FACTORS AFFECTING HAEMATOLOGICAL PARAMETERS BEFORE AND AFTER INTRODUCTION OF DOLUTEGRAVIR IN HIV-INFECTED CHILDREN AT GETRUDES CHILDRENS HOSPITAL COMPREHENSIVE CARE CLINIC

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DECLARATION

I certify that this dissertation is my original work and has not been presented or published by any other university.

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SUPERVISORS' APPROVAL

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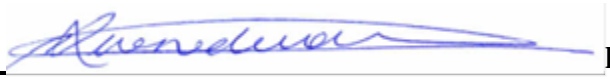
Signature:  **Date:** 22/05/2023

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DEDICATION

To my family members for their patience, perseverance and unwavering support.

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LIST OF ABBREVIATIONS

AIDS – Acquired Immunodeficiency Syndrome

HIV – Human Immunodeficiency Syndrome

ART – Anti-Retroviral Therapy

HAART – Highly Active Antiretroviral Treatment

CCC – Comprehensive Care Center

AZT – Zidovudine

d4T – Stavudine

NVP – Nevirapine

3TC- Lamivudine

EFV- Efavirenz

DTG- Dolutegravir

LPV/r- Lopinavir/ritanovir

CALHIV- Children and Adolescents Living with HIV

PLHIV- People living with HIV

MTB- Mycobacteria Tuberculosis

MAC- Mycobacterium Avium Complex

Hb- Haemoglobin

TWBC-Total White Blood Cell Count

PC(J)P –Pneumocytis carnii/jirovecii Pneumonia

IPT-Isoniazid Preventive Therapy

MUAC- Mid Upper Arm Circumference

W/H – Weight for height

PRCA- Pure Red Cell Anaemia

PCR- Polymerase Chain Reaction

MCV – Mean Corpuscular Volume

ITP- Immune Thrombocytopenic Purpura

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ABSTRACT

Haematological abnormalities frequently occur in HIV-infected individuals and are independent risk factors for mortality. Haematological abnormalities are a result of HIV infection and adverse drug reactions from antiretroviral therapy. Global research of antiretroviral therapies has led to the development of safer and less toxic antiretroviral drugs enabling withdrawal of the more toxic first-generation drugs. This study seeks to describe the haematological features of HIV-1 infected children on current combination antiretroviral medication.

Objectives: To describe and determine the prevalence of abnormal haematological parameters in children 0-18years who are on follow-up at Getrude's children's Hospital before and after switching to dolutegravir based protocols. Secondarily to describe demographic and clinical factors influencing these changes.

Methodology: Retrospective cohort study among HIV-infected children 0-18years on Antiretroviral Therapy at Gertrude's Children's Hospital Nairobi between January 2019-December 2022..

Results: Medical records of217 children meeting inclusion criteria were enrolled. The median age for diagnosis in less than 5 years was 10.8 months, between 6-12 years was 23.4months and in the over 13years was 34.4 months with a higher female to male ratio in the under 5s, and 6-12 year olds. Six months after switching to dolutegravir, haemoglobin levels had significant increase 0.001, MCV and MCH had a significant decrease of 0.01 and 0.02 respectively in the over 13 years. The prevalence of leucopenia rose significantly in the 6-12 age group and over 13 years from 10 to 21.6% and 27.6 to 42.1% respectively, neutropenia declined significantly in all age groups (under 5s 80 to 20% p 0.03, 6-12 21.6 to15% p 0.02 ,over 13 30.3 to 18.4% p 0.001) There was non-significant increase in the white cell count in 6-12age bracket and over 13. An increase in neutrophil count was significant at p 0.02 in the 6-12 years, the prevalence of thrombocytosis and thrombocytopenia after switch in the 6-12 age group and the over 13years declined significantly from 11.7 % to1.7% (p 0.03) and 3.9% to 1.3% (p 0.02) respectively with a non-significant increase of platelet count in the under 5s and 6-12 age group and decline in the over 13years. The prevalence of eosinophilia and eosinopenia declined significantly in the 6-12 years from 3.3 to 1.7% and for the over 13 years from 13.6 to 6.6% respectively and significant increase

in eosinopenia was seen in 6-12 age bracket from 1.7% to 10%. The eosinophil count had a non-significant increase in the under5s and over 13 years.

Conclusion: Heamatological parameters improved in all parameters except lymphocytes.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Acquired Immunodeficiency Disease (AIDS) is a chronic systemic disorder caused by Human Immunodeficiency Virus (HIV) characterized by a chronic immune activation that causes significant dysfunction and gradual damage to both humoral and cellular immune responses (1). Mother-to-child transmission of HIV can occur transplacentally, perinatally, and via breast milk. Without any intervention, at birth, the rate of transmission is 25-35%. Those who previously escaped infection at birth can later acquire HIV infection through the breast milk of an HIV positive mother without viral suppression 10-15% (2). According to 2019, UNAIDS DATA 2020, 1.9 million children were living with HIV, 90% of whom were from Sub-Saharan Africa. There were 150,000 new infections in children under the age of 15years, with 53% receiving antiretroviral therapy and 40% achieving viral suppression. The number of AIDS-related deaths in children less than 15years stood at 95000 (3). According to UNAIDS DATA 2020, Kenya was estimated to have 110,000 HIV-infected children with 6,800 new infections. In the same year, 4,300 AIDS-related mortality in children aged 0-14 years were reported (3).

Different forms of haematological abnormalities have been reported in both highly active antiretroviral treatment(HAART) naïve and HAART treated individuals with pancytopenia being the most significant. Anaemia is also the most common complication in HAART naïve children (4). Studies showed the prevalence of anaemia in treatment naïve children in Nigeria to be 38.2% while Kenya, India, and Tanzania showed a prevalence of 35.9%, 69% (5), and 44% respectively (6). Neutropenia affects about 10% of patients in the early asymptomatic HIV stages and far more than 50% of those with more advanced HIV infection. Commonly asymptomatic thrombocytopenia affects 20% to 33% of HIV-infected children, with the prevalence increasing with disease duration and Aids progression (7).

Haematological abnormalities which occur among HIV-infected children are strong independent risk factors for mortality (8), (9), and disease progression. According to W.H.O classification, clinical stage 3, defines unexplained **anaemia as haemoglobin less than 8gdl,** neutropenia less

than 500cells/microL, and or chronic thrombocytopenia of less than 50,000cells/microL for more than one month (10) as shown in appendix 2.

In 1990, W.H.O clinical staging for HIV/AIDS highlighted the use of clinical criteria to aid clinical decision-making(when to start, switch, stop antiretroviral therapy, assess clinical status, and response to treatment) in HIV/AIDS patients (10).

To prevent vertical transmission of HIV, WHO guidelines 2016 recommended the use of combined Zidovudine /Nevirapine prophylaxis for 6weeks (11) in infants. In 2019 WHO antiretroviral therapy guidelines dolutegravir was substituted for nevirapine and efavirenz for children less than 20kg but more than four weeks old (12). Dolutegravir is being widely used globally because it has fewer adverse effects, is more tolerable, has fewer drug to drug interactions, has a stronger genetic barrier to resistance, and is cheaper than other antiretroviral therapy regimens (13). There have been few studies conducted on the link between dolutegravir and haematological abnormalities in children (14).

This study seeks to describe the haematologic features of HIV-1 infected children on current combination antiretroviral therapy and to compare the status before and after the introduction of dolutegravir based combination antiretroviral therapy protocols. The study will also explore the factors associated with deranged haematological parameters. Reference standards in the Division of AIDS(DAIDS) table will be used to classify the haematologic parameters.

CHAPTER TWO

2.0 LITERATURE REVIEW

Acquired Immunodeficiency Disease (AIDS) is a chronic systemic disease caused by HIV infection, characterized by chronic immune activation that causes significant dysfunction and gradual damage to both cellular and humoral immune responses (1). Two severe effects of HIV infection are as follows:

Damage to the immune system specifically by loss of CD4+ lymphocytes and

Chronic immune activation

CD4+ lymphocytes play a role in both humoral and cell-mediated immunity. CD4+ depletion can occur as a result of HIV replication's direct cytotoxic effects, cell-mediated immune cytotoxicity, and thymic damage that hamper lymphocyte synthesis. CD4+ lymphocyte destruction rate is proportional to the plasma HIV levels (2). Early childhood infection is characterized by B cell hyperplasia causing hypergammaglobulinemia with elevated levels of anti-HIV antibodies reflecting both dysregulations of T cell suppression of B cell antibody synthesis as well as active CD4 enhancement of B lymphocyte (15).

HIV can infect a wide range of cells, it spreads beyond lymphoid organs to the lungs, gut, liver, central nervous system and kidneys occur late in the disease. During the first few weeks of infection, both humoral and cellular immune responses occur. Cell-mediated immunity is an important way of managing high levels of viral load in the early stages of infection, which later stabilize at a level (setpoint) at about 6months. The higher this setpoint, the faster the CD4 count drops to a point that substantially compromises immunity and culminates in the opportunistic infections and malignancies that characterize AIDS (2).

2.1 Classification of Anaemia

The Division of AIDS(DAIDS)(16) grading table as shown in Appendix 4

provides a meaningful uniform classification of anemia which will allow for more accurate comparisons of the prevalence and clinical outcomes of anemia in people living with HIV, it is made up of parameters, or adverse events, with a severity graded scale ranging from grade 1 to 5,

Grade 1 signifies a mild event, Grade 2 a moderate event, Grade 3 a severe event, Grade 4 a potentially life-threatening event and Grade 5 signifies death.

Neonatal haematological measurements differ greatly from those of children and adults. During the initial hours and days following delivery, the newborn infant's bone marrow and blood undergo dramatic changes, with rapid change in all levels of hematologic components. Most of the haematological parameters tested on the first day of life are elevated, particularly haemoglobin concentration, packed cell volume, reticulocyte count, and red cell indices. Between the third day and sixth weeks of life, a subsequent gradual decline is seen. Contributing factors to this decline is a drop in blood erythropoietin concentration shortly after birth, which reduces the erythropoietic rate and transient heamolysis common in the first few days or weeks after birth(18)explains the different ranges in haematological parameters as seen in the DAIDS table.

Table 1: Studies on Haematological Parameters in HIV Infected Children

Title, Author, Year	Type of ARV exposure – treatment, prophylaxis	Study population	Study design	Key haematologic findings
Risk factors for anaemia among HIV infected children attending care and treatment clinic at Muhimbili National Hospital in Dar es Salaam, Tanzania Pius Magesa et al 2012	AZT based and non-AZT based regimens	children aged 6 months to 59 months	cross-sectional study	Anaemia 44% Not being on HAART, CD4% less than 25%, history of TB, and hookworm infestation were independent factors for anaemia in children. Being positive more than or equal to 2.5 years had a low-risk factor for severe anaemia compared to being positive for less than 2.5years

Haematological and immunological abnormalities among children receiving highly active antiretroviral therapy at Hawassa University College of Medicine and Health Sciences, Southern Ethiopia DemissieAsseguFenta May 2020	AZT+3TC+EFV ABC+3TC+LPV/r TDF+3TC+LPV/r	273 children aged less than 15 years	Hospital-based Cross-sectional	The prevalence of Anaemia 11.4%, Thrombocytopenia 4%, Leukopenia 14.3%,pancytopenia 2.9%, neutropenia18.3% Abnormalities more prevalent in children with CD4-cell <15% and viral load > 150 viral copies /mm3.
Impact of highly active antiretroviral therapy on haematological indices among HIV-1 infected children at Kenyatta National Hospital. Kenya Kibaru et al 2008	Co-trimoxazole, dapsone AZT+3TC+NVP/ EFV ABC+3TC+NVP D4T+3TC+NVP/E FV	337 children Aged 5-144 months	retrospective study	Haematological abnormalities changed significantly within 6 months of antiretroviral therapy with significant Increase in haemoglobin level, MCV, MCH, and platelet and decrease in WBC and RBC.

2.2 Haematological Aspects of HIV

2.2.1 Anaemia

Anaemia is defined as a reduction in haemoglobin level in comparison to healthy individuals of similar age, gender, environment, and physiological state. This can be classified further according

to the aetiology and morphology of the red blood cell. Red cell size is determined by mean cell volume, (MCV).Mean cell volume is an essential initial test in the evaluation of anaemia and the gold standard for the diagnosis of micro and macrocytic anaemia (17). Anaemia in HIV usually presents as either normochromic normocytic or hypochromic microcytic (4). Reticulocytes are immature red cells seen in low concentrations in the blood, reticulocyte count measures bone marrow activity(whether or not there is an effective erythropoietic response when the number of red cells decreases owing to haemolysis or haemorrhage (18). Diagnosis and treatment of anaemia in HIV is important as it impairs the standard of living by causing symptoms such as chronic fatigue, congestive cardiac failure and has been associated with decrease in cognition, motor, socio-economic and neurophysiologic functioning and a higher risk of mortality in AIDS patients (8).

Anaemia in HIV can be caused by a variety of factors

1. **Decreased/ ineffective RBC production**

Is the most significant pathogenetic pathway causing anaemia in HIV Infected Children. It can be caused by **Neoplasia** such as Hodgkins and non-Hodgkin's disease, Kaposi sarcoma 29%, or infectious bone marrow infiltration(19) (20). **Drugs** such as zidovudine (21) (22) caused 41.6%of anaemia according to the Women's Interagency HIV Study (WIHS) report(23) ,and a progressive increase in erythrocyte mean corpuscular volume (18). Gancyclovir used for the treatment of cytomegalovirus in pregnant women was found to have bone marrow suppressive effect on neonates causing anaemia (24). The bone marrow of AIDS patients who take chloramphenicol is found to have a higher frequency of 90%hypocellularity as compared to 51.7% of those who had a negative, Trimethoprim inhibits the metabolism of folate in erythroid cells, whereas sulfamethoxazole, primaquine, and dapsone can promote hemolysis in those who lack the enzyme glucose-6-phosphate dehydrogenase (G6PD) (26). **HIV and its associated infections** (27) like mycobacterium avium intracellulare(MAC) observed to be prevalent in HIV-infected children globally causing 21% of anaemia, (25),(28),(29), while non-typhoid salmonella predominating the African setup (31)showed a prevalence of 65% and 5% for moderate and severe anaemia respectively(30). Parvovirus B19 (31) blocks erythroid maturation resulting in pure red cell aplasia and chronic anaemia (32). Malaria caused severe anaemia in children who are HIV co-infected (33) 65.2% and HIV exposed 35% (34) than in HIV negative19.1%. Anaemia of chronic

disease is a common complication of HIV; the process is complex and is induced, in part, by the release of cytokines, resulting in dyserythropoiesis and iron blockage (35). **Deficiencies** in folate, vitamin B12, and vitamin A, iron in a study done in India of children aged 2-12years reported a prevalence of 0.9% 8% and 26.6%, 65.5% respectively. Iron deficiency is caused by either reduced intake (nutritional) or increased loss (helminthic infestation, haemorrhage) whereas macrocytic anaemia is commonly linked to folate and vitamin B12 deficiency (36). In Nigeria, the prevalence of anaemia and malnutrition in the context of ART was estimated at 70% in children aged 18 months and above (36), (35), (37).

2. **Increased red blood cell destruction**

Red blood cell destruction can be caused by **haemolysis** due to thrombotic thrombocytopenic purpura (38), glucose-6-phosphate dehydrogenase deficiency in those taking primaquine, sulfamethoxazole, and dapsone. A study in Taiwan found the incidence of Autoimmune hemolytic anaemia in people living with HIV to be 23.76% and the incidence increased among those taking efavirenz or atazanavir (26), (39). **Drugs** such as ceftriaxone (40) and indinavir (41) have been associated with hemolytic anaemia in persons infected by HIV. **Infection** with cytomegalovirus (CMV), candida, **gastrointestinal bleeding** related to non-Hodgkin's lymphoma**,** Kaposi's sarcoma**, hypersplenism** induced by infection**,** haemophagocytosis**,** Cirrhosis(hepatits B virus, hepatits c virus), lymphoma (26) are all known to cause haemolysis in HIV.

Risk Factors associated with anaemia

Women, black race, increased viral load, zidovudine use, CD4 cell counts of less than 200cells/microL, advanced W.H.O stage (42),=- highly active antiretroviral treatment(HAART) naïve, children less than 15years and Living in rural areas, opportunistic infections,antiretroviral (ARV) regimen and length of HAART (43) use.

2.2.2 THROMBOCYTOPENIA

It is characterized by a low platelet count which causes bleeding disorders. Prevalence in HAART naïve is 4.1% to 26.7% (44).

In HIV infection, thrombocytopenia is caused primarily by ineffective/reduced platelet production and increased platelet destruction (45), (46), (47).

1. **Ineffective/ reduced platelet production**

Platelet production and thrombocytopenia are impaired by **direct virus infection of megakaryocytes**, **Neoplasia**, or unfavourable **drug** effects (26).

2. **Increased platelet destruction**

The destruction is usually antibody-mediated and caused by specific antiplatelet antibodies or deposition of nonspecific circulating immune complexes on platelets, **Immune thrombocytopenic purpura** which is the most common cause of thrombocytopenia, affects 30% or more of AIDS patients.

Thrombotic microangiopathy is significantly higher in patients who developed cryptosporidiosis or AIDS-related malignancies (48). A case report of severe thrombocytopenia of a 56year old Japanese man was noted while on a dolutegravir based regimen (14)

RISK FACTORS

Risk factors have been identified for the development of thrombocytopenia some immune mediated(chronic ITP, cryoglobulins, and antiplatelet autoantibodies HIV infection,) others hepatic in nature (hepatitis c co-infection, advanced liver disease, cirrhosis)

2.2.3 LEUCOPENIA

In HIV patients, leucopenia often corresponds with disease progression and is commonly caused by lymphopenia, primarily due to CD4+ depletion. The occurrence of neutropenia is dependent on several additional variables that are typical in people with advanced HIV illness. It can also arise as a result of viral toxicity to hematopoietic cells, opportunistic infections, associated malignancies, and myelosuppressive drugs (49).The severity of leucopenia and neutropenia reported by various studies varied as much as the clinical stage and geographical location with a prevalence of 11.7- 26.8% for leucopenia, and 10-85% for neutropenia (49).

2.3 EFFECT OF ARV ON HAEMATOLOGICAL PARAMETERS

Early administration of highly active antiretroviral treatment(HAART) boosts patients' immunological, clinical and haematological, profiles, delays disease development, and improves survival of individuals infected with HIV, which altogether leads to a decline in viral transmission (49), (1). The use of antiretroviral can have an impact on haematological parameters based on the type or combination used (1). Antiretroviral therapy regimens typically include a backbone of two nucleoside reverse transcriptase inhibitors (NRTIs) eg lamivudine/zidovudine/abacavir) and a core drug eg a protease inhibitor(PIs)(lopinavir,ritonavir,indinavir ,atazanavir), non-nucleoside reverse transcriptase inhibitors (NNRTIs eg nevirapine/efavirenz), and integrase strand transfer inhibitors (INSTIs eg dolutegravir). Little is known as to whether the risk of anaemia varies between routinely used ART classes in current treatment. One study observed the use of integrase strand transfer inhibitor(INSTI) on several core ART classes was linked to lower haemoglobin levels during follow-up as compared to non-nucleoside reverse transcriptase inhibitors based regimens (50)

Table 2: Combinations of ARVS Causing Changes in Haematological Parameters

ARV FORMULATION	ADVERSE EFFECT
Lamivudine and zidovudine combination	Neutropenia, anaemia, and, thrombocytopenia
Nevirapine	Eosinophilia and granulopenia
Zidovudine and Stavudine	Neutropenia, anaemia, and, thrombocytopenia

Monitoring anomalies of haematological parameters following HIV infection is critical for the early identification of immuno-haematological abnormalities and the implementation of essential therapeutic interventions to avoid future comorbidity (39).

2.4 OTHER DRUGS USED IN ROUTINE CARE OF HIV INFECTED CHILDREN AND THEIR EFFECT ON HAEMATOLOGICAL PARAMETERS

Co-trimoxazole- multiple cases of haematological problems have been reported related to co-trimoxazole use and the problems worsen if used with myelosuppressive or antifolate medication eg zidovudine (51). Co-trimoxazole has been known to impair folate metabolism (52). In Uganda, One study looked at the impact of co-trimoxazole on haematological markers in HIV infected adults and reported an increase of 2.1% in leucopenia,2.0% in neutropenia,2.3% in thrombocytopenia,5.4% in anaemia in patients before and after the start of co-trimoxazole prophylaxis, with a majority of the abnormalities observed with a CD4 cell count of 200 cells/L or higher (51).

Isoniazid(INH) prophylaxis-Isoniazid is used to treat tuberculosis and as a prophylaxis for latent TB causes, but it can cause hematologic side effects such as sideroblastic anaemia, pure red cell aplasia, and agranulocytosis (53).

FACTORS AFFECTING HAEMATOLOGICAL PARAMETERS

Table 3 Effect on haematological parameters based on age, WHO staging, and use of ARVs

FACTOR	EFFECT ON HAEMATOLOGICAL PARAMETERS
1. AGE	The younger the patient(<5years) the more likely anaemia is (54) due to low intake of iron from food, high iron requirement, and frequent infections (55).
2. WHO STAGING a) At diagnosis b) Current	As the WHO clinical stage advances the severity of anaemia increases whereby severe anaemia was found exclusively in stages 3 and 4 and mild anaemia is found in stages 1 and 2 (54).

3. Current ART use a)More suppressive vs Less suppressive I. Less suppressive II. VL< 1000 or < 50 b) Duration of treatment	HAART significantly improved heamatological parameters only after six months of use((23), (48). Fall of viral load is slightly lower in anaemia than non anaemia HIV infected infants (54). After using HAART for a longer period, there was a greater degree of anaemia resolution (23).

2.5 KENYA PAEDIATRIC GUIDELINES

Testing Services

HIV testing should be done ethically and voluntarily in a setting that ensures counselling, confidentiality, accurate results, consent, and linkage to support groups. All HIV-exposed babies (HEI) should be tested at delivery or within two weeks after delivery, followed by a DNA polymerase chain reaction at six weeks, six months, and twelve months if the results are negative (these changes before recommendations for newborn antibody testing at 9 months). At 18 months, an antibody test should be performed, followed by a 6months interval while breastfeeding, with the final antibody test done 6 weeks after full cessation of breastfeeding.

Monitoring of people living with HIV

CD4 monitoring is advised for all people living with HIV at the outset, those with a history of therapeutic failure, and on patients who are on continuous fluconazole therapy or on dapsone prophylaxis to ascertain when prophylaxis can be stopped.

Viral Load Monitoring

Monitoring of viral loads in polymerase chain reaction positive HIV exposed infants is done at the time of antiretroviral therapy initiation.

Those 0-24 years: every 6 months.

Before any drug is substituted (if no viral load is available from the prior 6 months) and 3months following any regimen change (including single drug substitution).

Initiation of ART

ART is offered to every person living with HIV irrespective of age, clinical stage, CD4 + cell count, pregnancy status, or comorbidities, and is ideally provided within two weeks of diagnosis, except for patients with pulmonary tuberculosis, TB meningitis, and Cryptococcus meningitis and the individual is willing to take antiretroviral therapy (versus in the past when treatment was given to those with CD4 counts ≤350cells/mm³ or clinical stage 3 or 4 regardless of their CD4 count).

Children with an interim positive HIV DNA polymerase chain reaction finding are assumed to be HIV positive and placed on antiretroviral therapy in accordance with the guidelines, with a corroborative baseline HIV DNA polymerase chain reaction and viral load obtained at the time of antiretroviral therapy introduction.

As of 2018 guidelines regimens used were (see appendix 3)

For HIV-infected and exposed infants, Co-trimoxazole should start at six weeks of age.

2.6 TRANSITIONING TO DTG

With guidance from WHO on optimization of ART recommending the use of Dolutegravir among Children and adolescents living with HIV weighing above 20kg in 2019, the Government Of Kenya through the Ministry Of Health embarked on a countrywide transition and optimization of dolutegravir which would potentially replace lopinavir/ritonavir and efavirenz based regimens for in children living with HIV aged less than 3 years that have been in use since 2013.

Dolutegravir 10mg became available and was found to be safe in use in children weighing less than 20kg.

The transition process was undertaken in three phases:

Phase 1: Phase-out of nevirapine based regimen.

Phase 2: Optimization of children and adolescents living with HIV(CALHIV) weighing above 20kg to dolutegravir based regimen.

Phase 3: Transition all children and adolescents living with HIV on 1st line and initiate those newly diagnosed weighing below 20kg to dolutegravir 10 mg.

New guidelines were introduced

1. **Initiating ART in newly diagnosed CALHIV less than 15years** (56)

Table 4: Recommended New Guidelines

Age/Weight	Preferred Regimen
Birth to 4 weeks	AZT+3TC+NVP/RAL
3-<20kgs	ABC+3TC+ped DTG
20kg- <35 kg	ABC +3TC+DTG
>35kg	TDF+3TC+DTG

AZT-Zidovudine, ABC- Abacavir,3TC-Lamivudine, RAL-Raltegravir, TDF- Tenofovir, DTG –Dolutegravir, NVP-Nevirapine

DTG will double the dose in children with rifampicin based anti TB treatment.

2. **The transition of CALHIV currently on 1st line ART who are virally suppressed (<1000 copies/ml)**

Table 5: Transitioning to DTG regimen in the virally suppressed (56)

Current regimen	Optimized ART Regimen		
	>_4 weeks<20kg	20-29.9kg	>_30kg
AZT+3TC+EFV/NVP	ABC+3TC+ DTG	ABC+3TC+DTG	TDF+3TC+DTG
ABC+3TC+EFV/NVP	ABC+3TC+ DTG	ABC+3TC+DTG	TDF+3TC+DTG
AZT+3TC+LPV/r/RAL	ABC+3TC+ DTG	ABC+3TC+DTG	TDF+3TC+DTG
ABC+3TC+LPV/r/RAL	ABC+3TC+ DTG	ABC 3TC DTG	TDF 3TC DTG

AZT-Zidovudine, ABC- Abacavir,3TC-Lamivudine, TDF- Tenofovir, DTG –Dolutegravir, EFV-Efavirenz, NVP- Nevirapine,LPV/r-Lopinavir /ritonavir.

3. Transitioning of CALHIV < 20kg currently in 1st line ART who are not virally suppressed (> 1000 copies/ml)

Table 6: Transitioning to dolutegravir in virally unsuppressed (56)

Current Regimen	Optimized ART Regimen
Contains ABC	Switch to AZT+ 3TC +DTG
Contains AZT	Switch to ABC +3TC+ DTG

AZT-Zidovudine, ABC- Abacavir,3TC-Lamivudine, TDF- Tenofovir, DTG –Dolutegravir.

Children on the 2nd line who are virally suppressed should not be transitioned and those not virally suppressed should be managed as per current national guidelines for the 3rd line.

CHAPTER THREE

3.0 STUDY JUSTIFICATION

Extensive global research has led to the introduction of new treatment guidelines which phased out some drugs such as stavudine and introduced new ones such as dolutegravir, and longer-acting PIs with a little less toxicities and thermal-stable ritonavir.

Since 2018, a test and treat strategy has been adopted whereby all persons infected with HIV regardless of their immune status are put on antiretroviral therapy.

Dolutegravir paediatric formulations are increasingly becoming available to children who are more than 35kg.

Haematological parameters are key monitoring tools for assessing treatment and outcome among highly active antiretroviral therapy experienced HIV positive children.

Early detection of abnormal parameters is important to prevent morbidity and mortality, improve living quality and prevent transmission of the virus to other uninfected individuals.

3.1 STUDY OBJECTIVES

3.2 STUDY QUESTION

1. What are the haematological parameters of HIV-infected children on current combination antiretroviral therapy?
2. What factors affect the haematological parameters of HIV-infected children?

3.3 OBJECTIVES

3.3.1 Specific Objectives

1. Describe and to determine the prevalence of abnormal heamatological variables (haemoglobin, mean corpuscular volume, platelets, total white blood cell count, and Lymphocytes count levels) among HIV-infected children [0-18years] followed at Gertrude's Children’s Hospital before and after switching to dolutegravir based protocols.

3.3.2 Secondary Objective

1. Describe the prevalence of viral suppression before and after switching to dolutegravir.
2. Determine demographic and clinical factors influencing abnormal haematological parameters of HIV infected children.

CHAPTER FOUR

4.0 METHODOLOGY

4.1 Study Design

A retrospective cohort study comparing baseline haematological parameters before the switch and 6 months after the switch to dolutegravir based protocol.

4.2 Study Setting

The study was carried out at the Comprehensive Care Centre in Gertrude's Children's Hospital which caters to 500 children drawn from Nairobi and its environs, referrals from other peripheral facilities within Nairobi, and self-referrals. The Comprehensive Care Centre provides diagnostic, treatment, prevention, and free counselling and testing services. It boasts of a laboratory, pharmaceutical, and HIV tuberculosis co-infection unit. Patients are attended to by paediatricians, clinical officers, nurses, social workers, nutritionists, and psychosocial counsellors.

4.3 Study Period

January 2019 to December 2022.

4.4 Study Population

All children and adolescents less than 18years who are HIV-infected on highly active antiretroviral therapy and attending the clinic at Gertrude's Children's Hospital

4.4.1 Inclusion Criteria

All children and adolescents less than 18 years and are HIV infected on highly active antiretroviral therapy attending the Comprehensive Care Clinic at Gertrude's Children's Hospital.

4.4.2 Exclusion Criteria

- Those known to have haematological disorders eg sickle cell or bleeding disorder.
- Absent baseline viral load and complete blood counts.
- Newly transfused in the last three months.

4.5 Case Definition

Cytopenia is a reduction of any of the peripheral cell lines, resulting in leucopenia, anaemia, neutropenia, and thrombocytopenia.

Anaemia is defined in appendix 4.

Leucopenia is defined in appendix 4.

Thrombocytopenia is defined in appendix 4.

Valid viral load six months before switch and six months after switch

4.6 Sample Size

All the observations that meet the inclusion criteria will be enrolled.

4.7 Sampling Method

Gertrude's Children's Hospital has an electronic medical data system – an EMR system. A query was designed for variables of interest. The birth date was used to identify the required age group. Keywords in the query used were date of birth, baseline viral load and CD4 counts, complete blood counts. Complete blood count were extracted from the electronic elephant system and inputted into a paper based record which was later exported to excel.

4.8 Study Procedure

Data collected by the principal investigator was from January 2019 to December 2022.

Data collected include:

Demographic Data: age, sex.

Clinical Data: WHO staging, presence of other co-rmobid conditions.

Nutritional Status Data: weight, BMI.

Details of Diagnosis: age of diagnosis.

Opportunistic infections: tuberculosis, helminthic infestation, lymphomas, hepatitis B and C.

Details of ARV: regimen used and if there were any switches and reasons given.

Details of other Drugs: co-trimoxazole prophylaxis, when iron supplementation started.

Haematological Parameters: heamoglobin, mean corpuscular volume, total white blood cell count, Lymphocyte, and Platelet count values before switching to dolutegravir and 6 months after switching.

Viral Loads: values six before the switch and 6 months after switching to dolutegravir based protocols

4.9 Data Analysis and Management

Data entered in excel spreadsheet was filtered for complete observations, cleaned for redundant variables, and duplicates. The data was then secured in password protected file and imported into R version 4.0.2. for analysis

Description of categorical variables was done using frequencies and percentages. Prevalence of the categories of haematological values e.g. for Haemoglobin(Hb), total white blood cell counts (TWBCs), and platelets were presented as percentages with a 95% confidence interval.

CHAPTER 5

5.0 RESULTS

A total of 217 records were abstracted from the Gertrudes' Children's Hospital CCC records from January 2019 to December 2022

5.1 Demographics

There were 5 subjects aged less than 5 years, 60 who were between 6 and 12 years, and 152 who were 13 years and above.

The median age of diagnosis in the under 5 years, 6-12 years, and over 13years was 10.8(IQR 6.33) months,23.4 months (IQR 27.3) and, 34.4months (IQR51.1) respectively which was statistically significance at 0.0006

The median duration in months between diagnosis and antiretroviral initiation was 1.5(IQR 8.51) in the under 5, 0.8 (IQR 10.2) in the 6-12years, and 2.1(IQR 20.2) in those over 13 years with the statistical non-significance of 0.4513

Median age in years at initiation of dolutegravir was 3(IQR 0.5) in under 5, 7.6(IQR 2.3) in the 6-12 age bracket and 12.8(IQR 3.1) in the over 13years with a significance of < 0.0001

More females than males were recruited in the under 5s and 6-12 age bracket than in the over 13age bracket

Table 7. Demographic and Clinical Characteristics

	< 5 years n=5	6-12years N=60	>13 years N=152	P value*
Age at diagnosis median (IQR)	10.8 months (6.33)	23.4 months (27.3)	34.4 (51.1)	0.0006
Median duration between diagnosis and ART initiation (months)	1.5 months (8.51)	0.8 months (10.2)	2.1 (20.2)	0.4513
Median age at initiation of DTG (years)	3 (0.5)	7.6 (2.3)	12.8 (3.1)	<0.0001
Male: female ratio	1:4	1:1.33	1.13:1	NA

**(Kruskal- Wallis test- ≥ 3 groups, non-parametric distribution with medians)*

5.2 Current and previous drug history

The nucleos(/t)ide reverse transcriptase inhibitors previously or currently used by the subjects included abacavir/lamivudine(ABC/3TC), tenofovir/lamivudine(TDF/3TC), zidovudine/lamivudine (AZT/3TC) and stavudine/lamivudineD4T/3TC. The mean duration of use was calculated and showed the duration as ABC/3TC (29.3months(SD 7.9), TDF/3TC(17.5 months(SD 30.2), AZT/3TC(69.9 months(SD 46.8)) and D4T/3TC(15 months(SD 6.8)).

The non-nucleoside reverse transcriptase inhibitor drugs previously used were efavirenz(EFV) and nevirapine(NVP). The mean duration of previous efavirenz(EFV) usage was 44.1 months (SD 38.6).

The protease inhibitors previously/currently used were lopinavir/ritonavir(LPVr) and atazanavir /ritonavir(ATVr). The mean duration of LPVr was 45.5 months(SD 34.2).

The history of use of iron supplements, antihelminthics, and other drugs of interest was largely unrecorded.

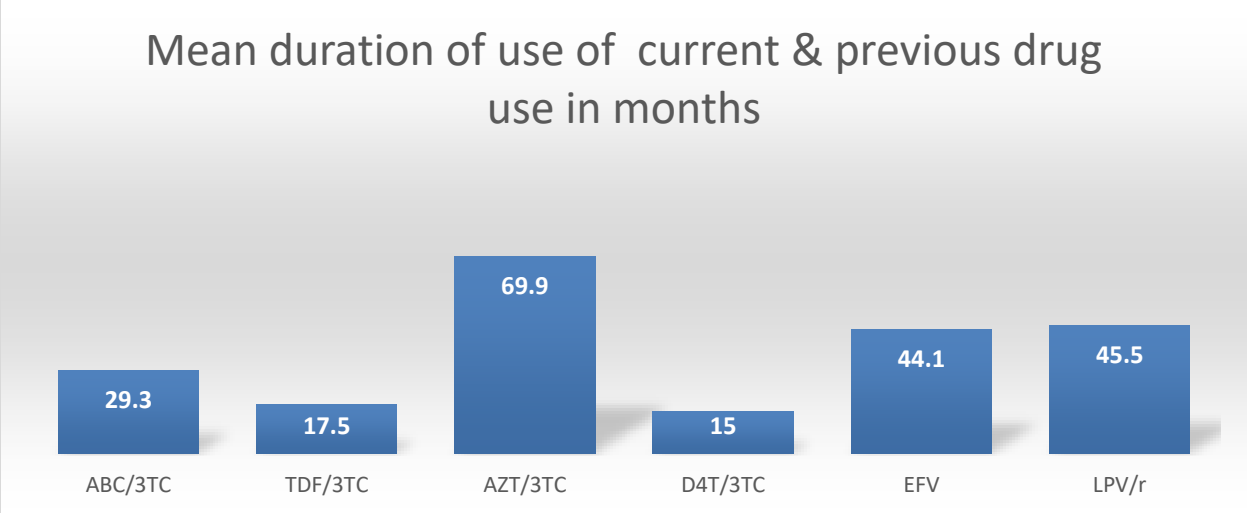


Figure 1. Baseline clinical characteristics

5.3 Access to viral load and complete blood counts

Before the DTG switch, there were 204(93.1%) valid viral load results while after DTG, there were 158(72.1%) available VL records.

Due to the discrepant distribution of missing data, a contingency table was made of missing data, and the Mantel-Haenszel test was done and was found to be significant ($X^2 = 14.3, p = 0.001$) for valid viral load. This would indicate a significant relationship between missing data and at least one of the variables. To investigate whether this difference translated to a demographic difference, various demographic indicators were compared at a 95% confidence level using Mann Whitney-U and Chi-square tests. These non-significant results allowed for the cautious comparison of the pre and post-DTG results for viral load despite the missing values.

The pre-DTG missing records were 2.3% and the post-DTG missing records were 37% for haematological parameters. The Mantel-Haenszel test for missing data was statistically significant showing a possible bias. To investigate if the missing data translated to a demographic bias, different variables were tested the most important being median age at DTG initiation which was tested for statistical significance using the Mann- Whitney U test and was not significant (*W = 13454, p-value = 0.4252*). This indicated that though the discrepancy in missing data was significant, it was not statistically significant with certain demographic characteristics.

Table 8. Access to viral load and complete blood count

	Pre-DTG	Post DTG	P value	Pre-DTG CBC	Post DTG CBC	P value
Count of available results	204	158	0.001[a]	214	137	0.0001[a]
Age (months) at diagnosis	31.07	33.48	0.6517[b]	30.2	2.2	0.2226[b]
Median duration between diagnosis and ART initiation	1.37	2.2	0.2663[b]	1.5	11.8	0.3001[b]
Median age at initiation of DTG	11.5	11.6	0.9239[b]	11.5	72	0.4252[b]
Male	101	76		106	65	0.058[c]
Female	105	79	0.124[c]	108		

[a] Mantel-Haenzel test [b] Mann Whitney U test [c] Chi square test

5.4 Red Blood Cell parameters before and after DTG

For the under 5years age group, the mean haemoglobin level increased from 11.97 g/dL to 12.17 g/dL after DTG treatment (Δ change = 0.2 g/dL, p-value = 0.5143). The mean corpuscular volume (MCV) decreased from 82.37 fL to 81.77 fL after DTG treatment (Δ change = -0.6 fL, p-value = 0.0216) while the mean corpuscular haemoglobin (MCH) decreased from 28.69 pg to 27.7 pg after DTG treatment (Δ change = -0.99 pg, p-value = 0.7105). The count of anaemia decreased from 1(20%) case to zero after DTG treatment (Δ change = -1, p-value = 0.2406

For the 6-12years age group, the mean haemoglobin level increased from 12.49 g/dL to 12.59 g/dL after DTG treatment (Δ change = 0.1 g/dL, p-value = 0.1223). The MCV decreased from 84.58 fL to 84.15 fL after DTG treatment (Δ change = -0.43 fL, p-value = 0.8359) while the MCH increased from 28.76 pg to 29.59 pg after DTG treatment (Δ change = 0.83 pg, p-value = 0.0584). The proportion of anaemia decreased from 7(11.6%) to 2(3.3%) after DTG treatment (Δ change = -5%, p-value = 0.6604).

For the more than 13years age group, the mean haemoglobin level increased from 13.06 g/dL to 13.45 g/dL after DTG treatment (Δ change = 0.39 g/dL, p-value < 0.0001). The MCV decreased from 85.59 fL to 84.97 fL after DTG treatment (Δ change = -0.62 fL, p-value = 0.0107) while the MCH decreased from 28.92 pg to 28.44 pg after DTG treatment (Δ change = -0.48 pg, p-value = 0.0016). The count of anaemia remained the same at 4(2.6%) after DTG treatment (Δ change = 0, p-value = 0.8428).

Overall, the results show that DTG treatment had a positive impact on the haemoglobin levels in all age groups, with significant improvements seen in the over 13years age group. The MCV decreased in all age groups, with significant changes seen in the under 5 years and over 13years age groups. The MCH decreased in the under 5 years and over 13years age groups, while increasing in the 6-12years age group. The proportion of anaemia decreased in the under 5 years and 6-12years age groups while remaining the same in the over 13years age group.

Table 9. Pre and Post DTG Red blood cell parameters and Anaemia in different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

		Haemoglobin (Mean)	Mean corpuscular volume(Mean)	MCH (Mean)	Anaemia (Count)
< 5yrs	Pre-DTG	11.97	82.37	28.69	1
	Post-DTG	12.17	81.77	27.7	0
	Δ change	0.2	-0.6	-0.99	1
	p-value*	**0.5143**	**0.0216**	**0.7105**	**0.2406**
6-12yrs	Pre-DTG	12.49	84.58	28.76	7
	Post-DTG	12.59	84.15	29.59	2
	Δ change	0.1	-0.43	0.83	5
	p-value*	**0.1223**	**0.8359**	**0.0584**	**0.6604**
>13yrs	Pre-DTG	13.06	85.59	28.92	4
	Post-DTG	13.45	84.97	28.44	4
	Δ change	0.39	-0.62	-0.48	0
	p-value*	**< 0.0001**	**0.0107**	**0.0016**	**0.8428**

*Paired 2 Sample T test

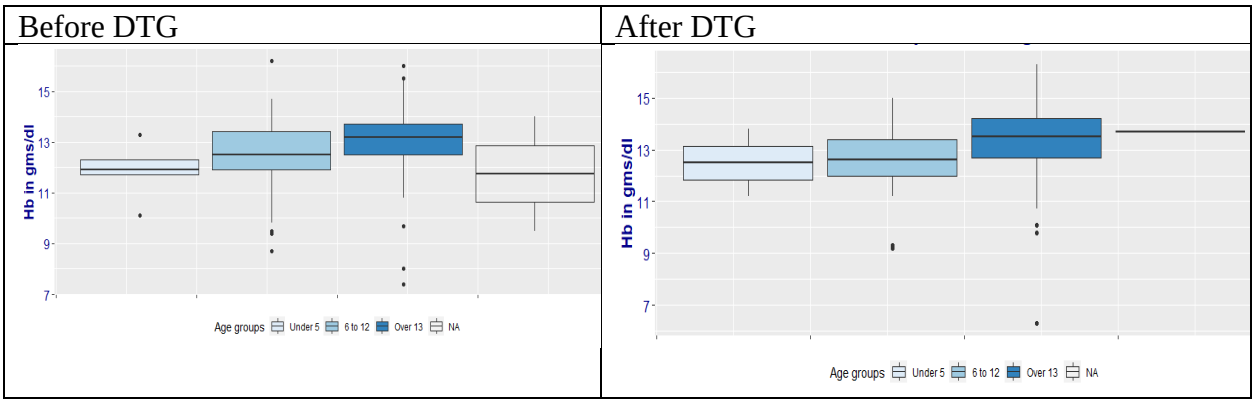


Figure 2. Box and whisker plot of pre and post-DTG haemoglobin levels in different age groups of CALHIV at Gertrudes Hospital CCC from 2019-2022

5.5 White blood cells before and after DTG

In the under 5years age group, there was a non-significant decrease in WBC counts from pre-DTG to post-DTG treatment ($6.81*10^9$ vs. $6.68*10^9$, p=0.5408). The percentage of patients with leukopenia decreased from 40% to 0% (p=0.0001), while no patients had leukocytosis before or after treatment.

In the 6-12years age group, there was a non-significant increase in WBC counts from pre-DTG to post-DTG treatment ($6.7*10^9$ vs. $6.99*10^9$, p=0.1806). The percentage of patients with leukopenia decreased from 10% to 21.6% (p=0.0001), while no patients had leukocytosis before or after treatment.

In the over 13years age group, there was a non-significant increase in WBC counts from pre-DTG to post-DTG treatment ($5.66*10^9$ vs. $5.8*10^9$, p=0.321). The percentage of patients with leukopenia decreased from 27.6% to 42.1% (p=0.0001), while the percentage of patients with leukocytosis remained the same (0.6%).

Table 10. Pre and Post-DTG white blood cell parameters, Leucopenia and Leucocytosis in different age groups among CALHIV at Gertrudes Hospital CCC from 2019 -2022

		WBC*(10^9)	Leukopenia	Leukocytosis
< 5yrs	Pre-DTG	6.81	2 (40%)	0 (0%)
	Post-DTG	6.68	0(0%)	0(0%)
	Δ change	-0.13	-2	0
	p-value*	0.5408	0.0001	0.9879
6-12yrs	Pre-DTG	6.7	6 (10%)	0 (0%)
	Post-DTG	6.99	13(21.6%)	0(0%)
	Δ change	0.29	7	0
	p-value*	0.1806	0.0001	0.8873
>13yrs	Pre-DTG	5.66	42 (27.6%)	1 (0.6%)
	Post-DTG	5.8	64(42.1%)	1(0.6%)
	Δ change	0.14	22	0
	p-value*	0.321	0.0001	0.9979

**Paired 2 sample T Test*

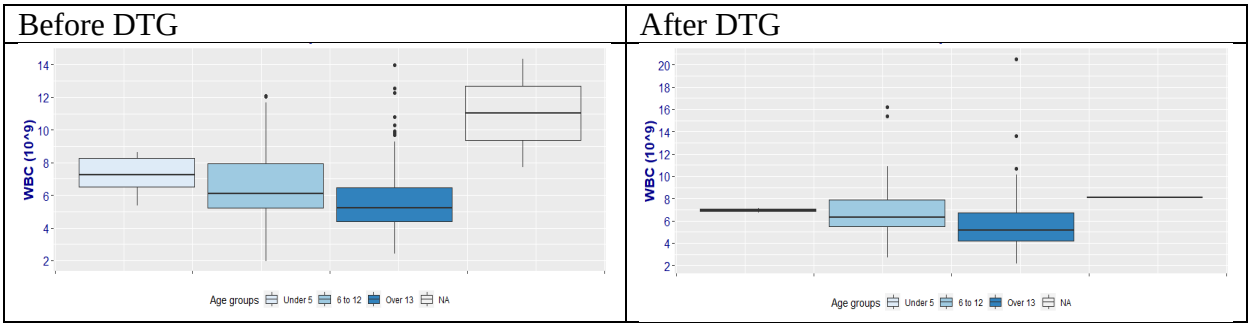


Figure 3 Box and whisker plot of pre and post-DTG WBC levels in different age groups among CALHIV at Gertrudes Hospital CCC

5.6 Neutrophil before and after Dolutegravir

In the under 5years age group, there was a non-significant increase in neutrophil counts from pre-DTG to post-DTG treatment ($1.53*10^9$ vs. $3.36*10^9$, p=0.5547). The percentage of patients with neutropenia decreased from 80% to 20% (p=0.0342), while no patients had neutrophilia before or after treatment.

In the 6-12years age group, there was a significant increase in neutrophil counts from pre-DTG to post-DTG treatment ($2.38 *10^9$ vs. $2.79*10^9$, p=0.0209). The percentage of patients with neutropenia decreased from 21.6% to 15% (p=0.0247), while the percentage of patients with neutrophilia increased from 0% to 1.7% (p=0.6727).

In the over13 years age group, there was a non-significant increase in neutrophil counts from pre-DTG to post-DTG treatment ($2.17*10^9$ vs. $2.38*10^9$, p=0.2232). The percentage of patients with neutropenia decreased from 30.3% to 18.4% (p=0.0001), while the percentage of patients with neutrophilia decreased from 3.9% to 1.9% (p=0.0578).

Table 11. Pre and Post DTG Neutrophil parameters in different age groups among CALHIV at Gertrudes Hospital CCC from 2019 -2022

		Neutrophil count	Neutropenia	Neutrophilia
< 5yrs	Pre-DTG	1.53	4(80%)	0(0%)
	Post-DTG	3.36	1(20%)	0(0%)
	Δ change	1.83	-3	0
	p-value*	0.5547	0.0342	0.9648
6-12yrs	Pre-DTG	2.38	13(21.6%)	0(0%)
	Post-DTG	2.79	9(15%)	1(1.7%)
	Δ change	0.41	-4	1
	p-value*	0.0209	0.0247	0.6727
>13yrs	Pre-DTG	2.17	46(30.3%)	6(3.9%)
	Post-DTG	2.38	28(18.4%)	3(1.9%)
	Δ change	0.21	-18	-3
	p-value*	0.2232	0.0001	0.0578

**Paired 2 sample T Test*

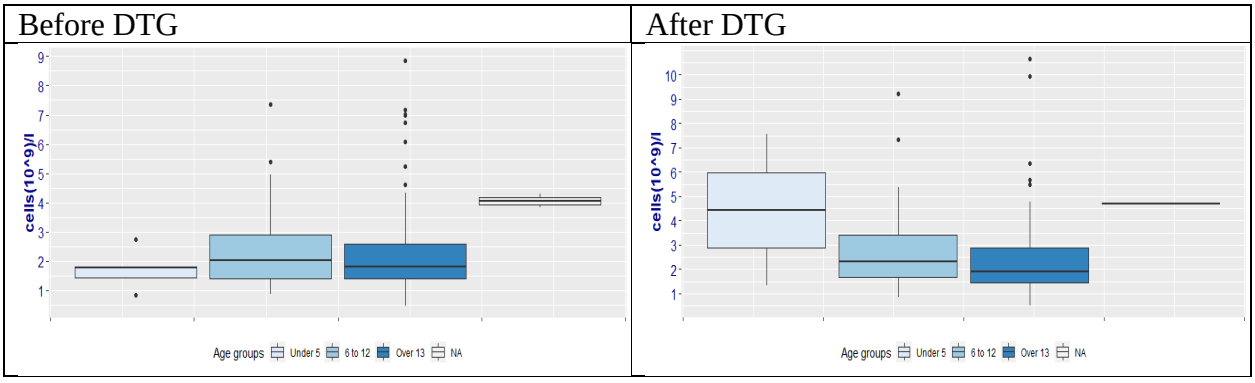


Figure4. Box and Whisker plot pre and post-DTG Neutrophil levels in the different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

5.7 Lymphocyte count

In under 5 year olds, before using DTG, the mean lymphocyte count was $4.65*10^9$, with none of the children having lymphopenia or lymphocytosis. After using DTG, the mean lymphocyte count decreased slightly to $4.46*10^9$, with no cases of lymphopenia or lymphocytosis reported. The delta change between pre- and post-DTG treatment was $-0.19*10^9$, indicating a slight decrease in lymphocyte count, although this was not statistically significant.

In those between 6 and 12 years prior to using DTG, the mean lymphocyte count was $3.54*10^9$, with 3.3% of the children having lymphopenia and 16.7% having lymphocytosis. After using DTG, the mean lymphocyte count decreased slightly to 3.35, with 1.7% of the children having lymphopenia and 11.7% having lymphocytosis. The delta change between pre- and post-DTG treatment was $-0.19*10^9$, indicating a slight decrease in lymphocyte count, although this was not statistically significant. The p-value for the change in lymphocyte count was 0.7357, indicating no significant difference in lymphocyte count between pre- and post-DTG treatment.

For 13 year olds and over, before DTG, the mean lymphocyte count was $2.9*10^9$, with 3.9% of the children having lymphopenia and 4.6% having lymphocytosis. After using DTG, the mean lymphocyte count decreased slightly to $2.74*10^9$, with 3.9% of the children having lymphopenia and 2.6% having lymphocytosis. The delta change between pre- and post-DTG treatment was -0.16, indicating a slight decrease in lymphocyte count, although this was not statistically significant

Table 12. Pre and Post Lymphocyte parameters, Lymphopenia, and Lymphocytosis in different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

		Lymphocytes*10^9	Lymphopenia	Lymphocytosis
< 5yrs	Pre-DTG	4.65	0(0%)	0(0%)
	Post-DTG	4.46	0(0%)	0(0%)
	Δ change	-0.19	0	0
	p-value*	0.9142	0.9765	0.9537
6-12yrs	Pre-DTG	3.54	2(3.3%)	10(16.7%)
	Post-DTG	3.35	1(1.7%)	7(11.7%)
	Δ change	-0.19	-1	-3
	p-value*	0.7357	0.4763	0.1753
>13yrs	Pre-DTG	2.9	6(3.9%)	7(4.6%)
	Post-DTG	2.74	6(3.9%)	4(2.6%)
	Δ change	-0.16	0	-3
	p-value*	0.171	0.9352	0.2762

**Paired 2 sample T Test*

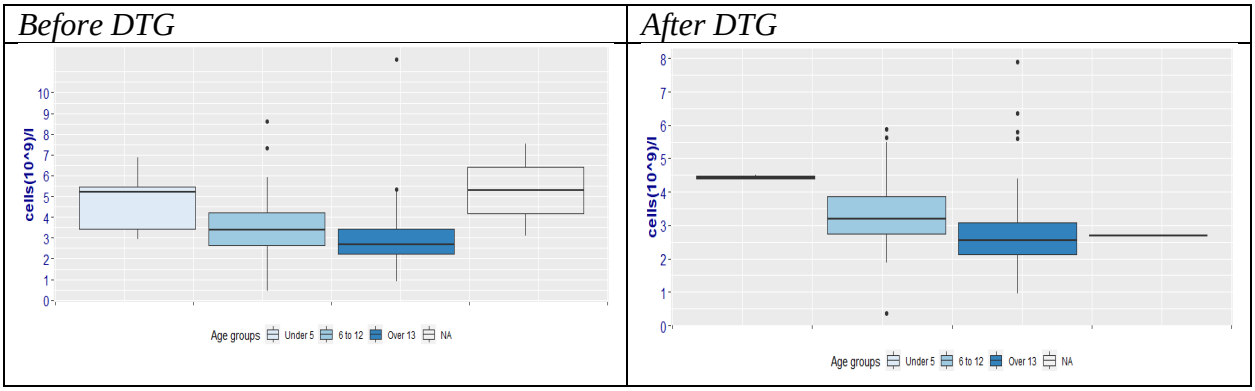


Figure 5. Box and whisker plots of pre and post-DTG Lymphocyte levels in different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

5.8 Eosinophil count before and after Dolutegravir

For children under 5 years old, there was an increase in eosinophil levels after treatment (from $0.13*10^9$ to $0.22*10^9$), but this change was not statistically significant (p-value = 0.8581). There were no cases of eosinopenia or eosinophilia in this group.

For children between 6-12 years old, there was no change in eosinophil levels after treatment ($0.23*10^9$ before and after), but there was a significant decrease in eosinopenia (from 6 to 1 case, p-value = 0.0341) and a significant increase in eosinophilia (from 2 to 19 cases, p-value = 0.0012).

For participants over 13 years old, there was a slight increase in eosinophil levels after treatment (from 0.2 to 0.21), but this change was not statistically significant (p-value = 0.2406). There was a significant decrease in eosinopenia (from 21 to 10 cases, p-value = 0.0112), but no significant change in eosinophilia (from 1 to 3 cases, p-value = 0.0891).

Table 13. Pre and Post Eosinophil parameters, Eosinopenia and Eosinophillia in different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

		Eosinophil	Eosinopenia	Eosinophilia
< 5yrs	Pre-DTG	0.13	2(40%)	0(0%)
	Post-DTG	0.22	0(0%)	0(0%)
	Δ change	0.09	-2	0
	p-value*	0.8581	0.3627	0.9382
6-12yrs	Pre-DTG	0.23	1(1.7%)	2(3.3%)
	Post-DTG	0.23	6(10%)	19(1.7%)
	Δ change	0	5	17
	p-value*	0.7357	0.0341	0.0012
>13yrs	Pre-DTG	0.2	21(13.8%)	1(0.6%)
	Post-DTG	0.21	10(6.6%)	3(2%)
	Δ change	0.01	-11	2
	p-value*	0.2406	0.0112	0.0891

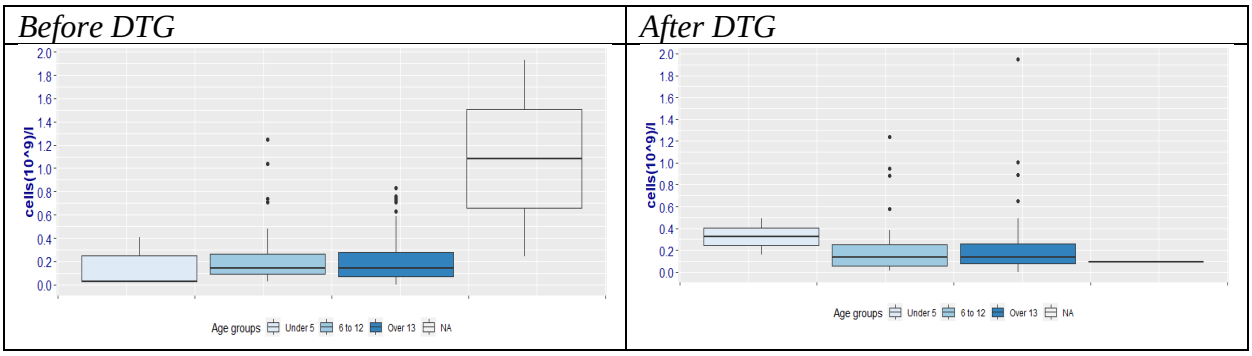


Figure 6. Box and whisker plot of pre and post eosinophil levels at different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

5.9 Basophil count

For the under 5years age group, there was a slight increase in basophil count from 0.02 to 0.03 after treatment with DTG, but the change was not statistically significant (p=0.7418). There was one patient with basopenia (20%) at baseline, but no patients had basopenia after treatment.

For the 6-12years age group, there was an increase in basophil count from 0.03 to 0.05 after treatment with DTG, but the change was not statistically significant (p=0.2722). Two patients had basopenia (3.3%) at baseline, and two patients had basopenia after treatment. One patient had basophilia (1.7%) at baseline, and no patients had basophilia after treatment.

For the over 13years age group, there was no change in basophil count after treatment with DTG, and the p-value was not significant (p=0.6831). Twenty-one patients had basopenia (13.8%) at baseline, and 18 patients had basopenia after treatment. Seven patients had basophilia (4.6%) at baseline, and no patients had basophilia after treatment. However, there was a significant reduction in the number of patients with basophilia after treatment (p=0.0023).

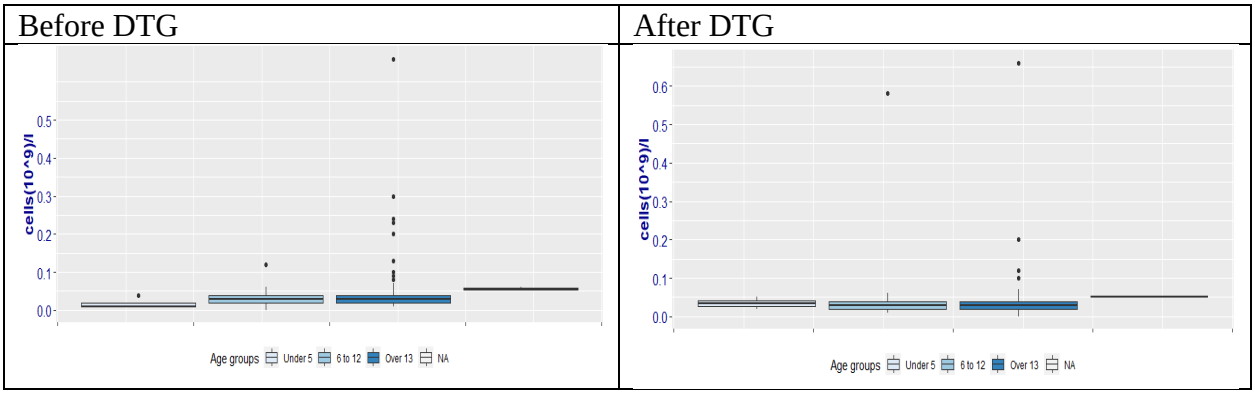


Figure 7. Box and whisker plot of pre and post basophil levels in different age groups among CALHIV at Gertrudes Hospital CCC from 2019- 2022

5.10 Monocyte count before and after Dolutegravir

In the under 5years age group, there was no significant difference in monocyte counts between pre-DTG and post-DTG treatment (0.56 vs. 0.61, p=0.9388). Furthermore, there was no change in the percentage of patients with monocytopenia (0%) or monocytosis (0%).

In the 6-12years age group, there was a slight increase in monocyte counts from pre-DTG to post-DTG treatment (0.64 vs. 0.65, p=0.1258), although this was not statistically significant. The percentage of patients with monocytopenia remained the same (0%), while the percentage of patients with monocytosis increased from 5% to 8.3% (p=0.1728).

In the over 13years age group, there was a decrease in monocyte counts from pre-DTG to post-DTG treatment (0.52 vs. 0.46, p=0.1627), although this was not statistically significant. There was no change in the percentage of patients with monocytopenia (0.7%), while the percentage of patients with monocytosis decreased from 2% to 0.7% (p=0.2182).

Table 14. Pre and Post Monocyte parameters, Monocytopenia and Monocytosis in different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

		Monocytes	Monocytopenia	Monocytosis
< 5yrs	Pre-DTG	0.56	0(0%)	0(0%)
	Post-DTG	0.61	0(0%)	0(0%)
	Δ change	0.05	0	0
	p-value*	0.9388	0.9462	0.9473
6-12yrs	Pre-DTG	0.64	0(0%)	3(5%)
	Post-DTG	0.65	2(3.3%)	5(8.3%)
	Δ change	0.01	2	2
	p-value*	0.1258	0.2938	0.1728
>13yrs	Pre-DTG	0.52	1(0.7%)	3(2%)
	Post-DTG	0.46	1(0.7%)	1(0.7%)
	Δ change	-0.06	0	-2
	p-value*	0.1627	0.8947	0.2182

Before DTG	*After DTG*

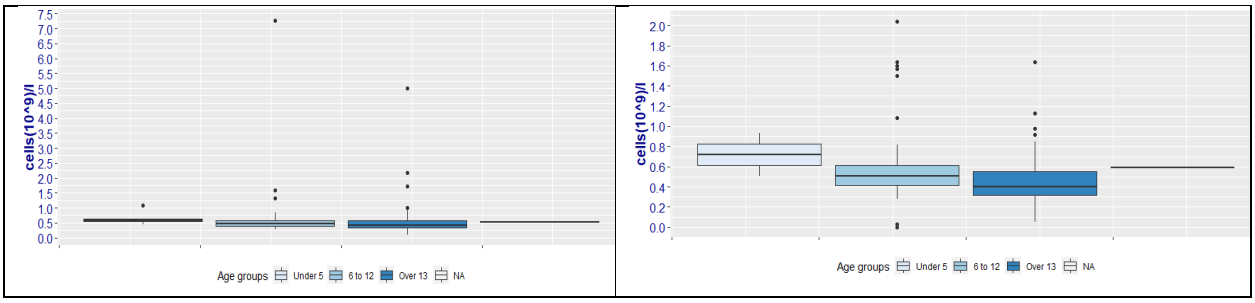


Figure 8. Box and whisker plot of pre and post-DTG monocyte count in different age groups among CALHIV at Gertrudes children Hospital CCC from 2019-2022

5.11 Platelet count before and after Dolutegravir

There were no significant differences in platelet counts before and after DTG treatment in the under 5-year-old age group. In the 6-12year-old age group, there was a significant decrease in thrombocytosis following DTG treatment, with a p-value of 0.0035. However, there were no significant changes in thrombocytopenia. In individuals over 13 years old, there was a non-significant decrease in thrombocytosis, with a p-value of 0.4373, and a significant decrease in thrombocytopenia with a p-value of 0.0023 following DTG treatment.

Table 15. Pre and Post DTG Platelet parameters, Thrombocytopenia and Thrombocytosis in different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

		Platelets	Thrombocytopenia	Thrombocytosis
< 5yrs	Pre-DTG	316.43	1(20%)	1(20%)
	Post-DTG	382.33	0(0%)	0(0%)
	Δ change	65.9	-1	-1
	p-value*	0.5582	0.3828	0.4573
6-12yrs	Pre-DTG	350.51	1(1.7%)	7(11.7%)
	Post-DTG	363.36	1(1.7%)	1(1.7%)
	Δ change	12.85	0	-6
	p-value*	0.4725	0.8974	0.0035
>13yrs	Pre-DTG	323.65	6(3.9%)	15(9.9%)
	Post-DTG	322.17	2(1.3%)	12(7.9%)

	Δ change	-1.48	-4	-3
	p-value*	0.5533	0.0023	0.4373

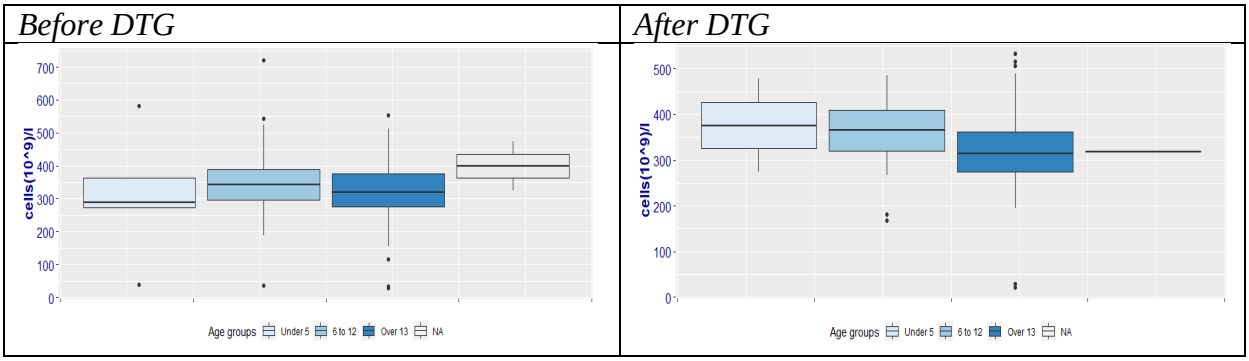
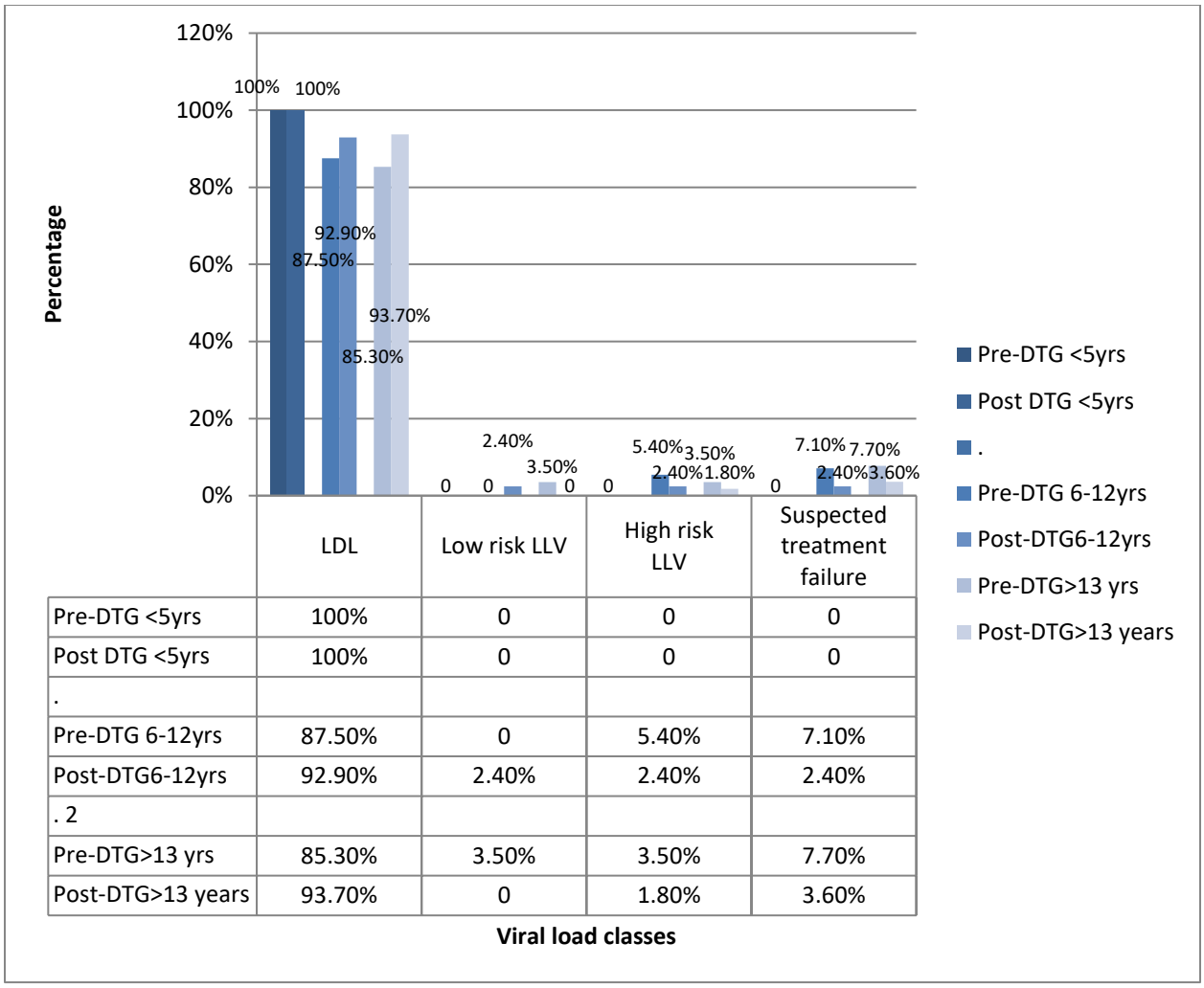


Figure 9. Box and whisker plot of pre and post-DTG platalet count in different age groups among CALHIV at Gertrudes Hospital CCC from 2019-2022

5.12 Viral load

For children aged under 5 years, all samples had viral loads that were below the detectable limit, both pre and post-DTG treatment, with no significant changes observed (Δ change = 0, P = 1 for all categories). For children aged 6-12 years, there was a significant decrease in the percentage of samples with High-risk Low Level Viremia post-DTG treatment compared to pre-DTG treatment (3% decrease, P = 0.0001). There was also a significant decrease in the percentage of samples with Suspected treatment failure post-DTG treatment compared to pre-DTG treatment (4.7% decrease, P = 0.0001). There were no significant changes observed for the other two categories (Lower Detectable Limit and Low-risk Low Level Viremia) with post-DTG treatment (P > 0.05)

Table 16. Pre and Post DTG Viral load classes in different age groups among CALHIV at Gertrudes Hospital from 2019-2022



	LDL	Low risk LLV	High risk LLV	Suspected treatment failure
Pre-DTG <5yrs	100%	0	0	0
Post DTG <5yrs	100%	0	0	0
.				
Pre-DTG 6-12yrs	87.50%	0	5.40%	7.10%
Post-DTG6-12yrs	92.90%	2.40%	2.40%	2.40%
. 2				
Pre-DTG>13 yrs	85.30%	3.50%	3.50%	7.70%
Post-DTG>13 years	93.70%	0	1.80%	3.60%

5.13 Simple linear regression of Haemoglobin

The simple regression had a statistically significant P value for age and sex. The intercept indicated that the mean haemoglobin in the reference group(under 5) is 12.56g/dl. The coefficient for "Over 13" is 0.89, indicating that on average, individuals in this age group have haemoglobin levels that are 0.89 units higher than those in the reference group. The coefficient for the 6 to 12 age group was -0.06, indicating that on average, individuals in this age group have haemoglobin levels that are 0.06 units lower than those in the reference group.

The p-values associated with the coefficients indicated the level of statistical significance of the under 5 and over 13 age group's effect on haemoglobin levels was statistically significant at the 0.05 level. The R-squared value (0.08057) indicated that only 8% of the variation in haemoglobin levels was explained by the age group variable. The F-statistic tested the overall significance of the model and has a p-value of 0.003912, indicating that the model was statistically significant.

Similarly, simple regression was done on white blood count and platelet count with no predictor variable having an adjusted R squared of over 0.3. Stepwise multiple linear regression was then done by adding the predictor variables to the model and analyzing the effect on the model's p-value and adjusted R squared. None of the multiple regression models had an adjusted R squared of more than 0.3. This would indicate that the chosen predictor variables in isolation or in combination did not adequately explain the variation in haemoglobin, white blood cells, or platelets. Thus no further inference could be drawn from the regression calculation.

Table 17. Simple regression estimates Post DTG heamoglobin and selected predictor variables observed among CALHIV at Gertrudes Hospital CCC from 2019-2022

Independent variable	Groups	Normal(%)	Abnormal (%)	Estimates	Estimate P values	Model Adjusted R squared	Model P value
Age	Under 5*	100%	0	12.5	0.9506	0.08	0.0039
	6 -12	98.5%	1.5%	0.06	<0.0001		
	Over 13	97%	3%	0.88	0.0012		
Sex	Male	89.9%	10.1%	0.60	0.0173	0.11	<0.0001
	Female*	91.1%	9.1%	12.9	<0.0001		
Viral Suppression	Suppressed*	86.6%	13.4%	13.2	<0.0001	0.01	0.5179
	Unsuppressed	87.5%	12.5%	-0.2	0.518		
AZT/3TC duration in months	Intercept	95%	5%	13.78	<0.0001	0.02	0.5236
	Slope			-0.01	0.524		
CPT	No	91%	9%	12.56	0.293	0.01	0.7236
	Yes			0.64	0.277		

*Reference group

CHAPTER 6

6.0 Discussion

Before dolutegravir initiation, the prevalence of anaemia in the under 5 was 20%, thrombocytosis was 20% and thrombocytopenia was 20%. After dolutegravir initiation, the prevalence reduced to zero percent. In the 6-12 age group, a decline in prevalence of 8.3% and 10% in anaemia and thrombocytosis is seen, respectively, with no change in prevalence of thrombocytopenia after dolutegravir initiation. In the over-13 age group, a decline of prevalence of 2% and 2.6% was noted in thrombocytosis and thrombocytopenia after dolutegravir initiation, and no change was noted in the prevalence of anaemia

Pre dolutegravir treatment the prevalence of leucopenia in age groups 6-12 and over 13 years was 10% and 27.6% respectively. Post-dolutegravir, the prevalence increased to 21.6% in the 6-12 years and 42.1% in over 13years. The IMPAACT trial 1093 found that children on dolutegravir treatment between the ages of 12 -18years had a prevalence of 8.7% of leucopenia(57).

Before dolutegravir initiation, neutropenia prevalence stood at 80% in the under 5,21.6% in the 6-12 years,and 30.3% in the over 13 years after treatment the prevalence decreased to 20% in the under 5,15% in the 6-12years and 18.4%in the over 13years these findings are lower than the IMPAACT trail 1093 which showed a prevalence of 35.29% in the 2 years-6years, 26.09% in the 6-12years and 17.39% in the 12-18years (57).

There is an increase in eosinophil prevalence in 6-12 years from 1.7% to 10%, as seen in a case report of a 44-year-old lady who developed a rash two days after switching to dolutegravir-based regimen(58)

Viral load findings show a shift is seen in all age groups from suspected treatment failure to lower detectable limits

6.1 Conclusion

Overall dolutegravir treatment showed a positive impact on heamatological parameters in all age groups

Once-daily dolutegravir, in combination with up to two other antiretroviral drugs, is well tolerated with a greater virological effect

6.2 Recommendations

The data collected supports the expedited roll-out of dolutegravir-based ART to all infants and children.

A study on the efficacy of dolutegravir-based ART in children with high viral loads at the time of switch in combination with extensive drug resistance should be done

A study on heamatological parameters of dolutegravir experienced children and adolescents should be done

6.3 Ethical Considerations

Ethical clearance was sought from U.O.N and KNH Ethics and Research Committee. A consent waiver obtained from Gertrude's Children's Hospital Ethics Review Board where the study was undertaken. Confidentiality was upheld by ensuring no personal identifying data was revealed to unauthorized persons by the use of passwords and study numbers instead of names.

All results will be disseminated at conferences and journals.

6.5 Study Strengths and Limitations

Missing data.

Included only children who were virally suppressed

Identified delay in dolutegravir initiation

locally no available data on heamatological parameters on dolutegravir

STUDY TIMELINE

Activity	Oct 21	Nov 21	Dec 21	Jan 22	Feb 22	Mar 22	Apr 22	May 22	June 22	July 22	Aug 22	Sept 22	Oct 22	Nov 22	Dec 23
Concept development															
Concept presentation to faculty															
Handing books for marking															
Approval by ERC															
Data collection															
Data entry															
Data analysis															
Poster Presentation															
First internal marking															
Student corrections															
Second internal marking															
Book sent to the external examiner															

Study Budget

1	**PREPARATION PHASE**			
	Cost item	**Quantity**	**Unit price (KSh)**	**Total Cost (KSh)**
	STATIONERY			
	Notebook	1	100.00	100.00
	Printing	5	5.00	25.00
	Pen	2	15.00	30.00
	Transport	1	60.00	60.00
	Communication	2	250.00	500.00
	Total Cost			**715.00**
2	IMPLEMENTATION			
	statistician	1	15000.00	15000.00
	Transport	3	60.00	180.00
	Thesis printing	1000	5.00	5000.00
	Printing drafts	500	5.00	2500.00
	KNH Ethics committee fee	1	2500.00	2500.00
	Getrude's Ethics committee fee	1	10000	10000
	Contingency			5009.25
	Total cost			**38,404.25**

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APPENDICES

APPENDIX 1 DATA TOOL- FACTORS AFFECTING HAEMATOLOGICAL PARAMETERS IN VIRALLY SUPPRESSED HIV INFECTED CHILDREN

Study No.	Sex	Date of Birth	Date of Diagnosis	Date of ART start	Date of DTG switch	WHO stage-enrollment	WHO stage-DTG switch
	M / F						
Diagnostic tests	DNA PCR(<6 wk)	DNA PCR(6wk)	DNA PCR(6 mnth)	DNA PCR(12 mnth)	Antibody(18m nth)	Antibody(24mnth)	Other
Date and result							

Prophylactic drugs	**Date started**	**Date stopped**	**ART**	**Date started**	**Date stopped**	**Other medications**	**Date started**	**Date stopped**
AZT +NVP			ABC+3TC			Iron		
NVP			AZT+3TC			Albendazole		
AZT			EFV					
CPT			LPVr					
IPT			DTG					

Date	Weight (kgs)	Height (cm)	Z score	Muac	OI reported	VL (c/mm3)	CD4 % if<5 years	WBC *10^9/L	Neut *10^9/L	Relative WBC Abn	HB g/dl	Retic Count %	MCV fl	Plt *10^9/L

Collected by -…… Date-……

APPENDIX 2 WHO CLINICAL STAGING OF HIV INFECTION IN INFANTS AND CHILDREN (56)

STAGE 1	**STAGE 2**
Asymptomatic Persistent generalised lymphadenopathy Unexplained , asymptomatic hepatosplenomegally	Papular pruritic eruptions Seborrheic dermatitis Fungal nail infections Angular chelitis Linear gingival erythema Extensive human papillomatous virus or molluscum infection (>5%body area/ face) Recurrent oral ulcerations (>2 episodes / in 6 months Parotid enlargement Herpes zosters Recurrent or chronic upper respiratory infection: otitis media, otorrhea, sinusitis(>2episodes/6months)
STAGE 3	**STAGE 4**
Unexplained moderate malnutrition (-2SD or Z score) not responding to standard therapy Unexplained persistent diarrhoea (>14 days) Unexplained persistent fever (intermittent or constant>1 month) Oral candidiasis (outside neonatal period) Oral hairy leucoplakia Pulmonary tuberculosis Severe recurrent presumed bacterial pneumonia (>2 episodes/12 months. Acute necrotizing ulcerative gingivitis/periodontitis Lymphoid interstitial pneumonitis Unexplained anaemia(<8g/dl), neutropenia(<1000/mm³), or thrombocytopenia (<30,000/mm³ for>1month HIV-related cardiomyopathy HIV-related nephropathy	Unexplained severe wasting or severe malnutrition (-3SD or Z score) not responding to standard therapy Pneumocystis pneumonia Recurrent severe bacterial infection (>2episodes/12months excluding pneumonia) Chronic orolabial or cutaneous human simplex virus Extra-pulmonary tuberculosis Kaposi's sarcoma Oesophageal candidiasis CNS toxoplasmosis Cryptococcal meningitis Any disseminated endemic mycosis Cryptosporidiosis or isosporiasis(with diarrhoea>1 month) Cytomegalovirus infection of an organ other than liver, spleen, lymphnodes (and onset age >1 month) Disseminated mycobacterial disease other than tuberculosis Candida of the trachea, bronchi, or lungs Acquired recto-vesicular fistula Cerebral or B-cell non-Hodgkins Lymphoma Progressive multifocal leukoencephalopathy HIV encephalopathy

APPENDIX 3 PREFERRED 1st and 2nd LINE ANTIRETROVIRAL TREATMENT REGIMEN FOR CHILDREN AND ADOLESCENTS (59)

Age	Preferred 1st line	Alternative 1st line	Preferred 2nd line
Birth to 4weeks	AZT+3TC+NVP	AZT+3TC+RAL	
4weeks –less than 3 years	ABC+3TC+LPV/r	AZT+3TC+LPV/r ABC+3TC+RAL/NVP AZT+3TC+NVP/RAL	DRT –based 2nd line AZT+3TC+LPV/r ABC+3TC+LPV/r
3-14years and less than 35kg body weight	ABC+3TC+EFV	ABC+3TC+RAL AZT+3TC+EFV/RAL ABC/AZT+3TC+LPV/r	AZT+3TC+LPV/r ABC+3TC+LPV/r DRT-based 2nd line
More than 15years of more than 35kg	TDF+3TC+DTG TDF+3TC+EFV	ABC+3TC+DTG AZT+3TC+DTG/EFV TDF/ABC/AZT+3TC+ATV/r/LPV/r	AZT+3TC+ATV/r TDF+3TC+ATV/r DRT-based2nd line
Pregnant and Breastfeeding	TDF + 3TC + DTG	ABC+3TC+EFV AZT+3TC+DTG/EFV TDF/ABC+3TC+ATV/r/LPV/r AZT+3TC+ATV/r/LPV/r	AZT + 3TC + ATV/r3 TDF+3TC+ATV/r AZT+3TC+DRV/r+RAL TDF+3TC+DRV/r+RAL

AZT-Zidovudine, ABC- Abacavir,3TC-Lamivudine, NVP-Nevirapine, RAL-Raltegravir, LPV/r-Lopinavir/ Ritonavir, TDF- Tenofovir, EFV- Efavirenz , DRT- Drug Resistant Testing, DTG –Dolutegravir, ATV/r-Atazanavir/Ritonavir, DRV/r-Darunavir/Ritonavir

REGIMEN USED IN TUBERCULOSIS/HIV and HIV HEPATITIS B COINFECTION (59)

HIV/HBV Coinfection		TDF+3TC+DTG	TDF+3TC+ATV/r
TB/HIV Co-infection	Age		Super-boost LPV/r or DRT-based 2ndline
	<4weeks	ABC+3TC+LPV/r super-boosted with RTV	
	3-14years	ABC+3TC+EFV	
		AZT+3TC+RAL	Double dose of RAL ABC+3TC+ super-boosted LPV/r
	>15years or Weight >_35kg	TDF+3TC+DTG orTDF+3TC+EFV	

HIV/HBV-Human Immunodeficiency Virus/Hepatitis B Virus, TB/HIV-Tuberculosis/Human Immunodeficiency Virus TDF- Tenofovir,3TC-Lamivudine, DTG –Dolutegravir, ATV/r-Atazanavir/Ritonavir, ABC- Abacavir, EFV- Efavirenz LPV/r-Lopinavir / Ritonavir, RTV-Ritonavir, DRT-drug resistance testing, RAL-Raltegravir.

PREFERRED 3rd LINE IN ANTIRETROVIRAL TREANTMENT REGIMENS (59)

	Possible 3rd line regimen
Children	RAL/DTG+3TC+DRV/r
	AZT RAL/DTG3TCDRV/r
	ABC/TDFRAL/DTG3TCDrv/r
	EFV 3TCDRV/r
Adults	DTG+3TC+DRV/r
	DTG+AZT+3TC+DRV/r
	DTG+TDF+3TC+DRV/r
	DTG+TDF/AZT+3TC
	EFV+3TC+DRV/r

RAL-Raltegravir, DTG –Dolutegravir, 3TC-Lamivudine, DRV/r-Darunavir/Ritonovir, AZT-Zidovudine, ABC-Abacavir, TDF- Tenofovir, EFV- Efavirenz .

APPENDIX 4 DAIDS TABLE (60)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
WBC, Decreased (*cells/L*) *> 7 days of age*	*2.000 x 10^9 to 2.499 x 10^9*	*1.500 x 10^9 to 1.999 x 10^9*	*1.000 x 10^9 to 1.499 x 10^9*	*< 1.000 x 10^9*
≤ 7 days of age	*5.500 x 10^9 to 6.999 x 10^9*	*4.000 x 10^9 to 5.499 x 10^9*	*2.500 x 10^9 to 3.999 x 10^9*	*< 2.500 x 10^9*
Absolute Neutrophil Count (ANC), Low (*cells/L*) *> 7 days of age*	*0.800 x 10^9 to 1.000 x 10^9*	*0.600 x 10^9 to 0.799 x 10^9*	*0.400 x 10^9 to 0.599 x 10^9*	*< 0.400 x 10^9*
2 to 7 days of age	*1.250 x 10^9 to 1.500 x 10^9*	*1.000 x 10^9 to 1.249 x 10^9*	*0.750 x 10^9 to 0.999 x 10^9*	< 750 *< 0.750 x 10^9*
≤ 1 day of age	*4.000 x 10^9 to 5.000 x 10^9*	*3.000 x 10^9 to 3.999 x 10^9*	*1.500 x 10^9 to 2.999 x 10^9*	*< 1.500 x 10^9*
Hemoglobin, Low				

(g/dL) *≥ 13 years of age(male only)*	10.0 to 10.9	9.0 to < 10.0	7.0 to < 9.0	< 7.0
≥ 13 years of age(female only)	9.5 to 10.4	8.5 to < 9.5	6.5 to < 8.5	< 6.5
57 days of age to < 13years of age (male and female)	9.5 to 10.4	8.5 to < 9.5	6.5 to < 8.5	< 6.5
36 to 56 days of age(male and female)	8.5 to 9.6	7.0 to < 8.5	6.0 to < 7.0	< 6.0
22 to 35 days of age(male and female)	9.5 to 11.0	8.0 to < 9.5	6.7 to < 8.0	< 6.7
8 to ≤ 21 days of age(male and female)	11.0 to 13.0	9.0 to < 11.0	8.0 to < 9.0	< 8.0
≤ 7 days of age (male and female)	13.0 to 14.0	10.0 to < 13.0	9.0 to < 10.0	< 9.0
Platelets, Decreased (*cells/L*)	*100.000 x 10^9 to < 125.000 x 10^9*	*50.000 x 10^9 to < 100.000 x 10^9*	*25.000 x 10^9 to < 50.000 x 10^9*	*< 25.000 x 10^9*

APPENDIX 5 VARIABLES TO BE COLLECTED

Variable	Format/ Units	Purpose
Study No.	AAA111	Anonymised study identifier
Sex	M/F	Predictor analysis
Date of Birth	ISO format-yyyy/mm/dd	Derive secondary variable
Date of Diagnosis	ISO format-yyyy/mm/dd	Derive secondary variable
Date of ART start	ISO format-yyyy/mm/dd	Derive secondary variable
Date of DTG switch	ISO format-yyyy/mm/dd	Before and After DTG analysis
WHO stage-enrollment	1,2,3,4	Predictor analysis
WHO stage-DTG switch	1,2,3,4	Predictor analysis
DNA PCR(<6 wk)	ISO format-yyyy/mm/dd	Predictor analysis
DNA PCR(6wk)	ISO format-yyyy/mm/dd	Predictor analysis
DNA PCR(6 mnth)	ISO format-yyyy/mm/dd	Predictor analysis
DNA PCR(12 mnth)	ISO format-yyyy/mm/dd	Predictor analysis
Antibody(18mnth)	ISO format-yyyy/mm/dd	Predictor analysis
Antibody(24mnth)	ISO format-yyyy/mm/dd	Predictor analysis
Prophylactic drugs-start date	ISO format-yyyy/mm/dd	Derive secondary variable
Prophylactic drugs-stop date	ISO format-yyyy/mm/dd	Derive secondary variable
ART drugs-start date	ISO format-yyyy/mm/dd	Derive secondary variable
ART drugs-stop date	ISO format-yyyy/mm/dd	Derive secondary variable
Other drugs-start date	ISO format-yyyy/mm/dd	Derive secondary variable
Other drugs-stop date	ISO format-yyyy/mm/dd	Derive secondary variable
Date of clinic visit	ISO format-yyyy/mm/dd	Temporal causation analysis
Weight	kgs	Before and After DTG analysis
Height	cms	Before and After DTG analysis
Z score for age	Standard deviations	Before and After DTG analysis
Muac	cm	Before and After DTG analysis
New Opportunistic infections	Categorical	Before and After DTG analysis
Viral load	Copies/mm^3	Before and After DTG analysis
CD4 count/percentage	Count/ % depending on age	Before and After DTG analysis
White blood cell count	$*10^9/L$	Before and After DTG analysis
Neutrophil Count	$*10^9/L$	Before and After DTG analysis
Relative WBC abnormality	Categorical	Before and After DTG analysis
Hemoglobin	g/dl	Before and After DTG analysis
Reticulocyte count	%	Before and After DTG analysis
Mean Corpuscular Volume-RBC	femtolitres	Before and After DTG analysis
Platelet count	$*10^9/L$	Before and After DTG analysis

Secondary variable	Original variable	Purpose
Age at HIV diagnosis	Date of birth, Date of diagnosis	Check if duration of HIV infection has a cumulative effect on hematologic parameters
Age at DTG switch	Date of birth, Date of DTG switch	Determine if the age at DTG switch has an effect on hematologic parameters
Duration of prophylaxis (x)	Prophylactic drug (x) -start date, Prophylactic drug (x) -stop date	Check if duration of prophylaxis usage has a cumulative effect on hematologic parameters
Duration of ART regimen (x)	ART regimen (x)-start date, ART regimen (x)-stop date	Check if duration of ART usage has a cumulative effect on hematologic parameters
Duration of any other drugs (x)	Other drugs-start date, Other drugs-stop date	Check if duration of other drugs has a cumulative effect on hematologic parameters

Appendix 6 APPROVAL FORM FOR GERTRUDE'S CHILDRENS HOSPITAL

Bella Juma,
P.O. Box 79567-00200,
Nairobi, Kenya,
0704761440.

Gertrude's children's Hospital,
Head of Clinical Services,
34 Muthaiga Road, Nairobi.
Attention: Dr. T.Ngwiri

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH AT YOUR FACILITY

My name is Bella Juma, a post graduate student in the Department of Paediatrics and Child Health at the University of Nairobi. As part of my training, I am conducting a research on how medicines used to treat HIV affect the blood in children, under the supervision of Professor Ruth Nduati, Lecturer School of Medicine University of Nairobi, Dr. Anne-Marie Macharia Peadiatric Infectious Disease Consultant Kenyatta National Hospital, and Dr. Joseph Mbuthia Paediatric Infectious Disease Consultant at Gertrude's Children's Hospital.

The study will only be conducted after approval from the Ethical Review Board of Kenyatta National Hospital and University of Nairobi. There will be no risks encountered as I will be looking at the results of blood tests routinely done at the clinic. The data set collected will be anonymized once it has been accrued and verified for accuracy.

Results of the study will be presented as an MMED dissertation, and also shared in conferences and journals. A copy of the data used for this analysis will be deposited with the institutions together with any publication accruing from it.

If in the course of the study we identify problems that need resolution, Dr.Mbuthia as a senior consultant for this HIV service will be informed. I am therefore kindly requesting your approval to proceed with the study.

Yours sincerely,

Bella Juma

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Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/358

22nd September, 2022

Dr. Bella Juma
Reg. No. H58/33975/2019
Dept. of Paediatrics and Child Health
Faculty of Health Sciences
University of Nairobi

APPROVED 22 SEP 2022

Dear Dr. Juma,

RESEARCH PROPOSAL: FACTORS AFFECTING HAEMATOLOGICAL PARAMETERS IN HIV-INFECTED CHILDREN WHO ARE VIRALLY SUPPRESSED (P255/03/2022)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P255/03/2022.** The approval period is 22nd September 2022 – 21st September 2023.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH-UoN ERC

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Assistant Director, Health Information Dept., KNH
The Chairperson, KNH- UoN ERC
The Chair, Dept. of Paediatrics and Child Health, UoN
Supervisors: Prof. Ruth Nduati, Dept. of Paediatrics and Child Health, UoN
Dr. Ann-Marie Macharia, Paediatric Infectious Disease Consultant, KNH
Dr. Joseph Mbuthia, Paediatric Infectious Disease Consultant, Gertrude's Children's Hospital

Protect to discover

FACTORS AFFECTING HAEMATOLOGICAL PARAMETERS BEFORE AND AFTER INTRODUCTION OF DOLUTEGRAVIR TO HIV-INFECTED CHILDREN AT GETRUDES CHILDRENS HOSPITAL COMPREHENSIVE CARE CLINIC

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