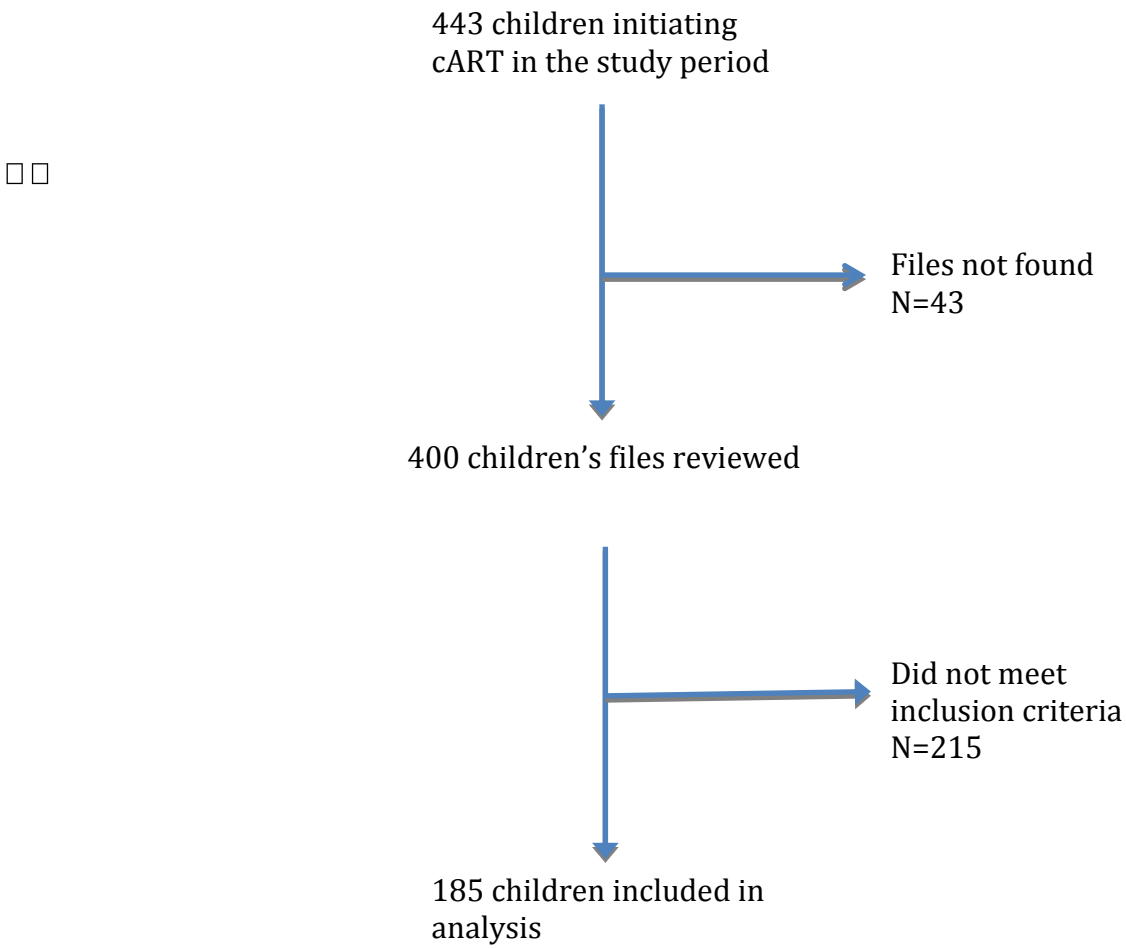
2.5 Sample size

A sample size calculation based on a preliminary audit of 45 paper-based records of infected children fitting the inclusion criteria were reviewed for the prevalence of cytopaenia, and found that 20/ 45 (44%) of children had anaemia, far greater than those with thrombocytopaenia and leukopaenia. This proportion was used to calculate the sample size Z^2 (1-p)(p)/d^2, and of note Z=1.96 & d=0.05 and an estimated sample size of 376 children to attain a targeted power of 80% was calculated. However, only 185 children in the clinic over the study period met eligibility criteria and could be included.

3.0 Results

3.1 Overall

Figure 3.1 Shows total number of children files that initiated cART in the study period down to those that fitted the inclusion criteria.



The 185 HIV-infected children eligible for this study had a mean age of 13 months (range 1–36) (Figure 3.1). At baseline 44% were underweight and stunted, and 31% were wasted (table 3.1). According to clinical staging, 92% were WHO stage III and IV and 75% of children presented with infection, 60% of which were lower respiratory tract infections. The CD4 count was <15% in 36%, and 85.5 % had viral load ≥100 000 copies/ml (table 3.2).

Table 3.1 Age, sex, anthropometry, Clinical features or diagnosis, World Health Organization (WHO) staging of HIV infection of children at baseline (n= 185)

	Number of patients (%)
Age in months, mean (SD) & Range	**14.8±9.5 (1-36)**
<12	78 (42.2)
≥12	107 (57.8)
Sex: Male	93 (50.3)
Female	92 (49.7)
Weight in kg, mean (SD) & Range	**7.84±2.54 (2.64-14.20)**
Normal (≥ -2 WAZ)	104 (56)
Underweight (<-2 WAZ)	81 (44)
Length in cm, mean (SD) & Range	**71.1±9.7 (46.0-90.0)**
Normal (≥ 2 HAZ)	104 (56)
Stunted (<2 HAZ)	81 (44)
Weight for Length: Normal (≥-2 WLZ)	128 (69)
Wasted (<-2 WLZ)	56 (31)
General	**45 (24.3)**
Failure to thrive	39 (21.1)
Encephalopathy	4 (2.2)
Eczema	2 (1.1)
Infections	**140 (75.7)**
Pulmonary TB	55 (75.7)
Community acquired pneumonia	44 (23.8)
Extra pulmonary	24 (13.0)
Pneumocystis Carinii pneumonia	6 (3.24)
Other (CMV, Septicaemia, LIP, meningitis, BCG adenitis)	11 (5.9)
WHO stage: Stage I/II	15 (8.1)
Stage III/IV	170 (91.9)

Table 3.2 Proportion of HIV infected children with leukopaenia or leukocytosis, anaemia, thrombocytopaenia, absolute CD4 count, CD4 percentages and HIV viral load of HIV-infected at baseline

Variable at baseline	Number (%)	Ranges
Haemoglobin (Hb) (g/dL) (Mean ± SD)	**9.89 ± 1.59**	**2.74-16.6**
Anaemia (Hb <11 g/dL)	140 (80.0)	
Normal (Hb =11-16 g/dL)	34 (19.4)	
Polycythaemia (Hb>16 g/dL)	1 (0.57)	
White cell count (wcc) (x 10^9/L) (Mean ± SD)	**11.8 ± 5.9**	**2.43-34.5**
Leukopaenia (wcc <4x 10^9/L)	2 (1.16)	
Normal (wcc = 4-11x 10^9/L)	104 (60.5)	
Leukocytosis (wcc >11x 10^9/L)	66 (38.4)	
Platelets (Plt) (x10^9/L) (Mean ± SD)	**354 ± 133**	**6-730**
Thrombocytopaenia (Plt <150x 10^9/L)	12 (6.86)	
Normal (Plt= 150-450x 10^9/L)	122 (69.7)	
Thrombocytosis (Plt >450**x 10^9/L)**	41 (23.4)	
Median Absolute CD4 counts (x10^6/L)	**1389**	**2-4752**
< 100	6 (3)	
100-349	28 (16)	
350-749	47 (26)	
>750	100 (55)	
Median CD4 count percentage	**28**	**3.8 - 61**
<15%	65 (36)	
16-25%	59 (33)	
>25%	57 (31)	
Median HIV Viral load	**741690**	**40-23600000**
<1000	3 (1.7)	
1000 -99 000	31 (17.8)	
≥100 000	140 (80.5)	

At baseline the prevalence of haematological abnormalities in HIV infected children were as follows- anaemia 80%; leukocytosis 38%, thrombocytosis 23%, thrombocytopaenia 7% and, leukopaenia 1%. At baseline anaemia was present in 80% of children, with a mean value of 9.78 g/dl, improving to a mean 10.9 g/dl at 6 months and 11.3 g/dl at 12 months (p-value <0.001 respectively). There was no difference in mean platelet levels at baseline, with a mean of 346 000 X 10^9 cells/microliter, compared to 371 000 X 10^9 cells/microliter at 6 months and 375 000 X 10^9 cells/microliter at 12 months with (p= 0.32 and 0.19 respectively). The proportion of children with anaemia improved to 45.8% at six and 32.2% at 12 months (p=<0.001). Thrombocytopaenia was present in 6.9% of children at baseline, 1.93% at 6 months (p=0.32) and 0.86% at 12 months (p=0.017). At baseline the mean WCC was 11.8 X 10^9 cells/microliter, 1% of children had leucopenia, improving to 0.5% at 6 months (table 3.3).

Table 3.3 Proportion of patients who had leukopaenia or leukocytosis, anaemia and thrombocytopaenia at baseline compared to after 6 and 12 months of treatment (cART)

Variable	No. of patients (%) at baseline	No. of patients (%) after 6 months of cART	No. of patients (%) after 12 months of cART	Baseline vs 6 months	Baseline vs 12 months
Haemoglobin (Hb) (g/dL)	**9.78 ±1.55**	**10.9±1.3**	**11.3 ±1.0**	<0.001	<0.001
Anaemia (Hb <11 g/dL)	140 (80.0)	76 (45.8)	39 (32.2)	<0.001	<0.001
Normal (Hb =11-16 g/dL)	34 (19.4)	90 (54.2)	82 (67.8)		
Polycythaemia (Hb>16 g/dL)	1 (0.57)	0 (0)	0 (0)		
White cell count (wcc) (x 10^9/L)	**11.7 ±6.2**	**10.1 ±4.2**	**9.91±3.7**	<0.001	0.006
Leukopaenia (wcc <4x 10^9/L)	2 (1.16)	1 (0.64)	0 (0)	0.387	0.500
Normal (wcc = 4-11x 10^9/L)	104 (60.5)	102 (65.4)	82 (70.1)		
Leukocytosis (wcc >11x 10^9/L)	66 (38.4)	53 (34.0)	35 (29.9)		
Platelets (Plt) (x10^9/L)	**346 ±148**	**371±106**	**375±108**	0.319	0.189
Thrombocytopaenia (Plt <150**)**	12 (6.86)	3 (1.93)	1 (0.86)	0.032	0.017
Normal (Plt= 150-450**)**	122 (69.7)	120 (77.4)	86 (74.8)		
Thrombocytosis (Plt >450**)**	41 (23.4)	32 (20.6)	28 (24.3)		

The secondary outcomes shown (table 3.4) anthropometrical improvements at 6 and 12 months with around 44% of children underweight at baseline, improving to 21% at 6 months and 14% at 12 months (p-value<0.001). Length for age at baseline showed less recovery with similar numbers of children stunted at each time point. Weight for length also showed

improvement with 21% of children wasted at baseline, improving to 12% at 6 months, maintained through 12 months (p-value =<0.001).

Table 3.4 Nutritional growth parameters from baseline to 6 and 12 months of cART

	Number of patients (%) at baseline	**Number of patients (%) after 6 months of HIV treatment**	**Number of patients (%) after 12 months HIV of treatment**	**Comparison between baseline and 6 months (p-values)**	**Comparison between baseline and 12 months (p-values)**
Weight	**7.84±2.55**	**9.85±2.26**	**11.3±2.04**		
Normal	104 (56)	143 (79)	126 (86)	<0.001	<0.001
Underweight	81 (44)	36 (21)	21 (14)	<0.001	<0.001
Length	**71.1±9.7**	**77.3±7.6**	**82.12±6.3**	<0.001	**<**0.001
Normal	104 (56)	102 (56)	76 (52)		
Stunted	81 (44)	78 (44)	71(48)	0.930	0.412
Weight for length					
Normal	129 (69)	163 (88)	139 (88)	<0.001	<0.001
Wasted	56 (21)	17 (12)	8 (12)		

CD4 absolute count and percentage improved significantly on cART, with CD4 absolute count and percentage increasing from baseline 976 cells/mm^3 and 20% to 1586 cells/mm^3 and 28% at 6 months and 1721 cells/mm^3 and 30% at 12 months follow up (p=< 0.001) (Table 3.5). The viral load copies reduced from a mean of 741690 copies/ml at baseline to 327.5 copies/ml at 6 months and 125 copies/ml at 12 months as shown in table 3.5. Despite this steady improvement, the proportion of children with viral loads < 1000 copies/ml at 6 months was 36.7% and at 12 months 43.2% (p=< 0.001).

Tabl 3.5 Comparisons in changes in CD4 absolute counts, CD4 percentages and HIV viral load at baseline and after 6 months and 12 months of cART

Variable	Number of patients (%) at baseline	Number of patients (%) after 6 months of cART	Number of patients (%) after 12 months of cART	Comparison between baseline vs 6 months	Comparison between baseline vs 12 months
CD4 Absolute Count (x10^6/L)	**976±742**	**1586±917**	**1721±782**	<0.001	<0.001
< 100	6 (3)	1 (1)	0 (0)	0.062	0.040
100-349	28 (16)	3 (2)	0 (0)		
350-749	47 (26)	17 (10)	8 (6)		
>750	100 (55)	146 (87)	125 (94)		
CD4 Percentage	**20.3±11.7**	**28.0±9.8**	**30.6±9.8**	<0.001	<0.001
<10%	31 (17)	5 (3)	1 (1)	<0.001	<0.001
10-15%	34 (19)	13 (7)	6 (4)		
16-25%	59 (33)	47 (26)	33 (25)		
>25%	57 (31)	102 (56)	93 (70)		
Viral load copies/ml median (Range)	**741690 (40-23600000)**	**327.5 (40-8832505)**	**125 (40-8832505)**	<0.001	<0.001
< 1000	3 (1.7)	61 (36.7)	61 (43.2)	<0.001	<0.001
1000 – 99 000	30 (17.3)	67 (40.4)	62 (44)		
≥100 000	140 (81.0)	38 (22.9)	18 (12.8)		

3.2 Discussion

In this study over 80% of HIV infected children have anaemia at baseline, which improves to 45.8% at 6 months and 32% at 12 months after cART initiation. Abnormalities in the other cell lines were less impressive at baseline with 6.9% having a thrombocytopaenia improving to 1.9% at 6 months and 0.9% at 12 months; and leukopaenia at baseline 1.2% improving to 0.6% at 6 months and none at 12 months after cART initiation. After 6 months of being on treatment the mean haemoglobin value improved significantly to 10.9 g/dl, p value <0.001

and the mean white cell count improved from baseline to 6 months p value <0.001 with not much difference at 6 and 12 months whereas mean platelets value remained normal at baseline, 6 months and 12 months after cART initiation. These findings are similar to those from a Belgian in 2001 by J Servais et al and also the recent Kenyan study of 2015 by EG Kibaru showing improvements in HIV associated haematologic disorders in adults at 6 months after cART initiation.[20,21] Multiple factors contribute to haematological abnormalities and each case needs careful consideration, with a meticulous, logical approach, including consideration of all possible contributors such as haematinic deficiencies and bone marrow infiltration.[12] The improvements in anaemia may have been due to a number of factors including treatment of infections, receiving supplementation with folate and iron and of course cART itself. Additionally, children accessing health care at HSCC may have been referred for nutritional support, may have been referred to access financial grant support which may have improved the family's nutritional and socioeconomic situation.

Children receiving cART also demonstrate improvement of other clinical and immunological parameters over time. Anthropometrically, there is an improvement in WAZ at each timepoint and for WFH improvement at 6 months which then plateaus at 12 months. At baseline there was stunting and underweight in 44% of children each, and wasting in 12% all which improved at 6 months except for the stunting and this is shown in other studies including the 2012 South African study by K Meera et al and Meyers et al in 2012 where the mean WAZ increased rapidly from −2.40 (95% CI: −2.48, −2.33) at baseline, to −1.40 (95% CI: −1.46, −1.33) at 12 months and continued to improve to −1.18 (95% CI: −1.29, −1.07) at 36 months but the improvement in the mean HAZ was less impressive, plateauing over time.[22,23] Additionally, Mary-Ann Davies at al showed an improvement in nutritional status in a longitudinal study between 2005 to 2010 where the number of children with WAZ <−3 decreased from 31 to 28% (p<0.001) over time and other nutritional indices showed similar trends.[24]

Immunologically, the CD4 count mean increased from 20.3% to 28% & 30.6% and the viral load copies reduced from a mean of 741690 copies/ml at baseline to 327.5 copies/ml at 6 months and 125 copies/ml at 12 months as shown in table 3.5. Despite this steady improvement, the proportion of children with viral loads < 1000 copies/ml at 6 months was 36.7% and at 12 months 43.2% (p=< 0.001) which is concerning but has been described in other studies describing viral load outcomes in young children who appear to take longer than 12 months to suppress virologically.[23]

Violari et al from the CHER study in 2008, showed similar immunological and virological responses to cART in young children. [6] Similarly, Davies et al showed a steady improvement in clinical outcomes, immunological outcomes in HIV-infected children, with those with severe WHO Stage III/IV disease decreasing from 75 to 62% in 6 months ($p<0.001$) and those with severe anemia decreasing from 12 to 7% ($p<0.001$). CD4% count also increased with a decrease in those with severe immunosuppression from 81 to 63% ($p<0.001$).[24]

In summary factors associated with anaemia in HIV infected children were multifactorial in nature.[25] Although our cohort had a high baseline prevalence of anaemia, cART initiation had an impact on improving cytopaenias, particularly anaemia and thrombocytopaenia in HIV infected children by 6 months, sustained through 12 months. Thrombocytosis and leukocytosis were common in this population, resolving over 12 months. There was also a significant improvement in nutritional and growth parameters and immunological response measured by CD4 count, and a reduction in viral load. This improvement may not only have been due to cART but it is also likely that many children would have received antibiotics and adjunctive treatment like multivitamin, folate and iron supplements, nutritional support and possible social support through increased access to child support grants.

3.3 Limitations

This study has some limitations. Firstly, there was a low total number of HIV infected children with baseline data mainly because of missing data elements on a number of variables. We were unable to include the number of children calculated by sample size for optimal analytical power, 376 children, but as 185 children were eligible, they were included even though this reduced the study power. This is a result of lower numbers of HIV infected children with effective PMTCT but also because SA DoH guidelines do not recommend follow-up FBC monitoring and children without follow-up FBC results were excluded from analysis.

The data was retrospectively analysed from a data base and paper based records and hence was reliant on accurate capturing which was not always the case. Particularly, data on co-morbidities, concomitant medications such as folate, iron supplementation and antibiotic treatment was not always well recorded so may be underrepresented however, the results are statistical significant and clinically there is improvement in both primary objectives and other outcomes in HIV infected children on cART.

3.4 Recommendations

Current SA NDoH guidelines recommends baseline FBC, repeated at 6 and 12 months if the child is receiving AZT and the improvement in each time point is reassuring. Given the high numbers of children with anaemia at baseline, it is important that a baseline FBC remains a recommendation in guidelines and that possible causes of anaemia are investigated and treated in HIV infected children. Increased effort required to test and diagnose HIV early in infants to initiate ART early and prevent associated morbidity and mortality in children.

3.5 Conclusions

Anaemia at cART initiation is common, occurring in 80% of children. Once cART was initiated an improvement, likely partly due to cART and improved clinical health, along with other factors was seen through 6 and 12 months. Additionally, children had improved anthropometrical measures, immunological parameters and viral load suppression, highlighting the positive impact of cART in young children. Ongoing efforts to diagnose and initiate cART early are likely to reduce these baseline cytopaenias improving children's cART outcomes even further.

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APPENDIX A: TURNIT IN REPORT

Turnitin Originality Report
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APPENDIX B: ETHICS CLEARANCE CERTIFICATE

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131042

NAME: (Principal Investigator)	Dr Theodore M Mabaso
DEPARTMENT:	Department of Paediatrics CH Baragwanath Academic Hospital
PROJECT TITLE:	A Description of the Full Blood Count Abnormalities in Human-Immunodefiency Virus Infected Children Less than Three Years of Age Before and After Being on Highly Active Antiretroviral Treatment
DATE CONSIDERED:	25/10/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Lee Fairlie
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/10/2013

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 Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized 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 the application to the Committee. **I agree to submit a yearly progress report**

Principal Investigator Signature M131042Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX C: PROTOCOL PAPER

Research protocol

Title: A description of the full blood count abnormalities in HIV-infected children less than three years of age before and after HAART initiation.

Investigator: Theodore Mlungisi Mabaso
MBCHB (Villa Clara-Medunsa)

Student Number: 9103081y
Degree: MMed (Paediatrics)

Supervisor: Dr Lee Fairlie
Consultant in Paediatrics
Wits Reproductive Health and HIV Institute (Wits RHI)
University of Witwatersrand Johannesburg
Co-Supervisor: Prof. Sithembiso Velaphi
Paediatrics head of department
Chris Hani Baragwanath Academic hospital (CHBAH)

Table of Content **page**

List of abbreviations

AIDS -acquired immunodeficiency disease syndrome

CHBAH -Chris Hani Baragwanath academic hospital

DAIDS –division of AIDS

DNA – deoxyribonucleic acid

ELISA – enzyme linked immunosorbent assay

FBC – full blood count

HAART - highly active antiretroviral treatment

Hb - hemoglobin concentration

Hct - hematocrit

HIV -human immunodeficiency virus

HSCC - Harriet Shezi HIV children clinic

MANOVA –multivariate analysis of variance

MCH - mean corpuscular hemoglobin

MCHC - mean corpuscular hemoglobin concentration

MCV - mean corpuscular volume

NDoH – National department of health

NHLS - National health laboratory services

PCR – polymerase chain reaction

Plt - platelet count

PMTCT -Prevention Mother to Child Transmission

RBC - red blood cell count

RDW - red cell distribution width

SAPMTCT - South African Prevention Mother to Child Transmission

SAPMTCTE- South African Prevention Mother to Child Transmission Effectiveness

SD – standard deviation

SSA - sub-Saharan Africa

T test –any statistical hypothesis test in which the test statistic follows a student's t distribution if the null hypothesis is followed, used to compare to sets of data.

UNAIDS –Joint United Nations programme on HIV and AIDS

WBC - white blood cell count

Wits RHI -Wits Reproductive Health and HIV Institute

Background

The human immunodeficiency virus (HIV) belongs to a large family of related retroviruses, known to cause immunodeficiency. In Africa 23 million people are HIV-infected constituting about 70% of the world's acquired immunodeficiency disease syndrome (AIDS) burden. South Africa is one of the most severely affected countries, currently having more than 5.6 million infected persons.[1] In 2009, 29.4 percent of all pregnant women were HIV infected, approximately 60 000 infants were born with HIV infection, and total of 330 000 children less than 15 years of age were living with HIV infection.[2] With the current Prevention Mother To Child Transmission (PMTCT) programmes, transmission rates at 6 week PCR test are around 3.5% according South African Prevention Mother To Child Transmission effectiveness (SAPMTCTE).[3] Every day in sub-Saharan Africa, about 1000 children acquire HIV-1 infection from their mothers. In 2007 it was estimated that 370 000 children became HIV infected globally, 270 000 children under 15 years of age died from HIV/AIDS (UNAIDS).[1] Almost 90 percent of all HIV infected children live in sub-Saharan Africa (SSA), with an estimated 2.2 to 2.3 million HIV infected children of which about 420 000 children are South Africans.[1]

Earlier HAART initiation in children particularly those under 12 months has been part of local and international guidelines since 2010, based on findings from the CHER study which showed a 76% reduction in mortality in children initiating HAART before they became symptomatic.[4] Among the estimated 2.2 to 2.3 million children younger than 15 years living with HIV in 2011, only 425 000 were receiving treatment. Treatment coverage for children must improve as a matter of urgency. In addition, the region needs to scale up programmes to diagnose, treat, and provide care and support to children living with HIV who survived through childhood into their teens.[5]

Acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) and is characterized by progressive damage to the body's immune system, which results in a number of opportunistic infections, immunological and haematological complications. The main immunological complication and hallmark of HIV infection is cellular CD4 T-lymphocyte depletion for which various mechanisms: HIV induced cytolysis; dysregulation of cytokines; cytotoxic T-lymphocyte responses and HIV induced autoimmune reactions which are not mutually exclusive have been suggested. Haematological complications have been documented to be the second most common cause of morbidity and

mortality in HIV patients and are generally marked with cytopenias such as anaemia, neutropenia, lymphopaenia and thrombocytopenia.[6]

The full blood count (FBC) consists of a panel of tests that examines whole blood and includes the following: total white blood cell count (WBC) and white blood cell differential, red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), platelet count (Plt), red cell indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW).[7]

The FBC provides important information about the types and numbers of cells in the blood, especially red cells, white cells, and platelets. Abnormalities of the FBC may result clinical features features such as weakness, fatigue, fever or bruising. The FBC may also diagnose conditions/ diseases such as anemia, infection, and other disorders and additionally may be used to determine the stages of a particular disease. A FBC is frequently obtained for evidence of infection and the initial response to infection, especially children, is leukocytosis with an initial neutrophilic response to both bacterial and viral infections. In general, bacterial infections are associated with greater neutrophil counts than viral infections. Transient lymphopaenia at the beginning of illness and lasting 24 and 48 hours has been described with many viral infections. If significantly abnormal FBC values are obtained, a peripheral blood smear should be prepared and examined (e.g., red cell morphology, WBC differential, platelet count estimation, identification of immature cells).[2]

Haematological abnormalities have been reported in HIV infected children and or adults' pre and post introduction of highly active antiretroviral treatment (HAART). These haematological manifestations of HIV are common and diverse, occurring at all stages of infection. Cytopenias are one of the most common complications of HIV and may be broadly classified as being due either to a bone marrow production defect associated with chronic illness or to increased peripheral loss or destruction of blood cells by the HIV virus itself, adverse effect of medications (zidovudine) or medications to treat opportunistic infections (ganciclovir) and nutritional status.[8]

Anaemia is the most common cytopaenia and occurs in up to 95% of HIV patients during their disease course which may be due to haemolysis or bone marrow suppression. The main mechanism of anaemia of chronic disease is a disturbed bone marrow cytokine homeostasis.

HIV is cytotoxic to T-helper lymphocytes, which in turn leads to dysregulation of B cells and altered release of cytokines. HIV-infected T cells directly suppress growth of bone marrow progenitors, thus suppressing haemopoeisis. CD4, the cell-surface receptor target of HIV, is carried by T-helper lymphocytes, monocytes and microvascular endothelial cells, which are prevalent in marrow.[9]

HAART is an important intervention in cytopaenic HIV patients, as it alleviates the cytokine disturbances. However, if patients develop a new cytopaenia while taking HAART, then a drug-induced cytopaenia must be considered and a change of HAART regimen may be required.[10]

JUSTIFICATION OF THE STUDY

In the literature the haematological abnormalities of HIV are commonly described in adults and they are a significant cause of morbidity and mortality. Early intervention with HAART is vital in correcting the haematological abnormalities associated with poor outcome. Descriptions of haematological abnormalities in HIV infected children are scarce, particularly in the era of earlier HAART initiation. Recently HAART is started much earlier than before, the effect of this early HAART on bone marrow has not been well documented regarding FBC changes.

STUDY AIM & OBJECTIVES

a. Aim:

The aim of the study is to describe the prevalence of haematological abnormalities pre-and post HAART in HIV infected children attending Harriet Shezi HIV Children Clinic (HSCC), Chris Hani Baragwanath Academic Hospital (CHBAH) SOWETO.

b. Objectives

1. To describe the baseline demographics and clinical characteristics of HIV infected children under the age of three initiating HAART at HSCC.
2. To describe the prevalence of anaemia, thrombocytopenia and white cell count changes in children pre-and post HAART at HSCC.
3. To determine the changes in anemia, thrombocytopenia and white cell count changes of HIV infected children who have been on HAART for 6 months and 12 months.

METHODOLOGY

a. Study design

A retrospective observational descriptive study.

b. **Study setting**

The HSCC is a secondary/tertiary level HIV clinic at Chris Hani Baragwanath Academic Hospital that cares for HIV-infected paediatric patients that are referred from inpatient wards and surrounding clinics as result of HIV related comorbidities.

c. Study Population & period

This study includes HIV-infected children under the age of 3 years attending HSCC, CHBAH SOWETO between the 1st January 2010 and 31st December 2012.

Standard of care for HAART initiation : During the study period management of children Harriet Shezi children clinic was according to the NDoH Guidelines as follows:

1) Children HIV infected (PCR positive) under 1 year of age regardless of CD4 or disease stage
2) Children between 1 to 5 years WHO stage III, IV or CD4 percentage < 25 or absolute count < 750 cells/mm^3.
3) Children over 5years WHO stage III, IV or CD4 percentage <15 or absolute count<350 cells/mm^3. [11]

(i) Inclusion criteria

- Confirmed HIV diagnosis by a positive HIV DNA-PCR test in a child under 18 months or a positive HIV ELISA in a child over 18 months of age. .
- Children initiating HAART during the study period with a baseline FBC (within 3 months before initiation) and at least 1 repeat FBC done at 6 months or 12 months or both.

(ii) Exclusion criteria

- HIV infected children under 3 years of age who were already receiving HAART before referral HSCC during the study period.

d. Definition of haematological abnormalities:

For this study the criteria for full blood count abnormalities in children is a reading of white cell count < 4.0 or > 10 cells/microliter, haemoglobin <11g/dl (-2SD for age) or haematocrit < 0.35 (-2SD), haemoglobin > 20g/dl or haematocrit > 0.60, lymphopaenia < 3cells/microliter or CD4 < 15% & platelets <150 000 or > 450 000 cells/microliter.[12] DAIDS grading tables will be used to grade haematologic abnormalities.

e. Data collection

A list of haematological/ laboratory FBC findings from HIV infected children under 3 years of age initiating HAART will be obtained from HSCC data system. Information that will be obtained from this data base, includes the demographic and clinical data, such as age, weight, height, sex, race, clinical presentation, clinical diagnosis, CD4 count and viral load during the study period.

f. Data handling

Information obtained from HSCC database will be captured in Microsoft Excel spread sheet and data sheet. In STATA the data sheet will contain FBC results (pre-and post HAART) limited demographic & clinic data. Anonymity will be ensured, by allocating each case study patient in the data collection sheet a number which will only be linked to patient identifiers in a password protected document.

g. Data processing and analysis

Data captured from Microsoft Excel spreadsheet will be imported to statistical software where all data management and statistical analysis will be performed. Appropriate descriptive statistical analysis will be carried out means and standard deviations will be used in continuous variables with normal distribution and median or quartiles will be used for non parametric continuous variables and categorical variables will be described using percentages.

The haemtological findings and other key outcomes will be reported using percentages along with 95% confidence intervals. In comparing the parameters before and after 6months of HAART the T test will be used and in comparing further to include period after 12 months of HAART an MANOVA will be used.

Implication of this study

This study will provide information to clinicians about the pre-HAART and post-HAART FBC abnormalities in HIV-infected children and will provide a description of these abnormalities and associated comorbidities.

Challenges

For the purpose of this study, haematological abnormalities of the full blood count, only includes presence of anemia, thrombocytopenia and white cell count changes. The other full blood count abnormalities such as reticulocytes & total red cell distribution width values have not been considered. Some or most of the laboratory full blood count results did not include differential count. As this study is a retrospective one it might be difficult to obtain all required data due to transcription errors in patient file numbers captured incorrectly making it difficult finding and retrieving results of FBC or to accessing the laboratory data.

Ethics approval

This study was approved by: The Human Research Ethics Committee of the university of the Witwatersrand and the Ethics clearance certificate number is M131042 and also the hospital CEO of CHBAH granted the permission to conduct the study in HSCC and make use of their data base. This data will only be used for the purpose of the study. No consent will be required from the parents since it is a retrospective study.

Budget

The cost (transport to & fro, stationery, printing, photocopying & binding) and funding of the study will be borne by the researcher +/- R1500.

Project Time Frame

Year	Aug	Sep	Oct	Nov	Dec	jan	feb	Mar	April To July
Research Protocol									
Protocol Assessment & etihics approval									
Data Collection									
Data Analysis									
Write up									

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APPENDIX D: DATA CAPTURING SHEET

Hospital #: _____ TE number: 64-

		Baseline (ART start day)	6months	12months
Demog	Date of Birth			
	Race (1=African; 2=Non-African)			
	Sex (1=Male; 2=female)			
	Date of 1st visit			
	Date HAART started			
	Date of Visit			
	Age			
	Weight (kg)			
	Height (cm)			
	BMI			
WHO	Clinical stage			
CD4	Absolute			
	Percentage			
	Date			
VL	VL			
	VL Date			
FBC	Wcc			
	Hb			
	Mcv			
	Plt			
	Neutrop			
	Lymph			
	FBC Date			
LFT	ALT			
	AST			
	LFT Date			
TB	AFB			
	Culture			
	TB Expert			

	Date			
	Reactive PPD (Y/N)			
ARV	(1) abc/3tc/kal (2) d4t/3tc/kal (3) d4t/3tc/kal/rit (4) abc/3tc/kal/rit			
Rx	(1) Bactrim			
	(2) MVT			
	(3) Anti-TB			
	(4) Other			
	If other specify			
Mom PMTCT	(1) Mom-AZT & NVP (2) Mom HAART (3) None			
Child PMTCT	(1) Child-NVP (2) Child-AZT & NVP (3) None			
Feeding history:	(1) Excl breastmilk (2) Formula fed (3) Mixed feeds (4) Unknown			
Dx & clin pres	Pneumonia (√)			
	PCP/PJP (√)			
	CMV (√)			
	GE (√)			
	Sepsis (√)			
	MAC (√)			
	Parvovirus (√)			
	EBV (√)			
	Hepatitis B (√)			
	Other, specify			
Admin	Date of completion			
	Date of capture			
	Captured by			

Comments:

APPENDIX E: PLAGIARISM DECLARATION

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY:

I, Theodore Mlungisi Mabaso (Student number: 9103081Y) am a student

registered for the degree of MMED IN PEDIATRICS in the academic year 2012.

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.

Signature: _____ Date: _____