Growth faltering and developmental delay in HIV-exposed uninfected infants

by

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Abstract

Background

Human Immunodeficiency Virus (HIV) is a major cause of mortality and morbidity globally. Vertical transmission is the primary mode of HIV acquisition among infants. Although successful interventions have reduced vertical transmission to less than <2.9% in low-to-middle income countries (LMIC), new concerns have emerged about the health outcomes of HIV-exposed uninfected (HEU) children. In 2018, there were an estimated 14.8 million children who were HEU, 90% of whom resided in Sub-Saharan Africa. HEU infants worldwide significantly outnumber infants that are infected with HIV and represent nearly 30% of the newborn population in certain HIV endemic nations. These HEU children have increased morbidity and mortality compared to HIV unexposed, uninfected (HUU) children. Among HEUs, studies have reported low birth weight (LBW), impaired early growth, impaired psychomotor and cognitive development, increased hearing loss, increased incidence of speech delay, immunological abnormalities, and increased susceptibility to infectious diseases.

This thesis has two objectives: (1) review published data on circulating biomarkers that are associated with growth faltering and neurodevelopmental delay in HEU infants (Chapter 2); and (2) evaluate growth faltering and neurodevelopment in a Ugandan HEU infant cohort (Chapter 3).

Methods

In Chapter 2, we performed a systematic review of the literature of studies to identify biomarkers that are associated with growth faltering and neurodevelopmental delay in HEU infants.

In Chapter 3, we prospectively followed a cohort of HEU infants from birth to 18 months of age, and measured growth parameters longitudinally. The Malawi Development Assessment Tool (MDAT) and the Color Object Association Test (COAT) were used for developmental assessments at 12 and 18 months of age. We examined the association between early growth faltering and subsequent neurodevelopment.

Results

In Chapter 2, we found that biomarkers of inflammation (acute phase reactant, proinflammatory cytokines, regulatory cytokines, chemokines), microbial translocation, growth factors, tissue remodelling, and neutrophil activation were associated with growth faltering and poor neurodevelopmental outcomes.

In Chapter 3, we found that LBW and failure to thrive (FTT) were associated with a lower MDAT score at 18 months of age.

Conclusion

This thesis addresses growth faltering and neurodevelopmental outcomes in HEU infants and biomarkers associated with these outcomes. These findings may contribute to identifying HEU infants at risk of having poor growth and neurodevelopment and target interventions to mitigate these adverse outcomes.

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LIST OF ABBREVIATIONS:

3TC lamivudine
ABC abacavir
AIDS acquired immune deficiency syndrome
AKI acute kidney injury
Angs angiopoietins
APC antigen-presenting cell
AZT zidovudine
BDNF brain-derived neurotrophic factor
BMI body mass index
BSID-III Bailey Scales of Infant Development-3rd edition
cART combination antiretroviral therapy
CD4 cluster of differentiation 4
cDNA complementary DNA
CIA collagen-induced arthritis
CMV cytomegalovirus
COAT Color Object Association Test
CRP C-reactive protein
CTX Co-trimoxazole
CXCL10/IP-10 C-X-C motif chemokine 10/interferon γ-induced protein 10 kDa
DBS dry blood spot
DTG dolutegravir
EFV efavirenz
ELISA enzyme linked immune sorbent assay
EPIC4 early pediatric initiation-canadian child cure cohort
FADD Fas-associated death domain
FPA N-terminal fibrinopeptide A
FTT failure to thrive
GH Growth hormones
GHRH growth hormone-releasing hormone
GM-CSF granulocyte-macrophage colony-stimulating factor
HCAZ head circumference-for-age z-score
HEU HIV-exposed uninfected
HIV human immunodeficiency virus
HUU HIV-unexposed uninfected
ICE IL-1β converting enzyme
I-FABP intestinal fatty acid binding protein
IFN interferon
IFN-γ interferon-gamma
IGF-1 insulin-like growth factor-1
IGFBP-1 insulin-like growth factor binding proteins-1
IGFBP-3 insulin-like growth factor binding proteins-3
IL- 1β interleukin-1β
IL-4 interleukin-4
IL-6 interleukin-6
IL-10 interleukin-10

IL-12p70 interleukin 12p70
INSTIs integrase strand transfer inhibitors
IQ intelligence quotient
IQR inter quartile range
IRT item response theory
IUGR intra uterine growth retardation
JAK Janus kinase
KT knowledge translation
LAZ/HAZ length-for-age/height-for age z-score
LBP LPS-binding protein
LBW low birth weight
LME linear mixed effects
LPV lopinavir
LPV/r ritonavir-boosted lopinavir
LPS lipopolysaccharide
LMICs low- and middle-income countries
LPS lipopolysaccharide
MAPK mitogen-activated protein kinase
MDAT Malawi Development Assessment Tool
MMP-9 membrane-type metalloprotease-9
MT microbial translocation
MUAC mid upper arm circumference
NAT nucleic acid testing
NFAT nuclear factors of activated T-cells
NGAL neutrophil gelatinase-associated lipocalin
NLHRS National Laboratory for HIV Reference Services
NK-cells natural killer cells
NNRTIs non-nucleoside reverse transcriptase inhibitors
NO nitric oxide
NRTIs nucleoside reverse transcriptase inhibitors
NVP nevirapine
PAMP pathogen-associated molecular pattern
PCh phosphocholine
PCR polymerase chain reaction
PI protease inhibitors
PYY peptide tyrosine–tyrosine
QUADAS-2 Quality Assessment of Diagnostic Accuracy Studies-2
REACH Regional East African Community Health
REG1B Regenerating gene 1β
S100B S100 calcium-binding protein B
sCD14 soluble CD14
sE-selectin soluble E-selectin
SD standard deviations
sICAM soluble intercellular adhesion molecule
sIL-6R soluble form of the IL-6R
sMCP monocyte chemoattractant protein

sP-selectin soluble P-selectin
STAT3 signal transducers and activators of transcription
STI sexually transmitted infection
sTNF-RI soluble tumor necrosis factor receptor I
sVCAM soluble vascular adhesion molecule
TACE metalloprotease TNF-converting enzyme
TDF tenofovir disoproxil fumarate
TGF-β transforming growth factor β
TNF tumor necrosis factor
mTNF transmembrane TNF
sTNF soluble TNF
TNF-RI tumor necrosis factor receptor type I
TNF-RII tumor necrosis factor receptor type 2
TRADD TNF receptor-associated death domain
Treg T regulatory
UMIC upper-middle-income countries
UNAIDS united nations program on HIV/AIDS
VEGF-A vascular endothelial growth factor A
WAZ Weight-for-age z-score
WISC-IV Wechsler Intelligence Scales for Children—4th Edition
WLZ/WHZ Weight-for-length/weight-for-height z-score
WHO World Health Organization

CHAPTER 1. INTRODUCTION

1.1 Human Immunodeficiency Virus (HIV)

1.1.1 Virology

Human Immunodeficiency Virus (HIV) causes a chronic infection characterized by immune dysfunction and life-threatening opportunistic infections [1, 2]. When opportunistic infections arise, the clinical condition is known as Acquired Immunodeficiency Syndrome (AIDS). HIV is classified in the genus lentivirus, a group of viruses characterized by a long duration of illness and a lengthy incubation period. There are two major lineages of HIV that have been characterized: HIV-1 and HIV-2 [3, 4]. HIV-1 is more virulent and infectious than HIV-2. It is also responsible for the majority of HIV infections globally, while HIV-2 is most prevalent in West Africa [3]. For simplicity, throughout this thesis, HIV-1 will be referred to as HIV. However, when describing the methodology for diagnostic testing in this thesis, we will specify the ability of the assay to detect HIV-1 and HIV-2.

HIV is an enveloped virus with a lipid bilayer surrounding the virus [1]. It has a roughly spherical shape with a diameter of 120 nm [5]. The outer envelope layer contains major glycoproteins which are responsible for direct interaction with the cell surface [6]. The two major glycoproteins are: (1) surface glycoprotein120 (gp120) which is responsible for primary attachment with CD4 receptors on T cells; and (2) transmembrane glycoprotein 41 (gp41), which helps to fuse with the cell membrane and enter into the host cells [7]. The virion core consists of matrix core (p17), capsid protein (p24) and nucleocapsid protein (p7) [8]. In the inner core, HIV is composed of two copies of positive-sense single-strand RNA enclosed by a conical capsid [1].

The single-stranded RNA codes for viral genes and is tightly bound to nucleocapsid and enzymes that are essential for viral replication (reverse transcriptase, proteases, integrase) [1].

1.1.2 Interaction with host cell

HIV infects host cells by targeting CD4+ T-lymphocytes and macrophages of the adaptive immune system by binding the CD4 receptor and chemokine co-receptors CCR5 and CXCR4 [7, 9]. The co-receptors (CCR5 and CXCR4) are responsible for the entry of viruses into lymphocytes and macrophages, respectively [7, 9]. This process of co-receptor binding following the interaction of gp120 to the CD4 receptor allows for viral entry and penetration [7]. The binding of gp120 to the CD4 receptor causes structural changes in gp120 and exposes gp41 [7]. This brings HIV closer to the target cells, ultimately precipitating the fusion of the viral envelope and the cell membrane [7, 10]. Upon fusion, the matrix core of the virus inserts into the host cell cytoplasm where the HIV capsid core disassembles, resulting in viral RNA release [11]. These RNA strands undergo conversion into viral DNA, mediated by the viral enzyme reverse transcriptase [12].

1.1.3 Immunity and host response (CD4+)

HIV infects CD4+ T-helper cells, which function to regulate the immune response [13]. T-helper cells induce the cellular immune response along with the production of anti-HIV antibodies and cytotoxic T cell production [13]. In primary infection, CD8+ T cells lyse HIV-infected cells and secrete cytokines and chemokines that inhibit viral replication and block viral entry into CD4+ T cells [14, 15]. During chronic infection, HIV replication increases and depletes CD4+ T-lymphocytes causing infected individuals to gradually become immunodeficient [15]. The

weakened immune system increases susceptibility to a wide range of infections, cancers, and AIDS [16].

1.1.4 Epidemiology

HIV is a major global health concern with 37.9 million people currently living with HIV in 2018 [17]. The global estimate of HIV incidence in children is 150,000 cases per year; most cases are reported to be vertically acquired [18, 19]. In 2018, there were an estimated 2.8 million children and adolescents under the age of 19 living with HIV, and 9 out of every 10 infected children were from Sub-Saharan Africa [20, 21].

HIV epidemics can be categorized as "concentrated" or "generalized" [22]. Epidemics are classified as concentrated when transmission takes place within a clearly defined vulnerable group [22]. Examples include sex workers, men who have sex with men, and injection drug users [22, 23]. On the other hand, in generalized epidemics, the transmission of HIV is sustained through sexual behaviours despite programs targeted towards vulnerable groups [24]. Southern and Eastern Africa has a generalized epidemic, while North America has a concentrated epidemic [24].

HIV continues to be highly prevalent in Sub-Saharan Africa, where over 25.7 million people are currently living with HIV [25]. In Eastern, Central and Southern Africa, there are approximately 678,000 new infections and 306,100 AIDS-related deaths annually [26]. Between 2005 and 2013, there was a 33% decline in new HIV infections in Sub-Saharan Africa, but the global burden of disease remains high in this region [27]. Sub-Saharan Africa makes up only 12% of

the world's population, yet it accounts for 71% of global HIV infection cases [28]. The countries that account for almost 80% of all people living with HIV are in Eastern and Southern Africa: South Africa (25%), Nigeria (13%), Mozambique (6%), Uganda (6%), Tanzania (6%), Zambia (4%), Zimbabwe (6%), Kenya (6%), Malawi (4%) and Ethiopia (3%) [28]. The largest number of new cases are mainly from South Africa (23%), Nigeria (15%), Uganda (10%), Mozambique (8%) and Kenya (7%) [28]. About 58% of the total number of people living with HIV are women, who also make up the largest group of people with HIV/AIDS-related deaths [28, 29]. For instance, in Uganda, the prevalence of HIV is 2.4% in men while it is 4.2% in women [28, 29].

In 2014, the Joint United Nations Program on HIV/AIDS (UNAIDS) and partners introduced the 90–90–90 targets to monitor progress toward mitigating the HIV/AIDS pandemic [30, 31]. The aim was to diagnose 90% of all HIV-positive individuals, provide combination antiretroviral therapy (cART) for 90% of those diagnosed with HIV, and reach viral suppression for 90% of those treated by the year 2020 [32]. Amongst the Eastern and Southern African population, as of 2018, nearly 82% of people living with HIV knew their status, 68% of people living with HIV were on cART, and 58% of HIV positive individuals on treatment had achieved viral suppression, falling short of the 90-90-90 target [32].

1.1.5 Transmission

1.1.5.1 *Modes of Transmission*

HIV transmission risk is dependent on the viral load of the source and the type of HIV exposure [33]. There are several modes of transmission: sexual, percutaneous blood exposure, mucous

membrane exposure, vertical transmission, and transfusion of contaminated blood products [33]. Sexual contact accounts for nearly 80% of HIV infections. Needlestick injuries (0.3%), mucous membrane exposure (0.1%), and contaminated injecting drug equipment (0.6%) make up smaller percentages of all infections [34-37]. Transfusion with contaminated blood has been nearly eliminated in North America due to highly effective blood screening methods [38]. However, in Sub-Saharan Africa, contaminated blood transfusion accounts for 5 to 10% of all HIV infections [39].

1.1.5.2 *Sexual Transmission*

Sexual intercourse accounts for over 90% of infections in Sub-Saharan Africa [40]. A substantial number of new HIV cases are linked to long-term heterosexual and HIV-discordant relationships [41]. Protective behaviours such as condom use have been an effective strategy to reduce HIV transmission by nearly 78%, yet studies continue to suggest that a significant percentage of individuals in these regions continue to engage in unprotected sex [40].

HIV transmission varies between upper-middle-income countries (UMIC) and low-and-middle-income countries (LMIC) [42]. For instance, in UMIC, male-to-female and female-to-male transmission is 0.08% and 0.04%, respectively [43]. This is lower than LMIC where male-to-female and female-to-male transmission is 0.38% and 0.30%, respectively [43]. One of the reasons that the risk of transmission is sex-specific may be due to women having greater mucus area exposed to HIV during penile penetration, underdeveloped cervix and low vaginal mucus production in women under 17 years of age, and exposure to gender inequalities and economic pressures in society [44-46].

1.1.5.3 *Vertical Transmission*

Vertical transmission is one of the leading causes of new HIV infections amongst children accounting for 50-80% of cases [47, 48]. The rate of vertical transmission in the absence of specific interventions ranges from 15-40% [48-51]. The risk factors for vertical transmissions include maternal viral load, duration of exposure, progression of the infection to AIDS, immune deficiency, mode of delivery, and duration of membrane rupture. Higher maternal viral load is associated with lower CD4+ T-lymphocyte count, and it serves as a prognostic marker for perinatal transmission risk [52].

Vertical transmission during pregnancy:

Of all HIV vertical transmission, one-third happens *in utero* (based on a South American study [49]) and two-thirds take place around or after delivery [49]. HIV can be transmitted to a fetus as early as eight weeks of gestation [53, 54]. HIV transmission occurs in low frequency with maternal viral loads <1,000 copies/mL [48]. However, *in utero* transmission may occur if the placental barrier is disrupted and blood travels from the maternal to fetal circulation [55].

Vertical transmission during delivery:

The greatest risk of vertical transmission occurs during the peripartum period (65%) where HIV may be present in maternal genital tract secretions [49, 56]. The duration of ruptured membranes, placental abruption, and vaginal route of delivery (versus cesarean delivery) increase the risk of peripartum transmission [57-59]. Furthermore, vaginal tears or prolonged rupture of membranes can increase the risk of perinatal HIV transmission [57-59]. Additionally, elevated levels of HIV

DNA in the cervicovaginal canal are associated with lower $CD4^+$ T-lymphocyte counts and vaginal discharge [56]. Elective cesarean section has been recommended as a method to prevent newborns from coming in contact with infectious fluids [48]. However, in settings where the burden of HIV is high, women may not have access to elective cesarean section [60].

Vertical transmission during breastfeeding:

Breastfeeding is one of the postnatal modes of HIV transmission [61]. In UMIC, transmission via breastfeeding ranges from 14 to 26%, while in LMIC, the risk ranges from 21 to 43% [48, 62]. The World Health Organization (WHO) generally recommends avoidance of breastfeeding by HIV-positive women where feasible [63]. The benefits of breastfeeding (e.g., prevention of diarrheal disease, respiratory illnesses, allergies, malnutrition) may outweigh the risk of HIV transmission in some settings [64]. In low-resource settings where replacement feeding may not be feasible, affordable, sustainable, or safe, WHO recommends that HIV-infected women adhere to exclusive breastfeeding for the first 6 months of life [63]. Of note, in HIV-infected women receiving cART, cell-associated virus can be detected in breastmilk, such that effective cART may not completely eliminate the risk of breastmilk transmission [65].

1.1.6 Clinical Manifestation

The symptoms of HIV vary with the stage of infection [66]. After primary HIV infection, individuals may be asymptomatic for the first few weeks or may develop influenza-like symptoms such as fever, headache, rash, or sore throat [67]. As the HIV infection progresses, infected individuals may develop a variety of symptoms, such as lymphadenopathy, weight loss, fever, diarrhea, and cough [68]. Without treatment, overt immune deficiency may arise, leading

to severe co-infections such as tuberculosis, cryptococcal meningitis, bacterial infections, and cancers [69].

Growth failure is a common clinical manifestation of HIV in infected children [70, 71]. These children have an increased risk of being born with low birth weight (LBW) (<2500g) [71, 72]. The prevalence of wasting and underweight is 17.5% and 18.5% respectively in HIV-infected children, which is 2 to 3 times higher risk than HIV uninfected children [73]. In a Zimbabwean study, 80% of infected children were moderate to severely stunted at 24 months of age [74]. Somatic growth retardation and malnutrition were prevalent among this population and were as high as 83.3% in some HIV endemic areas [75]. In HIV-infected children, poor growth is associated with disease progression and decreased survival [76]. Oral candidiasis can cause poor weight gain in children as it causes difficulty eating and swallowing food [71]. Chronic diarrhea, one of the major clinical manifestations of AIDS, is also associated with weight loss [71].

A spectrum of neurodevelopmental, cognitive and motor dysfunction has been reported among HIV-infected children [77]. Impacted children have an increased risk of lower head circumference at birth and slower neurodevelopment. Neurodevelopmental delays in infected children can be due to encephalopathy which affects speech and language, memory, learning, information processing, and motor functioning [78, 79]. The prevalence of neurodevelopmental delay in HIV-infected children ranges from 8% - 60% in the domains of cognition, motor function, speech and language [77].

1.1.7 Diagnosis

HIV infection can be identified by detection of HIV-specific antibodies or direct detection of the virus. Detection of virus can be performed with several techniques, including polymerase chain reaction (PCR) for detection of viral nucleic acids, p24 antigen testing, and virus-cell culture.

Direct detection of HIV nucleic acid can be useful for monitoring viral load for patients receiving cART and examining integrated provirus within host cells [80]. PCR-based assays may be qualitative (positive or negative) or quantitative (copies per volume of blood). Nucleic acid targets for PCR amplification may be viral RNA or cell-associated integrated proviral DNA. PCR tests target a specific genetic sequence; therefore, the assay sensitivity and specificity may differ for HIV-1 and HIV-2.

Antibody testing is the most commonly used screening assays, e.g., the highly sensitive HIV-1/HIV-2 enzyme immunoassay (EIA) tests. The EIA test can detect infection within two to three weeks of infection.

EIA-based p24 antigen tests use antibodies to capture the disrupted p24 antigen from serum [81]. With some who are seroconverting in the 'window period,' the p24 antigen may become positive before antibodies are detectable [81, 82]. Detectable p24 antigen may not be detected in all seroconverting patients, and it may not be consistently found in individuals who have HIV-positive antibodies [81, 82].

The dried blood spot (DBS) testing approach uses a sample of blood from a finger prick that is collected as a blot on a card. After the blood spot is dried at room temperature, it is sent to a public health laboratory for screening and confirmatory testing by PCR. DBS testing is convenient for use in rural and remote areas because the samples are stable without refrigeration after collection.

For HIV-infected infants, serological testing may not be clinically informative [83]. Virological testing has to be done using nucleic acid testing (NAT) technologies as transplacental transmitted maternal HIV antibodies may persist in children up to 18 months of age [84, 85].

1.1.8 Treatment

HIV replication can be suppressed with cART, generally consisting of three or more drugs [86]. The purpose of cART is to suppress viral replication, lower viral load and allow recovery of immune function.

Clinically, over 30 antiretroviral medications are licenced for clinical use. These medications can be classified according to their mechanism of action into several categories: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs) and entry inhibitors (e.g., fusion inhibitors and CCR5 antagonists) [87]. Combinations of these cARTs provide potent inhibition of viral replication. Although highly effective, cART regimens differ in their tolerability, long-term safety profile and barrier to the development of viral resistance [88].

In Uganda, cART is available free of charge to eligible patients through a national program [89]. The cART regimens currently in use are as follows. All eligible HIV-infected adolescents and adults weighing more than 30kg, as well as HIV-infected pregnant and breastfeeding women, are initiated on tenofovir disoproxil fumarate, lamivudine and dolutegravir (TDF+3TC+DTG) as a once-daily fixed-dose combination. All HIV-infected children weighing between 20kg to 30kg are initiated on abacavir + lamivudine+ dolutegravir (ABC+3TC+DTG). Children less than 20kg are initiated on abacavir + lamivudine + dolutegravir (ABC + 3TC + DTG) when appropriate DTG formulations and strengths (5mg, 10mg and 25mg) are available.

In the case that DTG formulations are not available, abacavir + lamivudine+ ritonavir-boosted lopinavir (ABC+3TC+LPV/r) should be initiated [89]. LPV/r syrup, pellets or tablets can be prescribed based on the child's ability to correctly take the specific formulation. As soon as the child can take pellets, these will be prescribed instead of syrup. Likewise, as soon as a child can swallow tablets without breaking, crushing, or chewing them, these will be prescribed instead of pellets.

1.2 HIV-Exposed Uninfected Infants

1.2.1 Epidemiology

The risk of vertical HIV transmission can be reduced significantly by cART. Globally, approximately 82% of HIV-infected pregnant women now have access to cART [90]. Successful interventions with cART have reduced vertical transmission of HIV to <1% in resource-rich areas [91]. Option B+ is a WHO strategy in which cART is started at diagnosis (before or during pregnancy) and is continued for life. This strategy has reduced HIV transmission to <2.9% in

LMICs such as Zambia [92]. Given that vertical transmission can be averted with cART, there are now an estimated 1.5 million HIV-infected mothers who give birth to children who are HIV-exposed but uninfected (HEU) annually [93].

In 2018, there were an estimated 14.8 million children who were HEU, 90% of whom resided in Sub-Saharan Africa and the remaining 5% in Asia and the Pacific region [90]. HEU newborns significantly outnumber HIV-infected infants worldwide and represent nearly 30% of the newborn population in some HIV-endemic nations [94]. Five countries account for 50% of the total HEU cases globally: South Africa (23.8%), Uganda (7.5%), Mozambique (6.6%), Tanzania (6.1%), and Nigeria (6.0%) [90]. In South African countries, HEU infants make up more than 15% of the total children population [90]. These include Eswatini (32.4%), Botswana (27.4%), South Africa (21.6%), Lesotho (21.1%), and Namibia (16.4%) [90]. Between 2000 and 2018, the prevalence of HEU children in Eastern and Southern African regions increased from 3.6% to 5.5% [90]. Within the same 18-year period, the population of HEU children doubled in the Asia and Pacific region and Eastern and Southern Africa regions [90]. The increasing prevalence of children who were HEU between 2000 and 2018 has been driven predominantly by the initial increase in HIV prevalence among pregnant women in countries with a high HIV burden [90]. Older children among this global cohort are less likely to have been exposed to antiretrovirals *in utero* during the early 2000s [90].

1.2.2 Poor health outcomes associated with HEU infants

Although HEU infants do not exhibit the severe manifestations of an HIV-infected infants, there may be clinical consequences of intrauterine exposure to both HIV and cART [95]. HEU infants'

health outcomes differ from those of HIV-unexposed uninfected (HUU) infants. At the population level in countries with a high prevalence of children who are HEU, infant HIV exposure has been associated with a substantial contribution to infant mortality [90]. For example, in resource-limited countries, HEU infants are two- to three times more likely to die than HUUs before their second birthday [95].

1.2.3 Growth Abnormalities in HEU infants

Growth faltering among HEU infants has been reported in diverse countries in Africa, as well as Brazil and the United States [90]. LBW, small gestational age, and preterm birth are more common in HEU infants than HUUs [95-97]. About 61% of low-birth-weight cases in HEU infants are associated with preterm birth [98].

Postnatally. HEU infants have high rates of stunting, being underweight, and smaller head circumference than HUU infants, each with a prevalence of 12-31%, 3-9% and 2.1-5.6%, respectively [97, 99, 100]. Additionally, HEU infants have three-fold higher odds of stunting compared to HUU until up to 12 months of age [95, 97, 101]. A longitudinal cohort in South Africa reported lower average weight-for-age (WAZ) and length/height-for-age (LAZ/HAZ) scores at all time points over the first 12 months [102].

Growth abnormalities in the first 12 months of life are reported consistently across studies; however, beyond infancy, results are inconsistent between studies. In some studies, early growth disparities in HEU infants decreased as the infants aged [103-105]. In a European study that followed HEU children to 10 years of age, growth patterns were similar to HUU infants [106]. In

contrast, in another study, HEU infants had lower WAZ, LAZ/HAZ and body mass index (BMI)-for-age Z-scores at 18 months and these differences persisted at 7 years of age [107].

Growth deficiencies are often associated with poor health outcomes. HEU infants born preterm have a 7-fold increase in mortality than those born at term [98]. LBW and small-for-gestational-age are associated with a 6-fold and 7-fold increase for infant mortality, respectively, when compared to HEU infants of average weight [98]. In a South African birth cohort, lower WAZ was associated with an increased risk of severe pneumonia in HEU infants [102]. As the WAZ scores increased, the risk of infectious disease hospitalization decreased by 28% in a Kenyan HEU cohort [108].

1.2.4 Neurodevelopmental outcomes in HEU infants

Exposure to HIV *in utero* impairs the neurodevelopment of HEU infants and this has been reported in diverse populations including countries in Uganda, Zaire, Democratic Republic of Congo, Botswana, South Africa, Malawi, and United States [76, 90, 99, 109, 110]. Although HEU infants do not exhibit severe immunodeficiency or frequent opportunistic infections like HIV-infected infants, HEU infants face biological and environmental risk factors that affect cognitive development [111]. HEU infants have poorer developmental outcomes compared to HUU infants, particularly in LMICs [76, 112, 113].

Several studies have reported impairments in cognitive, language, or motor function [93, 114]. In a US cohort, there was an increased risk of language impairments compared with population norms [115]. A Botswana study showed 2-year-old HEU infants had increased adverse

expressive language outcomes, and a Ugandan study showed receptive language impairment in HEU infants at 3 years of age [93, 116]. On the other hand, in a South African study examining the impact of HIV and cART exposure, no differences in any developmental domains between HEU and HUU infants at 6 months were apparent [109]. By 24 months, however, HEU infants had significantly poorer receptive and expressive language outcomes and had increased risks of delay [109]. A possible explanation for inconsistencies in results could be difficulty in assessing language skills in children at 12 months of age. More subtle language impairments might not be easily identified at such a young age before explicit verbal communication has developed.

HEU survivors have an increased risk of impaired psychomotor and cognitive development, hearing loss, and expressive language expression [92, 95, 117]. Neurodevelopmental delay in HEUs may be due to pre-and postnatal factors, including socioeconomic variables, cART exposure, subtle immune deficits, systemic inflammation, and exposure to infections [94, 118]. While some studies in Africa found no differences in cognitive, motor and language development between HEU and HUU infants, studies from Zaire, Democratic Republic of Congo and Zambia have reported cognitive, motor, and expressive language delays along with differences in scholastic performance [111, 117, 119, 120].

1.2.5 Immunologic differences in HEU infants

HEU infants are exposed to HIV and /or cART *in utero* during a critical period of immune system development [121]. Postnatally, medications such as oral nevirapine (NVP) or zidovudine (AZT) may be given to HEU infants as prophylaxis. Given HEU infants may

experience increased levels of infection susceptibility and have cART exposure pre-and postnatally, they may have an immunologic difference when compared to HUU infants [121]. A second reason for immunological abnormalities among HEUs is maternal HIV infection. The mechanism underlying the increased susceptibility to infections is unknown; however, the two key factors that differ between HEUs and uninfected infants are exposed to medications and maternal HIV.

Innate Immunity:

HEU infants have evidence of increased immune activation [122]. HEU infants have increased levels of systemic inflammatory markers [123-126]. In a South African HEU cohort, pathogen-associated molecular pattern (PAMP)-stimulated antigen-presenting cells (APCs) were present in a greater proportion when compared to HUU infants [96, 123]. There was also increased expression of monocyte, cDC-derived production of IL-12 and MHC-II on unstimulated APCs in HEUs [127]. This hyper response of innate immunity was observed at 2 and 6 weeks but normalized by 12 months of age [123]. Of note, hyperinflammatory states can lead to poor immune system management by causing immune paralysis, reducing APCs' ability to initiate a cell-mediated immune response and increased disease susceptibility [128].

Adaptive Immunity:

HEU infants appear to have increased activation of the cell-mediated compartment of the immune response [129]. Both T- and B- cells in HEU infants appear more similar to HIV-infected infants than to HUU infants between 3-6 months of age [129]. Despite not having HIV infection, HEU infants have a reduction in CD4+ T-lymphocyte counts when compared to HUU

infants [130]. These findings are not consistent across all studies, however, as some studies show variation in CD4+ T-lymphocyte count according to age, and others show variation with exposure to cART [131-133]. This suggests that environmental factors may explain, at least in part, the observed immunological abnormalities, in addition to HIV exposure *in utero*.

1.2.6 Mechanisms of growth faltering and neurodevelopmental delay

1.2.6.1 Pathogenesis of *in utero* growth restriction

Several interrelated factors affect intrauterine growth restriction in HEUs such as maternal immune activation, co-infections, maternal cART, and inadequate maternal diet.

Maternal Immune Activation/Inflammation

Maternal immune activation and inflammation may lead to *in utero* growth restriction in HEU infants [134]. Causes of immune activation and inflammation in HIV-infected pregnant women include uncontrolled HIV replication, latent replication-competent viral reservoir, microbial translocation, and co-infections [135-137].

The heightened inflammatory state in HIV-infected pregnant women can result in fetal growth restriction or fetal loss [134]. Inflammation in the placenta can cause utero- placental insufficiency and reduce nutrient exchange that is necessary for growth [138-140]. Secondly, inflammation in the placenta can increase fetal exposure to proinflammatory cytokines [138-140]. This may result in fetal inflammation which leads to growth hormone resistance and hinders growth [141].

Exposure to intrauterine co-infections

HIV-infected pregnant women are at increased risk of co-infections, which can affect intrauterine growth in HEU infants. Examples of co-infections include malaria and cytomegalovirus, which are prevalent in many HIV-endemic countries [142, 143]. Immune suppression in HIV and pregnancy may lead to increased frequency and severity of infections, as well as reactivation of latent infections [136]. Co-infections may give rise to systemic inflammation, with the aforementioned effects on placental development and fetal resistance to growth factors.

Exposure to maternal cART

Prenatal exposure to cART can lead to growth restriction or preterm birth, both of which lead to LBW. PI-based cART is associated with a 17-25% increased risk of premature delivery [144, 145]. Additionally, the NNRTI efavirenz is associated with a 13% increased risk of delivering infants that are small for gestational age [144, 145]. Several mechanisms have been proposed to explain the association between antiretrovirals and growth restriction, including endothelial injury leading to placental insufficiency, mitochondrial toxicity, production of reactive oxygen species, and inhibition of progesterone during pregnancy, which is essential for the growth and maintenance of the fetus [146-150].

Inadequate Maternal Diet

Food insecurity often coexists with HIV infection [151, 152]. In Uganda, households with HIV-infected women have lower dietary diversity and higher rates of food aid usage [153]. In other countries such as Kenya, 33.5% of the individuals accessing HIV clinics are food insecure [154].

Some studies report that many HIV-infected women have inadequate levels of vitamin B12 and poor weight gain during pregnancy [155-157]. Frequent illnesses may prevent women living with HIV from working, leading to limited financial ability to meet nutritional needs [152]. Poor gastrointestinal absorption or increased metabolic demand may further restrict calories available for fetal growth [158].

1.2.6.2 Pathogenesis of postnatal growth faltering

Several factors affect growth faltering in HEUs such as enteropathy, opportunistic infections, malnutrition, and growth hormone axis disruption.

Enteropathy

HEU infants have greater intestinal inflammation, microbial translocation and abundance of gut *Acidaminococcus sp* [159]. Some causes of enteropathy in HEU infants include repeated bouts of infectious diarrhea, which may lead to disruption of intestinal barrier function [160-162]. There are several ways that enteropathy can lead to growth faltering. Infectious diarrhea may lead to villous atrophy, which impairs gut barrier function and leads to microbial translocation [163, 164]. This results in systemic inflammation, thereby reducing circulating growth hormones and leading to linear growth faltering. Another proposed mechanism for growth failure stems from increased levels of *Acidaminococcus sp* [159]. This bacterial species appears to ferment glutamate, resulting in reduced levels of glutamate for amino acid metabolism, disruption of nitrogen balance and inadequate nutrient absorption [159, 165].

Opportunistic Infections

HEU infants are at increased risk for opportunistic infections such as diarrheal diseases, pneumonia, and tuberculosis. There is a 50% and 70% increased risk of diarrhea and pneumonia in HEU infants in the first 6 months of life, respectively [166]. Within this HEU infant population, there is also a 2-fold increased risk of contracting tuberculosis [167].

Opportunistic infections may cause growth faltering by decreasing nutrient transport to target tissues [168, 169]. Secondly, persistent diarrheal episodes can cause extensive fluid and electrolyte loss, as well as damage to the intestine, which decreases nutrient absorption [170] [171]. Lastly, elevated pro-inflammatory cytokines may suppress the growth hormone axis and reduce growth hormone bioavailability [169].

Malnutrition

Children born to HIV-infected mothers are more likely to be malnourished [172, 173]. While some studies have reported that malnutrition is higher in non-breastfeeding HEU than HUU infants, poor nutritional status is similar between HEU and HIV-infected infants [174, 175]. Poverty, low maternal education, unemployment, single motherhood, maternal food insecurity, and limited breastmilk supply can be risk factors for HEU infant malnutrition [176]. Additionally, malnutrition can cause villous blunting and reduction in mucus-secreting goblet cells [177-179]. As a result of the structural changes, nutrients are not absorbed properly leading to growth faltering [174, 179].

Growth hormone axis disruption

HEU infants have an increased risk of growth hormone-axis disruption relative to HUU infants. HEU infants have a 10% reduction in insulin-like growth factor-1 (IGF-1) in comparison to HUU infants at 6 weeks of age (29.5 ng/ml vs 32.6 ng/ml) [180]. Lower levels of IGF-1 may be explained by higher levels of inflammation in HEU infants, which disrupts the growth hormone axis [180, 181]. Zinc deficiency is also associated with reduced levels of IGF-1 [169]. Suppression of IGF-1 leads to disruption of chondrocyte proliferation at epiphyseal growth plates, thereby causing poor bone development [180, 182].

1.2.6.3 Pathogenesis of neurodevelopmental delay

Multiple factors may explain the developmental delay observed in HEUs such as neuronal injury, atypical brain growth, prenatal maternal stress, and reduced caregiver interaction.

Neuronal Injury

Diffusion tensor imaging of brains of HEU infants shows evidence of white matter damage [183]. These changes may be related to reduced axial diffusivity and poor cell migration during development [183-186]. Neuroinflammation, such as microglial activation, disrupts early brain development [187, 188]. In HEU infants, CCR2 expression on classical monocytes is increased and brain levels of monocyte chemoattractant protein-1 are higher, leading to increased recruitment of pro-inflammatory monocytes across the blood-brain barrier into the central nervous system [189-191].

Neuronal injury may be a result of exposure to pro-inflammatory and infectious molecules, or exposure to specific antiretrovirals [192, 193]. For example, dolutegravir causes neural tube

defects, and atazanavir and efavirenz are associated with lower language acquisition receptive language scores [194, 195].

Atypical brain growth

Atypical brain growth leading to microcephaly is linked to poor neurodevelopment [99, 196]. About 11% of HIV-infected infants, 6.8% to 7.5% HEU infants, and 5.4% HUU infants have microcephaly at birth [196]. Causes for microcephaly include increased incidence of co-infections in HEU infants and cART exposure [99]. For example, HEU infants taking cART that contains tenofovir and efavirenz have a 2-to-3-fold increased risk of microcephaly [196].

Prenatal maternal stress

Psychosocial stress in HIV-infected women can have negative neurodevelopmental impacts on their HEU offspring. About 85% of pregnant HIV-infected women experience symptoms of depression [197]. Intimate partner violence is also common [198]. Additional causes of maternal stress include gender and power inequity, economic vulnerability, gender-based violence, limited power to negotiate in intimate relationships, and stigma [199-203]. Maternal stress can give rise to increased circulating levels of cortisol and norepinephrine which may affect stress reactivity in HEU infants and lead to poor emotional and cognitive development [204].

Reduced caregiver interaction

Infants born to mothers living with HIV may experience reduced caregiver interaction [205]. This may result from psychological or economic stressors, changes in family composition, death of parents, and caregivers outside of the biological family [206]. Reduced caregiver interaction may also result from mental health challenges [110]. Reduced caregiver interaction limits stimulation and early learning opportunities for HEU infants, which may diminish cognitive development [110].

1.3 Biomarkers Associated with Growth and Neurodevelopment in HIV Infection

1.3.1 Definition and Clinical Utility of Biomarkers

The definition of a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [207]. An example is a host protein in the peripheral blood that participates in the host immune response and can be used as a prognostic indicator of a disease outcome [208]. Circulating biomarkers of host response to foreign pathogens have been shown to provide diagnostic and prognostic information in several infectious syndromes [209-213]. They can distinguish between a quiescent state and a state of immune activation, as occurs in HIV infection [214]. This may be useful in distinguishing HEU infants with normal versus altered growth and neurodevelopmental trajectories. In the following section, we describe several host biomarkers that participate in systemic inflammation and related pathways that have been studied in HEUs in relation to growth faltering and developmental delay. These are examined in greater detail in our systematic review (Chapter 3).

1.3.2 Acute Phase Reactants

Acute phase reactants are inflammatory markers that rise during inflammation, infection, fever, tissue injury, trauma or surgery, neoplastic growth or immunological disorders [215].

1.3.2.1 C-Reactive Protein (CRP)

CRP is a cyclic pentameric protein composed of five identical non-covalently attached subunits [216, 217]. Each subunit has an intra-disulfide bond, 206 amino acids long with a molecular mass of about 23 kDa each [216, 217]. The subunits have a phosphocholine (PCh)-binding site, which is the principal CRP ligand [218].

CRP is an acute-phase protein synthesized by hepatocytes in response to pro-inflammatory cytokines (inflammatory/infectious processes) [218]. The main function of CRP is to bind damaged cells of the host and microbial pathogens, and then mediate elimination by recruiting the complement system and phagocytic cells [219].

As injured or infected cells cause inflammation and express PCh on its surface, increased levels of CRP attach to the exposed PCh group to form a complex [218]. This activates the complement system through the classical pathway and leads to phagocytosis by macrophages that can clear infected host cells and bacterial cells [220]. During inflammatory states caused by bacterial infection, the expression of CRP is regulated at the transcriptional level with interleukin-6 being the principal inducer of the gene [218]. As the bacterial infection clears, CRP levels return to normal [221].

Inflammation arising from HIV has been known to be associated with increased CRP levels [222]. CRP levels increased over time in HIV-infected individuals independent of CD4 lymphocyte counts, viral RNA levels and progressed to AIDS [223]. Healthy individuals' CRP levels are usually less than 0.3 mg/L, while individuals with acute bacterial infection typically have CRP levels that are greater than 40 mg/L [224, 225]. Viral infections that lead to mild systemic inflammation are associated with elevations in CRP levels ranging between 10-40 mg/L [226].

1.3.2.2 Fibrinogen

Fibrinogen is a 340-kDa protein, and is the precursor of fibrin which is a soluble glycoprotein [227]. Fibrinogen is made up of three polypeptide chains called Aα, Bβ, and γ joined together in the N-terminal E domain by five symmetrical disulfide bridges [228]. Each fibrinogen Aα-chain contains an N-terminal fibrinopeptide A (FPA) sequence [228]. The cleavage of this N-terminal fibrinopeptide A (FPA) sequence by thrombin initiates fibrin assembly [228].

Fibrinogen is a coagulation factor synthesized in hepatocytes [229]. Fibrinogen is also a potent inducer of inflammation [230].

The expression of fibrinogen is increased in response to inflammatory stimuli by proximal promoters containing sites for hepatocyte transcription factors [231]. While the plasma concentration of fibrinogen ranges from 2-4g/L, this increases several folds during injury [232]. With tissue or vascular injury, fibrinogen is enzymatically converted to fibrin by thrombin [227]. Subsequently, fibrin-based blood clot forms via activated blood clotting factor XIII, containing

polymerized and cross-linked fibrin [233, 234]. This is crucial to occlude bleeding of blood vessels and promote wound healing [233, 234].

Elevated plasma fibrinogen levels can serve as a biomarker for the detection of vascular abnormalities in neurodegenerative disease, coronary artery disease, mechanical insult, infection, or immunological abnormalities [229, 235, 236].

It has been found that fibrinogen levels are 6-8% higher in HIV infected group when compared to uninfected controls [236].

1.3.3 Proinflammatory Cytokines

Proinflammatory cytokines are mainly produced by activated macrophages and are involved in the upregulation of cytokines to increase inflammatory responses [237]. These cytokines play an active role in the entry of HIV into host tissues, progression of disease and infections [237]. HIV infection is characterized by immune activation, with the production of proinflammatory cytokines as well as other cytokines [238]. During HIV infection, the virus depletes CD4+ T lymphocytes, impacts the function of T lymphocytes and macrophages, and dysregulates cytokine production [238].

1.3.3.1 Interleukin-1β (IL- 1β)

IL-1β is expressed as a 31 kDa pro-form in the cytoplasm [239]. It is processed and released from cells by a mechanism using IL-1β converting enzyme (ICE), also known as caspase-1

[239]. The conversion of the pro-IL-1β by ICE to its biologically activated mature form can take place in specialized secretory lysosomes in the cytoplasm [240].

IL-1β is a potent proinflammatory cytokine that can be produced by myeloid cells or inflammasomes at sites of tissue infection or injury [241]. This cytokine is crucial for host-defence responses to infection and can exacerbate damage during chronic disease, acute tissue injury, cell proliferation, differentiation, and apoptosis [239, 242]. IL-1β is known to be an important mediator during systemic and local inflammation, and autoimmune diseases [243].

IL-1β signalling is crucial to initiate inflammatory response; the IL-1β primary and accessory receptors are IL-1 receptor Type I (IL-1RI) and (IL-1RAcP), respectively [244]. Upon binding to IL-1RI, IL-1RAcP is recruited by binding to the composite surface of the cytokine and primary receptor, creating a ternary complex [240]. The binding affinity of IL-1RAcP is approximately 100-fold weaker than that of the IL-1β/IL-1RI complex [240]. The ectodomains of these receptors are attached to Toll/interleukin-1 receptor (TIR) domains that reside in the cytoplasm [244]. The cytoplasmic TIR domains of the two receptors are brought together to elicit downstream signalling [244].

IL-1β is responsible for the progression of atherosclerosis and secondary complications of neurological and hematological disorders in HIV disease progression [240, 245].

Mature IL-1β is secreted when inflammasome is activated during HIV infection [240]. As infection is detected, inflammasomes recognize threats and respond by activating the immune

system [241]. Inflammasomes are innate immune system receptors that regulate the activation of capcase-1 and induce inflammation [246]. These inflammasome signals for pro–IL-1β to activate into its mature form, and lytic programmed cell death of the infectious agent [241].

1.3.3.2 Interleukin-6 (IL-6)

IL-6 is a glycosylated protein with a four-helix bundle structure [247]. The IL-6 receptor–signalling system is made up of two receptor chains and downstream signalling molecules. Macrophages, neutrophils, $CD4^+$ T-lymphocytes, podocytes, and hepatocytes express IL-6R on their cell surface, and therefore can directly respond to IL-6 [248].

IL-6 plays a crucial role in acute phase response and regulating the transition from acute to chronic inflammation. It stimulates the synthesis of acute phase proteins such as CRP, fibrinogen and others in the liver, and also inhibits the production of albumin [247].

On target cells, IL-6 binds to membrane-bound IL-6R. This complex connects to two molecules of gp130 and initiates signal transduction [248]. JAK (Janus kinase)/STAT3 (signal transducers and activators of transcription) and SHP2/Gab/MAPK (mitogen-activated protein kinase) are the two major pathways involved in gp130 signalling [249]. Besides the membrane-bound, a soluble form of the IL-6R (sIL-6R) has been identified in body fluids such as blood and urine. sIL-6R binds to IL-6 with a similar affinity as the mbIL-6R. Subsequently, the complex of IL-6/sIL-6R can activate gp130 [248, 250].

IL-6 induces the production of acute phase reactants, including C-reactive protein, by hepatocytes [251]. IL-6 is involved in the regulation of T cell differentiation between regulatory

T (Treg) cells and T helper 17 (Th17) cells. IL-6 triggers the differentiation of Th17 cells together with TGF-βand dampens the generation of Treg cells via STAT3. Therefore, IL-6 has a clear implication in $CD4^+$ T cell differentiation and expansion and contributes to T-cell-mediated immune response [252].

Previously, IL-6 level has been characterized as a marker for metabolic disorder and cardiovascular disease [253]. Dysregulation of IL-6 production also contributes to the pathogenesis of chronic inflammation. HIV infection induces the expression and secretion of IL-6 by monocytes and macrophages [254].

1.3.3.3 Interleukin-12p70 (IL-12p70)

Cytokine IL-12p70 is a disulfide-linked heterodimeric 70-kDa cytokine composed of a 197 amino acid 35-kDa (p35) subunit and a 306 amino acid 40-kDa (p40) subunit [255]. This cytokine forms a link between innate and adaptive responses. It also binds to the IL-12 receptor, consisting of two subunits, IL-12Rβ1 and IL-12Rβ2, expressed on Th1 and NK cells [256].

IL-12p70 is a cytokine produced in myeloid cells and plays an important role in the differentiation of T-cells into Th1 cells [257]. The IL-1270 is in an interconnected pathway where it also stimulates the production of interferon-gamma (IFN-γ) and tumour necrosis factor (TNF) from T cells and natural killer (NK) cells and reduces IL-4 mediated suppression of IFN-γ [258]. The induction of Th1 cells is controlled by two types of T-lymphocytes expressing CD4: Th1 and Th2 [257]. The function of Th1-type cytokines is to produce proinflammatory responses

thereby killing intracellular parasites and spreading autoimmune responses [259]. On the other hand, the function of Th2-type cytokinesis to produce an anti-inflammatory response [259].

Upon activation, IL-12R-β2 becomes tyrosine phosphorylated and provides binding sites for kinases, Tyk2 and Jak2. These are important in activating critical transcription factor proteins [260]. For example, STAT4 is one of the transcription factor proteins and it is useful for IL-12 signalling in T cells and NK cells within the JAK-STAT pathway [260]. While IL-12p70 mainly acts through Th1 cells, its levels are impacted by antigenic stimulation [261].

It has been reported that IL-12p70 levels are increased in patients with acute HIV infection [261]. IL-12p70 concentrations are also elevated in genital tract fluids of women with acute, heterosexually acquired HIV infection [262].

1.3.3.4 Tumour Necrosis Factor (TNF)

TNF is translated as a 233 amino acid (26 kDa) polypeptide from transmembrane TNF (mTNF) [263]. This polypeptide is processed to a 157 (17 kDa) amino acid mature soluble TNF form by cleavage of a 76 amino acid signal peptide and mediation by the metalloprotease TNF-converting enzyme (TACE). Soluble TNF (sTNF) can exist as a homotrimer cleaved monomer (17 kDa) or as homotrimer uncleaved monomers (26 kDa). Each monomer is made up of two packed β-pleated sheets created by eight antiparallel β-strands arranged in a β-jellyroll topology [264]. TNF has two receptors that aids to carry out its function: tumour necrosis factor receptor type I (TNF-RI) and type 2 (TNF-RII) [265].

TNF is a proinflammatory cytokine, primarily produced by macrophages and is involved in inflammation, molecular proliferation, differentiation, and apoptosis [264]. The levels of TNF increase in serum and tissue during inflammatory and infectious conditions [218]. The main function of TNF is in the regulation of immune cells. Elevated TNF can cause fever, apoptosis, inflammation, inhibit viral replication, and respond to sepsis via IL-1 and IL-6-producing cells [266]. TNF receptor TNF-RI is responsible for proinflammatory and apoptotic actions and TNF-RII is anti-inflammatory and promotes cell proliferation [267].

The biological response to TNF is mediated by receptors TNF-RI and TNF-RII, which are present on most cell membranes [268]. TNF-RII is initially expressed intensively by T cells and endothelial cells [269]. Its functions involve recruiting or inhibiting specific cell types, such as lymphocytes and clastic lineages, when necessary, and induce a rapid and efficient response. Upon stimulation, the intracellular domain of TNF-RI binds to the TNF receptor-associated death domain (TRADD) protein to further activate apoptotic pathways. This can be done via the Fas-associated death domain (FADD) protein or proinflammatory pathway [270].

Increased TNF plasma concentration is associated with untreated HIV infection [271]. Persistent alteration of TNF levels in HIV-infected patients might cause tissue damage, even when HIV replication is long-term controlled by cART [272].

1.3.3.5 Interferon-γ (IFN- γ)

IFN- γ is a monomer is made up of a core of six α-helices with an unfolded sequence in the C-terminal region and when activated, the structure forms a dimer by antiparallel interlocking of the

two monomers [273]. Cellular responses to IFN-γ are mediated by its heterodimeric cell-surface receptor (IFN-γR), which activates downstream signal transduction cascades, ultimately leading to the regulation of gene expression [274, 275]. The receptor complex that mediates the full biologic function of IFN-γ consists of at least two species-matched chains: IFN-γR1, a 90-kDa glycoprotein α chain, and IFN-γR2, a 60–67-kDa glycoprotein accessory β chain [274]. The α chain is responsible for the binding of IFN-γ, but with the presence of an accessory β chain, IFN-γ binds with higher affinity [274, 275].

The IFN-γ is produced by natural killer (NK) cells and helper T cells CD4 Th1 and cytotoxic T lymphocytes in response to encountering foreign object detection. IFN-γ enhances autoimmune disorders by suppressing the inflammatory response IFN-γ attenuates the differentiation of T helper (Th) 17 cells and osteoclasts, whereas loss of IFN-γ has a protective effect in collagen-induced arthritis (CIA) [276]. This cytokine's signalling involves helping with antigen presentation, activating the innate immune system, regulating Th1/Th2 balance, controlling cellular proliferation and apoptosis [274, 277, 278]. Specific residues within the cytoplasmic domains of both the α and β chains of the IFN-γR are critical for transducing the IFN-γ signal from the cell surface to the nucleus through the activation of intracellular signalling pathways [274, 277].

Regulation of IFN-γ expression is driven by the transcription factor T-box expressed in T cells (T-bet). It can also be driven by nuclear factors of activated T-cells (NFAT) and inhibitor transforming growth factor beta (TGF-β) in CD4+ Type 1 helper T cells [279]. Upregulating IFN-γ expression requires phosphorylation of transcription factors, driven by activation of

multimeric receptors of cytokines linked to JAK activation [280]. IFN-γ expression can be downregulated by inhibition of the specific transcription factors that activate the signalling cascade [281].

IFN-γ is detected in HIV-infected individuals during the acute phase and throughout the course of infection [282, 283]. Initially, IFN-γ is produced along with other proinflammatory cytokines to fight the primary infection [284]. Together, these cytokines establish a chronic immune activation, which is responsible for the clinical diseases linked to AIDS [282].

1.3.3.6 Granulocyte-macrophage colony-stimulating factor (GM-CSF)

GM-CSF is composed of one α chain and one β chain that binds with low and high-affinity respectively, and the β chain is shared with IL-3 and IL-5 receptors [285]. After GM-CSF binds to its receptor, Janus-kinase-2 (JAK-2), it is recruited to the cytoplasmic domain of the β chain, and JAK-2 is activated [286]. This induces phosphorylation of STAT-5 and migration of STAT-5 dimers to the nucleus [286]. As a result, this signalling promotes the transcription of various genes to induce cell differentiation [287].

GM-CSF is a multipotent cytokine produced by activated T cells, monocytes, B cells, NK cells, endothelial, epithelial, and fibroblasts [287-289]. It stimulates the proliferation of bone marrow-derived macrophages and granulocytes [288-290]. GM-CSF can also boost the differentiation of M1-like macrophages and causes the production of higher levels of inflammatory cytokines such as IL-1, IL-6, and TNF α, all of which cooperate in the destruction of the myelin sheath [287]. The inflammasome processing of IL1β can be mediated by GM-CSF in myeloid cells such as

monocytes and macrophages, promoting the expansion of Th17 cells and more damage to the blood-brain barrier [287, 291].

Growing evidence supports that GM-CSF has a major role in some inflammatory and autoimmune reactions, augments the LPS-induced inflammatory response and in the host's response to pulmonary infection.[287, 290].

In previous studies, GM-CSF has been reported to inhibit HIV replication by binding to the β -chain of the GM-CSF receptor [292].

1.3.4 Regulatory Cytokines

Regulatory-inflammatory cytokines are immunoregulatory molecules that control the proinflammatory cytokine response, reduce inflammation, and promote healing [237, 293]. These cytokines are associated with specific inhibitors and soluble cytokine receptors to regulate immune responses. During HIV infection, the regulation of these cytokines is interrupted due to the impact of HIV on the immune cells [238].

1.3.4.1 Interleukin-4 (IL-4)

IL-4 is a small four-helix-bundle cytokine that is characterized by antiparallel juxtaposed helices and two long end-to-end loops, which are connected by a short β-sheet packed [294]. It has a molecular weight varying between 12 and 20 kDa and shares sequence homology, cell surface receptors, intracellular signalling, and some functional impact on cells with IL-13 [295].

IL-4 functions as a potent regulator of immunity, secreted primarily by mast cells, Th2 cells, eosinophils, and basophils [296]. This cytokine is well known for its key roles in type 2 immune responses, which result in resistance to helminth parasites and inactivation of toxins [297].

There are two different types of IL-4 and IL-13 receptors: type I and type II [298-300]. These receptors help IL-4 and IL-13 help to regulate cellular functions and activate transcriptional machinery. For IL-4, binding of the cytokine IL-4Rα generates a ligand complex. This requires the recruitment of a third receptor chain to become a functional receptor complex. The receptor formed by IL-4/IL-4Rα with γc is a type I L-4 receptor and the IL-4/IL-4Rα complex binding IL-13Rα1 is a type II IL-4 receptor [299]. Both types I and II are found in myeloid cells, but the former can be found in lymphocytes, while the latter can be found in non-hematopoietic cells.

IL-4 also plays an important role in the regulation of brain immunity, with measurable downstream effects on spatial learning, memory and implications for neurological disorders [296].

In HIV infection, IL-4 regulates HIV co-receptors CXCR4 and CCR5 in human peripheral blood mononuclear cells [301]. IL-4 modifies the levels of CXCR4 and CCR5 expression, which switches the phenotype of HIV from either rapid/high or syncytia-inducing (SI) and slow/low to non-SI (NSI) or vice versa [301]. NSI virus is found early in infection, while SI virus is present in later stages with high viral load. Individuals infected with HIV switch between these phenotypes and IL-4 regulates these changes [302, 303].

1.3.4.2 Interleukin-10 (IL-10)

The human IL-10 gene spans about 4.7 kb on chromosome 1q21-32 and contains 5 exons and 4 introns [304]. IL-10 predominantly exists as a homodimer, composed of two polypeptide chains of 160 amino acids each. The subunits within the dimer are non-covalently associated, although each subunit contains two intra-chain disulfide bonds [305].

IL-10 is a cytokine produced by myeloid cells that plays a central role in limiting host immune response to pathogens [304, 306-308]. IL-10 family cytokines are essential for maintaining the integrity and homeostasis of tissue epithelial layers [308]. This cytokine can also facilitate the tissue-healing process in injuries caused by infection or inflammation [308].

IL-10 production is regulated by changes in the chromatin structure, enhancement or silencing of IL10 transcription and post-transcriptional regulatory mechanisms [305]. Once IL-10 is produced, the assembly of the cell surface IL-10 receptor complex is the first step in initiating IL-10 signalling pathways [309]. These pathways regulate intestinal inflammation, viral persistence, and even tumour surveillance. IL-10 cellular responses require the specific recognition and assembly of a heterodimeric cell surface complex composed of IL-10R1 and IL-10R2 chains [310]. IL10R1 is expressed on most hematopoietic cells at a basal level but is upregulated by various cells upon activation, suggesting its importance in inhibitory pathways.

During HIV infection, IL-10 production is induced, and neutralization of endogenous IL-10 may improve defective antigen-specific T cell function in HIV-infected patients [311, 312]. The level

of IL-10 is significantly higher in patients with advanced clinical and immunological disease along with a high viral load [313].

1.3.5 Chemokine

A structurally related group of cytokines that are known to induce chemotaxis is known as chemokines [237]. This chemotactic cytokine group has low molecular weight proteins, whose function is to activate leukocytes and help them migrate to target cells. Chemokines have conserved cysteine residues and are categorized accordingly in four groups with various functions [314].

1.3.5.1 C-X-C motif chemokine 10/interferon γ-induced protein 10 kDa (CXCL10/IP-10)

CXCL10/IP-10 is a small-molecular-weight protein (10 kDa), whose gene is located on chromosome 4, contains 4 exons and 3 introns [315]. It encodes a protein of 98 amino acids, is functionally described as an ‘inflammatory' chemokine [316]. This is transcriptionally regulated in response to IFN-γ and lipopolysaccharides (LPS) by a region of 230 nucleotides upstream from the transcriptional start site [316, 317].

CXCL10/IP-10 is an inflammatory chemokine that induces chemotaxis, cell growth, angiogenesis, and apoptosis by binding to its surface chemokine receptor CXCR3 [315]. CXCL10/10 is mainly secreted from leukocytes, neutrophils, eosinophils, monocytes, epithelial, endothelial, and keratinocytes in response to IFN-γ.

CXCL10/IP-10 binds to several G-protein-coupled receptors and induces a variety of cellular effects such as inhibition of endothelial cell proliferation, inhibition of growth factor-dependent hematopoiesis, and tumour necrosis [318-320]. CXCL10/IP-10 is constitutively expressed at low levels in thymic, splenic, and lymph node stroma [320]. However, expression can be highly induced in a variety of cells, including endothelial cells, keratinocytes, fibroblasts, mesangial cells, astrocytes, monocytes, and neutrophils by stimulation with IFN-α, IFN-β, IFN-γ, or LPS and in T cells by Ag activation [320].

Chemokines are upregulated during neuroinflammatory activities or trauma to the central nervous system, while others are associated with growth-related oncogenes [237, 321, 322]. Chemokines are also upregulated in viral infection where viruses interact with receptors to infect cells [323, 324].

During the early phase of infection, HIV may induce increased chemokine secretion *via* the innate immune response, promoting immune activation [325, 326]. Chemokine CXCL10/IP10 is significantly elevated in 100% of HIV-infected individuals during early HIV infection and impacts the subsequent disease progression. These findings were also consistent amongst untreated HIV-infected patients.

1.3.6 Biomarkers of Microbial Translocation

Microbial translocation refers to the passage of gastrointestinal microbial products through the damaged gastrointestinal tract, reaching the mesenteric lymph nodes and then the bloodstream. This results in systemic immune activation without the presence of bacteria or infection [327].

Translocating microbes and microbial products are typically phagocytosed at the site of lamina propria and the mesenteric lymph nodes. However, this pathway fails when the immune system is compromised such as HIV infection [328].

Microbial translocation can be assessed by quantifying certain biomarkers circulating in the blood. Assays include direct detection of microbial products such as lipopolysaccharide (LPS), LPS-binding protein (LBP), and intestinal fatty acid binding protein (I-FABP) [329].

1.3.6.1 Lipopolysaccharide binding protein (LBP)

LBP is a 50 kDa glycosylated protein secreted into the bloodstream [330, 331]. The LBP structure is composed of three domains: (i) N-terminal domain barrel-barrel shaped structure, (ii) twisted, seven stranded antiparallel β sheets; and (iii) a C-terminal domain which is analogous to N-terminal but α helix is replaced by an "A" loop [331].

LBP is a protein produced by the liver and it is involved in the recognition, binding, and transport of LPS [330, 332]. LPS is a pathogen-associated molecular pattern found on the outer membrane of gram-negative bacteria and can initiate immune response during bacterial infections [333]. LBP and soluble CD14 (sCD14) are produced by the presence of LPS and initiate a potent innate immune response against the infection [333-335].

The role of LBP is to monomerize the LPS in the bloodstream, which leads to the formation of the monomer-LPS-sCD14 complex [336]. In low LBP concentration, LPS is delivered to the sCD14/ Toll-like receptor 4 receptor complex, which promotes the production of

proinflammatory stimuli [336, 337]. Excess levels of LBP neutralize LPS by shuttling it to the lipids to promote its elimination [336, 338]. But this mechanism is dysregulated in chronic infections. Reduction of LBP after effective cART is due to inflammation going down and partial restoration of gut-blood barrier [336].

LBP is elevated in the liver and systemic circulation in patients with sepsis and chronic liver disease due to the increased intestinal permeability and bacterial translocation [330].

LBP is also linked to persistent immune activation during HIV infection [330, 338].

1.3.6.2 Intestinal fatty acid binding protein (I-FABP)

I-FABP has a β-barrel structure consisting of 10 antiparallel β-sheets [339]. The small size (15 kDa) of I-FABP in the gut epithelium facilitates its leakage into the circulation from damaged enterocytes.

I-FABP is a cytosolic protein found in enterocytes of the gastrointestinal tract [340]. The role of I-FABP is to transport fatty acids from the apical membrane of enterocytes to the endoplasmic reticulum, where they are converted into triglycerides [341].

Elevated levels of circulating I-FABP are associated with disease conditions where the intestinal wall is injured or compromised [341]. Thus, I-FABP has an established role as a non-invasive marker for evaluating gut wall integrity loss and inflammation [342-345].

In HIV infection, there are several potential limitations of I-FABP as a biomarker of intestinal integrity [342-345]. As a fatty acid transport protein, I-FABP may be affected by some antiretroviral medications that cause metabolic lipid changes [346]. This suggests that metabolic changes independent of epithelial damage may confound the interpretation of I-FABP as a marker of microbial translocation [346].

1.3.7 Growth Factors and carrier proteins

Growth hormones (GH) are secreted by the somatotropic cells of the anterior pituitary gland when stimulated by hypothalamic growth hormone-releasing hormone (GHRH) [347, 348]. The GH axis regulates linear growth in infants through the hepatic synthesis of insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding proteins (IGFBP-1 and IGFBP-3) [349].

1.3.7.1 Insulin-like growth factor-1 (IGF-1)

The IGF-1 gene is on the long arm of chromosome 12q23–23 and consists of six exons, including two leader exons, and has two promoters [347]. It forms a small polypeptide weight 7.5-kDa, plays an important role in the regulation of somatic growth, metabolism, and cellular proliferation, differentiation, and survival [350]. It is made up of several chains, one of which is connected by disulphide bonds. Structurally, IGF-1 is similar to insulin and as a result, has shown low-affinity binding to insulin receptors [351].

IGF-1 is a major regulator of growth and organ development secreted by the liver and transported to tissues [352]. It plays a major role in neuronal plasticity and skeletal muscle formation [353]. IGF-I plays a pivotal role in many physiological processes as the mediator of the effects of GH and can function as both an endocrine and paracrine hormone [354].

The IGF receptor is expressed by almost all tissues and cell types during embryogenesis [352]. The IGF receptor is a heterotetramer composed of two extracellular spanning α subunits and transmembrane β subunits [352, 355]. The α subunits have binding sites for IGF-1 and are linked by disulphide bonds. The β subunit has a short extracellular domain, a transmembrane domain, and an intracellular domain. The intracellular part contains a tyrosine kinase domain, which constitutes the signal transduction mechanism [355]. The activated IGF-1 receptor is capable of phosphorylating other tyrosine-containing substrates.

They promote growth by both paracrine and endocrine pathways, their bioavailability being controlled by at least six IGF-binding proteins (IGFBPs) [356]. With IGF-1 being regulated by IGFBPs, only 1% of IGF-I is thought to circulate in a free or rapidly dissociable state and mediate effects on target tissues through an endocrine mechanism [357].

Infection with HIV is often associated with a decrease in the concentrations of IGF-I [347]. It was also shown that disturbances IGF-1- system in HIV-infected patients are associated with wasting and failure to thrive (FTT) [358].

1.3.7.2 Insulin-like growth factor binding protein-1 (IGFBP-1)

IGFBP-1 is a secreted 30-kDa protein [359]. Structurally, IGFBPs have six conserved disulfide bonds and a characteristic GCGCC motif in the N-domain.

IGFBP-1 is thought to be the major short-term modulator of IGF-I bioavailability [350, 360]. IGFBP-1 is less abundant than IGFBP-3, but appears particularly important in regulating IGF-I bioavailability, with strong inverse correlations between circulating IGFBP-1 and free IGF-I [360]. IGFBP-1 potentiates IGF's effect in certain cell systems, possibly as a result of the binding of IGFBP-1 to cell membranes through its Arg-Gly-Asp sequence [361]. The ability of IGFBP-1 to inhibit or potentiate IGF's action may depend on post-translational modifications of IGFBP-1 [362]. This appears to enhance the affinity of IGFBP-1 for IGF-1 and inhibit IGF's action. In addition to its regulatory function, IGFBP-1 can be involved in metabolism and reproduction. For instance, IGFBP-1 is the predominant binding protein species in the secretory endometrium and endometrial cells [362].

IGFBP-1 is mainly produced in the liver but can be expressed in other peripheral tissue [363].

IGFBP-1 production is inversely regulated by insulin [360]. Cachectic conditions, malnutrition, inflammatory cytokines, exercise, and oxidative stress all increase IGFBP-1 expression, which may, in turn, lead to inhibition of anabolic IGF-I effects by IGFBP-1 during catabolic states [360].

Infection with HIV is often associated with an increased concentration of IGFBP-1 [347].

1.3.7.3 Insulin-like growth factor binding protein-3 (IGFBP-3)

The *IGFBP-3* gene is located on chromosome 7 and contains four exons [364]. The mature, glycosylated IGFBP-3 protein has a molecular weight of approximately 29 kDa and comprises 264 amino acids composed of the conserved N and C terminal domains and a variable

midsection [365]. The midsection is also the site of phosphorylation, and it is the site responsible for interaction with cell surfaces.

IGFBP-3 is the main IGF transport protein in the bloodstream [366]. More than 80% of IGF-1 is bound to IGFBP-3 such that the half-life of IGF-1 is increased in circulation and only 1% of IGF-1 is biologically available for use.

IGFBP-3 is expressed in the kidney, stomach, placenta, uterus, and liver [365, 367]. The expression of IGFBP-3 action is GH dependent, either directly or through regulation by IGF. For example, serum IGFBP-3 is increased in acromegaly and low in GH-deficient children. IGFBP-3 expression can be induced by cell cycle regulators and growth inhibitory [364]. Many factors increase IGFBP-3 production by cells, including transforming growth factor-β (TGFβ), tumour necrosis factor-α, vitamin D, retinoic acid, IGF-1, and stimuli such as chemotherapy that activate tumour suppressor p53 [368].

Infection with HIV is associated with a decrease in the concentrations of IGFBP-3 [358].

1.3.8 Biomarkers of Tissue Remodelling

1.3.8.1 Matrix metallopeptidase 9 (MMP-9)

The Human MMP-9 gene is located at chromosome 20q13.12 [369]. This gene contains 13 exons and 12 introns. MMP-9 is synthesized as a pre-proenzyme with 19 amino acid N-terminal signal peptides within cells. Then, it is secreted into the extracellular environment as inactive pro-MMP-9 (about 92 kDa) [370]. Activation of MMP-9 requires cleavage by others [369]. Structurally, MMP-9 contains a hemopexin-like domain, catalytic domain, signal peptide, the

hinge region and propeptide region[369, 370] The catalytic domain of MMP-9 contains fibronectin type II (FN2) domains, active site and zinc-binding region [369]. The catalytic domain of MMP-9 plays a critical role in the catalytic activity of this enzyme [371]. Additionally, the fibronectin domain is important for some substrate binding and degradation [369].

Matrix metalloproteinases play a significant role in various human cancers and can be involved in the differentiation, morphogenesis and tissue remodelling during angiogenesis, tumour invasion and metastasis [369, 372]. Placental MMP is a proteolytic enzyme that plays a vital role in trophoblast invasion, regulation of vascular endothelial cell functions and placental angiogenesis [373, 374].

MMP-9 is secreted mainly by neutrophils and other cell types such as mesenchymal cells, fibroblasts, and several inflammatory cells like monocytes or lymphocytes [375].

MMP-9 could be used as a biomarker of cardiac disease, associated with severe inflammation and fibrosis [375-377]. It may also be a biomarker of inflammatory bowel disease (IBD) because it is the most abundant MMP in inflamed intestinal tissue [375].

In patients living with HIV, MMP-9 is also implicated in HIV associated neurological disorders such as dementia [378, 379]. Increased activity of MMP-9 may impair the integrity of the blood-brain barrier leading to enhanced monocyte infiltration into the CNS thereby causing dementia.

1.3.9 Biomarker of Neutrophil Activation

Neutrophils are immune effector cells that play an active role in host defence mechanisms by producing reactive oxygen species, initiating phagocytosis, and forming neutrophil extracellular traps (NETs) [380]. Neutrophils also partake in initiating inflammation, tissue destruction and erosion [380].

1.3.9.1 Neutrophil Gelatinase-Associated Lipocalin (NGAL)

NGAL is a 25 kDa protein. It contains a 20-amino acid signal peptide at the N-terminal end of the protein followed by a lipocalin domain [381]. This domain is responsible for the binding of lipocalins to their ligands and it is made up of eight stranded β barrels with its loops running in an antiparallel direction. Three of the β bulges present in this barrel have been suggested to contribute to the ligand-binding site for NGAL [382].

NGAL is a critical component of innate immunity to bacterial infection and is expressed by immune cells, hepatocytes, and renal tubular cells in various disease states [383]. Inflammatory and chronic disease conditions are known to increase NGAL expression in target cells [384].

NGAL is recognized as an early marker of acute kidney injury (AKI) [385-387]. This biomarker is specifically induced in the damaged nephron and then released into blood and urine, where it can be readily measured [383].

HIV-infected patients have higher levels of NGAL that are approximately 19% higher than uninfected patients. This suggests that HIV-infected patients may have more extensive kidney

injury than uninfected patients [388]. Alternatively, NGAL may be related to neuronal damage and neuroinflammation, as observed in the neocortex of brain tissues of HIV-infected patients [389, 390]. Increased level of NGAL was associated with cognitive impairment and reduced brain volume in HIV-infected patients.

1.4 Objectives of the Thesis

1.4.1 Objective of Chapter 2

The objective of chapter 2 was to review published data on circulating biomarkers that are associated with growth faltering and developmental delay in HEU infants. A small number of studies had examined biomarkers in HIV-infected infants, linking them to growth or neurodevelopmental outcomes. Some of these studies included HEUs as a control group. No previous study had systematically unified these studies. Furthermore, no studies had focused specifically on HEUs as a population of interest. We hypothesized that one or more host proteins in the peripheral blood would be predictive of subsequent growth faltering or developmental delay. To address this hypothesis, we conducted a systematic review of the literature.

1.4.2 Objective of Chapter 3

The objective of chapter 3 was to evaluate growth faltering and neurodevelopment in a Ugandan HEU cohort. Furthermore, we sought to explore the association between growth faltering (both intrauterine and postnatal) and neurodevelopment at 18 months of age. Several previous studies had examined growth outcomes and neurocognitive outcomes separately in HEUs. Other studies had compared HEUs to uninfected infants and/or children living with HIV. However, there was a gap in the literature with respect to the relationship between early growth faltering and later

neurodevelopmental delay. We hypothesized that early evidence of growth faltering would be associated with subsequent risk of poor neurodevelopment in HEU infants. To test this hypothesis, we conducted a prospective cohort study of HEU infants in Uganda. We performed longitudinal measurements of growth parameters and assessment of developmental milestones and we examined early growth disturbances (LBW and FTT) as potential predictors of neurodevelopment.

1.4.3 Importance of the study

Chapters 2 and 3 of this thesis represent two elements of a unified inquiry into key health outcomes in HEU infants. In Chapter 2, we synthesize the published literature on biomarkers that are associated with growth faltering and neurodevelopment, showing that there are gaps remaining in the existing knowledge. In Chapter 3, we take the first steps in the analysis of a prospective cohort of Ugandan HEUs, showing the association between early growth faltering and neurodevelopmental delay. Together, these chapters set the stage to test novel hypotheses using a biorepository of samples collected longitudinally from the prospective cohort (Chapter 3). This is further discussed in Chapter 4 under Future Directions of the research project. In brief, using the literature review (Chapter 2) as a launching point, we hypothesize that novel biomarkers of inflammation, endothelial activation, microbial translocation will be clinically informative predictors of well-characterized growth and neurocognitive outcomes in our prospective HEU cohort (Chapter 3).

CHAPTER 2: BIOMARKERS OF GROWTH FALTERING AND NEURODEVELOPMENTAL DELAY IN HIV-EXPOSED UNINFECTED INFANTS: A SYSTEMATIC REVIEW

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Contributions:

RS conducted the literature search, literature and database screening, data extraction, data analysis, quality appraisal, write-up of the manuscript, critically reviewed the manuscript, and revised the manuscript based on feedback from co-authors

ALC supervised write-up of the first draft of the manuscript and critically reviewed the manuscript

SN critically reviewed the manuscript

ROO critically reviewed the manuscript

UR literature and database screening and critically reviewed the manuscript

SF supervised write-up of the first draft of the manuscript and critically reviewed the manuscript

BOS supervised write-up of the first draft of the manuscript and critically reviewed the manuscript

MTH supervised literature and database screening, data extraction, data analysis, quality appraisal, write-up of the manuscript, critically reviewed the manuscript, and revised the manuscript based on feedback from co-authors

2.1 Abstract:

Introduction: HIV-exposed but uninfected infants (HEUs) are at risk of linear growth faltering and neurodevelopmental delay. Circulating biomarkers associated with these adverse outcomes may elucidate pathways of injury and may be clinically informative.

Methods: A systematic review of the literature found seven studies associating biomarker abnormalities and growth outcomes in HEUs and two studies on biomarker abnormalities and neurodevelopmental delay.

Results: Biomarker abnormalities associated with growth restriction were C-reactive protein (CRP), tumour necrosis factor (TNF), interferon-gamma (IFN-γ), interleukin (IL)-12p70, IFN-γ-induced protein-10 (CXCL10/IP-10), lipopolysaccharide binding protein (LBP), insulin-like growth factor-1 (IGF-1), and IGF-binding protein-1 (IGFBP-1). Biomarkers associated with motor, language, and cognitive delay were CRP, IFN-γ, IL-1β, -2, -4, -6, -10, -12p70, neutrophil gelatinase-associated lipocalin (NGAL), granulocyte-macrophage colony-stimulating factor (GM-CSF), and matrix metalloproteinase-9 (MMP-9).

Conclusion: In summary, elevated markers of inflammation (acute phase reactants, pro-inflammatory cytokines, chemokines), and intestinal microbial translocation are associated with growth faltering. Elevated markers of inflammation are associated with adverse neurodevelopment.

2.2 Introduction:

There are an estimated 14.8 million HIV exposed-uninfected (HEU) infants globally, nearly 90% of them from are Sub-Saharan Africa [90, 391]. Successful interventions, including combination antiretroviral therapy (cART) for HIV positive mothers, have reduced vertical HIV transmission to <2.9% in low-to-middle-income countries (LMIC) [91, 92]. HEU newborns significantly outnumber infected infants worldwide and represent nearly 30% of the newborn population in some HIV endemic nations [94]. Although HEUs do not exhibit severe immunodeficiency and opportunistic infections like HIV infected infants, they have adverse health outcomes that differ from those of HIV-unexposed uninfected (HUUs) infants [95]. In resource-limited countries, HEUs are 2-3 times more likely to die than HUUs before their second birthday and surviving HEUs have an increased risk of impaired linear growth and neurocognitive development [95, 117].

Low birth weight (LBW), reflecting poor intrauterine growth, is twice as common in HEUs as HUUs [95, 97]. Stunting in the first year of life, reflecting poor postnatal linear growth, is found in 12-31% of HEUs, representing three-fold higher odds relative to HUUs [74, 95, 97]. Growth faltering in the HEU population is associated with increased frequency of hospitalization, pneumonia, and diarrheal illness [101, 110, 392]. HEUs have increased risk of impaired psychomotor and cognitive development, hearing loss, and expressive language delay compared to HUUs [92, 95, 117]. Neurodevelopmental delay in HEUs may be due to pre- and postnatal factors, including socioeconomic variables, cART exposure, subtle immune deficits, systemic inflammation, and exposure to infections [94, 118].

The first 1000 days of life are critical periods for somatic and brain growth [110, 393]. UNICEF defines this as a "the time spanning roughly between conception and one's second birthday, a unique period of opportunity when the foundations of optimum health, growth, and neurodevelopment across the lifespan are established"[394]. Abnormalities arising in this period may be irreversible and are associated with subsequent impairments in cognition, executive function, and school attainment [393, 395-397]. Identifying at-risk HEUs before they complete this sensitive period of development may allow for targeted early interventions [398]. Thus, there is a need to identify biological indicators in infancy associated with later growth faltering and neurocognitive delay [97].

Circulating biomarkers can serve as a tool to interrogate pathways of disease progression [208, 399]. Growth and cognitive impairment are associated with increased inflammatory activity [102, 348]. There are several categories of inflammatory biomarkers that are commonly studied in HEUs: acute phase reactants, proinflammatory cytokines and chemokines [126]. Microbial translocation from gut lumen into the bloodstream is one possible cause of systemic inflammation in HEUs [117, 126, 134, 180, 400-402]. Systemic inflammation also negatively regulates growth hormone levels and inhibits growth at the epiphysis of long bones [141]. Measurement of one or more biomarkers in these interconnected pathways may provide clues to the pathogenesis of growth faltering and neurodevelopmental delay.

The objective of this review was to identify biomarkers that are associated with growth faltering and neurodevelopmental outcomes in HEUs. We performed a systematic review of the literature to summarize studies that measured biomarkers together with growth or neurocognitive outcomes in HEUs.

2.3 Methods:

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed for conduct and reporting of the study (http://www.prisma-statement.org/) [403]. The systematic review was registered on the International Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42021238363).

2.3.1 Search Criteria

A librarian-directed search was conducted of the electronic databases MEDLINE (1946-April 2021), EMBASE (1974-April 2021), Scopus (2004-April 2021), and PubMed (1985-April 2021). The search for growth and neurodevelopmental outcomes in HEUs was conducted on 11th April 2021 using the search terms shown in Table 2.1-2.3 (growth) and Tables 2.4-2.6 (neurodevelopment). There were no language or format restrictions for studies. All the studies were screened in Covidence software [404]. We also examined reference lists from identified studies for additional papers to include.

Table 2.1: Medline and Embase Database Search Strategy for Growth Outcomes. Search conducted on April 11th, 2021

Set	Search Statement
1	exp Child/ or exp infant/ or pediatrics/ or (pediatric* or paediatric* or child* or newborn* or infan* or baby or babies or neonat* or pre-term or preterm* or premature birth* or NICU or preschool* or pre-school* or toddler* or kindergarten* or kindergarden* or elementary school or school-age* or grade 1 or grade 2 or grade 3 or grade 4 or grade 5 or grade 6 or grade 7 or junior high or 3 months old or 6 months old or 12 months old or 18 months old or 24 months old or 30 months old or 36 months old or 1 y*-old or 2 y*-old or 3 y*-old or 4 y*-old or 5 y*-old or 6 y*-old or 7y* -old or 8 y*-old or 9 y*-old or 10 y*-old or 11 y*-old or 12 y*-old or 13 y*-old).mp. or (child* or adolesc* or pediat* or paediat*).jn.
2	exp HIV/
3	hiv infections/ or hiv seropositivity/
4	(human immunodeficiency or hiv).mp.
5	hiv exposed uninfected/ or HEU/
6	2 or 3 or 4 or 5
7	C-Reactive Protein/
8	(c-reactive protein or crp).mp.
9	orosomucoid/ or procalcitonin/ or blood sedimentation/ or Interleukin-8/ or interleukin-6/ or exp vascular endothelial growth factors/ or tumor necrosis factor-alpha/ or lymphotoxin-alpha/ or epidermal growth factor/ or insulin-like growth factor/ or exp somatomedins/ or exp insulin like growth factor binding proteins/ or Chitinase-3 like-protein-1/ or exp Angiopoietins/ or exp Monocyte chemoattractant proteins/ or intercellular adhesion molecule-1/ or vascular cell adhesion molecule-1/ or exp endothelins/
10	((Alpha-1 adj3 acid adj3 (glycoprotein or seromucoid)) or orosomucoid or AGP or procalcitonin or calcitonin-1 or pro-calcitonin or calcitonin precursor polyprotein or calcitonin related polypeptide alpha or PCT or Erythrocyte Sedimentation Rate or Interleukin-6 or IL-6 or (beta cell adj2 factor-2) or interferon-beta-2 or chemotactic factor or chemokine* or neutrophil-activating-peptide or cxcl8 or Interleukin-8 or IL-8 or (Vascular Endothelial adj2 Growth Factor) or VEGF or cachetin or Tumor Necrosis Factor or TNF* or lymphotoxin or Epidermal growth factor or urogastrone or EGF or insulin-like growth factor or IGF-1 or somatomedin* or insulin-like growth factor binding protein-3 or igf binding protein-3 or IGFBP-3 or Chitinase-3 like-protein-1 or CHI3L1 or ylk 40 protein or cgp-39 protein or gp-39 protein or Angiopoietin 1 or Ang-1 or Angiopoietin 2 or Ang-2 or monocyte chemoattractant protein* or MCP-1 or CD14 or sCD14 or soluble intercellular cell adhesion molecule-1 or ICAM-1 or sICAM-1 or soluble vascular cell adhesion molecule-1 or sVCAM-1 or VCAM-1 or Vascular cellular adhesion molecule or VCAM or endothelin or ET-1 or ET-2 or ET-3 or preproendothelin or proendothelin or endocan-2 or EC-2).mp.
11	Biomarkers/
12	(biomarker* or inflammatory marker* or systemic inflammation or low grade inflammation).mp.
13	7 or 8 or 9 or 10 or 11 or 12
14	Growth Disorders/
15	(stunt* or growth or failure-to-thrive or length*-for-age or height*-for-age or weight-for-age or length*-for-weight or height*-for-weight or head circumference or weight-for-age or mid-upper arm circumference or wasting or microcephaly or underweight).mp.
16	14 or 15

Table 2.2: Scopus Database Search Strategy for Growth Outcomes. Search conducted on April 11th, 2021

Set	Search Statement
1	TITLE-ABS-KEY ((human-immunodeficiency OR hiv))
2	TITLE-ABS-KEY ((c-reactive-protein OR crp OR alpha-1 AND adj3 AND acid AND adj3 OR glycoprotein OR seromucoid OR orosomucoid OR "AGP" OR procalcitonin OR "calci tonin 1" OR "procalcitonin" OR "calcitonin precursor polyprotein" OR "calcitonin related polypeptide alpha" OR "PCT" OR "Erythrocyte Sedimentation Rate" OR "Interleukin 6" OR "IL 6" OR "beta cell adj2 factor 2" OR "interferon beta 2" OR "chemotactic factor" OR "chemokine*" OR "neutrophil activating peptide" OR "cxcl8" OR "Interleukin 8" OR "IL 8" OR "Vascular Endothelial adj2 Growth Factor" OR "VEGF" OR "cachetin" OR "Tumor Necrosis Factor" OR tnf* OR lymphotoxin OR "Epidermal growth factor" OR "urogastrone" OR "EGF" OR "insulin like growth factor" OR "IGF 1" OR somatomedin* OR "insulin like growth factor binding protein 3" OR "igf binding protein 3" OR "IGFBP 3" OR "Chitinase 3 like protein 1" OR "CHI3L1" OR "ylk 40 protein" OR "cgp 39 protein" OR "gp 39 protein" OR "Angiopoietin 1" OR "Ang 1" OR "Angiopoietin 2" OR "Ang 2" OR "monocyte chemoattractant protein*" OR "MCP 1" OR "CD14" OR scd14 OR "soluble intercellular cell adhesion molecule 1" OR "ICAM 1" OR "sICAM 1" OR "soluble vascular cell adhesion molecule 1" OR "sVCAM 1" OR "VCAM 1" OR "Vascular cellular adhesion molecule" OR "VCAM" OR endothelin OR "ET 1" OR "ET 2" OR "ET 3" OR preproendothelin OR proendothelin OR "endocan 2" OR "EC 2") OR (biomarker* OR inflammatory-marker* OR systemic-inflammation OR low-grade-inflammation))
3	TITLE-ABS-KEY ((stunt* OR growth OR "height* for age" OR "failure to thrive" OR short-stature OR dwarfism OR "length*for age" OR "weight for age" OR "length* for weight" OR "height* for weight" OR "head circumference" OR "weight for age" OR "mid upper arm circumference" OR "wasting" OR "microcephaly" OR "underweight"))
4	TITLE-ABS-KEY(pediatric* OR paediatric* OR child* OR newborn* OR congenital* OR infan* OR baby OR ba bies OR neonat* OR "pre-term" OR preterm OR "premature birth*" OR nicu OR preschool* OR "pre-school*" OR kindergarten* OR "elementary school*" OR "nursery school*" OR schoolchild* OR "school age*" OR "grade 1" OR "grade 2" OR "grade 3" OR "grade 4" OR "grade 5" OR "grade 6" OR "grade 7" OR "junior high" OR "3 months old" OR "6 months old" OR "12 months old" OR "18 months old" OR "24 months old" OR "30 months old" OR "36 months old" OR "30 months old" OR "1 y* old" OR "2 y* old" OR "3 y* old" OR "4 y* old" OR "5 y* old" OR "6 y* old" OR "7y* old" OR "8 y* old" OR "9 y* old" OR "10 y* old" OR "11 y* old" OR "12 y* old" OR "13 y* old" OR toddler* OR boy OR boys OR girl* OR "middle school*" OR pubescen* OR juvenile* OR teen* OR youth* OR "high school*" OR adolesc* OR prepubesc* OR "pre-pubesc*") OR SRCTITLE (child* OR pediatric* OR paediatric* OR adolescent))
5	1 and 2 and 3 and 4

Table 2.3: PubMed Database Search Strategy for Growth Outcomes. Search conducted on April 11th, 2021

Set	Search Statement
1	(exp Child/ or exp infant/ or pediatrics/ or (pediatric or paediatrics or child or newborn or infant or baby or babies or neonatal or pre-term or preterm or premature birth or NICU or preschool or pre-school or toddler or kindergarten* or kindergarden* or elementary school or school-age* or grade 1 or grade 2 or grade 3 or grade 4 or grade 5 or grade 6 or grade 7 or junior high or 3 months old or 6 months old or 12 months old or 18 months old or 24 months old or 30 months old or 36 months old or 1 years old or 2 years old or 3 years old or 4 years old or 5 years old or 6 years old or 7 years old or 8 years old or 9 years old or 10 years old or 11 years old or 12 years old or 13 years old).mp. or (child* or adolescent or pediatric or paediatric))
2	(exp HIV/ or hiv infections/ or hiv seropositivity/ or (human immunodeficiency or hiv))
3	(C-Reactive Protein/ or (c-reactive protein or crp) or orosomucoid/ or procalcitonin/ or blood sedimentation/ or Interleukin-8/ or interleukin-6/ or exp vascular endothelial growth factors/ or tumor necrosis factor-alpha/ or lymphotoxin-alpha/ or epidermal growth factor/ or insulin-like growth factor/ or exp somatomedins/ or exp insulin like growth factor binding proteins/ or Chitinase-3 like-protein-1/ or exp Angiopoietins/ or exp Monocyte chemoattractant proteins/ or intercellular adhesion molecule-1/ or vascular cell adhesion molecule-1/ or exp endothelins/ or Alpha-1 adj3 acid adj3 (glycoprotein or seromucoid)) or orosomucoid or AGP or procalcitonin or calcitonin-1 or pro-calcitonin or calcitonin precursor polyprotein or calcitonin related polypeptide alpha or PCT or Erythrocyte Sedimentation Rate or Interleukin-6 or IL-6 or (beta cell adj2 factor-2) or interferon-beta-2 or chemotactic factor or chemokine* or neutrophil-activating-peptide or cxcl8 or Interleukin-8 or IL-8 or (Vascular Endothelial adj2 Growth Factor) or VEGF or cachetin or Tumor Necrosis Factor or TNF* or lymphotoxin or Epidermal growth factor or urogastrone or EGF or insulin-like growth factor or IGF-1 or somatomedin* or insulin-like growth factor binding protein-3 or igf binding protein-3 or IGFBP-3 or Chitinase-3 like-protein-1 or CHI3L1 or ylk 40 protein or cgp-39 protein or gp-39 protein or Angiopoietin 1 or Ang-1 or Angiopoietin 2 or Ang-2 or monocyte chemoattractant protein* or MCP-1 or CD14 or sCD14 or soluble intercellular cell adhesion molecule-1 or ICAM-1 or sICAM-1 or soluble vascular cell adhesion molecule-1 or sVCAM-1 or VCAM-1 or Vascular cellular adhesion molecule or VCAM or endothelin or ET-1 or ET-2 or ET-3 or preproendothelin or proendothelin or endocan-2 or EC-2 or Biomarkers/ or biomarker* or inflammatory marker* or systemic inflammation or low grade inflammation))
4	(Growth Disorders/ or stunt* or growth or failure-to-thrive or length-for-age or height-for-age or weight-for-age or length-for-weight or height-for-weight or head circumference or weight-for-age or mid-upper arm circumference or wasting
5	1 and 2 and 3 and 4

Table 2.4: Medline and Embase Database Search Strategy for Neurodevelopmental Outcomes. Search conducted on April 11th, 2021

Set	Search Statement
1	exp Child/ or exp infant/ or pediatrics/ or (pediatric* or paediatric* or child* or newborn* or infan* or baby or babies or neonat* or pre-term or preterm* or premature birth* or NICU or preschool* or pre-school* or toddler* or kindergarten* or kindergarden* or elementary school or school-age* or grade 1 or grade 2 or grade 3 or grade 4 or grade 5 or grade 6 or grade 7 or junior high or 3 months old or 6 months old or 12 months old or 18 months old or 24 months old or 30 months old or 36 months old or 1 y*-old or 2 y*-old or 3 y*-old or 4 y*-old or 5 y*-old or 6 y*-old or 7y* -old or 8 y*-old or 9 y*-old or 10 y*-old or 11 y*-old or 12 y*-old or 13 y*-old).mp. or (child* or adolesc* or pediat* or paediat*).jn.
2	exp HIV/
3	hiv infections/ or hiv seropositivity/
4	(human immunodeficiency or hiv).mp.
5	hiv exposed uninfected/ or HEU/
6	2 or 3 or 4 or 5
7	(C-reactive protein or CRP or Blood serotonin or Urine melatonin sulfate excretion or neuron-restrictive silencer factor or NRSF or brain-derived neurotrophic factor or BDNF or Neurofilament light polypeptide or NFL or repressor element 1-silencing transcription or REST or neuron-specific enolase or NSE or glial fibrillary acidic protein or GFAP or myelin basic protein or MBP or S100 calcium-binding protein B or S100B or Monocyte Chemoattractant Protein-1 or MCP-1 or interleukin-6 or IL-6).mp.
8	((Alpha-1 adj3 acid adj3 (glycoprotein or seromucoid)) or orosomucoid or AGP or procalcitonin or calcitonin-1 or pro-calcitonin or calcitonin precursor polyprotein or calcitonin related polypeptide alpha or PCT or Erythrocyte Sedimentation Rate or Interleukin-6 or IL-6 or (beta cell adj2 factor-2) or interferon-beta-2 or chemotactic factor or chemokine* or neutrophil-activating-peptide or cxcl8 or Interleukin-8 or IL-8 or (Vascular Endothelial adj2 Growth Factor) or VEGF or cachetin or Tumor Necrosis Factor or TNF* or lymphotoxin or Epidermal growth factor or urogastrone or EGF or insulin-like growth factor or IGF-1 or somatomedin* or insulin-like growth factor binding protein-3 or igf binding protein-3 or IGFBP-3 or Chitinase-3 like-protein-1 or CHI3L1 or ylk 40 protein or cgp-39 protein or gp-39 protein or Angiopoietin 1 or Ang-1 or Angiopoietin 2 or Ang-2 or monocyte chemoattractant protein* or MCP-1 or CD14 or sCD14 or soluble intercellular cell adhesion molecule-1 or ICAM-1 or sICAM-1 or soluble vascular cell adhesion molecule-1 or sVCAM-1 or VCAM-1 or Vascular cellular adhesion molecule or VCAM or endothelin or ET-1 or ET-2 or ET-3 or preproendothelin or proendothelin or endocan-2 or EC-2).mp.
9	Biomarkers/
10	(biomarker* or develop* marker* or neurodevelop* marker or neurocog* marker or cognitive marker or growth marker).mp.
11	7 or 8 or 9 or 10
12	(development* delay* or child developmental deviation or development* disorder* or developmental* disab* or mental deficiencies or retardation or developmental* abnormal* or motor skill* disability or learning disab* or neurocognitive disorder* or communication disorder* or child behavio* disorder* or neurodevelopmental disorder* or language delay* or delay* or speech delay* or Developmental Milestone* or "Bayley Scales of Infant and Toddler Development" or Bayley-III or "Ages and Stages Questionnaire" or Parenting Interactions with Children: Checklist of Observations Linked to Outcomes or Parenting Interactions with Children: Checklist of Observations Linked to Outcomes or Dyadic Parent-Child Interaction Coding System or "Ages and Stages Questionnaire: Social-Emotional" or Baby Pediatric Symptom Checklist or Brief Infant Toddler Social Emotional Assessment or Color object association test or Malawi Developmental Assessment tool or Early Childhood Screening Assessment or Preschool Pediatric Symptom Checklist or Young child PTSD screen or Behavior Assessment System for Children or Devereux Early Childhood Assessment or "Diagnostic Infant and Preschool Assessment" or Infant Toddler Social Emotional Assessment or behavioral assessment or Denver Developmental Screening Test).mp.
13	1 and 6 and 11 and 12

Table 2.5: Scopus Database Search Strategy for Neurodevelopmental Outcomes. Search conducted on April 11th, 2021

Set	Search Statement
1	("human-immunodeficiency" OR "hiv" OR "HIV exposed uninfected" OR "HEU")
2	("c-reactive-protein" OR crp OR "alpha-1 acid glycoprotein" OR seromucoid OR orosomucoid OR AGP OR procalcitonin OR calcitonin 1 OR procalcitonin OR "calcitonin precursor polyprotein" OR "calcitonin related polypeptide alpha" OR PCT OR "Erythrocyte Sedimentation Rate" OR "Interleukin 6" OR "IL 6" OR "beta cell adj2 factor 2" OR "interferon beta 2" OR "chemotactic factor" OR chemokine OR "neutrophil activating peptide" OR cxcl8 OR "Interleukin 8" OR "IL 8" OR "Vascular Endothelial adj2 Growth Factor" OR VEGF OR cachetin OR "Tumor Necrosis Factor" OR tnf OR lymphotoxin OR "Epidermal growth factor" OR urogastrone OR EGF OR "insulin like growth factor" OR "IGF 1" OR somatomedin OR "insulin like growth factor binding protein 3" OR "igf binding protein 3" OR "IGFBP 3" OR "Chitinase 3 like protein 1" OR CHI3L1 OR "ylk 40 protein" OR "cgp 39 protein" OR "gp 39 protein" OR "Angiopoietin 1" OR "Ang 1" OR "Angiopoietin 2" OR "Ang 2" OR "monocyte chemoattractant protein*" OR "MCP 1" OR CD14 OR scd14 OR "soluble intercellular cell adhesion molecule 1" OR "ICAM 1" OR "sICAM 1" OR "soluble vascular cell adhesion molecule 1" OR "sVCAM 1" OR "VCAM 1" OR "Vascular cellular adhesion molecule" OR VCAM OR endothelin OR "ET 1" OR "ET 2" OR "ET 3" OR preproendothelin OR proendothelin OR "endocan 2" OR "EC 2" OR biomarker OR "inflammatory-marker" OR "growth marker" OR "development marker" OR "C-reactive protein" OR "CRP" OR "Blood serotonin" OR "Urine melatonin sulfate excretion" OR "neuron-restrictive silencer factor" OR "NRSF" OR "brain-derived neurotrophic factor" OR "BDNF" OR "Neurofilament light polypeptide" OR "NFL" OR "repressor element 1-silencing transcription" OR "REST" OR "neuron-specific enolase" OR "NSE" OR "glial fibrillary acidic protein" OR "GFAP" OR "myelin basic protein" OR "MBP" OR "S100 calcium-binding protein B" OR "S100B" OR "Monocyte Chemoattractant Protein-1" OR "MCP-1" OR "interleukin-6" OR "IL-6")
3	("development delay" OR "child developmental deviation" OR "development disorder" OR "developmental disab" OR "mental deficiencies" OR "retardation" OR "developmental abnormal" OR "motor skill disability" OR "learning disab" OR "neurocognitive disorder" OR "communication disorder" OR "child behavio disorder" OR "neurodevelopmental disorder" OR "language delay" OR "delay" OR "speech delay" OR "Developmental Milestone" OR "Bayley Scales of Infant and Toddler Development" OR "Bayley-III" OR "Ages and Stages Questionnaire" OR "Parenting Interactions with Children: Checklist of Observations Linked to Outcomes" OR "Parenting Interactions with Children: Checklist of Observations Linked to Outcomes" OR "Dyadic Parent-Child Interaction Coding System" OR "Ages and Stages Questionnaire: Social-Emotional" OR "Baby Pediatric Symptom Checklist" OR "Brief Infant Toddler Social Emotional Assessment" OR "Color object association test" OR "Malawi Developmental Assessment tool" OR "Early Childhood Screening Assessment" OR "Preschool Pediatric Symptom Checklist" OR "Young child PTSD screen" OR "Behavior Assessment System for Children" OR "Devereux Early Childhood Assessment" OR "Diagnostic Infant and Preschool Assessment" OR "Infant Toddler Social Emotional Assessment" OR "behavioral assessment" OR "Denver Developmental Screening Test")
4	(pediatric OR paediatric OR child OR newborn OR congenital OR infan OR baby OR babies OR neonat OR "pre-term" OR preterm OR "premature birth*" OR nicu OR preschool OR "pre-school" OR kindergarten OR "elementary school" OR "nursery school" OR schoolchild OR "school age" OR "grade 1" OR "grade 2" OR "grade 3" OR "grade 4" OR "grade 5" OR "grade 6" OR "grade 7" OR "junior

	high" OR "3 months old" OR "6 months old" OR "12 months old" OR "18 months old" OR "24 months old" OR "30 months old" OR "36 months old" OR "30 months old" OR "1 year old" OR "2 year old" OR "3 year old" OR "4 year old" OR "5 year old" OR "6 year old" OR "7 year old" OR "8 year old" OR "9 year old" OR "10 year old" OR "11 year old" OR "12 year old" OR "13 year old" OR toddler OR boy OR boys OR girl* OR "middle school" OR pubescen OR juvenile OR teen OR youth OR adolesc OR prepubesc OR "pre-pubesc" OR child OR pediatric OR paediatric OR adolescent)
5	1 and 2 and 3 and 4

Table 2.6: PubMed Database Search Strategy for Neurodevelopmental Outcomes. Search conducted on April 11th, 2021

Set	Search Statement
1	("human-immunodeficiency" OR "hiv" OR "HIV exposed uninfected" OR "HEU")
2	("c-reactive-protein" OR crp OR "alpha-1 acid glycoprotein" OR seromucoid OR orosomucoid OR AGP OR procalcitonin OR calcitonin 1 OR procalcitonin OR "calcitonin precursor polyprotein" OR "calcitonin related polypeptide alpha" OR PCT OR "Erythrocyte Sedimentation Rate" OR "Interleukin 6" OR "IL 6" OR "beta cell adj2 factor 2" OR "interferon beta 2" OR "chemotactic factor" OR chemokine OR "neutrophil activating peptide" OR cxcl8 OR "Interleukin 8" OR "IL 8" OR "Vascular Endothelial adj2 Growth Factor" OR VEGF OR cachetin OR "Tumor Necrosis Factor" OR tnf OR lymphotoxin OR "Epidermal growth factor" OR urogastrone OR EGF OR "insulin like growth factor" OR "IGF 1" OR somatomedin OR "insulin like growth factor binding protein 3" OR "igf binding protein 3" OR "IGFBP 3" OR "Chitinase 3 like protein 1" OR CHI3L1 OR "ylk 40 protein" OR "cgp 39 protein" OR "gp 39 protein" OR "Angiopoietin 1" OR "Ang 1" OR "Angiopoietin 2" OR "Ang 2" OR "monocyte chemoattractant protein*" OR "MCP 1" OR CD14 OR scd14 OR "soluble intercellular cell adhesion molecule 1" OR "ICAM 1" OR "sICAM 1" OR "soluble vascular cell adhesion molecule 1" OR "sVCAM 1" OR "VCAM 1" OR "Vascular cellular adhesion molecule" OR VCAM OR endothelin OR "ET 1" OR "ET 2" OR "ET 3" OR preproendothelin OR proendothelin OR "endocan 2" OR "EC 2" OR biomarker OR "inflammatory-marker" OR "growth marker" OR "development marker" OR "C-reactive protein" OR "CRP" OR "Blood serotonin" OR "Urine melatonin sulfate excretion" OR "neuron-restrictive silencer factor" OR "NRSF" OR "brain-derived neurotrophic factor" OR "BDNF" OR "Neurofilament light polypeptide" OR "NFL" OR "repressor element 1-silencing transcription" OR "REST" OR "neuron-specific enolase" OR "NSE" OR "glial fibrillary acidic protein" OR "GFAP" OR "myelin basic protein" OR "MBP" OR "S100 calcium-binding protein B" OR "S100B" OR "Monocyte Chemoattractant Protein-1" OR "MCP-1" OR "interleukin-6" OR "IL-6")
3	("development delay" OR "child developmental deviation" OR "development disorder" OR "developmental disab" OR "mental deficiencies" OR "retardation" OR "developmental abnormal" OR "motor skill disability" OR "learning disab" OR "neurocognitive disorder" OR "communication disorder" OR "child behavio disorder" OR "neurodevelopmental disorder" OR "language delay" OR "delay" OR "speech delay" OR "Developmental Milestone" OR "Bayley Scales of Infant and Toddler Development" OR "Bayley-III" OR "Ages and Stages Questionnaire" OR "Parenting Interactions with Children: Checklist of Observations Linked to Outcomes" OR "Parenting Interactions with Children: Checklist of Observations Linked to Outcomes" OR "Dyadic Parent-Child Interaction Coding System" OR "Ages and Stages Questionnaire: Social-Emotional" OR "Baby Pediatric Symptom Checklist" OR "Brief Infant Toddler Social Emotional Assessment" OR "Color object association test" OR "Malawi Developmental Assessment tool" OR "Early Childhood Screening Assessment" OR "Preschool Pediatric Symptom Checklist" OR "Young child PTSD screen" OR "Behavior Assessment System for Children" OR "Devereux Early Childhood Assessment" OR "Diagnostic Infant and Preschool Assessment" OR "Infant Toddler Social Emotional Assessment" OR "behavioral assessment" OR "Denver Developmental Screening Test")
4	(pediatric OR paediatric OR child OR newborn OR congenital OR infan OR baby OR babies OR neonat OR "pre-term" OR preterm OR "premature birth*" OR nicu OR preschool OR "pre-school" OR kindergarten OR "elementary school" OR "nursery school" OR schoolchild OR "school age" OR "grade 1" OR "grade 2" OR "grade 3" OR "grade 4" OR "grade 5" OR "grade 6" OR "grade 7" OR "junior high" OR "3 months old" OR "6 months old" OR "12 months old" OR "18 months old" OR "24

	months old" OR "30 months old" OR "36 months old" OR "30 months old" OR "1 year old" OR "2 year old" OR "3 year old" OR "4 year old" OR "5 year old" OR "6 year old" OR "7 year old" OR "8 year old" OR "9 year old" OR "10 year old" OR "11 year old" OR "12 year old" OR "13 year old" OR toddler OR boy OR boys OR girl* OR "middle school" OR pubescen OR juvenile OR teen OR youth OR adolesc OR prepubesc OR "pre-pubesc" OR child OR pediatric OR paediatric OR adolescent)

2.3.2 Study Selection

Types of studies: Two independent reviewers (RS, UR) selected the studies: any primary research, sub-studies, experimental studies, prospective and retrospective cohort studies, chart reviews, observational studies, and longitudinal studies with an association between biomarker measurement and growth and/or neurodevelopmental outcomes.

Types of participants: HEUs under 13 years of age; studies with HIV infected and HUU infants were included if the results for HEUs were reported.

Types of predictors: Studies of any biomarker, defined as a biological molecule found in any easily accessible body fluid, which may serve as a measurable indicator of growth and/or neurodevelopmental outcomes were included. The biomarker measurement should ideally be performed prior to the growth or neurodevelopmental outcome; however, studies were included if the biomarker was measured concurrently.

Types of outcome measures: Studies in which growth or neurodevelopmental outcomes were assessed in at least one child. With respect to growth faltering, specific outcomes of interest included: weight, length/height, and head circumference at birth; weight-for-age z-score (WAZ), length/height-for-age z-score (LAZ/HAZ), weight-for-length/height z-score (WLZ/WHZ), and head circumference-for-age z-score (HCAZ); and body mass index (BMI)-for-age z-score.

Exclusion criteria: Studies were excluded if: i) participants had unknown HIV status; ii) the study was a review article, animal study, commentary or editorial; or iii) the study did not report an association between biomarkers and growth and/or neurodevelopmental outcomes.

2.3.3 Data Extraction

Initially, two reviewers (RS, UR) screened the titles and abstracts of the citations retrieved by the electronic search (first screen) on Covidence [404]. Citations that were identified as HEU biomarker studies moved on to review of full text. A third reviewer (MTH) independently reviewed the eligible included studies. Disagreements among reviewers were resolved by consensus.

2.3.4 Quality Appraisal

We used the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool, a validated quality checklist that assesses 4 domains: patient selection, index test, reference standard, and flow and timing. Each study was assessed for risk of bias and applicability by two investigators (MTH, RS). Disagreements were resolved by consensus.

2.4 Results

The included studies examined a variety of biomarkers (Table 2.7).

2.4.1 Systematic review of studies examining association between biomarkers and growth outcomes

2.4.1.1 Study Selection

The screening process for the review is reported in Figure 2.1. The database search and review of the reference lists resulted in seven studies for growth outcomes (Table 2.8).

Table 2.7: Description of biomarkers associated with growth and development.

	Functions
Acute phase reactants	
CRP	C-reactive protein. Acute-phase protein; produced in the liver; rises in response to tissue damage or inflammation [219].
Fibrinogen	Coagulation factor; Acute-phase reactant; inflammatory mediator in bacterial infections; close connections exist between the coagulation cascade and infection/inflammation [405].
Pro-inflammatory cytokines	
TNF	Tumor necrosis factor. Proinflammatory cytokine; secreted by inflammatory cells; plays a role in cell survival, proliferation, differentiation, and death [406].
IFN-γ	Interferon- γ. Proinflammatory cytokine; cytokine activated during inflammatory response; promotes macrophage activation; participates in antiviral and antibacterial immunity [274].
IL-6	Interleukin-6. Proinflammatory cytokine; produced at the site of inflammation; stimulates the production of acute phase proteins in the liver and neutrophils in the bone marrow [407].
IL-12p70	Interleukin-12p70. Proinflammatory cytokine; produced by activated monocytes following antigenic stimulation; promotes growth and function of T cells; induces the production of IFN-γ and TNF from T cells and natural killer cells [408, 409].
IL-1-α	Interleukin-1-α. Proinflammatory cytokine that promotes inflammation and fever [410].
IL-1β	Interleukin-1β. Pro-inflammatory cytokine; crucial for host-defense responses to infection; exacerbates tissue damage during acute and chronic disease; involved in monocyte activation and activation of proinflammatory signaling pathways in peripheral tissues and brain [240].
IL-2	Interleukin-2. Proinflammatory cytokine; produced primarily by recently activated T cells; influences various lymphocyte subsets during differentiation, immune responses and homeostasis [411].
GM-CSF	Granulocyte-macrophage colony-stimulating factor. Proinflammatory cytokine; role in inflammatory and autoimmune reactions and in the response to pulmonary infection [290]; produced by activated T cells, monocytes/macrophages, B cells, natural killer cells, endothelial, epithelial, and fibroblasts; major cytokine in chronic inflammatory and autoimmune diseases [412].
Anti-inflammatory cytokines	
IL-4	Interleukin-4. Anti-inflammatory cytokine; regulator of immunity; secreted primarily by mast cells, Th2 cells, eosinophils, and basophils; key role in type 2 immune responses to helminths and inactivation of toxins [296].
IL-10	Interleukin-10. Anti-inflammatory cytokine; cytokine with potent anti-inflammatory properties; central role in limiting host immune response to pathogens, thereby preventing damage and maintaining homeostasis [304].
Chemokine	
CXCL10/IP-10	Interferon- γ-induced protein. Chemokine; cytokine produced by a wide range of cells such as monocytes, neutrophil, leukocytes and others when triggered by IFN-γ with TNF or lipopolysaccharide; mediates immune responses through activation and recruitment of leukocytes (T cell, eosinophils, monocytes and natural killer cells) [315].

Marker of neutrophil activation	
NGAL	Neutrophil gelatinase-associated lipocalin. Marker of neutrophil activation; expressed in neutrophils; biomarker of kidney injury; role in cardiovascular and renal disease [384].
Marker of monocyte activation	
sCD-14	Soluble CD14. Marker of monocyte activation; nonspecific biomarker of monocyte activation; associated with morbidity and mortality in HIV infection [413].
Marker of tissue remodeling	
MMP-9	Matrix metalloproteinase (MMP)-9. Marker of tissue remodeling; inducible protease; marker of inflammation and fibrosis (e.g., cardiac tissue); abundant MMP in intestinal tissue of patients with inflammatory bowel disease [375].
Growth factors and circulating receptors	
IGF-1	Insulin-like growth factor-1. Growth factors; mediator of growth hormone (GH)-stimulated somatic growth; stimulates systemic growth [352].
IGFBP-1	Insulin-like growth factor binding protein-1. Growth factors; regulates metabolic and vascular homeostasis; regulates IGF signaling; binding to IGFBP-1 prolongs the half-life of the IGFs and alters their interaction with cell surface receptors [366].
IGFBP-3	Insulin-like growth factor binding protein-3. Growth factors; transports IGFs made in the liver through the circulation and in extracellular fluids; regulates metabolic and vascular homeostasis; dependent on growth hormone [349].
Markers of microbial translocation	
LBP	Lipopolysaccharide binding protein. Marker of microbial translocation; acute phase protein produced by the liver; binds lipopolysaccharide (LPS) to trigger immune response during microbial translocation [414].
I-FABP	Intestinal fatty-acid binding protein. Marker of microbial translocation; found in epithelial cells of small intestine tissue; released when intestinal mucosal damage occurs; noninvasive marker of gut wall integrity loss [340].

CRP C-Reactive Protein; TNF tumour necrosis factor; IFN interferon; IL interleukin; GM-CSF Granulocyte-macrophage colony-stimulating factor; CXCL10/IP-10 IFN-γ-induced protein 10; NGAL neutrophil gelatinase-associated lipocalin; sCD14 soluble CD14; MMP9 matrix metalloproteinase-9; IGF; IGFBP insulin-like growth factor binding protein; LBP lipopolysaccharide binding protein; I-FABP intestinal fatty-acid binding protein.

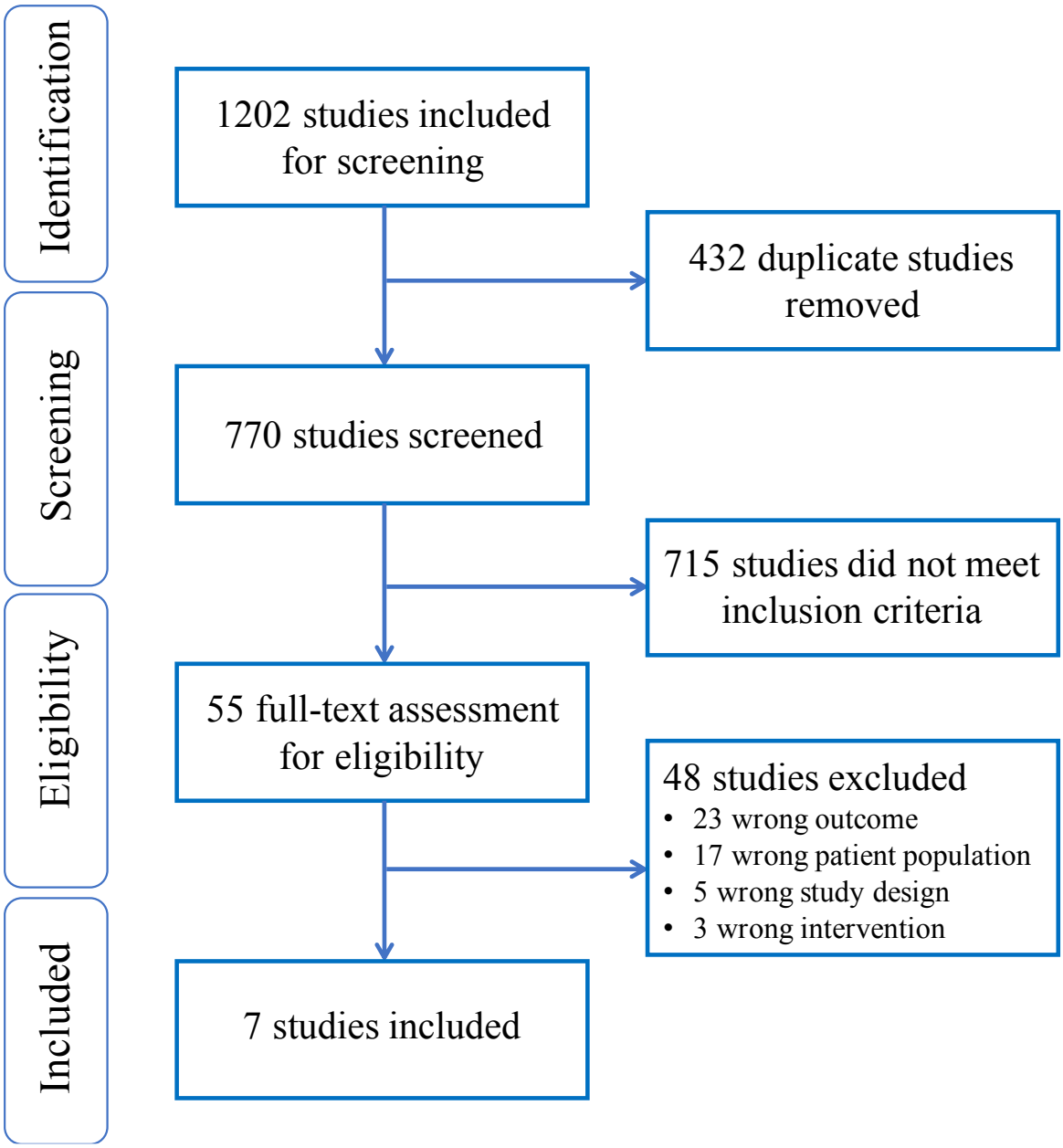

Figure 2.1. Study selection flow diagram for growth outcomes. From four databases, 1202 studies were exported to Covidence software for initial screening. A total of 432 duplicate studies were removed and 770 studies were screened by reading abstracts. From this, 715 studies did not meet the inclusion criteria and 55 studies were assessed in full-text format. About 48 studies were excluded after full text assessment, and the remaining 7 studies were included which examined the association between biomarker levels and growth outcomes.

Table 2.8: Characteristics and finding of included studies examining association between biomarkers and growth outcomes.

Author, Year	Year study was conducted	Study Country	Study Design	HEUs Sample Size	Biomarkers examined	Age at assessment of biomarkers	Growth Parameters	Age at assessment of growth parameters
Chantry et al. 2008 [415]	1994-1998	USA	Nested case-control study from a Prospective, observational, multicenter study	46	IGFBP-3	2.5-7 years	Weight-for-age, length/height-for-age, BMI-for-age	2.5-7 years
Kessler et al. 2013 [401]	1995-2010	USA	Sub-study including retrospective chart review from a cross-sectional retrospective analytical study	12	Serum IL-1 α, IL-6, TNF α, IGFBP-3, IGFBP-1, IGF-1	7 years	Length/height, BMI, Final Adult Height (FAH)	7 years
Nicholson et al. 2015 [117]	2005-2007, follow up 2014	Zambia	Sub-study from a prospective cohort study	111	CRP	6-12 years	Hip circumference, mid-upper-arm circumference, body mass index, thigh circumference, Height-for-age, BMI-for-age	6-12 years
Wilkinson et al. 2017 [134]	2012	Tanzania	Sub-study from a prospective cohort study	56	IFN-γ, IL-12p70, TNF-α, IL-10, IL-15, IL-2 IL-6, IL-13, IL-1β	Birth	Birthweight, birth length/height, head circumference, mid-upper arm circumference	Birth
Baroncelli et al. 2019 [400]	2008-2009	Malawi	Sub-study from a larger observational study	72	LBP, I-FABP, sCD14	1, 6 and 12 months	Length/Height-for-age, weight-for-age, weight-for-length/height, body mass index	1, 6 and 12 months
Dirajlal-Fargo et al. 2019 [126]	2004-2006	Brazil	Sub-study from a larger prospective cohort study	86	sTNF-RI, IL-6, CXCL10/IP-10, sCD14, oxidized LDL, hsCRP	Birth and 6 months	Weight, height, lower birth weight	Birth and 6 months
Evans et al. 2020 [180]	1997-2001	Zimbabwe	Sub-study from ZVITAMBO trial	243	IGF-1, CRP	Birth, 6 weeks, 3 months, 6 months	Length/height-for-age, weight-for-age, weight-for-length/height, head circumference-for-age	Birth, 6 weeks, 3 months, 6 months

2.4.1.2 Study characteristics

Two studies were conducted in a US population; the others were conducted in HIV-endemic LMICs: Zambia, Tanzania, Malawi, Brazil, Zimbabwe, and South Africa. Six [117, 126, 180, 400, 401, 416] of the seven included studies were small to moderate (n= 12 to 243) sub-studies nested within larger observational studies and one [134] was a small (n=56) prospective cohort study. HEU infants were not the focus of the research question for three of the seven studies; in these cases, HEUs were instead included as a comparator group for HUU and HIV infected infants. The primary research question for three of the seven studies was not the predictive value of biomarkers for growth faltering. Instead, multiple predictors (biomarkers, co-infections, environmental, socioeconomic and education level) and outcomes (growth, frequency of hospitalizations, school report cards) were the focus of these studies.

2.4.1.3 Biomarker Identification

The association between biomarkers and growth faltering is reported in Table 2.9.

Table 2.9. Studies investigating an association between circulating biomarkers and growth in HEUs

	Studies finding an association	**Studies finding no association**
Acute phase reactant		
CRP	Elevated hsCRP at birth was associated with lower weight at birth and at 6 mo of age [126].	CRP at 6 wk of age was not associated with LAZ/HAZ, WAZ, WLZ/WHZ, HCAZ at 6wk, 3mo, and 6mo of age[1] [180]. Not associated with HEU anthropometric outcomes such as hip circumference, mid-upper-arm circumference, body mass index, thigh circumference, Length/height-for-age, BMI-for-age [117].
Pro-inflammatory cytokines		
TNF	Higher TNF in cord blood was associated with lower length (β= –1.43, SE=0.66) and head circumference (β= –3.21, SE=1.10) at birth [134].	TNF in cord blood was not associated with birth weight (β= –273, SE=159) [134]. Not significantly correlated with height at mean age of 13 yr [401].
IFN-γ	Higher IFN-γ in cord blood was associated with lower birth weight (β= –482, SE=170) [134].	IFN-γ in cord blood was not associated with length/height (β= –1.13, SE=0.76) and head circumference (β= –0.18, SE=1.43) at birth [134].
IL-6		IL-6 in cord blood was not associated with weight (β= –241, SE=162), length (β= –0.70, SE=0.70) or head circumference (β=0.68, SE=1.28) at birth [134]. Not significantly correlated to length/height at mean age of 13 yr [401].
IL-12p70	Higher IL-12p70 in cord blood was associated with lower weight (β= –723, SE=196) and length (β= –2.40, SE=0.87) at birth [134].	IL-12p70 in cord blood was not associated with head circumference (β= –0.07, SE=1.72) at birth [134].
IL-1-α		Not significantly correlated to length/height at mean age of 13 yr [401].
IL-1β		IL-1β in cord blood was not associated with weight (β= –374, SE=192), length (β= –1.40, SE=0.82) and head circumference (β=0.22, SE=1.55) at birth [134].

Chemokine		
CXCL10/IP-10	Higher CXCL10/IP10 at birth was associated with lower weight at birth and 6 mo of age [126].	
Marker of monocyte activation		
sCD-14		Not associated with wasting or underweight at age 1, 6 and 12 mo [400].
Growth factors and circulating receptors		
IGF-1	Higher IGF-1 at 6 wk of age was associated with: higher LAZ/HAZ at 6 wk (β=1.45 x 10^{-2}) and 6 mo of age (β=1.82 x 10^{-2}); higher WAZ at 6 wk (β=2.53 x 10^{-2}), 3mo (β=2.36 x 10^{-2}), and 6 mo of age (β=2.28 x 10^{-2}); and higher WLZ/WHZ at 6 wk of age (β=1.67 x 10^{-2}) [180].	No association between IGF-1 at 6 wks of age and WLZ/WHZ scores at 3 mo (β=0.49 x 10^{-2}) and 6 mo of age (β=0.87 x 10^{-2}) or HCAZ scores at 6wk (β=0.95 x 10^{-2}), 3mo (β=1.01 x 10^{-2}), and 6 mo of age (β=0.28 x 10^{-2}) [180]. Not significantly correlated with length/height at mean age of 13 yr [401].
IGFBP-1	Inversely correlated with BMI (r=0.299) at mean age of 13 yr [401].	
IGFBP-3		Not significantly correlated with length/height at mean age of 13 yr [401]. Not associated with LAZ/HAZ, WAZ and BMZ between 2.5-7 yr [416].
Markers of microbial translocation		
LBP	Higher LBP was associated with stunting at mean age of 12 mo [400]. Inversely correlated with LAZ/HAZ at mean age of 12 mo (r= –0.347) [400].	
I-FABP		Not different in children with and without wasting and/or underweight at mean age of 12 mo [400].

CRP C-Reactive Protein; TNF tumour necrosis factor; IFN interferon; IL interleukin; CXCL10/IP-10 IFN-γ-induced protein 10; sCD14 soluble CD14; IGF insulin-like growth factor; IGFBP insulin-like growth factor binding protein; LBP lipopolysaccharide binding protein; I-FABP intestinal fatty-acid binding protein; LAZ/HAZ length/height-for-age z-score, WAZ weight-for-age z-score, WLZ/WHZ weight-for-length/height z-score, HCAZ head circumference-for-age z-score; mo month; wk week;
[1]HEU and HIV unexposed infants analysed together

Inflammatory biomarkers were generally associated with growth faltering, though this association was not consistent across all studies. With respect to intrauterine growth faltering, elevated levels of CRP, IFN-γ, IL-12p70, and CXCL10/IP-10, measured at birth, were associated with LBW [126, 180]. However, other inflammatory cytokines were not associated with LBW (TNF, IL-6, and IL-1β) [134]. Higher levels of TNF and IL-12p70 were associated with reduced birth length/height while other inflammatory cytokines (IFN-γ, IL-6 and IL-1β) were not [134]. Increased levels of TNF were associated with lower head circumference while IFN-γ, IL-6, IL-12p70 and IL-1β did not show a statistically significant correlation [134]. With respect to post-natal growth faltering, increased levels of CRP and CXCL10/IP-10 measured at birth were associated with lower weight at 6 months of age [126]. This association did not adjust for birth weight, which was also associated with elevated CRP and CXCL10/IP-10, and may represent an important confounding variable [126]. However, another study did not find an association between CRP measured at 6 weeks of age and WAZ, LAZ/HAZ, WLZ/WHZ, HCAZ, measured at 6 weeks, 3 months and 6 months of age [180]. Marker of monocyte activation (sCD14) was also not associated with WLZ/WHZ and WAZ at 1, 6 and 12 months of age [400].

Growth factor IGF-1 was associated with multiple growth parameters [180]. Specifically, elevated IGF-1 measured at 6 weeks of age was associated with LAZ/HAZ and WAZ at 6 weeks and subsequent time points [180]. At 13 years of age, IGF-1 was not associated with length/height but IGFBP-1 was correlated with reduced BMI (r=0.299) [401]. IGFBP-3 was also not associated with LAZ/HAZ, WAZ and BMZ in HEUs between 2.5-7 years of age and length/height at 13 years of age [401, 416].

With respect to biomarkers of microbial translocation, the level of LBP was inversely correlated with LAZ/HAZ at mean 12 months of (r = –0.347) [400]. On the other hand, I-FABP, an indirect marker of microbial translocation reflecting intestinal injury, was not different between children with and without wasting and/or underweight at mean 12 months of age [400].

2.4.1.4 Study quality

Systematic quality assessment identified methodologic concerns with all included studies (Tables 2.10-2.16). For six of the seven studies which examined a subset of a larger cohort, none described participants selection process (none explicitly mentioned random sampling) [134]. It is likely that a convenience sample based on availability of banked blood specimens was used, introducing the possibility of selection bias. The prospective cohort study did not describe consecutive sampling [134]. For three of the studies, data for HEUs was not reported separately, but was combined with HIV infected and/or HUU infants [134, 180, 401].

There was heterogeneity in the biomarkers tested and the age at which they were analyzed (Table 2.8). CRP and TNF were the only two that were examined in more than one study. For all seven included studies, it was unclear whether the results of biomarkers were interpreted without knowledge of the growth parameters.

There was also heterogeneity in the growth parameters reported, which included length/height, weight, head circumference, BMI and mid-upper arm circumference. One of the seven papers

reported on multiple growth parameters, whereas most studies looked at one growth outcome. It was not clear whether the measurement of growth parameters was performed blinded to the biomarker levels.

The strength of the association between biomarkers and growth was assessed using Pearson's correlation coefficient (two studies) or linear regression models (five studies). Two studies measured biomarkers at an earlier age than the growth outcome and could therefore be used to assess the predictive values of biomarkers. For the other five, a cross-sectional correlation was performed (same age for biomarker and growth measurement).

Table 2.10: QUADAS 2 Analysis of Chantry et al. (2008) Examining Growth Faltering Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST (biomarkers)	REFERENCE STANDARD (growth parameters)	FLOW AND TIMING
Description	This is a nested case-control from a larger prospective observational study examining history of HIV infection in mother and infants along with factors associated with maternal-infant transmission and disease progression (N=788 HIV-infected mothers and 657 HIV-infected infants).[417] The parent study was conducted in USA (1994 to 1998).[417] The sub-study included 46 HEU infants. Infants were included in the sub-study if they had stored samples collected between 2.5-7 years of age (convenience sample).	Biomarker: IGFBP-3 Sample: serum Timing: 2.5-7 years of age	Anthropometric measurements: weight-for-age, height-for-age, and body mass index-for-age Timing: 2.5-7 years of age	The biomarkers and the growth parameters were assessed at the same point between 2.5 to 7 years of age The strength of the association was determined using linear regression.
Signalling questions(yes/no/unclear)	Unclear. Sampling was prospective, but not necessarily consecutive	Unclear. Not stated explicitly that the testing was done blind to growth parameters.	Yes, the reference standard was likely to correctly classify the target condition	No. Biomarkers and growth parameters were measured at the same point in time. Predictive value of biomarkers cannot be assessed.
	No, case-control design was not avoided.	Not applicable. Thresholds were not used to assess correlations.	Yes (likely). Not stated explicitly that the growth parameters were measured blinded to the biomarker levels. However, growth measurements were likely done in a clinical setting with later laboratory assessment of biomarkers.	Yes, all patients received a reference standard
	Yes, inappropriate exclusions were avoided			Yes, all patients received the same reference standard
				Yes, all patients were included in the analysis

Risk of bias: high/ low/ unclear	Low risk of bias from patient selection	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns regarding applicability: high/ low/ unclear	Low. HEU infants and HIV infected infants were analysed separately.	Low. Only one growth hormone (IGFBP-3) was measured. Other markers would be necessary to more completely characterize growth in HEUs.	Low. Study examined height-for-age, weight-for-age, and body mass index-for-age. Other growth parameters such as head circumference, weight-for-height, and mid-upper arm circumference would be useful for characterizing growth.	

Table 2.11: QUADAS 2 Analysis of Wilkinson et al. (2017) Examining Growth Faltering Outcome

DOMAIN	PATIENT SELECTION	INDEX TEST (biomarkers)	REFERENCE STANDARD (growth parameters)	FLOW AND TIMING
Description	This study prospectively recruited pregnant women in semirural and rural Tanzania in 2012. Both HIV-positive (n=44) and HIV-negative (n=70) pregnant women were included. All HIV-exposed infants at 3 months of age were seronegative. Thus, infants in this study were HUU or HEU.	Biomarkers: TNF, IFN-γ, and IL-12p70. In addition, IL-10, IL-15, IL-13, IL-1β, IL-2, IL-6 were measured but did not report the association with growth Sample: cord blood Timing: birth	Anthropometric measurements: weight, length, and head circumference. Timing: birth	The biomarkers and the growth parameters were assessed at the same point in time (birth). The strength of the association was determined using linear regression, adjusting for infant sex.
Signalling questions(yes/no/unclear)	Unclear. Sampling was prospective, but not necessarily consecutive.	Unclear. Not stated explicitly that the testing was done blind to growth parameters.	Yes, the reference standard was likely to correctly classify the target condition	No. Biomarkers and growth parameters were measured at the same point in time. Predictive value of biomarkers cannot be assessed.
	Yes, case-control design was avoided	No. For abundant cytokines (TNF, IFN-γ, IL-12p70, IL-15 and IL-10), analytes were dichotomised based on the quartile distribution of the data into 'Higher' (highest quartile) or 'Lower' (lowest three quartiles combined). For low-abundance cytokines (IL-13, IL-1β, IL-2, IL-6), values were classified as either 'Higher' inf the analyte was above the lower limit of detection (LOD) or 'Lower' if the analyte was below the (LOD). These thresholds	Yes (likely). Not stated explicitly that the growth parameters were measured blinded to the biomarker levels. However, growth measurements were likely done in a clinical setting with later laboratory assessment of biomarkers.	Yes, all patients received a reference standard
	Yes, inappropriate exclusions were avoided			Yes, all patients received the same reference standard
				Yes, all patients were included in the analysis

		were determined *post hoc,* based on observed data.		
Risk of bias: high/ low/ unclear	Low risk of bias from patient selection	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns regarding applicability: high/ low/ unclear	High. HEU infants and HUU infants were not analysed separately; therefore, associations between cytokines and growth measurements at birth are pooled together.	Low. Only proinflammatory cytokines (TNF, IFN-γ, and IL-12p70) were measured. Other markers would be necessary to more completely characterize inflammation in cord blood.	Low. However, anthropometric measurements were obtained at birth only, not at later ages. Thus, this study is relevant for the association between inflammation and fetal growth, but not post-natal growth.	

Table 2.12: QUADAS 2 Analysis of Kessler et al. 2013 Examining Growth Faltering Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Description	Retrospective chart review from pediatric infectious diseases clinic, banked samples collected during routine clinic visits. Inner-city population in USA (1995 to 2010). The study included 34 HIV infected adolescents and 12 HEU (age matched siblings). Correlations between biomarkers and growth were reported in aggregate (HIV infected and HEUs pooled). Patients were excluded for endocrine abnormality or chronic disease.	Biomarkers: IL-1 α, IL-6, TNF α, IGFBP-3 and IGF-1 Sample: serum Timing: biomarkers were assayed from stored samples that were obtained at the child's peak growth velocity	Growth parameters were assessed longitudinally (retrospective chart review). Starting at age 7, height, weight, and BMI were recorded. Final Adult Height (FAH) was also recorded. Relevant data for the present analysis was reported only for height and BMI.	The biomarkers and the growth parameters were assessed at the same point in time (when the child reached peak growth velocity). Correlation between the biomarker levels and the growth parameters was assessed by Pearson's correlation coefficient. The correlation was cross-sectional (same age for assessment of biomarker and growth parameter).
Signalling questions (yes /no /unclear)	Unclear. Patient selection was not described, presumed convenience sample.	Unclear. Biomarkers were assayed from stored samples (after growth measurements were obtained). Blinding was not described.	Yes, reference standard likely to correctly classify the target condition	No. Biomarkers and growth parameters were measured at the same point in time (height, weight, BMI). Predictive value of biomarkers cannot be assessed for these growth parameters. For FAH, there was an appropriate interval between biomarker measurement (at the age of peak growth velocity) and growth parameter (FAH).
	Yes, case-control design was avoided		Yes (likely). Not explicitly stated but the growth parameters	Yes, all patients received a reference standard

	Yes. Patients were excluded if: growth hormone deficiency, non-compensated thyroid disorder, cerebral palsy, glucocorticoid treatment for >5 days, chronic diseases such as diabetes mellitus, cerebral palsy or non-ambulating patients. These are appropriate exclusions.	Not applicable. Thresholds were not used to assess correlations.	was measured during clinic visits, then biomarkers were analyzed from banked samples at a later time.	Yes, all patients received the same reference standard Yes, all patients were included in the analysis
Risk of bias: high/ low/ unclear	Unclear. The selection of study participants from among clinic patients for retrospective analysis was not reported.	Low risk of bias from index test	Low risk of bias from reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns Regarding applicability: high/ low/ unclear	High. Correlations between biomarkers and growth were reported in aggregate (HIV infected and HEUs pooled).	Low. Only three proinflammatory cytokines (IL-1 α, IL-6, TNF α) and two growth (IGF-1 and IGFBP-3) biomarkers were measured. Other markers would be necessary to more completely characterize inflammation.	Low. Study examined height and BMI. Other growth parameters such as head circumference, mid-upper arm circumference would be useful for characterizing growth.	

Table 2.13: QUADAS 2 Analysis of Baroncelli et al. 2019 Examining Growth Faltering Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Description	This is a sub-study from a larger observational study examining effect of cART during breastfeeding (N=311 HIV-infected mothers and 293 HEUs).[418] The parent study was conducted in Malawi (2008 to 2009).[418] The sub-study included 72 HEU infants. Infants were included in the sub-study if they had stored samples collected at 1 and 12 months of life (convenience sample).	Biomarkers sCD14, LBP and I-FABP Sample: Plasma Timing: 1 and 12 months of age	Anthropometric measurements: height-for-age, weight-for-age, weight-for-height, and body mass index-for-age Time: 1, 6 and 12 months of age.	The biomarkers and the growth parameters were assessed at the same point in time. To determine the strength of association, Pearson's correlation coefficient was used. In addition, biomarker levels were compared between HEUs with and without stunting.
Signalling questions (yes /no / unclear)	No. Participants in this study were a convenience sample of a larger observational study (based on sample availability).	Unclear. The growth parameter was likely known before the plasma sample was analyzed.	Yes, reference standard likely to correctly classify the target condition	No. Biomarkers and growth parameters were measured at the same time points (cross-sectional correlation). Predictive values of biomarkers cannot be assessed.
	Yes, case-control design was avoided	Not applicable. Biomarker thresholds were not used to determine the correlation.	Yes. The growth data were collected during the observational study and biomarkers were analyzed subsequently.	Yes, all patients received a reference standard
	Yes, inappropriate exclusions were avoided			Yes, all patients received the same reference standard
				Yes, all patients were included in the analysis

Risk of bias: high/ low/ unclear	Unclear. Convenience sampling based on sample availability could introduce bias.	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns Regarding applicability: high/ low/ unclear	Low. Study included HEU data only.	Low. Two microbial translocation (LBP and I-FABP) and one monocyte activation (sCD14) biomarkers were measures. Other markers would be necessary to more completely characterize inflammation.	Low. Study examined height-for-age, weight-for-age, weight-for-height, and body mass index-for-age. Other growth parameters such as head circumference, mid-upper arm circumference would be useful for characterizing growth.	

Table 2.14: QUADAS 2 Analysis of Dirajlal-Fargo 2019 Examining Growth Faltering Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Description	This is a sub study from larger prospective cohort studies (NISDI and LILAC) (n=1508).[419-421] The sub study included 86 HEU mother-infant pairs as well as HUUs. For the purpose of the current analysis, we included only data from HEUs. HEUs were included if they were product of a singleton pregnancy, had plasma sample from birth and 6 months of age, maternal plasma samples from close to delivery, birthweight>2500g, and GA>37 wk. Patients were excluded for cardiovascular disease, pulmonary disease, or birth defect.	Biomarkers: IP-10 and hsCRP. In addition, IL-6, sTNFR-I and II, sCD14, sCD163, VCAM, oxidized LDL, and D-Dimer were measured but the association with growth was not reported. Sample: Plasma Time: birth and 6 months of age.	Anthropometric measure: weight Time: birth and 6 months of age.	The biomarkers and the growth parameters were assessed at the same point in time. In addition, biomarkers measured at birth were examined as predictors of growth at 6 months of age. Linear regression models were used to examine the effect of biomarkers on infant growth parameters.
Signalling questions (yes /no /unclear)	Unclear. Patient selection from the larger parent study was not described, presumed convenience sample.	Unclear. The growth parameters were measured before the biomarkers were analyzed. Blinding was not described.	Yes, reference standard likely to correctly classify the target condition	Yes. Biomarkers measured at birth were examined as potential predictors of weight at 6 months. Predictive value of biomarkers was assessed.
	Yes, case-control design was avoided	Not applicable. There was no threshold used.	Yes (likely). The growth measurements were already recorded in the main study and biomarkers were	Yes, all patients received a reference standard
				Yes, all patients received the same reference standard

	Yes. The exclusion of preterm and low birth weight infants means that results are not generalizable to preterm and low birth weight infants.		analyzed after being selected for this sub study.	Yes, all patients were included in the analysis
Risk of bias: high/ low/ unclear	Unclear. Convenience sampling based on sample availability could introduce bias.	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns Regarding applicability: high/ low/ unclear	Low. Study included HEU data only.	Low. One acute phase reactant (hsCRP) and one chemokine (IP-10) were measured. Other proinflammatory markers would be necessary to characterize inflammation.	Low. Study examined weight only. Other growth parameters such as height, head circumference and mid-upper arm circumference would be useful to characterize growth.	

Table 2.15: QUADAS 2 Analysis of Evans et al. 2020 Examining Growth Faltering Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Description	This is a sub study from the ZVITAMBO trial (n=14,110, Zimbabwe) that included HEU infants. The main study tested the effect of single-large-dose maternal/neonatal vitamin A supplementation on VT, HIV-free survival, and mortality in HIV-exposed infants.[422] For the sub study, HEUs (n=243) and HUUs (n=100) were included. HEUs were included if the mother and infant survived up to 6 months of age, had growth measurements available, and had IGF measurement from 6 weeks of age. HEUs and HUU were assessed together, however, where there was a possible interaction with HIV exposure (P<0.2), results were stratified by maternal HIV exposure (HEUs and HUUs could be assessed separately).	Biomarkers: IGF-1, CRP Sample: Plasma Time: 6 weeks of age.	Anthropometric data: weight, length, and head circumference Time: birth, 6 weeks, 3 months, and 6 months of age	The biomarkers were assessed at 6 weeks, while growth was measured at multiple time points, including birth, 6 weeks, 3 months and 6 months of age. The strength of association between biomarkers and growth was determined using univariable linear and logistic regression.
Signalling questions (yes / no/ unclear)	No. The 243 included HEUs were selected based on sample availability.	Unclear. The growth parameters were measured before the biomarkers were analyzed. Blinding was not described.	Yes, reference standard likely to correctly classify the target condition	Yes. Biomarkers measured at 6 weeks, while growth measurements are available from 3 months and 6 months of age. Thus, this study can establish whether the biomarkers have predictive value for growth outcomes.
	Yes, case-control design was avoided	Not applicable. No threshold was used.	Unclear. The growth measurements extend to 6 months, where the biomarker data was already available. Blinding was not described.	Yes, all patients received a reference standard
	Yes, inappropriate exclusions were avoided			Yes, all patients received the same reference standard

				Yes, all patients were included in the analysis
Risk of bias: high/ low/ unclear	Low risk of bias from patient selection	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns Regarding applicability: high/ low/ unclear	High. The association between biomarker and growth among HEU infants was reported together with HUUs. Separate analysis among HEUs was available only if impact of HIV exposure was detected.	Low. One acute phase reactant (CRP) and one growth factor (IGF-1) were measured. Other markers would be necessary to more completely characterize systemic inflammation.	Low. Study examined height, weight and head circumference. Growth parameters such mid-upper arm circumference would be useful to characterize growth.	

Table 2.16: QUADAS 2 Analysis of Nicholson et al. 2015 Examining Growth Faltering Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Description	This is a prospective cohort study that included children born both to HIV infected and uninfected mothers from a Breastfeeding and Postpartum Health (BFPH) longitudinal cohort study (Zambia, 2001-4). The initial study recruited children at 6 months and follow up conducted in 2014. At follow-up, 111 HEU and 279 HUU children aged 6-12 years of age were included in the study. The current analysis focused only on the HEUs.	Biomarker CRP Sample: Plasma Time: children aged 6-12 years old	Anthropometry data: Height-for-age, BMI-for-age, mid-upper-arm circumference, hip circumference, thigh circumference Time: children aged 6-12 years old	The biomarkers and the growth parameters were assessed at the same time. For statistical analysis, the primary analyses used linear regression to compare CRP and anthropometry data among HEUs.
Signalling questions (yes /no /unclear)	Unclear. Parents were called in for check-up, HIV status was determined during the appointment. Information for school reports and blood sample was collected during this appointment. Likely a sample of convenience.	Unclear. The growth parameters were likely measured before the biomarkers were analyzed. Blinding was not described.	Yes, reference standard likely to correctly classify the target condition	Was there an appropriate interval between index test(s) and reference standard? No. The biomarkers and growth measurements were taken at the same time between 6-12 years of age. Predictive value of biomarkers cannot be assessed.
	Yes, case-control design was avoided	Not applicable. A threshold was not used.	Yes, likely. The growth measurements were taken before the biomarkers were analyzed.	Yes, all patients received a reference standard
	Yes, inappropriate exclusions were avoided			Yes, all patients received the same reference standard
				Yes, all patients were included in the analysis

Risk of bias: high/ low/ unclear	Unclear. Convenience sampling based on patient availability for follow-up could introduce bias.	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns Regarding applicability: high/ low/ unclear	Low. HEU and HUU were analyzed separately.	Low. One acute phase reactant (CRP) was measured. Other proinflammatory and growth biomarkers would be necessary to more completely characterize inflammation	Low. A comprehensive list of growth parameters was used.	

2.4.2 Systematic review of studies examining association between biomarkers and neurodevelopmental outcomes

2.4.2.1 Study Selection

The screening process for the review is reported in Figure 2.2 (neurodevelopmental outcomes). The database search and review of the reference lists resulted in two studies for neurodevelopmental outcomes (Table 2.17).

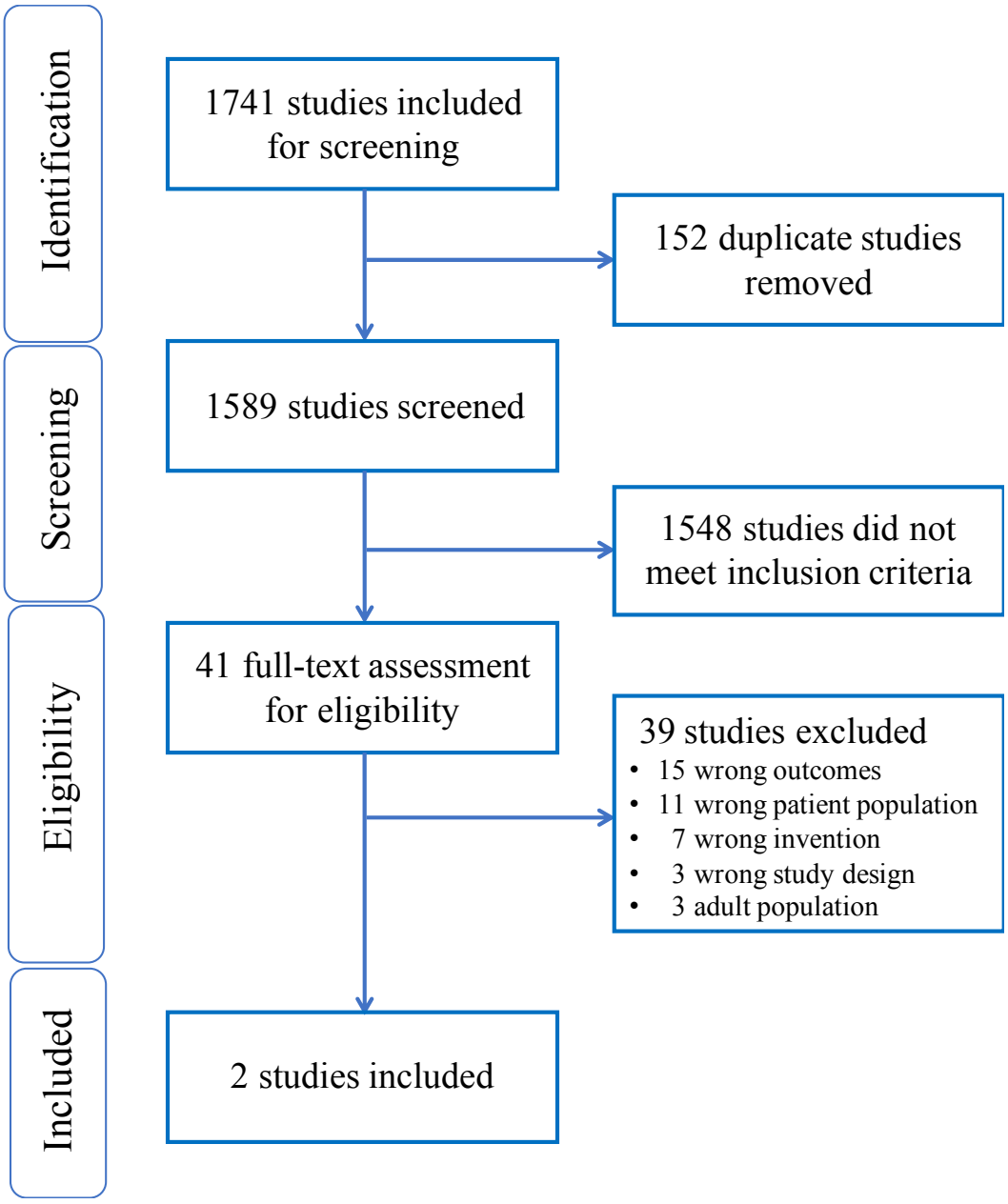

Figure 2.2. Study Selection flow diagram for developmental outcomes. From four databases, 1741 studies were exported to Covidence software for initial screening. A total of 152 duplicate studies were removed and 1589 studies were screened by reading abstracts. From this, 1548 studies did not meet the inclusion criteria and 41 studies were assessed in full-text format. About 39 studies were excluded after full text assessment, and the remaining 2 studies were included which examined the association between biomarker levels and developmental data.

Table 2.17: Characteristics and finding of included studies examining association between biomarkers and developmental outcomes.

Author, Year	Year the study was conducted	Study Country	Study Design	HEUs Sample Size (n=)	Biomarkers tested	Age at biomarker measurement	Developmental Tool	Age at development test
Sevenoaks et al. 2021 [423]	2009	South Africa	Sub-study of a population-based birth cohort study	63	GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL- 7, IL-8, IL-10, IL-12p70, IL-13, NGAL, MMP-9	6-10 weeks	Bayley Scales of Infant and Toddler Development, third edition (BSID-III)[1]	24-28 months
Kapetanovic et al. 2014 [424]	2007-2009	USA	Sub-study from a larger cross-sectional cohort study	130	CRP, fibrinogen, IL-6, sVCAM-1, sE-selectin, sMCP-1, sICAM-1, sP-selectin, adiponectin	mean 10.3 years (SD 2.39)	Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV), generates a Full-Scale IQ (FSIQ)[2]	mean 10.3 years (SD 2.39)

[1]cognitive, language and motor development

[2]Assesses general intellectual ability and provides five primary index scores: Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and Processing Speed Index.

2.4.2.2 Study characteristics

One study was conducted in the US and one in South Africa. Both of these studies were sub-studies nested within a larger study. Sample size was modest (n=77 and 130, respectively). The two papers used the neurodevelopmental tools Bayley Scales of Infant and Toddler Development, third edition (BSID-III), and Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV). The BSID-III is comprehensive (cognitive, language, and motor), but social development was not captured [423]. The WISC-IV captures verbal comprehension, visual-spatial, fluid reasoning, working memory, and processing speed, while motor and social development were not assessed [424].

2.4.2.3 Biomarker Identification

Biomarker levels and neurodevelopmental outcomes were studied at 6-10 weeks, 24-28 months and 11.4 years of age. The association of biomarkers with neurodevelopment are reported in Table 2.18.

Table 2.18. Studies investigating an association between circulating biomarkers and neurodevelopment in HEUs

	Studies finding an association	**Studies finding no association**
Acute phase reactants		
CRP	Inversely correlated with processing speed at 11 years of age [424].	Not correlated with full scale IQ, verbal comprehension, perceptual reasoning, or working memory at 11 years of age [424].
Fibrinogen	Inversely correlated with processing speed at 11 years of age [424].	Not correlated with full scale IQ, verbal comprehension, perceptual reasoning, or working memory at 11 years of age [424].
Pro-inflammatory cytokines		
TNF		Not correlated at 6-10 wk age with cognitive (r= –0.178), language (r= –0.152) or motor (r= –0.071) development scale at 24-28 mo of age [423]. Not correlated at 24-28mo age with cognitive (r= –0.054), language (r= –0.083) or motor (r= –0.027) development scale at 24-28 mo of age [423].
IFN-γ	Inversely correlated at 6-10 wk of age with motor development scale (r= –0.339) at 24-28 mo of age [423]. Inversely correlated at 24-28 mo of age with language development scale (r= –0.307) at 24-28 mo of age [423].	Not correlated at 6-10wk with cognitive (r=-0.178) and language scale (r=-0.106) of BSID-III at 24-28 mo[423] Not correlated at 24-28mo age with cognitive (r= –0.191) or motor scale (r= –0.073) of BSID-III at 24-28 mo [423].
IL-6	Inversely correlated at 6-10 wk age with motor development scale (r= –0.335) at 24-28 mo [423]. Inverse correlation with processing speed [424].	Not correlated at 6-10wk age with cognitive (r=-0.181) or language scales (r= –0.165) of BSID-III at 24-28 mo [423]. Not correlated at 24-28mo age with cognitive (r=-0.106), language (r= –0.012) and motor scales (r= –0.091) of BSID-III at 24-28 mo [423].
IL-12p70	Inversely correlated at 6-10 wk age with motor development scale (r= –0.379) at 24-28 mo of age [423]. Inversely correlated at 24-28 wk age with language development scale (r= –0.284) at 24-28 mo of age [423].	Not correlated at 6-10 wk age with cognitive (r= –0.089) and language scale (r= –0.055) of BSID-III at 24-28 mo [423].

		Not correlated at 24-28 wk age with cognitive (r= –0.166) and motor scale (r= –0.143) of BSID-III at 24-28 mo [423].
IL-1β	Inversely correlated at 6-10 wk of age with motor development scale (r= –0.491) at 24-28 mo of age [423]. Inversely correlated at 24-28 wk age with language scale (r= –0.241) at 24-28 mo [423].	Not correlated at 6-10 wk age with cognitive (r=-0.207) and language scale (r= –0.199) of BSID-III at 24-28 mo [423]. Not correlated at 24-28 wk age with cognitive (r= –0.148) and motor scale (r= –0.124) of BSID-III at 24-28 mo [423].
IL-2	Inversely correlated at 6-10 wk of age with motor development scale (r= –0.308) at 24-28 mo of age [423]. Inversely correlated at 24-28 wk age with language scale (r= –0.253) of BSID-III at 24-28 mo [423].	Not correlated at 6-10 wk age with cognitive (r=-0.126) and language scale (r=0.007) of BSID-III at 24-28 mo [423]. Not correlated at 24-28 wk age with cognitive (r=-0.148) and motor scale (r= –0.011) of BSID-III at 24-28 mo [423].
GM-CSF	Inversely correlated at 6-10 wk age with motor development scale (r= –0.309) of BSID-III at 24-28 mo [423].	Not correlated at 6-10wk with cognitive (r=0.005) or language scales (r=0.081) of BSID-III at 24-28 mo[423] Not correlated at 24-28mo age with cognitive (r=-0.032), language (r= –0.234) and motor (r= –0.050) scale of BSID-III at 24-28 mo [423].
Anti-inflammatory cytokines		
IL-4	Inversely correlated at 6-10 wk age with motor scale (r= –0.418) of BSID-III at 24-28 mo [423].	Not correlated at 6-10wk with cognitive (r= –0.164) or language scales (r= –0.182) of BSID-III at 24-28 mo [423]. Not correlated at 24-28mo age with cognitive (r= –0.118), language (r= –0.217) and motor scales (r= –0.154) of BSID-III at 24-28 mo [423].
IL-10	Inversely correlated at 6-10 wk age with motor scale (r= –0.451) of BSID-III at 24-28 mo [423]. Inversely correlated at 24-28 mo age with cognitive (r= –0.303) and language scale (r= –0.319) of BSID-III at 24-28 mo [423].	Not correlated at 6-10 wk with cognitive (r=-0.136) and language scales (r= –0.116) of BSID-III at 24-28 mo [423]. Not correlated at 24-28 age with motor scales (r= –0.215) of BSID-III at 24-28 mo [423].

Marker of neutrophil activation		
NGAL	Inversely correlated at 6-10 wk age with motor (r= –0.383) scale of BSID-III at 24-28 mo [423].	Not correlated at 6-10 wk age with cognitive (r= –0.153) or language (r= –0.169) scales of BSID-III at 24-28 mo [423]. Not correlated at 24-28 mo age with cognitive (r=0.207), language (r=0.159) and motor (r=0.112) scales of BSID-III at 24-28 mo [423].
Marker of tissue remodeling		
MMP-9	Inversely correlated at 6-10 wk age with motor (r= –0.289) and language (r= –0.305) scale of BSID-III at 24-28 mo [423].	Not correlated at 6-10 wk age with cognitive (r= –0.186) scales of BSID-III at 24-28 mo [423]. Not correlated at 24-28 mo age with cognitive (r= –0.007), language (r= –0.001) and motor (r=0.023) scales of BSID-III at 24-28 mo [423].
Adipokine		
Adiponectin		Not associated with full scale IQ score, verbal comprehension, perceptual reasoning, processing speed, working memory from the WISC-IV at 11.4 years of age [424].
Cell Adhesion Molecules		
sVCAM-1		Not associated with full scale IQ score, verbal comprehension, perceptual reasoning, processing speed, working memory from the WISC-IV at 11.4 years of age [424].
sE-selectin		Not associated with full scale IQ score, verbal comprehension, perceptual reasoning, processing speed, working memory from the WISC-IV at 11.4 years of age [424].
sMCP-1		Not associated with full scale IQ score, verbal comprehension, perceptual reasoning, processing speed, working memory from the WISC-IV at 11.4 years of age [424].
sICAM-1		Not associated with full scale IQ score, verbal comprehension, perceptual reasoning, processing speed,

		working memory from the WISC-IV at 11.4 years of age [424].
sP-selectin		Not associated with full scale IQ score, verbal comprehension, perceptual reasoning, processing speed, working memory from the WISC-IV at 11.4 years of age [424].

CRP C-reactive protein; TNF tumour necrosis factor; IFN interferon; IL interleukin; GM-CSF Granulocyte-macrophage colony-stimulating factor; NGAL neutrophil gelatinase-associated lipocalin; MMP9 matrix metalloproteinase-9; sVCAM soluble vascular adhesion molecule; sE-selectin soluble E-selectin; sMCP monocyte chemoattractant protein; sICAM soluble intercellular adhesion molecule; sP-selectin soluble P-selectin; BSID-III Bailey Scales of Infant Development-3rd edition; WISC-IV Wechsler Intelligence Scales for Children—4th Edition; mo month; wk week; IQ intelligence quotient

Increased inflammatory markers were generally associated with poor neurocognitive performance. CRP, fibrinogen and IL-6 were inversely correlated with processing speed [424]. Biomarkers IFN-γ, IL-6, IL-12p70, IL-1β, IL-2, GM-CSF, IL-4, IL-10, NGAL, MMP-9 measured at 6-10 weeks were inversely correlated with motor scores at 24-28 months of age (r = –0.289 to –0.491) [423, 425]. Biomarkers IFN-γ, IL-12p70, IL-1β, IL-2, IL-10, MMP-9, measured at 24-28 months of age were inversely correlated with language scores (r = –0.241 to –0.307) [423]. IL-10 measured at 24-28 months was inversely correlated with the cognitive scale of BSID-III at 24-28 months of age (r = –0.303) [423]. However, correlations were not statistically significant for numerous biomarkers and neurocognitive outcomes (Table 2.18).

2.4.2.4 Study Quality

Systematic quality assessment identified methodologic concerns with both included papers (Tables 2.19-2.20). Although these were nested sub-studies, the patient selection criteria from the parent study were not described (none explicitly mentioned random sampling). One of the studies excluded HEUs with CRP levels >10mg/L in the main analysis.

Both studies had heterogeneity in the biomarkers that were analyzed. For nine biomarkers, an association with neurodevelopment was not reported, leaving a subset of the fourteen biomarkers that could be assessed. Blinding to the neurodevelopmental assessments was not described in the papers.

One study measured biomarkers at an earlier age than the neurodevelopment outcome and could therefore be used to assess the predictive values of biomarkers. For the second study, a cross-sectional correlation was performed (same age for biomarker and neurodevelopment measurement). Regression models were used to determine the strength of the association between biomarkers and neurodevelopment. For one study, not all patients were accounted for in the analysis.

Table 2.19: QUADAS 2 Analysis of Sevenoaks et al. 2021 Examining Neurodevelopmental Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Description	This study was a sub-study of a population-based birth cohort study (n=1000, South Africa) [426, 427] 267 mother-child pairs from the parent study were included, 190 HIV negative and 77 HIV-positive mothers with 77 HEU infants (no cases of vertical transmission). In this study, we focus on the 77 HEUs.	Biomarkers: NGAL, MMP-9, GM-CSF, INF-γ, IL-1β, IL-2, IL-5, IL-6, IL-7, IL-8 and TNF-α, IL-4, IL-10, IL-12p70 and IL-13 Sample: Plasma Time: 6-10 weeks and 24-28 months of age	Neurodevelopment assessment: Bayley Scales of Infant and Toddler Development, third edition (BSID-III). Time: 24-28 months of age	At 6-10 weeks of age, biomarkers were reported for 63/77 (82%) of the HEUs. Reason for the missing data was not reported. All 77 HEUs were included at 24-28 months. Pearson's correlations were used to explore the associations between biomarkers at 6-10 weeks and 24-28 months of age with neurodevelopment measures at 24–28 months of age. Then, multivariable regression models were used on significant findings with the subsequent biomarkers as predictors and neurodevelopment measure as outcome.
Signalling questions (yes/no/ unclear)	Unclear. The authors report that a random sample of the parent study was included.	Unclear. Blinding was not described.	Yes. The BSID-III is a well-accepted developmental assessment tool.	Yes. The biomarkers measured at 6-10 weeks were examined for their predictive value of neurodevelopment at 24-28 months. Secondly, biomarkers measured 24-28 months were examined for their association with neurodevelopment at 24-28 months (same age). Predictive value of biomarkers cannot be assessed for this analysis.
	Yes. Although the authors sought to compare HEUs and HUUs in a case-control study, the present analysis was restricted to HEUs (correlation between biomarkers and neurodevelopment).	Not applicable. A threshold was not used.	Yes, likely. BSID-III testing was blind to HIV status and likely blind to biomarker levels.	Yes, all patients received a reference standard

	Unclear. The total number of infants (n=63) at 6-10 weeks of age differs from the HIV infected mothers (n=77) and infants included at 24-28 months of age (n=77), without explanation for the discrepancy.			Yes, all patients received the same reference standard No. The number of infants tested for biomarkers at 6-10 weeks is 63, while the number at 24-28 months who underwent neurodevelopmental testing is 77.
Risk of bias: High/low/ unclear	Low risk of bias from patient selection	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns Regarding applicability: High/low/ unclear	Low risk that patients do not match the review question	Low. A comprehensive list of inflammatory markers was tested, as well as MMP-9, a marker of tissue remodeling. Growth factors were not assessed.	Low. The BSID-III is comprehensive (cognitive, language, and motor). Social development is not captured.	

Table 2.20: QUADAS 2 Analysis of Kapetanovic et al. 2014 Examining Neurodevelopmental Outcome.

DOMAIN	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Description	This was a cross-sectional design within a longitudinal, prospective 15-site US-based cohort study conducted between March 2007 to November 2009. The study included HIV-positive (n=212) and HEU infants (n=130) who were between 7 to 16 years of age. The present analysis focused only on the HEUs.	Biomarkers: adiponectin, CRP, fibrinogen, IL-6, sVCAM-1, sE-selectin, sMCP-1, sICAM-1, and sP-selectin. Sample: Plasma Time: mean age 11.4 years	Neurodevelopmental tool: Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) with Full-Scale IQ Score (FSIQ). Time: mean age 11.4 years	The biomarkers and the growth parameters were assessed within 6 months of each other (11.4 years is mean age). Linear regression model was used to determine the association between biomarker and neurodevelopmental outcomes.
Signalling questions(yes/no/unclear)	No. Patients from the parent study were included only if they had a WISC-IV test and biomarker blood level taken less than 6 months apart. Likely a sample of convenience.	Unclear. The neurodevelopmental assessment and the biomarkers were conducted within 6 months of each other, but blinding was not described.	Yes. The WISC-IV is a well-accepted test.	No. The biomarkers and neurodevelopment were measured around the same age. Predictive value of biomarkers cannot be assessed.
	Yes, case-control design was avoided	Not applicable. No threshold was used.	Unclear. The neurodevelopmental assessment and the biomarkers were conducted within 6 months of each other, but blinding was not described.	Yes, all patients received a reference standard
	Yes. However, patients were excluded if CRP >10mg/L since these patients may have acute infection.			Yes, all patients received the same reference standard
				Yes, all patients were included in the analysis

Risk of bias: High/ low/ unclear	Low. However, patients with high CRP were systematically excluded.	Low risk of bias from the index test	Low risk of bias from the reference standard, its conduct, or its interpretation	Low risk of bias from patient flow
Concerns Regarding applicability: High/ low/ unclear	Low risk that patients do not match the review question	Low. A selection of inflammatory and endothelial activation markers was tested. On the other hand, growth factors were not assessed.	Low. The WISC-IV captures verbal comprehension, visual-spatial, fluid reasoning, working memory, and processing speed. On the other hand, motor and social development are not captured.	

2.5 Discussion

Our systematic review identified several biomarkers that are associated with growth faltering and neurodevelopmental outcomes among HEUs at different time points. Intrauterine growth was impacted by biomarkers of inflammation (TNF, IFN-γ, CRP, CXCL10/IP-10, and IL-12p70). Postnatally, biomarkers (CRP, fibrinogen IFN-y, IL-6, IL-12p70, IL-1β, IL-2, GM-CSF, IL-4, IL-10, MMP9, NGAL) showed association with growth and/or neurodevelopmental outcomes.

Low-levels of inflammation may arise following exposure to HIV, even in the absence of vertical infection [428]. The level of inflammation in HEUs is lower in comparison to patients with acute infections such as malaria [218, 429, 430] or sepsis [431]. For example, mean CRP levels are typically 42.0 to 44.5 mg/L in children with malaria [432], 18.5 to 105.8 mg/L in children with sepsis [433], and 0.43 to 1.49 mg/L in HEUs [117, 125, 181]. Levels of inflammation are also lower among HEUs than children chronically infected with HIV [124, 125]. On the other hand, some studies demonstrate a modest level of inflammation in HEUs, relative to HUUs [125, 181]. In one study, CRP concentrations in HEUs (0.43 mg/L) were higher than HUU (0.20mg/L) at 6 weeks of age [181]. CRP levels were comparable in HEUs and HUUs in some other studies [117, 122]. Low-level inflammation was associated with adverse growth and neurodevelopmental outcomes in our systematic review, albeit inconsistently across studies.

Prenatal inflammation is associated with intrauterine growth restriction [434]. In our study, an acute phase reactant (CRP), pro-inflammatory cytokines (TNF, IFN-γ, IL-12p70) and a chemokine (CXCL10/IP-10) increases were associated with LBW, length/height and head circumference (Table 2.9). In a previous report of newborns with intrauterine growth restriction,

poor growth was associated with high concentrations of pro-inflammatory cytokines (TNF and IL-6) [435-437]. The effect on growth may be due to HEUs' exposure to HIV virions, maternal inflammation, and medications, leading to increased circulating inflammatory cytokines, growth hormone resistance, and stunting [110, 163, 348, 423, 434]. Additionally, co-infections such as malaria may contribute to systemic inflammation since the majority of pregnancies affected by HIV occur in malaria-endemic countries in Sub-Saharan Africa [438, 439].

Systemic inflammation is also associated with poor postnatal linear growth [440]. In our study, an acute phase reactant (CRP), a chemokine (CXCL10/IP-10), a marker of microbial translocation (LBP), and a growth factor (IGF-1) were associated with lower weight and stunting between 6 weeks to 12 months of age (Table 2.9). Gastrointestinal infections and gut mucosal barrier disruption result in intestinal microbial translocation, which is a driver of systemic inflammation and subsequent growth restriction [159, 164]. Recurrent infections and systemic inflammation may result in decreased growth factor expression (IGF-1 and IGFBP-3), thereby suppressing long bone growth [163, 348, 434].

With respect to neurodevelopment, immune activation and inflammation in HEUs triggers cytokine release, directly impacting cell migration and axonal growth [110]. In our study, markers of inflammation, neutrophil activation (NGAL), and tissue remodeling (MMP-9) were inversely correlated with measures of motor and language development. In other studies of children with chorioamnionitis, preterm birth, and febrile illness, elevated pro-inflammatory cytokines in the umbilical cord, amniotic fluid, and/or fetal blood were linked to microcephaly, low hippocampal volumes, and impaired development [441-445]. Consistent with our findings,

HEUs have increased levels of migratory monocytes at birth, reduced head circumference, and poor motor and cognitive outcomes within 5 months of age compared to HUUs [446].

Our study has several limitations. We found only seven papers related to growth faltering and two papers related to neurodevelopment, which indicates a need for further research involving health outcomes of HEUs. The quality of the included studies was low. Eight of the nine were sub-studies from larger cohorts with potential selection bias due to convenience sampling. The sample size of included studies was small to moderate (n=12 to 243). There was heterogeneity in the biomarkers reported, growth parameters, neurodevelopment assessment tools used, and timing of measurements (including both intrauterine and postnatal timeframes). This made it challenging to summarize the association or predictive value of any one biomarker. On the other hand, taken together, multiple inflammatory biomarkers demonstrated similar associations with pre- and postnatal growth and neurodevelopment, suggesting a class effect.

There are potential clinical uses of our biomarkers. Validated biomarkers could be used early in life to predict impaired growth or neurodevelopment in HEUs. Abundant protein biomarkers could be adapted to a cost-effective immunochromatographic lateral flow platform to quickly identify infants at-risk in low-income settings [447, 448]. Early interventions during the critical developmental window in the first 1000 days of life (e.g., promotion of breastfeeding, nutrition [449], hygiene [450], psychosocial stimulation [451], and parenting skills) may have lifelong benefits [451]. Finally, identifying biomarkers in HEUs at risk may elucidate pathways of injury that could inform future therapeutics.

In conclusion, biomarkers of systemic inflammation are associated with growth faltering and neurodevelopmental delay in HEUs. Further primary research is warranted to investigate the relationship of biomarkers and growth faltering and/or neurodevelopmental delay in HEUs, given the substantial public health and economic implications.

CHAPTER 3. GROWTH FALTERING AND DEVELOPMENTAL DELAY IN HIV-EXPOSED UNINFECTED INFANTS: A PROSPECTIVE COHORT STUDY

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Contributions:

RS extracted the results from electronic databases, conducted the data and statistical analysis, write-up of the manuscript, critically reviewed the manuscript, and revised the manuscript based on feedback from co-authors

ALC collected infant growth and neurodevelopmental data, and critically reviewed the manuscript

SN collected infant growth and neurodevelopmental data, and critically reviewed the manuscript

ROO collected infant growth and neurodevelopmental data, and critically reviewed the manuscript

SL conducted the laboratory assays for dried blood spot and critically reviewed the manuscript

SF supervised write-up of the first draft of the manuscript and critically reviewed the manuscript

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MTH conceived the study, designed the prospective cohort study, obtained the funding, supervised the data collection, conducted the data and statistical analysis, write-up of the manuscript and critically reviewed the manuscript

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3.1 Abstract

Background: HIV exposed but uninfected (HEU) infants are at increased risk of impaired early linear growth and cognitive development. We examined associations between pre and postnatal growth and subsequent neurodevelopment in Ugandan HEUs, hypothesizing that early insults may explain alterations in both somatic growth and brain development.

Methods: We prospectively followed a cohort of HEUs from birth to 18 months of age, and measured length/height, weight, head and arm circumference longitudinally. The Malawi Development Assessment Tool (MDAT, 12 and 18 months) and the Color Object Association Test (COAT, 18 months) were used for developmental assessments.

Results: Among 170 HEUs, the prevalence of low birth weight (LBW) and failure to thrive (FTT) was 7.6% and 37%, respectively. HEUs had MDAT scores that were similar to the reference population. The mean (SD) score on the COAT was 5.5 (3.1) compared to 6.9 (5.3) in developmentally normal children. Developmental ability at 18 months of age showed strong cross-sectional correlation with weight- (ρ=0.36, p<0.0001), length/height- (ρ=0.41, p<0.0001), head circumference- (ρ=0.26, p=0.0011), and middle upper arm circumference (MUAC)-for-age (ρ=0.34, p=0.0014). There was a statistically significant correlation between birth weight and MDAT z-score at 18 months (ρ=0.20, p=0.010). FTT was associated with lower MDAT z-score (median -0.13 (IQR -0.75 to +0.14) *versus* +0.14 (IQR -0.44 to +0.63), p=0.042).

Conclusion: Growth faltering in HEUs was associated with lower attainment of developmental milestones at 18 months of age. Our findings point to a simple screening method for identifying HEUs at risk for developmental intervention.

3.2 Introduction

In addition to the 1.7 million children and adolescents living with HIV, there are 14.8 million HIV exposed but uninfected (HEU) infants globally, 90% of whom reside in Sub-Saharan Africa [90, 92]. Thanks to successful interventions such as maternal combination antiretroviral therapy (cART), vertical transmission of HIV has been reduced to <1% in resource-rich areas and <2.9% in low- and middle-income countries (LMICs) [91, 452-454]. HEU newborns significantly outnumber infected infants worldwide and represent nearly 30% of the newborn population in some HIV endemic nations [93].

Although HEUs do not exhibit severe immunodeficiency and opportunistic infections like HIV infected infants, HEU health outcomes differ from those of HIV-unexposed uninfected infants (HUUs). In LMICs, mortality among HEUs in the first 2 years of life is 2 to 3 times higher than HUUs [94]. HEU survivors have increased risk of impaired early linear growth, psychomotor and cognitive development, hearing loss, expressive language expression, and diarrheal disease [92, 95, 117, 118]. Growth faltering and neurodevelopmental delay in HEUs may be due to pre- and post-natal factors, including socioeconomic variables, cART exposure, subtle immune deficits, systemic inflammation, and exposure to infections [94, 455].

Early infant development is increasingly recognized as a determinant of health and productivity in adulthood and is listed among the United Nations Sustainable Development Goals [451, 456-458]. HEUs are among the estimated 219 million (39%) children younger than 5 years (under-5s) in LMICs at risk of not reaching their developmental potential [456]. The prenatal period and the first 2 years of life are the most sensitive times during which growth faltering is associated with

later cognition, executive function, and school attainment [395-397]. Identifying HEUs before they complete this sensitive period of somatic and brain growth may be crucial to foster optimal child development.

As both growth and neurodevelopment issues of HEU infants are recognized, our objective was to determine early growth challenges and indicators that can determine neurodevelopmental concerns later in infancy. Uganda is among the top 5 countries in the world with respect to total number of HEU children and represents an ideal context for the study of growth and development of HEUs in a LMIC [90]. We explored associations between prenatal and postnatal growth and subsequent development at 18 months of age.

3.3 Methods

3.3.1 Study design

This was a prospective cohort study of HEUs, conducted between March 2016 to December 2018, examining the relationship between growth faltering and neurodevelopment.

3.3.2 Study setting and participants

The prevalence of HIV in Uganda is 0.5% among children [459]. Our study was conducted at two facilities with labor and delivery services: Jinja Regional Referral Hospital (total catchment population 507,700) and Kambuga District Hospital (total catchment population 35,873). Both centers are characterized by high obstetrical volumes, high HIV rates, and numerous mothers with unknown HIV status presenting in labor. The National Program for prevention of vertical transmission in Uganda involves "Option B+" for the mother (lifelong cART initiated as soon as HIV detected in pregnancy), nevirapine from birth to 6 weeks of life for the infant, and co-trimoxazole prophylaxis from 6 weeks of age until HIV infection is excluded [89].
HIV-seropositive mothers of any age and their newborn infants were eligible for inclusion in the study. Exclusion criteria were: negative maternal HIV testing at delivery; vertical infection (one or more positive HIV PCR tests in infant); infant death before 18 months of age; and loss to follow-up at 18 months of age.

3.3.3 Study procedures

Consenting mothers with positive or unknown HIV status presenting in labor were tested with point-of-care rapid HIV serologic tests. The HIV testing was conducted in accordance with the Uganda National Policy Guidelines for HIV counselling and testing [460]. Specimens collected

were tested using rapid diagnostic tests in series. The test kits were validated by the national health reference library. The first test was done with Determine™ HIV–1/2 Ag/Ab Combo (Alere, USA). If positive, the result was confirmed by a second test, the HIV 1/2 STAT PAK® Assay (Chembio, USA). In case of a discordant result, a "tie-breaker" test was performed using the Uni-Gold™ Recombigen® HIV-1/2 (Trinity Biotech, Ireland).

Mothers testing positive were managed according to national guidelines [89]. Using standardized case report forms, demographic and clinical information was collected at birth. Follow-up clinic visits occurred at 6 weeks, 12 months and 18 months of age. Neurodevelopment assessments were conducted at 12 and 18 months of age.

3.3.4 Determination of HIV infection status

At each study visit, we collected a venipuncture blood sample and applied three aliquots of 10-20 µL to a filter paper (Whatman® FTA® DMPK-C Cards for Dried Blood Spot, GE Healthcare Life Sciences, USA). The filter paper was dried at room temperature and stored for subsequent shipping to the National Laboratory for HIV Reference Services (NLHRS) in Canada for HIV-1 DNA testing and analysis. For each dried blood spot (DBS) card, a whole spot (~75 µl) was cut and lysed in 2mL of NucliSENS® easyMag® Lysis buffer (bioMérieux, France). The samples were then placed on a shaker for 1 hour at room temperature. The lysed samples were extracted for total nucleic acid using the Generic 2.0.1 protocol on the NucliSENS® easyMag® platform. Extracted elutions were tested for HIV-1 using the NLHRS-molecular algorithm, which includes in-house PCRs targeting DQ-alpha and pol (integrase). Sample quality was first assessed by the in-house DQ-alpha PCR (Taq PCR Core Kit, QIAGEN, Germany). RNA from positive DQ-

alpha samples were synthesized into complementary DNA (cDNA) using the Superscript IV VILO Master Mix (ThermoFisher Scientific, USA) kit. The detection of HIV-1 in the DBS samples was assessed by using an in-house nested pol (integrase) PCR (Taq PCR Core Kit, QIAGEN, Germany). A positive band of 175 bp using a QIAxcel instrument (QIAGEN, Germany) defined HIV-1 infection.

3.3.5 Clinical definitions

Failure to thrive (FTT) was defined as downward crossing of two or more major percentile lines on the World Health Organization (WHO) weight-for-age growth chart between 6 weeks and 18 months of age [461, 462]. Major percentile were defined as the 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, 95^{th} lines [461]. Because catch-up or catch-down growth can occur with transition from the intrauterine to the extrauterine environment, the birthweight was excluded in the assessment of FTT.

Growth velocity was determined as the change in weight, height, and head circumference from the 12 months visit to the 18 months visit. This change was compared to the WHO standards to obtain the growth velocity z-score [462].

Stunting was defined as infant length/height-for-age two standard deviations or more below the mean using WHO growth standards [462]. The z-score for length/height-for-age was calculated using the package *zscorer* in the R statistical environment [463].

Wasting was defined as infant weight-for-length two standard deviations or more below the mean using WHO growth standards [462]. The z-score for weight-for-length was calculated using the package *zscorer* in the R statistical environment [463].

Neurodevelopmental ability (rank) was assigned based on the standardized score of Malawi Developmental Assessment Tool (MDAT) milestones achieved at 18 months of age (see Statistical Analysis below).

Small for gestational age was defined as weight below the tenth percentile for gestational age at birth, based on Ugandan norms [464].

3.3.6 Neurodevelopmental assessment

The MDAT is a culturally appropriate, reliable developmental assessment tool for use in African settings including Uganda for children from birth to 6 years of age [465, 466]. This test examines milestones in the domains of gross motor, fine motor, language, and social development through direct observation of the child and questions to the caregiver. Items are scored as pass/fail. Reference norms for this tool are derived from a population of 1,513 normally healthy children (excluding those with malnutrition, significant medical problems, prematurity, or known neurodisability) from birth to 6 years of age, recruited from clinics at rural and urban sites in Malawi [466]. The MDAT testing protocol was modified for this study by selecting age-appropriate milestones for 12 and 18 months old infants (Table 3.4). Three Ugandan study nurses were trained to administer the MDAT in one half-day training session. The nurses, who had tacit linguistic and cultural understanding, observed infant behavior, and administered the MDAT

questionnaire in the local languages (Luganda, Lusoga, and Rukiiga) as a verbal interview, allowing them to provide clarification, when necessary, around the meaning of the questions

The Color Object Association Test (COAT) measures declarative (explicit) memory at 18 to 36 months of age and has been used in Ugandan infants in previous studies [467-469]. This visually paired association memory test directs children to correctly place a familiar toy/object in its color-associated wooden hinged lid box (e.g., toy car in pink box). After a child underwent learning phase, we conducted 4 trials with increasing number of objects to remember per trial. Two points were given for correctly putting toys in the correct color-pair box, 1 point was given for self-correction, and 0 points were given for incorrect responses. The COAT has demonstrated strong reliability and validity (discriminant and convergent) when compared to established memory tools for children across various ages [467]. Reference norms for the COAT were derived from a US cohort of 281 toddlers aged 18-36 months from a multi-ethnic community of mostly lower- and lower middle-class socioeconomic backgrounds [467].

During neurodevelopmental testing, the examiner evaluated and rated the child's test behavior as follows: (1) overall typical or atypical behavior; (2) compliance (complies, usually complies, rarely complies); (3) interest in surroundings (alert, somewhat disinterested, seriously disinterested); (4) fearfulness (none, somewhat fearful, very fearful); and (5) attention span (attentive, somewhat distractable, very distractable) [470]. These were subjective ratings, intended to systematically note the child's style of interacting with his or her environment [470].

3.3.7 Statistical analyses

In our primary analysis, we used FTT as the predictor variable and neurodevelopmental ability (rank) at 18 months of age as the outcome variable [461, 471]. Neurodevelopmental ability was computed using item response theory (IRT), a psychometric model of a trade-off between test taker's ability and the difficulty of the test [472]. The Rasch Model, a 1-parameter logistic model to determine an infant's ability based on their responses to multiple test items was used [472]. A standardized (mean zero, unit standard deviation) score was determined for each infant for each MDAT developmental domain and an overall developmental score. This allowed ranking of individuals by neurodevelopmental ability within the cohort.

Secondary neurodevelopmental outcomes included scores on the MDAT at 12 months of age and the declarative memory, as assessed by the COAT at 18 months of age [467]. In secondary analyses, the associations between neurodevelopmental outcomes and the following growth parameters were assessed: birth weight, weight-for-age, length/height-for-age, weight-for-length/height, growth velocity between 12 and 18 months of age, mid-upper arm circumference, and head circumference. For secondary analyses involving multiple statistical comparisons, the Holm-Bonferroni correction was used to adjust for the family-wise type 1 error rate.

To examine associations between variables, non-parametric methods (Mann–Whitney U test) were used for continuous data. The two-tailed Pearson Chi-Square or Fisher's exact test were used for categorical data, as appropriate. Correlations between continuous variables were assessed using Spearman's rank correlation coefficient (ρ). To compare milestones achieved at two different time points (MDAT at 12 and 18 months of age), we used the McNemar test for

paired nominal data. To determine if pre-natal (birth weight) and post-natal (FTT) growth parameters were independently associated with MDAT score, we used a multivariable linear regression model. We included the MDAT score as the continuous dependent variable and the birth weight (continuous) and FTT (binary) as independent variables. Variable selection for the multivariable model was guided by theoretical considerations (pre- and post-natal growth measurements), rather than statistical model selection. To determine whether growth faltering increased with age, we used a linear mixed effects (LME) model to account for repeated measurements over time using *lme4* [473]. The z-score (weight-for-age, length/height-for-age, or weight-for-length/height) was entered as a continuous dependent variable into the model and age was entered as a fixed effect. We modeled intercepts and slopes for each subject as random effects. P-values were obtained by likelihood ratio tests of the full model including age as fixed effect against the reduced model without age. Data analyses were performed using GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA), and the R statistical environment [474].

3.3.8 Sample size calculation

A standard sample calculation showed that we would need 139 patients to detect a difference of 0.5 standard deviations (SDs) in the mean MDAT score between children with and without FTT with 80% power, at the α=0.05 level of significance [475]. This sample size calculation assumed that 35% of children in our cohort would have FTT and that a difference of 0.5 SDs ("medium" effect size) would represent a clinically significant difference in outcome [76, 476, 477].

3.3.9 Ethical approval and consent to participate

This study was reviewed and approved by the Makerere University School of Biomedical Sciences Research Ethics Committee (REC Protocol #SBSREC 295) and the University of Alberta Research Ethics Committee (Study reference Pro00057175). Regulatory approval for the study was obtained by the Uganda National Council of Science and Technology (registration number HS 1985). All participating children had a parent or caregiver that provided written informed consent.

3.4 Results

A total of 1239 pregnant HIV positive mothers and their infants were screened and 375 were enrolled (Figure 3.1). Eight infants subsequently tested positive by DNA PCR for HIV. Three infants died at the age of 7, 8 and 10 weeks before the primary outcome could be assessed (MDAT at 18 months of age). A further 194 children were lost to follow-up at 18 months of age (Figure 3.1). Younger maternal age, lower gravidity and parity, fewer antenatal clinic visits, and prematurity were factors associated with loss to follow-up (Table 3.1). The final cohort consisted of 170 HEU infants who completed the MDAT at 18 months of age. The baseline characteristics of the cohort are reported in Table 3.2, disaggregated by the primary outcome (developmental ability at 18 months of age).

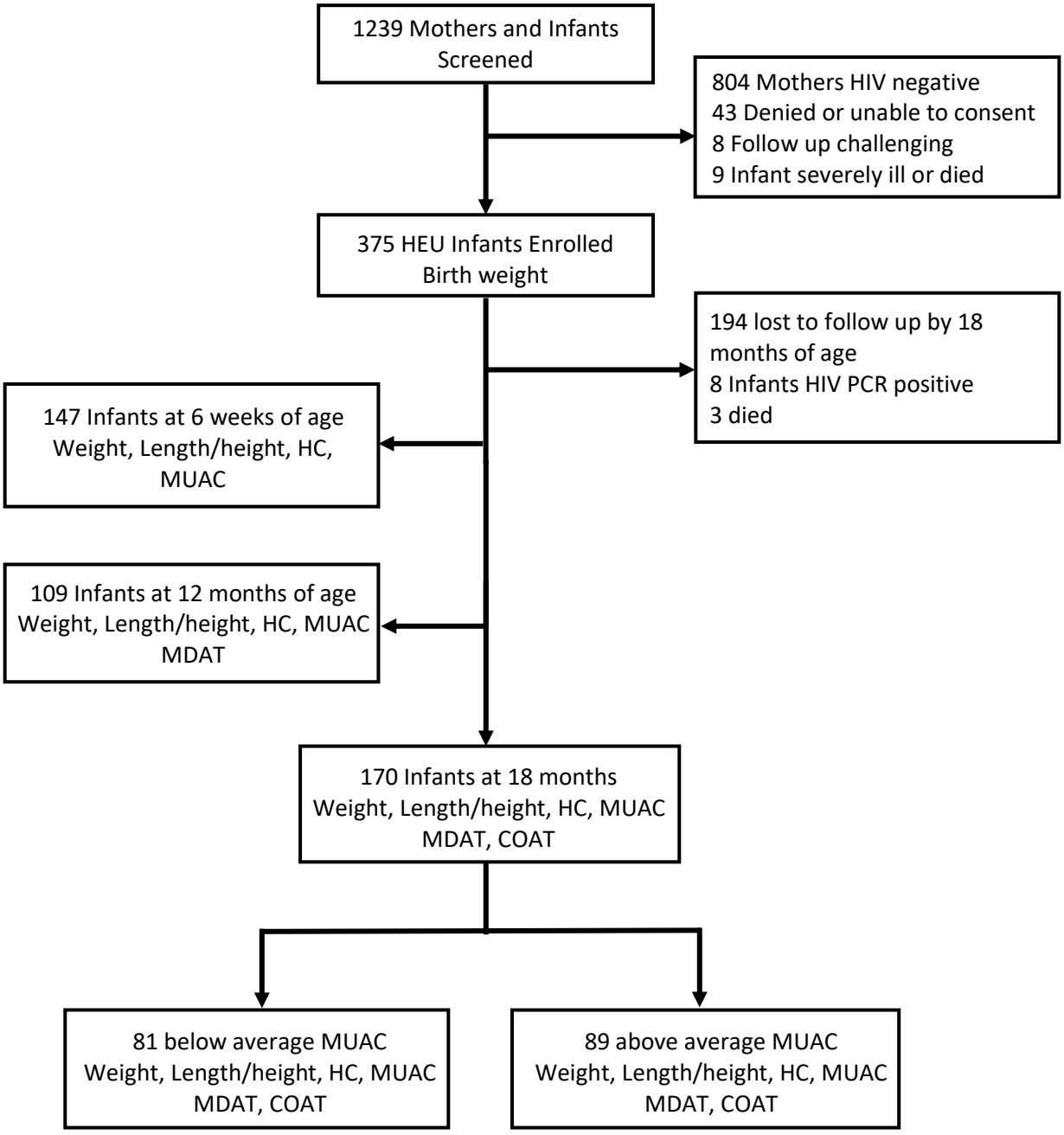

Figure 3.1. Trial flow diagram. 1241 mothers-infant pairs at the Jinja Regional Referral Hospital or Kambuga Hospital were screened and 170 were included. Anthropomorphic data (weight, length/height/height, head circumference (HC), and mid-upper arm circumference (MUAC)) were recorded at 6 weeks, 12 months and 18 months of age. Neurodevelopment assessment was performed using the Malawi Development Assessment Tool (MDAT) at 12 and 18 months; and the Color Object Association Test (COAT) at 18 months. The cohort with complete growth and neurodevelopment data (n=170) was dichotomized based on the average MDAT score at 18 months of age.

Table 3.1: Baseline characteristics of HIV positive mothers and HIV exposed, uninfected infants in Uganda

Characteristics	Entire Cohort (N=375)	Included (N=170)	Lost to follow up (N=194)	HIV infected (N=8)	Fatal outcome (N=3)	P-value[1]
Maternal, n (%)						
Age [yr], median (IQR)	27 (23-31)	28 (24-33)	25 (23-30)	26 (22-27)	21,25,38	0.00018
Gravidity	3 (2-4)	4 (2-5)	3 (2-4)	2 (1-3)	2,9,2	0.00069
Parity	3 (2-4)	4 (2-4)	3 (2-4)	2 (1-2)	2,9,2	0.0056
CD4+ T-lymphocyte count ($\times 10^6$/L)[2]	554 (396-788)	547 (397-696)	548 (395-810)	794[3]	-	0.48
Number of Antenatal Clinic Visits	4 (3-4)	4 (3-5)	4 (3-4)	4 (3-5)	1,2,3	0.014
STI during pregnancy	65 (17)	30 (18)	33 (17)	0	2 (67)	>0.99
HIV Drug Regimen during pregnancy						0.44
TDF/3TC/EFV	305 (81)	134 (79)	166 (86)	5 (63)	0	
AZT/3TC/NVP	31 (8.3)	17 (10)	13 (6.7)	1 (13)	0	
Other[4]	37 (9.9)	19 (11)	15 (7.7)	2 (13)	3	
WHO Clinical Stage at Delivery						0.90
Stage 1	364 (97)	166 (97)	188 (97)	7 (100)	3 (100)	
Stage 2	7 (1.9)	3 (1.8)	4 (2.1)	0	0	
Stage 3	2 (0.80)	1 (0.60)	2 (1.0)	0	0	
Infant, n (%)						
Sex[5]						0.079
Male	197 (53)	79 (47)	112 (58)	4 (50)	2 (66)	
Female	173 (46)	90 (53)	78 (40)	4 (50)	1 (33)	
Gestational age [wk], median (IQR)	39 (38-41)	40 (38-41)	39 (38-41)	39 (36-38)	36,36,37	0.44
Premature	51 (17)	18 (12)	31 (20)	2 (29)	2 (67)	0.047
Low Birth Weight	29 (7.9)	13 (7.8)	16 (8.3)	0	0	>0.99
Mode of Delivery						0.59
Spontaneous Vaginal Delivery	297 (79)	136 (80)	153 (79)	5 (63)	3 (100)	
Caesarean Section	76 (20)	32 (19)	41 (21)	3 (38)	0	
Breastfed within 1 hour	287 (77)	132 (78)	151 (79)	3 (38)	1 (33)	0.59
APGAR Score at 1 min						0.26
>8	222 (62)	103 (61)	117 (59)	2 (33)	1 (100)[6]	
≤8	133 (37)	58 (34)	71 (36)	4 (67)		
APGAR Score at 5 min						0.64
>8	333 (93)	152 (94)	176 (94)	4 (67)	1 (100)[5]	
≤8	21 (6.4)	9 (5.6)	11 (5.9)	2 (33)		

Suctioned	64 (17)	32 (19)	31 (16)	1 (13)	0	0.50
Bag-mask ventilation	6 (1.7)	3 (1.9)	2 (1.1)	1 (13)	0	0.47
Infant Feeding Option						0.55
Exclusive Breast Feeding	365 (98)	167 (98)	188 (98)	7 (100)	3 (100)	
Replacement Feeding	6 (1.6)	2 (1.1)	4 (2.1)	0	0	
Exclusive breast feeding						
6 weeks	273 (73)	150 (99)	117 (98)	3 (75)	3 (100)	0.33
12 months	2 (1.3)	2 (1.2)	0	0	0	-[7]
18 months	0	0	0	0	0	-[7]
Weaned (no longer breast feeding)						
6 weeks	0	0	0	0	0	-[7]
12 months	68 (44)	56 (50)	10 (24)	2 (67)	0	0.015
18 months	172 (93)	149 (88)	21 (95)	2 (100)	0	0.40
Trimethoprim-sulfamethoxazole prophylaxis						
6 weeks	210 (77)	112 (76)	94 (80)	3 (75)	1 (50)	0.68
12 months	122 (83)	82 (95)	37 (90)	3 (100)	0	0.54
18 months	73 (40)	61 (39)	10 (43)	2 (100)	0	0.53

STI sexually transmitted infection; WHO World Health Organization

[1]P-value compares infants included in the analysis (N=170) to those who were excluded (lost to follow-up, HIV infected, and deceased, N=205).

[2]CD4+ T-lymphocyte count was available for 84 (22%) mothers

[3]CD4 count was available for only one of the eight mothers.

[4]other cART regimens included 1. for whole cohort: TDF/3TC/NVP (n=6), AZT/3TC/EFV (n=7), ABC/3TC/ATZ (n=1), ABC/3TC/LPV/r (n=1), and unknown (n=22). 2. For included cohort: TDF/3TC/NVP (n=3), AZT/3TC/EFV (n=3), ABC/3TC/ATZ (n=1), ABC/3TC/LPV/r (n=1), and unknown (n=11). 3. For loss to follow up cohort: TDF/3TC/NVP (n=3), AZT/3TC/EFV (n=4), and unknown (n=8). 4. HIV positive: TDF/2TC/EFV (n=1), and unknown (n=1). 5. For Fatal Outcome cohort: unknown (n=3).

[5]Sex was missing for 5 infants

[6]APGAR score was available for one of the three infants.

[7]No cases found.

Table 3.2: Baseline characteristics of 170 HIV positive mothers and their HIV exposed, uninfected infants, disaggregated by primary outcome (neurodevelopmental score at 18 months of age)

Characteristics	Entire Cohort (N=170)	Below average MDAT 18 (N=81)	Above average MDAT 18 (N=89)	P-value
Maternal, n (%)				
Age (yr]), median (IQR)	28 (24-33)	28 (24-34)	29 (24-33)	0.77
Gravidity	4 (2-5)	4 (2-5)	4 (2-5)	0.99
Parity	4 (2-4)	4 (2-4)	3 (2-4)	0.75
Number of Antenatal Clinic Visits	4 (3-5)	4 (3-5)	4 (3-5)	0.81
STI during pregnancy	30 (17)	14 (17)	16 (18)	0.91
CD4+ T-lymphocyte count ($\times 10^6$/L)	550 (400-700)	520 (400-650)	560 (390-820)	0.40
HIV Drug Regimen during pregnancy				0.60
TDF/3TC/EFV	134 (79)	66 (81)	68 (76)	
AZT/3TC/NVP	17 (10)	8 (9.9)	9 (10)	
Other[1]	19 (11)	7 (8.6)	12 (13)	
WHO Clinical Stage at Delivery				0.37
Stage 1	166 (97)	80 (99)	86 (97)	
Stage 2	3 (1.8)	0 (0)	3 (3.4)	
Stage 3	1 (0.60)	1 (1.2)	0 (0)	
Infant, n (%)				
Sex[2]				0.84
Male	79 (47)	39 (48)	40 (45)	
Female	90 (53)	42 (52)	48 (55)	
Gestational age [weeks], median (IQR)	40 (38-41)	40 (38-41)	39 (38-41)	0.67
Premature	18 (11)	8 (9.9)	10 (11)	0.89
Low Birth Weight (<2500g)	13 (7.6)	10 (12)	3 (3.4)	0.034
Mode of Delivery				0.37
Spontaneous Vaginal Delivery	136 (80)	62 (77)	74 (84)	
Caesarean Section	32 (19)	18 (22)	14 (16)	
Breastfed within 1 hour	132 (78)	64 (79)	68 (76)	0.96
APGAR Score at 1 min				0.63
>8	103 (61)	49 (60)	54 (61)	
≤8	58 (34)	27 (33)	31 (35)	
APGAR Score at 5 min				0.32
>8	152 (89)	72 (89)	80 (90)	
≤8	9 (5.3)	4 (4.9)	5 (5.6)	
Suctioned	32 (19)	17 (21)	15 (17)	0.49
Bag-mask ventilation	3 (1.8)	2 (2.5)	1 (1.1)	0.51
Infant feeding option at birth				0.96
Exclusive Breast Feeding	167 (98)	80 (99)	87 (98)	
Replacement Feeding	2 (1.2)	1 (1)	1 (1.1)	
Exclusive breast feeding				
6 weeks	150 (99)	72 (89)	78 (88)	0.99
12 months	2 (1.9)	0	2 (2.2)	0.50
18 months	0	0	0	- [3]
Weaned (no longer breast feeding)				
6 weeks	0	0	0	- [3]

12 months	56 (33)	25 (31)	31 (35)	0.70
18 months	149 (88)	74 (91)	75 (84)	0.24
Trimethoprim-sulfamethoxazole prophylaxis				
6 weeks	112 (66)	56 (69)	56 (63)	0.49
12 months	82 (48)	40 (49)	42 (47)	0.90
18 months	61 (39)	29 (36)	32 (36)	>0.99

Numbers are n (%) unless otherwise indicated

Abbreviations: MDAT 18, Malawi Developmental Assessment Tool at 18 months of age; WHO, World Health Organization; STI, sexually transmitted infection

[1]Other cART regimens included: TDF/3TC/NVP (n=3), AZT/3TC/EFV (n=3), ABC/3TC/ATZ (n=1), ABC/3TC/LPV/r (n=1), and unknown (n=11).

[2]Data on sex was missing for one patient.

[3]No cases found.

3.4.1 Growth faltering in HEU infants

Weight-for-age, length/height-for-age, weight-for-length/height, mid-upper arm circumference (MUAC)-for-age, and head circumference-for-age are shown in Figure 3.2. The proportion of HEU infants who were stunted, wasted, and underweight at each follow-up visit are shown in Table 3.3. Low birth weight (LBW) was correlated with lower weight, MUAC, and head circumference at 18 months of age, suggesting that small newborns tended to remain small in later infancy. However, an increasing proportion of underweight, stunting, and wasting with increasing age was also observed (Table 3.3). LME models confirmed that the z-scores increasingly deviated from the mean with increasing age: change in weight-for-age z-score -0.39 per year (95% CI -0.51 to -0.28, $p<0.0001$); length/height-for-age z-score -0.94 per year (95% CI -1.2 to -0.72, $p<0.0001$); weight-for-length/height z-score -0.41 per year (95% CI -0.70 to -0.13, $p=0.0045$). With respect to growth velocity between 12 and 18 months of age, 22%, 52%, and 37% of infants had weight, height, and head circumference velocity less than -2SD, based on WHO growth charts, respectively. FTT was observed in 44 infants (37%).

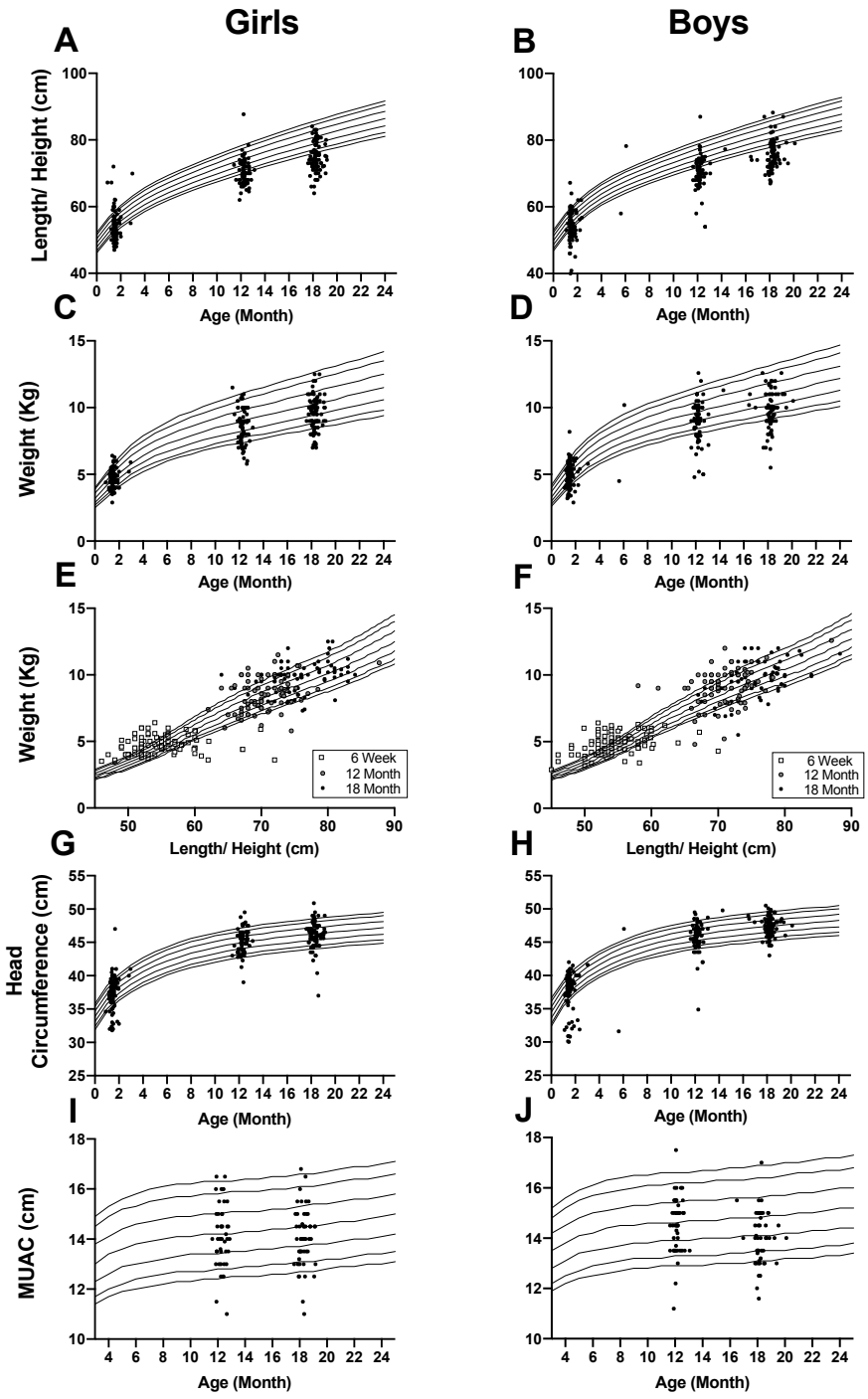

Figure 3.2. Growth of 170 Ugandan HEU infants. Growth parameters of girls and boys at 6 weeks, 12 and 18 months of age plotted on World Health Organization growth charts, including length/height-for-age (**A** and **B**), weight-for-age (**C** and **D**), weight-for-length/height (**E** and **F**), head circumference-for-age (**G** and **H**) and Mid-Upper Arm Circumference (MUAC)-for-age (**I** and **J**). Lines indicate major percentiles (5th, 10th, 25th, 50th, 75th, 90th, 95th). Points indicate individual measurements on HEU infants. Two boys had their measurements taken at 5.6 and 6.1 months of age (**B, D, F** and **H**).

Table 3.3: Anthropomorphic measurements of 170 HIV exposed uninfected infants from week 6 to 18 months of age.

Growth Measures	6 weeks	12 months	18 months
Weight [kg], median (IQR)	4.7 (4.2 to 5.3)	9.0 (7.9 to 9.8)	9.8 (9.0 to 10)
Length/height [cm], median (IQR)	55 (52 to 58)	70 (68 to 73)	75 (72 to 79)
Head Circumference [cm] , median (IQR)	38 (37 to 39)	46 (44 to 47)	47 (46 to 48)
Underweight, n (%)	18 (7.4)	23 (15)	26 (15)
Stunting, n (%)	60 (32)	65 (43)	102 (58)
Wasting, n (%)	26 (13)	10 (6.7)	9 (5.1)
Microcephaly, n (%)	29 (12)	7 (4.7)	9 (4.9)

3.4.2 Neurodevelopmental outcomes in HEU infants

At 12 and 18 months of age, 109 and 170 HEU infants, respectively, underwent MDAT testing (Figure 3.3). The proportion of HEU infants who had achieved the selected MDAT milestones was similar or superior to the reference range for normal infants, with the exception of two social milestones at 18 months of age (Figure 3.3 and Table 3.4). Girls had higher language achievement than boys at 18 months of age ($p=0.0065$, significant after Holm Bonferroni correction for multiple comparisons). Of note, achievement in the four developmental domains were significantly correlated with each other, suggesting that infants strong in one domain tended to be strong in all domains, as opposed to isolated and independent developmental strengths (Table 3.5 and 3.6). Furthermore, the MDAT social domain was correlated with the COAT score ($\rho=0.21$, $p=0.042$, Table 3.7). Comparing the MDAT at 12 and 18 months of age, achievement of overlapping milestones in fine motor, language, and social domains increased between 12 to 18 months, as expected ($p<0.05$ for all comparisons, Table 3.8). Test behavior (typical/atypical, interest in surroundings, compliance, distractibility, fearfulness) showed statistically significant associations with MDAT ability (Figure 3.4). The mean (SD) score on the COAT (declarative memory) at 18 months of age was 5.5 (3.1) compared to 6.9 (5.3) in a reference population in the USA [467].

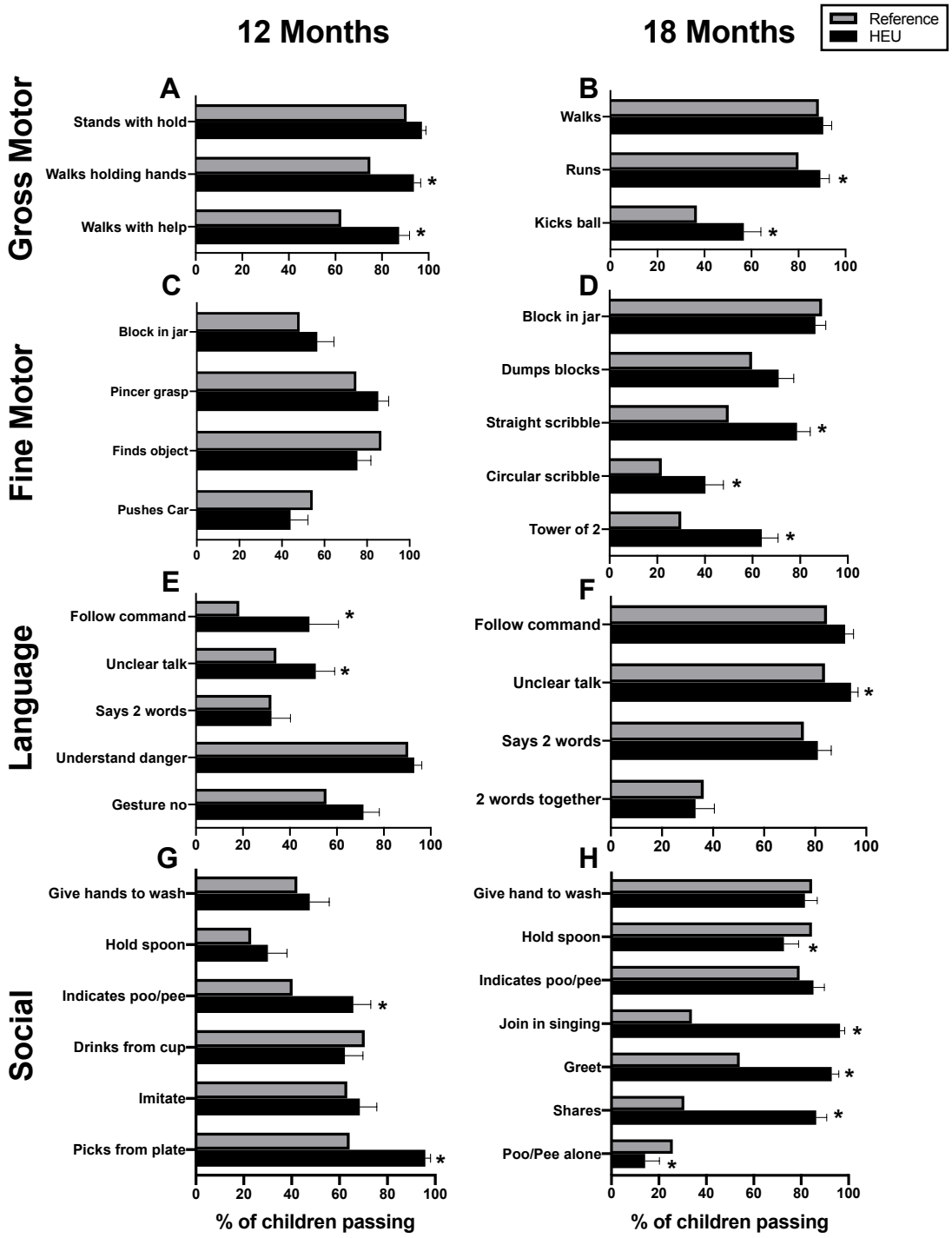

Figure 3.3. Attainment of milestones at 12 and 18 months of age among 170 HEU infants. The proportion of HEUs who had achieved age-appropriate Malawi Developmental Assessment Tool (MDAT) milestones is plotted and compared to a normative population [466]. The tool assesses four developmental domains: gross motor (**A** and **B**), fine motor (**C** and **D**), language (**E** and **F**) and social (**G** and **H**). The MDAT was assessed at 12 and 18 months of age.

Table 3.4: Comparison of Ugandan HEU infants and normative population with respect to MDAT milestones at 12 and 18 months of age

	Correct (N=170)	Observed (%)	Expected (%)[1]	P-value
12 months				
Gross Motor				
Ability to stand if holding on to things	100	98	91	0.016
Walks using both hands of someone	99	97	75	**<0.0001***
Walks with help using some's hand or furniture	92	90	63	**<0.0001***
Fine Motor				
Neat pincer grasp, picks up maize or bean with thumb and one finger	90	88	75	0.0030
Puts blocks into jar in imitation. Puts	60	59	48	0.043
Finds object under piece of cloth	82	80	87	0.084
Pushes a little car along	45	45	54	0.059
Language				
Understands when being cautioned about danger	95	93	90	0.44
Indicates by gesture to say "No."	71	70	56	0.0059
Follows simple commands (1 stage) eg. "give me the cup"	35	35	18	**<0.0001***
Unclear talk/jabber in sentences - pretends to talk but does not actually make sense	51	50	34	**0.00087***
Says 2 words, but words other than mama/dada	35	34	32	0.72
Social				
Drinks form a cup well without spilling	63	62	71	0.067
Is able to indicate, by pointing, that they want something	69	67	63	0.41
Can the child eat by picking posho/kaloo from a plate in morsels that mum has made?	97	95	64	**<0.0001***
Puts hands out to have them washed by mum	47	46	42	0.51
Can hold a spoon and take food by self, but spills some	30	29	23	0.16
Indicates in some way that they need to go for a poo/pee	67	66	40	**<0.0001***
18 months				
Gross Motor				
Walks well	153	92	89	0.34
Runs, but basic running – may fall over at times	151	90	80	**0.0011***
Kicks a ball in any way/tries to kick a ball	96	57	37	**<0.0001***

Fine Motor				
Puts blocks into jar in imitation	145	88	89	0.67
Dumps blocks out of jar purposefully	119	72	60	0.0018
Scribbles on paper (straight scribble)	133	81	50	**<0.0001***
Scribbles on paper (circular scribble)	68	41	22	**<0.0001***
Tower of 2 blocks	107	65	30	**<0.0001***
Language				
Follows simple commands (1 stage) e.g., "give me the cup"	154	92	85	0.015
Unclear talk/jabber in sentences - pretends to talk but does not actually make sense.	158	94	84	**0.00046***
Says 2 words, but words other than mama/dada	135	80	76	0.18
Says 2 words together	55	33	37	0.40
Social				
Puts hands out to have them washed by mum	136	81	85	0.23
Can hold a spoon and take food by self, but spills some	124	74	85	**0.00020***
Indicates in some way that they need to go for a poo/pee	144	86	79	0.050
Wants to join in with singing games	162	97	34	**<0.0001***
Able to greet either by extending hand or verbally	156	93	54	**<0.0001***
Sharing things, including food with others	145	86	31	**<0.0001***
Does a poo or pees by themselves without wetting their pants	24	14	26	**0.00092***

*statistically significant using the Holm Bonferroni correction for multiple comparisons

MDAT Malawi Developmental Assessment Tool

[1]Based on normative population of Malawian infants

Table 3.5: Correlation between developmental domains MDAT (12 months)

	Gross motor	Fine motor	Language	Social
Gross motor				
Fine motor	ρ=0.28 **p=0.0010***			
Language	ρ=0.39 **p<0.0001***	ρ=0.45 **p<0.0001***		
Social	ρ=0.23 **p=0.0075***	ρ=0.17 **p=0.043***	ρ=0.29 **p=0.00054***	

*statistically significant using the Holm Bonferroni correction for multiple comparisons
MDAT Malawi Developmental Assessment Tool

Table 3.6: Correlation between developmental domains MDAT (18 months)

	Gross motor	Fine motor	Language	Social
Gross motor				
Fine motor	ρ=0.43 **p<0.0001***			
Language	ρ=0.34 **p<0.0001***	ρ=0.25 **p=0.0012***		
Social	ρ=0.069 p=0.38	ρ=0.18 **p=0.022***	ρ=0.19 p=0.016	

*statistically significant using the Holm Bonferroni correction for multiple comparisons
MDAT Malawi Developmental Assessment Tool

Table 3.7: Correlation between developmental domains of the MDAT (18 months) and the COAT (also conducted at 18 months of age)

MDAT (18 months)	COAT
Gross Motor	ρ=0.053 p=0.61
Fine Motor	ρ=0.12 p=0.23
Language	ρ=0.051 p=0.62
Social	ρ=0.21 p=0.042

MDAT Malawi Developmental Assessment Tool
COAT Color Object Association Test

Table 3.8: Comparison of MDAT Milestones assessed at both 12 and 18 months of age

Category	Milestone	12 months	18 months	p-value
Fine Motor	Puts blocks into jar in imitation. Puts at least one block into the jar when shown by the examiner	81/141 (57)	147/167 (88)	**<0.0001***
Language	Follows simple commands (1 stage) e.g., "give me the cup"	45/141 (32)	155/170 (91)	**<0.0001***
	Unclear talk/jabber in sentences - pretends to talk but does not actually make sense	72/141 (51)	159/170 (94)	**<0.0001***
	Says 2 words, but words other than mama/dada	45/142 (32)	137/170 (81)	**<0.0001***
Social	Puts hands out to have them washed by mum	68/142 (48)	138/170 (81)	**<0.0001***
	Can hold a spoon and take food by self, but spills some	43/142 (30)	124/170 (73)	**<0.0001***
	Indicates in some way that they need to go for a poo/pee	94/142 (66)	145/170 (85)	**0.0023***

*statistically significant using the Holm Bonferroni correction for multiple comparisons
MDAT Malawi Developmental Assessment Tool

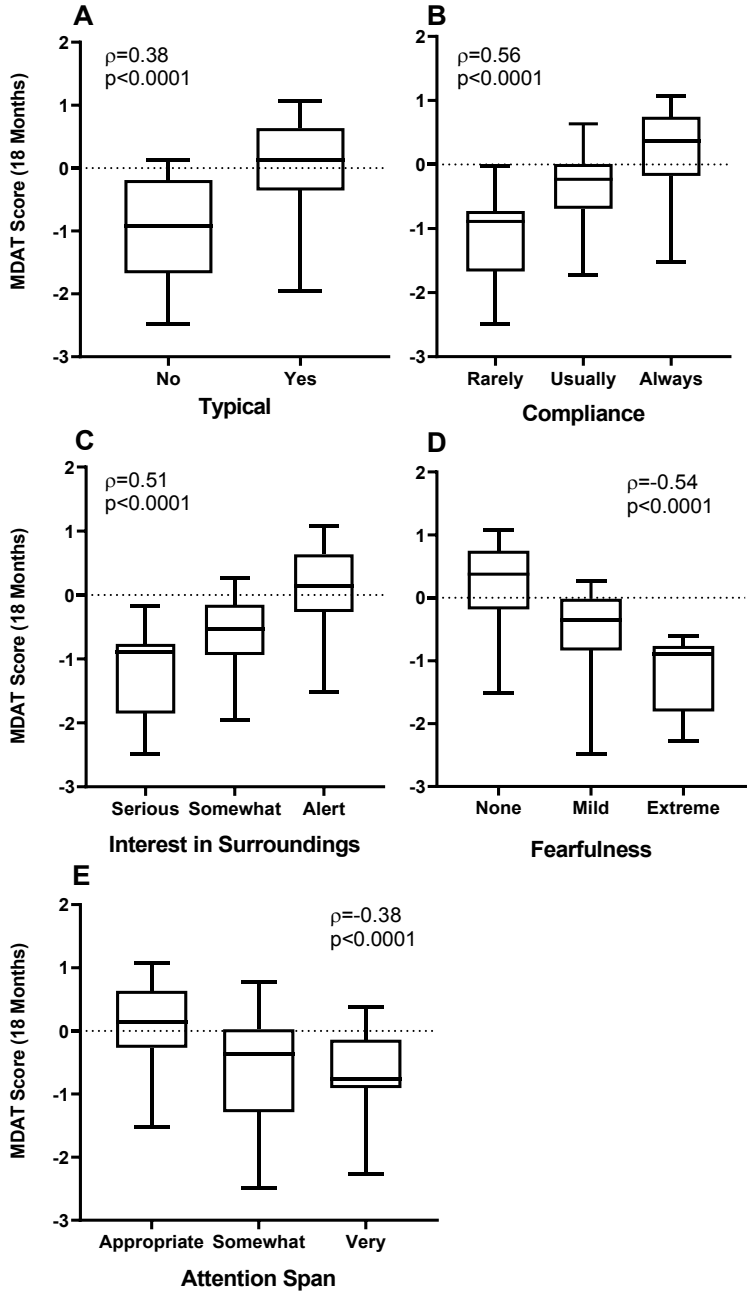

Figure 3.4. Association between MDAT score at 18 months of age and Denver Development Test Behaviors. The test behavior examines four domains: **A)** Typical, **B)** Compliance, **C)** Interest in Surroundings, **D)** Fearfulness and **E)** Attention span at 18 months of age. Each domain compares 2 (**A**) or 3 levels (**B-E**). The x-axis compares these different extremes of behavior for each domain and the y-axis shows the MDAT Score standard deviations at 18 months age. The results are statistically significant using the Holm Bonferroni correction for multiple comparisons.

3.4.3 Growth faltering is associated with lower neurodevelopmental attainment

Cross-sectional correlations between neurodevelopmental scores at 12 and 18 months of age and anthropometric parameters measured at the time of the assessment and are shown in Table 3.9. Weight-for-age, length/height-for-age, and MUAC-for-age z-scores were statistically significantly associated with the MDAT scores at 12 and 18 months of age (Table 3.9). Stunting at 18 months of age was strongly associated with lower MDAT score (median -0.19 (IQR -0.89 to -0.19) *versus* +0.38 (IQR -0.18 to +0.75), p<0.0001). Similarly, wasting was associated with lower MDAT score (median -0.94 (IQR -1.5 to -0.42) *versus* +0.025 (IQR -0.45 to +0.62), p=0.0074). In addition, the head circumference-for-age z-score was correlated with the MDAT score at 18 months of age. The weight-for-length/height z-scores were correlated with the COAT score at 18 months of age (Table 3.9).

Table 3.9: Cross-sectional correlation between growth and developmental assessments

	MDAT (12 months)	**MDAT (18 months)**	**COAT (18 months)**
Weight-for-age z-score	ρ=-0.038 p=0.012	ρ=0.36 **p<0.0001***	ρ=0.14 p=0.18
Weight-for-length/height z-score	ρ=-0.12 p=0.69	ρ=0.086 p=0.29	ρ=0.32 **p=0.002***
Length/height-for-age z-score	ρ=0.22 **p=0.00015***	ρ=0.41 **p<0.0001***	ρ= -0.23 p=0.024
Head circumference-for-age z-score	ρ=0.17 p=0.19	ρ=0.26 **p=0.0011***	ρ=-0.094 p=0.36
MUAC-for-age z-score	ρ=0.46 **p=0.0019***	ρ=0.34 **p=0.0014***	ρ=-0.072 p=0.68

*statistically significant using the Holm Bonferroni correction for multiple comparisons
Abbreviations: MDAT Malawi Developmental Assessment Tool; COAT Colour Object Association Test

Birth weight (reflecting intrauterine growth), as well as height velocity between 12 to 18 months of age (reflecting postnatal growth) were predictive of developmental scores at 18 months of age. There was a statistically significant correlation between birth weight and lower MDAT score at 18 months (ρ=0.20, p=0.010, Figure 3.5). Infants with LBW (<2500 g) had lower MDAT scores than children with normal birth weight (median -0.69 (IQR -1.1 to -0.18) *versus* +0.025 (IQR -0.44 to +0.62), p=0.0065, Figure 3.5). On the other hand, preterm infants (<37 weeks gestational age at birth) had similar MDAT scores compared to term infants (p=0.92). Infants who were small for gestational age had similar MDAT scores compared to infants who were average or large for gestational age (p=0.12). The height velocity between 12 and 18 months of age was also correlated with the MDAT z-score at 18 months (ρ=0.22, p=0.026).

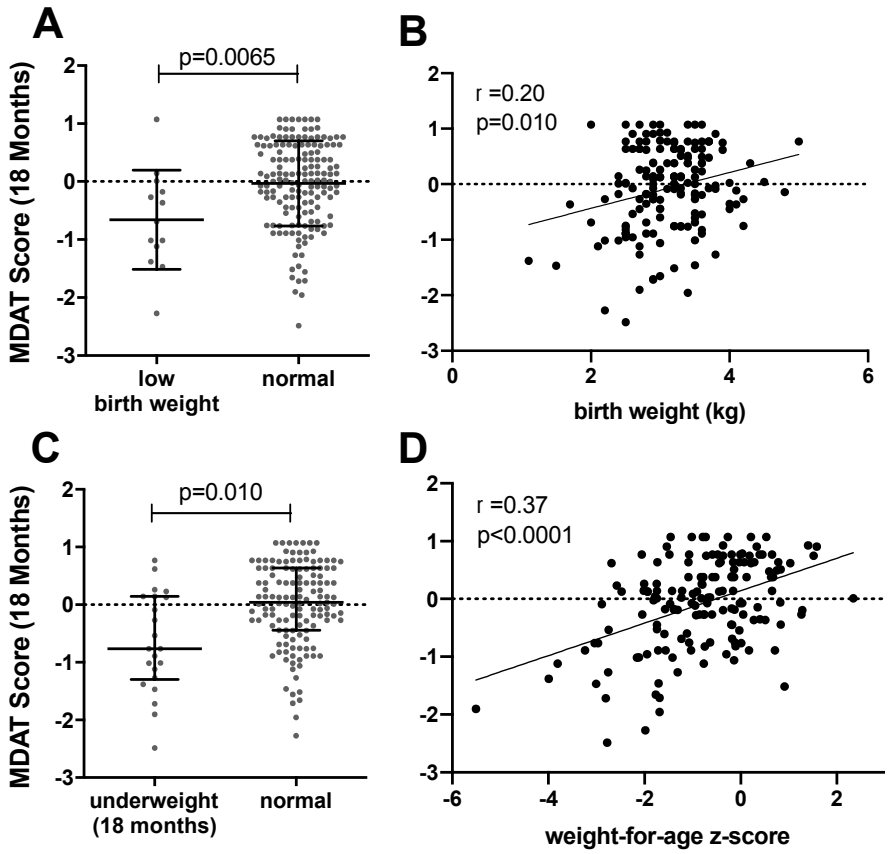

Figure 3.5. Association between growth and neurodevelopment among HEU infants. Lower neurodevelopmental ability (MDAT score at 18 months of age) was associated with low birth weight (**A** and **B**) and underweight at 18 months of age (**C** and **D**).

With respect to the primary outcome, FTT was associated with a lower MDAT score (median -0.13 (IQR -0.75 to +0.14) *versus* +0.14 (IQR -0.44 to +0.63, p=0.042, Figure 3.6). Infants with FTT had a relative risk of 1.5 (95%CI 1.04-2.1, p=0.035) of below-average neurodevelopment attainment compared to infants without FTT. In a multivariable linear regression model, both birth weight (p=0.026) and FTT (p=0.030) were statistically significant independent predictors of MDAT score at 18 months of age.

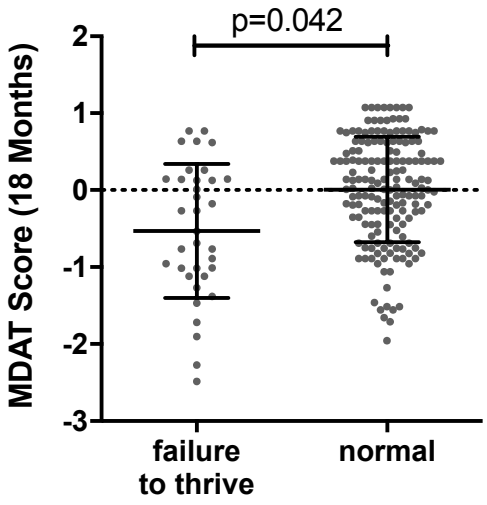

Figure 3.6. Association between failure to thrive (FTT) and neurodevelopment among HEU infants. Infants with FTT from 6 weeks to 18 months of age had median MDAT standardized ability score of -0.13 (IQR -0.75 to +0.14) compared to infants without FTT, median +0.14 (IQR -0.44 to +0.63, p=0.042).

3.5 Discussion

Here we show that growth faltering is common in HEUs and predicts lower neurodevelopmental achievement at 18 months of age. Both LBW, reflecting intrauterine growth restriction, and FTT, reflecting postnatal growth faltering, were independently associated with subsequent lower neurodevelopmental scores.

Our cohort of HIV positive mothers and HEUs was a relatively uniform group of mother-infant pairs, typical of a resource-limited, HIV-endemic setting. Overall, 80% of the mothers received the same publicly available cART regimen (tenofovir disoproxil/lamivudine/efavirenz), 99% of infants were breastfed at 6 weeks of age with 94% weaned by 18 months of age, and all infants were prescribed nevirapine syrup for 6 weeks and co-trimoxazole from week 6 until HIV infection was excluded. The rate of vertical transmission was 8/375 (2.1%) and the child

mortality was 3/375 (0.8%) during the follow-up period. This is similar to another study in African mothers with HIV, in which vertical transmission was 3.6% and mortality was 2.2% among HEUs in the first 2 years of life [478].

The proportion of LBW among HEUs in our study (8%) was similar to that previously reported (8-18%) [74, 101, 479]. HEUs have a higher prevalence of LBW than HUUs, suggesting that growth faltering begins at the fetal stage [95, 97, 110, 480]. With respect to postnatal growth faltering, stunting, wasting, underweight, and microcephaly are more prevalent in HEUs than HUUs [74, 76, 95, 97]. In a recent study, HEUs, relative to HUUs, had a higher risk of stunting at 24 months of age (adjusted odds ratio 1.32 and 1.67 for Malawian and Ugandan infants, respectively) [481]. In our study, HEUs had high prevalence of stunting, which increased with age (32%, 43% and 58% at 6 weeks, 12 months, and 18 months of age, respectively) (Table 2.3), a finding that has been documented in three previous longitudinal cohorts in LMICs [74, 76, 95]. The prevalence of wasting (5-13%) and underweight (7-15%) in our study were similar to other LMIC cohorts [74, 76, 95]. Likewise, the prevalence of microcephaly in our study was similar to another study in Zimbabwe [99].

HEUs (relative to HUUs) have subtle neurocognitive deficits that vary between studies and neurodevelopment domains [109, 111, 482]. Most studies have found that HEUs are delayed in receptive and expressive language, although cognitive delays may only be detectable at 30-42 months of age [74, 76, 100, 102, 109-111, 483-485]. Delays in school performance, lower IQ, language, and fine motor development are apparent in HEUs 2-12 years of age [110, 486, 487]. In several studies, HEUs did not show any significant motor and social delays relative to HUUs

[102, 109, 111, 483]. In a recent prospective cohort study from Uganda and Malawi, ante-partum and post-partum exposure to HIV and cART did not result in greater developmental risks for the HEU children through age 60 months, relative to HUUs [488]. Our findings in young infants are consistent with previous studies showing that subtle language and cognitive delays may only appear at later age; nonetheless, by ranking the cohort by developmental ability, we were able to discern associations between growth parameters and neurodevelopment.

In our study, LBW, stunting, wasting, lower height velocity between 12 and 18 months of age, and FTT were associated with lower neurodevelopmental scores at 18 months of age. Other studies have established that stunting is a marker of chronic malnutrition and a strong predictor of poor neurodevelopment in HEU infants [95, 111, 489, 490]. Findings from our study confirm the association between stunting and MDAT scores at 12 and 18 months and may suggest that chronic malnutrition was a cause of both poor linear growth and lower developmental achievement. The COAT score was associated with lower weight-for-length/height but was not associated with stunting. This may suggest that infant memory is less susceptible to effects of chronic undernutrition than other developmental domains. Lastly, some studies showed that microcephaly was linked to poorer school performance and cognitive and memory deficits in school aged HEU children [192, 490]. Likewise, in our study, head circumference at 18 months of age was correlated with MDAT scores at 18 months of age ($p=0.001$). Our study is noteworthy for finding multiple associations between prenatal and postnatal growth with cognitive ability in infancy. Taken together, these results suggest that early insults in HEU infants affect both somatic growth and brain development.

Several mechanisms may explain the association between growth faltering and neurodevelopmental delay in some HEUs. HEU infants are exposed in utero to HIV virions and proteins, co-infections such as cytomegalovirus, maternal medications, low-grade systemic inflammation, mitochondrial or immunological perturbations, or dysregulation of bone metabolism or mineralization [110, 163, 468, 481, 491, 492]. Fetal exposure to chronic inflammation results in both abnormal growth trajectory, structural brain abnormalities, including low hippocampal volumes, and developmental delay [493-495]. Postnatally, HEUs have more frequent infections, may not be breastfed as long as HUUs, and are exposed to prophylactic medications (NVP and CTX) [492, 496-498]. Although molecular details are not fully elucidated, subtle immune deficits, repeated infections, and elevated levels circulating inflammatory biomarkers may lead to growth hormone resistance and stunting [348, 495]. Socioeconomic determinants such as the home environment, maternal education level, and household income would likely influence both growth and development [110]. Households affected by HIV are often food insecure, which may lead to protein-energy malnutrition with deleterious effect on child growth and cognitive development [499, 500].

Growth disturbances early in infancy predicted subsequent developmental ability, suggesting a screening mechanism to identify susceptible infants for developmental interventions. Strategies for low-income settings that can be used to mitigate the developmental problems include cognitive stimulation with storytelling, singing, and playing with household objects [501]. Platforms for development services can be through home visits, clinic attendance, community-based group sessions, community health workers, and broadcast media such as radio or television [456, 502]. Our findings suggest that a subgroup of HEUs at risk of developmental

delay can be identified using simple methods such as monitoring early growth (LBW and FTT). This may allow us to introduce early childhood development interventions during the critical first 1000 days of life and track neurodevelopmental trajectory using performance measures such as the MDAT [398, 466].

Our study has several strengths and limitations. Our prospective cohort design allowed us to demonstrate predictive value of growth parameters but was subject to significant loss to follow up. Loss to follow-up is common in early infant programs for prevention of vertical HIV transmission in Sub-Saharan Africa, ranging from 19% to 89% of mother-infant pairs in published studies [503]. We had a loss to follow-up rate of 194/375 (51%) by 18 months of age, despite our best attempts to ensure that mothers returned to the clinic (e.g., transportation reimbursement, telephone reminders). We found that frequent causes of loss to follow-up were: inability to contact the mother (e.g., wrong telephone number provided); mother had moved out of the community; or attendance at a different follow-up clinic. Anecdotally, stigma associated with the HIV diagnosis prevented many mothers from openly seeking follow-up care for their infants. This study also did not include a group of healthy controls (HUU) or HIV positive infants to compare the growth and neurodevelopment assessments; however, relative growth faltering and neurodevelopmental delay has been well documented in previous studies. We used the culturally validated tool MDAT which was specifically developed and validated in an African population along with the COAT memory tool. Other studies have employed a wide range of psychometric tests, which limits the comparability of findings between studies.

In summary, we have demonstrated associations between growth faltering (both prenatal and postnatal) and neurodevelopmental ability at 18 months of age. These findings suggest common mechanisms, with onset in utero but persisting after birth, that affect both somatic and brain growth. Future directions of this research include examining biomarkers of inflammation, growth hormone axis, and neuronal injury, in an attempt to shed light on the mechanism affecting impaired growth and development in some HEUs. By demonstrating the association between LBW, FTT, and subsequent developmental ability, our findings point to a simple screening method that could be used to identify children at risk for developmental intervention.

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CHAPTER 4. CONCLUSIONS

4.1 Summary of Research

Our studies in chapters 2 and 3 address early growth, neurodevelopment, and their predictive biomarkers in HEU infants. One of the greatest success stories of the HIV pandemic is the global reduction in vertical transmission, which has led to the growth of HEU population [504, 505]. The risk of growth faltering and neurodevelopment delay has become a new challenge amongst the increasing HEU population. While these adverse outcomes among HEUs are not as severe as untreated HIV infection, the burden of morbidity is shifting to a growing HEU infant population.

In chapter 2, I conducted a systematic review to identify biomarkers that may be associated with growth faltering and/or neurodevelopmental delay in HEU infants. I identified biomarkers linked with pre-and postnatal growth and neurodevelopment. Elevated markers of inflammation (acute phase reactants, proinflammatory cytokines, chemokines), and biomarkers of intestinal microbial translocation were associated with growth faltering. Elevated markers of inflammation were also associated with adverse neurodevelopment.

In chapter 3 of this thesis, I examined growth parameters, neurodevelopment, and the association between these variables in HEU infants. Results showed a high prevalence of stunting and being underweight, which increased from 6 weeks to 18 months of age. The MDAT and COAT scores for the HEU infants were similar to the reference population's (HUU infants) scores but showed wide variability. Examining the association between growth and neurodevelopment, we found that LBW, being underweight and FTT, at 18 months of age were significantly associated with

poor MDAT scores at 18 months of age. This suggests a possible clinically applicable method to identify infants at risk for poor neurodevelopment by monitoring growth parameters.

4.2 Recommendations arising from this research

Our results in chapters 2 and 3 have clinical implications and may be actionable. These findings logically lead to the following recommendations: (1) further study of predictive biomarkers; (2) use growth parameters to monitor HEU fetal and postnatal growth in the critical first 1000 days of life; and (3) increase the frequency and intensity of visits to healthcare clinics for HEU infants with LBW.

4.2.1 Recommendation 1: Conducting further research in biomarkers predictive of poor health outcomes for HEU infants

Our systematic review highlighted the paucity and low quality of published data on clinically informative biomarkers in HEUs. Some of the gaps in the literature include using a sample of convenience, not having HEU infants as the focus of study and examining a limited number of biomarkers, growth parameters and neurodevelopmental domains. These gaps in the literature warrant further study. We outline additional biomarkers that could be measured in HEU infants that may be associated with growth and neurodevelopment in section 4.4.1 (Future Directions).

4.2.2 Recommendation 2: Using growth parameters to monitor HEU infants' neurodevelopment in the critical first 1000 days of life.

Our second recommendation is to use growth parameters such as birth weight, weight-for-age, and FTT because these have predictive value for later neurodevelopmental outcomes. The first

1000 days of life are a critical period during which the brain develops rapidly. The hippocampus, visual and auditory cortices, language processing areas, and prefrontal cortex grow rapidly during this time. These areas control "higher processing" such as attention, inhibition, and flexibility. If this critical sensitive period is interrupted, it can have short- and long-lasting consequences on brain health and function [506].

Once growth faltering is identified in a clinical setting, healthcare workers may target these at-risk infants for neurodevelopmental assessment and/or supportive intervention. A simple tool such as the MDAT could be used in the African setting. If a child is not meeting his/her developmental milestones, early interventions may be introduced. For instance, enhancing the home environment in terms of variability of experience (e.g., offering a wide range of stimulation to the child) and learning materials (e.g., presence of books or appropriate toys) can improve cognitive outcomes [507]. Other interventions include age-appropriate physical activities for motor skill development such as reaching for or grasping toys or objects, and playing or rolling on the floor [508]. These interventions can be useful to promote optimal neurodevelopment in the first 1000 days of life. Additionally, these interventions could be implemented in a low-resource setting through maternal education or through programs involving community health workers as agents to foster early infant development.

4.2.3 Recommendation 3: Increase the frequency and intensity of visits to healthcare clinics for HEU infants with LBW

Our third recommendation is for healthcare clinics in LMICs to provide frequent checkups for HEU infants are born with LBW (<2500g). Our results indicate that HEU infants born with LBW

have poor neurodevelopment later on. Increasing the frequency of visits will allow healthcare workers to pinpoint at-risk children whose weight continues to cross weight-for-age percentiles in a downward direction, or who show delays in achievement of developmental milestones. Simple tools such as the WHO growth curves and the MDAT could be used for this purpose. Increasing the frequency of follow-up visits could allow healthcare practitioners to identify children as early as possible for corrective interventions.

Once growth faltering or developmental delay are identified, interventions could be considered. One example is nutritional therapy, which can enable "catch up" weight gain for infants with LBW [509]. Nutritional counselling (i.e., advising patients to feed food that is higher in protein and energy) can be beneficial [510, 511]. Higher protein and energy consumption during the first week after birth in lower birth weight infants results in a lower risk of growth faltering at later ages.

These visits to healthcare clinics should ideally continue until a child demonstrates normal growth velocity or in the average weight range for their age compared to the WHO growth charts. Once the benefit (improvement in weight and development of HEU infants) is quantified, the cost-effectiveness of this strategy could be formally evaluated. Limitations of this strategy include HIV-impacted families living in remote regions where frequent visits to clinics may not be feasible, since this strategy relies on having a weight measurement taken by healthcare workers. One way to mitigate this issue would be providing community health workers with training and tools to identify risk factors in HEU infants and have weight scales to keep track of

their health. In case of concerns, the community health worker could refer the infant to a nearby clinic for further testing.

4.3 Significance and impact of findings

In addition to actionable findings from chapters 2 and 3, which lead to the recommendations above (Section 4.2), this thesis provides noteworthy contributions to the understanding of growth, neurodevelopment, and predictive biomarkers in HEU infants.

4.3.1 Inflammatory biomarkers point to possible translational strategies in HEUs: diagnostics and interventions

Chapter 2 synthesized the published evidence on biomarkers associated with growth faltering and developmental delay in HEUs. Our systematic review found that markers of systemic inflammation may lead to some poor health outcomes in HEU infants. The association of inflammatory biomarkers with growth and neurodevelopment may have potential diagnostic and/or therapeutic implications [512, 513]. Abundant circulating protein biomarkers are amenable to translation to lateral flow immunochromatographic bedside tests (analogous to a malaria rapid diagnostic test). If adequately predictive and adapted to a low-income environment, these tests could provide objective screening tools for use in healthcare settings to identify children at risk. By implicating inflammation as a key pathogenic pathway associated with linear growth and developmental abnormalities, targeted interventions could be contemplated to limit excessive inflammation and improve outcomes in children [512].

4.3.2 Awareness of adverse outcomes among HEUs

Chapter 3 contributes to the growing recognition of adverse health outcomes in HEUs. Despite success in preventing vertical transmission of HIV using cART, poor growth outcomes and developmental delay among HEU infants have not been widely recognized and addressed. HEUs represent a growing population, such that understanding and anticipating their health outcomes may help to nurture and monitor the growth and development of HEU infants. This study highlights the importance of continued vigilance of health care providers monitoring the growth of HEU infants so that interventions can be introduced early on (e.g., nutritional supplements for malnutrition). Data on infant neurodevelopment are also scarce in Sub-Saharan Africa, and this study provides additional data using a practical tool (MDAT).

The association between linear growth abnormalities and cognition has been previously recognized but has not been frequently reported in HEUs. As noted above (Recommendation 2, section 4.2.2), this finding suggests a clinically actionable method for early detection of HEUs at risk of developmental delay.

4.4 Future Directions

4.4.1 Further studies to elucidate the pathogenesis of growth faltering and developmental delay in HEUs

In Chapter 2, we analyzed existing research on the association between biomarkers and growth and neurodevelopment in HEUs and found an association between systemic inflammation and growth faltering and developmental delay. In Chapter 3, we laid the groundwork (description of a prospective cohort study) that would allow us to test additional biomarkers that may be

determinants of growth and neurodevelopment. A cryopreserved biobank of venipuncture from cord blood and blood samples from birth, 6 weeks, 12 months and 18 months of age is available from this cohort study for biomarker assays. Given that this cohort has detailed growth and neurodevelopmental data, quantifying levels of biomarkers using enzyme linked immune sorbent assay (ELISA) on these samples may allow us to test novel hypotheses.

Building upon the set of biomarkers identified in our systematic review (Chapter 2), additional biomarkers could be measured to provide a more comprehensive understanding of systemic inflammation, growth factors, endothelial activation, microbial translocation, and neuronal injury.

For systemic inflammation, our study identified a set of cytokines and chemokines (CRP, IL-6, TNF and CXCL10/IP10) that were associated with growth faltering. Additional biomarkers could be measured to provide a more comprehensive picture of systemic inflammation. Examples include monocyte chemotactic protein-1, macrophage inflammatory proteins −1α and −1β, procalcitonin and alpha-1-acid glycoprotein [514].

For growth parameters, we identified biomarkers IGF-1 and IGFBP-3 as biomarkers of future growth faltering. While these are promising, a recent study found that regenerating gene 1β (REG1B) protein in stool early in life correlates with later growth deficits [515]. REG1 proteins are known for their involvement in cell growth, tissue repair, and regeneration [516-519]. In a Bangladeshi cohort, higher REG1B concentrations at 3 months were independently associated with stunting at 9 through 24 months in a linear regression model [515]. The emergence of stool REG1B concentration as a significant predictor of stunting may be useful for providing early

interventions to prevent stunting in at-risk children [515]. REG1B has been known to interact with gut enteropathy [515]. This further supports our idea that biomarkers may be showing a class effect rather than a singular biomarker being associated with a singular parameter.

Endothelial dysfunction and capillary leak, which occur commonly in systemic inflammatory syndromes, may play a role in these HEU infants [97, 520]. Candidate biomarkers to interrogate endothelial activation include soluble ICAM-1 (sICAM-1), soluble P-selectin, and Angiopoietins (Angs). The sICAM-1, from the endothelial surface, is increased in chronic HIV-1 infection [521]. Soluble P-selectin has been found to correlate with neurodevelopmental outcomes in HIV-infected children [522]. Angs are dysregulated in children living with HIV [523]. Given that these biomarkers of endothelial activation are altered in children living with HIV, it would be informative to examine if these biomarkers in HEU infants are predictive of growth faltering or developmental delay.

We found that the biomarker LBP, which is a marker of microbial translocation, was associated with growth faltering. Another biomarker that may be useful to test is I-FABP, but it has limitations as a biomarker of microbial translocation. This is because I-FABP levels may be affected by metabolic lipid changes due to the disease process or medications [75]. Additional biomarkers of interest include LPS, sCD14 and zonulin, which are markers of enterocyte damage [524-527]. These could readily be measured in blood samples from HEU infants.

Finally, with respect to neurodevelopmental outcomes, we did not identify any neuronal-specific biomarkers in HEUs. Therefore, it would be valuable to examine established biomarkers of

neuronal development, for example, S100 calcium-binding protein B (S100B), brain-derived neurotrophic factor (BDNF), and vascular endothelial growth factor A (VEGF-A) [528].

We hypothesize that levels of these biomarkers will differ in infants with normal versus delayed trajectories in linear growth and neurodevelopment.

4.4.2 Knowledge translation for community health workers, nurses, clinicians, and policymakers in HIV-endemic managing HEUs

Chapter 3 provides practical tools to monitor growth to identify children at risk that may be amenable to knowledge translation (KT) in LMICs. For HEU infants, it would be important to outline a protocol for monitoring growth and neurodevelopment, similar to protocols for the prevention of vertical transmission. A KT plan would be needed to implement such a protocol. Key stakeholders for KT include community health workers, nurses managing women living with HIV, and practitioners providing maternity and child health care. Key messages for KT include: (1) risk of growth faltering and developmental delays in HEU infants; (2) careful measurement and documentation of growth parameters (LBW, underweight and FTT) to assess neurodevelopmental outcomes; and (3) awareness of the risk period and need for timely intervention. Methods for KT may include engaging at individual-level, community-level, and changes in policies for decision making.

At an individual level, it is necessary to implement programs that support the physical and mental health of expecting HIV-infected mothers in LMIC [529]. Providing information sheets

in the local language for mothers who are able to read, or verbal counselling to mothers who are not able to read, would be a crucial step. Regardless of the modality (in print or by mouth), it would be important for a healthcare worker to explain the growth and neurodevelopmental risks that face HEU infants. Early education and knowledge may allow healthcare facilities to connect these expecting mothers with mental and physical health support.

The published literature demonstrates that current community-based HIV programs may not be fully effective because community health workers lack HIV-specific training, may have low levels of baseline knowledge, and may hold stigmatizing attitudes [530-533]. Thus, one strategy to translate knowledge to a community setting can be providing community health workers with training materials and providing in-person training in the local languages. Additionally, there is growing interest to standardize community health worker programmes within and between countries, including the initial and ongoing training to improve community health [534, 535].

At the policy level for decision making, the Regional East African Community Health (REACH) policy initiative was established as a knowledge advisor to bridge the gap between research and health policy decision-making in East African countries [529]. As such, when researching with HEU infants, it would be important to engage organizations such as REACH that can assist in creating system-level changes. With REACH, there may be potential to disseminate our findings to a large number of clinics in the East Africa region to promote monitoring LBW, underweight and FTT in HEU infants as a regular protocol.

4.5 Concluding Remarks

In summary, this thesis identifies potential ways to predict neurodevelopmental outcomes in HEU infants using inflammatory biomarkers and growth parameters. Our findings may be translated to a diagnostic strategy for identifying HEU infants at risk before they surpass the critical first 1000 days of life. Our findings open new avenues for research into predictive biomarkers and molecular targets for the prevention of poor health outcomes.

Thanks to advances in the prevention of vertical HIV transmission, more and more women living with HIV are giving birth to uninfected infants. With this tremendous success comes the growth of a population of HEU infants, now numbering more than 14.8 million worldwide and accounting for nearly 30% of all newborn infants in some HIV-endemic countries [90, 94]. The magnitude of the HEU population globally demands increased attention to their subtler morbidities in terms of growth faltering and neurodevelopment. Our work sheds light on the determinants of growth and neurocognitive outcomes, toward an improved understanding of the pathogenesis of developmental abnormalities in HEUs. Ultimately, advances in the understanding of the developmental trajectories of HEUs will equip the global community to nurture this important group to reach their maximum potential.

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