Zambian newborns exposed to HIV and antiretroviral drugs: Evolution of neutrophil cell count in the first 6 months

Sub-study of the ANRS 12174 trial in Zambia

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ABSTRACT

Background: The efficacy of antiretroviral drugs (ARVs) for the prevention of HIV transmission from mother to child is one of the greatest successes of antiretroviral therapy. However, concern has been expressed in several studies about potential side effects of antenatal and neonatal exposure to antiretroviral drugs such as the occurrence of low neutrophil cell in infants during the early months of life. A number of children were potentially eligible for the ANRS 12174 trial but were finally not included because their absolute neutrophil counts was low. These children are provided with nevirapine according to WHO recommendations and the national programme.

Objectives: To investigate evolution of the neutrophil cell count during the first 6 months of life in HIV-exposed, uninfected children subjected to antiretroviral drugs perinatally and with low neutrophil count.

Design: A prospective longitudinal cohort study related to the ANRS 12174 trial.

Subjects and methods: We enrolled 56 uninfected infants born to HIV-positive mothers on antiretroviral drugs (zidovudine, lamivudine, nevirapine) during pregnancy and child birth, 18 with neutrophil count too low to be included in the trial ("study group", receiving nevirapine during breastfeeding as 'standard of care') and 38 with neutrophil count just above the cut-off for inclusion in the trial ("comparative group", receiving one of the two study drugs, lamivudine/ Kaletra®). Maternal CD4+ cell counts were assessed before delivery and neonatal characteristics include gender, gestational age, weight and length. Clinical condition, growth and absolute neutrophil count were followed up to 26 weeks of age. Non-improvers were compared to improvers.

Results: The mean gestational age at birth was 38.0 (SD ± 1.7) weeks and the mean birth weight was 3.0 (±0.4) kg. There was gender balance in the group of 56 enrolled infants, 27 girls and 29 boys. The mean absolute neutrophil counts at day 7 and at week 26 were: 1) in the study group, 1.0 (±0.2) and 1.6 (± 0.7), respectively; and 2) in the comparative group 1.4 (±0.2) and 2.1 (±1.2), respectively. The non-improvers in terms of absolute neutrophil count (= deteriorating, remaining low or very slight improvement) in the study and the comparative group were 5/18 and 6/38 infants,

respectively). No statistical association was found between non-improvement and selected potential predictors. All infants were apparently thriving with no apparent concurrent conditions and did not receive any other medications except for septrim and the follow-up drugs. There was no statistically significant difference in growth between the non-improvers and the improvers.

Conclusion: With the limitations of this study, such as small sample size, lack of appropriate control group, low follow-up intensity, short duration of follow-up and the question mark around the usefulness of the DAIDS tables, we conclude that 1) infants exposed to HIV-1/ART antenatelly and who received nevirapine or lamivudine/Kaletra® from birth up to age week 26 may be at risk for transient low absolute neutrophil count; 2) such neutropenia can be moderate to severe and still not generate any apparent morbidity in the form of hospitalisations or other severe illnesses; 3) over the first 6 months, there is a good chance that the child recovers spontaneously and 4) these infants as a group present a growth as expected which is an indication that they are doing OK.

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LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ANC	Absolute neutrophil count
ART	Antiretroviral treatment
ARVs	Antiretroviral drugs
AZT	Zidovudine
BAN study	Breastfeeding, Antiretroviral and Nutrition study
BEN	Benign ethnic neutropenia
CBC	Complete blood count
DAIDS	Division of AIDS, National Institutes of Health, USA
DBS	Dry blood spot
D7	Day 7
EBF	Exclusive breastfeeding
EDTA	Ethylenediaminetetraacetic acid
HAART	Highly Active Anti-Retroviral Therapy
HIV	Human immunodeficiency virus
LPV	Lopinavir
LPV/r	Lopinavir boosted with ritonavir
MTCT	Mother-to-child transmission
NVP	Nevirapine
OIs	Opportunistic infections
PCP	Pneumocystis pneumonia
PMTCT	Prevention mother-to-child transmission
VCT	Voluntary counseling and testing
WBC	White blood cells
WHO	World Health Organisation
W26	Week 26

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1. BACKGROUND AND LITERATURE REVIEW

1.1 White blood cells and neutrophils

All blood cells, white and red blood cells and platelets are produced in the bone marrow. They all originate from pluripotent hematopoietic stem cells. The white blood cells (WBC) or leukocytes, which are the focus of this thesis, are important components of the host defence system, responsible for protection against invading bacteria, fungi, virus, and parasites. Leucocytes fall in two categories: granulocytes and lymphoid cells (agranulocytes). The granulocytes include neutrophils, eosinophils and basophils; the lymphoid cells include the lymphocytes and monocytes. The average white blood cell count for an adult may vary between 5-10 x 10^9 cells/L of blood (Table 1). The SI unit for blood cell count is x 10^9/L, which is the same as x 10^3/mm^3 or x 10^3/µL. But many countries still use the count per cubic millimetre, in which case the 5-10 x 10^9 cells/L is equivalent to 5,000-10,000/mm^3. New-born infants have higher WBC count at birth (9-30 x 10^9 cells/L). The WBC count decreases with age, initially rapidly then more slowly. Children under the age of one year have a WBC count of 6-18 x 10^9 cells/L of blood. After a slow decline, it reaches adult levels at around 4 years. After this age the adult WBC count is used as reference for a healthy child.

Of the 5-10 x10^9 leukocytes/L in the blood of an adult the subgroups are present in different proportions : neutrophils 50-70% (2.5-7 x 10^9 cells/L), eosinophils 1-3% (0.1-0.3 x 10^9 cells/L), basophils 0.5-1% (0.05-0.1 x 10^9 cells/L), lymphocytes 20-40% (2-4 x 10^9 cells/L) and monocytes 2-8% (0.2-0.8 x 10^9 cells/L).The largest subgroups of white blood cells are thus neutrophils and lymphocytes. The number of neutrophils present in a blood sample is measured by the absolute neutrophil count (ANC).

Among the lymphocytes, there are two major types, B-cells and T-cells. T-cells develop from T-lymphoid precursor cells and the earliest progenitors of T-cells are produced in the bone marrow. The development and "training" to mature immunologically competent T-cells occurs in the thymus. During their residence in the thymus, precursors of T-cells develop into thymocytes. Thymocytes pass from the cortex of the

thymus to the medulla and the predominant thymocyte subsets in the medulla are the immune-competent CD4+ and CD8+ cells, which subsequently leave the thymus to

Table 1. Overview of the white blood cells and subtypes as well as the age-specific normal values and their main role

			Normal range	Unit	Main role
White blood cell count (WBC)	**Total**	**Over 21 years/Adult**	**5-10**	**x 10^9/L x 10^3/mm^3 or x 10^3/µL**	**Component of the host defence system**
		8-16 years	**5.0-13.5**		
		6 years	**5.0-14.5**		
		4 years	**5.5-15.5**		
		6 months-2 years	**6-18**		
		Newborn	**9-30**		
	Neutrophils	Adult	2.5-7.0	x 10^9/L	Very active in phagocyte of bacteria
		Newborn	6-26	x 10^9/L	
	Eosinophils	Adult	0-0.5	x 10^9/L	Modulate allergic inflammatory responses
		Newborn	0.02-0.85	x 10^9/L	
	Basophils	Adult	0.04-0.9	x 10^9/L	Release histamine for inflammatory responses
		Newborn	0-0.6	x 10^9/L	
	Lymphocytes	Adult	0.7-4.8	x 10^9/L	B cells: release antibodies and assists activation of the T cells T cells: participate in cell-mediated immune responses
		Newborn	2-11	x 10^9/L	
	Monocytes	Adult	0.1-0.8	x 10^9/L	migrate from the bloodstream to other tissues and differentiate into macrophages
		Newborn	0.4-3.1	x 10^9/L	
	Cd4+ cells	Adult	0.4-1.6	x 10^9/L	Helper-inducer T cells
		Newborn	0.5-2.0	x 10^9/L	
	CD8+ cells	Adult	0.2-1.1	x 10^9/L	Cytotoxic-suppressor T cells
		Newborn	0.35-1.75	x 10^9/L	

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populate the peripheral blood and the body to perform their respective functions: CD4+ cells serving primarily as T helper (Th) cells, and CD8+ cells serving primarily as cytotoxic T lymphocytes.(1) T lymphocyte subsets vary with age in childhood, but different conclusions have been reached regarding these age-related changes, possibly because of few community-based studies of CD4+ and CD8+ T cells in children not at risk of HIV infection.(2)

1.2 Natural variation in white blood cells

The distribution of white blood cells in a normal human population may be influenced by a number of factors. Fluctuations occur during the day; lower values are obtained during rest and higher values during exercise. Intense physical exertion may cause the count to exceed 20 x 10^9/L. White cell count also may increase in response to convulsions, strong emotional reactions, pain, pregnancy and labour. Other factors include age, gender, race or ethnicity.

Benign ethnic neutropenia (BEN) has been suggested as a name used to describe individuals of African descent with absolute neutrophil counts less than 1.5x 10^9 cells/L in the absence of other causes.(3) Approximately 25-50% of persons of African descent and some ethnic groups in the Middle East have benign ethnic neutropenia.(4) A closer look at this condition would offer an opportunity to re-evaluate the normal range of the absolute neutrophil count (ANC) for all ethnic groups and if the lower level for normal value should be placed below the current, conventional 1.5 x 10^9 cells/L, large numbers of people now considered to have benign ethnic neutropenia will automatically fall within the normal range, and the diagnosis of benign ethnic neutropenia could no longer be justified.(4)

1.3 HIV

Human immunodeficiency virus (HIV) is a lentivirus that infects vital cells in the human immune system such as lymphocytes. Two types of HIV have been characterised HIV-1

and HIV-2. Both are believed to have originated from non-human primates in West-Central Africa and were transferred to humans. (5)

HIV-1 is more virulent and infective and is the cause of the majority of HIV infections globally, while HIV-2 has relatively poor capacity for transmission and is largely confined to West Africa. The spread of the virus is via sexual intercourse, shared intravenous needles, blood transfusion, pregnancy and breastfeeding. HIV testing is initially by an enzyme-linked immunosorbent assay (ELISA) to detect HIV antibodies; and polymerase chain reaction test (PCR test) which is useful test to detect the genetic material of the virus itself.

Three stages of HIV infection have been described:
- The initial stage of infection (primary infection), which occurs within weeks of acquiring the virus, and often characterised by a flu-like illness that generally resolves within weeks;
- The stage of chronic asymptomatic infection which lasts an average of 8-10 years;
- And the stage of symptomatic infection in which the body's immune system has been suppressed and opportunistic infections (OIs) occur known as the acquired immunodeficiency syndrome (AIDS).
The absolute number and proportion of CD4 T lymphocytes and the CD4/CD8 ratio are used as markers for infection progression.(2)
A normal CD4 count is from 500 to 1,500 cells/ mm^3. The ratio CD4/CD8 is approximately 1.5-2 CD4 cells to one CD8 in healthy individuals and falls as CD4 cells counts fall in individuals with HIV infection.
Antiretroviral therapy (ART) is the recommended treatment for HIV infection.

1.4 Antiretroviral drugs

Antiretroviral drugs, called ARVs, are medications for the treatment of infection by retroviruses, primarily HIV. The development of these drugs has been exceptionally rapid with less than 20 years from discovery of the virus to the arrival of a functional drug regimen to allow infected individuals to have a near-normal life and life-span.

Usually new drugs in medicine are first tested and used in adults before being tested in adolescent patients above 12 years of age. If ok, they are thereafter tested in children 6-12 years, then 2-6 years and only thereafter tested in children. The use of new drugs in pregnancy is extremely restrictive to protect the developing foetus. However, unlike most other drugs used in medicine; this process was fast-tracked for ARVs due to the urgent need. Even their use during pregnancy was approved extremely rapidly. This means that their potential side-effects have less extensively studied.

Table 2. Groups of antiretroviral drugs

Antiretroviral drug class	Abbreviations	Drugs	Mode of action
Nucleoside Reverse Transcriptase Inhibitors	NRTIs	abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zidovudine, delavirdine, efavirenz, etravirine, nevirapine, rilpivirine	NRTIs interfere with the action of the HIV enzyme reverse transcriptase, which makes DNA copies of the viral RNA strands.
Non-nucleoside Reverse Transcriptase Inhibitors	NNRTIs	efavirenz, etravirine, nevirapine, rilpivirine	NNRTIs also stop HIV from replicating within cells by inhibiting the reverse transcriptase protein
Protease Inhibitors	PIs	atazanavir, darunavir fosamprenavir, indinavir lopinavir/ ritonavir, nelfinavir, ritonavir saquinavir, tipranavir	PIs inhibit protease, which is another enzyme involved in the HIV replication process.
Fusion Inhibitors		enfuvirtide	Fusion or entry inhibitors prevent HIV from binding to or entering human immune cells.
Integrase Inhibitors		raltegravir	Integrase inhibitors interfere with the integrase enzyme, which HIV needs to insert its genetic material into human chromosomes.

There are different classes of antiretroviral drugs that act on different stages of the HIV life-cycle: the nucleoside reverse transcriptase inhibitors, the non-nucleoside reverse

transcriptase inhibitors, the protease inhibitors, the fusion inhibitors and the integrase inhibitors.

1.4.1 Groups of antiretroviral drugs

The table 2 represents the different groups of antiretrovirals, the drugs in each group and the mode of action of the drugs.

1.4.2 Drug combinations

Some antiretroviral drugs have been combined into one pill, which is known as a 'fixed dose combination'.

Table 3. Examples of fixed-dose combinations.

Brand name	Content
Triomune	Stavudine + lamivudine + nevirapine
Combivir	zidovudine + lamivudine
Trizivir	abacavir + zidovudine + lamivudine
Kaletra	lopinavir + ritonavir
Epzicom (in USA) / Kivexa (in Europe)	abacavir + lamivudine
Truvada	tenofovir + emtricitabine
Atripla	Efavirenz + tenofovir/emtricitabine

1.4.3 Adverse events associated with antiretroviral drugs

The ARVs are drugs with frequent side effects, which is due to the fact that the viral metabolism and the human metabolism are close to each other and when a drug intercepts the function of a viral enzyme, it is likely also to interfere to some extent with similar human enzymes. The following table summarizes the most common and most serious adverse events associated with antiretroviral medications.

Table 4. ARVs and side effects

Class	Drug	Side-effects
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Abacavir (ABC)	-Hypersensitivity syndrome (fever, myalgia, malaise, nausea, vomiting, diarrhoea) -Rash
	Didanosine (ddl)	-Pancreatitis -Peripheral neuropathy -Nausea, diarrhoea
	Emtricitabine (FTC)	-Headache, nausea, diarrhoea, insomnia -Hyperpigmentation of palms and soles
	Lamivudine (3TC)	-Headache, dry mouth
	Stavudine (d4T)	-Peripheral neuropathy -Pancreatitis -Dyslipidaemia -Diarrhoea
	Tenofovir (TDF)	-Flatulence, nausea, diarrhoea, abdominal discomfort -Asthenia -Acute/Chronic renal insufficiency,
	Zidovudine (ZDV/AZT)	-Anaemia, neutropenia -Fatigue, malaise, headache, -Nausea, vomiting -Myalgia, myopathy -Hyperpigmentation of skin and nail
Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Delavirdine (DLV)	-Fatigue -Elevations in liver function tests, hepatitis -Nausea, diarrhoea
	Efavirenz (EFV)	-Drowsiness, dizziness, confusion, mood changes -Elevations in liver function tests -Hyperlipidaemia
	Etravirine (ETV/ETR)	-Elevations in liver function tests
	Nevirapine (NVP)	Elevations in liver function tests, hepatitis, liver failure
	Rilpivirine (RPV)	-Insomnia, depression -Elevations in liver function tests -Elevations in serum creatinine
Protease Inhibitors (PIs)	Atazanavir (ATZ)	-Hyperbilirubinaemia, jaundice -Elevations in liver function tests -PR interval prolongation
	Darunavir (DRV)	-Rash -Elevations in liver function tests
	Fosamprenavir (FPV/r)	-Diarrhoea, nausea, vomiting

Class	Drug	Side-effects
		-Elevations in liver function tests -Rash -Hyperlipidaemia
	Indinavir (IDV/r)	-Nephrolithiasis, flank pain -Hyperbilirubinaemia -Elevations in liver function tests -Alopecia, dry skin, ingrown nails -Insomnia -Taste perversion
	Lopinavir/ ritonavir (LPV/r)	-Diarrhoea, nausea, vomiting -Dyslipidaemia -Elevations in liver function tests -Taste perversion
	Nelfinavir (NFV)	-Diarrhoea -Nausea, vomiting -Elevations in liver function tests -Fatigue
	Ritonavir (RTV)	-Nausea, vomiting, diarrhoea, abdominal pain -Elevations in liver function tests -Fatigue -Peripheral numbness -Taste perversion -Hyperuricemia
	Saquinavir (SQV/r)	-Nausea, vomiting, diarrhea -Elevations in liver function tests -Headache -Oral ulcerations
	Tipranavir (TPV)	-Nausea, vomiting, diarrhoea -Elevations in liver function tests -Increased total cholesterol and triglycerides -Rash -Intracranial haemorrhage
Fusion Inhibitors	Enfuvirtide	-Injection site reactions; erythema, cysts, and nodules at injection sites -Neutropenia -Possible increased frequency of pneumonia
Integrase Inhibitors	Raltegravir (RAL)	-Nausea, diarrhoea, flatulence -Elevations in amylase and liver function tests -Headache -Dizziness, -Pruritus, rash -Fatigue, muscle pain

1.4.4 Potential haematological side-effects of ARVs

The efficacy of antiretroviral drugs (ARVs) for the prevention of HIV transmission from mother to child has been clearly demonstrated and remains one of the greatest successes of antiretroviral therapy. (6) Correctly administrated, ARVs have the potential to reduce this transmission to less than 1%. (7) However, these drugs have been reported to have side-effects. They are all targeting the replication of the virus which means that its interference in the human cells is most pronounced in rapidly replicating cells such as for instance the blood cells. Therefore, haematological toxicities such as anaemia, neutropenia and thrombocytopenia are among the most common adverse events of antiretroviral drugs. In neonates, zidovudine monotherapy, or zidovudine in combination with lamivudine, was reported to induce short-term changes in haematological parameters. (8)

A longer-term reduced neutrophils in children have also been associated to ART. (7) The table below shows the potential haematological side effects caused principally by zidovudine.

Table 5. ARVs and haematological toxicities

Adverse effects	Associated ARVs	Onset + Clinical manifestations	Estimated frequency	Risk factors
Anaemia	Principally AZT	Onset: Variable, weeks to months Presentation: Most commonly asymptomatic or mild fatigue, pallor, rarely tachypnoea, Congestive heart failure	HIV-exposed newborns: Severe anaemia HIV-infected children on ARVs: 2-3 times more common with AZT	HIV-exposed newborns: premature birth HIV-infected children on ARVS: underlying hemoglobinopathy, Poorly controlled HIV
Neutropenia	Principally AZT	Onset: variable Presentation: most commonly asymptomatic		

Concern has been expressed in several studies about potential side effects of antenatal and neonatal exposure to antiretroviral drugs such as the occurrence of low neutrophil cell in infants during the early months of life.

1.4.5 Studies of potential side-effects of ARVs on the exposed infants

In a study evaluating the effect of HIV-1 antiretroviral prophylaxis on hepatic and haematological parameters of African infants, infants born to women who received or did not receive standard intrapartum nevirapine (NVP) dosing were randomized to receive orally either single dose NVP alone or NVP plus zidovudine (twice daily for a week). An additional group of untreated infants, born to HIV-uninfected women was enrolled as a control group. The investigators found that haematological parameters did not differ between groups at birth but at 6 weeks of age the levels of granulocytes were lower among antiviral drug-treated groups compared with controls. They concluded that hematologic abnormalities associated with short-term neonatal antiretroviral treatment among African children are minimal. (8) However a nested cohort study within a randomized clinical trial (the Mashi Study) was conducted to assess haematological toxicities associated with in utero and breastfeeding exposure to maternal antiretroviral therapy among infants in Botswana. Absolute neutrophil level was recorded from birth to 7 months of age in infants and grade 3 and 4 toxicities were compared by infant antiretroviral exposure status. It was found that antenatal exposure to ARVs was associated with increased risk for neutropenia in infants up to one month of age. (9) The same was found in a prospective observational study that investigated haematological alterations during the first 3 months of life in HIV-exposed uninfected infants subjected to antiretroviral medication before and after birth. Two hundred twenty-one uninfected infants born to HIV-positive mothers on antiretroviral medication during pregnancy were included and the perinatal prophylaxis comprised zidovudine. Full blood counts and differentials were determined at birth and at 2, 4, 6, and 12 weeks of age and haematological toxicity was graded according to paediatric toxicity scales. The investigators observed that antiretroviral prophylaxis was associated with significant anaemia and neutropenia in HIV-uninfected infants during the first 3 months of life. (10)

A study conducted to evaluate the effect of maternal HIV-1 status and antiretroviral drugs on haematological profiles of South African infants in early life concluded that maternal HIV-1 disease status and antiretroviral drug exposure influence infant haematological parameters which may have implications for infant immune response capacity. (11) In a study looking at the levels and patterns of neutrophil cell count over the first 8 years of life in children of HIV-1-infected mothers (31) the authors evaluated the influence of maternal immune status on infant neutrophil levels. The findings were that in the first 6 months of life, uninfected children of mothers with CD4 cells count <250 cells/µL had lower neutrophil counts than those of mothers with 250-500 cells/µL or > 500 cells/µL.

To investigate haematological parameters in infants born to HIV-1-infected mothers and exposed to ART, a prospective observational study was carried out in Netherlands. The researchers concluded that antiretroviral therapy is successful in preventing MTCT (mother-to-child transmission), but alterations in haematological parameters may persist for a long period and the clinical implications remain uncertain. (12) With the increasing use of antiretroviral drugs to prevent mother-to-child transmission of HIV, large numbers of infants are exposed, with potential subsequent toxicity. (13)

1.4.6 Toxicity Tables and related concern for African children

The Toxicity Tables (table 6) elaborated by the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Disease, National Institute of Health commonly referred to as "the DAIDS tables", includes parameters for grading both adult and paediatric adverse events and one of them is absolute neutrophil count.

These tables are being used to evaluate potential toxicities in multisite trials around the world, which include participants from diverse racial/ethnic backgrounds. (14) Although use of standardized norms helps provide a common base for comparisons, reliance on laboratory norms based on data from US populations alone could lead to misclassification of normal values as "abnormal" among other populations such as in Africa and East Asia. (14) A number of researchers have raised concern about the validity of the reference values for hematologic and immunologic indices currently used

in Africa. In a cross-sectional study in a rural parish in eastern Uganda (15), researchers collected blood samples from more than 3,000 HIV-negative Ugandans to assess the validity of current reference values of hematologic and immunologic indices for an African population. The neutrophil counts in this population were lower than standard values, and there appeared to be regional variations in reference values and the range of hematologic and immunologic indices between the African population and the population in the USA.

Table 6. Toxicity tables

DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009 LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
HEMATOLOGY *Standard International Units are listed in italics*				
Absolute CD4+ count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	$300 – 400/$ mm^3 *300 – 400/μL*	$200 – 299/$ mm^3 *200 – 299/μL*	$100 – 199/$ mm^3 *100 – 199/μL*	$< 100/$ mm^3 *< 100/μL*
Absolute lymphocyte count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	$600 – 650/$ mm^3 *0.600 x 10^9 – 0.650 x 10^9 /L*	$500 – 599/$ mm^3 *0.500 x 10^9– 0.599 x 10^9 /L*	$350 – 499/$ mm^3 *0.350 x 10^9 – 0.499 x 10^9 /L*	$< 350/$mm^3 *< 0.350 x 10^9 /L*
Comment: Values in children ≤ 13 years are not given for the two parameters above because the absolute counts are variable.				
Absolute neutrophil count (ANC)				
Adult and Pediatric, > 7 days	$1,000 – 1,300/$ mm^3 *1.000 x 10^9 /L – 1.300 x 10^9 /L*	$750 – 999/$mm^3 *0.750 x 10^9 /L – 0.999 x 10^9 /L*	$500 – 749/$mm^3 *0.500 x 10^9 /L – 0.749 x 10^9 /L*	$< 500/$mm^3 *< 0.500 x 10^9 /L*
Infant †, **2 – ≤ 7 days**	$1,250 – 1,500/$ mm^3 *1.250 x 10^9 /L – 1.500 x 10^9 /L*	$1,000 – 1,249/$ mm^3 *1.000 x 10^9 /L – 1.249 x 10^9 /L*	$750 – 999/$ mm^3 *0.750 x 10^9 /L – 0.999 x 10^9 /L*	$< 750/$mm^3 *< 0.750 x 10^9 /L*
Infant †, **≤1 day**	$4,000 – 5,000/$ mm^3 *4.000 x 10^9 /L – 5.000 x 10^9 /L*	$3,000 – 3,999/$ mm^3 *3.000 x 10^9 /L – 3.999 x 10^9 /L*	$1,500 –2,999/$ mm^3 *1.500 x 10^9 /L – 2.999 x 10^9 /L*	$< 1,500/$ mm^3 *< 1.500 x 10^9 /L*
Comment: Parameter changed from "Infant, < 1 day" to "Infant, ≤1 day"				
Fibrinogen, decreased	$100 – 200$ mg/dL *1.00 – 2.00 g/L* OR $0.75 – 0.99$ x LLN	$75 – 99$ mg/dL *0.75 – 0.99 g/L* OR $0.50 – 0.74$ x LLN	$50 – 74$ mg/dL *0.50 – 0.74 g/L* OR $0.25 – 0.49$ x LLN	< 50 mg/dL *< 0.50 g/L* OR < 0.25 x LLN OR Associated with gross bleeding

*Values are for term infants. Preterm infants should be assessed using local normal ranges.

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Table 7. Neutrophil counts for the different classifications

Absolute neutrophil count	Infant 2 - ≤ 7 days	Adult and Paediatric > 7 days
Grade 1 Mild	1.25 - 1.50 x 10^9 /L	1.00 - 1.3 x 10^9 /L
Grade 2 Moderate	1.00 - 1.24 x 10^9 /L	0.75 - 0.99 x 10^9 /L
Grade 3 Severe	0.75 - 0.99 x 10^9 /L	0.50 - 0.74 x 10^9 /L
Grade 4 Potentially life-threatening	< 0.75 x 10^9 /L	< 0.50 x 10^9 /L

The same concern was addressed in a cross-sectional laboratory study (14) aiming to determine normal hematologic and selected blood chemistry values among healthy, full-term, non-HIV-exposed infants during the first 6 months of life in Uganda and Malawi, and to use this information to assess the proportion of healthy babies who would be labelled as having an "abnormal" or toxicity-graded laboratory value based on US reference norms using the 2004 DAIDS toxicity tables. The authors found that after 7 days of life, about 20% of healthy infants at both sites would have been classified as having a low neutrophil count. According to the researchers, the findings underscore the importance in the conduct of international paediatric clinical trials to gather relevant local norms for infant hematologic values to avoid mislabelling laboratory values of healthy infants taking part in a study as abnormal or assuming possible drug toxicity based on inaccurately describing a normal value as representing a toxicity grade.

During the initial stages of conduct of a clinical trial of an antiretroviral and a nutritional intervention in HIV-infected breastfeeding mothers and their infants in Malawi (16), it was noted an unexpected high number of infants with absolute neutrophil counts that would have been classifiable as neutropenic using the National Institutes of Health's Division of AIDS toxicity tables. Among the 206 infants who were recruited some months after the start of the study, there were 86 infants with neutropenia at any time point (42%). The authors argue that the relevant Division of AIDS table does not take into account the available evidence of low absolute neutrophil count in African infants. As part of the European Collaborative Study, a prospective birth cohort study, a cross-sectional study (17) was conducted to investigate racial immuno-haematological differences in HIV-uninfected children with or without HIV exposure in Uganda and Europe. The researchers compared cross-sectional data from children in Uganda to data on children residing in Europe (black and Caucasian), and noted that during infancy, the total lymphocyte count (TLC) , CD4+, CD8+ counts were lower in Ugandan children

compared to black children in Europe. The interesting finding was that in Ugandan children, CD4+ count and neutrophil count were also lower than in children born to Ugandan mothers in Europe. The DAIDS tables are based on normal blood counts for white infants and are used in sub-Saharan Africa, although there is evidence that black African infants may have lower absolute neutrophil counts (ANCs) than white infants. (18)

Concerns have been also expressed about the validity of the reference values for hematologic and immunologic indices currently used in Africa. In the Breastfeeding, Antiretroviral and Nutrition (BAN) study (19) conducted in Malawi in order to evaluate strategies to reduce HIV transmission through breast milk and optimize both maternal and infant health among HIV-infected women and their infants, the investigators end up changing the infant neutropenia criteria for their protocol in light of benign ethnic infant neutropenia. At the beginning of the study, it was noticed that of 206 newborns enrolled, 22 had grade 3 or 4 neutropenia (10%) without any clinical manifestations and with spontaneous recovery of the absolute neutrophil count within weeks. Some details of the above mentioned studies regarding the side-effects of ARVs on the exposed infants and the use of the Toxicity Tables are shown in table 8.

Table 8. Studies on hematologic toxicity associated with perinatal exposure to ARVs and population-based absolute neutrophil count

Reference Location Duration	Study design Follow up time	Sample	Study groups	Results Percentage or Neutrophils (x 10^9/L)
Bae et al (9) Botswana, March 2001- October 2003	Nested cohort study within a randomized clinical trial. CBC for 1 and 2 at birth and at 1,2,3,4,5,6,7 months	N=1,200 infected pregnant women	1 **Exposed** HAART mother-infant pairs(n=69) 2 **Control** mother-infant pairs (n=109)	Grade 3 or 4 ANC 1 5.8% at **birth**, 15.9% **between birh-1month**, 2.9% **between 2-7 months** 2 1.8% at **birth**, 3.7% **between birth-1month**, 8% between **2-7 months**
Taha et al (8) Malawi,	Ancillary study to a randomized clinic trial. CBC for 1 and 2 at birth and 6 weeks Follow up 12 months	N=1,000 infants	1 **Exposed** Intra-partum NVP alone or NVP plus AZT (n=800) 2 **Control** (n=200)	**At birth**: No haematological differences between 1 and 2 **At 6 weeks**: 1 Grade 1(mild) toxicity 75% (600)

Schramm et al (11) Chris Hani Baragwanath hospital and Coronation women and Children Hospital, South Africa	Cohort study Follow up: 12 weeks	N=601	1 **Exposed** No drug (n=28), NVP only (n=424) HAART (n=31) 2 **Control** (n=118)	Neutrophils 1 **At birth** 9.4×10^9 /L **6weeks** 2.3×10^9 /L **12 weeks** 1.7×10^9 /L 2 **At birth** 9.1×10^9 /L **6 weeks** 1.8×10^9 /L **12 weeks** 1.7×10^9 /L
Bunders et al (12) Emma Children's Hospital, Netherlands 1997-2002	Prospective cohort study	N=201	1 **Exposed** ART(n=92) 2 **Control** (n=72)	Neutrophils 1 **At birth** 6.4×10^9 /L **5 weeks** 2.1×10^9 /L **8 months** 2.6×10^9 /L **20 months** 2.7×10^9 /L 2 **At birth** 7.8×10^9 /L **5 weeks** 3.0×10^9 /L **8 months** 3.9×10^9 /L **20 months** 3.2×10^9 /L
Cornelia et al (10) Charité University Medical Centre, Germany 1997-2004	Prospective Observation study. CBC at birth and at 2,4,6,12 months	N=215 mothers and 221 infants	1 38% exposed to ZDV, 62% exposed to HAART 2 None	**Neutropenia Grade 2 or higher at least once** 106 infants (48%)
Lugada et al (15) Eastern Uganda, Jan.-Sep.2002	Population based cross-sectional survey	N=3,421	HIV-negative Ugandans Age group (yr) (no. of subjects) <1 year (n=373) 1-5 years (n=518) 6-12 years (n=731) 13-18 years (n=346) 19-24 years (n=235) >24 years (n=845)	Median neutrophils Cell count [10^9 /L (90% reference interval)]by age group 2.1(0.9-4.4) $\times 10^9$ /L 2.1(1.0-3.9) $\times 10^9$ /L 1.8 (0.9-3.6) $\times 10^9$ /L 1.8 (0.9-3.5) $\times 10^9$ /L 1.8 (1.0-3.5) $\times 10^9$ /L 1.8 (0.84-3.37) $\times 10^9$ /L

Lubega et al (14) 1.The Makerere University-Johns Hopkins University (MU-JHU) Core Laboratory, Uganda 2.The Johns Hopkins University-College of Medicine Research Project (JHU/COM RP) Laboratory, Malawi	Cross-sectional laboratory studies	N=561 (Uganda, n1=254; Malawi, n2=307)	Healthy full-term infants born to HIV-negative mothers at Mulago Hospital (Uganda) and Blantyre Hospital (Malawi)	Low absolute neutrophil count based on DAIDS Toxicity Tables 2004 **At birth** n1: 2/10 (20%); n2: 13/45 (29%) **1-7 days** n1: 3/65 (4.6%); n2: 1/37 (2.7%) **>7 days** n1: 35/179 (19.5%) n2: 42/206 (20.4%)
Kourtis et al (16) Kamuza Central Hospital-Lilongwe, Malawi, Mar.2004-Jan. 2010	Clinical Trial-the Breast-feeding, Anti-retroviral and Nutrition (BAN) study Follow up to 40 weeks of life	N=206 infants (by 2005)	1 **Exposed** to ART 2 **Control**	**Preliminary finding (2005)** Low absolute neutrophil count based on standard DAIDS criteria (Grade 1-4) **At birth** n=25 **Days 3-7** n=4 **Weeks 2-18** n=57 Total: 86
van der Horst et al (19) Kamuza Central Hospital-Lilongwe, Malawi Mar.2004-Jan. 2010	Clinical Trial(Factorial study design)-the Breast-feeding, Anti-retroviral and Nutrition (BAN) Study.	N=2,418 HIV-Infected mother-infant pairs	n=403 per each of the 6 types of treatment	**Revised** **DAIDS toxicity table (20)** **Day2-7** Grade1: 1250-1500 Grade 2: 1000-1250 Grade 3: 750-1000 Grade4: < 750 **Amendment to protocol** **BAN modification of toxicity table** **Day2-7** Grade1: 750-1200 Grade2: 400-750 Grade3: 250-400 Grade 4: < 250

| Bunders et al (17)

1. Uganda

2. Europe | Cross-sectional study | N=3,622 HIV-uninfected children born to HIV-infected mothers | N1=1,633 living in Uganda

N2=1,959 (n2=604 black Ugandan ; n2'=1,355 Caucasians) living in Europe | **Patterns of TLC,CD4+,CD8+ and neutrophil counts over age**

Z-scores* for all 4 markers were Between (0.3; 0.7)higher in n2 than N1 in first 2 years; and between (-0.7;-0.2) lower after infancy (2-12 years)

* Z-score of 0 is the 50th centile line. In this analysis, n2 were the reference group against which the others were compared. |

1.5. Cotrimoxazole prophylaxis

HIV-positive children are at increased risk of getting Pneumocystis pneumonia (PCP) and cotrimoxazole (septrim) is the most effective drug to prevent the occurrence of this potentially fatal illness. In 2000, the World Health Organisation (WHO) recommended cotrimoxazole as prophylaxis against PCP for all HIV-positive patients in sub-Saharan Africa with a CD4 cell count below 500 cells/mm³. This recommendation was modified in 2004 to include all HIV-infected or -exposed children, until immune restoration has occurred or HIV-negativity is demonstrated. (21) In 2006, WHO reinforced this suggestion in a guideline document recommending cotrimoxazole prophylaxis for all infants exposed to HIV infection. (22) One of the well-known effects of this drug is low white blood cell count, particularly if administrated in association with certain medicines such as zidovudine (AZT). The impact of oral administration of cotrimoxazole on the white blood cells in association with other antiretroviral drugs has not been studied. (22)

1.6 The PROMISE PEP clinical trial

The PROMISE PEP trial or the ANRS 12174 trial is an on-going multisite, randomised controlled clinical trial that compare the efficacy and safety of prolonged infant peri-

exposure prophylaxis with lopinavir/ritonavir (LPV/r) versus lamivudine to prevent HIV-1 transmission through breast milk in children born to HIV-1-infected mothers not eligible for HAART and having benefited from the national prevention of mother to child transmission (PMTCT) program during pregnancy and/or delivery. The study closed recruitment by 30 April 2012 with 1269 mother-infant pairs recruited in African countries (Burkina Faso, Uganda, South Africa and Zambia). The study population is constituted by HIV-uninfected infants at day 7 (± 2 days) born to HIV-1 infected mothers and the child is excluded if her/his Hb < 12 g/dL, birth weight < 2.0 kg or presents with serious congenital malformation(s). Initially, haematological criteria for exclusion were based on the earlier version of the DAIDS tables, where grade 1 (mild adverse event AE, ANC < 1.75 x 10^9 cells/L) was used as exclusion criteria. Especially in the Zambian site this criteria lead to a high proportion of seemingly healthy babies being excluded. This meant that some infants were excluded from the study because of their low neutrophil count, below 1.75 x 10^9 cells/L at the beginning of the study. Later during the study, the exclusion criteria was adjusted in view of the review above, and from 15 January 2011, the lower cut-off for neutropenia was readjusted to 1.2 x 10^9 cells/L. The further development of their neutrophil count remains unknown.

2. RESEARCH QUESTION

A number of children were potentially eligible for the trial (ANRS 12174) but finally not included because their absolute neutrophil counts was low. These children are provided with nevirapine according to WHO recommendations (23) and the national programme. In the Zambian site, these children were/are followed at the clinic and we were interested to follow the natural development of this neutropenia. What is the evolution of their neutrophil count in comparison to the children in the study who receive lamivudine or Kaletra ®?

3. OBJECTIVES OF THE STUDY

The objectives of the study were

a) To investigate evolution of the neutrophil cell count during the first 6 months of life in HIV- exposed, uninfected children subjected to antiretroviral drugs antenatally, born with low neutrophil count and receiving nevirapine postnatally; and

b) To compare this evolution to that of infants born with a neutrophil cell count above the threshold receiving any of the study drugs lamivudine or Kaletra®.

4. SUBJECTS AND METHODS

4.1 Study area

This study was conducted in Lusaka (Zambia) at the University Teaching Hospital (UTH) in the Department of Paediatrics and Child Health. This site is one of the 4 where the main trial is conducted, selected because of its high proportion of children with low neutrophil count.

4.1.1 Zambia

4.1.1.1 Overview

Zambia is a landlocked country in Southern Africa with a total land area of 750,000 km² which is about twice that of Norway and an estimated population of about 13 million inhabitants in 2009. Approximately 35 % of the population lives in urban areas and 65% in rural areas. The Zambian population has increased over the past decades from only 2.3 million inhabitants in 1950 to the actual estimate. The neighbouring countries are the Democratic Republic of the Congo to the north, Tanzania to the north-east, Malawi to the east, Mozambique, Zimbabwe, Botswana and Namibia to the south, and Angola to the west. (24)

Figure 1: Location of Zambia within the African continent

Figure 2: Map of Zambia

4.1.1.2 Economic profile

The country's economy has historically been based on the copper mining industry. Once a middle-income country, Zambia began to slide into poverty in the 1970s when the copper prices declined on world markets. To reduce the economy's reliance on the copper industry, the Zambian government has been undertaking economic diversification by promoting agriculture, tourism, and hydro-power. The economy registered a real gross domestic product (GDP) growth rate of 6% in 2008. (25) In spite of this; the country faces challenges of poverty, slow-economic growth and corruption. The current annual per capita income at US$ 1,230 is well below their levels at independence in 1964 and place the country among the world's poorest nations.

4.1.1.3 Health indicators

Generally there have been some improvements in the health status of the Zambian population over the period 1950-2010.In the first 30 years, life expectancy at birth increased from 42 (1950) to 51 years (1980).The period between 1980 and 2000 saw a decline in life expectancy back to 42 years in 2000 due to the emergence of HIV.
In the last decade life expectancy has again increased and is currently estimated at 49 years. (26) Zambia's infant mortality has declined from 109 death/1,000 live births in 1990 to the current 69/1,000 live births (2010). However, it is still above the global average of 40/1,000 births. Similarly, the under-five mortality has also declined, from more than 200 deaths per 1,000 live births in 1960 to the current 111/1,000 live births (2010). The major causes of mortality among under-five children in Zambia are malaria, respiratory infections, diarrhoea, malnutrition and anaemia. The maternal mortality levels are high in Zambia, currently estimated to 590 per 100,000 live births, a decline from 729 per 100,000 births in 2001. (27)

 4.1.1.4 HIV in Zambia

Zambia, like the majority of the countries in Southern Africa, has one of the world's most devastating HIV epidemics. More than one in seven adults in the country is living with HIV. Heterosexual sex remains the major mode of transmission of HIV in Zambia accounting for 78% of new HIV infections.

A review of the estimates of trends in HIV incidence suggests that the HIV incidence in adults aged 15-49 years has halved since 1990 and is estimated to be at a stable level at 1.6% in 2009 (2% in women and 1.2% in men). Over 900,000 people are estimated to be living with HIV in Zambia, of which nearly 80,000 were newly infected in 2009, that is about 200 new infections each day. (27) The ZDHS data (24) show that the HIV prevalence in Zambia is 14.3%, a decline from 15.6% in 2001/2. The prevalence rates in Zambia vary significantly according to age, sex and geographical area. Of the approximately 1 million Zambians who are living with HIV and AIDS, 12% or 120,000 are children aged 0-14 years. (26)

The current HIV prevalence in adults according to data from ZDHS, 2007 (figure 3) shows a low prevalence in the 15-19 years age group (4.7%), the highest prevalence 23.6% for the age group of 35-39 years and lower 15.1% for the 45-49 years age group. Of particular concern is the HIV prevalence among young women. Young women between 15-19 years are more likely to be infected (5.7%) compared to young men of the same age group 3.6%. For the age group 20-24 years, this gap is wider with women at 11.8% twice that of the men (5.2%) of the same age group at and this gap remains for several age groups.

Figure 3: HIV prevalence by sex and age based on the ZDHS 2007

Source: ZDHS, 2007

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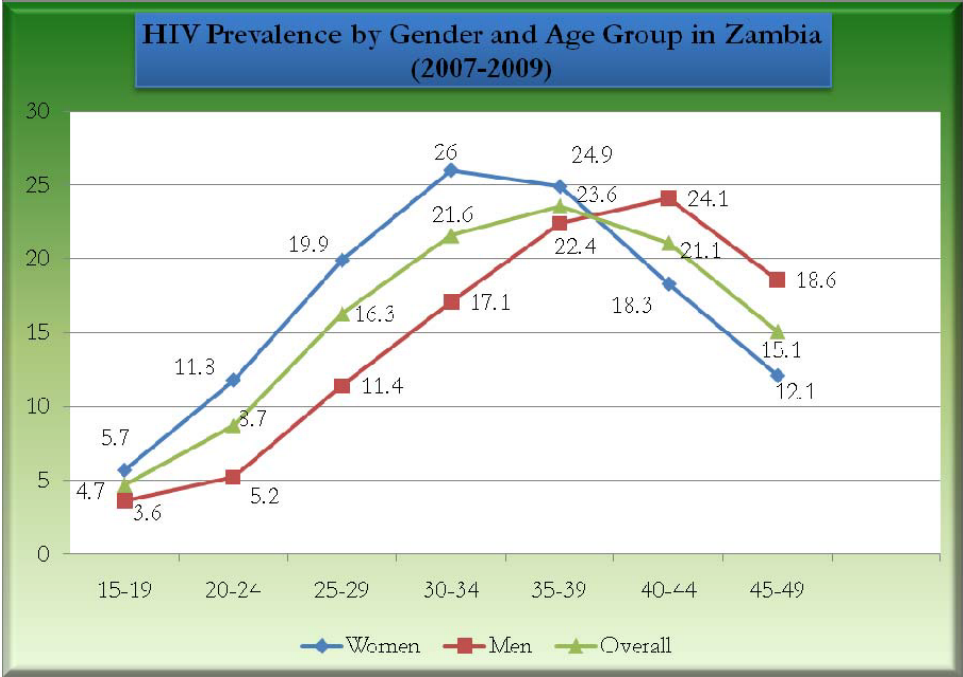
The HIV prevalence in Zambia varies dramatically by province, ranging from 6.8% in Northern to 20.8% in Lusaka. (28)

4.1.1.5 PMTCT in Zambia

In Zambia, mother to child transmission accounts for 21% of HIV infections during pregnancy, at birth or while breastfeeding. The availability and access to Prevention of Mother to Child Transmission (PMTCT) services continue to increase with a number

Table 9. Summary of WHO PMTCT and breastfeeding guidelines 2010

Mother:	
All women identified as HIV positive during pregnancy will receive Antiretroviral drugs	
CD4 count less or equal to 350 cells/mm^3 Mother takes ARVs for her own health	CD4 count more than 350 cells/mm^3 Mother takes ARVs for her infant's health
A recommended course of triple ARVs to be started as soon as possible and taken indefinitely.	Option A (maternal AZT) . AZT from 14th week gestation. Single dose NVP . AZT+3TC at onset of labour . AZT+3TC through 7 days postpartum Option B (maternal triple ARV prophylaxis) . A recommended course of triple ARVs from the 14th week of pregnancy until 1-week after cessation of breastfeeding.
Infant:	
All infants, whose status is unknown or negative, will receive daily NVP	
If breastfeeding	If not breastfeeding
Exclusively breastfeed for 6 months then complementary feed and continue breastfeeding for the first 12 months of life Option A (maternal AZT) If the mother is taking AZT for the infant, then the infant should have daily NVP until 1 week after cessation of breastfeeding Option B (maternal triple ARV prophylaxis) If the mother is taking ARVs for her infant's health then the infant should have daily NVP or AZT for 6 weeks.	The infant should have daily NVP or AZT for 6 weeks.

ANC facilities providing HIV testing and counseling for pregnant women and antiretroviral (ART) for PMTCT. Zambia's PMTCT initiative was launched in 1999, beginning with a three-year pilot programme in Copperbelt Province. In 2004 it had expanded so that 74 health facilities in 4 provinces offered antiretroviral drugs (primarily single dose nevirapine) to expectant mothers and newborn infants, reaching

939 facilities at the end of 2008. (27) In 2007 an estimated 47% of pregnant women living with HIV received ARVs for the prevention of mother-to-child transmission. By the end of 2009 this had increased to 69%. (29)

Zambia's PMTCT programme is based on the World Health Organisation's (WHO) guidelines. Previous guidelines, issued in 2006 (30) recommended that only women with a low CD4 count should receive ARVs to prevent mother to child transmission and all HIV positive mothers were advised to exclusively breastfeed for 6 months. Under the 2010 guidelines (30) all HIV positive mothers, identified during pregnancy, should receive a course of antiretroviral drugs to prevent mother to child transmission. All infants born to HIV positive mothers should also receive a course of antiretroviral drugs and should be exclusively breastfed for 6 months and complementary fed up to a year (table 9).

4.1.2 Lusaka

Lusaka, the capital city is located in the south-central part of the country and its population is estimated to about 2.2 million inhabitants in 2010 and 40 % of the population is constituted of children less than 15 years old. (25) It is the largest city in Zambia, which serves as the large commercial centre as well as the centre of government administration. The city is among the places most heavily burdened by HIV. At the four sentinel surveillance sites in Lusaka, the seroprevalence among pregnant women is being tested. The rates are high in all age groups, ranging from 10-24% among 15-19 years old. The highest rates was found among 25-29 years old at Kalingalinga (41%), in the 35-39 age group at Matero and Chilenje (45%), and among 40-44 year olds at Chelstone (33%). (28)

Figure 4: View of central Lusaka

4.1.3 University Teaching Hospital (UTH)

 The University Teaching Hospital (UTH) is the main medical institution in Zambia, located in Lusaka approximately 4 km east of the city centre.

Figure 5: View of the University Teaching Hospital in Lusaka

It has 1655 beds and 250 baby cots and provides a full range of primary, secondary, and tertiary health and medical services to in-and out-patients. It serves as the country's specialist centre receiving referrals from all over the country.

4.2 Study design and participants

We performed a prospective longitudinal cohort study in close relation to the ANRS 12174 trial. The data collection was carried out from 15 August 2011 to 15 November 2011 on site in Lusaka.

The primary population of this study were newborns that were screened for possible enrolment in the clinical trial ANRS 12174 in the Zambian site but failed to be included due to a low neutrophil count of less than 1.20×10^9 cells/L. A comparative group of children included in the trial with a neutrophil count of $1.20 - 1.75 \times 10^9$ cells/L was also selected from all four sites of the trial.

4.2.1 Inclusion criteria

The inclusion criteria were:
- Screening visit between 15 January 2011 to 15 November 2011
- Maternal CD4 count above 350 cells
- HIV-1 DNA PCR test negative at day 7 (+/-2)
- Perinatal exposure to ART
- Absolute neutrophil count $< 1.20 \times 10^9$ cells/L or $1.20\text{-}1.75 \times 10^9$ cells/L for the comparative group
- Informed written consent

4.2.2 Exclusion criteria

- Low birth weight (< 2.0kg)
- Multiple pregnancies
- Serious congenital malformation
- Severe illness

We included 18 HIV-1-unifected infants from January to December 2011, and a comparative group of 38 infants with a neutrophil count between $1.20 - 1.75 \times 10^9$ cells/L, was identified in the same time period from all four sites of the trial.

4.3 Intervention with antiretroviral drugs

The treatment of the mother with antiretroviral drugs during pregnancy and of the child after birth was taken into consideration. Women received zidovudine (AZT), lamivudine

(3TC) and nevirapine (NVP) for a certain number of weeks as chemoprophylaxis before delivery.

During follow-up, enrolled infants received NVP according to WHO recommendations (2010) and the national programme, while infants in the comparative group received the study drug.

4.4 Co-intervention

All infants in this study received cotrimoxazole prophylaxis from the age of 6 weeks until cessation of breastfeeding according to WHO and national guidelines.

4.5 Follow-up visits

The newborns that could not be enrolled in the ANRS 12174 trial were offered to be followed according to the same schedule as children in the ANRS 12174 trial with monthly follow-ups for refill of drugs. After being screened right after delivery, infants were examined to detect any clinical exclusion criteria, and thereafter followed up in the outpatient clinic at day 7, week 6, week 10, week 14 and week 26. At each visit, blood samples were analysed for absolute neutrophil count and the mothers were informed about the result. HIV tests were performed at birth, at day 7 and at 3 and 6 months of age. They were informed about the scheduled clinic visits and encouraged to bring the child to the research clinic whenever he/she is sick. Electronic and paper-based files were used during data collection. For the data collected within the ANRS trial, OpenClinica was used (www.openclinica.com), whereas the data on the non-included children was collected on paper.

4.6 Clinical and laboratory monitoring

Maternal laboratory tests, including CD4 cell count were assessed during pregnancy. Neonatal characteristics, including gender, gestational age, weight and length were recorded at birth. Infants were examined to detect any clinical exclusion criteria and followed at least until the age of week 26 according to the protocol. White blood cell

count and differentials were evaluated at day 7 and at week 26. Deviation from age-dependent normal ranges was assessed, and neutrophil values outside the normal range were graded according to the revised 2004 paediatric toxicity tables developed by the National Institute of Allergy and Infectious Diseases, Division of AIDS (DAIDS). (20)

An HIV DNA Polymerase chain reaction (PCR) assay was performed at birth and infants were considered uninfected if this HIV PCR test was negative.

Phlebotomy/venepuncture was performed on the infants via venepuncture using either the antecubital vein or the dorsal vein, and a total of 2-3 ml was collected in EDTA Vacutainer® tubes. Blood was then transported to the site laboratory where a complete blood count (CBC) was performed within 12 hours of blood collection; using ABX pentra XL 80 automated haematology analyzer (Horiba medical) according to the manufacturer's protocol.

The table below shows the number of samples used in this substudy.

Table 10. Samples used in this substudy

Age	Blood collection	Blood storage
<7 days	Heparin 2.5 ml EDTA 2ml	1 Filter paper (4 spots)
Day 7	Heparin 2.5 ml EDTA 2ml	1 Filter paper (4 spots)
Week 26	Heparin 5 ml EDTA 5ml	1 Filter paper (4 spots)

4.7 Statistical methods

To define our dependent variable, normal value of absolute neutrophil count and grade 1 toxicity were classified together as "normal ANC or mild toxicity" and grade 2, grade 3 and grade 4 toxicity were classified together as "moderate or severe toxicity". Descriptive statistics were used to characterize the study population. For continuous variables, means and ranges were reported; and the non-parametic Mann-Whitney U test was used to determine whether there were any significant differences in the proportions and absolute neutrophils cells count between the infants in the study groups. For categorical variables, frequencies and percentages were reported. Differences in proportions were analysed using the [Chi] ^{2} test and Fisher's exact test when appropriate. For uni-or multivariate analyses, logistic regression was applied to

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obtain odds ratios (ORs) and 95% confidence intervals (CIs) to assess the association between perinatal exposure to ARV drugs and neutrophil cell count.

The associations were examined at 2 time points: at age day 7 and at age week 26; the models were adjusted for age, infant's birth weight, infant's sex and maternal CD4+ cell count at delivery.

Data analyses were performed using SPSS software for windows (version 18.0, SPSS Inc., Chicago, IL). All statistical tests were two-tailed and considered significant at $p < 0.05$

4.8 Ethical considerations

The ANRS 12174 Trial Steering Committee and the local Ethic Committee approved the study. Written informed consent was obtained from the mother of the participant following the trial informed consent form (see annex)

5. RESULTS

5.1 Study participants

Between 15 January and 31 December 2011, a total of 56 HIV-1-uninfected infants born from HIV-1-infected mothers were included, 18 with initial neutrophil count below 1.2 x 10^9 cells/L receiving nevirapine and the remaining 38 with an initial neutrophil count between 1.2-1.75 x 10^9 cells/L receiving the study drug. Of the infants, 27 (48.2%) were girls, and 29 (51.8%) were boys. Selected characteristics are summarized below in table 11. Most characteristics were similar between the infants in the study group and in the comparative group. There were small differences in the median weight and length at day 7 in favour of the comparative group. At week 26 this difference had increased in the study group on nevirapine compared to the trial infants on the study drug with a p-value of 0.04 and 0.03, respectively. So the differences were statistically significant. The mean gestational age at start of AZT appears to be somewhat lower in the comparative group compared to the study group (Table 12).

Table 11. Selected characteristics of mothers and their infants.

	Mothers		
	Study group N=18 Mean (±SD)	Comparative group N=38 Mean (±SD)	All N=56 Mean (±SD)
Maternal age (years)	28.4 (±6.8)	27.1 (±5.5)	27.5 (±5.9)
CD4 at enrolment (cells/μL)	597 (±237)	568 (±198)	578 (±210)
Gestational age at start of AZT (weeks)	38.1 (±1.3)	29.0 (±3.6)	29.3 (±3.2)
Length of antenatal AZT (weeks)	8.2 (±2.1)	8.7 (±3.4)	8.5 (±3.0)
	Infants		
	Study group N=18 Mean (±SD)	Comparative group N=38 Mean (±SD)	All N=56 Mean (±SD)
Gestational age (weeks)	38.1 (±1.25)	37.9 (±1.9)	38.0 (±1.7)
Birth weight (kg)	2.9 (±0.4)	3.1 (±0.4)	3.0 (±0.4)
Weight at age day 7 (kg)	2.9 (±0.5)	3.1 (±0.5)	3.1 (±0.5)
Length at age day 7 (cm)	48.4 (±1.7)	49.2 (±2.1)	49.0 (±2.0)
Absolute neutrophils count at day 7 (x 10^9 cells/L)	1.0 (±0.2)	1.4 (±0.2)	1.3 (±0.3)
Weight at age week 26 (kg)	6.8 (±0.5)	7.3 (±1.0)	7.1 (±0.9)
Length at age week 26 (cm)	64.2 (±2.2)	65.6 (±2.2)	65.2 (±2.3)
Absolute neutrophils count at week 26 (x 10^9 cells/L)	1.6 (±0.7)	2.1 (±1.2)	2.0 (±1.1)

5.2 Neutrophil count parameters

Of the 18 study infants, 15 (83%) had a neutrophil count at day 7 classified according to the DAIDS tables as a toxicity grade 2 or more. At 26 weeks of age, only 3 infants (17%) fell into the category of grade 2 toxicity or more on neutrophils while the remaining 15 (83%) had higher neutrophil counts, table 13.

Of the 38 infants in the comparative group, 6 (12%) had a grade 2 toxicity or more at week 26. No statistically significant difference in the absolute neutrophil count values at day 7 was observed between males and females (p = 0.98; Mann-Whitney Test).

Table 12. Characteristics (median and IQR) of HIV-1/ART exposed infants with moderate-to-severe neutropenia receiving nevirapine and characteristics of HIV-1/ART exposed infants with mild-to-moderate neutropenia receiving study drug (lamivudine or Kaletra®).

Characteristics	Neutropenic moderate-to-severe HIV-1/ART-exposed infants on nevirapine (Study group)		Neutropenic mild-to-moderate HIV-1/ART- exposed infants on study drug (Comparative group)	
		IQR (25-75%)		IQR (25-75%)
Number of infants	18		38	
Gender ratio (M/F)	0.8 (8/10)		1.2 (21/17)	
Birth weight (kg)	2.8	2.7-3.3	3.0	2.8-3.4
Gestational age (wk)	38.0	37.0-38.5	38.0	36.0-40.0
Weight at day 7 (wk)	2.8	2.7-3.4	3.3	2.8-3.5
Length at day 7 (cm)	48.0	47.0-49.6	49.0	47.7-51.0
Absolute neutrophil count at day 7 (10^3 cells/mm^3)	1.0	0.9-1.1	1.5	1.3-1.6
Weight at week 26 (kg)	6.9	6.2-7.2	7.2	6.4-7.7
Length at week 26 (cm)	64.0	62.0-66.0	66.4	63.7-67.0
Absolute neutrophils count at week 26 (10^3 cells/mm^3)	1.5	1.1-2.0	1.9	1.5-2.5
Maternal age at delivery (years)	28.0	22.8-36.3	28.0	22.0-29.0
Maternal CD4+ count at enrolment (cells/µL)	551.5	445.3-646.5	519.0	407.0-656.8
Gestational age at start of AZT (wk)	30.0	28.8-31.3	29.0	27.0-32.0
Length of antenatal AZT (wk)	8.0	6.0-10.0	8.5	7.0-10.0

Tables 14 and 15 and their corresponding figures 6 and 7 shows the ANC evolution of each infant in the study and the comparative group at the 2 time points, day 7 and week 26. In the study group one infant (no 10) deteriorated from moderately low ANC at age day 7 to severe low ANC at age week 26 and four infants (no 12, 13, 14 and 15) improved from severe low ANC to mild or moderate low ANC; while four infants

improved from severe low ANC to moderate (no 12 and 14) or to mild low ANC (no 13 and 15). The improvement rate in this group by 26 weeks of age was 72% (13/18 cases). On the other hand, in the comparative group three infants deteriorated from mild low ANC at age day 7 to moderate (no 33 and 35) or severe low ANC (no 26) at week 26. One infant deteriorated from moderate to severe low ANC (no 55). In this group, 2 infants with a normal ANC at day 7 ended up with moderate (no 27) or severe low ANC (no 56) at week 26. The improvement rate in this group was 84% (32/38 cases). The number of cases with positive outcome in the study and the comparative groups were 13 and 32 respectively, while the number of cases with negative outcome is respectively 5 and 6.

Table 13. Number of infants in the two groups with neutrophil counts equivalent to toxicity at day 7 and week 26.

Absolute neutrophil count grading	Infants receiving nevirapine N=18		Infants receiving study drug N=38	
	Age day 7 n (%)	Age week 26 n (%)	Age day 7 n (%)	Age week 26 n (%)
Normal	0 (0)	13 (72)	16 (42)	32 (84.2%)
Grade 1 (mild)	3 (17)	2 (11)	16 (42)	0 (0)
Grade 2 (moderate)	5 (28)	2 (11)	6 (16)	3 (8%)
Grade 3 (severe)	9 (50)	1 (6)	0 (0)	3 (8%)
Grade 4 (potentially life threatening)	1 (6)	0 (0)	0 (0)	0 (0)

Table 14. Individual ANC evolution of infants in the study group from age day 7 to week 26.

Figure 6.Individual ANC evolution of infants in the study group from age D7 to W26

No and sex	Day 7		Week 26	
	ANC	Grd	ANC	Grd
1 m	0.79	3	3.12	R*
2 f	1.28	1	2.43	R
3 f	1.02	2	1.76	R
4 m	1.33	1	3.08	R
5 m	0.97	3	1.56	R
6 f	0.86	3	1.73	R
7 f	1.07	2	2.33	R
8 f	0.92	3	1.53	R
9 f	0.82	3	1.65	R
10 m	1.07	**2**	0.67	**3**
11 m	1.16	2	1.54	R
12 m	0.96	**3**	0.81	**2**
13 f	0.87	**3**	1.15	**1**
14 m	0.76	**3**	0.82	**2**
15 f	0.50	**4**	1.10	**1**
16 f	1.10	2	1.42	R
17 m	0.87	3	1.37	R
18 f	1.25	1	1.34	R
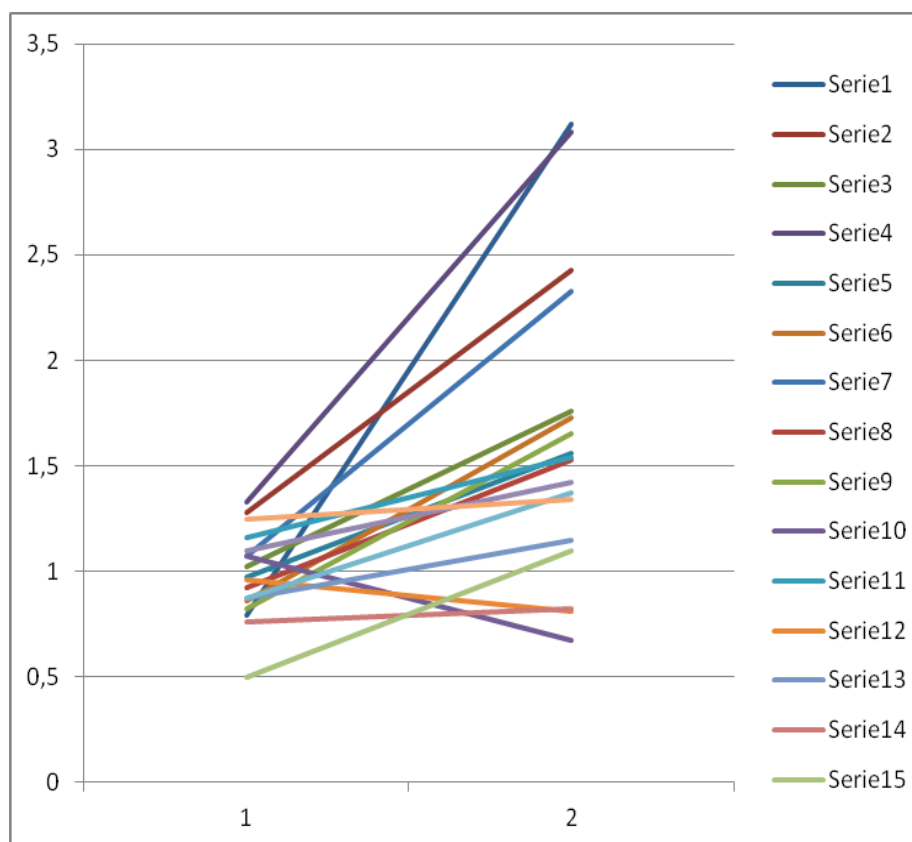
ANC in 10^9 cells/L

Grd: AIDS Grading

*R: Recovered, ANC normal

Number of cases/series with positive outcome: 13

Number of cases/series with negative outcome: 5

Table 15. Individual ANC evolution in the comparative group from day 7 to week 26.

No and sex	Day 7 ANC	Day 7 Grd	Week 26 ANC	Week 26 Grd
19 m	1.25	1	1.51	R*
20 m	1.67	N*	1.88	N*
21 m	1.65	N	2.31	N
22 m	1.30	1	3.06	R
23 f	1.30	1	3.93	R
24 f	1.54	N	1.95	N
25 f	1.00	N	2.90	N
26 m	1.37	**1**	0.66	**3**
27 m	1.53	**N**	0.96	**2**
28 f	1.51	N	1.35	N
29 m	1.46	1	4.61	R
30 f	1.55	N	4.21	N
31 m	1.40	1	1.49	R
32 f	1.21	2	1.32	R
33 m	1.42	**1**	0.93	**2**
34 f	1.50	1	2.70	N
35f	1.40	**1**	0.84	**2**
36m	1.68	N	2.44	N
37f	1.24	2	1.80	R
38 m	1.23	2	2.09	R
39 f	1.50	1	2.05	R
40 m	1.21	2	1.45	R
41 m	1.44	1	1.58	R
42 f	1.70	N	1.66	N
43 m	1.32	1	2.62	R
44 m	1.59	N	2.04	N
45 f	1.41	1	1.59	R
46 m	1.37	1	3.56	R
47 f	1.46	1	1.66	R
48 f	1.61	N	1.52	N
49 m	1.59	N	1.92	N
50 f	1.67	N	2.05	N
51m	1.22	2	1.95	R
52 m	1.49	1	2.41	R
53 m	1.61	N	7.08	N
54 f	1.61	N	1.93	N
55 m	1.20	**2**	0.68	**3**
56 f	1.68	**N**	0.72	**3**

Figure 7.Individual ANC evolution of infants in the comparative group from Day 7 to Week 26
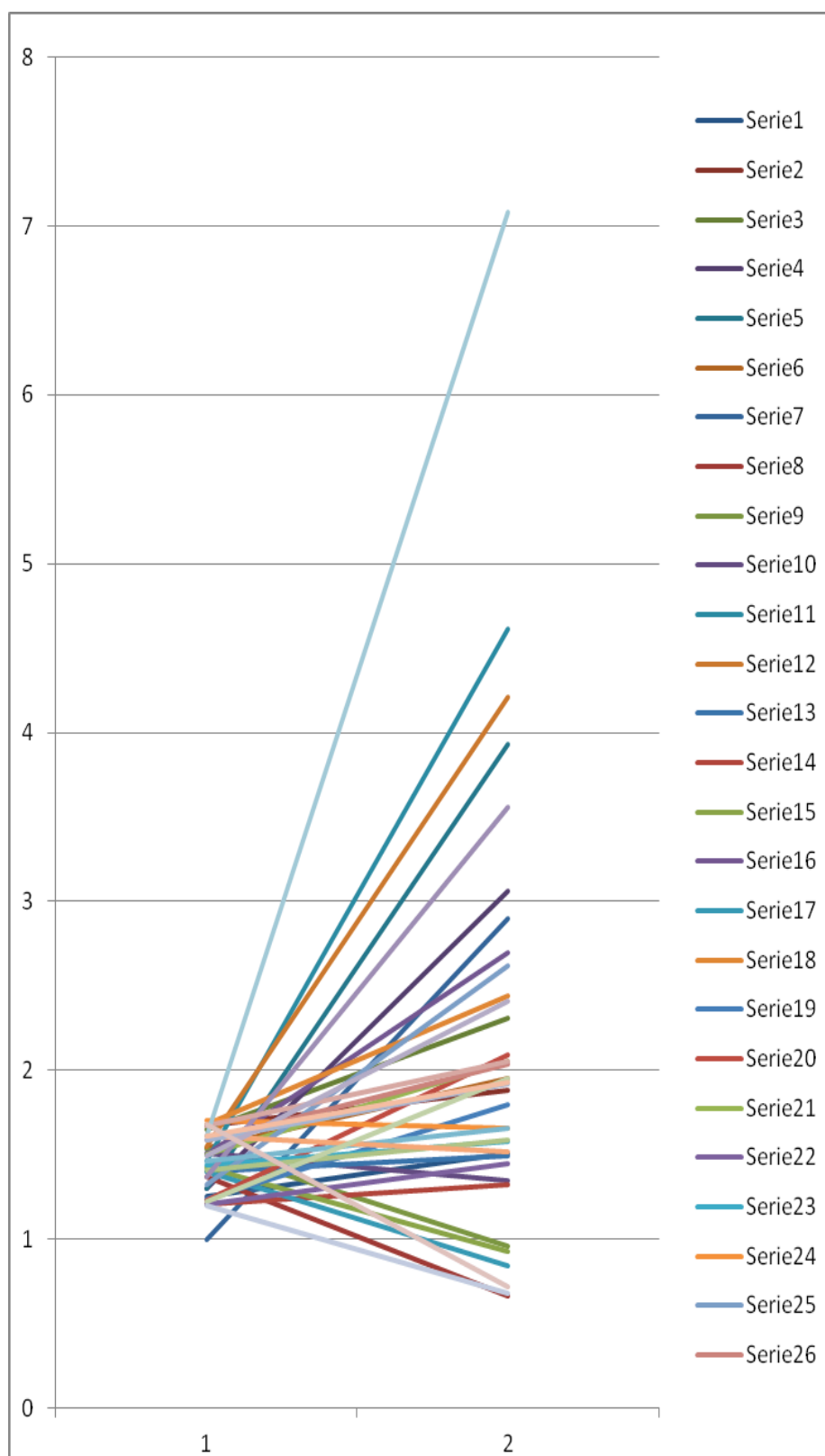
Serie 1 represents infant no 19, serie 2 infant no 20,...

ANC in 10^9 cells/L

Grd: AIDS Grading

*R: Recovered

*N: Normal ANC

5.3 Comparison of risk factors for neutropenia in the two groups (study and comparative group)

We categorised the infants according to whether or not their neutrophil count improved over time or not. Children who deteriorated, remained low (status quo) or had a very slight improvement were categorised as "non-improvers", and children whose neutrophil count rose as "improvers". This categorisation yielded 5 non-improvers and 13 improvers in the study group, and 6 non-improvers and 32 improvers in the comparative group.

The individual non-improving children in the study group were (table 14), 1 child who is deteriorating (no 10), 2 remaining on a low status quo (no 12, 14), 2 had a very modest improvement (no 13, 15) and the rest all had marked improvements in their ANC. In the comparative group (table 15), 4 children had deteriorating ANC (no 26, 27, 55 and 56), and 2 children remained on a low status quo (no 33 and 35) and the rest all had marked improvements in their ANC.

Table 16. Comparison of evolution of ANC in the 2 groups

		Absolute neutrophil count		
		Improved "improvers"	Remained low or deteriorated "non-improvers"	Totals
Group	Study group	(a) 13	(b) 5	(a+b) 18
	Comparative group	(c) 32	(d) 6	(c+d) 38
Totals		(a+c) 45	(b+d) 11	56

Odd ratio (OR) = (a/b)/ (c/d) = (13/5)/ (32/6) = 0.5 (95% CI: 0.12-1.88)

This OR suggests that there is a positive relationship between being in the study group and improvement of low absolute neutrophil count. However, this OR is not statistically significant.

Table 17. Comparison of risk factors for neutropenia

	Study group N=18		Comparative group N=38		0R (95% CI)	p[a]
Characteristics	Improvers n= 13	Non-improvers n= 5	Improvers n= 32	Non-improvers n= 6		
Male gender	5	3	17	4	0.4 (0.06-2.3)	0.35
Low birth weight (< 2.5 kg)	2	2	3	1	0.3 (0.01-6.6)	0.99
Gestational age (<37wk)	1	1	11	1	0.1 (0.003-2.8)	0.27
Maternal CD4+ count at enrolment (< 500 cells/µL)	7	1	13	4	2.1 (0.2-23.1)	0.64
Gestational age at start of AZT (< 27 wk.= before 3rd trimester)	1	1	10	0		0.16

[a] p-value calculated using Fisher's Exact Test, two-tailed

Potential predictors of persistent low neutrophil count were studied by comparing the improvers and non-improvers in the two groups (study and comparative group, table 17). We did not find any positive statistical association between the selected potential predictors and the recovery of children with low neutrophil count. Surprisingly, none of the commonly identified reasons for low neutrophil count like exposure duration to ART in utero or an earlier start with ART during pregnancy were related to the occurrence of low ANC or to poor recovery. Neither was maternal CD4 cell count at enrolment of less than 500 cells/µL, a predictor of low neutrophil count or a clinical condition in any of the groups.

In the medical records of infants with neutrophil toxicity grade 2 or more we did not find an increased frequency of infection as reported by parents or clinician. None of the exposed infants were admitted to the hospital for an infectious episode that required antibiotic treatment. All infants were apparently thriving with no apparent concurrent conditions and did not receive any other medications except for septrim and the follow-up drugs.

5.4 Growth of non-improving children

Infants are expected to grow and growth failure is commonly used in paediatrics to assess if an infant is thriving. We therefore studied the growth of each individual non-improver to see if we could identify any signs of growth failure. The assessment of their growth was done using the WHO growth standards for weight-for-height/length (WFH), height/length-for-age (HFA) and weight-for-age (WFA) and calculating the standard deviation score (Z-score) of each variable on day 7 and at week 26 (table 18) and its change during the mentioned growth period, A child growing well is supposed to have little change in Z-scores for the 3 variables over time, alternatively an increase in z-score may indicate a catch-up growth if the z-score was low in the first place. A z-score below -2 is considered a moderate deviation and below -3 is a severe deviation. For WFH the deviation is named 'wasting', for HFA it is named 'stunting' and for WFA it is called 'underweight'.

Table 18. Weight and height development of the affected children (non-improvers) in the study and the comparative group

No and sex	Z-score								
	WFH D7	WFH W26	WFH Change	HFA D7	HFA W26	HFA Change	WFA D7	WFA W26	WFA Change
10 m	-2.1	-0.7	1.4	-2.3	-1.0	1.3	-2.9	-1.2	1.7
12 m	-1.5	-1.7	-0.2	-0.5	-1.7	-1.2	-1.3	-2.3	-1
13 f	-3.0	0	3.0	-0.6	-1.9	-1.3	-2.3	-1.3	1
14 m	-0.4	-0.1	0.3	0.1	-1.4	-1.5	-0.3	-1.0	-0.7
15 f	-1.1	-1.4	-0.3	-0.6	-0.9	-0.3	-1.2	-1.7	-0.5
26 m	-0.8	-0.9	-0.1	1.1	-0.5	-1.6	0.1	-1.0	-1.1
27 m	-0.1	1.3	1.4	0.1	-0.4	-0.5	-0.1	0.6	0.7
33 m	0.6	1.8	1.2	-0.5	-0.5	0	-0.1	1.0	1.1
35 f	0	-1.4	-1.4	-0.1	0.8	0.9	-0.2	-0.6	-0.4
55 m	-0.2	-0.1	0.1	-1.3	-2.9	-1.6	-1.2	-2.0	-0.8
56 f	-1.3	0.3	1.6	-2.1	-1.0	1.1	-25	-0.4	2.1

WFH, weight for height/length; HFA, height for age; WFA, weight for age

Table 19. Comparison of the change in anthropometric indices between the improvers and non-improvers in the first 6 months of life.

| Change in growth from D7 to W26 | Improvers N=45 | Non-improvers N=11 | |
	Mean (±SD)	Mean (±SD)	p[a]
Weight for height change	0.06 (±1.7)	0.6 (± 1.2)	0.31
Height for age change	-0.3 (±0.9)	-0.4 (± 1.1)	0.87
Weight for age change	-0.2 (±1.1)	0.2 (±1.1)	0.33

[a]p-value calculated using unpaired t test, two-tailed.

We have compared the anthropometric parameters of the non-improvers to the ones of the improvers, table 19. First, we see that the change is not far away from zero, which indicates that the children on average grow as expected. Second, the differences in change in the growth parameters between the non-improvers and the improvers are not statistically significant. Table 20 in appendix shows the Z-score of the growth parameters of the improvers.

6. DISCUSSION

The main aim of the present study was to investigate neutrophil cell count evolution during the first 6 months of life in HIV-uninfected children subjected to antiretroviral drugs antenatally, born with low neutrophil count and receiving nevirapine postnatally; and to compare this evolution to infants born with a neutrophil cell count above the threshold receiving lamivudine or Kaletra®. One important conclusion is that such neutropenia can be moderate to severe and still not generate any apparent morbidity in the form of hospitalisations or other severe illnesses. The second conclusion is that over the first 6 months, there is a good chance that the child recovers spontaneously, in study group 13/18 children had recovered at 26 weeks of age. The third conclusion is that these infants as a group present a growth as expected, an indication that they are doing OK. Any long-term consequences of this low neutrophil count remain unexplored in our study.

Several studies have previously showed that perinatal AZT exposure is associated with transient neutropenia while some others failed to demonstrate any effect of this drug on haematological parameters. In our study group and comparative group, all children were exposed to antenatal AZT for shorter or longer duration, this means that our study is unable to assess whether or not this variable had any impact on the neutropenia. However, our results suggest that low neutrophil count in infants associated with antenatal and postnatal exposure to antiretroviral drugs may be transient in a good number of children.

In our study, we found that a number of children in the comparative group had an ANC grade 3 toxicity at week 26. Since infants in the comparative group were part of the double-blind randomized trial, we do not know which drug – lamivudine or Kaletra® – they were receiving. Of course, it is crucial to reassess this issue as soon as the randomisation code of the clinical trial has been broken to see if there is any relation of this late toxicity with any of the drugs.

Infant mortality and morbidity has been significantly associated with HIV-1 infected mothers with advanced disease (CD4 counts < 200 cells/ μL). However, infants born to infected mothers with high CD4 counts (> 500 cells/μL) with or without NVP exposure tends to have normal haematological parameters (11). The mothers in our study had relatively high median maternal CD4+ count at enrolment (> 500 cells/μL) and at this level maternal CD4+ does not seem to be a risk factor for neutropenia in the infants particularly at day 7 in both the study. In our small study, we were unable to identify any risk factor for infant low neutrophil count.

Low neutrophil count in children was defined by the DAIDS toxicity tables, as specified in the protocol. However, recent studies have indicated that neutrophil counts of African children are generally lower than those of Caucasian infants, and thus it raises the question whether the DAIDS toxicity tables are applicable in African children. (3) In our study group, children with grade 2 or more toxicity at day 7 were clinically healthy and a large number grew well up to week 26. The design of our study makes it impossible to contribute to the question of whether the DAIDS toxicity tables are valid in Africa.

The median birth weight was different between the study and the comparative groups (table 12). Even if the difference is not significant from our results, we can argue that low birth weight can be associated with neutrophil toxicity due to HIV-1-infection/ART exposure. Some studies have previously supported that children born with lower birth weight eliminate zidovudine more slowly, increasing the toxic exposure of zidovudine on the bone marrow. Results from infants in the study group show a median birth weight of 0.20 kg less than infants in the comparative group. Low birth weight maybe a risk factor for low neutrophil cell count in the study group, but not a predictor of poor recovery (table 17).

7. METHODOLOGICAL ISSUES AND LIMITATIONS

7.1 Study design

Our study was designed with a prospective longitudinal cohort design. However, the follow-up of the two groups, study group and comparative group, was not exactly the same and the data from the comparative group came from the trial data base and originated from all four trial sites. However, the follow-up was very similar and we do not consider this difference a threat to the internal validity.

7.2. Choice of comparative group

The children we are studying are doubly exposed: to HIV and to antiretrovirals antenatally. It is therefore extremely difficult to identify a suitable control group as it is unethical to organise a study that has a control group that is only HIV exposed or only exposed to antiretrovirals without being exposed to HIV. In this particular case, there is no way to obtain a real control group. We therefore decided to use children that were as similar as possible, that is with a (mild) Grade 1 neutropenia to try to look at the "natural history" of neutropenia under these conditions. It is obvious that our comparative group in no way can replace a control group or be used as a control group.

The consequence of this is that disentangling the effect of each of the two (HIV and ARVs) is therefore very difficult.

7.3 Sample size

Our study had a small population size of 18 mother-infant pairs and of 38 pairs in the comparative group, more like a pilot study. The reason for the small sample size is the fact that the study was piggy-backed onto the trial, and this was done only after realising that low neutrophil count posed a real problem for the trial. Because of this late start-up of this study, we missed out on a large number of children excluded from the trial, who had already completed a full year of nevirapine administration prior to initiation of our data collection. The small sample size limits the possibility to draw solid conclusions.

7.4. Follow-up intensity

The follow-up of the infants excluded from the trial receiving nevirapine was not as intensive as for those in the trial. In our study we collected blood for ANC assessment at D7 and W26. Children excluded from the trial were not scheduled for further blood collection after day 7. We added one blood collection at week 26 but of course it would have been even more informative to have blood samples taken at different time point, for instance at day 7, week 10, week 14, week 20 and week 26 but this is ethically challenging as it is difficult to claim that such repeated blood sampling will benefit the individual child. Repeated sampling would have allowed us to know more about at which point the children recovered from their low neutrophil count.

7.5. Short duration of follow-up

The duration of follow-up was limited to 26 weeks. It is obvious that a longer duration would have been beneficial for understanding more about the 'natural history' of the neutropenia seen in the study, especially in the non-improving children .

8. CONCLUSION

With the limitations of this study, such as small sample size, lack of appropriate control group, low follow-up intensity, short duration of follow-up and the question mark around the usefulness of the DAIDS tables, we conclude that 1) infants exposed to HIV-1/ART antenatelly and who received nevirapine or lamivudine/Kaletra® from birth up to age week 26 may be at risk for transient low absolute neutrophil count; 2) such neutropenia can be moderate to severe and still not generate any apparent morbidity in the form of hospitalisations or other severe illnesses; 3) over the first 6 months, there is a good chance that the child recovers spontaneously and 4) these infants as a group present a growth as expected which is an indication that they are doing OK. These findings may provide some reassurance for women in the PMTCT programme who expose their infants to antiretroviral drugs perinatally.

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10. ANNEXES

Annex 1.Table 20. Weight and height development of the improving children in the study and the comparative group.

No and sex	Z-score								
	WFH D7	WFH W26	WFH Change	HFA D7	HFA W26	HFA Change	WFA D7	WFA W26	WFA Change
1 m	2.5	-1.3	-3.8	-1.5	0.2	1.7	0.3	-0.9	-1.2
2 f	0.5	-0.5	-1	-1.4	-1.8	-0.4	-0.9	-1.6	-0.7
3 f	-3.2	-0.4	2.8	-0.1	-0.8	-0.7	-2.0	-0.8	1.2
4 m	-0.1	-0.3	-0.2	-1.5	-3.1	-1.6	-1.3	-2.4	-1.1
5 m	0.7	-1.3	-2	-0.2	0.2	0.4	0.2	-0.9	-1.1
6 f	0.8	-0.1	-0.9	-0.6	-1.6	-1.0	-0.1	-1.2	-1.1
7 f	0	0.2	0.2	-1.2	-1.0	0.2	-1.0	-0.5	0.5
8 f	-2.3	0.1	2.4	0.5	0.6	0.1	-1.1	0.3	1.4
9 f	-0.8	0.5	1.3	-1.2	-1.9	-0.7	-1.5	-0.9	0.6
11 m	0.2	-0.9	-1.1	0.2	-0.7	-0.9	0.1	-1.1	-1.2
16 f	1.1	0.6	-0.5	-0.1	-0.7	-0.6	0.6	-0.1	-0.7
17 m	0.7	0	-0.7	1.3	-0.9	-2.2	1.3	-0.6	-1.9
18 f	0	1.0	1	-1.2	-0.8	0.4	-1.0	0.2	1.2
19 m	-1.2	-1.7	-0.5	-3.1	-2.2	0.9	-2.7	-2.7	0
20 m	-1.7	1.8	3.5	0.6	0.5	-0.1	-0.6	1.6	2.2
21 m	-0.8	-1.4	-0.6	0.9	0	-0.9	0.1	-1.1	-1.2
22 m	-0.3	-3.0	-2.7	1.1	-0.5	-1.6	0.6	-2.5	-3.1
23 f	-0.2	0.9	1.1	1.4	2.3	0.9	0.8	1.2	0.4
24 f	-1.6	-0.5	1.1	2.1	0.8	-1.3	0.6	0	-0.6
25 f	-1.8	-0.1	1.7	-0.9	-1.6	-0.7	-2.0	-1.2	0.8
28 f	0.1	1.7	1.6	1.0	0.6	-0.4	0.8	1.5	0.7
29 m	2.2	-0.6	-2.8	-0.5	0.6	1.1	1.0	-0.2	-1.2
30 f	0.2	-1.1	-1.3	0.5	-0.2	-0.7	0.5	-1.1	-1.5
31 m	1.5	-0.8	-2.3	-1.0	0.4	1.4	-0.1	-0.9	-0.8
32 f	-1.4	-1.5	-0.1	0	-1.2	-1.2	-1.0	-1.9	-0.9
34 f	1.4	-0.2	-1.6	0.9	0.3	-0.6	1.5	-0.2	-1.7
36 m	0.9	-0.2	-1.1	-0.2	-0.5	-0.3	0.3	-0.5	-0.8
37 f	-0.3	0	0.3	-1.6	-1.9	-0.3	-1.6	-1.3	0.3

38 m	0.2	-2.8	-3	0.6	0.2	-0.4	0.5	-1.1	-1.6
39 f	-0.7	1.1	1.8	-1.2	-0.5	0.7	-1.4	0.4	1.8
40 m	1.4	-0.9	-2.3	-0.8	-0.2	0.6	0	-0.1	-0.1
41 m	0.9	2.8	1.9	0.1	-0.8	-0.9	0.5	1.7	1.2
42 f	0.1	0.7	0.6	-0.3	-1.2	-0.9	-0.3	-0.3	0
43 m	-0.8	-0.2	0.6	0.6	-0.6	-1.2	-0.1	-0.6	-0.5
44 m	-1.4	-0.4	1	0.1	-0.3	-0.4	-0.8	-0.5	0.3
45 f	0.9	-0.2	-1.1	-1.3	-1.6	-0.3	-0.6	-1.3	-0.7
46 m	-1.1	1.8	2.9	1.1	-1.3	-2.4	0.1	0.5	0.4
47 f	-2.3	0.9	3.2	-0.3	-0.3	0	-1.7	0.4	2.1
48 f	-0.1	0.4	0.5	-0.6	0.2	0.8	-0.6	0.3	0.9
49 m	-2.0	-0.5	1.5	-1.1	-1.0	0.1	-2.1	-1.2	0.9
50 f	-1.0	-0.2	0.8	-0.9	-1.3	-0.4	-1.3	-1.1	0.2
51 m	-1.7	-0.7	1	-0.5	-0.4	0.1	-1.4	-0.9	0.5
52 m	0.2	-1.3	-1.5	-0.5	-1.7	-1.2	-0.3	-2.1	-1.8
53 m	0.8	-0.1	-0.9	-1.5	-1.8	-0.3	-0.7	-1.3	-0.6
54 f	-1.4	0.5	1.9	2.1	0.4	-1.7	0.7	0.5	-0.2

Annex 2.ANRS 12174 Consent form post-delivery screening

TITLE OF THE RESEARCH PROJECT:

ANRS 12174: A randomised controlled trial comparing the efficacy of infant peri-exposure prophylaxis with Lopinavir/Ritonavir (LPV/r) versus Lamivudine to prevent HIV-1 transmission by breastfeeding

Declaration by Participant

By signing below, I ………………………………..…………….. agree to take part in a research study entitled

ANRS 12174 Post-Delivery Screening Survey.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.

- I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.

- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) …………………..………..…………... on (*date*) ………….…..……….. .2011

....
Signature (or Thumbprint) of Participant   Signature of Witness (if applicable)

Declaration by Investigator

I *(name)* ……………………………………….………… declare that:
- I explained the information in this document to ………………………………..
- I encouraged her to ask questions and took adequate time to answer them.
- I am satisfied that she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) …………………..……....……………... on (*date*) …………....………... 2011.

……………………………………………………………….
Signature of Investigator

………………………………………………………………………
Signature of Witness (if applicable)

Declaration by Interpreter (if applicable)

I *(name)* …………………………………………..………… declare that:
- I assisted the Investigator (*name*) ……………………………………… to explain the information in this document to (*name of participant*) ………………..………………using the language medium of ………………….... ……..(specify language used).
- We encouraged her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all her questions satisfactorily answered.

Signed at (*place*) …………………..……....……………... on (*date*) …………....………... 2009.

…………………………………………………………..
Signature of Interpreter