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Community and Home-Based Care HIV Service Delivery Model in the Context of Paediatric HIV Management and Contributing to Health Systems Strengthening in a Resource-Limited Setting (Uganda): Operational Research

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DEDICATION

I dedicate this thesis to my Godfather; Mr. James Osei Kojo Sekyi. He 'implanted' the idea of a PhD in my mind and has guided, inspired, and nurtured me, leaving indelible imprints on my soul. I am most grateful…

Table of Contents

i. Summary:

This thesis is about the Tukula Fenna Project (TFP) that was set up at the Home Care Department of St. Raphael of St Francis Hospital (Nsambya Hospital) in Kampala, Uganda. In 2003, Associazione Casa Accoglienza alla vita "Padre Angelo" (ACAVPA) or "HOUSE FOR LIFE, Father Angelo" and other Italian partners; in particular, PENTA Foundation and University of Padova, Department of paediatrics collectively signed a **memorandum of understanding** (MoU) with Nsambya Hospital. The aim of the MoU was to collaborate with the hospital in the fight against HIV particularly in children and adolescents, orphans and vulnerable children (OVC) and their families in Kampala and three surrounding districts (Mukono, Wakiso and Mpigi). Thus, the MoU officially established the children's HIV programme at Nsambya Hospital, Home Care Department in 2003. The programme was then called the "PCP Project" because the initial intervention was among other things, providing Cotrimoxazole prophylaxis against Pneumocystis Carinii pneumonia (PCP, also known as Jiroveci Pneumonia). As more resources including provision of antiretroviral drugs (ARVs) from external sources and expertise became available over the years, the project evolved into a full-blown HIV programme for infants, children and adolescents as well as their families and caretakers. Additionally, the name "PCP", was replaced by "Tukula Fenna", which means "growing up together" in the local language (Luganda).

The project was implemented at the Home Care Department within an existing community home-based care (CHBC) model that evolved in response to the HIV epidemic in Uganda, and other high-burden resource-limited settings. The TFP provides comprehensive HIV care, treatment and psychosocial support services (PSS) and apart from operating at the Home Care department of Nsambya Hospital, it also operates at Ggaba Parish Outreach Clinic and 3 other outreach clinics in and around Kampala.

This thesis describes the research outcomes of the project that was managed by Dr. Massavon from 2008 to 2013. It reviews the published literature from the key milestones of the HIV epidemic to the post-conflict health reforms in Uganda and their relevance to current health outcomes, the national AIDS response and health systems strengthening. The literature review also examines the human resources for health (HRH) crisis and task shifting in the scaling up of ART in high-burden resource-limited settings. In addition, the review looks at the evolution of complementary HIV service delivery models like community and home-based care as a spontaneous response to the HIV epidemic in many resource-limited settings including Uganda. Finally, the literature documents that, there are relatively few paediatric

HIV services in the country, leading to poor geographical access and a low antiretroviral therapy (ART) coverage for children and that, HIV-infected children and in particular, AIDS orphans are an underserved and an understudied population.

At the time of this thesis, approximately 2,100 infants, children and adolescents had been enrolled into care in the TFP; about 1140 were active in care, and about 60% were on ART. Approximately, 47% of children and adolescents in the project are orphans.

This thesis therefore aims at contributing to improving paediatric HIV management through operational research in the context of a CHBC model in Kampala, Uganda. The findings cover key outcomes such as retention in care, attrition and loss to follow up (LTFU), treatment failure, mortality on antiretroviral therapy (ART) and operationalization of dried blood spots (DBS) for viral load testing among HIV-infected children. The thesis also included a specific study on HIV-Epstein-Barr Virus (EBV) co infections in children and adolescents, considered relevant to the project setting.

Except for study 5 (EBV study) which was a cross-sectional study, the studies were generally retrospective cohort studies conducted at the Home Care Department of Nsambya Hospital in Kampala, Uganda. The methodology of the operational research was based on an implementation schema derived from the ART guidelines of the WHO and Uganda (Figure 17). The selection of the outcomes for the operational research was based on the rationale that, they have direct bearings on implementation and potentially could improve the same.

The findings and implications from the six studies that constitute the chapters of the thesis are summarized as follows:

Study 1:

This retrospective observational study compared HIV and TB outcomes from adults and children in the Nsambya CHBC with national averages from 2007-2011. The core findings show that Nsambya CHBC activities enhance and complement national HIV and TB management, and resulted in better outcomes when compared to the national averages.

This approach may hold the potential for chronic disease management in resource-limited settings. Scaling up CHBC could have wider positive impacts on the management of not only HIV and TB, but also other chronic diseases as well as the general health system. A long-standing “faith-based solidarity” among international donors and partners has been pivotal to the survival and evolution of the Nsambya CHBC.

Study 2:

This is a retrospective cohort analysis of attrition and LTFU and their predictors among children and adolescents aged 0-20 years. Over the study period, 5.34% (62) of patients died, 37.61% (437) were LTFU, and thus overall attrition was 42.94% (499).

Generally, attrition and LTFU were relatively high among children and adolescents in the TFP. Not receiving ART was the single factor significantly associated with attrition in the cohort, while both baseline BMI z-scores and receipt of ART were protective against LTFU among HIV positive children and adolescents. Efforts should be made to initiate ART among all paediatric patients as soon as possible, and to provide aggressive follow-up for those not yet receiving ART. Orphans need more nutritional support to reduce the burden of malnutrition and improved access to early ART, which could also promote growth responses in this vulnerable and understudied group.

Study 3:

This retrospective cohort study reviewed records from HIV positive children age 0 to 18 years engaged in a CHBC and a Facility-based, family-centred approach (FBFCA) from 2003 to 2010 focussing on retention in care, loss to follow-up, mortality, use of ART, and clinical characteristics.

Irrespective of model of care, children receiving ART had better retention in care and therefore long-term survival. Encouragingly, if children were on ART, then their survival was as good, if not slightly better, in the CHBC compared to the FBFCA. Based on our observations, substantial improvement in child survival can be achieved in either a community-based or a family-care model as long as HIV- infected children are identified early and begun on ART. To ensure this occurs, early identification of HIV infected children requires strong linkages of pregnant HIV- infected women to prevention of mother to child transmission (PMTCT) services; active tracking to ensure all HIV exposed infants receive Polymerase Chain Reaction-based early infant diagnosis. Additionally, rapid early initiation of ART among HIV infected infants and children are essential.

Study 4:

This is an observational study that included HIV-infected children attending the Beira Central Hospital (Mozambique) and the Nsambya Hospital, Home Care Department (Uganda), and evaluated clinical and immunological failure according to the WHO 2006 guidelines.

Two hundred and eighteen of 740 children with at least 24 weeks follow-up experienced treatment failure ((29% 95%CI (26-33)), with crude incidence of 20.0 events per 100 person-years (95%CI 17.5-22.9). Having tuberculosis co-infection or WHO stage 4, or starting a non-triple cART significantly increased the risk of failure. Drug toxicity (18.3%), drug availability (17.3%) and anti-tuberculosis drug interactions (52, 25.7%) were the main reported reasons while only 9 (4%) patients switched cART for clinical or immunological failure.

Considerable delay in switching to second line cART may occur despite an observed high rate of treatment failure. Our findings reinforce the need for simplification of more effective clinical and immunological criteria for prompt recognition of cART treatment failure. Children presenting with advanced disease and TB co-infection should be targeted for closer and more sensitive monitoring of treatment response. This should be matched with a constant provision of appropriate antiretroviral drugs with optimization of first line drugs and treatment sequencing. Supply of new paediatric formulations for second line regimens and drug optimization should be considered as critical milestones to allow scaling up of early cART and reduction of treatment failure in children.

Study 5:

In this cross-sectional study, dried blood spot (DBS) samples from 213 HIV-1 infected children were collected and EBV DNA was extracted and analysed for quantification of EBV types 1 and 2 and for quantification of 16S ribosomial DNA (16S rDNA), a marker of microbial translocation.

Ninety-two of 140(66%) children on ART and 57 of 73(78%) ART-naive children had detectable EBV levels. Co-infection with both EBV types was significantly less frequent in ART-treated than in ART-naïve children (OR=0.54, 95%CI 0.30;0.98, p=0.042). HIV-1 inducing microbial translocation and a state of persistent immune activation, may lead to EBV replication and expansion of EBV-infected B-cells, thus increasing the EBV-DNA load. Super-infection by both types of EBV in HIV-1 infected subjects may represent an additional risk for the onset of EBV-related malignancies. ART, by limiting HIV-1 replication, microbial translocation and related immune activation, may prevent super-infection by both EBV types and keep EBV viremia down, thus reducing the risk of EBV-associated lymphomas.

Study 6:

This was a retrospective study to evaluate viral load (VL) using DBS and to explore the accuracy of clinical and immunological criteria for treatment failure (TF) in a cohort of HIV-1-infected children. In this cohort, immunological and clinical criteria as per WHO 2010 guidelines poorly predicted the presence of a viral load greater than either 1000 cp/ml or 5000 cp/ml (whole blood) from DBS. The low sensitivity and positive predictive values for immunological and/or clinical failure confirm those reported by the literature. This finding further supports the WHO recommendations that VL monitoring should be implemented and used to identify cases of treatment failure earlier.

Policy implications of key findings of thesis

Scaling up CHBC could have wider positive impacts on the management of not only HIV and TB, but also other chronic diseases as well as the general health system.

In this thesis, and in line with the literature, Early ART initiation was associated with improved survival and retention in both community-based and facility-based approaches.

ART is potentially protective against EBV-related lymphoproliferative disorders in HIV-EBV co infected children. This calls for early ART initiation and close monitoring in such children.

Operationalization of the use of DBS in viral load monitoring in HIV-infected children in low and middle-income countries is feasible and should be encouraged to improve the quality of paediatric HIV management in such settings.

The low ART coverage among children calls for urgent, greater and more effective decentralization of paediatric ART services within primary health care services at the district and sub-district levels in the general health system in Uganda.

Children presenting with advanced HIV disease and TB co-infection should be targeted for closer and more sensitive monitoring of treatment response.

Orphans need more nutritional support to reduce the burden of malnutrition and improved access to early ART, which in turn could promote growth responses in this vulnerable and understudied group.

ii. Riassunto della tesi in italiano

Questa tesi descrive il Progetto Fenna Tukula (TFP) in corso presso il Home Care Department dell'Ospedale St. Raphael e St. Francis (Nsambya Hospital) a Kampala (Uganda).

Nel 2003, l'Associazione Casa Accoglienza alla Vita "Padre Angelo" (ACAVPA) insieme ad altri Partner (in particolare la Fondazione PENTA e l'Università di Padova), hanno firmato una lettera di intenti con il Nsambya Hospital. L'obiettivo di questo documento era di collaborare con l'ospedale nella lotta all'AIDS nei bambini ed adolescenti, orfani (OVC) e le loro famiglie a Kampala e nei distretti circostanti di Mukono, Wakiso e Mpigi.

Il progetto è stato chiamato inizialmente "PCP project" in quanto l'intervento consisteva essenzialmente nella profilassi con il Cotrimoxazole per la prevenzione della polmonite da Pneumocystis Carinii (conosciuta anche come Jiroveci Pneumonia). Dopo due anni dall'inizio del progetto grazie ad una aumentata disponibilità di risorse è stato possibile fornire ai bambini che ne avevano necessità la terapia con farmaci antiretrovirali (ARVs) da e quindi il progetto si è indirizzato verso un programma 'tout-court' di lotta all'AIDS pediatrico con un approccio globale, che includeva anche le famiglie e non solamente i bambini. Di conseguenza, il nome "PCP" è stato rimpiazzato da "Tukula Fenna", che significa "crescere insieme" nella lingua locale (luganda).

Il progetto si è caratterizzato con l'implementazione di un modello di cure domiciliari (CHBC) adattato alla realtà dell' Uganda andando quindi oltre i confini dello NHC fino a comprendere delle strutture periferiche tra cui la Clinica della Parrocchia di Ggaba ed altre 3 cliniche nei dintorni di Kampala.

Questa tesi descrive i risultati dell'attività di ricerca svolta nell'ambito del progetto che è stato coordinato dal Dr. Massavon tra il 2008 e il 2013. La tesi si articola in una prima parte di revisione della letteratura con particolare riferimento alla realtà ugandese sia da un punto di vista dell' epidemiologia dell' HIV che dell' organizzazione sanitaria nel paese con particolare riferimento all'evoluzione dei modelli sanitari finalizzati alla lotta all'AIDS, come modelli di cura comunitaria o domiciliari. L'analisi della letteratura ha documentato che, in Uganda vi sono relativamente pochi servizi specialistici sull' HIV pediatrico. Tale aspetto ha come conseguenza una disparità tra le varie regioni del paese e

un limitato accesso alla terapia antiretrovirale per i bambini soprattutto coloro che sono senza genitori naturali.

A dicembre 2013 circa 2.100 bambini ed adolescenti sono stati arruolati nel TFP. 1.140 sono seguiti regolarmente e il 60% di loro sono in terapia con ART. Il 47% dei bambini è orfano.

La finalità ultima della tesi è quello di contribuire al miglioramento delle cure nei bambini HIV positivi in Uganda attraverso la valutazione di un modello di assistenza domiciliare. In quest'ottica l'attività di ricerca si è articolata nella valutazione delle caratteristiche dei pazienti persi al follow-up, dell'outcome della terapia antiretrovirale e, in un ambito più prettamente clinico, nello studio dell' impatto della infezione da EBV sulla progressione della malattia da HIV.

L'attività si è sviluppata attorno diverse linee di ricerca i cui risultati sono stati pubblicati (o in corso di pubblicazione) nei lavori i cui elementi fondamentali sono riassunti di seguito:

Studio 1:

Studio osservazionale retrospettivo che analizza i risultati del follow-up dei pazienti con HIV e TB (adulti e bambini) seguiti presso lo Nsambya Hospital confrontandoli con i dati nazionali tra il 2007 e il 2011. I risultati mostrano che il modello seguito allo Nsambya ha prodotto migliori risultati in termini di morbilità e mortalità rispetto alle medie nazionali. Il modello descritto basato sull'assistenza domiciliare potrebbe essere utilizzato anche in altri contesti nei paesi in via di sviluppo.

Studio 2:

Analisi di coorte retrospettiva per la valutazione delle caratteristiche dei pazienti persi al follow up (LTFU) e dei fattori di rischio associati, nei bambini ed adolescenti tra 0 e 20 anni. Nel corso del periodo di follow up considerato, il 5,3% dei pazienti è deceduto, il 37,6% è stato perso al follow-up con un "attrito" globale del 42,9%.

In generale, LTFU sono stati relativamente alti tra i bambini e gli adolescenti nel TFP. La terapia con ARV e la crescita regolare sono stati fattori associati con la permanenza in follow up e con la sopravvivenza. Tali osservazioni suggeriscono come gli sforzi dovrebbero essere indirizzati ad iniziare la ART nei pazienti pediatrici il prima possibile,

e a fornire un follow-up regolare a coloro che non sono ancora in terapia. Particolare attenzione va data agli orfani che necessitano di un supporto alimentare particolarmente attento e di un follow up regolare per definire il momento migliore quando iniziare la ART.

Studio 3:

Studio di coorte retrospettivo che ha studiato i bambini HIV positivi tra 0 e 18 anni inseriti in un programma di assistenza domiciliare con un approccio centrato sulla famiglia (FBFCA) dal 2003 al 2010, focalizzandosi sulla perdita al follow-up, la mortalità, l'uso di ART e le caratteristiche cliniche.

A prescindere dal modello di cura, i bambini che ricevevano l'ART sono seguiti piu' regolarmente e di conseguenza hanno una sopravvivenza a lungo termine maggiore. Basandosi sulle nostre osservazioni, un miglioramento sostanziale nella sopravvivenza dei bambini può essere raggiunto sia con un modello basato sulla assistenza domiciliare che sul coinvolgimento attivo della comunità.

Studio 4:

Studio osservazionale prospettico che ha incluso bambini HIV positivi assistiti presso il Beira Central Hospital, in Mozambico e lo Nsambya Hospital, che ha valutato il rischio di fallimento immunologico e clinico secondo le linee guida del WHO del 2006.

218 su 740 bambini con almeno 24 settimane di follow-up ha avuto un fallimento della terapia ((29% 95% CI (26-33)), con una incidenza di 20.0 eventi su 100 anni-persona (95%CI 17.5-22.9). La coinfezione con la TB, la presenza di AIDS (WHO stadio 4), o l'inizio della ART con uno o due farmaci aumenta significativamente il rischio di fallimento terapeutico.

Un ritardo considerevole nel passaggio alla seconda linea di cART si è osservato nonostante un alto tasso di fallimento terapeutico. Tali osservazioni sottolineano ancora una volta l'importanza di garantire un efficace monitoraggio clinico e immunologico per poter modificare la terapia prima che insorgano ceppi virali resistenti. Insieme alla necessità di un corretto monitoraggio va sottolineata l'importanza di garantire una fornitura di farmaco regolare senza interruzioni e le formulazioni pediatriche per i bambini più piccoli

Studio 5:

Studio trasversale, effettuato su campioni raccolti in cartoncini assorbenti (DBS) prelevati da 243 bambini affetti da HIV-1 da cui è stato estratto il DNA del EBV per analisi e quantificazione dei tipi 1 e 2, e per la quantificazione di 16s DNA ribosomiale (16S rDNA), un marker di traslocazione microbica.

92 su 140 (66%) dei bambini in terapia con ART e 57 su 73 (78%) di bambini non trattati sono risultati positivi all' EBV. La coinfezione con entrambi i tipi di EBV è stata significativamente meno frequente in coloro in terapia con ART (OR=0.54, 95%CI 0.30; 0.98, p=0.042). Tale osservazione è compatibile con il fatto che ' HIV-1, che induce una traslocazione microbica e uno stato di persistente attivazione immunitaria, può portare a una replicazione di EBV ed ad una espansione di cellule B infette, aumentando di conseguenza il DNA dell'EBV.

La co-infezione da EBV in soggetti affetti da HIV-1 può rappresentare un rischio addizionale per lo scatenarsi di tumori (linfomi) associati all'EBV. Il trattamento con ART, riducendo la replicazione dell'HIV-1, la traslocazione microbica e la relativa attivazione immunitaria, può prevenire la super infezione da EBV e mantenere la viremia EBV bassa, riducendo il rischio di linfomi ad esso associata.

Studio 6:

Studio retrospettivo per valutare la carica virale dell'HIV (VL) su campioni raccolti in DBS e per esplorare l'accuratezza dei criteri clinici ed immunologici per la definizione del fallimento terapeutico. La bassa sensibilità e valore predittivo del fallimento clinico e/o immunologico, da noi osservate, confermano quanto riportato in letteratura. Questa osservazione supporta ulteriormente la raccomandazione del WHO che il monitoraggio della carica virale debba essere implementato ed utilizzato per identificare precocemente casi di fallimento del trattamento.

Implicazioni dei risultati della tesi e messaggi chiave

Il modello assistenziale centrato sull' assistenza domiciliare è risultato molto efficace per ridurre il rischio di perdita al follow up. Tale modello potrebbe quindi essere considerato anche per l'assistenza dei malati di TB o con altre malattie croniche.

Le nostre osservazioni supportano quanto già riportato in letteratura che l'inizio precoce dell'ART è era associato non solo aduna migliore sopravvivenza ma anche ad un minor rischio di perdita al follow up.

Il trattamento ART è potenzialmente protettivo contro patologie linfoproliferative correlate al EBV nei bambini con coinfezione da HIV ed EBV.

L'uso del DBS per il monitoraggio della carica virale nei bambini HIV positivi si è rivelato fattibile sia da un punto di vista organizzativo che della qualità dei campioni da testare. Tale metodica dovrebbe quindi essere incoraggiata per migliorare la qualità della gestione pediatrica dell'HIV soprattutto nei paesi in via di sviluppo

La bassa copertura di ART tra i bambini richiede un urgente, maggiore e più efficace decentramento dei servizi pediatrici centrali e la loro integrazione con i servizi sanitari di base a livello distrettuale e sub-distrettuale in Uganda.

I bambini che presentino uno stadio avanzato di infezione HIV e coinfezione da TB dovrebbero essere sottoposti a monitoraggio più serrato per iniziare il trattamento ART appena ciò si renda necessario.

Gli orfani necessitano un particolare attenzione sia per quanto riguarda il supporto nutrizionale che il monitoraggio clinico e immunologico necessario per iniziare correttamente la ART.

iii. Contributors:

This thesis project was made possible by the collaboration of the following:

- **University of Padova, Department of Paediatrics, Padova, Italy**:-provided the funding (PhD research grant), academic framework and part of the supervision of the research project,
- **TFP**: the thesis is based on the activities and studies set up within the project setting. The analyses are based wholly or partly on data from the project. The candidate was the project manager of the TFP. Since 2006, the project has been funded by *Provincia Autonoma di Trento, Regione Trentino Alto Adige* and supported by PENTA Foundation.

- **Home Care Department of St. Raphael of St. Francis Hospital (Nsambya Hospital), Kampala, Uganda**: the department is the main local partner of the project. It provided

the framework (the community home-based care model) within which the TFP was implemented in Kampala, Uganda. The Department also provided access to study participants (including adults) within its catchment area and data for some of the research activities

- **Associazione Casa Accoglienza alla vita, Padre Angelo (ACAVPA) and PENTA Foundation**: both were instrumental in establishing and supporting the evolution of the TFP as well as the research activities documented in this thesis. ACAVPA was also the recipient of funding from *Provincia Autonoma di Trento and Regione Trentino Alto Adige.*
- **Makerere University, Department of Child Health, Kampala, Uganda**: provided part of the local supervision through Prof. James K. Tumwine.
- **Makerere University-Johns Hopkins University Research Collaboration**: Dr. Mary Glenn Fowler (CEO) also provided part of the local supervision. Additionally, this collaboration enabled a joint study comparing survival and retention among children and adolescents receiving ART in two different ART delivery models in Kampala (Paper 3).

iv. List of tables

Table and figures not presented in the 6 papers.

v. List of figures

vi. Preface:

This thesis is based on the following six papers:

Study 1: Nsambya Community Home-Based Care complements national HIV and TB management efforts and contributes to Health Systems Strengthening in Uganda: an observational study. Authors: Massavon William, Mugenyi Levi, Nsubuga Martin, Lundin Rebecca, Penazzato Martina, Nannyonga M Maria, Namisi P Charles, Ingabire Resty, Kalibbala Daniel, Kironde Susan, Costenaro Paola, Bilardi Davide, Mazza Antonio, Criel Bart, Tumwine K James, Seeley Janet, Giaquinto Carlo

In press: ISRN Public Health

Study 2: Attrition and loss to follow-up among children and adolescents in a community home-based care HIV programme in Uganda- Authors: Massavon William[1§2], Lundin Rebecca[1], Costenaro Paola[1], Penazzato Martina[1], Namisi P. Charles[2], Ingabire Resty[2], Nannyonga Musoke Maria[2], Bilardi Davide[1], Mazza Antonio[3], Giaquinto Carlo[1]

In press: Journal of Paediatrics and Therapeutics

Study 3: Survival and Retention among HIV-infected Children and Adolescents in a Community Home-Based Care and a Facility-Based Family-Centred Approach in Kampala, Uganda: a cohort study. Authors: Massavon W, Barlow-Mosha L, Mugenyi L, McFarland W, Gray G, Lundin R, Costenaro P, Nannyonga M M, Penazzato M, Bagenda D, Namisi P C, Wabwire D, Mubiru M, Kironde S, Bilardi D, Mazza A, Fowler MG, Musoke P, Giaquinto C.

In press: *ISRN* AIDS

Study 4: **Predictors of treatment failure in HIV positive children receiving combined antiretroviral therapy: cohort data from Mozambique and Uganda**- Authors: Costenaro P, Penazzato M, Lundin R, Rossi G, Massavon W, Patel D, Nabachwa S, Franceschetto G, Morelli E, Bilardi D, Nannyonga MM, Atzori A, Mastrogiacomo AL, Mazza A, Putoto G, Giaquinto C.

In press: Journal of Paediatric Infectious Disease Society

Study 5: Epstein-Barr Virus load in children infected with Human Immunodeficiency Virus type 1 in Uganda:-Authors: Maria Raffaella Petrara, Martina Penazzato, William Massavon, Sandra Nabachwa, Maria Nannyonga, Antonio Mazza, Ketty Gianesin, Paola Del Bianco, Rebecca Lundin, Colin Sumpter, Marisa Zanchetta, Carlo Giaquinto and Anita De Rossi.

Under review- Journal of Paediatric Infectious Diseases

Study 6: **Viral load detection using dried blood spots in a cohort of HIV-1-infected children in Uganda:** outcomes and correlations with clinical and immunological criteria for treatment failure. Authors: Costenaro P, Lundin R, Petrara MR, Penazzato M, Massavon W, Kizito S, Nabachwa S, Nannyonga M, Morelli E, Bilardi D, Mazza A , Zanchetta M, Giaquinto C , De Rossi A.

Under review- Journal of Clinical Microbiology

The candidate, William Massavon:

- Designed or contributed to the design of all the studies included in this thesis,
- Implemented, coordinated and supervised the studies in a project setting,
- Obtained ethical approval for the studies,
- Obtained Material Transfer Agreement (MTA) approvals for the EBV and viral load studies, including packaging of dried blood spots (DBS) for periodic shipments to the laboratory in Italy, where the analyses were done,
- Collected data or supervised data collection for the other studies,
- As a first author: conceptualized the primary study questions and design with input from other study team members, analyzed some of the datasets, wrote the first drafts, circulated drafts to all co-authors, and captured the reviewing co-authors' comments and suggestions into various versions of the manuscripts as appropriate and approved the final drafts,
- As a co-author: the candidate participated in the data analyses, reviewing of various versions of the drafts, editing and approval of the final drafts

vii. Abbreviations & Acronyms

3TC	**Lamivudine**
ABC	**Abacavir**
ACAVPA	**Associazione Casa Accoglienza, alla vita Padre Angelo**
AFB	**Acid Fast Bacilli**
AIC	**Akaike Information Criterion/ AIDS Information Centre**
AIDS	**Acquired Immunodeficiency Syndrome**
ANC	**Antenatal Care**
ART	**Antiretroviral Therapy**
ARVs	**Antiretroviral (Drugs)**
AUC	**Area Under Curve**
AZT	**Zidovudine**
BCH	**Beira Central Hospital**
BL	**Burkett's Lymphoma**
BMI	**Body Mass Index**
CARAP	**Comitato Assistenza Ricerca AIDS Pediatrico**

cART	**Combination Antiretroviral Therapy**
CBTBC	**Community-Based Tuberculosis Care**
CDC	**Centers for Disease Control and Prevention**
CF	**Clinical Failure**
CHBC	**Community Home-Based Care**
CHIVSDM	**Complementary HIV Service Delivery Models**
CSW	**Commercial Sex Workers**
DBS	**Dried Blood Spots**
DNA	**Deoxyribonucleic acid**
DOT	**Directly Observed Therapy Short Course**
DPT3	**Diphtheria, Pertussis and Tetanus (vaccine, third dose)**
EBV	**Epstein-Bar Virus**
EDTA	**Ethylenediaminetetraacetic acid**
EFV	**Efavirenz**
EID	**Early Infant Diagnosis (of HIV)**
FCA	**Family-Centred Approach**
FBFCA	**Facility-Based Family Centred Approach**
GDP	**Gross Domestic Product**
GFATM	**Global Fund to fight AIDS, Tuberculosis and Malaria**
GHIs	**Global Health Initiatives**
GoU	**Government of Uganda**
HBC	**Home-Based Care**
HIV	**Human Immunodeficiency Virus**
HPT	**Hypertension**
HRH	**Human Resources for Health**
HSSP	**Health Sector Strategic Plan**
HSS	**Health Systems Strengthening**
ICF	**Intensified Case Finding**
IF	**Immunological Failure**
IPT	**Isoniazid Preventive Therapy**
IUD	**Injection Drug Users**
JCRC	**Joint Clinical Research Centre**
KS	**Kaposi's Sarcoma**
LMIC	**Low-Middle Income Countries**

LPV/r	**Lopinavir/ritonavir**
LTFU	**Loss to Follow Up**
MAP	**Multiple ART Programmes for Africa**
MDD	**Music Dance and Drama**
MDGs	**Millennium Development Goals**
MICE	**Multiple Imputation by Chain Equation**
MoH	**Ministry of Health**
MoU	**Memorandum of Understanding**
MSF	**Médecins Sans Frontières**
MSM	**Men who have SEX with Men**
MTCT	**Mother to Child Transmission of HIV**
MU-JHU Collaboration	**Makerere University-Johns Hopkins University Research**
NACP	**National AIDS Control Programme**
NGO	**Non-Governmental Organization**
NHC	**Nsambya Home Care (Department)**
NH	**Nsambya Hospital**
NHL	**Non-Hodgkin's Lymphoma**
NNRTI	**Non-Nucleoside Reverse Transcriptase Inhibitor**
NOP	**National Operational Plan**
NSF	**National Strategic Framework**
NSP	**National Strategic Plan for HIV/AIDS**
NTLP	**National TB and Leprosy Programme**
NVP	**Nevirapine**
OOP	**Out of Pocket (health expenditure)**
OPD	**Out Patients' Department**
OVC	**Orphans and Vulnerable Children**
PAMPs	**Pathogen-Associated Molecular Patterns**
PCP	**Pneumocystis Carinii Pneumonia (Jiroveci Pneumonia)**
PCR	**Polymerase Chain Reaction (test)**
PENTA	**Paediatric European Network for Treatment of AIDS**
PEPFAR	**US President's Emergency Plan for AIDS Relief**
PHC	**Primary Health Care**
PI	**Protease Inhibitor**

PLHA	**People Living with HIV/AIDS**
PMTCT	**Prevention of Mother To Child Transmission of HIV**
PNFP	**Private-Not-For-Profit**
PSS	**Psychosocial Support Services**
RNA	**Ribonucleic acid**
SMC	**Safe Male Circumcision**
SSA	**Sub-Saharan Africa**
SWAp	**Sector Wide Approach**
TASO	**The AIDS Support Organization**
TB	**Tuberculosis**
TERT	**Telomerase Reverse Transcriptase**
TF	**Treatment Failure**
TFP	**Tukula Fenna Project**
TLR	**Toll-Like Receptors**
UAC	**Uganda AIDS Control**
UBoS	**Uganda Bureau of Statistics**
UCMB	**Uganda Catholic Medical Bureau**
UDHS	**Uganda Demographic Health Surveillance**
UNAIDS	**The United Nations Joint Programme on HIV/AIDS**
UNCST	**Uganda National Council for Science and Technology**
UNFPA	**United Nations Population Fund**
UNICEF	**United Nations Children's Fund**
VCT	**Voluntary Counselling and Testing**
VL	**Viral Load**
WHO	**World Health Organization**

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Dr. Mary Glenn Fowler: Professor, Dept. of Pathology, Johns Hopkins University School of Medicine, Baltimore MD USA; and onsite Executive Director Makerere University- Johns Hopkins University Research Collaboration , Kampala, Uganda.
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1.0. Brief overview of the HIV epidemic milestones

This section of the literature review looks at some key milestones on the path of the HIV epidemic through time, as they contribute to the context within which this thesis project was developed.

In 1981, the U.S. Centers for Disease Control and Prevention (CDC) reported an outbreak of opportunistic infections and Kaposi's sarcoma among a small number of homosexual men in San Francisco and New York in its weekly morbidity and mortality reports [1, 2]. A hallmark of the cases was severe immunosuppression, and the disease eventually became known as the acquired immunodeficiency syndrome (AIDS) [3-6]. Reports show that, the disease was also identified among injection drug users, patients with hemophilia, transfusion recipients and infants of infected mothers within the first year of its description [3, 7]. However, little did the world know that those cases would explode into an epidemic that would decimate populations around the globe.

With the reporting of the first cases of Pneumocystis pneumonia and Kaposi's sarcoma, scientists and researchers raced to find the cause of the disease and within two years, the human immunodeficiency virus (HIV) was identified as the cause of AIDS [2, 8]. With that discovery in 1983, scientists and researchers switched gears to develop diagnostic tests and treatment for HIV/AIDS [9]. In 1985, the first test to diagnose HIV was licensed; the first International AIDS Conference was held in Atlanta (USA), and a National AIDS Response was launched in Uganda. A year later, the WHO launched the AIDS Control Programme [10, 11]. By the mid-1990s, the first brands of antiretroviral drugs (ARVs) were available in the rich northern countries. In addition, those countries were able to implement preventive measures such as counselling and testing, condom distribution; risk-reduction programmes, for example, needle–exchange programmes and safe blood transfusions [9, 12]. Nevertheless, the HIV epidemic gained momentum and by the end of the first decade, about 10 million people had been infected, including a heterosexual epidemic affecting more women in Central Africa [2, 3].

By the second decade, AIDS-related deaths started declining in the advanced and resource-rich countries as well as a substantial reduction in mother-to-child HIV transmission. The situation was however, very different to other parts of the world [13]. Indeed, time, inaction, and the absence of ART / PMTCT intensified the epidemic in the high burden resource-limited settings such as sub-Saharan Africa, resulting in one of the worst epidemics in recent

times; by all measures. It is reported that, in 2001, there were 36 million people living with HIV, some 20 million people had already died, and sub-Saharan Africa remained the epicenter of the HIV epidemic [14].

As the global epidemic gained more attention, United Nations (UN) member states made a historic declaration of commitment on HIV/AIDS in 2001. That declaration led to what have become the global health initiatives (GHI), global partnerships and regional collaborations to respond to the epidemic. The Joint United Nations Programme on HIV/AIDS (UNAIDS) was set up to coordinate the global response to the HIV epidemic, in line with that declaration. That was soon followed by the Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM), the United States President's Emergency Plan for AIDS Relief (PEPFAR) and the World Bank's Multiple ART Programmes (MAP) for Africa. The UN political declaration on HIV/AIDS was revised in 2011 setting the targets for 2015 [12, 15-17].

By the third decade, ART / PMTCT became available in some resource-limited settings as a result of the GHIs [9, 12, 18]. However, new challenges soon emerged. Evaluation studies showed that, various set targets were either not met or could not be achieved principally because of weak health systems and shortages of professional health workers [19-21]. Additionally, many health systems in SSA countries are witnessing a demographic transition with high burdens of communicable and chronic non-communicable diseases [22-25].

The fourth decade has seen some progress in all spheres of prevention, care and treatment [9, 18]. In the last few years, researchers and scientists cautiously discuss possible 'functional cure' of HIV, as demonstrated by the cases of the Berlin patient [26], the Massachusetts baby [27, 28] and the Visconti cohort of France [29].

The HIV pandemic will certainly be remembered for the great devastation in terms of loss to human life and HIV-related morbidities. It will also be remembered for breaking down barriers and uniting communities, civil society, all kinds of leaders, activists, researchers and scientists to work together for a crucial humane cause, resulting in a global solidarity and one of the most notable worldwide responses in modern times [9, 18, 30].

1.1. Burden of HIV Disease

Globally, 35.3 million (32.2-38.8 million) people were living with HIV by the end of 2012. It is estimated that, 2.3 million (1.9-2.7million) new HIV infections and 1.6 million (1.4-1.9 million) AIDS-related deaths occurred in the same period. Overall, 10.6 million people

received antiretroviral therapy (ART), with 9.7 million (91.5%) in low- and middle-income countries (LMIC) in 2012, representing 61% of those eligible for ART according to the 2010 WHO treatment guidelines. However, going by the 2013 WHO consolidated guidelines, this ART coverage only represents 34% (32-37%) of the 28.3 million eligible people in LMIC in 2013 [31].

Approximately, seventy percent of all people living with HIV were in SSA, including 1.4 million (1.2-1.5million) pregnant women, 3.0 million (2.7-3.3 million) children less than 15 years and 1.7 million of the global 2.1 million (1.7-2.8 million) adolescents aged 15-19 years. Among the adolescents, about two-thirds of the new infections were among girls aged 15-19 years. SSA recorded 210,000 new HIV infections with women making up 59% (56–63%), 97,000 AIDS-related deaths among adolescents aged 10-19 years and about 85% of the 17.8 million (16.1-21.6 million) AIDS orphans [11, 32, 33].

Globally, by the end of 2012, there was a 35% decline in new infections among children aged less than 15years compared with 2009. In the same period, 260,000 new infections occurred among children less than 15years in LMIC in 2012, whereas 850,000 new infections were averted in the same age group between 2005 and 2012 in LMIC. Only 39% of infants were tested for HIV within two months of birth in 2012 in LMIC. Additionally, the majority of children diagnosed with HIV in 2012 did not start ART, resulting in 210,000 (190,000-250,000) AIDS-related deaths among children. Furthermore, children under 15 years old who needed ART were less likely than adults to receive it. That is a major concern, as in the absence of timely initiation of ART, one-third of infants living with HIV will die before their first birthday, and more than half will die before the age of two years [11, 31, 34].

Overall, ART coverage for children aged less than 15 years was 34% compared to 64% in adults in LMIC. Whereas the coverage for PMTCT reached 62% in the 22 Global Plan Priority countries, compared with 57 per cent in 2011 and 49 per cent in 2010 [21, 32].

In Uganda, the national HIV prevalence was 7.3% (6.4-8.4%) among adults aged 15-49years in 2012, and an estimated 1.5 million (1.4-1.8 million) people were living with HIV, including 100, 000 (88,000-120,000) pregnant women, and 190,000 (160,000-230,000) children aged less than 15 years, approximately 58% of whom needed ART. ART coverage was 33% (28-41%) for children aged less than 15 years, 70% (64-78%) among adults and 72% (62-82%) for PMTCT. Adolescents aged 10-19 years made up 24.5% of the total

population and 7% of all people living with HIV in the country. About 10.0% (6,300)of all AIDS-related deaths were among adolescents aged 10-19years in that year [31, 32, 35].

1.2. Global Trends in new infections and AIDS-related deaths among children adolescents and young people

Figure 1: Estimated number of new HIV infections in children (aged 0–14): Global trend and projections, 2001–2015

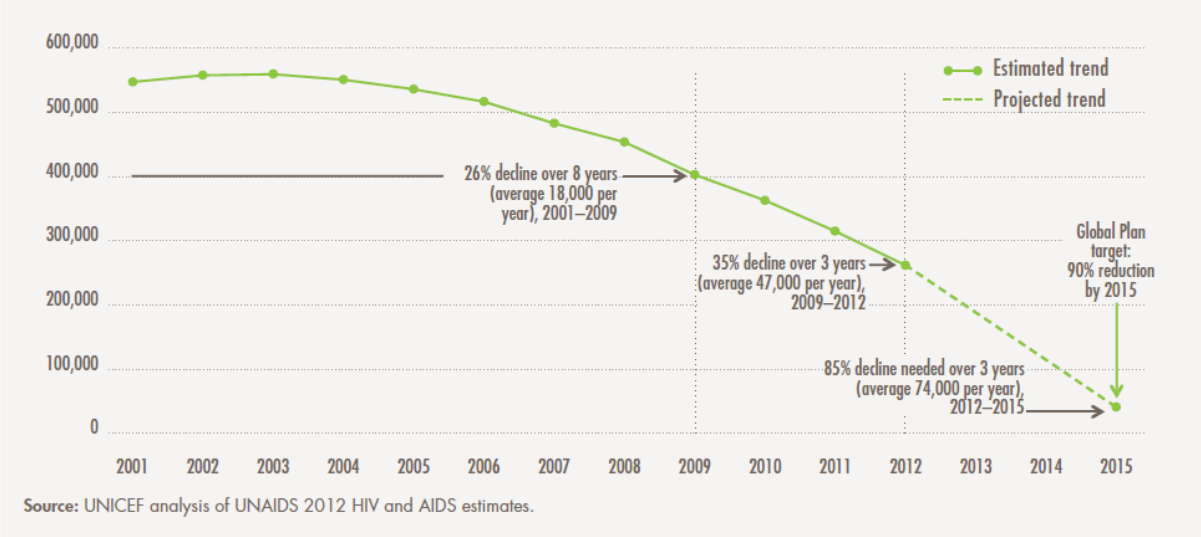


Figure 1 above, shows an important decline in new HIV infections in children aged 0-14years. The trajectory of the projected trend provides even steeper decline during the period between 2012 and 2015, the timeline for achieving the Global Plan target of 90% reduction in new infections. The observed trend is encouraging and most likely due to the impact of PMTC.

Figure 2: Estimated number of new HIV infections among children aged 0–14, adolescents aged 15–19 and young people aged 20–24, 2000–2012

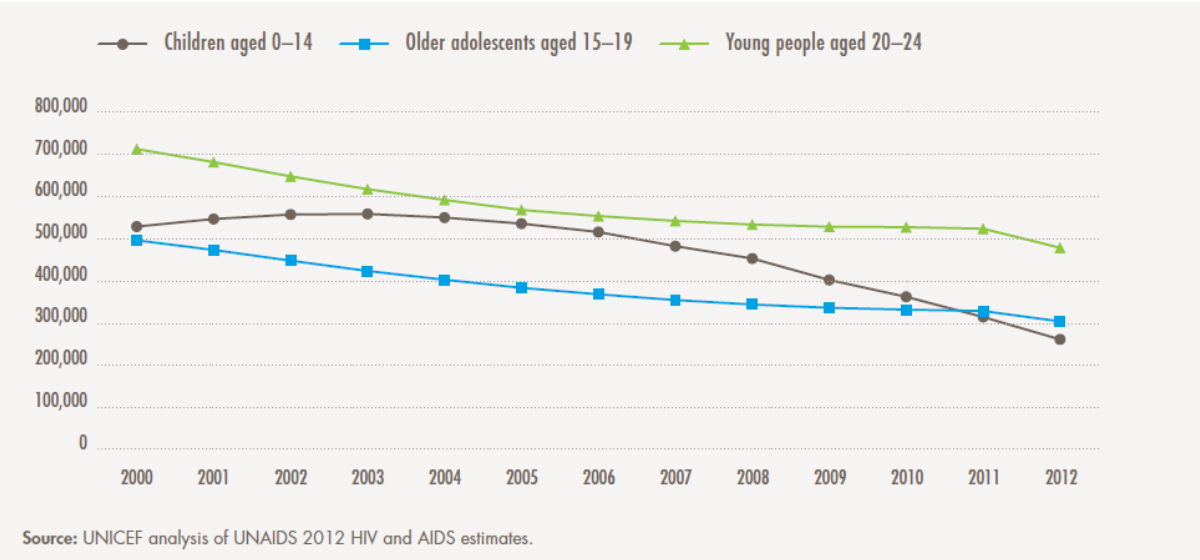
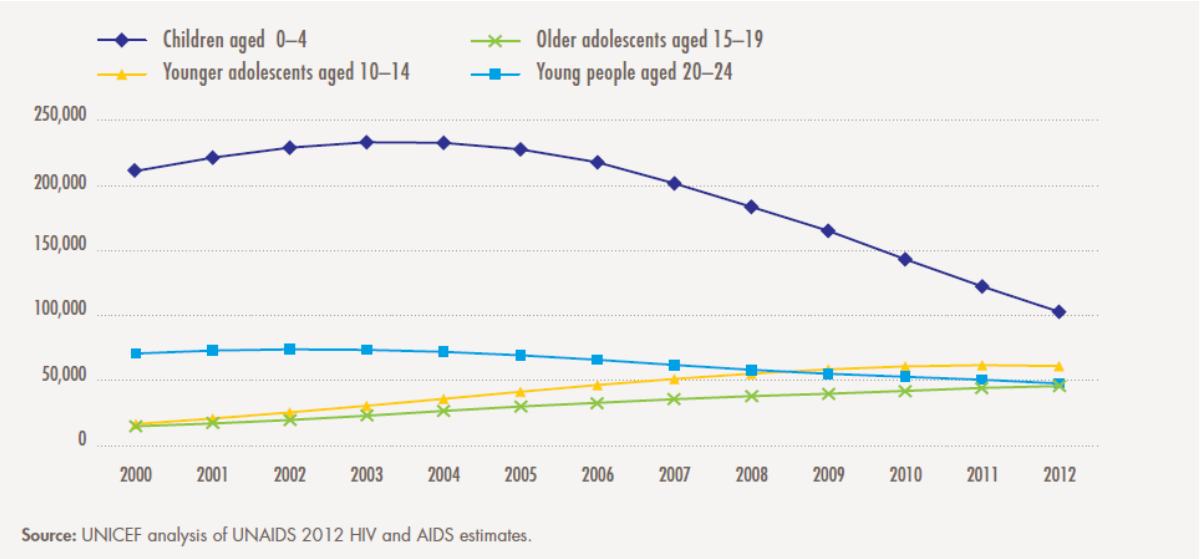


Figure 3: Estimated number of AIDS-related deaths among children aged 0–4, younger adolescents aged 10–14, older adolescents aged 15–19 and young people aged 20–24, 2000–2012



Overall, the global trends in Figures 1 and 2 illustrate declines in new infections in children, adolescents and young people. Although Figures 3 shows declines in AIDS-related deaths from 2004 onwards for age groups 0-4, and young people aged 20-24 years, AIDS-related deaths are gradually increasing among adolescents (10-19 years). The literature reports of barriers to HIV treatment access for adolescents, including increased vulnerability and may be contributing to the increased deaths among adolescents.

On the other hand, despite the slow increase in ART coverage among children aged, 0-14years, AIDS-related deaths have declined most probably due to the impact of PMTCT programmes. By lowering the levels of viral loads within populations, ART is slowing down HIV transmission [32] and directly promoting survival through treatment.

1.3. Paediatric HIV and co infections

Studies have consistently shown that, the intertwined relationship between HIV infection and other co infections, such as tuberculosis, malaria, hepatitis, Epstein - Barr virus (EBV) or herpes virus infection, have a great impact on immune response, treatment efficacy and correct diagnosis. This is particularly important in infants and young children living in resource-limited settings where disease epidemiology widely differs from western countries [36-39].

The highest TB incidence rates occur in sub-Saharan Africa and South-East Asia where antenatal HIV prevalence is high and interventions to prevent mother-to-child transmission of HIV infection are not widely implemented. The literature also documents that infants and young children of less than three years are particularly at risk of developing TB disease following exposure [40-42]. Apart from HIV infection; other important risk factors for acquisition of TB infection and disease in children include poverty, overcrowding and malnutrition. With this background, current research in paediatrics in high burden countries aims at optimizing co treatment and the use of Isoniazid preventive therapy (IPT) to cure latent tuberculosis, while working towards the identification of context-appropriate approaches for intensive case finding (ICF) [43, 44].

2.0. Overview of Uganda Health Outcome Indicators: the Health Systems Reforms

The HIV epidemic has put the spotlight once more on the health systems in high-burden resource-limited settings, including Uganda. This section, therefore, presents an overview of the health systems reforms that were pursued in the post-conflict era and their relevance to current health outcomes, the national response to the HIV epidemic and health system strengthening in the Uganda.

2.1. Background to Uganda Health Systems Reforms

Prior to independence from Britain in 1962, Ugandans depended mainly on traditional medical practice (non-western) for their health care needs. After independence, Uganda inherited a colonial health system that needed expansion in terms of capacity, redesign and strengthening to meet the health needs of the growing population. Consequently, several hospitals and health centres were built throughout the country. That initiative was accompanied by the setting up of medical training institutions, resulting in more native Ugandans being trained in the allied health sciences from within the country. However, political and economic upheavals led to the collapse of the health system and many services in the1970s and 1980s. For instance, government funding was chronically insufficient, leading to late and meager salaries for health workers, permanent shortages of drugs and supplies and worn-out health infrastructure. That situation triggered an exodus among professional health workers; and traditional medicine once more became the main form of health care [45-49].

The quest for solutions encouraged Uganda to embrace the concept of primary health care (PHC) following the WHO's Alma Ata Declaration in 1978. Like many other developing nations, Uganda adopted a 'selective PHC' due to scare resources. However, implementation of the PHC concept was not as effective as expected and only minimal improvements were achieved in health services delivery [50-52].

Even though, the HIV epidemic affected almost every sector, the health sector bore the brunt. Like in other countries with high HIV burden, the health system in Uganda was faced with increased demands for services, translating into increased workloads for health workers, aggravating an existing HRH crisis, and increased pressure on health infrastructure. On the other hand, the GHIs brought in significant resources, including funding into the health

sector, but such funds were often channeled through projects that operated parallel health systems [14, 30, 53, 54].

2.2. Rationale for the health systems reforms

Indeed, the poor health indicators in the 1980 /90s and inefficient use of scarce resources prompted the government of Uganda (GoU) and development partners to embark on an extensive health system reform to improve the performance of the sector. For example, a large proportion of the government budget (66 % in 1999/2000) was allocated to large hospitals and the central ministry of health (MoH), whose activities did not benefit the district health facilities providing primary care for the large population of the rural poor [55]. Although international donors provided the greater part of development assistance to Uganda, donor projects had high overheads, focused on investment goods, and were inefficient in providing basic health care inputs. User fees did not raise appreciable revenue as intended, on the contrary, they became a significant barrier to the poor in accessing health care services, and exemption policies failed to protect the vulnerable populations [47]. Additionally, the disproportionate allocation of health budgets with urban biases left meager funds for basic health care inputs such as medicines, health workers' salaries and health facility maintenance in the rural areas, where ~84% of the population lives. Furthermore, the HIV epidemic exposed the fragile linkages and weaknesses within the health system and presented another reason for health systems strengthening [56-59].

2.3. The Uganda Health Systems Reforms

The reforms were implemented from the year 2000 and included the following:

-The sector-wide-approach (SWAp) reform that was characterized by government ownership and a holistic platform for the coordination of development assistance for the health sector with emphasis on: policy design, strategic and operational management, pooling of financial resources, overall resources allocation and common arrangements for monitoring and evaluation. The blueprint for the SWAp was the Health Sector Strategic Plan (HSSP) of 2000/01 - 2004/05, which has been revised and updated over the years [60-62]. The principles governing this reform were described in a memorandum of understanding (MoU) between the government of Uganda (GoU) and development partners. They encouraged government development partners to align their support for a common government-led 'basket funding' mechanism. The main goal of the SWAp was to improve the performance of the health systems [63-65].

-Abolition of user fees in public health facilities: this triggered an immediate increase in the utilization of out-patients' services in government public health facilities as well as private-not-for-profit (PNFP) facilities [66];

-The Medicines' reform improved management systems, particularly in the finance, purchase and supply of drugs and medical supplies [67];

-Public-private partnerships reforms brought on board private providers and harnessed some of the potentials of the private health sector [68];

-Decentralization of health services delivery reform: It led to more resources and capacity building to strengthen district health systems [69];

-Improved resources allocation: larger shares of resources were allocated to district PHC services, as well as PNFP providers [55];

-Health financing reform: donors switching from project-based funding to budget support and less reliance on user fees [47, 70];

-Political leadership: the roles of the president, ministers of health and finance in working towards and through the reforms [47].

2.4. Results of the health systems reforms in Uganda

Overall, the findings from evaluating the impact of the reforms suggest that there have been improvements in all four key functions of the health systems according to the WHO definition; notably, stewardship, service delivery, resource generation and financing. As an illustration, GoU budget for the health sector increased by a modest 18% during the reforms [47].

Apart from being a reform on its own, the SWAp served as a catalyst that provided the needed platform, management systems, processes and mechanisms to facilitate the other reforms that were launched concurrently in the country. Hence, it could be argued that, the SWAp may have contributed directly and indirectly to the post-reforms health outcomes and indicators.

Even though, the abolition of user fees was associated with an immediate increase in the utilization of ambulatory health services at the GoU public health facilities and the PNFP units (Figure 1), there were also issues with quality of services. Drug stocks-outs were

frequent at many public facilities resulting in clients seeking health care at private facilities, including the poor. On the contrary, financial support from the GoU to many PNFP facilities enabled them to reduce user fees, to some extent, while continuing to provide some of the basic drugs and medical supplies. That scenario encouraged an increase in demand of their services. Similarly, the medicines' reform was associated with an increased demand for services. That observation has been linked with the notion that having drugs at health facilities signifies quality in the health services. However, that state of affairs was soon replaced by persistent drug stock-outs leading to shorter and fewer notable effects of the reform, particularly in the rural areas [71-73].

In terms of maternal and child health, the results showed stagnation of maternal health outcomes and minimal improvement in infant mortality rates. They fell below the United Nations' millennium development goals (MDGs) targets as depicted in Table 1 and Figure 3).

An important product of the SWAp was the tracking studies. They played vital roles in monitoring and evaluation in addition to providing evidence to inform policy and decision-making. This was particularly important to the case of the flow of funds from the central ministry to the peripheral health systems and drugs and other medical supplies.

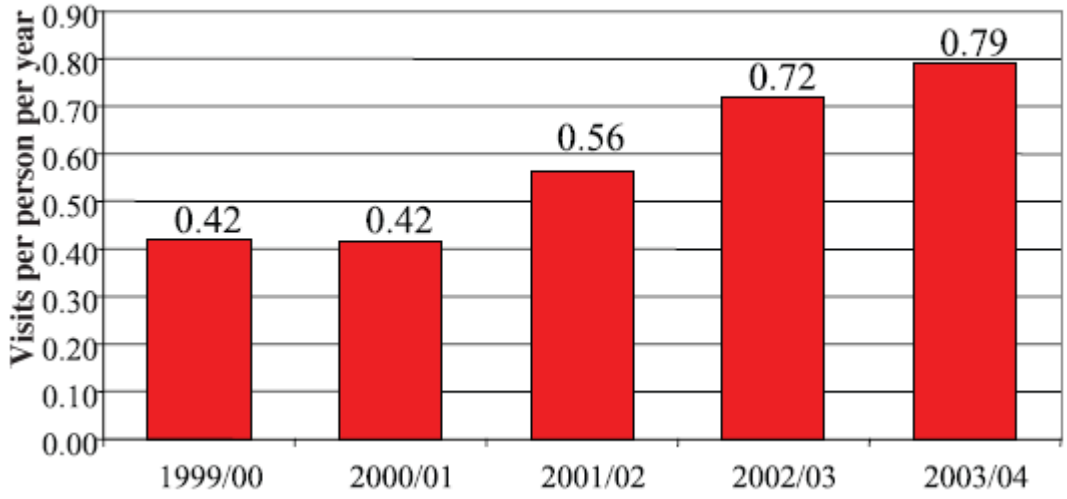
Table 1: Stagnating health outcome indicators in Uganda in the 1990s

Indicator	1995	2000	PEAP[1] Target (2005)	MDG[2] Target (2015)
Infant Mortality Rate (Deaths <1 year per 1000 live births)	81	88	68	41
Maternal Mortality Rate (Deaths per 100,000 live births)	527	505	345	131

Source: Tashobya et al (2006): Health Systems Reforms in Uganda: processes and outputs

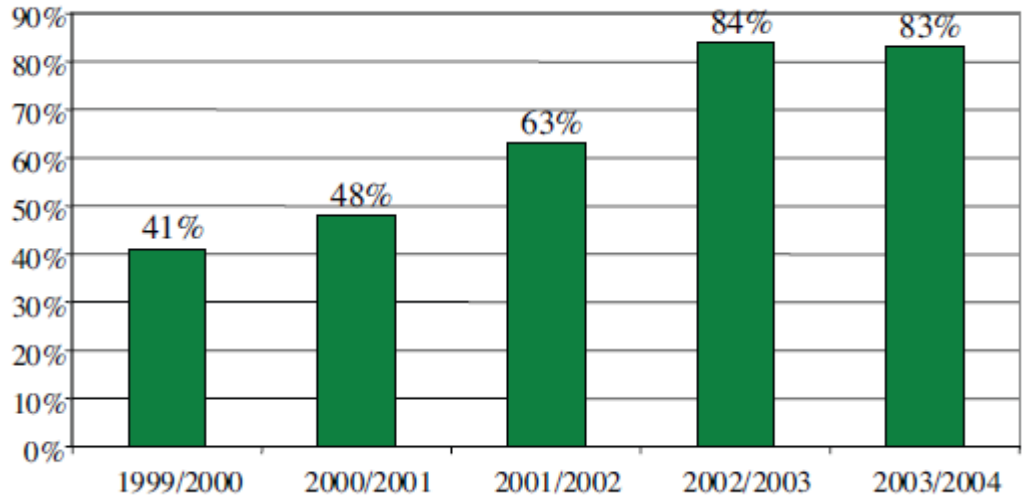
[1]Poverty Eradication Action Plan., [2]Millennium Development Goal.

Figure 4: Utilization Rates of New Outpatient Attendances in Government of Uganda and Private-Not-For-Profit Health Units



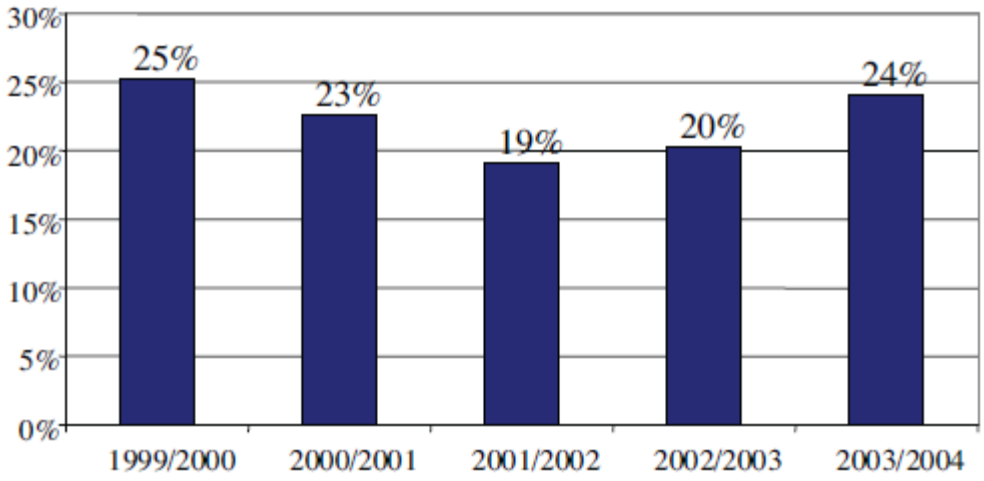
Source: Tashobya et al (2006): Health Systems Reforms in Uganda: processes and outputs

Figure 5: DPT3 Immunization Rates DPT3 for Children under One Year



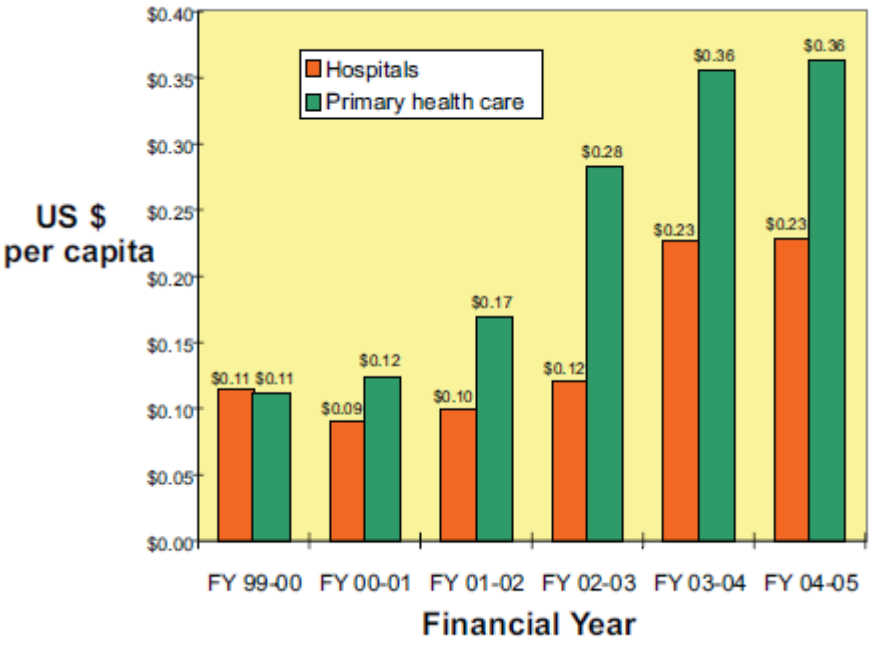
Source: Tashobya et al (2006): Health Systems Reforms in Uganda: processes and outputs

Figure 6: Proportion of Babies delivered in Government and Private-Not-For-Profit Health Units



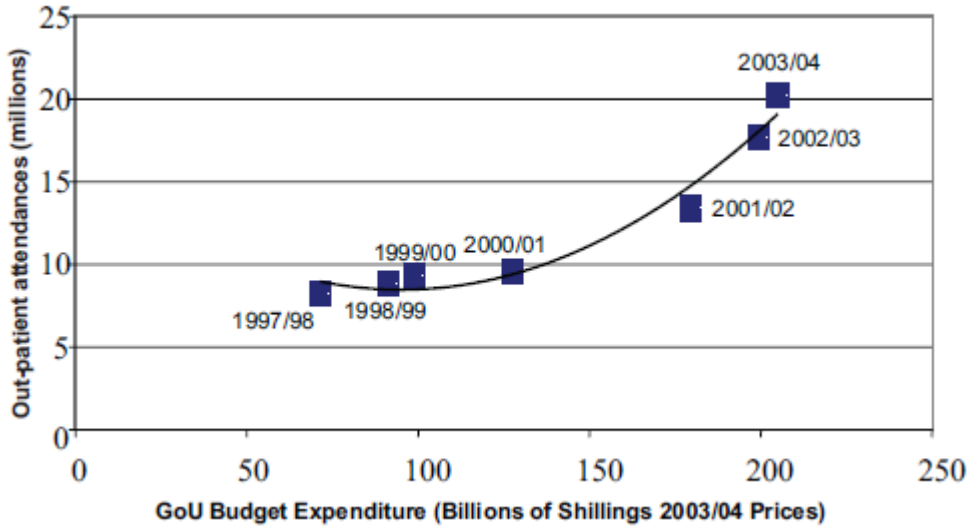
Source: Tashobya et al (2006): Health Systems Reforms in Uganda: processes and outputs

Figure 7: Government of Uganda Budget Allocations for Medicines



Source: Tashobya et al (2006): Health Systems Reforms in Uganda: processes and outputs

Figure 8: Government of Uganda Budget Expenditure and Total Outpatient Attendances



Source: Tashobya et al (2006): Health Systems Reforms in Uganda: processes and outputs

2.5. Uganda Health Systems Outcomes: a decade after reforms (2013)

- Per capita out patients department (OPD) utilization rate was 1.1 above the annual HSSP target of 1.0 but less than the 1.2 in 2011/12.
- According to the Uganda Demographic Health Surveillance of 2011, Maternal Mortality Ratio (maternal deaths per 100,000 live birth) increased from
- 435/100,000 to 438/100,000
- 31% of pregnant women attended 4 ANC sessions in 2012/13. HSSP target 55% for 2012/13.
- 41% of pregnant women delivered in health facilities. HSSP target 65% for 2012/13.
- Infant Mortality Rate (per 1,000 live births) was 54
- 87 % of children less than one year old were immunized with 3rd dose pentavalent vaccine (DPT3). HSSP 2012/13 target (85%)
- 13.5% (228) of health facilities experienced drug stock-outs in 2012/13

***Source**: MoH Uganda, Annual Health Sector Performance Report 2013*

2.6. Have the reforms contributed to improve health outcome indicators?

Approximately 10 years after the health reforms, many of the indicators listed above have maintained positive trends compared to the baseline values reported in Table 1 and Figures 1-4 above. For instance, per capita OPD utilization rate has increased steadily from 0.42 in

2000 to 1.1 in 2012/13. Similarly, DPT3 for children under one year old increased from 41% in 2000 to 87% and above the HSSP target of 85%; the proportion of pregnant women who delivered at health facilities increased modestly from 25% in 2000 to 41% in 2012/13 but 24% points below the HSSP target of 65%. Although maternal mortality rate decreased from 505/100,000 live births in 2000 to 435/100,000 live births in 2011/12, it increased to 438/100,000 live births in 2012/13. Infant mortality rate stood at 88/1000 live births in 2000 and decreased slightly to 54 in 2012/13. The pace of decline remains well below the targets for MDG 4 and 5.

With respect to drug stock-outs, 13.5% of health facilities experienced drug stock-outs in 2012/13.

Generally, the recent health outcome indicators may be described as a 'mixed bag' when compared to the post-reforms outcome indicators of 2000/01. As shown above, the changes in maternal deaths and infant mortality have been very minimal and at a very slow pace or near stagnation.

Additionally, some gains of the reforms appear to have been reversed over the years. Recent studies report of the re-introduction of user fees at some public health facilities and persistent shortages of drugs and basic supplies virtually at all levels of the health systems [70, 74-77]. With these findings, one wonders if the Medicines reforms and decentralization of health services to district and sub-district levels have been effective.

Undoubtedly, the reforms established policy frameworks, developed partnerships, and brought about coordination, important mechanisms, and development of processes and systems. As an illustration, the SWAp processes literary 'redefined' health funding as they promoted coordination of international donor and partner aid for government budget support and overall resource allocation to the health sector. These measures and the resultant synergies may have facilitated the implementation of the different specific reforms.

In conclusion, the health reforms in Uganda raised awareness and provided vital inputs for the health sector resulting in improvements in some service delivery as reflected in some health outcome indicators. However, recent studies show that maternal and infant mortality figures have not improved much after the reforms. In addition, some gains of the reforms appear to have been eroded.

2.7. Have the Uganda Health Reforms contributed to the National AIDS Response?

While the global response to the HIV/AIDS epidemic may have exposed the weaknesses of the existing health systems, it has also galvanized concerted efforts to deal with systemic challenges such as HRH crisis, physical infrastructure, drugs and supply chains as well as health financing and health information systems [56].

Uganda adopted a multi-sectoral approach to its national AIDS response. The response combined prevention, care and treatment with impact mitigation in line with the global and African AIDS responses [3, 78-80]. The impact mitigation component focused largely on poverty eradication programmes as the impact of the epidemic is experienced most severely at the household level where it exacerbates poverty [81-83].

The health systems reforms established policy frameworks, brought in huge funding mainly from international donor sources and developed systems as described earlier. These in turn facilitated the establishment of the pillars of the national AIDS response. In a chronological order, the pillars included:

- 1986, formation of the AIDS Control Programme,
- 1987, creation of the AIDS Support Organisation (TASO) and other community home-based programmes (CHBC) such as Kitovu Mobile HIV programme and Nsambya Home Care to support people living with HIV,
- 1988, establishment of a number of research collaborations between Ugandan and international university partners including Medical Research Council Uganda; the Makerere University- Johns Hopkins University Research Collaboration and the Joint Clinical Research Centre (JCRC) to conduct HIV/AIDS-related research.
- 1992, establishment of the AIDS Information Centre (AIC) to provide voluntary counselling and testing services; the Uganda AIDS Commission (UAC) to coordinate the multi-sectoral response to HIV and the multi-sectorial National Operational Plan and HIV/AIDS Policy Guidelines,
- 1993, formation of the multi-sectoral National Operational Plan (NOP) and HIV/AIDS Policy Guidelines,
- 1997, development of a Five-year National Strategic Framework (NSF) for HIV/AIDS that was extended to 2005 and

- 2007/2008- 2011/2012, Implementation of the national HIV prevention strategy, within in the National Strategic Plan for HIV/AIDS (NSP) [9, 11, 18].

According to the UNAIDS' Benchmark study and some reports, the Uganda AIDS response has made appreciable progress, but much remains to be achieved. A few selected response outcomes show that in 2012, Uganda had ART coverage of 33% for children aged less than 15 years, 70% among adults and 72% for PMTCT (please see under HIV burden of disease, p29.paragraph 3). Safe male circumcision (SMC) coverage was 11% as of December 2012 [31, 78, 84].

Generally, the Uganda National AIDS response has been characterized by strong political commitment, supportive policies, open dialogue, involvement of religious and community leaders, multi-sectoral and decentralized interventions and coordination, with emphasis on the involvement of local communities, investment in research and impact mitigation [85, 86].

Nonetheless, the gains of the Uganda National AIDS Response may be threatened by several factors. The recent Uganda Demographic and Health Survey (UDHS) in 2011 showed that HIV prevalence has increased from 6.4% in 2004 to 7.3% in 2011 [87]. This observation shows that treatment access has improved and even if incidence remained the same, or reduced a bit, prevalence would still go up as more people are surviving because of ART. Additionally, there may be issues with behavioural change campaign activities, and lack of resources. Although the policies appear elaborate, the national AIDS response does not seem to have adequate provisions for key populations such as commercial sex workers (CSW), men who have sex with men (MSM), prisoners and injection drug users (IDU) who have the potential to sustain or drive the epidemic for a longtime. There are already signs of donor fatigue especially in the wake of the global financial crises [88, 89]. As illustrated under the reforms, GOU budget increased was a modest 18%, which may not be sufficient to continue, expand and sustain the national response for a considerable period.

To conclude, the Uganda health reforms set the stage for the establishment of the pillars of the national response, which in turn facilitated the strategies used to implement the various interventions. As illustrated by the literature, significant progress has been made, but future progress will depend on greater inclusion of key populations, substantial increment in GoU budget support for the national response and dealing with the low ART coverage for children,

the slow pace of SMC, persistent shortages of drugs and medical supplies and emerging challenges.

2.8. Have the health reforms strengthened the Uganda health systems?

The WHO defines health systems strengthening as building capacity in critical components of health systems (policy, funding, human resources, service management and information and monitoring systems) in order to achieve more equitable and sustained improvements across health services and improved health outcomes [57, 90].

The results of the Uganda health reforms were associated with GoU leadership, stewardship roles, responsiveness and some improvements in service delivery all of which contribute to health systems strengthening. As shown under the national AIDS response, the reforms established vital policy guidelines, provided platforms, developed processes and mechanism and coordinated resources that resulted in the re-organization of health service's delivery.

Specifically, the abolition of user fees, decentralization of health services to primary care facilities at the district and sub-district levels, medicines reform and HRH and infrastructural development interventions seem to have promoted financial and geographical accessibility to some extent.

Nevertheless, recent studies from Uganda and elsewhere have demonstrated that, access, equity and coverage for health services remain important challenges, and that implementation of the health reforms may have inadvertently resulted in impoverishing care as well as fragmented care [91-95]. Indeed, in the absence of adequate health insurance, there are catastrophic out of pocket (OOP) health expenditures for households that on average has been quoted as 54% of total health expenditure [74, 96], for a setting where 38% live below the poverty line [47]. The recent renewed interest in project-based funding by powerful GHIs could mean reversing of international donor support for government budget and weakening of the SWAp initiatives. The re-introduction of user fees in public health facilities [59] and the persistent shortages of drugs including ARVs, anti-tuberculosis drugs and essential or basic drugs, raise questions about the effectiveness of the related reforms. Additionally, the reforms have been associated with paradoxes. As an illustration, the post-reforms results showed that, there were trained health workers who could not be employed because of some policy decisions. Moreover, in the district and sub-district levels, the reports mention a mismatch between health infrastructure development and the planning of health services, vis a vis, the availability of health workers. Consequently, there were newly constructed health

infrastructure at the sub-district levels that were underutilized [73, 97]. Finally, the data management system is weak, fragmented and unable to provide real-time warning advice [98].

In conclusion, the health systems reforms were timely and created favourable conditions that channeled a wide range of resources into the health sector and contributed to health systems strengthening in one way or the other. About a decade after the reforms, stock-outs of drugs and medical supplies and issues with access, equity and coverage for various health services remain.

The renewed interests in GHIs call for collective efforts towards effective and functional decentralization of vertical programmes such as HIV voluntary counselling and testing, ART prescription and refills, PMTCT/EID and TB services by integration at the primary care level. Such an approach could promote geographical accessibility especially for the rural majority and indirectly, universal access. However, the success of such a measure will depend among other factors on the availability of polyvalent health workers, motivated, regularly trained and supervised at the primary care level. Some of the countries that have successfully followed such a path include Swaziland, Mozambique and Cambodia [99-101].

2.9. Challenges to the Uganda health reforms

- The renewed interests in project-based funding by powerful GHIs have potentially destabilizing effects on the SWAp processes and mechanisms. The quest for rapid results could mean a return to priority disease-specific interventions that may have geographical biases. Clearly, such approaches lead to parallel systems with high administrative costs and inefficiencies, fragmentation and duplication of efforts, none of which support health systems strengthening, as defined by the WHO.
- The SWAp was a unifying mechanism and a catalyst for other health reforms, but because of this intrinsic influence it was difficult to disentangle the impacts of other reforms implemented simultaneously,
- Reliance on aid funds raises concerns about predictability and sustainability of the flow of aid funds into the country in an era of global financial crisis,
- an important limitation of the analyses of the health outcomes following the reforms was the use of intermediate measures of performance because demographic and health statistics are collected every five years in Uganda,

- another limitation was the relatively small number of indicators used in the immediate post-reforms analyses, which raises issues with extrapolation of the outcomes,
- furthermore, the zeal for the reforms seems to have waned over the years, for instance the tracking studies that tracked progress through periodic monitoring and evaluations have either stopped all together, or are being pursued with little interest.

3.0. Evolution of Complementary HIV service delivery models (CHIVSDM)

Complementary HIV service delivery models (CHIVSDM), as the name suggests are various approaches aimed at providing a wide range of support to households and communities infected and affected by the HIV epidemic. Such models may be owned by local people, for example, The AIDS Support Organization (TASO) of Uganda, local and or international faith-based organizations and local or international non-governmental organizations (NGOs) collaborating with local communities or health institutions. In terms of structural design, they may be community-based, comprehensive home-based care (HBC), community and home-based care, facility-based with community outreach clinics or various configurations dictated by their missions, visions and objectives. CHIVSDM may operate within existing health facilities, serves as bridges between traditional health facilities and community initiatives or operates as 'stand-alone' community-based models at the community levels. [83, 102, 103].

The services provided by such initiatives cover a wide range and are often dictated by their objectives and more importantly, available resources. Generally, the services include, pre-enrolment care like, VCT, pre and post-test counselling, linkage to care and treatment, follow up while in care or on treatment, psychosocial support (various form of counselling, material assistance, including food supplements, assistance for income generating ventures and orphans and vulnerable children support programmes). Additionally, some organizations provide orphans care, palliative care, and community volunteer support and participate in HIV-related research [104-106].

Apart from developing their own infrastructure or 'nesting' in traditional health facilities, an enabling feature that appears to be common to many of these CHIVSDM is their ability to use existing community resources such as public places (e.g. markets and church premises) and engaging non-professional health workers through task shifting to provide HIV/TB services. This innovative approach does not only overcome some of the shortages of health infrastructure and professional health workers, but it also provides some basic services relatively closer to communities thus promoting geographical accessibility at the same time. As illustrations, a local NGO, Uganda Cares operates an HIV clinic within the Balekudembbe Market in Kampala, while the Home Care Department of Nsambya Hospital operates outreach clinics on the premises of Christ-The-King and Ggaba parishes in Kampala under its community and home-based care HIV service delivery model. These approaches often complement their workforce by engaging PLHA as expert patients or as community

volunteers in task shifting. The mother-2-mother peer-support for PMTCT is a special case where HIV positive mothers who have experienced PMTCT are trained as mentors to support other HIV positive women in PMTCT interventions (from antenatal care through delivery to post-delivery care) by offering counselling, home visits and other forms of psychosocial support such as adherence support. The literature suggests these initiatives are culturally acceptable [99, 107, 108]) and some have been recognized both nationally and internationally with policy guidelines to support, monitor and regulate their operations. A case in point is the community and home-based care HIV service delivery model in Uganda [109].

CHIVSDM may have evolved in response to the HIV epidemic in many resource-limited settings, particularly settings with high HIV disease burdens. However, the literature presents considerable variations in their structural compositions, objectives (some overlapping), scope and reach, as shown above. These findings seem to suggest that, the various CHIVSDM may not have all evolved from a particular design but rather spontaneously with respect to contextual needs. It is also possible that some modifications have occurred over time reflecting changes in trends in service provision, as dictated by the available resources and programme objectives.

3.1. Community Home Based-Care HIV service delivery in Resource-limited settings

Community and Home-Based Care includes any form of care (physical, psychosocial, palliative and spiritual) given to the sick and the affected in their own homes and care extended from the hospital or health facility to their homes through family participation and community involvement [110, 111]. CHBC has also been defined as the care given to an individual in his/her own environment (home) by his/her family and supported by skilled welfare officers and communities to meet not only the physical and health needs, but also the spiritual, material, and psychosocial needs [103]. Generally, CHBC has been described as "the programme that offers health care services to support the care process in the home of the HIV infected person".[112].

The global scaling-up of ART has undoubtedly saved or prolonged the lives of millions by transforming HIV/ AIDS: a deadly disease into a manageable chronic condition. This positive impact comes with additional challenges for the already weak health systems in SSA. Some of the challenges include: the worsening of the HRH crisis (please see literature review on 'HRH and Task shifting' below), exacerbation of pressure on health infrastructure, and the

need for innovative healthcare delivery approaches to maintain a large and growing population of PLHA on ART for life [113-115].

The impact of the HIV/AIDS epidemic on healthcare delivery systems in SSA [116-119] has indeed demonstrated that, the traditional healthcare delivery model; the facility-based model, needs modification, strengthening and an extended service delivery capacity to provide and ensure continuity of care, for a large and growing population of PLHA in their homes and communities beyond the traditional health facilities.

In 1987, home care programmes, providing a variety of services to PLHA and their families were developed in Africa. In 1989, the World Health Organization's (WHO) Global Programme on AIDS conducted a descriptive study of six programmes selected from Uganda and Zambia. The objective of the study was to learn lessons from those programmes that could be used and adapted by policymakers and programme planners in their own settings when deciding on "their" model of home care [120].

Various configurations of Hospital-outreach HBC models were the first to be established but these were costly and unable to provide the types of non-medical services needed by large numbers of clients in the communities. Five additional models of CHBC programmes have been developed. They include : NGO-based, Faith-based, Community-rooted, Support groups for PLHAs and Self-help groups" [83].
CHBC is culturally acceptable to the communities and mitigates some important challenges such as: distance, transport costs to health facilities and the opportunity costs of time spent to seek health care at health facilities. A Cochrane systematic review [121] found in two studies from Uganda and Zambia, very high acceptability and uptake of VCT when testing and or results were offered at home, compared to the standard (facility-based testing and results). A study from Botswana [122], documented an increase in community home-based care (CHBC) following a shift from hospital care to CHBC because of the HIV/AIDS epidemic. However, there is the need for CHBC programmes to strengthen the capacity of the home and communities to care for PLHA by building on traditional family structures which support all chronically ill people [123].

The CHBC model has demonstrated the potential to extending health care delivery services beyond the traditional health facilities to PLHA in their homes and communities. This is a potential for the design of Chronic Disease Management Model in resource-limited settings,

and it is crucial that such a model integrates the management of other chronic diseases like TB, hypertension (HPT) and Diabetes in HIV/AIDS management. Various studies [124-127] have shown that, such an approach would not only minimize stigma to HIV and TB patients and improve treatment outcomes, but also avoid further fragmentation of health systems; characteristic of vertical programmes and promote Health Systems Strengthening.

CHBC may be promising but there are also significant challenges. Although recognised, currently, CHBC does not appear integrated in the government healthcare delivery systems and remains mainly an initiative of NGOs, missionary institutions and faith-based organizations with little or no support from governments, which also explains the very low coverage with respect to the burden of HIV/AIDS [128].

3.2. CHBC and Health Systems Strengthening: bridging the gaps in the health systems in resource-limited settings including Uganda

As the number of PLHA increases, the gap continues to widen between the demand for, and the availability of health care services. Relying mainly on the family and community as caregivers, community home-based care (CHBC), has become a significant contributor in the treatment, care and support of those infected and affected by HIV/AIDS [103]. **CHBC** provides an approach that goes beyond the traditional facility-based health service delivery to response to the demand of the growing population of PLHA in their homes and communities, thus, maintaining the continuum of care [56].

Infrastructural limitations have often dictated the capacity or scope of services that could be delivered at health facilities [129]. By adopting the CHBC model, communities can provide PLHA with basic services such as VCT, palliative care and ART refills through outreaches closer to their homes. Additionally, the literature demonstrates that CHBC reduces bed occupancy rates in hospitals, that in turn benefits patients and their families and contributes to decongesting the health systems [111, 130].

Appointment systems and ambulatory clinics have proven suitable and vital for stable patients on therapy.-This means that with basic infrastructure vital services could be provided and perhaps at even lower costs to clients [131]. According to the WHO Global TB Report for 2010, in ensuring community involvement in TB care and prevention, community-based approach was 40-50% more cost-effective compared to the traditional facility-based approach[132].

HRH crisis and Task shifting: as shown under the literature review on 'HRH crisis and Task shifting' below, the concept of Task shifting neither started with HIV nor is it confined to HIV. However, the HIV epidemic has made Task shifting perhaps more visible through an explosion of publications linking Task shifting in HIV care and ART programmes globally. Some of the headlines that highlighted the crisis are captured below:

1. "Help Wanted"[133],
2. "The real challenges for scaling up ART in sub-Saharan Africa"[134],
3. "Task shifting for antiretroviral treatment delivery in sub-Saharan Africa: not a panacea"[135],
4. "Scaling up access to ART programmes in Southern Africa: who will do the job?"[136],
5. "Task shifting: rational redistribution of tasks among health workforce teams: Global recommendations and guidelines"[137].

Perhaps, among other things, Task shifting may have made a difference between implementing and not implementing important HIV programmes in many high-burden resource-limited settings with critical HRH shortages.

Promoting geographical access to services: outreach services and community-based programmes are practical ways of expanding access to care at the periphery of health systems and promoting universal access at primary care levels. The CHBC takes services closer to clients and somehow deals with some of the challenges of geographical accessibility and mitigates the opportunity costs of long waiting times at health facilities [116, 138]. VCT in the communities and home delivery of results have often resulted in improvements in the uptake of those services [139].

WHO Reports and independent studies have demonstrated that **community involvements** in chronic diseases management are often associated with improvements in outcomes. Uganda is one of the 22 high-burden countries with respect to TB and provides an example. In 1990, the country implemented a community-based TB care (CBTBC) strategy to promote access to TB services. By 2002, it had achieved 100 percent DOTS population coverage. That achievement led the Ministry of Health (MoH) to formally adopt the community-based TB care (CBTBC) strategy in the country [40, 104, 140].

The CHBC framework has crucial components in the communities that promote decentralization of ART, TB and other chronic disease services in primary care. That feature makes CHBC a vital model for community and public health interventions. In 2008, the WHO described community-directed treatment of Onchocerciasis (river blindness) as one of the most successful public health campaigns ever conducted in the developing world. Recently, community involvement in Brazil's HIV treatment programmes has been reported to yield visible impacts [23, 141].

Several studies in Uganda and other high-burden resource-limited settings have shown that HIV-infected patients managed in community-based approaches were more likely to achieve viral suppression, have better adherence, better retention and survival outcomes, when compared to patients managed at hospitals. In addition, community-based programmes were slightly more cost-effective compared to patients treated in a hospital setting [142].

From the foregoing points and findings from Paper1, clearly, the CHBC model has the potential to integrate and decentralize interventions to support community or public health approaches, which is crucial for both chronic disease management and health systems strengthening in resource-limited settings [111, 143].

4.0. Literature review on HRH crisis and Task shifting

Africa has 12% of the global population, 24% of the global disease burden, only 3% of the global workforce and commands less than 1% of the world health expenditure [144, 145]. Africa remains the global epicentre of the AIDS epidemic, and sub-Saharan Africa (SSA) with an adult HIV prevalence of 5 % (4.7%-5.2%) is the region with the largest AIDS burden [31, 146].

The 2006 World Health Report estimated that there was a global health workforce deficit of more than four million trained health care workers. The deficits in many of the countries of SSA, and in parts of Asia and the Americas were critical. The report also documented that thirty-six of the fifty-seven countries with critical shortages of HRH were in SSA. For instance, from 2005-2010, Africa had a health workforce density of 2 physicians and 11 nurses and midwives per 10,000 of people, and Malawi's shortage of health workers was so extreme that there was only around one doctor for every 10,000 people [144, 145, 147].

In 2000, the United Nations Millennium Development Goals (MDGs) initiative was set into motion [148, 149]. The initiative soon triggered a global reaction among world leaders. Subsequently, in 2001, leaders from 189 United Nations member states met in an historic session of the United Nations General Assembly to adopt the Declaration of Commitment on HIV/AIDS. The declaration aimed at providing comprehensive, time-bound targets for the delivery of effective HIV prevention, treatment, care and support needed to stop as well as mitigate the impacts of the global epidemic by 2015 [15, 150]. With time, the declaration galvanized the transformation of international response into Global Health Initiatives (GHIs) [3].

Consequently, in December 2003, the WHO's "3 by 5" initiative was also launched[90]. Other partners such as the Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM) [151, 152], The United States President's Emergency Plan For AIDS Relief (PEPFAR) [153], and The World Bank's Multi-country AIDS Programme for Africa (MAP) [16] soon followed suite in providing resources for the mass scaling-up of ART/PMTCT services. Primarily, the GHIs aimed to save and prolong the lives of the millions in need and in so doing mitigate some of the devastating impacts such as the increased death rates, morbidity and escalating demands for health services in many countries. Certainly, the global scaling-up of ART is the single largest global health intervention; there are simply no precedents on a similar scale in SSA ([54, 154].

Years after the implementation of some of these GHIs, evaluation studies have shown that many sub-Saharan African countries were not on track with respect to many of the set targets [90, 149, 155]. Apart from weak health systems and lack of absorptive capacity, prominent among the reasons for the failures was the lack of HRH. There were just not enough skilled health workers to do the job. Many SSA health systems currently do not have the capacity to provide the minimum health care delivery packages let alone the added burden from HIV/AIDS [136, 156]-. Studies have also shown that there have been significant advances recently in terms of global AIDS funding and that the real challenge was the severe HRH crisis facing health systems in SSA [114, 157, 158].

4.1. SSA and the HRH situation

The WHO's "Health for ALL" standard of one doctor per 5,000 population is not met by thirty-one SSA countries and at least twenty of those countries have no more than one doctor per 20,000 population. The WHO has documented that thirty-six of the fifty-seven countries with critical shortages of health workers are in SSA. Some of the reasons for the critical shortages of professional health workers include: limited capacity to train the needed health staff [61], HRH policies that are not coordinated, often leading to inconsistencies such as multiple staffing norms, varieties of contradicting organizational charts, uncoordinated in-service training and conflicting instructions to health training institutions [47, 159] and maldistribution. Also notable among the policy defects are policy decisions, which do not take into account the dynamics of the global labour market [160]. Furthermore, the adoption of macroeconomic policies under structural adjustments reforms often associated with widespread capping of public sector spending placed added limitations on the training and recruitment capacities of MoH facilities in the 1980s and 90s . It is thus paradoxical to find trained health workers unemployed in some countries with severe or critical shortages of health workers [145, 161]. In terms of attrition, brain drain accounts for between 1% to over 70% of African health professionals abroad [162], poor salaries, burnt-out syndrome and generally demotivating working environments are other reasons for health workers leaving the health sector [46].

Adding to the existing chronic shortage of health workers are the impacts of HIV/AIDS on the health workforce. They include: increased workload from dwindling numbers of health workers from death, absenteeism from work for own sickness or attending to sick relatives or funerals and early retirements from fear of contracting HIV from the workplace [163, 164].

Another twist to the HIV/AIDS-HRH crisis in SSA is the fact that the global scaling-up of ART has transformed HIV/ AIDS; a deadly disease into a manageable chronic condition ([119, 165-168] with implications not yet fully known for the health systems involved. Although HIV-related admissions may have decreased compared to the late 1980s to early 2000s, the population of PLHA on chronic care keeps growing in many SSA countries [169-171]. Clearly, the health workforce may not be keeping pace with the increasing demands for health care delivery services.

4.2. How many health workers are required to scale-up ART?

Based on doctor-centred ART delivery model (the model by default in most countries), copied from industrialized countries, various studies estimate that SSA will have to triple its current workforce in order to come close to reaching the Health Millennium Development Goals [172, 173]. Kurowski et al comment that, Tanzania and probably many low-income countries in SSA will require human resources far in excess of the number likely to be present in 2015 for the scaling-up of priority interventions to achieve health improvements similar to the Millennium Development goals [173, 174]. For Zambia and Mozambique, it was projected that to scale –up ART to all clinically eligible within the next ten years or so would require, two and four times as many doctors, respectively, as their current total stock of doctors [134]. These estimates clearly demonstrate that a doctor-centred ART delivery model is labour intensive and not feasible for most SSA countries with high HIV/AIDS burden and severe or critical health worker shortages.

4.3. What are the proposed solutions for the shortages of HRH?

The proposed solutions include: increasing the numbers and capacities of the training institutions [175-177], increasing recruitments of trained health workers, introducing retention schemes and capacity building programmes of health workers and importation of foreign health workers from abroad [178, 179].

Another option is utilizing the available human resources for health through the delegation of tasks or task shifting. Compared to the other proposed solutions, task shifting is relatively rapid. For instance, it takes approximately 6 years to train a batch of doctors and 3 to 4 years to train a batch of nurses in Uganda, while the epidemic rages on [180]. From the estimations of the required human resources for the scaling-up of ART, the proposed solutions and the current human resources crises, there is certainly the need for an alternative ART delivery model, which should be relatively less labour intensive and context specific [181].

4.4. In practical terms, what are the high burden countries doing to scale-up ART/PMTCT?

The WHO 2007 Report shows that more than 25 sub-Saharan African countries are already implementing task shifting as a pragmatic response to dealing with their HRH crisis and to varying degrees in the provision of HIV/AIDS services as well as general health care delivery services [137, 182, 183].

4.5. What is task shifting?

The WHO defines task shifting as a process which involves the rational redistribution of tasks among health workforce teams whereby specific tasks are moved, where appropriate, from highly qualified health workers to health workers with shorter training and fewer qualifications in order to make more efficient use of the available human resources for health [182]. Task shifting thus expands the pool of HRH and in so doing increases access to services. Reorganization and decentralization of services using task shifting could promote universal access through increasing access to services at the peripheral levels of care [182]. From the existing evidence task shifting can be classified broadly into four types, as follows:

Task shifting type I – The extension of the scope of practice of non-physician clinicians in order to enable them to assume some tasks previously undertaken by more senior cadres (e.g. medical doctors).

Task shifting type II – The extension of the scope of practice of nurses and midwives in order to enable them to assume some tasks previously undertaken by senior cadres (e.g. non-physician clinicians and medical doctors).

Task shifting type III – The extension of the scope of practice of community health workers (often called non-professional health workers or lay providers), including people living with HIV/AIDS, in order to enable them to assume some tasks previously undertaken by senior cadres (e.g. nurses and midwives, non-physician clinicians and medical doctors).

Task shifting type IV – People living with HIV/AIDS, trained in self-management, assume some tasks related to their own care that would previously have been undertaken by health workers.

In the classification of task shifting, the defining factor for task shifting types is the cadre that assumes the new task. For example, any extension of the scope of practice of

nurses and midwives is defined as task shifting type II [182]. Figure 9 below provides a graphic illustration of the concept of task shifting.

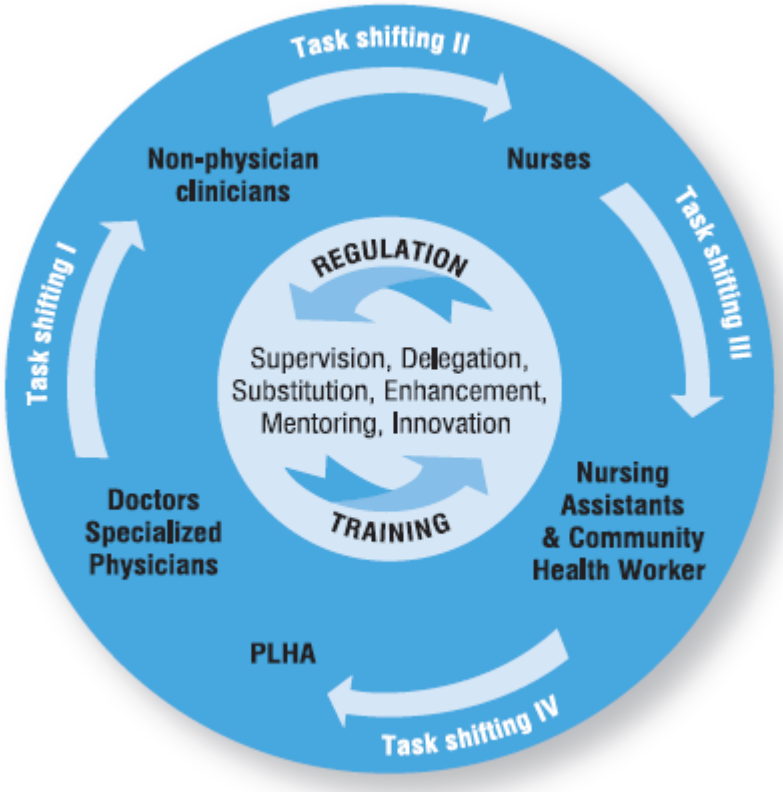


Figure 9: **Graphic illustration of the concept of Task shifting to expand the pool of human resources for health**
Source: WHO 2007

4.5.1. Task shifting type 1:

The Ministry of Health (MoH) in Zambia scaled-up HIV/AIDS care and treatment services at the primary care clinics using predominantly Non-physician clinicians [184, 185]. Non-Physician Clinicians play crucial roles in the decentralization of ART. They have been trained to diagnose, prescribe, initiate and follow up patients on ART in Ethiopia, Kenya, Malawi and Uganda [104, 147, 186, 187].

4.5.2. Type shifting type II:

Shifting ART initiation to nurses has been central in the rapid scale-up of HIV treatment and care with high coverage at the primary health care level in the Lusikisiki sub-district (Eastern Cape Province of South Africa). The National Strategic Plan predicted that by 2011 most people in need of ART would receive their treatment from nurses at the primary healthcare clinics and not from doctors in hospitals [188]. In 2004 when the health authorities in

Botswana realized that a physician-centred ART delivery model was not feasible, a nurse-centred ART delivery model: where ART-trained nurses clinically manage stable ART patients was piloted in two projects. The findings show increased access reflected in shortening of waiting lists [137, 158].

4.5.3. Task shifting type III:

Medecins Sans Frontieres (MSF) in the Thyolo district of Malawi piloted community participation in HIV care, treatment and support by involving community caregivers. Their findings have shown clinical outcomes to be better when community caregivers were involved in the provision of services [189]. Partners in Health (USA) and a Haitian Organization (Zanmi Lasante) working together to provide ART have integrated ART into the primary health care delivery services. Using the Directly Observed Therapy Short Course (DOT) approach for both TB and ART patients, community health workers (accompagnateurs) provide ART at the community level with supervision. Patient outcomes have been reported to be good and adherence, impressive [137, 190].

4.5.4. Task shifting is not new

Historically, many nations both rich and poor have used health care providers who were not trained as physicians but who are capable of many of the diagnostic and clinical functions of medical doctors. Depending on the countries or regions and roles of the health cadres involved, they may be known as Non-Physician Clinicians, "mid-level cadres", "substitute health workers", Community Health Workers and others [137, 182, 186, 187, 191-193]. The Alma Ata Declaration of 1978 [50], the Kasongo project of the Institute of Tropical Medicine (Antwerp) and the expert patients concepts provide further examples of the involvement of non-professional health workers in the provision of health care [165, 194, 195].

4.5.5. Why the renewed interests in task shifting practice?

Task shifting is promising and appears most pragmatic given the current HRH crisis and HIV/AIDS scenario in SSA [135, 179, 196]. The renewed interests call for research to explore further the potentials of task shifting as an innovative strategy in finding solutions to the mismatch between HRH crisis and health services utilization in SSA.

4.5.6. In summary, what can be said about Task shifting?

Task shifting may be promising, but that potential will be better harnessed when there are clearly established career development pathways for non-professional health workers to progress through further training, regular supportive supervision, some form of remuneration

and a formal recognition. In that way, task shifting may serve as an entry point for interested but non-professional health workers to become professional health workers. Consequently, task shifting may serve the dual purpose of expanding the pool of non-professional as well as professional workers, while promoting the rational redistribution of the few skilled professionals.

5.0. The Tukula Fenna Project setting:

The TFP is a paediatric HIV programme that was set up at the Home Care Department of St. Raphael of Francis Hospital (Nsambya Hospital) in Kampala, (Uganda) in 2003. Uganda is a land-locked country on the equator. It is located in East Africa and shares frontiers with Kenya to the east, Tanzania, Rwanda and Lake Victoria to the south, and the Democratic Republic of Congo to the west and South Sudan to the north (please see Figure 10 below). According to the 2013, State of Uganda Population and the UNFPA Reports, Uganda had a population of 35.4 and 37.6 million respectively. Approximately 51% were children aged less than 15 years, 33% were adolescents aged 0-19 years and the youth (people aged < 30 years made up 78%. The average annual population growth rate was 3.2% and 3.3% respectively. About 84% of the population lives in rural settings. Kampala, the capital city has about 2.0 million inhabitants. The gross domestic product (GDP) was (US $) 533 [197, 198]. HIV, Malaria and TB are major public health problems for the country.

Figure 10: Map of Uganda

Source: World Atlas (www.worldatlas.com)

St. Raphael of St. Francis Hospital (Nsambya Hospital) is a Catholic Missionary hospital located in the Nsambya area of Kampala. It belongs to the private-not-for-profit (PNFP) health facilities coordinated by the Uganda Catholic Medical Bureau (UCMB). It is a tertiary referral hospital and offers services in all the basic specialties including primary health care.

Figure 11: Front view of Nsambya Hospital, Kampala, Uganda

5.1. Nsambya Hospital, Home Care Department (Nsambya Home Care)

The Home Care Department; popularly called Nsambya Home Care (NHC) is dedicated to disease control (vertical) programmes (HIV, ART, PMTCT/EID and TB). Although NHC has a management team to ensure effective management and coordination of the vertical programmes, the hospital administration provides oversight. Thus, the arrangement between the Home Care Department and the hospital provides an example of integrated vertical and horizontal health systems at the same health facility (hospital) effectively operating within the general health system of the country. Whereas this arrangement may not be designed, it provides a feasible contextual adaptation to the health needs of the country. The Mulago hospital complex and many large and referral hospitals with collaborations running vertical programmes provide further examples, suggesting that both systems are necessary [199, 200].

Figure 12: New building for the Home Care Department of Nsambya Hospital (NHC) supported by the TFP.

Figure 13: Inauguration of new TB clinic provided by TFP to strengthen HIV/TB co infection management in the Nsambya CHBC model.

5.2. Community involvement in Community Home Based-Care:

Community involvement and linkage is one of the crucial pillars of the Nsambya CHBC model, and different methods have been employed to achieve that. They include a community

volunteer network, training sessions and workshops for community leaders, media involvements like announcements in churches and mosques and other public places, AIDS awareness campaigns and the NHC music dance and drama (MDD) club. It may be worth noting that some members of the volunteer network and the MDD club are expert patients who play roles in the fight against HIV in the communities. The volunteer network is made up of 50 individuals carefully selected from their communities of residence to cover the entire catchment area of the model. They are trained in basic health care and communication skills and assisted with bicycles and a mobile phone network to facilitate their work. Their primary role is working closely with the multidisciplinary team (counsellors, social workers, nurses, and sometimes clinicians) from the department, traditional leaders, religious leaders, and local council (local government) leaders to sensitize and mobilize communities to access HIV services. Additionally, community volunteers do home visits, track defaulting clients (HIV and TB), link them back to care and refer clients from the communities to the outreach clinics as well as the department for the needed services. Other roles of the volunteers include working with counsellors and social workers to coordinate and provide PSS to identified clients as well as mobilizing clients and caregivers for various workshops. The volunteers also perform various tasks at the ambulatory and outreach clinics from time to time. As a reward, they receive monthly stipends as well as financial assistance in the form of starting capitals for income generating activities. One is aware of the debates surrounding stipends for "volunteers". However, it is argued that, in a setting where a significant proportion of the population lives below the poverty line [201], where the traditional social safety nets may have developed "holes" [202], and HIV/AIDS has impoverished many [82, 203, 204], perhaps stipends and financial assistance for income generating activities are the way forward. In the Nsambya CHBC experience, these measures have somehow reduced the turnover of the community volunteers over the years. Indeed, a recent analysis of data on the volunteers showed that 46% of them have served the Nsambya CHBC model since the volunteer programme started, 11 years ago. From interactions with colleagues operating similar programmes, this duration may be slightly longer than observed for many similar programmes in and around Kampala. Perhaps, it is time to review the label "volunteer" in this context.

Apart from the primary function of linking the department to the communities in the catchment area, over the years, community involvements have indeed extended the reach of the model significantly. Additionally, it has been observed that as more communities got

sensitized and engaged the level of stigma also reduced considerably. From the community perspective, the volunteers can be seen as the 'front-liners' of the model.

While in the communities, the multidisciplinary team supervises the volunteers, carries out advocacy, does VCT and provider initiated HIV counselling and testing. The team also supports the satellite or outreach clinics, which were designed to decentralize basic services to the communities and thus promote geographical access to services at the periphery. Of note, when the outreach clinics are utilized they ease congestions as well as reduce waiting times at the ambulatory clinics at the department. The community activities often target pregnant women, OVC, men and groups considered hard to reach.

Figure 14: **Renovation of Ggaba outreach clinic for decentralization of HIV/TB services into the communities (work-in-progress)**

Figure15: Inauguration of Ggaba outreach clinic, an important outreach facility for surrounding communities.

6.0. History and evolution of the Tukula Fenna Project

6.1. Background of Tukula Fenna Project

Associazione Casa Accoglienza alla vita "Padre Angelo" or "HOUSE FOR LIFE, Father Angelo" is an Italian Health NGO and a partner to the Home Care Department of Nsambya Hospital. Prior to its registration as an NGO in 2009, "HOUSE FOR LIFE" together with other Italian partners; in particular, PENTA Foundation and University of Padova, Department of paediatrics worked to establish the children's HIV programme at Nsambya Hospital, Home Care Department in 2003. That collaboration was established through a **memorandum of understanding (MoU)** aimed at supporting the hospital in the fight against HIV particularly in children and adolescents, orphans and vulnerable children (OVC) and their families in Kampala and three surrounding districts (Mukono, Wakiso and Mpigi). The programme was then called the "PCP Project" because the initial intervention was providing Cotrimoxazole prophylaxis against Pneumocystis Carinii pneumonia (PCP). As more resources including expertise became available over the years, the project evolved into a full-blown HIV programme for infants, children and adolescents as well as their families and caretakers. The name "PCP" became inappropriate and was replaced by "Tukula Fenna", which in Luganda means "growing up together". The TFP provides comprehensive HIV care, treatment and PSS and operates at the Home Care department of Nsambya Hospital, Ggaba Parish Outreach Clinic and 3 other outreach clinics in and around Kampala. To date, approximately 2,100 infants, children and adolescents have ever been enrolled into care, about 1140 are active in care and about 60% are receiving antiretroviral therapy (ART) Figure 16 below is a flow chart showing the number enrolled into care, those active in care, those on ART, lost to care and deaths, since the project started.

6.2. CORE Project Activities and Services include:

i. HIV education, counselling and testing of infants, children and adolescents
ii. enrolment of infants, children and adolescents into the programme
iii. screening children for TB and enrolling positive ones into the TB programme
iv. provision of Isoniazid prophylaxis to HIV infected children after screening to exclude active TB
v. provision of Septrin prophylaxis for all HIV positive children
vi. provision of nutritional supplements for all children in care
vii. psychosocial support for children and their families and caretakers

viii. antiretroviral therapy (ART) for medically eligible children
ix. monthly clinical and laboratory follow up of the children
x. home visits for defaulter tracing, monitoring adherence to ART and anti TB treatment, family therapy support, adherence support counselling and psychosocial support for Orphans and Vulnerable Children
xi. in-patient care (paying medical bills of children admitted to Nsambya Hospital) whose caregivers cannot afford
xii. workshops for children and guardians to provide health education on a wide range of health issues and promote adherence to ART
xiii. early infant diagnosis (EID) and
xiv. adolescents' transition clinic activities
xv. research studies

The literature review has shown that there are relatively few paediatric HIV services in Uganda, translating into limited access for children and vulnerable populations like orphans and vulnerable children (OVCs). In comparison to adults, HIV-infected children are an underserved and understudied population [205-207].

6.3. Aims of the Tukula Fenna Project:

To contribute to paediatric HIV services in Uganda, and conduct research studies in children and adolescents infected with HIV to improve their management.

6.4. Objectives of the Tukula Fenna Project:

To provide comprehensive and quality paediatric HIV services, for HIV-infected infants, children and adolescents and their families in four districts in Uganda, including Kampala, Mukono, Wakiso and Mpigi and

To conduct relevant HIV-related research studies that can inform and improve operations and contribute to scientific knowledge in the field of HIV paediatric management in a resource-limited setting (Uganda).

6.5. Research

The types of research are detailed below:

6.5.1. Operational research:

This operational research focused on the following broad themes:

- Retention in care,
- Survival on ART,
- Attrition and LTFU,
- Treatment failure,
- Dried blood spots (DBS) and virological outcomes among children on ART: potential for operationalization of virological monitoring using DBS in resource-limited settings

6.5.2. Specific study on HIV-EBV co infections in children and adolescents in the Tukula Fenna Project

7.0. Objectives of this thesis:

7.1. Main objective:

To contribute to improvement of paediatric HIV management through operational research in the context of a community home –based care model, in a resource-limited setting (Kampala, Uganda)

7.2. Specific objectives

a) To explore factors associated with treatment failure among children receiving ART in two ART programmes one in Mozambique and the other in Uganda (**Paper 4**)

b) To investigate attrition and lost to follow up and their baseline predictors among children and adolescents in a community home-based care ART programme in Uganda (**Paper 2)**

c) To examine the effects of the Nsambya Community Home-Based Care Model on National HIV and Tuberculosis management and health system strengthening in Uganda (**Paper 1**)

d) To compare retention and survival among HIV-infected children and adolescents in two different ART delivery models in Kampala; the Nsambya Community Home-based Care of Nsambya Hospital, and the Facility-Based Family-Centred Approach of the Makerere University-Johns Hopkins University Research Collaboration (**Paper 3)**

e) To explore HIV-EBV co infection in children and how it influences HIV disease progression and optimization of care (**Paper 5**),

g) To investigate the relationships between viral loads measured from dried blood spots and clinical and immunological treatment failure criteria among ART-experienced HIV-infected children in Uganda (**Paper 6**)

7.3. Research questions:

a) What are the factors and reasons associated with treatment failure among children receiving ART in ART programmes in Mozambique (Beira) and Uganda (Kampala)?

b) What are the baseline predictors of attrition and lost to follow up among children and adolescents in the Nsambya community home-based care ART programme in Uganda?

c) What are the effects of the Nsambya Community Home-Based Care model on National HIV and Tuberculosis management and health systems strengthening in Uganda?

d) Among HIV-infected children and adolescents on ART, are there any differences in retention and survival between the Nsambya Community Home-based Care model of Nsambya Hospital and the Facility-Based Family-Centred Approach of the Makerere University-Johns Hopkins University Research Collaboration?

e) What are the effects of HIV-EBV co infection on HIV disease progression in children and optimization of care and treatment?

g) In using dried blood spots to measure viral loads among ART-experienced HIV-infected children in Uganda, what relationships emerge between virological outcomes and clinical treatment failure criteria on one hand, and immunological treatment failure criteria on the other?

8.0 Methodology:

This section provides an overview of the methods sections of the six studies. Additional details are provided under the methodology of each study.

Generally, the studies were observational, specifically, retrospective cohort studies, except for study 5, which was a cross-sectional study (please see Paper 5). Except for studies 3 and 4 that involved two centres, all the studies were conducted at the Home Care Department of Nsambya Hospital in Kampala, Uganda.

Apart from the chart reviews, literature reviews provided context for the studies. That was complemented by review of documents such as monthly and annual programme activity reports and other relevant documents.

Study subjects were mainly HIV-infected children and adolescents enrolled in the TFP for periods related to the individual studies. The only exception was study 1, which included adults in the Nsambya CHBC, where the TFP operates.

Two main tools were used for data collection; patients' files and the databases. Paper 2 provides detail description of the data collection system and tries to answer data-related questions such as, who collects data?, what type of data are collected?, how are the data collected and when are the data collected?.

The **outcomes for the operational research** were: a) retention in care, b) attrition and LTFU, c) survival on ART, d) treatment failure and e) operationalizing the use of Dried blood spots for viral load measurements in children in a resource-limited setting.

Rationale for selected themes for operational research

The selected themes have direct bearings on implementation as well as potentials to contribute to improvement of HIV paediatric management in the project setting.

The statistical methods, ethical clearance and funding sources were detailed in each study.

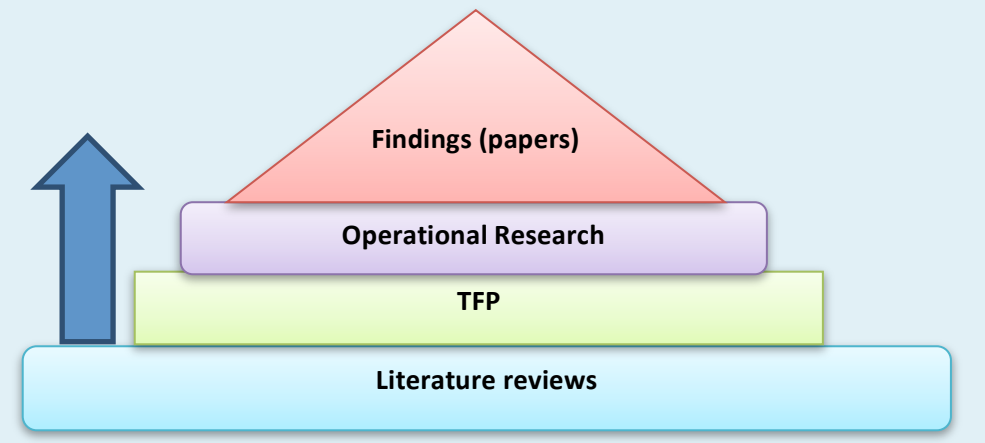


Figure 17: Simplified conceptual framework of thesis showing progression from literature reviews through implementation of the TFP and research to findings of the thesis.

The TFP used a schema derived from the WHO and Ugandan ART guidelines for the implementation of interventions. The schema is presented in Figure 18 below.

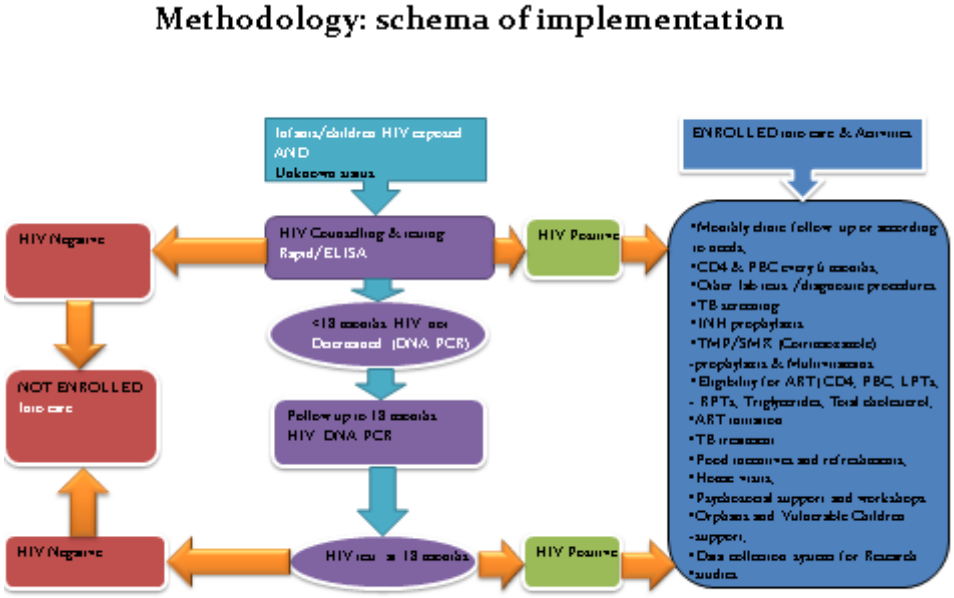


Figure 18: Schema for implementation of HIV/ ART programmes at Nsambya Home Care Department (Nsambya Hospital, Kampala). This schema was applied in the TFP

9.0. Results: original papers as chapters of this thesis:

All the papers have been placed in the Appendix

9.1. Chapter 1: Nsambya Community Home-Based Care complements National HIV and Tuberculosis management in Uganda, and contributes to health systems strengthening

9.2. Chapter 2: Attrition and LTFU among children and adolescents on ART in a community home-based care programme in Kampala, Uganda

9.3. Chapter 3: Survival and retention in care of children and adolescents on ART in two different ART delivery models in Kampala; Community Home-Based Care and Facility-Based Family-Centred Approach

9.4. Chapter 4: Treatment failure among cohorts of children on ART in Uganda and Mozambique,

9.5. Chapter 5: Epstein-Barr Virus load in children infected with Human Immunodeficiency Virus type 1 in Uganda.

9.6. Chapter 6: Virological outcome from dried blood spots testing among ART-experienced HIV-infected children routinely monitored with clinical and immunological criteria in Uganda

9.7. Abstracts presented at international conferences and year of publication:

9.7.1. Abstracts published in 2013:

1. **Poster #: (P_47): Baseline predictors of attrition and loss to follow-up among children and adolescents in a community home-based care HIV programme in Uganda**, accepted for a poster display at the 5th International Workshop on HIV Paediatrics, 28 – 29 June 2013, Kuala Lumpur, Malaysia: Authors: Massavon William[1§2], Lundin Rebecca[1], Costenaro Paola[1], Nabachwa Sandra[2], Penazzato Martina[1], Kayiwa Joshua[5] , Namisi P. Charles[2], Ingabire Resty[2], Tumwine K. James[3], Nannyonga Musoke Maria[2], Morelli Erika[1], Bilardi Davide[1], Kalibbala Daniel[2], Mazza Antonio[4], Giaquinto Carlo[1]

2. **Abstract #: 2428251**: **Operational Challenges of Isoniazid Preventive Therapy Following WHO 2011 Recommendations For Children Living With HIV/AIDS: The Case Of Uganda"** accepted by for oral presentation at the 17th ICASA conference in Cape Town, South Africa, 7-11 December, 2013. Authors: *Costenaro P, Lundin R, Massavon W, Giaquinto C, Nabachwa S M, Kizito S, Alowo A, Morelli E, Nannyonga M, Namisi C, Bilardi D, Mazza A and Penazzato M*

3. **Abstract #: 2427977: Survival And Retention Among HIV-infected Children and Adolescents in a Community Home-Based Care and a Facility-Based Family-Centred Approach in Kampala, Uganda: A Cohort Study,** accepted as a Poster Display, at the 17th ICASA Conference, 7 – 11 December 2013, Cape Town, South Africa. Authors: Massavon W[12], Barlow-Mosha L[3], Mugenyi L[5], McFarland W[6], Gray G,[7] Lundin R[1], Costenaro P[1], Nannyonga M M[2], Penazzato M[1], Bagenda D[3,8], Namisi P C[2],Wabwire D[3], Mubiru M[3], Kironde S[2], Bilardi D[1], Mazza A[4],Fowler MG[3,9], Musoke P[3], Giaquinto C[1].

4. **Poster #: P_13**: **Virological outcomes of dried blood spots testing of combination-ART experienced HIV-infected children routinely monitored with clinical and immunological criteria in Uganda**. Accepted as a poster display at the 5th International Workshop on HIV Paediatrics in Kuala Lumpur, Malaysia, 28-29 June 2013. Authors: *Costenaro P, Lundin R, Petrara MR, Penazzato M, Massavon W,*

Kizito S, Nabachwa S, Nannyonga M, Morelli E, Bilardi D, Mazza A , Zanchetta M, Giaquinto C , De Rossi A.

9.7.2. Abstracts published in 2012

1. **Abstract #: P-01: Implementing the 2011 WHO Intensified Case Finding and Isoniazid Preventive Therapy Algorithm in children and adolescents in a resource-limited setting: a programme perspective,** accepted for poster presentation at the 13TH Annual Conference of the Uganda Society of Health Scientists, Silver Springs Hotel, Kampala, Uganda, 21-22 June, 2012. Authors: Massavon W, Costenaro P, Nannyonga M, Nabachwa S, Namisi CP, Penazzato M, Kizito S , Bbaale Kusiima J, Nantongo R, Morelli E, Bilardi D, Mazza A, Giaquinto C.

2. **Abstract #:P_07: Nsambya Community/Home-Based Care Model: complementing national HIV and TB Management and contributing to Health Systems Strengthening in Uganda,** accepted for poster presentation at the 6TH International Workshop on HIV Treatment, Pathogenesis and Prevention Research in Resource Limited Settings (INTEREST) in Mombasa, Kenya, 8-11 May 2012. Authors: Massavon W[1§2], Nannyonga M M[2], Namisi C[2], Ingabire R[2], Nsubuga M[2], Kalibbala D[2], Penazzato M[1], Kizito S[2], Sozzi F[2], Criel B[3], Tumwine JK[4], , Morelli E[1], Bilardi D[1], Mazza A[5], Costernaro P[1], Giaquinto C[1].

3. **Abstract #: P_27: Feasibility and challenges of Isoniazid Preventive Therapy and Intensified Case Finding in the paediatric HIV care package in a low-income setting**, accepted for poster presentation at the 2012 International AIDS Society (IAS) HIV Paediatric Workshop in Washington DC (USA), July 20-21. Authors: *Costenaro P, Penazzato M, Franceschetto G, Massavon W, Nabachwa S M, Morelli E, Kizito S, Nannyonga M, Namisi C, Bilardi D, Mazza A and Giaquinto C.*

4. **Abstract #: 8594: Validation of WHO 2010 immunologic criteria in predicting paediatric first-line antiretroviral treatment (ART) failure in ART- experienced children in Uganda**: CD4 is a poor surrogate for virologic monitoring of paediatric

ART failure. Accepted for poster exhibition XIX International AIDS Conference in Washington D.C. (USA), 22-27 July 2012. Authors: Linda Barlow-Mosha[1], Peter Mudiope[1], William Massavon[4], Danstan Bagenda[1], Enid Kabugho[1], Monica Etima[1], Mary Glenn Fowler[1, 3], Maria Nannyonga[4], Carlo Giaquinto[5], Philippa Musoke[1,2].

5. **Abstract #: 6970: Integration of legal service aid into HIV/AIDS service delivery, the experience of Nsambya Home Care, Uganda**, was selected for presentation in the poster exhibition at the XIX International AIDS Conference held in Washington, D.C. (USA), 22-27 July 2012. Authors: R.Kamahoro Ingabire[1], C. Giaquinto[2], W.Massavon[1&2], A.Mazza[3], M. Nannyonga Musoke[1], I.Owomugisha[4], E. Morelli[2], D. Bilardi[2], Costenaro P[2], M. Penazzato[2.]

6. **Abstract #: A-452-0408-10719: Management of acute malnutrition using plump nut among HIV infected and exposed children in Uganda**. Accepted as poster presentation at the XIX International AIDS Conference held in Washington, D.C. (USA), 22-27 July 2012. Authors: Nalutaaya AJ[1], Musoke I[1], Namisi CP[1], Massavon W[1&2], Giaquinto C[2], Nannyonga MM[1], Mazza A[3], Morelli E[2], Bilardi D[2], Costernaro P[2], Penazzato M[2].

9.7.3. Abstracts published in 2011:

1. **Abstract #:1.2-038: Retention in care of children and adolescents in an HIV programme in Kampala, Uganda: the impact of a multifaceted approach**. Accepted as a poster and presented at the 7TH ECTMIH in Barcelona, 3-6 Oct 2011. Authors: Massavon W[1,2], Nannyonga M[2], Penazzato M[1], Nabachwa S[2], Kayiwa JA[3], Namisi CP[2], Morelli E[1], Bilardi D[1], Mazza A[4], Giaquinto C[1].

2. **Abstract #:MOPE258: Dynamics of Epstein-Barr virus in HIV-1-infected children in Uganda.** Accepted for oral presentation at the 6TH IAS Paediatric HIV Workshop in Rome, Italy, July 15-16, 2011. Authors: Petrara MR[1], Penazzato M[2,3], Massavon W[3], Nabachwa S[4], Nannyonga M[4], Mazza A [3,5], Zanchetta M[6], Giaquinto C[2,3] , De Rossi A[1,6].

3. **Abstract #:P_90: The challenges of Isoniazid Preventive Therapy (IPT) and Intensified Case Finding (ICF) algorithm implementation in the Paediatric HIV care package**. Accepted as a poster and presented at the Paediatric HIV Workshop in Rome (Italy), July 15-16, 2011. Authors: *Costenaro P,* 2 *Penazzato M,* 2 *Morelli E,* 2 *Massavon W,* 3 *Nabachwa S M,* 3 *Namisi C,* 3 *Nantie R C,* 3 *Nannyonga M,* 2 *Bilardi D,* 4 *Mazza A,* 2 *Giaquinto C.*

4. **Abstract #:P_52: Treatment failure in children living in resource-limited settings: the experience from two paediatric cohorts from Beira (Mozambique) and Kampala (Uganda).** Accepted as a poster and presented at the Paediatric HIV Workshop in Rome (Italy), 15-16 July, 2011. Authors: Costenaro P, Penazzato M, Lundin R, Rossi G, Massavon W, Patel D, Nabachwa S, Franceschetto G, Morelli E, Bilardi D, Nannyonga Musoke M, Atzori A, Putoto G, Mastrogiacomo ML, Mazza A, Giaquinto C.

10.0. Synopsis:

This section focuses on the operational aspects of the findings and relevant observations that contribute in developing the complete picture of this thesis, and covers the following components:

a) *CHBC and paediatric HIV management in a resource-limited setting*

b) Missing data and the operational setting

c) ART and the potential protective effect against EBV-related lymphoproliferative disorders in HIV-EBV co infected children

d) Operationalization of the use of DBS in Viral load monitoring in HIV-infected children in low and middle-income countries

e) LTFU in an HIV programme: some operational interpretations and

f)) TFP: contributing to national AIDS response and health systems strengthening

10.1. Community Home-Based Care and Family-Centred Approach in paediatric HIV management in a resource-limited setting: potentially powerful synergies

Implementing the TFP within the existing CHBC model for adults promotes the concept of family-centred approach (FCA). The literature shows that FCA builds family support for adherence and promotes retention in care , and enhances family uptake of HIV services including voluntary counselling and testing (VCT) prevention of mother to child transmission (PMTCT) and early infant diagnosis [208, 209]. Despite these benefits, inherent barriers in implementation have not encouraged the realization of the full potentials of FCA within the TFP. They include patient information systems that are not synchronized for families, which translates into different clinic appointments days for children and adolescents and their care givers or parents, difficulties in arranging care for a family unit and tracking family members missing appointments

What are the possible solutions?

- Ensuring complete and regularly updated patients' contact information,

-Synchronizing health information of family members as a unit,

-Arranging same-day clinical appointments for the family members as a unit

-Arranging ‘snow-ball’ tracking of family members missing appointments, to promote retention in care. By this concept, family members and friends in the social networks of patients work with health workers to help track other family members who have defaulted.

Family-centred approach (FCA) functioning optimally within a community home-based care is feasible and could lead to powerful synergies to provide and enhance HIV services uptake and treatment outcomes for families with benefits for children and adolescents in resource-limited settings [210-213].

10.2. Missing data and the operational setting

Although capturing laboratory and clinical data in line with national monitoring guidelines may be considered a proxy for quality of care [214], this may not always be the case because of missing data. Apart from the data related challenges described in Paper 2 [215], other reasons for missing data in our programme included:

-variables either not measured due to lack or defective equipment, for example weight and height, or the variable may be measured but data not captured in patients’ medical records or transferred to the electronic databases for storage and future analysis,

-lack of expertise for instance WHO clinical staging by less qualified staffs,

-Observations from Paper 2 show that, changes in treatment guidelines that do not require routine measurements of some variables (e.g. baseline CD4 and viral loads), may lead to missing data [215],

-Policy decisions may compromise data collection and lead to missing data, affect data quality, reporting and programme evaluation. For instance, in times of financial crises, laboratory investigations were often stopped, or done selectively which led to missing data over time,

-As clinic days get busier with increasing patient numbers staffs often forget or omit recording variables in some of the patients,

- Some patients have been on ART for many years and often have two or more versions of their paper-based medical files. Some of the files may not be available, particularly in retrospective record reviews, where access to medical records may be selective .This could lead to potential bias [216, 217].

-Furthermore, it is expected that with time, some adolescents will transfer to adults' programmes, and as paediatric ART services gradually expand nationwide, some children and adolescents will transfer to other sites for different reasons. Currently, health information systems are disjointed; this could mean that, as patients transfer out, their baseline characteristics at ART initiation may not be available to the receiving sites. Patients should be able to move with their health information to facilitate continuity of care as well as verification when needed at the receiving facilities [218].

10.3. ART and the potential protective effect against EBV-related lymphoproliferative disorders in HIV-EBV co infected children

As demonstrated by the literature, children co infected with HIV and EBV have a higher risk of developing EBV-related lymphoproliferative disorders. In paper 5, we found that recipients on ART had relatively lower levels of EBV DNA, suggesting possible suppression of replication of EBV in HIV-EBV co infected children on ART. This means that, ART theoretically could protect against the development of EBV-related lympho-proliferative disorders in such children. For that to happen, HIV-EBV co infected children will require early detection and ART initiation as well as close monitoring over time.

10.4. Operationalization of the use of DBS in Viral load monitoring in HIV-infected children in low and middle-income countries

For a longtime, children on ART in low and middle-income countries were monitored using clinical and immunological criteria. That was primarily due to the prohibitive cost of viral load testing and lack of expertise in such settings. In paper 6, we demonstrated that virological measurements were feasible from dried blood spots (DBS), in line with the literature [219, 220]. Generally, filter papers are relatively cheap, DBS is easy to process, store, package and ship to distant laboratories with the expertise and resources to conduct virological testing on such children. The associated potentials are many and make the use of DBS cost-effective. This simple technique could expand and improve the quality of paediatric HIV care and treatment, particularly, early detection of treatment failure, the need for switching and possible resistant viral strains testing in resource-limited settings [221, 222].

10.5. LTFU in an HIV programme: some operational interpretations

Poor quality of care and long distances to health facilities are important determinants of LTFU particularly in remote rural areas of Uganda [223]. This situation may encourage internal migration to seek HIV and other medical services in the urban areas.

Among patients loss to follow up (LTFU), a common theme that emerged from home visit reports, counsellors' reports, quarterly ART reports, was "relocation to original village" after some improvement in health conditions. This suggests some internal migration for health care services, especially for children and adolescents who have less choice compared to adults. LTFU may therefore be partially explained by 'internal migration for health care' because of the disproportionate distribution of health facilities, with the majority concentrated in urban settings. As an example, Kampala has about 30% of all HIV facilities in the country [78, 224], while the majority of Ugandans are rural dwellers. Hence, to promote geographical and universal access, there is urgent need for greater and more effective decentralization of paediatric ART services within primary health care facilities at the district and sub-district levels in the general health system.

10.6. Tukula Fenna Project: contributing to national AIDS response and health systems strengthening in Uganda

The TFP (TFP) can be seen as a contribution to the Uganda national AIDS response as it helps in expanding access to paediatric HIV services in Uganda. The project is one of the relatively few HIV paediatric programmes providing comprehensive care, treatment, PSS including orphans, and vulnerable children (OVC) support in the country. All the services are free of charge owing to external financial support. Findings from research studies from the project add to knowledge in the field of paediatric HIV management in a resource-limited setting.

As shown earlier, the TFP was integrated and implemented within an existing community and home-based care model that was originally designed for adults at the home care department of Nsambya Hospital. In terms of contributions to the health system, the TFP provided additional funding from external sources, particularly from Italian partners and donors such as House for Life, Father Angelo (Italian NGO), PENTA Foundation and CARAP. The project also supported health infrastructure development. Examples include the new building for the Home Care Department (Figure 12), the TB clinic to strengthen integrated HIV-TB management (Figure 13) and the renovation and upgrading of the Ggaba Outreach Clinic (Figure 14). It also provided additional HRH, as well as transportation and logistic support for clinical services and community-based activities like outreaches and home visiting.

HRH management followed common policy guidelines facilitated by administrative and operational integration. In addition, administrative and operational integration encouraged common approaches to health worker trainings, supervisions and appraisals. The measures have been described in detail in paper 1 [111].

Decentralization of basic HIV services is a cardinal feature of the Nsambya community and home-based care (CHBC) model. Decentralized services include VCT, ART refills, collection of laboratory specimens and PSS through outreach clinics in the communities. This approach seems to have promoted geographical access to HIV services at the periphery of the health system. In the Nsambya CHBC model, community volunteers play 'frontline roles' in terms of home visiting, tracking of defaulting patients in the communities and sensitizing and mobilizing communities to access HIV and TB services. To that extent, the TFP has worked closely and supported community volunteers with bicycles and mobile phone lines to motivate as well as facilitate their work.

Conclusions:

-Strengthening FCA in the Nsambya CHBC model could generate powerful synergies to improve uptake of HIV, TB and other medical services for families as units with benefits for children and adolescents;

-Improving data management systems could have potentially positive impacts on programme outcomes and reporting,

-Apart from bringing in additional resources to the Home Care Department of Nsambya Hospital, the TFP adopted an integrated approach to the implementation of HIV paediatric care, treatment and PSS within the existing Nsambya CHBC model. That is quite atypical of projects and the approach avoided the creation of a parallel health system and contributed to health systems strengthening.

-although the TFP implementation approach may not have been a perfect arrangement, for over a decade, the project provided comprehensive HIV care, treatment and PSS for children and adolescents, including OVCs free of charge. Certainly, over a decade of free services may have removed part of the financial barriers and promoted access to HIV services for this underserved population. To this end, the TFP can be considered as an important contribution to the Uganda national AIDS response in terms of expanding access to paediatric HIV services and indirectly contributing to health systems strengthening in the country.

11.0. Conclusions from thesis; paper by paper:

Paper 1

The Nsambya CHBC complements national HIV and TB management efforts, and resulted in more positive outcomes when compared to the national averages. This approach may hold the potential for chronic disease management in resource-limited settings. Scaling up CHBC could have wider positive impacts on the management of not only HIV and TB, but also other chronic diseases as well as the general health system. A long-standing "faith-based solidarity" among international donors and partners has been pivotal to the survival and evolution of the Nsambya CHBC.

Paper 2

Overall, attrition and LTFU were relatively high among children and adolescents in the Tukula project. Not receiving ART was the single factor significantly associated with attrition in the cohort, while both baseline BMI z-scores and receipt of ART were protective against LTFU among HIV positive children and adolescents enrolled in the TFP. Efforts should be made to initiate ART among all paediatric patients as soon as possible, and to provide aggressive follow-up for those not yet receiving ART. Orphans need more nutritional support to reduce the burden of malnutrition and improved access to early ART, which could also promote growth responses in this vulnerable and understudied group.

Paper 3

Irrespective of model of care, children receiving ART had better retention in care and therefore long-term survival. Encouragingly, if children were on ART, then their survival was as good, if not slightly better, in the CHBC compared to the FBFCA. Based on our observations, substantial improvement in child survival can be achieved in either a community-based or a family-care model as long as HIV- infected children are identified early and begun on ART. To ensure this occurs, early identification of HIV infected children requires strong linkages of pregnant HIV- infected women to PMTCT services; active tracking to ensure all HIV exposed infants receive Polymerase Chain Reaction-based early infant diagnosis. Additionally, rapid early initiation of ART among HIV infected infants and children are essential. We anticipate the move to early initiation of ART in all HIV-infected

children and adolescents in resource-limited settings, irrespective of their CD4 cell counts, will improve survival.

Among ART patients in both models, attrition was significantly associated with model of care, mild immunosuppression and being underweight. In the CHBC, attrition was significantly associated with CD4 cell count, WHO clinical stages III-IV and absence of ART.

Paper 4

In conclusion, our data reinforce the need for simplification of more effective clinical and immunological criteria for prompt recognition of cART treatment failure. Children presenting with advanced disease and TB co-infection should be targeted for closer and more sensitive monitoring of treatment response. This should be matched with a constant provision of appropriate antiretroviral drugs and with optimization of first line drugs and treatment sequencing. Supply of new paediatric formulations for second line regimens and drug optimization should be considered as critical milestones to allow scaling up of early cART and reduction of treatment failure in children.

Paper 5

In conclusion, HIV-1, inducing microbial translocation and a state of persistent immune activation, may lead to EBV replication and expansion of EBV-infected B-cells, thus increasing the EBV-DNA load. Super-infection by both types of EBV in HIV-1 infected subjects may represent an additional risk for the onset of EBV-related malignancies. ART, by limiting HIV-1 replication, microbial translocation and related immune activation, may prevent super-infection by both EBV types and keep EBV viremia down, thus reducing the risk of EBV-associated lymphomas.

Paper 6

In our cohort immunological and clinical criteria as per WHO 2010 guidelines poorly predict the presence of a viral load greater than either 1000 cp/ml or 5000 cp/ml (whole blood) from DBS. The low sensitivity and positive predictive values for immunological and/or clinical failure confirm those reported by literature [225] This finding further supports the WHO recommendations that VL monitoring should be implemented and used to earlier identify cases of treatment failure [226].

This study provides data on virological outcome in a program setting among children on cART routinely monitored by clinical and immunological criteria alone. We confirm that VL monitoring using DBS is feasible in LMIC. Studies are needed to improve the accuracy of viral load determination from DBS to increase test accuracy and detect early virological failure.

11.1. Policy implications of thesis findings

Scaling up CHBC could have wider positive impacts on the management of not only HIV and TB, but also other chronic diseases as well as the general health system.

In this thesis and in line with the literature, Early ART initiation was associated with improved survival and retention in both community-based and facility-based approaches.

ART is potentially protective against EBV-related lymphoproliferative disorders in HIV-EBV co infected children. This calls for early ART initiation in such children.

Operationalization of the use of DBS in Viral load monitoring in HIV-infected children in low and middle-income countries is feasible and should be encouraged to improve the quality of paediatric HIV management in such settings.

The low ART coverage among children calls for urgent, greater and more effective decentralization of paediatric ART services within primary health care services at the district and sub-district levels in the general health system in Uganda.

Children presenting with advanced HIV disease and TB co-infection should be targeted for closer and more sensitive monitoring of treatment response.

Orphans need more nutritional support to reduce the burden of malnutrition and improved access to early ART, which could also promote growth responses in this vulnerable and understudied group

11.2. Further research

-The CHBC model demonstrates the potential for chronic disease management in resource-limited settings, further studies could validate this potential.

-Community involvement has been shown to improve HIV and TB treatment outcomes. Yet there are no clear tools to objectively measure community engagements.

-There is urgent need for studies to improve the accuracy of viral load determination from DBS to improve detection of early virological failure.

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13.0. ANNEX 1: Structure of Ugandan Health Sector, Populations and Services Provided

Level		Health Centre	Population (approx.)	Services Provided
District	Health Sub-District	I	Village - 1,000	Community-based preventive and promotive health services. Village Health Committee or similar status.
		II	Parish - 5,000	Preventive, promotive and out-patient curative health services, and outreach care.
		III	Sub-county - 20,000	Preventive, promotive, out-patient curative, maternity and in-patient health services and laboratory services.
		IV	County - 100,000	Preventive, promotive, out-patient curative, maternity, in-patient health services, emergency surgery, blood transfusion and laboratory services.
		V	General Hospital – 500,000	In addition to services offered at health centre level IV, other general services are provided including in-service training, consultation and research for community-based health care programmes.
Regional		VI	Regional Referral Hospital - 2,000,000	In addition to services offered at the general hospital, specialist services are offered, such as psychiatry, Ear, Nose and Throat (ENT), ophthalmology, dentistry, intensive care, radiology, pathology, higher level surgical and medical services.
National		VII	National Referral Hospital – 24,700,000	These provide comprehensive specialist services and are also involved in teaching and research.

Source: Tashobya et al (2006): Health Systems Reforms in Uganda: processes and outputs

APPENDIX CONTAINING ALL SIX PAPERS IN THIS THESIS

Nsambya Community Home-Based Care complements national HIV and TB management efforts and contributes to Health Systems Strengthening in Uganda: an observational study

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Abstract:

Community Home-Based Care (CHBC) has evolved in resource-limited settings to fill the unmet needs of people living with HIV/AIDS (PLHA).We compare HIV and tuberculosis (TB) outcomes from the Nsambya CHBC with national averages in Kampala, Uganda. This retrospective observational study compared HIV and TB outcomes from adults and children in the Nsambya CHBC, with national averages from 2007-2011. Outcomes included numbers of HIV and TB patients enrolled into care, retention, loss to follow up (LTFU) and mortality among patients on antiretroviral therapy (ART) at 12 months from initiation; new smear-positive TB cure and defaulter rates, and proportion of TB patients tested for HIV. Chi-square test and trends analyses were used to compare outcomes from Nsambya CHBC with national averages. By 2011, approximately 14,000 PLHA had been enrolled in the Nsambya CHBC, and about 4,000 new cases of TB were detected and managed over the study period. Overall, retention and LTFU of ART patients 12 months after initiation; proportion of TB patients tested for HIV, and cure rates for new smear-positive TB scored higher in the Nsambya CHBC compared to national averages. The findings show that Nsambya CHBC complements national HIV and TB management, and resulted in more positive outcomes.

Keywords: Community Home-Based Care, Nsambya, HIV/AIDS, TB, Health Systems Strengthening, Resource-limited Settings, Sub-Saharan Africa, Uganda

Background

In the wake of the human immunodeficiency virus (HIV) epidemic in Sub-Saharan Africa (SSA), alternative service delivery models like the Community Home-Based Care (CHBC) [1-6] have evolved to fill the gap left by over-stretched and under-resourced health systems. CHBC includes any form of care (physical, psychosocial, palliative and spiritual) given to the sick and the affected in their own homes and care extended from the hospital or health facility to their homes through family participation and community involvement [7, 8]. CHBC provides for the unmet needs of the large and growing population of PLHA in many resource-limited settings [7, 9, 10]. However, the effects of CHBC on national HIV and TB outcomes have not been examined in detail.

In Uganda, the first CHBC programmes were established in 1987 in response to increasing numbers of acutely ill HIV/AIDS patients leading to congestion of hospital wards, increased staff workload and excessive pressure on infrastructure. Three different organizations pioneered this approach: Kitovu Mobile HIV programme, The AIDS Support Organization (TASO) and Nsambya Hospital Home Care Department; popularly known as Nsambya Home Care (NHC). TASO was started by local people, whereas, Kitovu Mobile and Nsambya Home Care were pioneered by catholic missionary sisters from Ireland.

HIV and TB present important public health problems and health systems challenges for the country. According to the 2011/12 Uganda AIDS Commission Progress Report, Uganda has a generalized HIV epidemic and prevalence has increased from 6.4% in 2004 to 7.3% in 2011[11] . Uganda is also one of the 22 high-burden countries with respect to TB [12], and the 2012 WHO Global TB Control Report, shows that Uganda had a TB prevalence of 183 (95-298) and an incidence rate of

193(156-234) per 100, 000 population respectively. In the same report, 53% of TB patients tested positive for HIV [13]. To deal with the dual epidemics, the ministry of health (MoH) developed national policy guidelines for the integrated management of TB/HIV co infection. The guidelines aim among other things, to reduce the burden of both diseases, by improving detection, quality of care and promoting access through decentralization of services to the lower levels of the health systems, where the majority of the population lives [14].

The Ugandan guidelines for scaling up ART [15] outline a primary care approach and make provisions for a CHBC model, in line with the WHO framework for action on CHBC in resource-limited settings[7]. This encourages synergies in the implementation of interventions for both HIV and TB. By mandate, CHBC is expected among other functions to provide palliative care, pain management, implement HIV-related interventions and serve as a bridge to extend HIV care, treatment and psychosocial support services beyond the traditional health facilities to the large and growing population of PLHA in their homes and communities [16] . Clearly, CHBC is consistent with the designed national response, and as a service delivery model, it could promote integrated management of the dual epidemics in Uganda and other settings with similar problems [17-20].

In this paper, we describe the Nsambya CHBC; examine the results and their effects on national HIV and TB outcomes, and their contribution to health systems strengthening in Uganda. Additionally, we highlight some challenges and recommend practical steps to strengthen implementation of CHBC in a resource-limited setting.

Methods

Study design, setting and population

This retrospective observational study compared HIV and TB outcomes from the Nsambya CHBC with national averages reported by the National TB and Leprosy Programme (NTLP) and the National AIDS Control Programme (NACP) over five years (2007-2011). The study was conducted at St. Raphael of St. Francis Hospital (Nsambya Hospital), Home Care Department in Kampala, Uganda. Nsambya Hospital is a Faith-based Private-Not-For-Profit facility owned by the Catholic Archdiocese of Kampala and accredited by both the ministry of health (MoH) and the Uganda Catholic Medical Bureau (UCMB). It is a general tertiary referral hospital with a bed capacity of 361, and involved in research and training of postgraduate doctors, nurses, midwives and laboratory technicians.

The study population consisted of adults and children receiving HIV and TB care, treatment and psychosocial support services over the study period.

Description of CHBC

NHC was established to extend basic health services into patients' homes, to reduce pressure on hospital workers and infrastructure and encourage family members to participate in the care of their relatives. This service was also intended to promote early hospital discharge, follow-up after discharge and community involvement. What started as a team of three health workers providing palliative care to patients in their homes has evolved into a specialized HIV and TB Centre. NHC has a catchment area stretching across four districts in and around Kampala, and covers approximately 21 km in radius. The estimated population of the catchment area was about 4 million in 2012 [21] . Over the years, NHC has evolved into a CHBC with the development of

community components, which include community engagements, a community-based volunteer programme, community outreach programmes and outreach clinics.

The Nsambya CHBC is a blend between facility-based care and home-based care with the community serving as an important intermediary. It employs task shifting to overcome some of the shortages in the workforce, and uses home visits and outreach clinics to get services closer to patients. In addition, psychosocial support services help patients to deal with some of the challenges posed by HIV positive status, and poverty in accessing healthcare in poor-resource settings. The pillars of the CHBC and how they function, patient enrolment practice, tracking of defaulters and other interventions have been described in detail in a previous study [22].

Programmes implemented with CHBC

Prior to implementing programmes with the CHBC, donors and partners made concerted efforts to operate within existing national policy guidelines as much as possible. That understanding paved the way for establishing a framework of administrative and operational integration among donors and partners aimed at coordinating resources, promoting efficiency and avoiding measures that could potentially damage the health system.

Within that framework, several closely related programmes were implemented with the CHBC: HIV prevention education, counselling and testing, ART, HIV chronic and palliative care, TB treatment, Intensified TB Case Finding (ICF) and Isoniazid Preventive Therapy (IPT). The programmes were vertical owing to the weak state of the general health system, and the approach can be considered as a contextualized solution [23-26]. Nonetheless, the Nsambya CHBC has extensive and important functional linkages to the general health system and all the relevant stakeholders. For instance, the MoH supports the TB clinic within the Nsambya CHBC to function as a

national referral facility that provides TB treatment for the public, participates in surveillance activities and national TB-HIV studies. The various interrelationships of the Nsambya CHBC are illustrated in Figure 1, and the key players, processes and linkages to the observed outcomes are summarized in Figure 2.

The Nsambya CHBC was funded mainly by non-governmental initiatives through a long-standing faith-based solidarity, and minimal support from the MoH. The faith-based solidarity also provided vital technology and technical assistance to achieve a common goal. The goal was to provide comprehensive HIV care, treatment and psychosocial support services for HIV-infected patients and their families and affected communities. Services were generally free of charge; however, adults paid a user fee of 1,000 Uganda shillings, the equivalent of 38 cents of a US dollar at the time of this study, per visit.

Data collection

Data from routine programme activities, programme reports, patients' records, HIV and TB registers at NHC were collected for the study. Country-level data were obtained from the NACP and NTLP reports as well as from global HIV and TB reports. Some of the data were incomplete from the three institutions in the study. Consequently, the analyses were limited to periods with complete data, and that has been provided under the results section.

Statistical methods and data analysis

Primary study outcomes included the proportions of ART patients retained in care, LTFU and mortality at 12 months from ART initiation, proportion of TB patients tested for HIV, and cure and defaulter rates for new smear-positive cases. Secondary

outcomes included HIV-TB co infection and ART status among defaulters, and bed occupancy rate for HIV-related hospital admissions within 12 months of starting the CHBC. Bed occupancy rate was determined from a hospital report (unpublished). The data were analyzed with Microsoft Excel programme version 2007, and STATA version 12. Chi-square tests were used to determine the differences and trends between the mean outcomes from the Nsambya CHBC and national outcomes. In addition, Chi square test and Fisher's exact test were used to determine differences in the proportions of TB defaulters co infected with HIV, not co infected, receiving ART, and not on ART.

The Uganda National Council for Science and Technology granted ethical approval for the study (UNCST Ref: HS 1383). The relevant authorities waived informed consent.

Results

Effect on HIV patient outcomes

It is estimated that about 14,000 PLHA and their families have been enrolled in the Nsambya CHBC since its inception in 1987. Overall, about 91.6% were adults, 67.7% were females and 8.4% were children. From January 2009 to December 2011, on average, 90% (89%-91%) of the Nsambya CHBC patients on ART were retained in care, 12 months after ART initiation, compared to 83.3% (83.2%-83.4%) for the national average. The difference was significant (Chi-square =60.3, $p<0.001$) and the trends were significantly different (chi-square for trend=26.8, $p<0.001$), with the Nsambya CHBC having more positive trends than National (Table 1 and Figure 3).

The Nsambya CHBC recorded an average LTFU rate of 5.7% (4.9%-6.4%) for ART patients 12 months after initiation, compared to the national figure of 8.7% (8.6%-8.8%). Overall, LTFU differed significantly (Chi-square =95.8, p<0.001), as well as the trends (chi-square for trend=95.7, p<0.001), with the Nsambya CHBC having a significantly decreasing trend compared to the national trend (Table 1 and Figure 4).

The proportion of ART patients that died, 12 months after ART initiation was 5.4% (4.7%-6.1%) for the Nsambya CHBC and 4.6% (4.5%-4.7%) for the national figure. Overall, the difference in mortality (Chi-square = 38.9, p<0.001) and the trends were significant (chi-square for trend= 35.8, p<0.001). However, the trend was significantly decreasing under the Nsambya CHBC compared to the National (Table 1 and Figure 5).

Effect on TB patient outcomes

Approximately 4,000 new TB cases were detected and managed from 2007 to 2011. Adults constituted 92.3%, females 51.0%, and children 7.7% of the cases. On average, 95% of TB patients from the Nsambya CHBC were tested for HIV as against 72% for the national value. From 2007 to 2010, the Nsambya CHBC recorded an average cure rate of 54.6% for new smear-positive TB patients, while the figure for the national average was 30.8%. The difference was significant (Chi-square =21.2, p=0.001), but there was no difference in the trends (chi-square for trend=3.0, p=0.083, Table 2 and Figure 6). New smear-positive TB defaulter rates were 10.1% and 10.7% for the Nsambya CHBC and National respectively. The difference was not significant (Chi-square =2.1, p=0.541) and the trends did not differ (chi-square for trend=0.02, p=0.877), Table 2 and Figure7.

Overall, there were 110 TB defaulters, 54.5% (60/110) were enrolled in care in the Nsambya CHBC and the rest were referrals from other facilities. Majority of the TB defaulters were HIV-TB co infected (72%, $p<0.001$), and included all the TB defaulters enrolled in care in the Nsambya CHBC, who accounted for 76% ($p<0.001$) of all the HIV-TB co infected defaulters. Overall, minority of the TB defaulters were receiving ART (39%), and when stratified by source of patients, the proportions were similar: 38% versus 42%, ($p=0.769$) for Nsambya CHBC patients and referrals respectively (Table 3).

Effect on bed occupancy

The immediate impact of the Nsambya CHBC was a remarkable reduction in bed occupancy from an average of three months to two weeks for HIV/AIDS-related hospital admissions in 1987, within 12 months of starting the CHBC; long before ART became publicly accessible in the country (data not presented).

Discussion

Overall, the core findings from this study demonstrate that the Nsambya CHBC complements national HIV and TB management, and resulted in a higher proportion of ART patients retained in care and a lower LTFU rate, 12 months after initiation. We believe the higher retention in care and lower LTFU rates seen among the ART patients could be revealing the results of 25 years of evolution of the Nsambya CHBC, from preparing PLHA for death in the pre-ART era to keeping them alive through ART and long-term follow up measures. The process entailed regular review of CHBC design to make it sensitive to some key challenges faced by patients while seeking health care. That translated into additional psychosocial support services such as the OVC support programme, food supplements to help with food insecurity,

economic empowerment, particularly of adolescents through sponsorships for vocational trainings, and some caregivers to enable them deal with poverty and other negative impacts of HIV/AIDS [22]. Other factors include strategies such as tracking of defaulting patients, community involvements, task shifting to community volunteers, nurses, counsellors and social workers [27], and using outreaches to promote geographical access to some services. Furthermore, the evolutionary process involved the adoption of measures that have contributed to health system strengthening, as a stronger health system [5] is crucial for effective service delivery.

We think that, the slightly higher mortality rate seen among Nsambya CHBC patients on ART might be due to improved tracking of patients considered "lost to follow up". Indeed, a variable proportion of ART patients labelled as "lost to care" were actually dead upon tracking, and that observation is consistent with the literature [28-30]. Our mortality rate may also be reflecting improved documentation and reporting of deaths from the communities by community volunteers, who are residents of the communities. Overall, the mortality trend was reducing much more in the Nsambya CHBC, as depicted in Figure 5.

We also found that a higher percentage of TB patients were tested for HIV in the Nsambya CHBC and the average cure rate for new smear-positive TB patients was higher than the national average. However, the defaulter rates were similar. Various factors may explain the higher proportion of TB patients tested for HIV and the improved TB cure rates in the Nsambya CHBC. The Nsambya CHBC has a TB clinic and a laboratory for various tests including sputum microscopy on the same premises as the main HIV clinic. That structural arrangement coupled with training of health workers on policy guidelines for the integrated management of HIV-TB co infection may have strengthened management of the two diseases. That arrangement may also

have raised awareness among health workers and patients as well as facilitated screening of TB patients for HIV and vice versa. Moreover, patients see the arrangement as convenient and cost-saving to have HIV and TB screening and treatment at the same facility [31]. The high TB defaulter rate was unexpected, particularly when compared to LTFU among ART patients in the Nsambya CHBC. Nevertheless, that finding might be linked to the overall small proportion of TB defaulters started on ART (table 3), which is in keeping with the literature [32, 33]. In addition, TB patients referred to receive treatment but not enrolled in the Nsambya CHBC could not be tracked because of disjointed health information systems and logistic challenges. This observation could be reflecting a wider problem, and calls for early initiation of ART in all HIV-TB co infected patients in line with the recent revisions of the treatment guidelines [34, 35], and concerted efforts to track all TB patients receiving treatment in the Nsambya CHBC.

The remarkable reduction in bed occupancy was feasible because of early discharge from hospital, home-based care provided by outreach staffs, and support from family members and friends of patients and community involvements. Studies from Uganda [36, 37] and elsewhere in SSA [38, 39] reported high HIV-related hospital admissions, sometimes to the exclusion of non-HIV patients in the 1990s and early 2000s. Undoubtedly, the reduction in bed occupancy translates into freeing up beds for non-HIV patients, decreased workload on health workers and reduced pressures on health infrastructure, all of which have gains for the health system [26]. There may also be gains for the patients and their caregivers from the shorter stays, such as overall costs. With the advent of ART, the reduction in bed occupancy has been sustained, as the health conditions of many HIV patients improved and relatively fewer patients were admitted to hospital and for shorter durations, in line with the

literature [40-42]. Over the years, community involvements seem to have raised awareness about HIV and reduced stigma to some extent, as reported by some patients, their caregivers as well as community volunteers, some of whom are expert patients. These positive effects could contribute to the building blocks for chronic disease management in resource-limited settings, particularly, where the default healthcare delivery models were not designed for chronic disease management [16] .

We have observed some of the positive impacts of home visits and the various forms of psychosocial support, including lessons on self-management on patient outcomes, and believe they could go beyond HIV and TB management to benefit patients with other chronic conditions such as diabetes and hypertension, to mention a few [43]. Yet, that scenario may be dictated by additional funding and other resources to scale up, policy guidelines for regulation and the political commitment to support and sustain the approach, as well as a change in the mind-sets of programme coordinators and managers. Currently, CHBC is largely a 'donor-partner' funded initiative operating on a miniature scale [8, 44], compared to the existing HIV and TB disease burdens and unmet needs.

Impact of long standing faith-based solidarity

To a large extent, the findings from the Nsambya CHBC illustrate what could be achieved when a common goal is backed by some form of 'solidarity'; in this case, 'a complex and powerful long standing faith-based solidarity' involving international donor-partnerships and local partners. To accomplish the common goal for the solidarity, a wide range of resources and several programmes were envisaged. Somehow, the donors and partners directly or indirectly, supported most of the essential pillars of health system strengthening [45, 46] through funding, drugs,

equipment, materials, infrastructure development and vital technologies like electronic databases to support health information systems. The donor- partnership synergies also provided technical assistance to build and maintain systems; train, supervise, monitor and evaluate performance against set standards periodically. Thus, gradually developing some of the needed systemic capacities [47], as well as a viable service delivery mechanism over time. It is estimated that, at the time of this study, the existing key donors and partners had each supported the Nsambya CHBC for at least eight years covering different periods or with some overlap, and the oldest partner for over 25 years and on-going. This 'longevity of faith-based solidarity' has been pivotal to the survival, evolution and expansion of the Nsambya CHBC.

Administrative and operational integration

One of the important achievements of the Nsambya CHBC was the establishment of administrative and operational integration among donors and partners. The measures allow some resources to be pooled together; utilized after collective decisions and accounted for in a transparent manner. Administrative and operational integration have facilitated and somehow harmonized implementation of some common policy guidelines. For instance, there are common policy guidelines for human resources for health management in place. In practice, they translate into common procedures for advertising vacancies, standardized selection criteria and the use of Ugandan national salary scales. This avoids disparities in salaries and working conditions for health workers with similar qualifications and experiences, but working on different programmes. The measures have reduced duplication, minimized wastage and administrative costs, poaching of health workers, and seem to have promoted efficiency and synergies with better programme outcomes.

Positive 'spill over' effects

Even though, the original goal of the Nsambya CHBC was to provide care, treatment and psychosocial support for PLHA and their families, with time, the additional resources from the HIV programmes appear to have benefited other programmes and the general health system. Notably, TB control, nutritional support for children, OVC support including sponsorships for vocational training and support for caregivers [22]. These positive "spill over" effects are consistent with findings from studies in Ethiopia and Malawi [48] and elsewhere [25, 26, 49] globally.

Challenges to be addressed

Despite the achievements of the Nsambya CHBC, some important challenges remain, and they can be viewed from the level of CHBC, from that of the implementing organization, donor-partner demands and preferences and the general health system. Documentation and data capturing from community activities need to improve in order to contribute to operational research in the future. The referral networks linking the communities to the outreach clinics and to the department and hospital require strengthening in order to be effective. Community volunteers play vital roles in the referral networks, but they may not be adequately resourced to function effectively. Budgeting must also be clear and stable in order for programmes to be designed in a feasible manner and implemented consistently. This can be difficult to manage with multiple donors and partners providing varying portions of funds over varying periods. Supplies such as drugs and medical products are also received on a variable basis from a range of sources. In order to prevent waste and actively identify areas of both overage and shortage, efficient and timely recording systems for supplies are essential.

Although, the Nsambya CHBC has an organizational know-how in place that could be extended to benefit other health problems other than HIV and TB, some donors and partners prefer to fund only specific aspects of the programmes. That state of affairs, creates some difficulties among the donor-partner relationships, and somehow does not contribute to the realization of the full potentials of CHBC. With respect to the general health system, referral networks are generally fragile, operational guidelines lack visibility, and the disjointed nature of health information systems makes it a daunting task tracking patients lost to care, especially when they relocate to different cities, towns or villages.

We recommend the following steps for the relevant authorities to consider in strengthening and expanding implementation of CHBC programmes:

i. Government co-funding and political commitment to scale up CHBC and ensure continuity of support in the face of changes in the donor-partner relationships,

ii. Streamlining the existing patient tracking system to make it sensitive for tracking all patients in care in the Nsambya CHBC,

iii. Strengthening of the referral networks through national guidelines and resource allocation as well as research and,

iv. A critical assessment of how the CHBC models impact on the general health system.

Limitations of study

Potential limitations of this study include issues with documentation (incomplete data), availability and data quality. Consequently, we believe the reported number of

patients ever enrolled in the Nsambya CHBC could be an underestimation, possibly due to missing data from worn out paper-based registers, before electronic databases became available. To deal with missing data , we relied on reported data from the NACP and NTLP, presented in global HIV and TB reports for the comparisons, whenever possible. That meant, only periods with available reported data could be compared. These limitations were accommodated for, by providing the periods for the various analyses in the text under results.

Data on the average bed occupancy rate was from a secondary source (unpublished hospital report) which did not provide the standard deviation for the mean reported. In addition, portions of the relevant paper-based registers for 1987-88 hospital admissions have worn out over the years, resulting in missing data. Therefore, the primary data could not be accessed for analyses.

We also recognize that the national averages level off diversity in data and their sources and therefore believe that, the observed differences in outcomes may not be due solely to the CHBC approach but possibly some other factors, which we were unable to explore.

Conclusions

We conclude that, the Nsambya CHBC complements national HIV and TB management efforts, and resulted in more positive results for several HIV and TB outcomes, when compared to the national averages. The findings could be reflecting the results of 25 years of evolution of the Nsambya CHBC, from preparing PLHA for death in the pre-ART era, to keeping them alive through life-prolonging ART and long-term follow up measures. A process that entailed regular review of the approach, community involvements, additional interventions to mitigate some of the

negative impacts of HIV/AIDS, while adopting measures and strategies that have contributed to health system strengthening in the country. This approach may hold the potential for chronic disease management in resource-limited settings. Scaling up CHBC could have wider positive impacts on the management of not only HIV and TB, but also other chronic diseases as well as the general health system. A complex and powerful long-standing "faith-based solidarity" among international donors and partners has been pivotal to the survival and evolution of the Nsambya CHBC.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MW conceived of the study and participated in the drafting and editing of the manuscript, data analysis and interpretation of the findings. NMM, KS and IR participated in the drafting of the manuscript. PM and NC participated in the drafting, and editing of the manuscript and interpretation of findings. NM, PC, BD and MA participated in the editing of the manuscript. ML and KD participated in the data analysis and interpretation of the findings. RL, CB, SJ, GC and TJK participated in the editing of the manuscript and interpretation of the findings. All authors read and approved the final manuscript.

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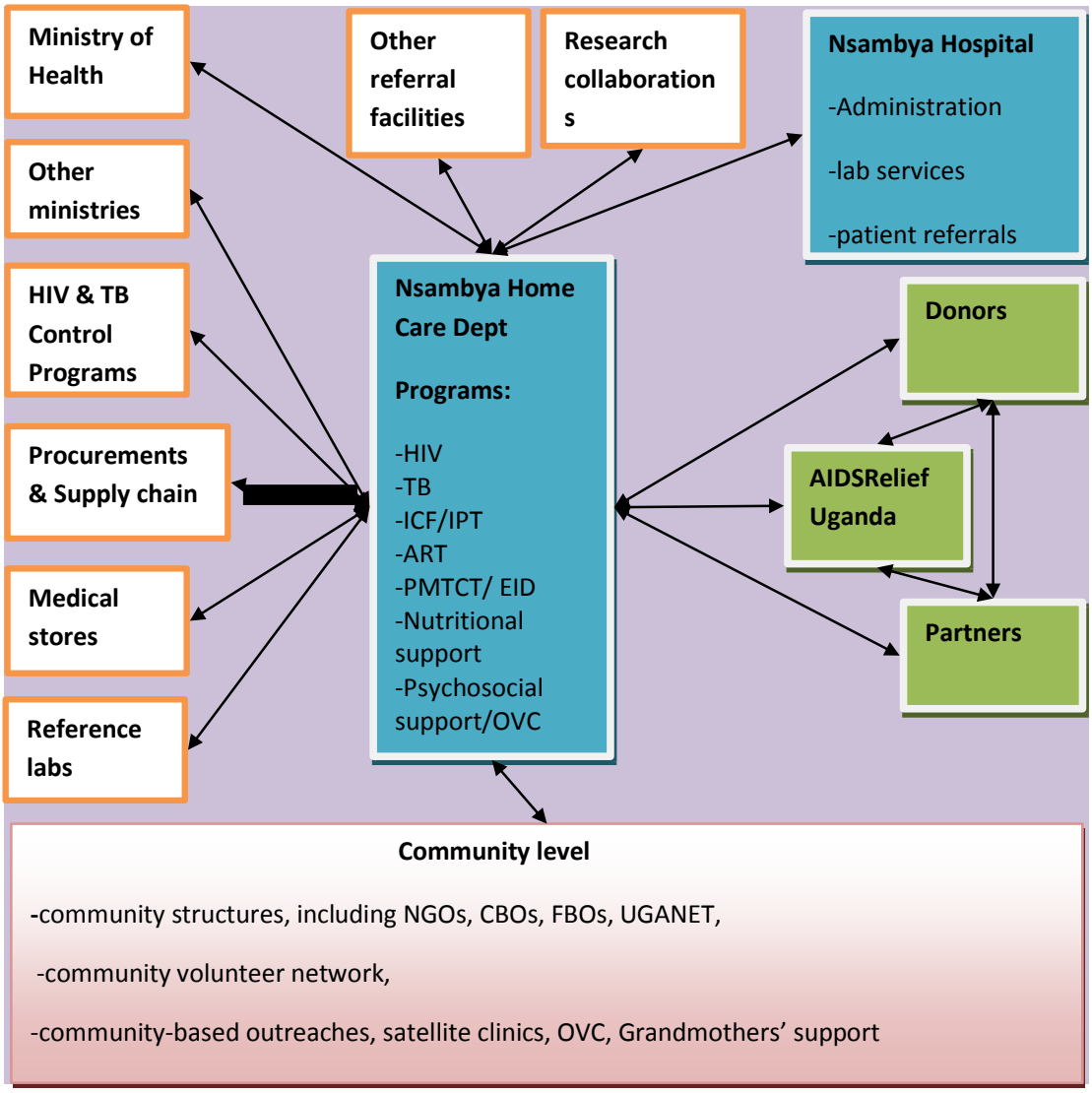

Figure 1: Nsambya Community Home-Based Care and linkages to the general health system, stakeholders and communities.

NGO: non-governmental organization; CBO: community-based organizations; FBO: faith based organizations; UGANET: Uganda network on law, ethics and HIV/AIDS; OVC: orphans and vulnerable children; TB: tuberculosis; ICF/IPT: intensified case finding and Isoniazid preventive therapy; ART: antiretroviral therapy; PMTCT: prevention of mother to child transmission; EID: early infant diagnosis

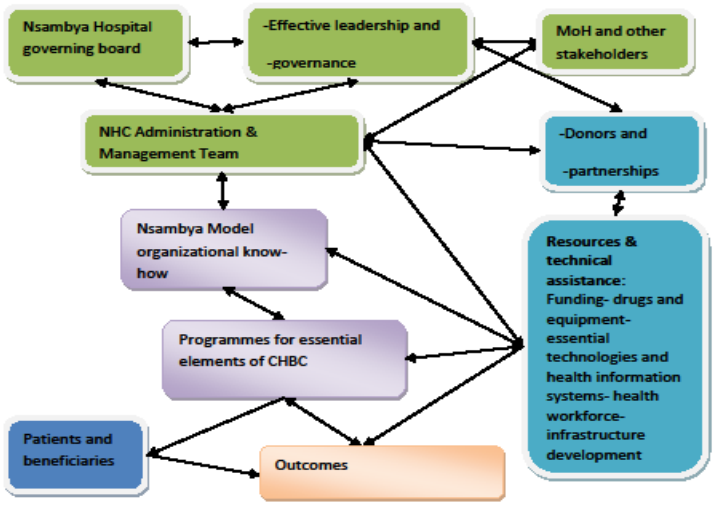

Figure 2: Illustration of the key players in the Nsambya model, the vital components and linkages to outcomes.

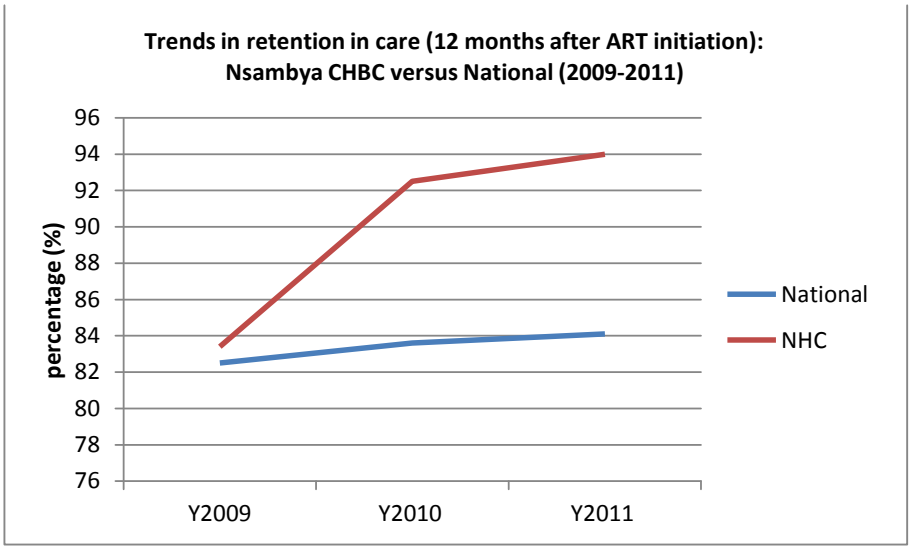

Figure 3: Trends for ART patients retained in care, 12 months after initiation, Nsambya CHBC and National (2009-2011)

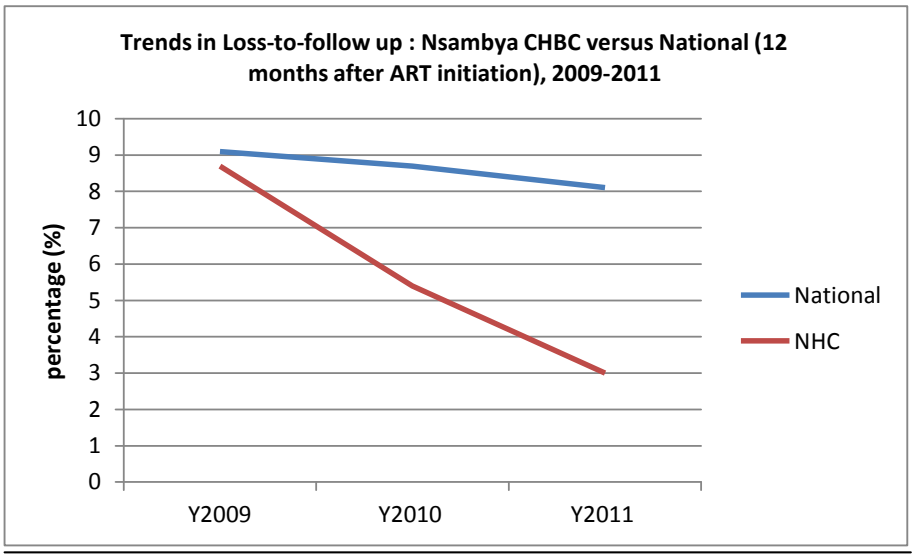

Figure 4: Loss to follow up trends for Nsambya CHBC and National (2009-2011)

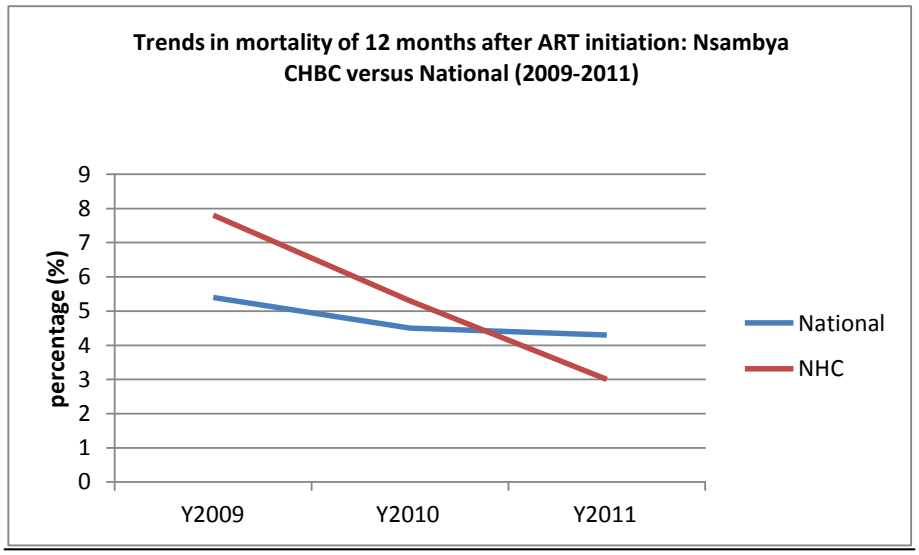

Figure 5: Mortality trends after 12 months of ART initiation, Nsambya CHBC and National (2009-2011)

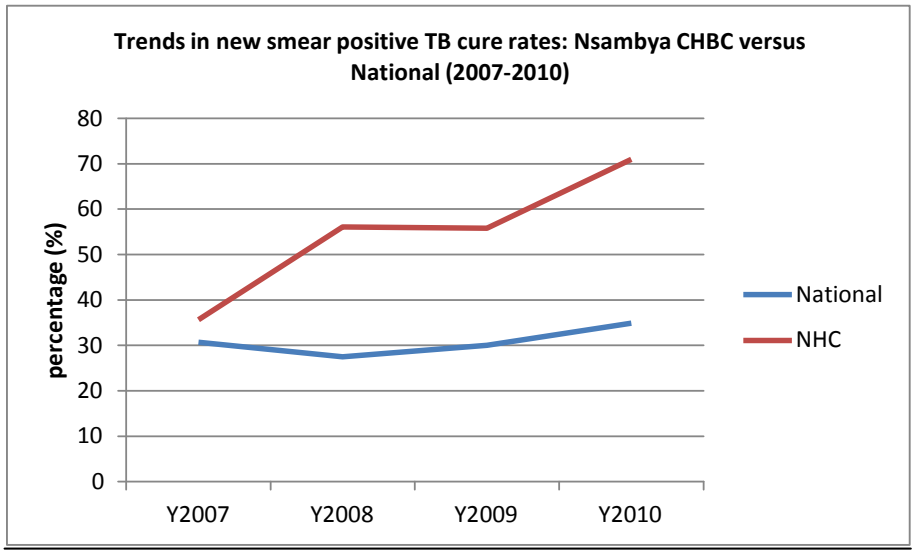

Figure 6: Trends for new smear-positive TB cure rates, Nsambya CHBC and National (2007-2010)

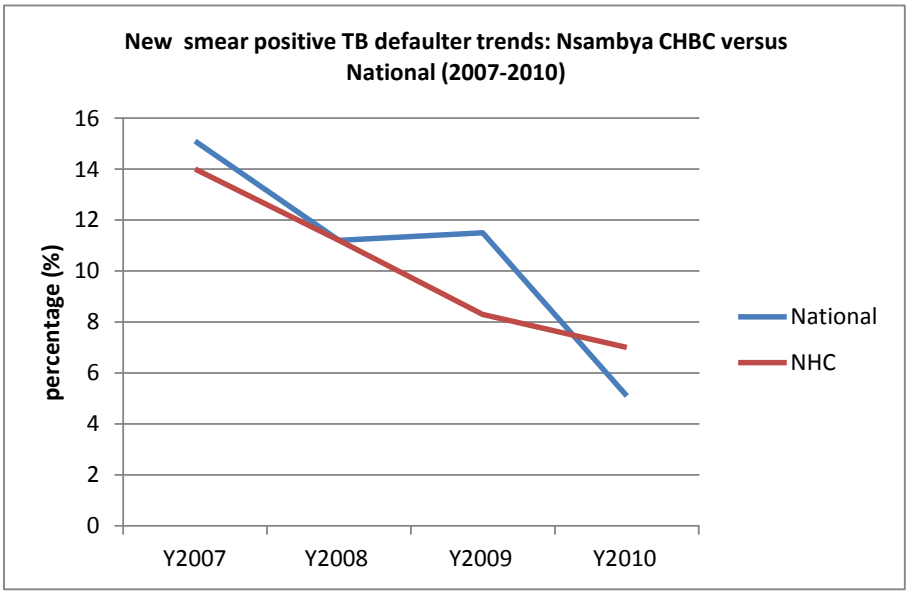

Figure 7: Trends for new smear-positive TB defaulter rates, Nsambya CHBC and National (2007-2010)

Table 1: comparison of HIV treatment outcomes from the Nsambya CHBC with National averages, 12 months after ART initiation, Kampala, (2009-2011)

Year	National % (n/N)	Nsambya CHBC % (n/N)	Overall chi-square P - value	Chi-square for trend P - value
Retained in care				
2009	82.5 (252155/305642)	83.4 (2946/3532)		
2010	83.6 (310435/371334)	92.5 (4352/4705)	<0.001	<0.001
2011	83.9 (386693/460898)	94.0 (5185/5516)		
Loss to follow up				
2009	9.1 (27813/305642)	8.7 (307/3532)		
2010	8.7 (32306/371334)	5.4 (254/4705)	<0.001	<0.001
2011	8.2 (37794/460898)	3.0 (166/5516)		
Mortality				
2009	5.4 (16505/305642)	7.8 (276/3532)		
2010	4.5 (16710/371334)	5.3 (249/4705)	<0.001	<0.001
2011	3.9 (17975/460898)	3.0 (166/5516)		

Table 2: comparison of new smear-positive TB cure and defaulter rates from the Nsambya CHBC with National averages, Kampala, (2007-2010)

Year	National % (n/N)	Nsambya CHBC % (n/N)	Overall chi-square P - value	Chi-square for trend P - value
New smear-positive TB cure rates				
2007	30.7 (6540/21303)	35.6 (99/278)		
2008	27.5 (6261/22766)	56.1 (165/294)	0.001	0.083
2009	30.0 (6934/23113)	55.8 (168/301)		
2010	34.9 (8186/23456)	71.0 (171/241)		
New smear-positive TB defaulter rates				
2007	15.1 (3217/21303)	14.0 (39/278)		
2008	11.2 (2550/22766)	11.2 (33/294)	0.541	0.877
2009	11.5 (2658/23113)	8.3 (25/301)		
2010	5.1 (1196/23456)	7.0 (17/241)		

Table 3: Comparison of TB defaulters (adults and children) receiving treatment in the Nsambya CHBC by HIV-TB co infection and ART status, Kampala, (2007-2010)

Characteristic	Referred patients N=50	NCHBC patients N=60	Total N=110	Fisher's exact/ Chi square-tests P - value
HIV-TB co infected				
	n(%)	n(%)		
Yes	19(38)	60(100)	79(72)	<0.001*
No/unknown	31(62)	0 (0)	31(28)	
HIV-TB co infected on ART				
Yes	8(42)	23 (38)	31(39)	0.769**
No	11(58)	37(62)	48(61)	

* Fisher's exact test, ** Chi square test

Data sources:

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3. Status of Antiretroviral Therapy Services in Uganda. Quarterly ART Reports for January-June 2011,
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12. WHO: TB_outcomes_2012-06-15.csv, http://www.who.int/tb/country/data/download/en/index.html

13. Nsambya Home Care TB registers

Figure captions and legends

1.Figure 1: **Nsambya Community Home-Based Care and linkages to the general health system, stakeholders and communities**

Legend: NGO: non-governmental organization; CBO: community-based organizations; FBO: faith based organizations; UGANET: Uganda network on law, ethics and HIV/AIDS; OVC: orphans and vulnerable children; TB: tuberculosis; ICF/IPT: intensified case finding and Isoniazid preventive therapy; ART: antiretroviral therapy; PMTCT: prevention of mother to child transmission; EID: early infant diagnosis

2. Figure 2: Illustration of the key players in the Nsambya CHBC model, the vital components and linkages to outcomes

Legend: Conceptual framework of the Nsambya CHBC, the context within which it operates and functional connections to all stakeholders including beneficiaries

3. Figure 3: **Trends in retention in care (12 month's after ART initiation): Nsambya CHBC versus National (2009-2011)**

Legend: NHC=NCBHC, Y- on x-axis of the graph represents year

4. Figure 4: **Trends in Loss-to-follow up : Nsambya CHBC versus National (12 month's after ART initiation), 2009-2011**

Legend: NHC=NCHBC, Y-on x-axis represents year

5. Figure 5: Trends in mortality at 12 months after ART initiation: Nsambya CHBC versus National (2009-2011)
Legend: NHC=NCHBC, Y-on x-axis represents year

6. Figure 6: Trends in new smear positive TB cure rates: Nsambya CHBC versus National (2007-2010)

Legend: NHC=NCHBC, Y-on x-axis represents year

7. **Figure 7**: **New smear positive TB defaulter trends: Nsambya CHBC versus National (2007-2010)**

Legend: NHC=NCHBC, Y-on x-axis represents year

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Research Article Open Access

Attrition and loss to follow-up Among Children and Adolescents in a Community Home-Based Care HIV Programme in Uganda

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Abstract

Background: We examine attrition and loss to follow-up (LTFU) and their baseline predictors among HIV-infected children and adolescents in a Community Home-Based Care (CHBC) model in Kampala (Uganda).

Methods: We conducted a retrospective cohort analysis of attrition and LTFU and their predictors among children and adolescents aged 0-20 years in the Tukula Fenna project. The project operates at the Home Care Department of Nsambya Hospital and four outreach clinics, located in Kampala and three surrounding districts in Uganda. The project uses community home-based care to provide free Antiretroviral Therapy (ART), other medical treatment as necessary, nutritional support, psychosocial support, and home visits. Kaplan-Meier curves were used to assess attrition and LTFU, and multivariate Cox proportional hazard regression models were used to identify their predictors.

Results: 1162 children and adolescents with confirmed positive HIV status were enrolled in the Tukula Fenna project between October 2003 and August 2012. Over this period, 5.34% (62) of patients died 37.61% (437) were LTFU, and overall attrition was 42.94% (499). This resulted in overall incidence of death of 18 per 1000 person-years, of LTFU of 126 per 1000 person-years, and of attrition of 144 per 1000 person-years. The single factor significantly associated with overall attrition among the 1162 patients was absence of ART (HR: 0.11, 95% CI: 0.09,0.14). Both baseline BMI z-score (HR: 0.96, 95% CI: 0.91, 1.00) and receipt of ART (HR: 0.12, 95% CI: 0.10, 0.15) were significantly negatively associated with LTFU among all 1162 patients in this cohort.

Conclusion: Not receiving ART was the single factor significantly associated with overall attrition. Both baseline BMI z-scores and receipt of ART were protective against LTFU among HIV positive children and adolescents enrolled in the Tukula Fenna project. Orphans need more nutritional support and improved access to early ART initiation.

Keywords: Attrition; LTFU; Retention; Children; Adolescents; CHBC; Nsambya; Uganda

Introduction

The availabilty and rapid scaling up of Antiretroviral Therapy (ART) programmes for infants and children with Human Immunodeficiency Virus (HIV) infection in Low-Middle Income Countries (LMIC) has enabled many to survive and grow into adolescents and adults [1-3]. However, retention in care of children and adolescents with HIV remains a major operational challenge requiring innovation and creativity [4-6]. Particularly, challenges associated with data collection systems may influence reporting of important outcomes such as attrition and loss to follow-up (LTFU) in these settings.

Various approaches have been employed to improve retention in HIV programmes in LMIC with varying degrees of success [7-9]. A number of studies have documented improved retention in care as well as better clinical outcomes using community-based or Community Home-Base Care (CHBC) models in Malawi, Haiti and elsewhere [10-14]. In general, CHBC includes any form of care (physical, psychosocial, palliative and spiritual) given to the sick and the affected in their own homes and care extended from the hospital or health facility to their homes through family participation and community involvement [15,16]. Home delivery of HIV counselling and testing, defaulter tracking, adherence counselling and monitoring in the home have also been shown to be associated with improved retention in care [8,17]. Other studies have demonstrated positive impacts of psychosocial support services on retention in HIV programmes in LMIC [18,19].

Thus, home delivery of basic HIV services, community-based or CHBC approaches seem to improve retention in care. However, the majority of the studies cited above were conducted in adults, and little is known about retention of children and adolescents from such approaches in LMIC. In this paper, we examine attrition and LTFU and their baseline predictors among children and adolescents in the Nsambya CHBC HIV service delivery model in Kampala, Uganda.

Methods

Study design

This retrospective cohort study utilized data routinely collected from the Tukula Fenna project between October 2003 and August 2012.

Study setting

According to the United Nations Children's Fund, by 2010, AIDS orphans accounted for 39% of all children orphaned in Uganda [20]. Approximately half of the patients in the Tukula Fenna project are orphans and recipients of Orphans and Vulnerable Children's (OVC) support. The Tukula Fenna project operates at the Home Care

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Department of St. Raphael of St. Francis Hospital (Nsambya Hospital) and four outreach clinics, located in Kampala and three surrounding districts in Uganda. Nsambya Hospital is a tertiary referral hospital in the Nsambya area of Kampala. Although Nsambya Hospital is a private-not-for-profit facility, all paediatric HIV services are free of charge due to external support from international partners (House for Life, Father Angelo, Paediatric European Network for Treatment of AIDS Foundation and the US President's Emergency Plan For AIDS Relief) and local support from the Ugandan Ministry of Health.

The Tukula Fenna project started in 2003 and provides standard and comprehensive HIV services for infants, children and adolescents within the framework of the Nsambya CHBC model. The components of the Nsambya CHBC framework include monthly on-site appointments either at the Home Care Department or at the four outreach clinics added to decentralize Voluntary Counselling and Testing (VCT), ART refills and Prevention of Mother-To-Child Transmission (PMTCT) and Early Infant Diagnosis (EID) services. Other components include a nutritional support clinic, home visits, psychosocial support workshops and services provided by a multidisciplinary outreach team (counsellors, social workers, nurses and sometimes clinicians) and an adolescents' transition clinic to prepare adolescents and their caregivers for a smooth transition to adults' HIV care. The outreach team also works with community volunteers and community leaders to identify OVC for support, as well as sensitize and mobilize communities to access HIV services.

Study population

The study included all infants, children and adolescents aged 0-20 years enrolled in the Tukula Fenna project between October 2003 and August 2012 with confirmed HIV infection and with a valid enrollment date.

Pre-ART care package

Patients were enrolled into care after VCT either at the Home Care Department or at the outreach clinics or as referrals from various sources, such as other hospitals or centres, community HIV testing sites, and self-referrals. Infants and children less than 18 months of age known or suspected to be HIV-exposed were diagnosed through HIV-1 DNA polymerase chain reaction test. Other services included cotrimoxazole prophylaxis, multivitamins supplements, screening for tuberculosis (TB), and Isoniazid Preventive Therapy (IPT) as prophylaxis and treatment for latent TB, and treatment of opportunistic infections. Laboratory evaluation involved 6-monthly CD4 cell counts and percentages and full blood counts and other tests as necessary. Nutritional status was assessed through monthly age-appropriate anthropometric measurements (weight, height and mid upper arm circumference) taken and recorded by attending nurses or nutritionists at the nutritional support clinic.

In terms of psychosocial support, paediatric patients and their care givers received various forms of counselling and social services, including monthly food incentives, free medical services, and sponsorships for basic and vocational education for some orphans and vulnerable children. Other forms of psychosocial support included children and adolescents' peer- support groups and quarterly psychosocial support workshops for paediatric patients and their caregivers. Peer-support groups consisted of 5-10 children or adolescent peers carefully selected to form a group. The group provided an atmosphere of belonging which enabled children and adolescents to share life experiences related to being HIV positive, including peer-counselling on adherence to clinical appointments and medications. In addition, drama performances by some of the adolescents in the project were used to further educate and sensitize paediatric patients on HIV prevention, positive living and other themes from time to time.

The quarterly psychosocial support workshops provided opportunities for further interactions between health care providers and paediatric patients and their caregivers outside the traditional health facilities. Discussion topics commonly included nutrition, adherence issues, self-management in HIV/AIDS and age-appropriate sexual and reproductive health issues. Sometimes psychosocial support entailed initiating child protection procedures involving legal assistance for OVC, when necessary.

Patients have been followed up through regular monthly visits since starting the project, either at the outreach clinics or at the Home Care Department. As part of the follow up process, pre-ART patients were home visited to map their homes for future visits, assess psychosocial issues that could affect adherence during pre-ART preparations, and evaluate the need for other psychosocial support services. Home visiting provided vital first hand impressions about the homes and family settings of the children and adolescents visited. The strategy was also used to track defaulters.

ART care package

Generally, ART care was an "add-on" to the pre-ART care package described above. All eligible patients and their care givers were taken through two to three weeks of pre-ART preparations before starting ART. Issues with disclosure, drug stock-outs and other logistic challenges may have delayed ART initiation. Follow up visit schedules for clinical evaluation and laboratory tests for ART patients were similar to pre-ART patients. Additional tests were performed anytime if clinically required.

Generally, patients on ART had a more aggressive tracking through phone calls and home visits, when they missed appointments or when treatment failure was suspected. Such patients received additional adherence support counselling and other forms of support depending on the needs and available resources. For example, health care providers also made efforts to build family support for therapy. In special cases, the outreach team delivered ART and food supplements to patients in their homes to promote adherence and improve treatment outcomes. Such special cases usually involved orphans and vulnerable children with little or no social support networks. Although such services were usually provided on a monthly basis, the frequencies depended largely on the needs and available resources.

Data collection system

Data collection involved different categories of health workers, different types of data and data collection tools, and selective linkage of data from paper-based registers and patient files to an electronic database, all of which were associated with challenges.

Patient data were routinely collected by attending clinicians, nurses, counsellors, social workers, laboratory staffs and data clerks at all five locations and recorded in physical patient files and registers or pre-designed reporting templates. Data collected included socio-demographic information, clinical records, laboratory tests and results and information on counselling and other psychosocial support services including OVC support, workshops and home visits. Data were collected during the clinics, counselling sessions, workshops, home visits and community-based activities, where community volunteers played important roles in data collection, through monthly reports.

Although large amounts of data were collected, owing to design and limited capacity, it was mainly quantitative socio-demographic data, clinical records and laboratory data that were selectively entered into an electronic database by trained staffs. Such data were stored in password protected Microsoft Access files and updated on a monthly basis (Microsoft Corporation, Virginia, USA).

Generally, data collection was associated with many operational challenges, particularly documentation and capturing laboratory data. Ordering laboratory tests and receiving results entailed a complex chain of activities often involving about three different categories of staffs and a number of intermediate steps. Not all the players and intermediate steps were effectively coordinated, resulting in missing data at various points along the chain, which in turn may influence reporting.

Outcome measurements and statistical analysis

Outcomes of interest were death, LTFU, and attrition. Among patients receiving ART, LTFU was defined as no patient contact for more than 3 months before the end of the study period among patients who had not died or transferred out of care; patients not receiving ART were considered LTFU after 6 months with no patient contact. Attrition was defined as LTFU and death combined. Time to attrition was calculated as the difference between date of last patient visit, date of patient transfer to other treatment center, or date of death and date of first patient visit. One day of follow-up was added for patients with a recorded first visit but no other recorded visits or death.

Baseline characteristics were compared between groups using Pearson's χ^2 for comparisons between two binomial categorical variables and Kruskal Wallis tests for comparisons between categorical variables with two or more categories. In addition to gender, comparisons were made by age category (0-2, >2-5, >5-10, >10-15, and >15-20 years), orphan status, whether or not the child had initiated ART, and whether or not the child had recorded CD4 values within one year before to one month after ART initiation. Kaplan-Meier estimates of death, LTFU, and attrition were calculated. The logrank test was used to compare Kaplan-Meier estimates of attrition between children receiving ART and those not receiving ART.

Multivariable Cox proportional hazard regression models were used to identify predictors of LTFU or attrition among all children in the cohort and among the subset of patients receiving ART. Models were adjusted for baseline characteristics including gender, orphan status, age, BMI, CD4 count and percent, whether or not ART was received, initial ART regimen and year of ART initiation. CD4 count and percent were only included in the model among the subset of patients receiving ART, as three quarters of the full cohort were missing CD4 data while fewer (55%) were missing CD4 data among the subset of children on ART. Multiple imputation by chained equations (MICE) was used to estimate missing covariate values in all models. Data were analyzed in Stata version 12.0 (Stata Corporation, College Station, TX, USA).

Ethical approval

The Uganda National Council for Science and Technology granted ethical approval (Ref #: HS 1021) for the study.

Results

Baseline patient characteristics

Between October 2003 and August 2012, 1498 patients were enrolled in the Tukula Fenna project. Of the 1498 enrolled, 1162 patients had confirmed positive HIV status. Total follow-up was 3466 person-years, median follow-up time from enrollment was 24.97 months (IQR 6.21-65.61), and median follow-up time from ART initiation was 36.57 months (IQR 17.48-61.24) among the 625 patients who initiated ART.

Baseline characteristics for these 1162 patients stratified by gender are summarized in Table 1. Significant differences were found between age categories in orphan status, with higher proportions of orphans in the >5-10 and >10-15 years groups (38.10% and 36.80%, respectively) than in the 0-2 (5.02%), >2-5 (17.47%), or >15-20 (2.60%), p for trend<0.01) years age groups. In addition, median BMI z-score was lower among children>5-10 and >10-15 years old (-2.39 and -2.40 versus -0.81, -0.97, and -1.50 than those 0-2, >2-5, and >15-20, respectively, p for trend<0.01). Initiation of ART and timing and make-up of initial ART regimens also differed by age category, with a higher percentage of children receiving ART in the >5-10 and >10-15 years age groups (33.92% and 30.40% versus 13.76%, 19.84%, and 2.08% than those 0-2, >2-5, and >15-20 years, respectively, p for trend<0.01). Children in the >2-5 years age group made up a large percentage of ART recipients only in 2009-2010 (31.55% versus 16.33%, 13.29%, 17.44%, and 14.85% in 2003-2004, 2005-2006, 2007-2008, 2011-2012, respectively). On the other hand, those in the 0-2 years age group made up a large percentage of ART recipients only in 2011-2012 (28.71% versus 4.08%, 10.49%, 13.37%, and 11.76% in 2003-2004, 2005-2006, 2007-2008, and 2009-2010, respectively, p for trend<0.01). Orphans in this cohort were older (median age 8.77 years versus 4.36 years for non-orphans, p<0.01, 28.05% and 28.33% of non-orphans in 0-2 and >2-5 years age groups, respectively versus 5.02% and 17.47% of orphans, p<0.01), had lower BMI z-scores (median -2.40 versus -1.53 among non-orphans, p<0.01), and were less likely to receive ART (51.3% orphans received ART versus 59.5% non-orphans, p=0.02). In addition to orphan status and age, children receiving ART differed from those not on ART by CD4 z-score, with a lower median CD4 z-score (-0.18) among ART recipients than among non-recipients (1.28, p<0.01). Patients missing CD4 count data were more often orphans, (62.9% orphans versus 52.2% among those with CD4 data, p=0.01) and younger (median 6.27 years versus 7.21 years among those with CD4 data, p=0.02). They also weighed less (median BMI z-score -1.93 versus -1.36 among those with CD4 data, p<0.01), and were much less likely to receive ART (39.4% received ART versus 96.6% of those with CD4 data, p<0.01).

Attrition and loss to follow-up

Throughout the entire study period, 5.34% (62) of patients died, 37.61% (437) were LTFU, and overall attrition was 42.94% (499). This resulted in overall incidence of death of 18 per 1000 person-years, of LTFU of 126 per 1000 person-years, and of attrition of 144 per 1000 person-years. At 12, 24, and 36 months after enrollment, mortality was 4.13%, 4.65%, and 4.99%, LTFU was 21.34%, 26.76%, and 29.52%, and overall attrition was 25.47%, 31.41%, and 34.51%, respectively. Attrition, LTFU and mortality were lower among the 625 patients receiving ART, with 12, 24, and 36 month mortality of 1.60%, 2.08%, and 2.24%, LTFU of 3.84%, 7.04%, and 8.48%, and overall attrition of 5.44%, 9.12%, and 10.72%. Figure 1 shows the Kaplan-Meier failure curves for death, LTFU, and attrition throughout the duration of the study among all 1162 patients. While mortality ceases to increase after the first year or so, LTFU and overall attrition continue to increase after 12 months, albeit at a slower rate.

Predictors of attrition and loss to follow-up

Cox proportional hazards ratios for attrition and LTFU among all patients and those receiving ART are shown in (Table 2 and 3), respectively. The single factor significantly associated with overall

Table 1: Baseline characteristics of 1162 children enrolled in Tukula Fenna HIV programme between October 2003 and August 2012, by gender.

	Overall	Male	Female	p-value for difference¥
	n=1162 (100.00%)	n=562 (48.36%)	n=600 (51.64%)	
Orphaned*, *n* (%) (*n*=891)[1]	538 (60.38)	259 (59.82)	279 (60.92)	0.74
Age in years, *median* (IQR)	6.52 (7.31)	6.26 (7.15)	6.82 (7.40)	0.41
Age group categories, *n* (%)				0.72
0-2 years	213 (18.33)	103 (18.33)	110 (18.33)	
>2-5 years	247 (21.26)	125 (22.24)	122 (20.33)	
>5-10 years	380 (32.70)	184 (32.74)	196 (32.67)	
>10-15 years	302 (25.99)	138 (24.56)	164 (27.33)	
>15-20 years	20 (1.72)	12 (2.14)	8 (1.33)	
CD4 count± z-score, *median*(IQR), (*n*=293)[2]	-0.13 (1.61)	-0.06 (1.72)	-0.18 (1.44)	0.47
CD4 percent± z-score, *median*(IQR), (*n*=228)[3]	-0.21 (1.56)	-0.33 (1.63)	0.00 (1.61)	0.06
WHO clinical stage, *n* (%), (*n*=1158)[4]				0.28
I/II	983 (84.89)	468 (83.72)	515 (85.98)	
III/IV	175 (15.11)	91 (16.28)	84 (14.02)	
BMI z-score, *median* (IQR),	-1.74 (2.85)	-1.74 (2.88)	-1.73 (2.81)	0.21
(*n*=999)[5]				
Receiving ART, *n* (%)	625 (53.79)	302 (53.74)	323 (53.83)	0.97
Initial ART regimen by key component, *n* (%) (*n*=625)[6]				
NNRTI-based	591 (94.56)	284 (94.04)	307 (95.05)	0.58
PI-based	28 (4.48)	16 (5.30)	12 (3.72)	0.34
Including d4t	180 (28.80)	91 (30.13)	89 (27.55)	0.48
Including ZDV	407 (65.12)	200 (66.23)	207 (64.09)	0.58
Year starting ART, *n*(%), (*n*=625)[7]				0.3
2003/2004	49 (7.52)	20 (6.31)	29 (8.66)	
2005/2006	143 (21.93)	72 (22.71)	71 (21.19)	
2007/2008	172 (26.38)	80 (25.24)	92 (27.46)	
2009/2011	187 (28.68)	101 (31.86)	86 (25.67)	
2011/2012	101 (15.49)	44 (13.88)	57 (17.01)	

* Child considered orphaned if either mother or father was reported deceased at baseline
± CD4 count and percent results from within 1 year prior to or 1 month after ART initiation
¥ Pearson's chi square or Kruskal Wallis chi square tests used, as appropriate
1 missing orphan status for 271 (23.32%) of children
2 missing CD4 count for 869 (74.78%) of children
3 missing CD4% for 934 (80.38%) of children
4 missing WHO stage for 4 (0.00%) of children
5 missing BMI z-score for 163 (14.03%) of children
6, 7 missing information on initial ART regimen and year starting ART for 537 (46.21%) of children

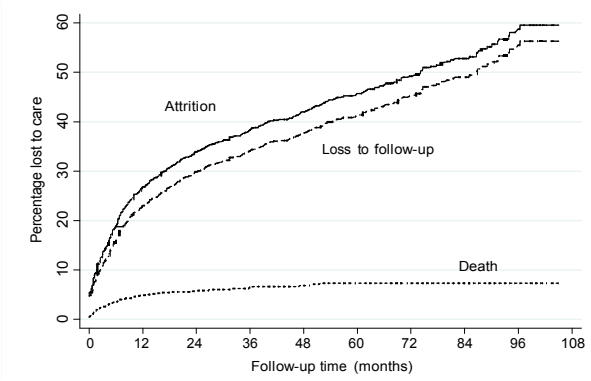

Figure 1: Kaplan-Meier failure curves for death, LTFU, and attrition throughout the duration of the study among all 1162 patients.

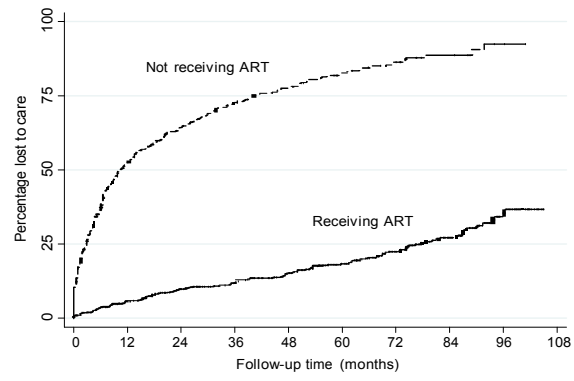

Figure 2: Kaplan-Meier failure curves for overall attrition among those patients receiving and not receiving ART.

attrition among 1162 eligible patients was absence of ART (HR: 0.11, 95% CI: 0.09, 0.14). Both BMI z-score (HR: 0.96, 95% CI: 0.91, 1.00) and receipt of ART (HR: 0.12, 95% CI: 0.10, 0.15) were significantly negatively associated with LTFU among all 1162 patients in this cohort.

Figure 2 shows the Kaplan-Meier failure curves for attrition among children who were receiving ART and those who were not yet on ART. Attrition was significantly higher among those not receiving ART (logrank χ^2=516.27, p<0.01). In the subgroup of 625 patients receiving

Table 2: Cox proportional hazard ratio of risk of attrition* among 1162 children enrolled in Tukula Fenna HIV programme and 625 receiving ART between October 2003 and August 2012.

	All patients (*n*=1 162)		Patients receiving ART (*n*=625)	
	Hazard ratio (95% confidence intervals)	p-value	Hazard ratio (95% confidence intervals)	p-value
Male	1.12 (0.94-1.34)	0.21	0.89 (0.68-1.44)	0.95
Orphaned	1.03 (0.81-1.30)	0.82	1.03 (0.65-1.63)	0.9
Baseline age in months	1.00 (1.00-1.00)	0.68	1.00 (1.00, 1.01)	0.41
Baseline BMI z-score	0.96 (0.91-1.00)	0.04	1.02 (0.92-1.01)	0.69
Baseline CD4 count± z-score	-	-	0.88 (0.61-1.26)	0.48
Baseline CD4 percent± z-score	-	-	1.03 (0.72-1.48)	0.86
Receiving ART	0.12 (0.10-0.15)	<0.01	-	-
Initial ART regimen				
NNRTI-based	-	-	Reference	
PI-based	-	-	0.87 (0.36-2.07)	0.75
Including d4t	-	-	0.69 (0.32-1.48)	0.34
Including ZDV	-	-	0.85 (0.42-1.70)	0.64
Year starting ART				
2003/2004	-	-	0.98 (0.39-2.47)	0.96
2005/2006	-	-	0.69 (0.30-1.59)	0.39
2007/2008	-	-	0.75 (0.30-1.87)	0.54
2009/2010	-	-	0.75 (0.35-1.63)	0.47
2011/2012	-	-	Reference	

*Attrition defined as death or no contact prior to August 7, 2012 for at least 6 months among children not receiving ART and for at least 3 months among those receiving ART
± Baseline CD4 count and percent results from within 1 year prior to or 1 month after ART initiation

Table 3: Cox proportional hazard ratio of risk of loss to follow-up* among 1162 children enrolled in Tukula Fenna HIV programme and 625 receiving ART between October 2003 and August 2012.

	All patients (*n*=1 162)		Patients receiving ART (*n*=625)	
	Hazard ratio (95% confidence intervals)	p-value	Hazard ratio (95% confidence intervals)	p-value
Male	1.15 (0.94-1.40)	0.09	0.98 (0.66-1.47)	0.94
Orphaned	1.11 (0.85-1.45)	0.74	1.06 (0.63-1.76)	0.84
Baseline age in months	1.00 (1.00-1.00)	0.7	1.00 (1.00, 1.01)	0.35
Baseline BMI z-score	0.97 (0.93-1.03)	0.09	1.01 (0.89-1.13)	0.91
Baseline CD4 count± z-score	-	-	0.90 (0.63-1.26)	0.52
Baseline CD4 percent± z-score	-	-	1.03 (0.71-1.49)	0.89
Receiving ART	0.11 (0.09-0.14)	<0.01	-	-
Initial ART regimen				
NNRTI-based	-	-	Reference	
PI-based	-	-	0.89 (0.34-2.30)	0.8
Including d4t	-	-	0.72 (0.32-1.62)	0.43
Including ZDV	-	-	0.87 (0.42-1.83)	0.72
Year starting ART				
2003/2004	-	-	0.76 (0.29-2.03)	0.59
2005/2006	-	-	0.52 (0.22-1.27)	0.15
2007/2008	-	-	0.68 (0.26-1.75)	0.42
2009/2010	-	-	0.73 (0.33-1.64)	0.45
2011/2012	-	-	Reference	

*Loss to follow-up defined as no contact prior to August 7, 2012, excluding any deaths, for at least 6 months among children not receiving ART and for at least 3 months among those receiving ART
± Baseline CD4 count and percent results from within 1 year prior to or 1 month after ART initiation

ART, no baseline characteristics were significantly associated with either attrition or LTFU.

Missing data

High percentages of children were missing CD4 data 74.78% (CD4 count) and 80.38% (CD4%). In addition, 46.21% of ART regimen information, 23.32% of data on orphan status and 14.03% of data on BMI z-score were missing.

Discussion

We found that among children and adolescents receiving HIV care through the Tukula Fenna project, overall incidence of death was 18 per 1000 person-years, of LTFU was 126 per 1000 person-years, and of attrition was 144 per 1000 person-years. Not receiving ART was the single factor significantly associated with overall attrition among the 1162 patients studied, while both baseline BMI z-score and receipt of ART were significantly negatively associated with LTFU among all patients in this cohort. The higher rate of LTFU and relatively lower death rate observed in this study are in line with the literature, and most likely due to considerable misclassification of LTFU, that is, many deaths among patients considered LTFU [21,22]. We also think that the high LTFU rate may be due in part to delays in obtaining and entering data, such that patients considered LTFU may actually have unrecorded visits or unrecorded deaths. A considerable proportion of attrition occurred during the first 12 months or so after enrolment into care, which is consistent with the literature [23,24] and depicted in Figure 1. Potential explanations include patient factors, such as enrolling into care with comorbidities and advanced disease, providers factors such as delayed linkage to care and or failure of timely initiation of ART in eligible patients, and health system factors such as stock-outs of drugs and poor geographical access to HIV paediatric services.

Our data also support studies that have demonstrated that ART is protective against LTFU and attrition. That is illustrated by lower rates of deaths, LTFU and attrition among patients receiving ART, compared to those not receiving ART (Figure 2) over the study period [4,25]. Children on ART may enjoy better overall health including improved growth responses [26-28], better enabling them to come in for their scheduled visits. There may also be greater motivation on the part of caregivers to promote retention in care as part of promotion of adherence to ART, such that children not on ART would not receive the extra attention paid to those on ART. Studies have also demonstrated that healthy HIV infected children have a lower risk of attrition [29,30] compared to sick and malnourished children and may explain the protective effect of baseline BMI z-scores against LTFU observed in the current study. Additionally, as described under the ART care package, the existing patient tracking system seems to favour those receiving ART, compared to pre-ART patients, due to rationing of resources. Every effort should be made to initiate ART as early as possible among HIV positive paediatric patients, as well as streamline linkage to care and tracking of HIV positive children not yet receiving ART in order to improve retention rates among this sub-group.

There were notable variations in the timing, proportions and trends in ART initiation with respect to the age categories. For instance,

children in the age group >2-5 years made up a large percentage of ART recipients only in 2009-2010, while those in 0-2 years constituted a large proportion of ART recipients mainly in 2011-2012. It is important to recognize that patients in this study have been enrolled from 2003-2012 and many changes have occurred over the period in terms of general access, treatment protocols, and in particular, eligibility for ART among children, following the serial revisions of the treatment guidelines. Thus, the observed trends could be reflecting increasing access with improvements in some services overtime, and increasing eligibility for ART associated with the serial revisions [31-35] of the guidelines (Table 1). In addition to the changes in the treatment guidelines, PMTCT / EID, programmes were introduced in the project in 2011 and may explain the observed peak in ART initiation among the 0-2 year age group by 2011-2012.

We also saw differences between orphans and non-orphans in terms of age, nutritional status and receipt of ART. Ophans were generally older, more malnourished and were less likely to receive ART. This finding could mean that, despite food incentives and the community home-based care approach, some orphans have late or limited access to ART with resultant poor growth responses. Additionally, and perhaps more importantly, this finding may be reflecting issues related to custody of some orphans in our setting. Studies conducted in Uganda and elsewhere in sub-Saharan Africa have shown that most orphans are cared for by grandmothers and or older siblings who generally have little or no formal education and usually have no training or financial support for the care and management of People Living With HIV/AIDS (PLHA) [36-39]. Furthermore, sometimes depending on the socio-cultural events in the lives of the caretakers, some orphans may have to change caretakers and or relocate to different dwelling places. These scenarios and unreliable addresses or telephone contacts make it quite difficult tracking some orphans in the communities to provide them with psychosocial supports services in real-time. There is urgent need to strengthen the orphans and vulnerable children's programme to indentify and support such children and promote their access to health care and linkage to the nutritional support clinic.

Missing data was an important challenge in this study, and the main reasons have been described under the data collection system. Further to that, certain patient characteristics seem to be at play in explaining the observed trends. For example, patients missing CD4 count data were more often orphans, younger, in poorer health (more malnourished) and were much less likely to receive ART. These patient characteristics were found to be associated with attrition in the literature [24,30,40] and may partly explain our observations. Alternatively, this finding could be reflecting poor access to HIV services among these categories of patients.

Limitations of this study include using routine programmatic data that are based on observational information for research, issues with data collection, missing data and their potential impacts on the study outcomes. Some of the challenges with observational data are that, they may not be designed to answer specific research questions, and operational definitions may not be universal, making it difficult to compare some outcomes from different studies. In addition, the scope of observational data may predispose to bias, which in turn could limit the extent to which results can be extrapolated. The current analysis has revealed important gaps in data collection, fragmented and uncoordinated data collection tools and selective linkage of mainly quantitative data from the clinics and laboratory into the electronic database. These challenges translate into poor data capturing and missing data for some variables, including those related to attrition and LTFU. Missing data about reason for exiting the programme may also contribute to these rates, as some patients considered lost to follow-up may have transferred to other facilities or tested negative for HIV after an initial positive test and terminated from care. Other studies from similar LMIC have documented the negative impacts of incorrect or missing contact information on LTFU and attrition rates [21,41].

Multiple Imputation by Chained Equations (MICE) was used to estimate missing covariate values in all models in this study. Additionally, differences in baseline characteristics were all adjusted for, in order to minimize any potential confounding.

We believe that regular training of data collecting staffs, simplifying and streamlining data collection tools, together with regular updates of patient information could improve the data capturing system and minimize missing data. In addition, including qualitative data on home visits, psychosocial support services and orphans and vulnerable children support in the electronic databases would facilitate analysis and usage of such data for research. Such a measure would explore the value of qualitative data and show how they may contribute to explain outcomes such as attrition and LTFU in this under studied population. Despite the limitations, we believe that our findings remain valid and relevant.

Conclusion

Overall, attrition and LTFU were relatively high in this study. Not receiving ART was the single factor significantly associated with attrition in this cohort, while both baseline BMI z-scores and receipt of ART were protective against LTFU among HIV positive children and adolescents enrolled in the Tukula Fenna project. Efforts should be made to initiate ART among all paediatric patients as soon as possible, and to provide aggressive follow-up for those not yet receiving ART. Orphans need more nutritional support to reduce the burden of malnutrition and improved access to early ART, which could also promote growth responses in this vulnerable and understudied group.

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Factors determining Survival and Retention among HIV-infected Children and Adolescents in a Community Home-Based Care and a Facility-Based Family-Centred Approach in Kampala, Uganda: **a cohort study**

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Abstract

We describe factors determining retention and survival among HIV-infected children and adolescents engaged in two health care delivery models in Kampala, Uganda: one a community home-based care (CHBC), and the other a facility-based family-centred approach (FBFCA). This retrospective cohort study reviewed records from HIV-positive children age 0 to 18 years engaged in the two models from 2003 to 2010 focussing on retention/loss to follow-up, mortality, use of antiretroviral therapy (ART), and clinical characteristics. Kaplan Meier survival curves with log rank tests were used to describe and compare retention and survival, stratified by model of care and age. Overall, 1,623 children were included, 90.0% (1460/1623) from the CHBC. Children completed an average of 4.2 years of follow-up (maximum 7.7 years). Median age was 53 (IQR: 11-109) months at enrolment. In the CHBC, retention differed significantly between those on ART and patients not receiving ART (log-rank test, adjusted, $p<0.001$). Comparing patients on ART in both models, there was no significant difference in long-term survival (log-rank test, $p=0.308$, adjusted, $p=0.489$), while retention was higher in the CHBC; 94.8% versus 84.7% in the FBFCA (log-rank test, $p<0.001$, adjusted $p=0.006$). Irrespective of model of care, children receiving ART had better retention in care and survival.

Key words: Antiretroviral therapy, HIV care, children, adolescents, retention, survival, Uganda

Background

Sub-Saharan Africa (SSA) is home to the vast majority of infants, children, and adolescents living with HIV and morbidity and mortality remain high [1-3]. For example, mortality among HIV-infected children has been measured at 4.3% per year in East Africa and 8.3% in West Africa [4,5]. A recent meta-analysis conducted in SSA reported a higher risk of early death among perinatally infected children [6]. Studies have also shown that substantial proportions of children and adolescents initiate treatment in SSA with advanced disease (46.3%-72.0%), and comorbidities such as TB (5.7%-34.0%) and malnutrition (33%-54%) that tend to be associated with early mortality and poor clinical outcomes [7-10].

Significant child mortality can be averted if antiretroviral therapy (ART) is started early [11-14]. However, despite overwhelming evidence demonstrating the benefits of ART, in practice high mortality and loss to follow up persist among HIV-infected children and adolescents in care in the resource-limited settings of SSA. In addition to scarce resources for programmes for children, the situation is compounded by a combination of factors including late HIV diagnosis, missed opportunities to initiate ART, health care programmes not tailored to the needs of the infected child and their family, and logistic bottlenecks in implementation of care and treatment programmes [15,16]. Initiation of ART even among children known to be eligible may be missed. For instance, in a study of ART-eligible children in The Gambia, only 32.7% started ART, 47.1% were lost to follow-up, and 13.5% died before initiating ART [17].

Retention in care while awaiting ART eligibility can also be a challenge. As an illustration, retention varied from 71% to 95% and 62% to 93% at 12 and 24 months, respectively, among children and adolescents in ART programmes in several

countries of SSA [18]. A prior study in Uganda showed that even with frequent CD4 monitoring, HIV-infected children experienced significant clinical events while ineligible for ART according to the 2006 WHO guidelines [19]. Another study in Uganda showed that mortality was highest among HIV-infected children under two years [20]. Given this situation, it is important to assess factors that determine survival and retention in care among HIV-infected children and adolescents in care in resource-limited settings.

The present study focuses on retention and survival in two different ART delivery models for HIV-infected children and adolescents in Kampala, Uganda. One is a facility-based, family-centred approach (FBFCA) adopted by the mother to child transmission (MTCT)-Plus programme of the Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration. The other is a community home-based care (CHBC) implemented by the children's HIV programme of the Home Care Department of Nsambya Hospital. The American Academy of Paediatrics defined family-centred care as based on the understanding that the family is the child's primary source of strength and support [21]. Beyond that, the family provides an enabling environment for using index HIV patients to reach other infected and affected family members, build family support for therapy and chronic care, integrate other medical needs for the family and thus enhance uptake of HIV and other medical services for the family as a unit [22]. In general, CHBC includes any form of care (physical, psychosocial, palliative and spiritual) given to the sick and the affected in their own homes and care extended from the hospital or health facility to their homes through family participation and community involvement [23,24].

Factors that determine retention and survival of HIV-infected children and adolescents in these health care models are not well understood. The present study therefore aims at identifying factors that determine these outcomes for the CHBC of Nsambya Hospital and the FBFCA of MU-JHU. We also examine pre-ART deaths among children and adolescents in the CHBC to compare mortality rates prior to and after ART initiation and to ascertain whether children experiencing mortality prior to initiating ART had met the 2010 WHO [25] or the 2011 updated United States (US) [26] treatment guidelines for initiating ART or not.

Methods

Study design, setting, and population

This retrospective cohort study covered eight years of records review (2003 to 2010) from two facilities implementing HIV paediatric programmes in Kampala, Uganda. They included the children's HIV programme of the Home Care Department of Nsambya Hospital, which uses community home-based care (CHBC) and the MTCT-plus programme of MU-JHU Research Collaboration, which adopts a facility-based family-centred approach (FBFCA). Prior to the study, all children in the FBFCA had been initiated on ART, thus the record review at the FBFCA involved only children on ART. The facilities are both private-not-for-profit, but differ in service delivery approaches, including catchment areas and enrolment practices. The study population included all HIV positive infants, children and adolescents aged 0-18 years, enrolled in both programmes over the study period. Services are generally free of charge under both models.

Description of health care models

The FBFCA of MU-JHU Collaboration was established in 2003 with funding from Columbia University. Its catchment area includes Kampala and Wakiso districts and covers approximately 20 km radius from Mulago hospital in Kampala. Enrolment into the FBFCA occurred between 2003 and 2005 and targeted all HIV-infected family members as a unit. However, HIV- infected pregnant women in PMTCT served as the starting point for identifying other infected family members such as infants, children and spouses or partners to be enrolled into care. Eligibility of the women included being pregnant, testing HIV positive, attendance of PMTCT clinic, disclosure of HIV status to spouse or partner, willingness to be home visited and living within 20 km radius from Mulago Hospital. The FBFCA offered comprehensive HIV care, including early infant diagnosis (EID), treatment and psychosocial support services, other medical services, and routine follow up to eligible women and their families. All children enrolled in the study had been initiated on ART prior to the study start date, in contrast to the CHBC. The programme has a uniform design that has been implemented in many countries. Although funding ended in December 2011, the families continue to be followed in a family care approach with funding from the US President's Emergency Plan for AIDS Relief (PEPFAR).

The CHBC also started in 2003 and it integrates facility-based care with home-based care using community involvements as important linkages to decentralize HIV services. It has a catchment area covering four districts: Kampala, Wakiso, Mukono and Mpigi within 21 km radius from Nsambya hospital. In contrast to the FBFCA, children and adolescents in the CHBC were identified directly for participation. The components of the CHBC, details of enrolment practice, pre-ART care and ART care packages, as well as patient tracking system have been described in an earlier work

[27]. The CHBC was funded by international donors and partners, and indirectly supported by PEPFAR.

Both health care models share some common elements such as providing additional support including nutritional supplements, patient education, and counselling of patients and their caregivers. Additionally, peer support groups for both adults and children have been developed to promote emotional support. Other components of psychosocial support include financial assistance for income generating activities, a music, dance and drama group and home visiting to track defaulting patients. Furthermore, the FBFCA trains peer- educators to provide support and help in the clinics.

Clinical and laboratory follow up

Patients were followed up routinely using similar appointment systems, standard guidelines and procedures. Generally, children on ART had monthly clinic visits under both models, while visits for pre-ART patients depended on their clinical conditions and varied between one month under the CHBC and 3-6 months under the FBFCA. Initiation of ART was based on the Ugandan National ART guidelines that are adopted from the WHO guidelines [25,28-30]. The latter have changed over time, especially with the more recent move in 2013 to initiate treatment in all children under 5 years of age, irrespective of clinical or immune status [11]. During visits, patients were evaluated clinically using WHO clinical staging, age, weight, height, ART status, and laboratory investigations like haemoglobin levels and 6-monthly CD4 cell counts to monitor response to therapy. Adherence to clinical appointments was assessed using appointment schedules, while adherence to medication was assessed by caregivers and self-reports in addition to pill counts. Apart from ART,

patients in care received universal Cotrimoxazole prophylaxis for opportunistic infections, and secondary prophylaxis for cryptococcal meningitis. In addition, patients in the CHBC received isoniazid preventive therapy (IPT) for prophylaxis and treatment of latent TB infection.

Study outcomes

The main study outcomes were: a) retention in care, b) deaths among patients on ART, and c) pre-ART deaths (deaths before ART initiation) among patients in the CHBC programme. Death was ascertained through medical records and verbal autopsies carried out by trained community volunteers and counsellors. Retention was defined as the proportion of patients known to be alive (either, by patient record review or by telephone calls or home visits) and in care at the end of the follow up period. We defined loss to follow up (LTFU) as 90 days or more (if on ART) and 180 days or more (if not on ART) without contact since the last clinic appointment. Attrition included deaths and LTFU. Known transfers to continue ART or care at other facilities were not considered as attrition.

Statistical analysis

We analysed factors that determined retention and mortality among children and adolescents enrolled in the two HIV service delivery models described above. We used frequency distributions, medians, and interquartile range (IQR) to describe baseline characteristics and compared these using Chi-square and Wilcoxon Rank-Sum tests, respectively. The baseline characteristics included age groups (at enrolment), gender, CD4 cell counts, CD4 per cent, growth responses (weight-for-age and height-for-age z-scores), WHO disease stages, ART status, and age at ART initiation. Because of differences in baseline characteristics in the two study groups, all analyses were adjusted for age at ART initiation, CD4 per cent, CD4 cell counts,

proportions on ART, nutritional status and WHO clinical staging using Cox regression. In addition, Cox regression was used to determine factors associated with attrition among patients on ART in both models, and among patients in the CHBC separately, in unadjusted and adjusted analyses. Kaplan Meier curves with log-rank tests were used to describe and compare retention and survival, stratified by model of care as well as by age groups. Data on CD4 cell count and CD4 per cent were log transformed because of skewed distribution. Finally, we used Chi-square test to examine the number and proportions of children dying prior to ART initiation in terms of whether they met or did not meet the 2011 US or 2010 WHO guidelines for initiating ART. All statistical testing was two-sided and conducted at the 5% significance level. Data from both programmes were extracted from databases, merged and analysed with Intercooled STATA software version 12.

Ethical clearance was approved by the MildMay Institutional Review Board and Ethics Committee, and the study was registered by the Uganda National Council for Science and Technology (UNCST, ref#: HS 1021). The relevant committees waived informed consent. The study was funded by the University of Padova, Department of Paediatrics and supported by *Casa Accoglienza alla vita padre Angelo*.

Results

Baseline characteristics

Overall, 1,623 infants, children, and adolescents were included in the analyses, 90.0% (1460/1623) were in the CHBC (Table1). There were slightly but not significantly more females compared to males. At enrolment, 47.1% in the CHBC and 38.9% in the FBFCA were over 60 months of age (p=0.097). Baseline median CD4 cell counts

were 393 cells/mm3 in the CHBC versus 727 cells/mm3 in FBFCA (p <0.001) and median CD4 per cents were 5.8 % in CHBC versus 17.0% in FBFCA (p <0.001). By WHO clinical staging, 86.4% and 96.9% were in stages I-II in the CHBC and FBFCA, respectively, versus 13.6% and 3.1% in stages III-IV in the CHBC and FBFCA respectively (p<0.001). ART was initiated among 30.2% in the CHBC model compared to 100% in the FBFCA (p<0.0001). Median age at ART initiation was 91.0 months for children in the CHBC versus 45.9 months in the FBFCA (p <0.001). In terms of growth response, 37.4% in the CHBC versus 16.9% in the FBFCA had weight-for-age z-scores of ≤-2SD (p<0.001), while 55.7% in the CHBC versus 69.7% in the FBFCA had height-for-age z-scores of >-2SD, (p=0.001).

Retention in care

An overall average of 4.2 years of follow-up was observed (maximum 7.7 years). Retention in care was substantially and significantly higher among children on ART compared to those not on ART within the CHBC model (p<0.001, adjusted, p<0.001). A total of 266 children were lost to follow-up, all were within the CHBC model (18.2%) with only two (0.5%) on ART lost to follow-up. Among children on ART, retention was higher in the CHBC (94.8%, 95% CI: 92.7% - 96.8%) compared to (84.7%, 95% CI: 79.1% - 90.2%, p=0.001, adjusted p=0.006) in the FBFCA (Figures 1A and 1B).

Factors associated with attrition among children and adolescents on ART in both models

In univariate analysis, attrition was significantly associated with model of care (HR: 0.40, 95%CI: 0.23-0.70, p=0.002); mild immunosuppression (HR: 4.17, 95% CI: 1.31-13.31, p=0.016); severe immunosuppression (HR:3.14, 95% CI: 1.10-8.94,

p=0.032); age at ART initiation (HR:0.74, 95% CI: 0.61-0.90, p=0.003); and weight-for–age z-scores of >-2SD (HR: 0.40, 95% CI: 0.21-0.77, p=0.006).

At multivariate modelling, the risk of attrition was significantly associated with model of care (HR: 0.29, 95% CI: 0.12-0.70, p=0.006) with the CHBC model promoting retention; mild immunosuppression (HR: 4.66, 95% CI: 1.21-17.98, p=0.026); and weight-for-age z-scores of >-2SD (HR: 0.31, 95% CI: 0.15-0.65, p=0.002, Table 2).

Factors associated with attrition among children and adolescents in the CHBC (30% on ART)

At univariate analysis, the risk of attrition in the CHBC was significantly associated with age group 36-59 months (HR: 0.46, 95% CI: 0.33-0.64, p<0.001); age group 60+ months (HR: 0.44, 95% CI: 0.35-0.56, p<0.001); CD4 cell count (HR: 0.85, 95% CI: 0.78-0.93, p<0.001); CD4 per cent (HR: 0.80, 95% CI: 0.66-0.96, p=0.016) and severe immunosuppression (HR: 1.87, 95% CI: 1.44-2.43, p<0.001). The risk of attrition was also significantly associated with WHO clinical stages III-IV (HR: 1.84, 95% CI: 1.47.-2.29, p<0.001); weight-for-age z-scores of >-2SD (HR: 0.76, 95% CI: 0.62-0.93, p=0.007); height-for-age z-scores of >-2SD (HR: 0.69, 95% CI: 0.56-0.84, p<0.001) and receipt of ART (HR: 0.06, 95% CI: 0.04-0.09, p<0.001).

At multivariate analysis, the risk of attrition was significantly associated with CD4 cell count (HR: 0.84, 95% CI: 0.74-0.95, p=0.006); WHO clinical stages III-IV (HR: 1.94, 95% CI: 1.34-2.80, p<0.001), and receipt of ART (HR: 0.04, 95% CI: 0.02-0.08, p<0.001, Table 3).

Retention stratified by age groups

When retention was stratified by age groups among children and adolescents in the two models, the difference was only significant in the age group 12 -35 months

(P=0.015) and borderline in age group 36 -59 months (P=0.06). This means that age has a confounding effect on retention in care (Figure 2).

Mortality on ART

There was no significant difference in survival of children and adolescents receiving ART between the two models (Figure 3A, p=0.308, adjusted p=0.489). However, there was a significant difference between pre-ART deaths (deaths that occurred before ART initiation) and deaths among ART patients in the CHBC, (Figure 3B: log-rank test p=0.001, adjusted p=0.001). Overall, 34 children died while on ART, 4.8% (21/441) within the CHBC and 8.0% (13/163) within the FBFCA. The estimated time-point mortality for ART patients in the CHBC were 1.9% at 12 months, 5.9% at 60 months, and 5.9% at 96 months. For the FBFCA, estimated time-point mortality at 12, 60, and 96 months were 1.2%, 8.3%, and 9.3%, respectively.

Survival stratified by age groups

When survival was stratified by age groups in the two models, there was no significant difference, except a borderline effect for age group 36-59 months (log rank test: p=0.060, Figure 4). Thus, there was not enough evidence to suggest that age had a confounding effect on survival.

Pre-ART Mortality

Sixty deaths were recorded among children in the CHBC who were not on ART (Table 4). Most were known to have met the 2011 US (81.7%) and the 2010 WHO (80.0%) treatment initiation guidelines. All recorded infant deaths (under 12 months) were among those who had met both treatment guidelines before they died.

Discussion

We found that by far the most significant factor determining retention in care among infants, children, and adolescents with HIV was being on ART. Once on ART, there was no difference in survival between the CHBC and FBFCA. In addition to ART initiation directly promoting survival [31-33], we observed a substantial indirect survival effect by ART dramatically enhancing retention (Figure 1B). The CHBC model therefore stands to improve child survival greatly if ART is initiated early. Furthermore, patients can benefit from ancillary interventions to promote health and well-being such as food supplements, adherence counselling, and psychosocial support also effective only if retained in the programmes [34-36]. Of note, the CHBC and FBFCA models reach two different populations of HIV-infected children (Table 1). The FBFCA engages the children at or nearer to birth, whereas the CHBC attempts to find them in the communities, thus complementing each other. Operational synergies between the two models could result in a wider reach and greater ART coverage among HIV-infected children and adolescents.

We also observed that the majority of the pre-ART deaths occurred in children and adolescents who had met the 2011 US and the 2010 WHO treatment guidelines but did not initiate therapy for various reasons. This finding illustrates the failure of timely initiation of ART, which in turn may be linked to programmes that are not tailored to meet the needs of the infected child and family. For instance, at the time of this study, there were no PMTCT/EID services within the CHBC. That scenario, coupled with delayed HIV diagnosis, drug stock-outs, logistic challenges and fragile linkages to ART initiation and psychosocial support services may have resulted in considerable LTFU and deaths (Figures 1B and 3B) between testing and initiating care and treatment [37-40] in the CHBC. There is urgent need for concerted efforts to

ensure timely initiation of ART in children and adolescents in the CHBC. To that effect, simplification of treatment guidelines to universal treatment regardless of disease stage would not only help such efforts, but also eliminate missed opportunities to initiate ART, while awaiting eligibility criteria to be met [3].

We also saw that nearly one out of every five deaths occurred in children and adolescents who had not yet met either guideline for initiation. As shown by the literature, significant clinical events do occur in HIV-infected children and adolescents even before meeting the previous guidelines [19,20]. Our data therefore support the 2013 WHO consolidated guidelines recommending early ART initiation for children less than five years old regardless of CD4 cell count [41]. However, the new guidelines may not address substantial mortality for children over five. Given the number of deaths we observed in children over five who had not met the guidelines, coupled with substantial LTFU when not on ART, we believe the benefits of early ART outweigh the risk of delayed ART for this older group as well. We therefore endorse extending initiation of ART to all HIV-infected children and adolescents irrespective of their CD4 cell counts as a means to avert child deaths.

Among patients on ART in both models, retention was higher in the CHBC. Factors that may explain this observation include decentralized voluntary counselling and testing and ART refills involving outreach clinics within the communities. In addition, ART patients in the CHBC have a 'priority' tracking system within the clinics compared to patients not receiving ART, due to scanty resources [27]. However, the 'priority' tracking may not have promoted retention of children not on ART. This disparity calls for an integrated approach towards patients tracking.

Although all the children were on ART in the FBFCA and retention was relatively stable, the services were not decentralized. Hence, we believe that geographical access may have been a barrier to retention over time. We also think that socioeconomic factors and education level of caretakers may have contributed to the differences in retention.

We also noted that, age was a confounder for retention but not survival (Figures 2 and 4). Indeed, when retention was stratified by age groups, more children in the CHBC than in the FBFCA were retained in the age groups 12 -35 months and 36-59 months. Many of the children in the FBFCA in those age groups were 'graduates' of the PMTCT programme and were exposed to single dose Nevirapine (sd NVP) for PMTCT and were on NVP-based first line ART regimens. Studies from Uganda and elsewhere have shown that, exposure to sd NVP during PMTCT and initiating NVP-based first line ART regimens in HIV-infected children was associated with suboptimal clinical outcomes [42-45] and may partly explain this observation. Additionally, maternal health issues could be contributory factors. On the contrary, the CHBC had no PMTCT programme and most probably had more non NVP-exposed children for those age groups and thus better outcomes on ART. For the age group 5 years plus, retention was not different between the two models, as they were either far removed from exposure or were never exposed to sd NVP.

Additionally, we found that among patients receiving ART, attrition was significantly associated with model of care, mild immunosuppression and being more underweight (Table 2), whereas in the CHBC, the risk of attrition was significantly associated with

CD4 cell count, WHO clinical stages III-IV and absence of ART (Table 3). These findings have been described consistently in the literature [10,13,27].

In terms of growth responses, we saw that children in the CHBC were significantly more underweight, whereas, those in the FBFCA were significantly more stunted with some overlap. Although, it is well recognized that HIV infection in children compromises growth responses, in Uganda, 39% and 16% of all children less than 5 years are stunted and underweight respectively, demonstrating that important non-HIV contributors to stunting and wasting exist, in particular high background rates of TB and other coinfections, food insecurity and malnutrition [46,47]. Children in the FBFCA were relatively younger, mainly enrolled through PMTCT/ EID programmes and were all on ART. Thus, the observed growth responses could be related to complications of perinatal HIV infection, younger age as well as background factors. In contrast, children in the CHBC were older and only 30% were on ART. In an earlier study [27], we noted that about 46% of the patients in the CHBC were orphans and majority were malnourished, older, and less likely to receive ART. We therefore think that these factors could be contributing to the observed growth responses in that cohort.

We note potential limitations to our data and conclusions. First, the data were observational and based on programmatic information. Second, the two programmes should be interpreted in the context of reaching different populations of children and the direct comparisons are cautious. Patients were not randomized to receive one or the other model, but rather circumstances (such as distance to facilities) dictated what options were available. Third, some aspects of the interventions were shared by both models, such as guidelines for initiating ART and nutritional support and could be

potentially confounding. Fourth, the high level of loss to follow-up among patients not on ART in the CHBC leaves much doubt on the final disposition of the children in that programme. We expect that many of the children lost to care may have died; however, the characteristics of those who died and those who may have accessed care elsewhere are not known [48-50]. Additionally, there were differences in the study population sizes, proportions in age groups and baseline characteristics, all of which could be potential confounders. Consequently, the analyses were adjusted, including stratification of retention and survival by model of care and age groups, in order to minimize any potentially confounding effects.

We also acknowledge that our findings reflect data from programmes in one urban setting in East Africa, and therefore may not be generalizable to family–centred approach and community home-based care models in other settings. Despite the limitations, we firmly believe that our findings remain valid and relevant.

Conclusion

We conclude that, irrespective of model of care, children receiving ART had better retention in care and therefore long-term survival. Encouragingly, if children were on ART, then their survival was as good, if not slightly better, in the CHBC compared to the FBFCA. Based on our observations, substantial improvement in child survival can be achieved in either a community-based or a family-care model as long as HIV-infected children are identified early and begun on ART. To ensure this occurs, early identification of HIV infected children requires strong linkages of pregnant HIV-infected women to PMTCT services; active tracking to ensure all HIV exposed infants receive Polymerase Chain Reaction-based early infant diagnosis. Additionally, rapid early initiation of ART among HIV infected infants and children are essential. We anticipate the move to early initiation of ART in all HIV-infected children and

adolescents in resource-limited settings, irrespective of their CD4 cell counts, will improve survival.

Among ART patients in both models, attrition was significantly associated with model of care, mild immunosuppression and being underweight. In the CHBC, attrition was significantly associated with CD4 cell count, WHO clinical stages III-IV and absence of ART.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MW[1, 2] conceived of the study and participated in the drafting and editing of the manuscript, data analysis and interpretation of the findings. BML[3], ML[5], MW[6] and GG[7] participated in the data analysis, editing of the manuscript and interpretation of findings. RL[1], PC[1], MNM[2], CPN[2] and DW[3] participated in the drafting and editing of the manuscript. BD[3] and MM[3] participated in the data analysis. AM[4], SK[2] and DB[1] participated in editing the manuscript. MP[1], PM[3], MGF[3] and CG[1] participated in editing of the manuscript and interpretation of findings. All authors read and approved the final manuscript.

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Table1:Baseline characteristics of HIV-infected children and adolescents in a community home-based care model and a facility-based family-centred approach, Kampala, Uganda, 2003-2010

Characteristic	Community-based home care approach N = 1, 460	Facility-based family-centred approach N = 163	Total N = 1,623	P values
	n (%)	n (%)	n (%)	
Age (months)				
< 12	371 (25.4)	54 (33.1)	425 (26.2)	0.097
12 – 35	228 (15.6)	21 (12.9)	249 (15.3)	
36 – 59	173 (11.9)	23 (14.1)	196 (12.1)	
60+	688 (47.1)	65 (38.9)	753 (46.4)	
Gender				
Female	746 (51.1)	87 (53.4)	833 (51.3)	0.581
Male	714 (48.9)	77 (46.6)	790 (48.7)	
Cd4count& percent				
Median CD4 (IQR)	393 (51 – 762)	727 (442 - 1348)	438 (78 - 824)	<0.001
Median CD4 % (IQR)	5.8 (0.1 – 16.9)	17.0 (10.0 – 25.0)	8.1 (0.2 – 18.1)	<0.001
Level of immunosuppression by CD4				
Not significant	273 (24.4)	39 (25.0)	312 (24.4)	0.424
Mild	122 (10.8)	21 (13.5)	143 (11.1)	
Advanced	131 (11.6)	12 (7.7)	143 (11.1)	
Severe	605 (53.5)	84 (53.8)	689 (53.5)	
On ART				
Yes	441 (30.2)	163 (100)	604 (37.2)	<0.001
No	1019 (69.8)	0 (0.0)	1019 (62.8)	
Age at ART initiation (months)				
Median (IQR)	91.0 (48.9 – 135.5)	45.9 (6.5 – 85.0)	78.6 (34.0 – 124.6)	<0.001
WHO clinical staging				
I – II	1258 (86.4)	157 (96.9)	1415 (87.5)	<0.001
III – IV	198 (13.6)	5 (3.1)	203 (12.5)	
Growth response				
Weight for Age				
≤ -2SD	430 (37.4)	24 (16.9)	454 (35.2)	<0.001
>-2SD	719 (62.6)	118 (83.1)	837 (64.8)	
Height for Age				
≤ -2SD	508 (44.3)	43 (30.3)	551 (42.8)	0.001
>-2SD	639 (55.7)	99 (69.7)	738 (57.2)	

Table 2: Factors associated with attrition among HIV-infected children and adolescents on ART in a community home-based care model and a facility-based family-centred approach, Kampala, Uganda, (2003-2010) using Cox regression

Characteristic	Unadjusted (Univariate analysis)		Adjusted* (Multivariate analysis)	
	HR‡ (95% CI)	P	HR‡ (95% CI)	P
Model of care				
FBFCA	1		1	
CHBC	0.40 (0.23 – 0.70)	0.002	0.29 (0.12 – 0.70)	0.006
Age (months)				
< 12	1		1	
12 – 35	0.73 (0.31 – 0.70)	0.465	1.95 (0.39 – 9.89)	0.419
36 – 59	0.29 (0.09 – 0.87)	0.028	1.63 (0.18 – 15.17)	0.664
60+	0.43 (0.22 – 0.85)	0.015	3.19 (0.27 – 37.03)	0.354
Gender				
Female	1			
Male	1.13 (0.64 – 1.99)	0.675		
Cd4count & percent				
CD4 †	0.86 (0.69 – 1.06)	0.162		
CD4 % †	1.00 (0.57 – 1.77)	0.992		
Level of immunosuppression by CD4				
Not significant	1		1	
Mild	4.17 (1.31 – 13.31)	0.016	4.66 (1.21 – 17.98)	0.026
Advanced	0.87 (0.16 – 4.73)	0.868	0.89 (0.09 – 9.12)	0.921
Severe	3.14 (1.10 – 8.94)	0.032	3.16 (0.81 – 12.39)	0.099
Age at ART initiation				
Months†	0.74 (0.61 – 0.90)	0.003	0.67 (0.31 – 1.44)	0.309
WHO clinical staging				
I – II	1			
III – IV	1.52 (0.68 – 3.38)	0.309		
Growth response Weight for Age				
≤ -2SD	1		1	
>-2SD	0.40 (0.21 – 0.77)	0.006	0.31 (0.15 – 0.65)	0.002
Height for Age				
≤ -2SD	1			
>-2SD	0.54 (0.28 – 1.03)	0.060		

‡ Hazard of attrition; † Log transformed due to skewed data;
*Only factors significant at univariate or borderline were considered into multivariate

Table 3: Factors associated with attrition among HIV-infected children and adolescents in the CHBC model, Kampala, Uganda (2003-2010) using Cox regression

Characteristic	Unadjusted (Univariate analysis)		Adjusted* (Multivariate analysis)	
	HR‡ (95% CI)	P	HR‡ (95% CI)	P
ART				
No	1		1	
Yes	0.06 (0.04 – 0.09)	<0.001	0.04 (0.02 – 0.08)	<0.001
Age (months)				
< 12	1		1	
12 – 35	0.85 (0.65 – 1.10)	0.218	2.18 (0.97 – 4.92)	0.060
36 – 59	0.46 (0.33 – 0.64)	<0.001	1.65 (0.74 – 3.69)	0.225
60+	0.44 (0.35 – 0.56)	<0.001	1.38 (0.63 – 3.01)	0.418
Gender				
Female	1			
Male	1.11 (0.93 – 1.33)	0.243		
Cd4count & percent				
CD4 †	0.85 (0.78 – 0.93)	<0.001	0.84 (0.74 – 0.95)	0.006
CD4 % †	0.80 (0.66 – 0.96)	0.016		
Level of immunosuppression by CD4				
Not significant	1			
Mild	0.99 (0.66 – 1.49)	0.968		
Advanced	0.89 (0.59 – 1.34)	0.590		
Severe	1.87 (1.44 – 2.43)	<0.001		
Age at ART initiation				
Months†	0.85 (0.66 – 1.10)	0.209		
WHO clinical staging				
I – II	1			
III – IV	1.84 (1.47 – 2.29)	<0.001	1.94 (1.34 – 2.80)	<0.001
Growth response Weight for Age				
≤ -2SD	1			
>-2SD	0.76 (0.62 – 0.93)	0.007		
Height for Age				
≤ -2SD	1			
>-2SD	0.69 (0.56 – 0.84)	<0.001	0.81 (0.60 – 1.09)	0.162

‡ Hazard of attrition; † Log transformed due to skewed data;
*Only factors significant at univariate or borderline were considered into multivariate

Table 4: Deaths among children and adolescents in community home-based care who were not on ART classified according to whether the US 2011 or WHO 2010 initiation guidelines were met or not , Kampala, Uganda, 2003-2010.

Age group for US (months)	N	US CDC guidelines[1] for ART met	Age group for WHO (months)	N	WHO guidelines[2] for ART met
<12	11	11 (100%)	<12	11	11 (100%)
12-35	13	10 (76.9%)	12-24	9	9 (100%)
36-59	4	3 (75.0%)	24-59	8	5 (62.5%)
60+	32	25 (78.1%)	>60	32	23 (71.9%)
Overall	**60**	**49 (81.7%)**	**Overall**	**60**	**48 (80.0%)**

Source: *WHO and US treatment guidelines*

1. **US CDC 2011** criteria for ART initiation: <12 months all should be on ART; 12 – 35 months if CD4 <1000 or <25%; 36 – 59 months if CD4 <750 or <25%; 60+ months if CD4 <500.

2. **WHO 2010** criteria for ART initiation: <12 months all should be on ART; 12 – 24 months all should be ART; 24 – 59 months if CD4 <750 or <25%; 60+ months if CD4 <350.

Figure 1: Retention in care, HIV-infected children and adolescents in a facility-based family centred approach (FBFCA) and a community home-based care (CHBC) model, on and not on ART, Kampala, Uganda, 2003-2010

Fig 1A: FBFCA (all on ART) versus CHBC (on ART)

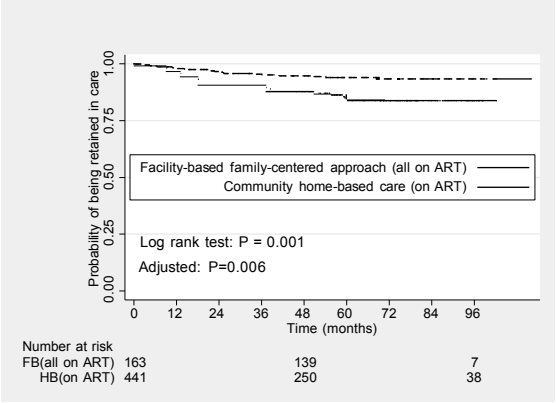
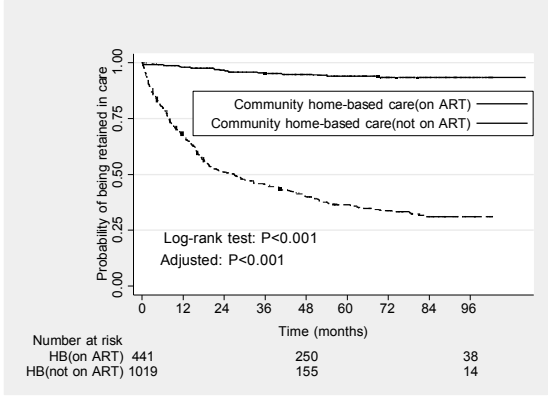
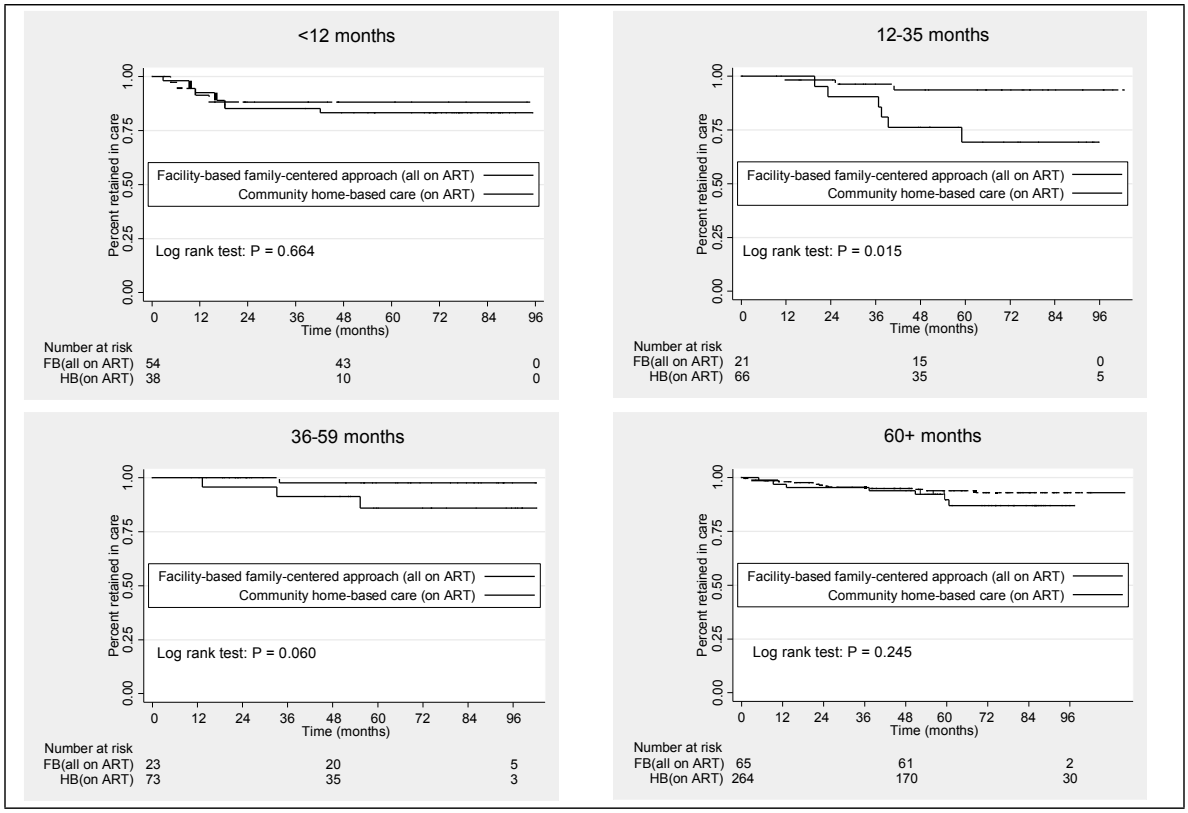


Fig 1B: CHBC (on ART) versus CHBC (not on ART)



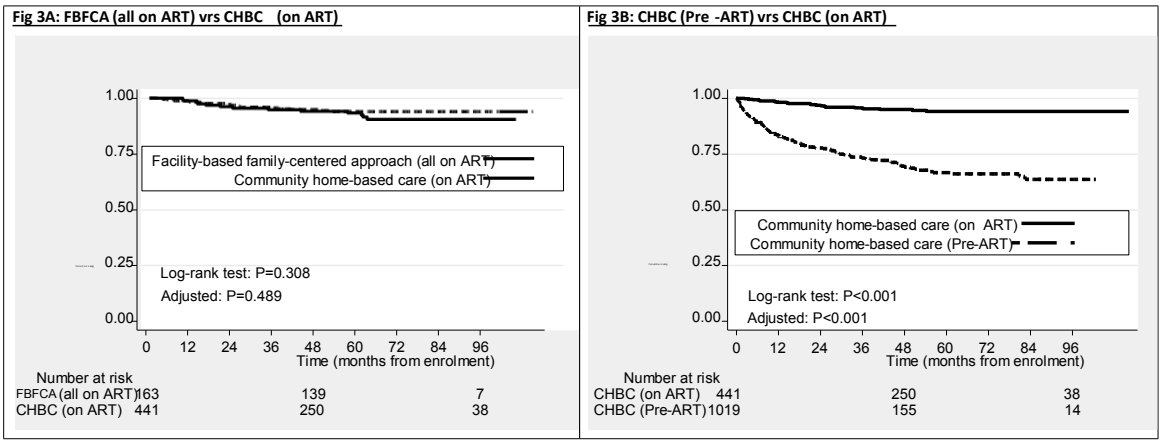
Legend: Figure 1 compares retention among patients on ART in the two models, and it was higher in the CHBC.

Figure 2: Retention stratified by age group among HIV-infected children and adolescents in a CHBC and a FBFCA in Kampala, Uganda (2003-2010)



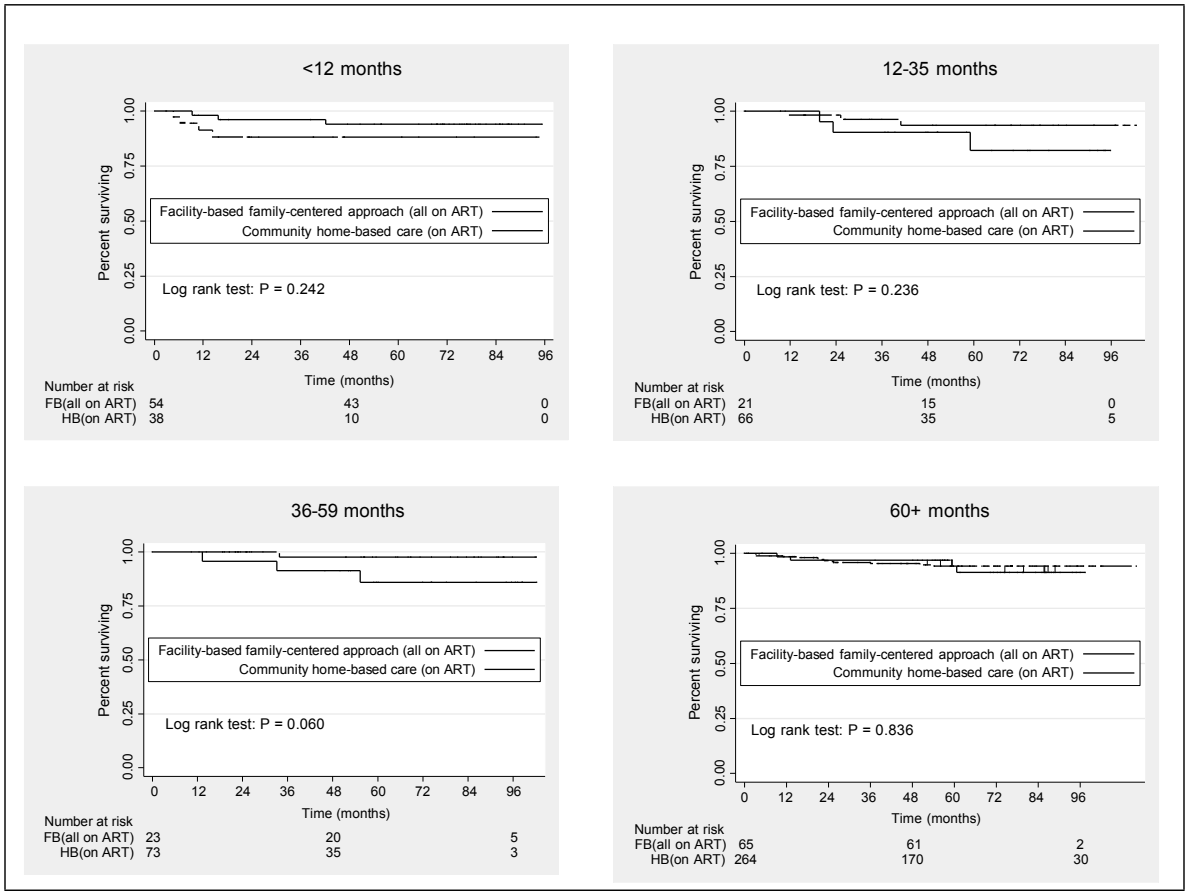
Legend: Figure 2 stratifies retention by age groups in the two models. Age groups 12-35months and 36-59 months appear to be confounders for retention.

Figure 3: Survival on anti-retroviral therapy (ART), HIV-infected children and adolescents in a facility-based family-centred approach (FBFCA) and a community home-based care (CHBC) model, Kampala, Uganda, 2003-2010



Legend: Fig 3A compares survival trends among children and adolescents on ART in both models, and shows that they were not significantly different. On the other hand, Fig 3B shows that survival trends differed significantly between patients on ART in the CHBC and those not receiving ART (pre-ART).

Figure 4: Survival stratified by age group among HIV-infected children and adolescents in a CHBC and a FBFCA in Kampala, Uganda (2003-2010)



Legend: Figure 4 stratifies survival by age groups in the two models. Apart from a borderline effect for age group 36-59 months (p=0.060), there was not enough evidence to suggest that age had a confounding effect on survival in the two models.

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Predictors of treatment failure in HIV positive children receiving combination antiretroviral therapy: cohort data from Mozambique and Uganda

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ABSTRACT

Background. Delays detecting treatment failure and switching to second line combination antiretroviral therapy (cART) are often observed in HIV-infected children of low-middle income countries (LMIC).

Methods. An observational study included HIV-infected children attending the Beira Central Hospital (Mozambique) and the St. Raphael of St. Francis Nsambya Home Care (Uganda) evaluated clinical and immunological failure according to WHO 2006 guidelines. Baseline predictors for cART failure and for drug substitution were explored in unadjusted and adjusted Cox proportional hazard models.

Results. 218 of 740 children with at least 24 weeks follow-up experienced treatment failure (29% 95%CI [26-33]), with crude incidence of 20.0 events per 100 person-years (95%CI 17.5-22.9). Having tuberculosis co-infection or WHO stage 4, or starting a non-triple cART significantly increased risk of failure.

202 of 769 (26.3%) children receiving cART substituted drug(s), with crude incidence of 15.4 events per 100 person-years (95% CI 13.4-17.7). Drug toxicity (18.3%), drug availability (17.3%) and tuberculosis drugs interaction (52, 25.7%) were main reported reasons while only 9 (4%) patients switched cART for clinical or immunological failure. Children starting lamivudine-zidovudine-nevirapine or lamivudine-stavudine-efavirenz or lamivudine-zidovudine-efavirenz were more likely to substitute drugs. Increased substitution was found in children with mild immunosuppression and tuberculosis co-infection at cART initiation as well as poor adherence before drug substitution.

Conclusions. Considerable delay in switching to second line cART may occur despite an observed high rate of failure. Factors including WHO clinical stage and tuberculosis co-infection should be evaluated before starting cART. Toxicity and drug adherence should be monitored to minimize drug substitution in LMIC.

MANUSCRIPT

Background:

The global scaling up of treatment and care for people living with HIV (PLWH) has led to a 43% decline in new HIV paediatric infections since 2003, with 330,000 newly infected children in 2011. Despite the efforts to expand access to combination antiretroviral therapy (cART), only 28% of eligible children have received it (1). Expansion of early HIV diagnosis coverage, prompt cART initiation and better retention in care remain major goals (2, 3) and the lack of laboratory monitoring frequently observed in low and middle-income countries (LMIC) should not represent a barrier to cART distribution in children (4). However, optimization of the clinical management of PLWH and prompt diagnosis of treatment failure are becoming more and more critical in the context of life-long treatment and limited drug availability.

Although virological failure is widely considered the gold standard to detect treatment failure, clinical and immunologic parameters are often the only criteria available in LMIC (5, 6). CD4 cell monitoring has been shown to be a poor predictor of virological failure in treatment experienced children (7-9), particularly when severely immune-compromised (10). Studies in LMIC have reported high rates of virological suppression in children up to 5-6 years after treatment initiation (11, 12), however treatment failure rates of 10-34% were observed among children after two to three years of cART (13-18). Program reports suggest that only a small proportion of patients on treatment are receiving a second line therapy, an estimated 4% of adults and 1-14% of children (16, 18-20). Delays in detecting treatment failure and switching to second line therapy lead to the development of HIV drug-resistance, compromising subsequent regimens (6, 21). This is particularly relevant for children, due to the lack of paediatric formulations.

Randomized trials were conducted to evaluate the optimal first antiretroviral regimen for reducing the risk of treatment failure. Findings from the P1060 trial reported an increased risk of failure starting a nevirapine (NVP)-based cART in infants and young children (13, 22, 23). This was not confirmed by the PENPACT1 trial, where no difference in clinical and virological outcomes were shown between NNRTI

and PI-based regimens in older children (24). Data to inform the most durable NRTI backbone in the context of a triple therapy is still limited. Conflicting results were reported concern the use of abacavir (ABC) as first line regimen: Green et al. suggested that abacavir (ABC) may be preferable to zidovudine (AZT) combination with lamivudine (3TC) (25), while poorer early virological outcomes were recently observed in children starting ABC/3TC-based first line regimens, compared to d4T/3TC (26, 27). Identifying optimal regimens is particularly relevant for children with HIV/TB co-infection living in LMIC, where NVP is widely preferred to EFV or a triple NRTI-based regimen, due to its better acceptability and relatively low cost (28).

Drug substitution is often required to optimize antiretroviral treatment (19, 29). Results from observational studies estimate a probability of cART discontinuation or modification ranging between 2.8-20% in adults of LMIC (19, 30-34). A randomized study conducted in children shows a cART switching/discontinuation rate up to 29% (24). Acute and chronic toxicity, drug intolerance, poor adherence and treatment failure remain the major determinants of cART modification (35-38). Drug costs and/or stock outs due to challenges in adequately forecasting and maintaining an effective supply chain have been cited as further reasons for cART discontinuation in LMIC (31, 33).

The aim of this study is to estimate the rate and predictors of cART treatment failure in two pediatric cohorts from Mozambique and Uganda during a five years follow-up period, and to explore the rate of and factors associated with drug-substitution.

Methods.

Setting and study design.

We conducted a retrospective cohort study among children starting cART between January 2005 and December 2009 at the Beira Central Hospital (HCB) in Mozambique and the Nsambya Home care (NHC) department of St. Raphael of St. Francis Hospital in Uganda. Two Italian non-governmental organizations, Doctors with Africa Cuamm (Mozambique) and Associazione Casa Aiuto alla Vita Padre Angelo (Uganda), partnered with these hospitals to provide pediatric HIV care.

Both programs provided HIV counseling and testing, cotrimoxazole prophylaxis, cART, laboratory investigations and management of opportunistic infections. Infants and children under 18 months of age, known or suspected to be exposed to HIV, were diagnosed through HIV-1 DNA testing. Patients were considered eligible for cART according to WHO 2006 guidelines (39).

Laboratory examinations including full blood count, liver function tests, creatinine and CD4 count were required before starting cART, as well as a chest x-ray and acid fast bacilli (AFB) testing to exclude TB if suspected. In the absence of contraindications, written consent was collected when enrolling in the programme and before starting cART. Throughout the study, patients were switched to second line cART when treatment failure was identified following WHO 2006 guidelines (39).

The study was approved by the Uganda National Council for Science and Technology and the Nsambya Hospital and HCB ethics committees (Uganda) and by the Gabinete Do Director Gerar , Ministerio Da Saudè of HCB (Mozambique) .

Data collection.

In Mozambique, data were collected from clinical charts and paper registries and entered in the hospital's electronic patient database system. Similarly, in Uganda, routine clinical data were recorded in paper-based patient files and registries and entered into an electronic interface by trained staff.

Children were examined at least monthly during the first six months of cART and then every three months in Mozambique, while in Uganda monthly visits were maintained throughout the follow-up according to the project design. Weight and height were measured at every clinic visit. Full blood count, liver function tests and glucose assays were performed every 6 months, and CD4 counts every 6 to 12 months. Adherence to cART was assessed at every follow-up visit and defined as “good” or “poor” if the self-reported number of doses was more or less than 95% of expected monthly number of doses. HIV related clinical events were diagnosed with or without biological confirmation, depending on lab facilities available, while immunodeficiency was classified as mild, advanced and severe according to the WHO 2006 thresholds (39). For the treatment failure analysis the period of follow-up was from cART initiation

up to the treatment failure outcome, while follow-up was from treatment initiation to first cART drug substitution for drug substitution analysis. For children without treatment failure or drug-substitution, follow-up was censored at date of death, loss to follow up (LTFU, defined as missing follow-up visits for more than 6 months), transferred to other clinic, confirmed HIV-negative or aged more than 18 years old, last CD4 measurement, or last anthropometric or adherence record, whichever occurred latest.

Endpoint Definitions and Study Population.

Drug-substitution was defined as substitution of one or more drugs of the first antiretroviral regimen for any reason. Reasons for drug substitution were classified retrospectively from the inspection of what was reported by clinicians in patient's clinical charts. Clinical and immunological failure were defined according to the WHO 2006 criteria, using CD4 measurements and WHO disease stage from at least 24 weeks after cART initiation (39). Treatment failure, when both clinical and immunological failure were observed, was considered to occur at the earliest of the two events.

For analysis of treatment failure, only children with at least 24 weeks of follow-up post-cART initiation were included to ensure sufficient time for treatment response.

For analysis of cART drug substitution, children who received an ABC component in their initial cART regimen were excluded, as first line ABC treatment was systematically administered to children diagnosed with active TB and all patients initially on ABC were routinely switched to EFV once the TB infection cleared.

Statistical analyses.

In this intent-to-treat analysis, all children were included from cART initiation, regardless of subsequent modifications. All analyses were conducted in R version 2 (R Development Core Team, Vienna, Austria) and Stata version 12.0 (Stata Corporation, College Station, TX, USA).

For both treatment failure and drug substitution analyses, frequency distributions and median and interquartile range (IQR) were used to describe baseline patient characteristics. Baseline characteristics of

interest were gender, age at treatment initiation, Body Mass Index (BMI, weight(kg)/height^2(m)) for age z-score, WHO disease stage, initial cART treatment regimen (also by most potent component), adherence to cART, CD4 count and percent, and immunodeficiency classification. All descriptive analyses were stratified by hospital. Differences on all key variables at baseline between these strata were determined using Pearson's chi-squared test for categorical variables, the t-test for difference in means for baseline BMI for age z-score, and the Wilcoxon rank sum test for all other continuous variables.

Unadjusted Cox proportional hazards models were used to determine the odds of treatment failure and cART drug substitution. The following variables were considered in a multivariate adjusted Cox proportional hazards model of treatment failure: cART treatment regime, age, adherence, gender, country of treatment, baseline disease stage, immunodeficiency status, and BMI for age z-score. The following variables were considered in a multivariate adjusted Cox proportional hazards model of cART drug substitution: cART treatment regime, adherence, classification of immunodeficiency status, WHO disease stage, and age group. A backward selection procedure was used to create these adjusted models with a variable being included in the model if it resulted in an improvement in the model fit as defined by the Akaike Information Criterion (AIC).

Results

Between January 2005 and December 2009, 1075 HIV-infected children less than 15 years old began cART in HCB and NHC. Two hundred and thirteen (20%) children were excluded from the study due to missing data (table 1). Children excluded from both treatment failure and drug substitution analyses were more likely to be Ugandan ($p<0.01$), female ($p=0.049$), younger ($p<0.01$) and enrolled and starting cART later (p 0.01 and <0.01, respectively) than children included in the study.

Treatment failure analyses

Among 862 children eligible for analysis, 740 children (492 from Mozambique and 248 from Uganda) with at least 24 weeks of follow-up were included for a total of 1088.5 person/years of follow-up. At the time of data collection, 24/740 (3.24%) children died, 68 (9.19%) were LTFU, 7 (0.95%) were transferred to other clinic and 1 (0.14%) was confirmed HIV-negative. A total of 218 treatment failure events (29% 95%CI [26-33]) occurred, with a crude incidence rate of 20.0 events per 100 person-years (95%CI 17.5-22.9). Median time to treatment failure was 379 days (IQR 229-649). Immunological failure alone occurred in 100 (46%) children while clinical failure alone was found in 116/218 (53%) cases. Two children (1%) had concomitant clinical and immunological failure. Baseline characteristics are shown in table 2.

The adjusted Cox proportional hazards model of treatment failure with the lowest AIC included age, treatment type and baseline disease stage. Incidence rates and crude and adjusted relative hazards from the model are shown in table 3.

Patients with TB and those with other WHO stage 4 defining diseases were significantly more likely to experience treatment failure (HR 2.27, 95%CI 1.5-3.4, p <0.001 and HR 1.57, 95%CI 1.02-2.4, p 0.04, respectively) compared to children with WHO stage 3 disease without TB. As expected, starting cART with an unconventional regimen (not containing an NRTI backbone in combination with EFV, NVP, LPV/r, or ABC) was also significantly associated with risk of treatment failure (HR 3,37, 95%CI 1.12-11.89, p=0.03).

Drug substitution analysis.

Among 862 eligible children, 4 with unknown ART regimen and 89 who received ABC in their initial cART regimen were excluded from the cART drug substitution analysis. The remaining 769 children had an overall follow-up of 1499 person/years. Throughout the study period, 202 (26%, 95% C.I. 23-30%) patients substituted treatment, with median time to substitution of 9.69 months (IQR 25.82). Overall incidence of substitution was 15.4 events per 100 person-years (95% CI 13.4-17.7). Reported reasons for substitution included any toxicity (37, 18.3%), of which 3 were d4T toxicity (1.5%), 25 (12.4%) AZT

toxicity and 9 (4.5%) NVP toxicity, clinical and immunological failure (9, 4.5%), drug availability (35, 17.3%), drug interaction (1, 0.5%), provider preference for a better option (32, 15.8%), simplification associated with non-adherence (4, 2%), caregiver health problem (1, 0.5%) and TB drugs interaction (52, 25.7%). Among the 9 patients with drug substitution for clinical or immunological failure, median time to substitution was 26.65 months (IQR 23.95). Reasons for substitution were unknown for 31 (15.3%) children. Baseline characteristics are provided in table 4.

The adjusted Cox proportional hazards model of cART drug substitution with the lowest AIC included age, adherence, treatment type, immunodeficiency status, and baseline disease stage. Incidence rates and crude and adjusted relative hazards from the model are shown in table 5.

Drug substitution was significantly more likely among patients starting treatment with 3TC-AZT-NVP (HR 3.29, 95%CI 2.27-4.76, p <0.001), 3TC-d4T-EFV (HR 3.22, 95%CI 2.02-5.13, p <0.0001) or 3TC+AZT+EFV and (HR 1.74, 95%CI 1.03-2.95, p=0.037) compared to those starting on 3TC-d4T-NVP. Drug substitution was also more likely among mildly immunosuppressed patients (HR 2.23, 95%CI 1.24-4.02, p <0.01) compared to those with severe immunodeficiency, in infants (HR 2.74, 95%CI 1.54-4.90, p <0.01) compared to children ≥5 years, in children with TB (HR 3.38, 95%CI 2.28-5.01, p<0.0001) compared to those with WHO stage 3 or 4 disease without TB or those with stage 1 or 2 disease, and among those with good treatment adherence before drug substitution (HR 0.53, 95% CI 0.37-0.77, p <0.001) compared to those with poor adherence.

Discussion.

In this study, a notable proportion (29%) of HIV positive children experienced clinical and/or immunological cART failure, with a crude incidence rate of 20.0 events per 100 person years. Our findings appear in line with evidence from the literature referring on immunological failure (16-18) . Considering that virological failure tends to precede clinical and immunological failure, this figure could underestimate a greater impact of virological failure.

Over a 5 year period, among the 202 patients who substituted cART only 4% switched to second line regimen due to treatment failure after a median time of 26.65 months, indicating a significant delay in switching to second line despite the high rate of failure retrospectively observed in the cohort. Several studies reported low proportion of children on second-line cART in LMICs (19, 20). Our switch rate appears even lower than those observed by Davies et al (16) and by two other observational studies showing that around 14% children switched to second-line due to clinical and/or immunological failure (17, 18). Reasons explaining the alarming gap between a recognized clinical and/or immunological failure and the initiation of a second line cART were not well identified. In our programme we hypothesize that limited availability and costs of second line drugs may be major barriers to second line therapy. Furthermore the trade-off that clinicians are facing when considering the limited options for children failing first line and the risk of maintaining them on a failing regimen can be very challenging and may result in further delays in switching to second line cART. Under-diagnosis of treatment failure may also have contributed to the low rate of switching observed. It should be noted that in the drug substitution analysis reasons for switching may be misclassified, as these data were collected retrospectively and are based on clinician report.

WHO clinical stage 4 and TB co-infection at cART initiation were significantly associated with treatment failure. Poor clinical status has been observed to negatively affect treatment response, in particular malnutrition and chronic diarrhea independently increase the risk of treatment failure as much as baseline low immunity, high VL and younger age (15-17). As suggested by Hermans et al (40, 41), TB co-infection may impair immune recovery after cART initiation in adults. In addition, poor adherence may occur as a result of high pill burden, and interaction with rifampicin may affect the bioavailability of HIV drugs, particularly for NVP and LPV/r (42). Development of better options for TB co-treatment appears to be critical to prolong effectiveness of first line regimens.

As expected, unconventional regimens were associated with treatment failure compared to triple cART

validity of this finding may be questionable considering that only a few children were receiving a PI-based regimen at the time of the study. Few randomized trials investigated the most effective first-line cART regimen in HIV-positive children. The P1060 trial (13, 23) showed an increased risk of virological failure in children (<3 years) on NPV-based cART, regardless of PMTCT exposure, however this was not confirmed by the PENPACT trial conducted in older children of high income countries (24). Due to the nature of our cohort's age and lack of reliable PMTCT exposure data, our observational retrospective findings are not comparable to those from either controlled trial.

Determining when to switch to second line cART is a critical decision in settings where virological monitoring is not available. Although evidence shows that viral load (VL) is not essential to identify treatment failure (34), using clinical and immunological parameters leads to delays in switching to second line therapy (17), resulting in longer exposure to failing regimens which contributes to development of drug-resistant HIV strains (6). In our study reasons for delays to cART switching were not completely clarified, in particular we were unable to understand if clinicians didn't switch cART in children with recognized treatment failure or if clinical/immunological criteria were too complicated to recognize treatment failure. Earlier cART initiation and VL monitoring are currently recommended by WHO 2013 consolidated guidelines (3). Based on our data, advanced disease and TB co-infection should be considered as warning signals requiring closer follow-up and counseling to improve treatment outcomes and prolong duration of first line therapy. Adherence to cART was found to be a poor indicator of treatment failure, maybe due to the low accuracy of self-reporting adherence monitoring.

About 26% (203/769) of patients substituted treatment with an overall incidence rate of 13.5 events per 100 person years and 95% of these were for causes other than treatment failure. This figure is consistent with previous observational studies among HIV positive children (17, 24, 43) living in LMIC. Toxicity/intolerance was one of the main reasons reported for substitution (18.3%), mostly related to AZT toxicity (12.4%), as reported in other studies (17, 38, 44). Due to high prevalence of HIV/TB co-infection

(88/769, 11,4%), drug interaction in TB/HIV co-treatment (25.7%) was another major reason to substitute drugs. Drug availability (17.3%) was another considerable reason, reflecting the importance of ensuring adequate and continuous supply of cART in settings where drug costs are still a major barrier for PLWHA. Reasons for drug substitution were not classified prospectively but assessed from inspection of patient clinical charts, potentially leading to inaccurate classifications.

Higher rates of drug substitution were observed among children starting AZT-containing or EFV-based regimens. Increased drug substitution while on AZT is often the result of AZT-related anemia as well-described previously (23, 31, 36). AZT toxicity was more prevalent among the Mozambique cohort, where children were younger and malnutrition and/or more advanced WHO disease stages were observed, suggesting that AZT anemia may have been exacerbated. Despite the lack of more robust evidence, our findings suggest that AZT may not be the preferred NRTI to be used in these settings, particularly in younger children.

Further description of EFV substitution was not possible in this dataset due to the limited number of children receiving this drug, and we could not rule out specific EFV-related toxicity.

As previously mentioned, our results may be confounded by country specific differences. Mozambique patients were younger, had a more advanced WHO stage and a lower BMI z-score at cART initiation. These differences may reflect clinicians' preference in first-line treatment choice, accounting for the wider use of AZT and EFV in Uganda as much as for the increased choice of NVP-based regimen observed in children from Mozambique. Country specific differences may potentially confound the relationships seen between cART regimen and treatment failure and drug substitution. In terms of follow-up visits, the Ugandan children were followed up much more frequently (monthly) than those in Mozambique (every 3 months). This difference between program performances may have provided further confounders, potentially influencing the trends observed in older children at lower risk of failure and the higher rate of drug substitution observed in infants.

In conclusion, our data reinforce the need for simplification of more effective clinical and immunological criteria for prompt recognition of cART treatment failure. Children presenting with advanced disease and TB co-infection should be targeted for closer and more sensitive monitoring of treatment response. This should be matched with a constant provision of appropriate antiretrovirals and with optimization of first line drugs and treatment sequencing. Supply of new paediatric formulations for second line regimens and drug optimization should be considered as critical milestones to allow scaling up of early cART and reduction of treatment failure in children.

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All authors have significantly contributed to the manuscript and agreed with the content. They also declare that they have no conflicts of interest.

Table 1. Baseline characteristics of 862 children included and 213 children excluded from analyses due to missing data.

Variable	Value	Included children n=862	Excluded children n=213 (20%)	p-value
Gender	Male	449 (52)	99 (46)	0.049*
	Female	413 (48)	113 (53)	
Country	Mozambique	538 (68)	102 (48)	<0.01*
	Uganda	279 (32)	111 (52)	
Year of birth	Median (IQR)	2002 (1998-2005)	2001 (1996-2005)	
	< 1995	76 (9)	34 (16)	0.02¥
	1995-1999	219 (25)	54 (25)	
	2000-2004	289 (34)	58 (27)	<0.01*
	2005-2009	278 (32)	59 (28)	
	Missing	0 (0)	8 (4)	
Age at treatment initiation (years)	Median (IQR)	4.73 (2.09-9.11)	6.33 (2.62-11.56)	
	< 12 months	62 (7)	11 (5)	<0.01¥
	12- 35 months	256 (30)	48 (23)	
	36-59 months	120 (14)	22 (10)	<0.01*
	5-8 years	157 (18)	40 (19)	
	>8 years	267 (31)	92 (43)	
	Missing	0 (0)	9 (4)	
WHO clinical stage at treatment initiation	Stage I or II	75 (9)	14 (7)	
	Stage III with TB	36 (4)	11 (5)	
	Stage III w/o TB	57 (7)	22 (10)	0.14*
	Stage IV with TB	10 (1)	6 (3)	
	Stage IV w/o TB	47 (6)	13 (6)	
	Unknown	637 (74)	147 (69)	
Age at enrolment (years)	Median (IQR)	3.94 (1.48-8.09)	5.57 (1.61-9.47)	0.01*
	Missing	0 (0)	8 (4)	
Initial treatment regimen	3TC+4DT+NVP	369 (43)	70 (33)	
	3TC+AZT+NVP	231 (27)	60 (28)	
	3TC+AZT+EFV	80 (9)	35 (16)	
	3TC+4DT+EFV	57 (7)	15 (7)	0.06*
	3TC+AZT+ABC	48 (6)	15 (7)	
	Other triple	68 (8)	15 (7)	
	Other dual	3 (0)	1 (<1)	
	missing	6 (1)	2 (1)	

* Pearson chi square test
¥ Kruskal-Wallis test

Table 2. Baseline characteristics of children included in the treatment failure analysis – demographics and treatment.

Variable	Value	All children n=740 (100.00%)	Mozambique n= 492 (66.49%)	Uganda n= 248 (33.51%)	p-value ®
Gender	Male	382 (51.62)	260 (52.85)	122 (49.19)	0.348
	Female	358 (48.38)	232 (47.15)	126 (50.81)	
Age at treatment initiation	Median (IQR)	5.05 (7.00)	3.42 (5.49)	8.22 (7.13)	<0.001
(n=205)	< 12 months	51 (6.89)	44 (8.94)	7 (2.82)	<0.001
	12- 35 months	216 (29.19)	186 (37.80)	30 (12.10)	
	36-59 months	97 (13.11)	68 (13.82)	29 (11.69)	
	≥ 5 years	376 (50.81)	194 (39.43)	182 (73.39)	
BMI for age z-score	Median (IQR)	-0.92 (1.94) (n=564)	-1.10 (1.95) (n=327)	-0.75 (1.82) (n=237)	0.019
WHO disease stage	Stage I or II	174 (23.51)	46 (9.35)	128 (51.61)	<0.001
	Stage III with TB	83 (11.22)	68 (13.82)	15 (6.05)	
	Stage III w/o TB	305 (41.22)	224 (45.53)	81 (32.66)	
	Stage IV with TB	52 (7.03)	48 (9.76)	4 (1.61)	
	Stage IV w/o TB	101 (13.65)	81 (16.46)	20 (8.06)	
	Unknown	25 (3.38)	25 (5.08)	0 (0.00)	
Initial treatment regimen	3TC+d4T+NVP	325 (43.92)	269 (54.67)	56 (22.58)	<0.001
	3TC+AZT+NVP	195 (26.35)	114 (23.17)	81 (32.66)	
	3TC+AZT+EFV	69 (9.32)	13 (2.64)	56 (22.58)	
	3TC+d4T+EFV	50 (6.76)	24 (4.88)	26 (10.48)	
	3TC+AZT+LPV/r	18 (2.43)	0 (0.00)	18 (7.26)	
	3TC+d4T+LPV/r	6 (0.81)	0 (0.00)	6 (2.42)	
	3TC+d4T+ABC	25 (3.38)	25 (5.08)	0 (0.00)	
	3TC+AZT+ABC	40 (5.41)	39 (7.93)	1 (0.40)	
	Other*	12 (1.62)	8 (1.63)	4 (1.61)	
Initial treatment regimen (by most potent component)	EFV-containing	120 (16.22)	37 (7.52)	83 (33.47)	<0.001
	NVP-containing	523 (70.68)	383 (77.85)	140 (56.45)	
	LPV/r-containing	24 (3.24)	0 (0.00)	24 (9.68)	
	ABC-containing **	67 (9.05)	66 (13.41)	1 (0.40)	
	Other *	6 (0.81)	6 (1.22)	0 (0.00)	
Adherence	Good	485 (65.54)	294 (59.76)	191 (77.02)	<0.001
	Poor	255(34.46)	198(40.24)	57(22.98)	
CD4 percent (mean, 95% CI)	<12 months	15.95 (9.60)	15.60 (9.20)	18.93 (4.60)	<0.001
	12-35 months	14.50 (8.70)	15.00 (8.20)	10.62 (8.12)	
	36-59 months	12.16 (7.90)	12.85 (7.05)	11.81 (8.77)	
	>5 years	9.60 (10.00)	11.05 (10.00)	8.47 (9.76)	
CD4 count (mean cells/mm^3, 95% CI)	<12 months	784.00 (971.00)	746.50 (765.50)	1404.00 (1145.00)	<0.001
	12-35 months	721.00 (606.00)	730.50 (585.00)	554.00 (765.00)	
	36-59 months	467.00 (395.50)	420.50 (292.50)	551.00 (509.00)	
	>5 years	239.00 (286.00)	265.00 (363.00)	226.00 (224.00)	
CD4 count z-score	Median (IQR)	-0.30 (1.07) (n=736)	-0.14 (1.12) (n=492)	-0.54 (0.69) (n=244)	<0.001

Classification of Immunodeficiency **	Not significant	58 (7.84)	40 (8.13)	18 (7.26)	0.092
	Mild	35 (4.73)	19 (3.86)	16 (6.45)	
	Advanced	66 (8.92)	37 (7.52)	29 (11.69)	
	Severe	581 (78.51)	396 (80.49)	185 (74.60)	

* Other regimens include mono or dual therapies and those with missing information on cART regimen
** ABC-containing regimen include a 3 NRTI regimen containing ABC
- Other regimens include only those without an EFV, NVP, LPV/r, or ABC component, regardless of number of components
-- Immunodeficiency was classified as mild (CD4% of 30-35, 25-30, 20-25 and CD4 cell count of 350-499 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively), advanced (CD4% of 25-29, 20-24, 15-19 and CD4 cell count of 200-349 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) and severe (CD4% <25, <20, <15 and CD4 cell count < 200/<15% for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) according to the WHO 2006 thresholds
® P-values refer to differences between Mozambique and Uganda sub-cohorts on baseline characteristics

Table 3. Relative hazards for treatment failure in children from Mozambique and Uganda (n=740 children)

Variable		Person time (years)	Events	Crude Incidence rate† (95%CI)	Unadjusted Relative Hazard	95%CI	p-value	Adjusted Relative Hazard	95%CI	p-value
Treatment type	NVP-containing	781.2	150	19.2 (16.4,22.5)	Reference			Reference		
	ABC-containing *	62.0	18	29.0 (18.3,46.1)	1.38	(0.84,2.25)	0.20	0.76	(0.43,1.34)	0.34
	EFV-containing	187.5	37	19.7 (14.3,27.2)	1.09	(0.76,1.56)	0.65	0.95	(0.64,1.41)	0.80
	LPV/r-containing	53.8	10	18.6 (10.0,34.5)	1.08	(0.56,2.08)	0.81	1.03	(0.53,2.02)	0.93
	Other **	3.9	3	76.1 (24.6,236.1)	3.32	(1.05,10.43)	0.04	3.73	(1.17,11.89)	0.03
BMI for age z-score tertiles	Lowest tertile	410.7	96	23.4 (19.1,28.6)	Reference					
	Middle tertile	0.6	42	16.1 (11.9,21.8)	0.69	(0.48,0.99)	0.04			
	Highest tertile	54	38	24. 7 (17.9,33.9)	1.04	(0.71,1.51)	0.85			
	Unknown	263.1	42	16.0 (11.8,21.6)	0.68	(0.48,0.98)	0.04			
Gender	Female	540.7	1	18.5 (15.2,22.5)	Reference					
	Male	547.8	1	1.5 (18.0,25.8)	1.17	(0.89,1.52)	0.26			
Country of treatment	Mozambique	681.5	143	21. (1 8,24.7)	Reference					
	Uganda	407.0	75	18.4 (.7 3.1)	0.91	(0.69,1.21)	0.51			
Adherence	Good	1027.3	202	19.7 (17.1, 22)	Reference					
	Poor	61.1	16	26.2 (16.0, 42.7)	1.24	(0.74,2.06)	0.41			
Classification of immunodeficiency **	Not significant	78.6	10	12.7 (6.8,23.6)	0	(0.31,1.10)	0.10			
	Mild	57.9	11	19.0 (10.5,34.3)	0.	(0.49,1.64)	0.72			
	Advanced	90.3	13	14.4 (8.4,24.8)	0.64	(0.36,1 2)	0.12			
	Severe	861.7	184	21.4 (18.5,24. 7)	Reference					
Age group	0-11 months	46.0	13	28.2 (16.4,48.6)	1.28	(0. 2,2.)	0.41	1.08	(0.59,1.96)	0.80
	12-35 months	275.5	64	23.2 (18.2,29.7)	1.12	(0.83,1. 2)	.46	1.08	(0.77,1.50)	0.66
	35-59 months	176.8	23	13.0 (8.6,19.6)	0.67	(0.43,1.05)	0.	0.64	(0.41,1.00)	0.05
	>= 5 years	590. 1	118	20.0 (16.7,24.0)	Reference			Reference		
WHO disease stage at baseline	Stage 1 or 2	289.0	52	18.0 (13.7,23.6)	1.12	(0.79,1.58)	0.52	1.17	(0.81,1.67)	0.40
	Stage 3 or 4 with TB	145.8	50	34.3 (26.0,45.2)	1.96	(1.38,2.79)	<0.001	2.27	(1.50,3.42)	<0.001
	Stage 3 w/o TB	510.9	83	16.2 (13.1,20.1)	Reference	(0.78,7.7)	0.045	Reference		
	Stage 4 w/o TB	106.3	29	27.3 (19.0,39.3)	1.54	(1.01,2.35)	0.40	1.57	(1.02,2.414)	0.04
	Unknown	36.5	4	11.0 (4.1,29.2)	0.65	(0.24,1.78)		0.67	(0.24,1.82)	0.43

† - per 100 years

* ABC-containing regimen include a 3 NRTI regimen containing ABC

** Other regimens include only those without an EFV, NVP, LPV/r, or ABC component, regardless of number of components

** Immunodeficiency was classified as mild (CD4% of 30-35, 25-30, 20-25 and CD4 cell count of 350-499 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively), advanced (CD4% of 25-29, 20-24, 15-19 and CD4 cell count of 200-349 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) and severe (CD4% <25, <20, <15 and CD4 cell count < 200/<15% for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) according to the WHO 2006 thresholds

Table 4. Baseline characteristics of children included in the drug substitution analysis– demographics and treatment.

Variable	Value	All children n=769 (100.0%)	Mozambique n= 491 (63.9%)	Uganda n= 278 (36.15%)	p-value
Gender	Male	400 (52.0)	261 (53.2)	139 (50.0)	0.400
	Female	369 (48.0)	230 (46.8)	139 (50.0)	
Age at treatment initiation (N=205)	Median (IQR)	5.2 (7.0)	3.7 (5.5)	8.2 (7.2)	<0.001
	< 12 months	50 (6.5)	43 (8.8)	7 (2.5)	<0.001
	12- 35 months	209 (27.2)	175 (35.6)	34 (12.2)	
	36-59 months	112 (14.6)	76 (15.5)	36 (13.0)	
	≥ 5 years	398 (51.8)	197 (40.1)	201 (72.3)	
BMI for age z-score	Median (IQR)	-0.8 (2.0) (n=598)	-1.0 (2.0) (n=335)	-0.8 (1.9) (n=263)	0.110
WHO disease stage	Stage I or II	190 (24.7)	51 (10.4)	139 (50.0)	<0.001
	Stage III with TB	62 (8.1)	45 (9.2)	17 (6.1)	
	Stage III w/o TB	343 (44.6)	249 (50.7)	94 (33.8)	
	Stage IV with TB	26 (3.4)	21 (4.3)	5 (1.8)	
	Stage IV w/o TB	124 (16.1)	101 (20.6)	23 (8.3)	
	Unknown	24 (3.1)	24 (4.9)	0 (0.0)	
Initial treatment regimen	3TC+d4T+NVP	369 (48.0)	303 (61.7)	66 (23.7)	<0.001
	3TC+AZT+NVP	231 (30.0)	141 (28.7)	90 (32.4)	
	3TC+AZT+EFV	80 (10.4)	16 (3.3)	64 (23.0)	
	3TC+d4T+EFV	57 (7.4)	28 (5.7)	29 (10.4)	
	3TC+AZT+LPV/r	18 (2.3)	0 (0.0)	18 (2.3)	
	3TC+d4T+LPV/r	7 (0.9)	0 (0.0)	7 (2.5)	
	Other*	7 (0.9)	3 (0.6)	4 (1.4)	
Initial treatment regimen (by most potent component)	EFV-containing	138 (18.0)	44 (9.0)	94 (33.8)	<0.001
	NVP-containing	603 (78.4)	444 (90.4)	159 (57.2)	
	LPV/r-containing	25 (3.3)	0 (0.0)	25 (9.0)	
	Other**	3 (0.4)	3 (0.6)	0 (0.0)	
Adherence	Good	542 (70.5)	321 (65.4)	221 (79.5)	<0.001
	Poor	227 (29.5)	170 (34.6)	57 (20.5)	
CD4 percent (mean, 95% CI)	<12 months	15.7 (9.2)	14.3 (9.3)	18.9 (4.6)	<0.001
	12-35 months	15.0 (9.5)	15.6 (9.4)	10.7 (8.1)	
	36-59 months	12.1 (8.7)	12.9 (9.0)	11.9 (8.3)	
	>5 years	9.7 (9.9)	11.3 (9.7)	8.6 (9.9)	
CD4 count (mean, 95% CI)	<12 months	857 (879)	784 (888)	1404 (1145)	<0.001
	12-35 months	759 (617)	767 (624)	554 (697)	
	36-59 months	455 (428)	411.5 (431.5)	524 (583)	
	>5 years	242 (270)	260 (337)	232 (230.5)	
CD4 count z-score	Median (IQR)	-0.26 (0.81) (n=765)	-0.14 (0.96) (n=491)	-0.42 (0.53) (n=274)	<0.001

Classification of Immunodeficiency **	Not significant	65 (8.5)	42 (8.6)	23 (8.3)	0.650
	Mild	42 (5.5)	25 (5.1)	17 (6.1)	
	Advanced	74 (9.6)	43 (8.8)	31 (11.2)	
	Severe	588 (76.5)	381 (77.6)	207 (74.5)	

* Other regimens include mono or dual therapies and those with missing information on cART regimen

** Other regimens include only those without an EFV, NVP, LPV/r, or ABC component, regardless of number of components

** Immunodeficiency was classified as mild (CD4% of 30-35, 25-30, 20-25 and CD4 cell count of 350-499 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively), advanced (CD4% of 25-29, 20-24, 15-19 and CD4 cell count of 200-349 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) and severe (CD4% <25, <20, <15 and CD4 cell count < 200/<15% for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) according to the WHO 2006 thresholds

Table 5. Unadjusted and adjusted Cox proportional hazards ratios for cART drug substitution by baseline characteristics among 769 children included in the drug substitution analysis

Variable		Person time (years)	Events	Crude Incidence rate† (95%CI)	Unadjusted Relative Hazard	95%CI	p-value	Adjusted Relative Hazard	95%CI	p-value
TreatmentType	NVP-containing	981.9	131	13.3 (11.2, 15.8)	Reference					
	EFV-containing	241.4	66	27.3 (21.5, 34.8)	2.02	1.50, 2.72	<0.001			
	LPV/r-containing	80.2	4	5.0 (1.9, 13.3)	0.28	0.10, 0.78	0.014			
	Other *	6.8	1	14.7 (2.1, 104.6)	1.24	0.17, 8.86	0.83			
TreatmentType 2	3TC+D4T+NVP	667.7	48	7.2 (5.4, 9.5)	Reference			Reference		
	3TC+AZT+NVP	3[illegible].4	82	26.5 (21.3, 32.9)	3.53	2.47, 5.04	<0.001	3.29	2.27, 4.76	<0.001
	3TC+D4T+EFV	98.8	42	42.5 (31.4, 57.5)	5.55	3.66, 8.42	<0.001	3.22	2.02, 5.13	<0.001
	3TC+AZT+EFV	139.5	2[illegible]	17.2 (11.5, 25.7)	2.37	1.45, 3.88	<0.001	1.74	1.03, 2.95	0.037
	3TC+D4T+LPV/r	22.7	[illegible]	1[illegible] ([illegible]3, 41.0)	1.56	0.48, 5.05	0.46	1.68	0.51, 5.53	0.39
	3TC+AZT+LPV/r	57.5	1	[illegible] (6.4, 12.3)	0.17	0.02, 1.27	0.09	0.15	0.02, 1.09	0.06
	Other **	14.6	2	13[illegible] (3.4, [illegible]8)	2.10	0.51, 8.64	0.31	1.25	0.29, 5.35	0.76
BMI for age z-score tertiles	Lowest tertile	303.6	60	19.8 (1[illegible]3, 2[illegible]5)	Reference					
	Middle tertile	376.7	50	13.3 (10.1, 17.5	0.69	0.48, 1.01	0.06			
	Highest tertile	341.0	47	13.8 (10.4, 18.3)	1.41	0.48, 1.04	0.08			
	Unknown	289.0	45	15.6 (11.6, 20.9)	1.[illegible]	0.56, 1.21	0.33			
Gender	Female	635.4	98	15.4 (12.7, 18.8)	Refe[illegible]					
	Male	674.9	104	15.4(12.7, 18.7)	1.00	0.76, 1.32	0.99			
Country of treatment	Mozambique	785.0	114	14.5 (12.1, 17.4)	Reference					
	Uganda	525.4	88	16.8 (13.6, 20.6)	1.15	0.8[illegible] 1.52	0.33			
Adherence	Good	884.0	166	18.8 (16.1, 21.9)	Reference			Reference		
	Poor	426.4	36	8.4 (6.1, 11.7)	0.45	0.31, 0.6	<0.001	0.53	0.37, 0.77	<0.001
Classification of immunodeficiency **	Not significant	96.6	14	14.5 (8.6, 24.5)	0.95	0.55, 1.65		1.23	0.70, 2.16	0.47
	Mild	63.8	13	20.4 (11.8, 35.1)	1.32	0.75, 2.33		2.23	1.24, 4.02	<0.001
	Advanced	109.6	17	15.5(9.6, 24.9)	1.06	0.64, 1.75		1.16	0.70, 1.93	0.57
	Severe	1040.4	158	15.2 (13.0, 17.7)	Reference			Reference		
Age group	0-11 months	46.3	15	32.4 (19.5, 53.8)	2.19	1.28, 3.72		2.74	1.54, 4.90	<0.001
	12-35 months	319.9	47	14.7 (11.0, 19.6)	1.02	0.72, 1.44		1.03	0.70, 1.50	0.89
	35-59 months	200.7	32	15.9 (11.3, 22.6)	1.05	0.71, 1.56		0.77	0.51, 1.18	0.24
	>= 5 years	743.5	108	14.5 (12.0, 17..5)	Reference			Reference		
WHO disease stage at baseline	Stage 1 or 2	362.0	52	14.4 (10.9,18.8)	1.27	0.88, 1.81		1.21	0.83, 1.77	0.32
	Stage 3 or 4 with TB	119.2	60	50.3 (39.1, 64.8)	4.45	3.14, 6.30		3.38	2.28, 5.01	<0.001
	Stage 3 w/o TB	624.2	70	11.2 (8.9, 14.2)	Reference			Reference		
	Stage 4 w/o TB	155.5	18	11.6 (7.3, 18.4)	1.01	0.60, 1.69		0.96	0.56, 1.63	0.87
	Unknown	49.3	2	4.1 (1.01, 16.2)	0.41	0.10, 1.67		0.47	0.11, 1.94	0.30

† - per 100 years

* Other regimens include only those without an EFV, NVP, LPV/r, or ABC component, regardless of number of components

** Other regimens include mono or dual therapies and those with missing information on cART regimen

‡‡ Immunodeficiency was classified as mild (CD4% of 30-35, 25-30, 20-25 and CD4 cell count of 350-499 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively), advanced (CD4% of 25-29, 20-24, 15-19 and CD4 cell count of 200-349 for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) and severe (CD4% <25, <20, <15 and CD4 cell count < 200/<15% for children <=11 months, 12-35 months, 36-59 months or >=5 years, respectively) according to the WHO 2006 thresholds

Title: Predictors of treatment failure in HIV positive children receiving combination antiretroviral therapy: cohort data from Mozambique and Uganda

Dear Editor,

We thank you and the Reviewers for the helpful comments on our manuscript. We have responded to each point in turn, and include both the Reviewers' comments (in italics) and our responses below

Reviewer: 1

Comments to the Author

The authors have done an excellent job of compiling outcomes on a large cohort of children initiating ART in two NGO-supported programs in Mozambique and Uganda. There are limited data on outcomes of ART-treated outcomes, and as pointed out by the authors, a need to better understand predictors of treatment failure as well as how children are managed outside of research studies. Please see specific comments by page and, when appropriate, by line.

1. Page 1: title. I believe that the commonly used term is 'combination antiretroviral therapy' rather than 'combined antiretroviral therapy.' If the authors have intentionally chosen the word 'combined' it would be important to both define and justify in the text.

   Thank you for pointing this out. We revised the text using the most common definition "combination antiretroviral therapy".

2. Page 4 line 52. The description of the studies provided in this paragraph would benefit from more specificity. P1060 examined optimal starting regimen in infants and young children, for example. Also the authors may want to also include reference to the recently published Technau paper (PIDJ 2013) which suggests somewhat lower field efficacy of ABC.

   As suggested, the following sentence was added to the text: "Findings from the P1060 trial reported an increased risk of failure starting a nevirapine (NVP)-based cART in infants and young children" AND "Conflicting results were reported concerning the use of abacavir (ABC) as first line regimen: Green et al. suggested that abacavir (ABC) may be preferable to zidovudine (AZT) combination with lamivudine (3TC) (25), while poorer early virological outcomes were recently observed in children starting ABC/3TC-based first line regimens, compared to d4T/3TC (26, 27)".

3. Page 6 line 12. The authors note that cART was prescribed after counseling and written consent by the caregiver. Can they please discuss the consent more fully? Were they consented to participate in a study? Or consented to start ART? . The previous paragraph section describes this study as a retrospective chart review and no mention is made of consenting parents/caregivers. Also were older children asked to sign an assent?

A written consent was collected when enrolling in the programme and before starting cART but was not collected to participate to this study, as we conducted a retrospective analysis of the routine clinical records.

4. Page 6, line 52. It would be useful to restate the definitions of immunodeficiency as described in the WHO 2006 guidelines. Given the passage of time and changing guidelines most readers can no longer remember these definitions. They can be included as a footnote in the table or in the text.

   As suggested, WHO 2006 criteria for immunodeficiency were included in the tables, as a footnote.

5. Page 7, line 29. Please explain further why these children were excluded from the analysis?

   Children with ABC were excluded from the drug substitution analysis because the majority of ABC-related drug substitution was done systematically at the end of TB treatment for which ABC had been started to reduce drug-drug interaction. In the text: "For analysis of cART drug substitution, children who received an ABC component in their initial cART regimen were excluded, as first line ABC treatment was systematically administered to children diagnosed with active TB and all patients initially on ABC were routinely switched to EFV once the TB infection cleared."

6. P7 statistical analyses. Did the authors consider including calendar year as a variable in the multivariate analysis? Given the duration of the observation period, general changes and improvements in care over the time period, and clinician learning that likely occurred, year of enrollment may be an important factor to include.

   Calendar year was initially considered but was not found to be associated with either switching or failure, at univariate analysis. For this reason it was not included in the multivariate analysis.

7. Page 8 results. I would recommend that the authors first describe the characteristics of the cohort they analyzed followed by those who were excluded from study.

   Differences between children excluded and included in the study are shown in revised table 1 and are summarized in the text as follows: "Two hundred and thirteen (20%) children were excluded from the study due to missing data (table 1). Children excluded from both treatment failure and drug substitution analyses were more likely to be Ugandan ($p<0.01$), female ($p=0.049$), younger ($p<0.01$) and enrolled and starting cART later (p 0.01 and <0.01, respectively) than children included in the study." We chose to describe characteristics of the treatment failure and drug substitution cohorts separately, as the two analyses were conducted among slightly different cohorts due to differing inclusion and exclusion criteria (refer to page 7, lines 12-34). We think that adding a detailed description of the entire population, with both the treatment failure and drug substitution cohorts combined, would become lengthy and confusing without adding much information.

8. Given all the information in Table 2, I would recommend that the authors summarize the characteristics of cohort and also identify any meaningful differences between those in Uganda and those in Mozambique. It would also be important to reflect back to the reader how the excluded children differ from those studied and consider commenting on these differences and their implications in the discussion, especially if viewed as a limitation. Other studies have identified HIV positive patients with missing key data (particularly CD4) at enrollment as particularly vulnerable to poor outcomes.

   Differences between Uganda and Mozambique were reported in table 2 and table 4. In particular, in both analyses, children from Mozambique were the majority (66% in treatment failure (TF) cohort, 64% in drug substitution (DS) cohort). Mozambique patients were younger at treatment initiation ($p<0.010$ in TF cohort, $p<0.01$ in DS cohort), had higher baseline WHO disease stage ($p<0.010$ in TF cohort, $p<0.010$ in DS cohort), were more likely to have a baseline cART regimen containing NVP ($p<0.01$ in TF cohort, $p<0.010$ in DS cohort) and poor adherence to cART ($p<0.010$ in TF cohort, $p<0.010$ in DS cohort), and had less negative CD4 count z-scores ($p<0.010$ in TF cohort, $p<0.010$ in DS cohort) in both treatment failure and substitution analyses. In the treatment failure cohort, children from Mozambique also had more negative baseline BMI z-scores ($p=0.02$). As this information is provided in tables 2 and 4, we decided not to add an extensive paragraph on country differences to the results section in order to devote more text to key results and discussion. In the discussion section country differences were highlighted as follow: "As previously mentioned, our results may be confounded by country specific differences. Mozambique patients were younger, had a more advanced WHO stage and a lower BMI z-score at cART initiation. These differences may reflect clinicians' preference in first-line treatment choice, accounting for the wider use of AZT and EFV in Uganda as much as for the increased choice of NVP-based regimen observed in children from Mozambique. Country specific differences may potentially confound the relationships seen between cART regimen and treatment failure and drug substitution. In terms of follow-up visits, the Ugandan children were followed up much more frequently (monthly) than those in Mozambique (every 3 months). This difference between program performances may have provided further confounders, potentially influencing the trends observed in older children at lower risk of failure and the higher rate of drug substitution observed in infants."

9. The authors should also consider including a table and/or description of how children met treatment failure criteria – how many were immunologic, how many were clinical, what were the most common clinical findings, etc. This information would be very useful to the reader and might help to understand the discrepancy between what was determined on retrospective chart review and how children were clinically managed.

   As suggested, rate of immunological failure was added in the "Treatment failure analysis" paragraph: "A total of 218 treatment failure events (29% 95%CI [26-33]) occurred, with a crude incidence rate of 20.0 events per 100 person-years (95%CI 17.5-22.9). Median time to treatment failure was 379 days (IQR 229-649). Immunological failure alone occurred in 100 (46%) children while clinical failure alone was found in 116/218 (53%) cases. Two children (1%) had concomitant clinical and immunological failure."

10. Page 8 line 53. Please distinguish between 'dropped out' and LTF.

    Children LTFU were those who missed follow-up visits for more than 6 months while children were dropped out of care if confirmed HIV-negative, or if aged more than 18 years old (in this case they were referred to adult HIV clinic). In the text we modified as follows(refer to "Methods - Data collection" paragraph): "For children without treatment failure or drug-substitution, follow-up was censored at date of death, loss to follow up (LTFU, defined as missing follow-up visits for more than 6 months), transferred to other clinic, confirmed HIV-negative or aged more than 18 years old, last CD4 measurement, or last anthropometric or adherence record, whichever occurred latest."

11. While the numbers are small, drug substitution analyses often excluded ARV changes for treatment failure. Why have the authors decided to include treatment failure cases in the drug substitution analysis?

    In the drug substitution analyses, 'treatment failure' was as reported by the treating physician, rather than assessed using CD4 counts and WHO disease staging. As physician report in this case may be incomplete or at times inaccurate, we did not feel it appropriate to drop these children from the analysis. In addition, we think that it could be interesting to show both reported and calculated treatment failure rates.

12. The authors do not comment on the baseline characteristics or difference in characteristics between countries. The table is probably unnecessary. A table, however, providing more information on the drug substitutions including more descriptive information on the toxicities of each drug and the substitution decisions would be of great interest.

    We agree with this suggestion but unfortunately JPIDS allows no more than 5 tables. In addition, unfortunately at this time we cannot provide detailed information for all patients that switched for toxicity, due to missing data.

13. Furthermore, the authors should clarify whether there were specific clinic protocols to identify and manage drug toxicities – in particular, was Hg monitored for children on AZT and where there specified indications for discontinuation/change?

    Toxicity to antiretroviral drugs was managed following the WHO guidelines available at the time of the study. In particular, clinicians mainly refer to WHO 2006 guidelines where severe anaemia was defined as Hb<7.5 g/dl. In both countries full blood count was routinely assessed every 6 month or more frequently if any clinical suspect of anaemia, independently of the type of cART regimen (refer to methods, page 6 line 43).

14. Discussion
Page 10, line 54. Please clarify whether the reasons provided for delayed switching are actual reasons provided by clinic staff or hypotheses provided by the authors.

Reasons provided in the discussion (page 10, line 54) are author's hypotheses, based on the program setting. In the text: "In our program we hypothesize that limited availability and costs of second line drugs may be major barriers to second line therapy."

15. The authors hypothesize that death or lost-to-follow-up prior to switch may have contributed to the low rate of switching. Few children in the study died and the lost-to-follow-up rate among the cohort of children included in the analysis was comparatively low. Were these children over-represented among those who met treatment failure criteria? Can the authors examine this in their analysis?

"Unfortunately we cannot provide detailed information on mortality and/or LTFU events occurred after the recognition of treatment failure, therefore this sentence was removed from the manuscript: "Under-diagnosis of treatment failure or the occurrence of death or lost to follow-up before switching cART in children with treatment failure may have contributed to the low rate of switching observed."

16. Page 11, line 10. Please clarify this sentence.

We have clarified the sentence as follows: "It should be noted that in the drug substitution analysis reasons for switching may be misclassified, as these data were collected retrospectively and are based on clinician report. "

17. Page 11, line 36. While the findings in this paper reflect the realities of clinical care in low resource settings, I question the validity of the comparison with the clinical trials. In table 2 it is noted that only 24 children of the 740 children included in the treatment failure analysis initiated a PI-based regimen and over 95% initiated NNRTI therapy. There may be a comparison to be had between EFV and NVP but I suggest that any conclusions about PI-based treatment be reconsidered.

We agree with this comment and we therefore revised the paragraph as follows: "Treatment failure was not different between PI-based and NNRTI-based regimens, however this finding is of difficult interpretation since only a limited number of children were receiving a PI-based regimen at the time of the study. Few randomized trials investigated the most effective first-line cART regimen in HIV-positive children. The P1060 trial (13, 23) showed an increased risk of virological failure in children (<3 years) on NPV-based cART, regardless of PMTCT exposure, however this was not confirmed by the PENPACT trial conducted in older children of high income countries (24). Due to the nature of our cohort's age and lack of reliable PMTCT exposure data, our observational retrospective findings are not comparable to those from either controlled trial."

18. Page 11 line 59. The authors make an important point about the role of viral load monitoring to identify treatment failure and the potential delays in switching to second line with clinical and immunologic criteria. However, the primary findings of the paper don't speak to either viral load monitoring or the sensitivity and specificity of clinical or immunologic criteria. Rather, they demonstrated that in the field clinicians did not switch to second line when children met existing treatment failure criteria. One major drawback of the analysis is that the authors are unable to determine whether the clinicians applied and/or recognized that the children met criteria and decided not to change treatment or whether the criteria were too complicated or difficult to use in the clinical setting so that clinicians could not recognize/diagnose treatment failure.

    We completely agree with this comment. Our results suggest the need of a qualitative assay to better explore reasons of delay in cART switching among children with immunological/clinical treatment failure. In the text we modified as follows: "Determining when to switch to second line cART is a critical decision in settings where virological monitoring is not available. Although evidence shows that viral load (VL) is not essential to identify treatment failure (34), using clinical and immunological parameters leads to delays in switching to second line therapy (17), resulting in longer exposure to failing regimens which contributes to development of drug-resistant HIV strains (6). In our study reasons for delays to cART switching were not completely clarified, in particular we were unable to understand if clinicians didn't switch cART in children with recognized treatment failure or if clinical/immunological criteria were too complicated to recognize treatment failure. Earlier cART initiation and VL monitoring are currently recommended by WHO 2013 consolidated guidelines (3)."

19. page 12 line 45. As noted earlier, it would be helpful to know if there were criteria for monitoring hemoglobin and changing drugs based on anemia at the sites. Also, while the authors suggest that ABC may better tolerated, they have minimal experience in their cohort with ABC. Rather, their data suggests that D4T is better tolerated compared with AZT. While no longer recommended because of long term metabolic complications, the experience in the field with D4T, particularly for young children and for initiating treatment, has been good.

    In both cohorts Hb levels were monitored every 6 months or more frequently, if indicated by clinicians (page 6 line 43). We agree with the comment on ABC and we modified as follow: "Higher rates of drug substitution were observed among children starting AZT-containing or EFV-based regimens. Increased drug substitution while on AZT is often the result of AZT-related anemia as well-described previously (23, 31, 36). AZT toxicity was more prevalent among the Mozambique cohort, where children were younger and malnutrition and/or more advanced WHO disease stages were observed, suggesting that AZT anemia may have been exacerbated. Despite the lack of more robust evidence, our findings suggest that AZT may not be the preferred NRTI to be used in these settings, particularly in younger children."

Reviewer: 2

Comments to the Author

The authors present some interesting data from the 2 cohorts in Africa. Treatment failure in children is particularly topical as the WHO roll out the updated consolidated 2013 guidelines, where viral load monitoring and stricter criteria for virologoc failure are defined. The topic suggests that the subject is treatment failure, however, analysis of drug substitution is also a big focus. In my opinion, these 2 topics should be dealt with separately. I suggest that for this paper adhering to the topic of treatment failure will help focus it better. It is of course important to refer to the data on drug substitution to demonstrate the fact that only small proportion of children switch therapy because of treatment failure.

Thank you very much for this comment. There was much discussion on whether to include treatment failure and drug substitution in the same paper or to separate the two topics. We decided to combine treatment failure and drug substitution for several reasons. First, we thought it was extremely important to look at the rate of switching to second line therapy after analyzing treatment failure. It was also interesting to look at cases of treatment failure reported by clinicians, together with the retrospective analysis of treatment failure based on clinical and immunological findings. Despite the complexity of combining these two topics, we think that this combination gives a wider perspective of management of cART in HIV-positive children of low-middle income countries.

Some specific comments:

1. I think table 1 is not very helpful on its own, it might be more interesting to compare those who were included to those who were excluded.

   Thank you for this comment. Kindly refer to new TABLE 1.

2. In table 2 it is not clear what the p-values refer to, maybe this needs to be more specific, could have footnotes and mention how these were derived.

   The followed footnote was add to TABLE 2: “p-values refer to differences between Mozambique and Uganda sub-cohorts on baseline characteristics”

3. Since it appears there is some relationship between TB and treatment failure, I would like to see if possible, more detailed analysis of the initial regimens used together with TB treatment, to assess whether drug regimens used during TB treatment might be associated with treatment failure?

   We agree that TB treatment may interact with cART, and that the proposed analysis could provide very useful information for clinicians. Unfortunately, data available on TB treatment in this cohort is incomplete and, in our opinion, not reliable enough to conduct this analysis.

4. WHO/Unicef usually use weight-for-age z-scores to assess malnutrition particularly for young children and I wonder why the authors have chosen to use BMI z-scores?

   In our study we chose to use BMI for age z-scores instead of weight-for-age z-scores because BMI provides a more appropriate assessment for chronic malnutrition, as it reflects both weight and height. As weight for age z-scores, BMI for age z-score has been used in several studies conducted in HIV-

positive children (3Cs4kids Analysis and Writing Committee, AIDS 2008; Landes M et al, PLoS One. 2012; Jacobson DL et al, Am J Clin Nutr. 2011).

5. The authors discuss the updated 2013 WHO guidelines in terms of earlier treatment start for children but fail to discuss the criteria for switching. If viral load were available, treatment failure would likely be detected earlier and implications for these 2 countries needs to be discussed.

   Both countries mainly referred to WHO 2006 guidelines for cART switching, however we do not have information on adherence to these guidelines at the sites where data were collected for this study. Regarding implications of VL testing in Uganda and Mozambique, please refer to the following text in the background section of the manuscript: “Delays in detecting treatment failure and switching to second line therapy lead to the development of HIV drug-resistance, compromising subsequent regimens (6, 21). This is particularly relevant for children, due to the lack of paediatric formulations” and “Earlier cART initiation and VL monitoring are currently recommended by WHO 2013 consolidated guidelines (3). Our results suggest that more efforts should be done to qualitatively assess reasons of delay in switching to second line cART, particularly in light of the scaling up of VL monitoring in LMIC.” This implies that more prompt switching to second line therapy when appropriate would reduce the development of HIV drug resistance and the compromise of subsequent regimens. Viral load monitoring would probably anticipate the diagnosis of treatment failure, but efforts should be done to better explore any reason of delay/not starting second line cART, in the program setting.

6. Finally, there are some grammatical errors. In the first line of the abstract "delays detecting... is" should be "are", and line 12, "evaluated for..." delete "for".

   Thank you for pointing this out, all these grammatical errors were corrected.

Epstein-Barr Virus load in children infected with Human Immunodeficiency Virus type 1 in Uganda

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Running head: EBV in HIV-1 infected children in Uganda

Keywords: EBV, HIV-1, African children, immune activation, antiretroviral therapy

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Short summary

This study analysed for the first time EBV types and load in HIV-1-infected African children. Findings indicate that antiretroviral treatment, by limiting HIV-1 replication, microbial translocation and related immune activation, prevents super-infection with EBV types and keeps EBV viremia down.

Abstract

Background: Epstein-Barr Virus (EBV) is involved in a wide range of malignancies, particularly in immunocompromised subjects. Besides immunodepression, immune activation may also affect dynamics of EBV infection. In Africa, EBV primary infection occurs during early childhood, but little is known about EBV load in HIV-1 infected children.

Methods: Dried Blood Spot samples from 213 HIV-1 infected children, 140 on antiretroviral therapy (ART), were collected at the Nsambya Hospital in Kampala, Uganda. DNA was extracted and analysed for quantification of EBV types 1 and 2 by multiplex real-time PCR, and for quantification of 16S ribosomial DNA (16S rDNA), a marker of microbial translocation, by real-time PCR. RNA was extracted and used to quantify HIV-1 RNA in peripheral blood.

Results: Ninety-two of 140(66%) children on ART and 57 of 73(78%) ART-naive children had detectable EBV levels. Co-infection with both EBV types was significantly less frequent in ART-treated than in ART-naïve children (OR=0.54, 95%CI 0.30;0.98, p=0.042); EBV-DNA levels were significantly lower in the former (3.99±0.59 vs 4.22±0.54 $\log_{10}$ copies/ml; p=0.0059) and tended to be inversely associated with time on ART. EBV-DNA levels were also significantly higher in children with HIV-1 RNA>3 $\log_{10}$ copies/ml of blood (regression coefficient=0.32 [95%CI 0.05;0.59]; p=0.0197) and correlated with circulating 16S rDNA levels (r_s=0.250 [95%CI 0.02;0.46]; p=0.0310).

Conclusions: These findings suggest that ART, by limiting HIV-1 replication, microbial translocation and related immune activation, prevents super-infection with both EBV types and keeps EBV viremia down, thus potentially reducing the risk of EBV-associated lymphomas.

1. Introduction

Epstein-Barr Virus (EBV) is associated with a wide range of malignancies, particularly in immunocompromised subjects, ranging from lymphoproliferative disorders to B-cell non-Hodgkin's lymphomas (NHL) [1,2]. Besides immunodepression, chronic immune activation, a hallmark of Human Immunodeficiency Virus type 1 (HIV)-1 pathogenesis [3], may play a critical role in the genesis of B-cell lymphomas [4,5]. Cell activation driven by HIV-1 antigens, together with impaired immunosurveillance against EBV, may result in chronic B-cell stimulation and expansion of EBV-infected B-cells [6-9], thus increasing the risk of EBV-related malignancies. The factors contributing to HIV-1-induced B-cell activation and expansion of EBV-infected cells are largely unknown. Massive HIV-1-induced T-cell depletion causes damage to intestinal mucosa, promoting translocation of microbial products into circulation. Pathogen-associated molecular patterns (PAMPs), such as 16S ribosomal DNA (16S rDNA), trigger a potent innate immune response through the engagement of several Toll-like receptors (TLRs), which also involve B-cells, causing polyclonal B-cell activation [10].

Antiretroviral therapy (ART) has greatly modified the natural course of HIV-1 infection, resulting in decreased HIV-1 load, increased CD4+ T cells, and decreased HIV-1-associated opportunistic infections, indicating the restoration of immune function [11]. Although the incidence of HIV-1 related malignancies, such as Kaposi sarcoma (KS), has declined markedly following expanded access to ART, the incidence of NHL still remains elevated [12]. Two EBV types are recognized, type 1 and type 2. EBV type 1 is more common in most populations; 80% to 90% of EBV isolates from Caucasian and Southeast-Asian populations are type 1. EBV type 2 shows a prevalence almost equal to EBV type 1 in populations in Africa and New Guinea [13]. HIV-1 infectedsubjects may have either EBV type 1 or type 2, and co-infection with both types is also possible [13].

In Africa, EBV primary infection occurs during infancy and early childhood, and EBV-associated lymphomas represent a substantial cause of morbidity and mortality in children. NHL commonly occurs in African children, endemic Burkitt's Lymphoma (BL) being the most common type of cancer [14]. Endemic BL is closely associated with EBV and accounts for up to 75% of all childhood malignancies [15], reaching 5-10 cases per 100000 of annual incidence in Central Africa [16]. Other tumors, such as EBV-associated immunoblastic lymphomas, may occur in HIV-1 infectedchildren [17,18].

To date, there are no available data about EBV types and viremia in the context of HIV-1 infection in African children. This absence may partly be due to the lack of adequately equipped laboratories and expertise in developing countries. The use of Dried Blood Spots (DBS) sampling may represent a more feasible method to collect and store blood samples and may be instrumental in expanding studies in resource-limited settings.

2. Material and Methods

2.1 Patients and sample collection

This is a cross-sectional study, conducted in the Home Care Department of the St. Raphael of St. Francis Hospital at Nsambya in Kampala, Uganda. Blood samples from 213 HIV-1 infected children (0-18 years) who attended to Home Care Department from March 2010 to July 2010 were collected. At the time of sampling for this study, 140 (66%) children were on ART. Ninety-seven children received Nevirapine (NVP)-based regimens, 34 Efavirenz (EFV)-based therapy, and 8 a Lopinavir/ritonavir (LPV/r)-based treatment (Table 1). Blood from each child was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes by trained nurses. Fifty µl of this blood were spotted onto each of the five circles of Protein Saver TM 903 Card (Whatman GmbH, Hahnestra, Germany). DBS were dried at room temperature overnight, stored in individual ziplock

bags containing a desiccant, and shipped to the laboratory of the Viral-Oncology Unit and AIDS Reference Center, Section of Oncology and Immunology, Padova, Italy. The study was approved by the Internal Review Board and Ethics Committee of Nsambya Hospital and registered by the Uganda National Council for Science and Technology (Ref. HS724).

2.2 DNA elution

From each 50µl DBS, three 3 mm-diameter circles, equivalent to 5 µl of whole blood each (15 µl total), were used to extract DNA with the Qiagen DNA MicroKit (Qiagen, Hilden, Germany) and were resuspended in a final volume of 50 µl. To check whether the eluted DNA could be amplified, 5µl of final elution were amplified for the human telomerase reverse transcriptase (TERT) gene (Gen Bank accession: AF128893), employed as housekeeping gene. Amplification was carried out as previously described [19].

2.3 EBV-DNA typing and quantification

A quantitative method, based on multiplex real-time PCR assay, was employed to quantify EBV types 1 and EBV type 2, as described elsewhere [20]. Each PCR was performed in a 25µl reaction mix containing 5µl of eluted DNA (corresponding to 1.5µl of blood). A standard reference curve was obtained by five-fold serial dilution of two amplicons, one for EBV type 1 and one for EBV type 2, and amplification was performed as already described [20]. The multiplex assay showed a dynamic range from 5 to $2x10^5$ copies. The lower limit of detection was up to 1 copy and was established by analysing replicate dilution samples containing 1 EBV copy. According to Poisson distribution, repeated measurement of these samples gave positive results for EBV detection in 9 out of 15 replicates. As amplification was carried out in a final volume of 1.5 µl blood, a sample was positive when there are at least 660 EBV-DNA copies/ml blood. Results are expressed as $\log_{10}$ EBV-DNA copies/ml.

2.4 16S rDNA quantification

A quantitative method based on real-time PCR assay was performed to quantify 16S rDNA, with the primer pair and probe as described [21]. A standard curve was generated from five-fold serial dilutions of plasmid DNA containing known copy numbers of the template. The assay showed a dynamic range from 3 to $2.5x10^5$ copies. Levels of 16S rDNA were quantified in 109 available samples. Results are expressed as $\log_{10}$ 16S rDNA copies/µl.

2.5 HIV-1 RNA quantification

For each sample, two entire DBS, corresponding to 100 µl of whole blood, were used to extract RNA with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). RNA was eluted with 75 µl of the elution buffer heated to 70°C. HIV-1 RNA levels were determined with the Amplicor HIV-1 Monitor Test (Roche Diagnostic Systems, Branchburg, NJ, USA). The lower limit of detection with the DBS samples was 170 HIV-1 RNA copies/ml of blood. Results are expressed in $\log_{10}$ HIV-1 RNA copies/ml of blood. As HIV-1 RNA of up to 3 $\log_{10}$ copies/ml plasma is currently considered the most appropriate cut-off to define treatment failure [22], and also for prevention of mother-to-child transmission of HIV ART was recommended for all women with HIV-1 RNA levels of $\geq$3 $\log_{10}$ copies/ml of plasma [23], the relationship between EBV and HIV-1 was estimated according to this cut-off value.

2.6 Statistical analyses

EBV-DNA, HIV-1 RNA and 16S rDNA data were $\log_{10}$ transformed to obtain more normal distributions. Chi-square test for categorical variables and Kruskal-Wallis test for continuous variables were employed. Logistic regression analysis was employed to assess the relationship between EBV detection status and whether or not ART was received, after adjustment for multiple

confounders such as CD4 Z-score, WHO stage, age, gender. Logistic regression analysis and Spearman's correlation coefficient were used to evaluate relationships between status and levels of EBV-DNA and time on ART. Linear regression analysis was used to assess the relationship between HIV-1 RNA load and EBV-DNA levels in univariate analysis and after adjustment for multiple confounders. The association between EBV-DNA and circulating bacterial 16S rDNA levels was evaluated by Spearman's correlation and by a linear regression model. All statistical analyses were performed with software SPSS v.18 and STATA v.12.

3. Results

3.1 EBV-DNA levels in children

Table 1 lists the baseline characteristics of the 213 HIV-1 infected children. A total of 149 children, 92 out of 140 (66%) on ART and 57 out of 73 (78%) ART-naive had detectable EBV in blood. EBV types 1 or 2 were detected in 31 (22%) and 21 (15%) children on ART, and in 15 (21%) and 11 (15%) ART-naive children, respectively. Co-infection with both EBV types was observed in 40 (29%) ART-treated and 31 (42%) ART-naive children. Children on ART had less probability of being co-infected with both EBV types than ART-naive children (OR=0.54 [95% CI 0.30;0.98]; p=0.0423), and overall ART-treated children were less likely to carry detectable EBV-DNA than ART-naive children (OR=0.54 [95% CI 0.28;1.04]; p=0.0637) (Table 2). These findings were confirmed in the multivariate analysis, after adjustment for CD4-Z score, age, gender, and WHO stage (OR=0.50 [95% CI 0.23;1.08]; p= 0.0696, and OR=0.40 [95% CI 0.1;0.97]; p=0.0426, respectively) (Table 2).

Mean (± Standard Deviation) levels of EBV-DNA were similar in children infected with either EBV type 1 (3.80±0.52 $\log_{10}$ copies/ml) or type 2 (3.92±0.54 $\log_{10}$ copies/ml), but they were higher in children co-infected with both viruses (4.34±0.54 $\log_{10}$ copies/ml) (Figure 1A). No significant

differences in mean levels of EBV types 1 or 2 were found between ART-treated or ART-naive children, whereas e mean levels of co-infection with both EBV types were significantly lower in children on ART (4.23±0.54 vs 4.47±0.50 log_{10} copies/ml; p=0.0210). Overall, total EBV-DNA load was significantly lower in ART-treated than in ART-naive children (3.99±0.59 vs 4.22±0.54 log_{10} copies/ml; p=0.0059) (Figure 1B). EBV-DNA load tended to be inversely associated with the time on ART *(rs=-0.201 [95% CI -0.394;0.009]; p=0.061*). In particular, after adjustment for WHO stage and CD4-Z score, the detection of EBV type 2 was less frequent according to the time on ART (OR=0.811 [95% CI 0.659;0.997]; p=0.048).

3.2 EBV-DNA levels in relation to HIV-1 load

Total HIV-1 RNA (cell-associated and plasma) levels were evaluated in the DBS samples of 204 HIV-1 infected children. One hundred and ninety-one (94%) children, 125 out of 134 (93%) on ART and 66 out of 70 (94%) ART-naive, had detectable HIV-1 RNA. Such levels were significantly lower in ART-treated than in ART-naive children (3.55±0.66 vs 4.18±0.83 log_{10} copies/ml; p<0.0001). EBV-DNA levels were significantly higher in children with HIV-1 RNA >3 log_{10} copies/ml of blood, in both univariate analysis (regression coefficient=0.32 [95% CI 0.05;0.59]; p=0.0197) and multivariate analyses, after adjusting for age, gender, WHO stage, CD4 Z-score, and ART (regression coefficient=0.33 [95% CI 0.30;0.63]; p=0.0318) (Table 3).

3.3 EBV-DNA levels in relation to markers of immune activation

Levels of 16S rDNA were significantly lower in ART-treated than in ART-naive children (2.09±0.23 vs 2.16±0.25 log_{10} copies/µl; p=0.007) (Figure 2A). In a total of 109 children, 72 had detectable EBV, 30 had EBV type 1, 13 type 2, and 29 were co-infected with both EBV types. Levels of 16S rDNA were similar in children infected with EBV type 1 (2.09±0.27 log_{10} copies/µl) or EBV type 2 (2.14±0.11 log_{10} copies/µl), but higher in children co-infected with both EBV types (2.22±0.12 log_{10}

copies/μl) (Figure 2B). Levels of 16S rDNA significantly correlated with EBV-DNA levels (r_s=0.250 [95% CI 0.02;0.46]; p=0.0310) (Figure 2C). A linear regression model confirmed the significant relationship between 16S rDNA and EBV (regression coefficient=1.01 [95% CI 1.00;1.02]; p=0.0197).

Discussion

This is the first study describing the relationship between HIV-1 infection, markers of microbial translocation, and EBV types and viremia in HIV-1 infected African children. First of all, we found that HIV-1 infected children were more co-infected with both EBV types than with only type 1 or type 2. Although healthy individuals usually harbor a single EBV type, HIV-1 infected subjects may be infected with both, as observed in this study. The fact that EBV type 1 transforms B cells *in vitro* more efficiently than type 2 has suggested type-specific differences in oncogenic activity *in vivo* [13,24]. However, both types have been found in HIV-1-related lymphomas [7,13,24,25], indicating that both viruses may play an oncogenic role in the context of a weakened immune system. In addition, LMP1, the master oncoprotein of EBV, differs among the EBV strains, according to the number of 33 base-pair repeat elements, but not specifically between the two types [26,27].

We found that children co-infected with both EBV types had higher EBV levels than those infected with only type 1 or type 2. Co-infection and levels of EBV are also related to blood levels of HIV-1 RNA and 16S rDNA, a marker of microbial translocation [10]. These results suggest that HIV-1 replication and circulating microbial products may lead to EBV replication and expansion of EBV-infected B-cells, thus increasing the EBV-DNA load. Super-infection with both EBV types may represent an additional risk.

Our results show that ART affects EBV infection and levels. Overall, ART-treated children were less likely to have detectable EBV levels. In particular, they had a lower probability of being co-infected with both EBV types and, overall, their EBV levels were significantly lower than in ART-naïve children. Of interest, EBV-DNA load tended to be inversely associated with time on ART, and children with a prolonged time on ART had lower EBV-DNA levels. These findings indicate that ART constrains EBV super-infection and keeps EBV viremia down.

As expected, HIV-1 RNA load in total blood (cell-associated and plasma) was significantly lower in ART-treated than in ART-naive children, but surprisingly, many children on ART (93%) had detectable HIV-1 RNA levels. This finding has two possible explanations. First, poor adherence to therapy may lead to a weak virological response, with persistent detectable plasma viremia. Second, the amount of HIV-1 RNA extracted from DBS is due to plasma viremia and cell-associated viral RNA. Cell-associated HIV-1 RNA may persist even in patients with undetectable plasma viremia [28,29] and may be considered as an indicator of residual virus replication in ART-treated patients [30]. The introduction of ART has resulted in a significant reduction in HIV-1 load and has reduced circulating levels of bacterial 16S rDNA [21]. As expected, we found that levels of circulating bacterial 16S rDNA were significantly lower in ART-treated than in ART-naive children. Overall, the relationships among EBV, HIV-1 and 16S rDNA indicate that ART, by limiting HIV-1 replication and reservoirs, microbial translocation and chronic immune activation, impedes super-infection by both EBV types, preventing stimulation of B-cells and expansion of EBV-infected B-cells, thus reducing the risk of developing EBV-associated B-cell lymphomas.

DBS filter papers are commonly used to diagnose and monitor different DNA/RNA viral loads [31]. However, to date only one study [32] has investigated EBV-DNA levels from DBS. EBV viral load assessment in peripheral blood may be an important instrument for diagnosing and monitoring

EBV-associated lymphoproliferative diseases, particularly in some regions of Africa, such as Uganda, where EBV sero-prevalence is nearly universal, with acquisition of infection in early childhood. Our results show that it is possible to quantify EBV-DNA, HIV-1 RNA and 16S rDNA by DBS sampling, thus improving the diagnostic and monitoring capabilities in resource-limited settings.

Our findings should be interpreted in the light of some potential limitations. First, the DBS specimens were collected in Uganda, stored at room temperature, and shipped to Italy in a median time of 49 days (range 34-64 days). While amplification failure has been described when specimens are stored at room temperature for more than 30 days [33], the influence of storage conditions on the EBV amplification is unknown. Second, although multivariate analyses were carried out, potential additional confounders, such as comorbities or type of therapy, cannot be enterely ruled out.

In conclusion, HIV-1, inducing microbial translocation and a state of persistent immune activation, may lead to EBV replication and expansion of EBV-infected B-cells, thus increasing the EBV-DNA load. Super-infection by both types of EBV in HIV-1 infected subjects may represent an additional risk for the onset of EBV-related malignancies. ART, by limiting HIV-1 replication, microbial translocation and related immune activation, may prevent super-infection by both EBV types and keep EBV viremia down, thus reducing the risk of EBV-associated lymphomas.

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Conflict of interest

None of the authors declares any conflict of interest

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Table 1. Baseline characteristics of HIV-1 infected children

Characteristics*		No. children (%)
Children on ART (n=213)	No	73 (34)
	Yes	140 (66)
WHO Stage (n=209)	I	60 (29)
	II	120 (57)
	III	27 (13)
	IV	2 (1)
WHO stage of children on ART (n=140)	I	23 (16)
	II	97 (69)
	III	18 (13)
	IV	2 (2)
ART type (n=140)	NVP Based	97 (69)
	EFV Based	34 (24)
	LPV/r Based	8 (6)
	3-NRTI	1 (1)
CD4 cell/µl (n=177)	< 350	18 (10)
	350-750	68 (38)
	750-1000	41 (23)
	>1000	50 (29)

*All characteristics referred to the time of sample collection

Table 2. EBV detection among HIV-1 infected ART-treated vs ART-naive children

EBV detection	ART-treated	ART-naive	OR (95% CI)		*p*-value	
	n (%)	n (%)	univariate	multivariate*	univariate	multivariate*
EBV type 1	31 (22)	15 (21)	1.10 (0.55;2.20)	0.85 (0.35;2.06)	0.7884	0.7278
EBV type 2	21 (15)	11 (15)	0.99 (0.45;2.20)	1.00 (0.37;2.71)	0.9894	0.9971
EBV type 1+ 2	40 (29)	31 (42)	0.54 (0.30;0.98)	0.50 (0.23;1.08)	0.0423	0.0696
EBV total	92 (66)	57 (78)	0.54 (0.28;1.04)	0.40 (0.17;0.97)	0.0637	0.0426

* Covariates considered for multivariate model: age, gender, CD4 Z-score, WHO stage

Table 3. Multivariate regression analyses of EBV-DNA levels in HIV-1 infected children

EBV detection	Covariate[a]	Regression coefficient (95% CI)		*p*-value	
		univariate	multivariate*	univariate	multivariate*
EBV type 1	HIV-1 RNA >3 log_{10}[b]	0.30 (-0.08;0.69)	0.38 (-0.11;0.87)	0.1216	0.1233
EBV type 2	HIV-1 RNA >3 log_{10}[b]	0.06 (-0.51;0.63)	0.15 (-0.45;0.76)	0.8274	0.6004
EBV type 1+ 2	HIV-1 RNA >3 log_{10}[b]	0.25 (-0.19;0.69)	0.27 (-0.21;0.75)	0.2548	0.2639
EBV total	HIV-1 RNA >3 log_{10}[b]	0.32 (0.05;0.57)	0.33 (0.30;0.63)	0.0197	0.0318

[a] Other covariates in multivariate model: gender, age, CD4 Z-score, WHO stage, ART
[b] HIV-1 RNA load is expressed as log_{10} copies/ml of blood

Legends to Figures

Figure 1. EBV-DNA load in HIV-1 infected children. EBV-DNA levels in HIV-1 infected children sub-grouped by A) type of infection with EBV and B) therapy. Each plot represents one child. Lines indicate the mean values.

Figure 2. 16S rDNA levels in HIV-1 infected children. 16S rDNA levels in HIV-1 infected children sub-grouped according to A) therapy: on ART or ART-naive, B) type of infection with EBV, and C) correlation with EBV-DNA levels. Each plot represents one child. Lines indicate the mean values.

Figure 1

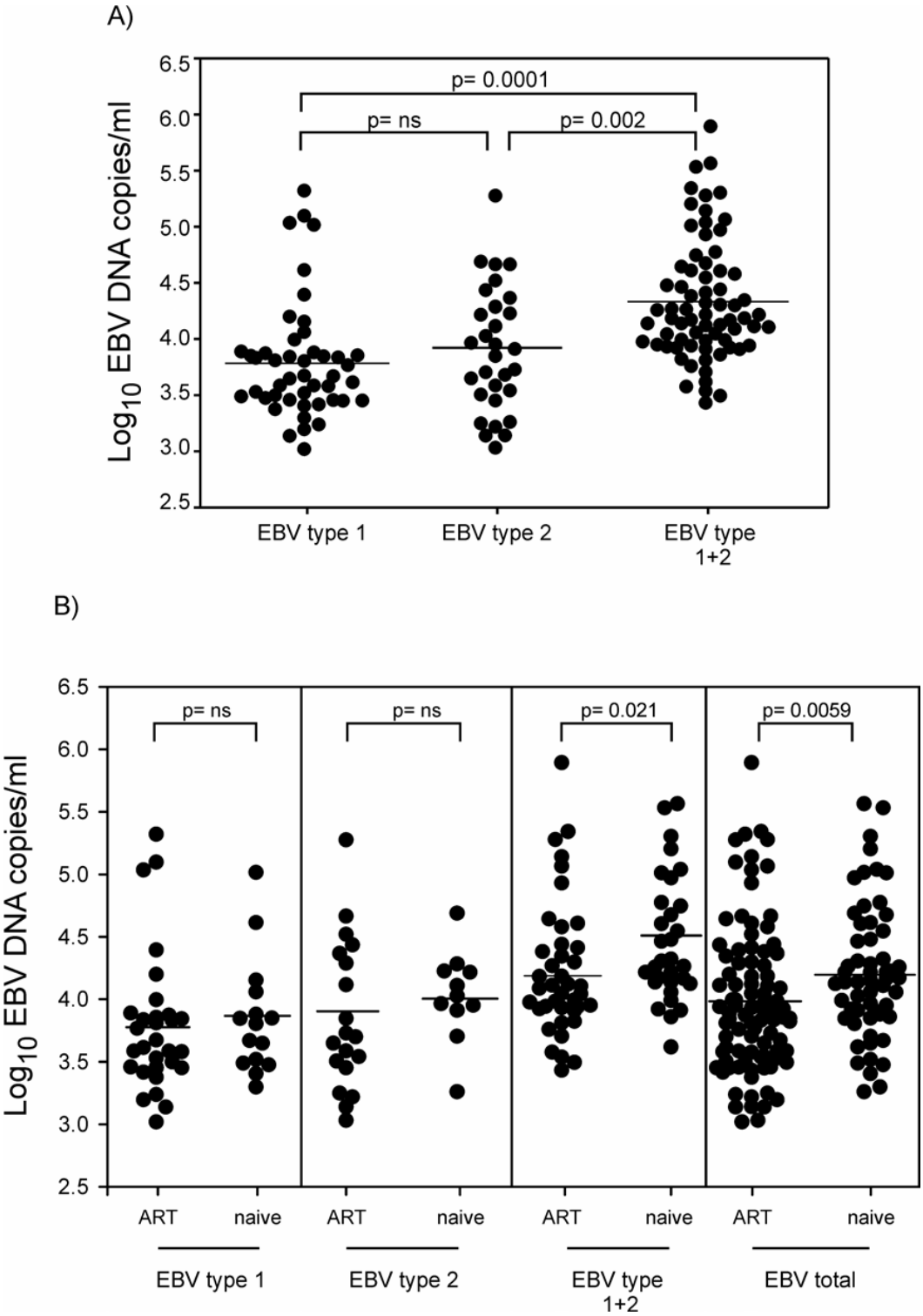
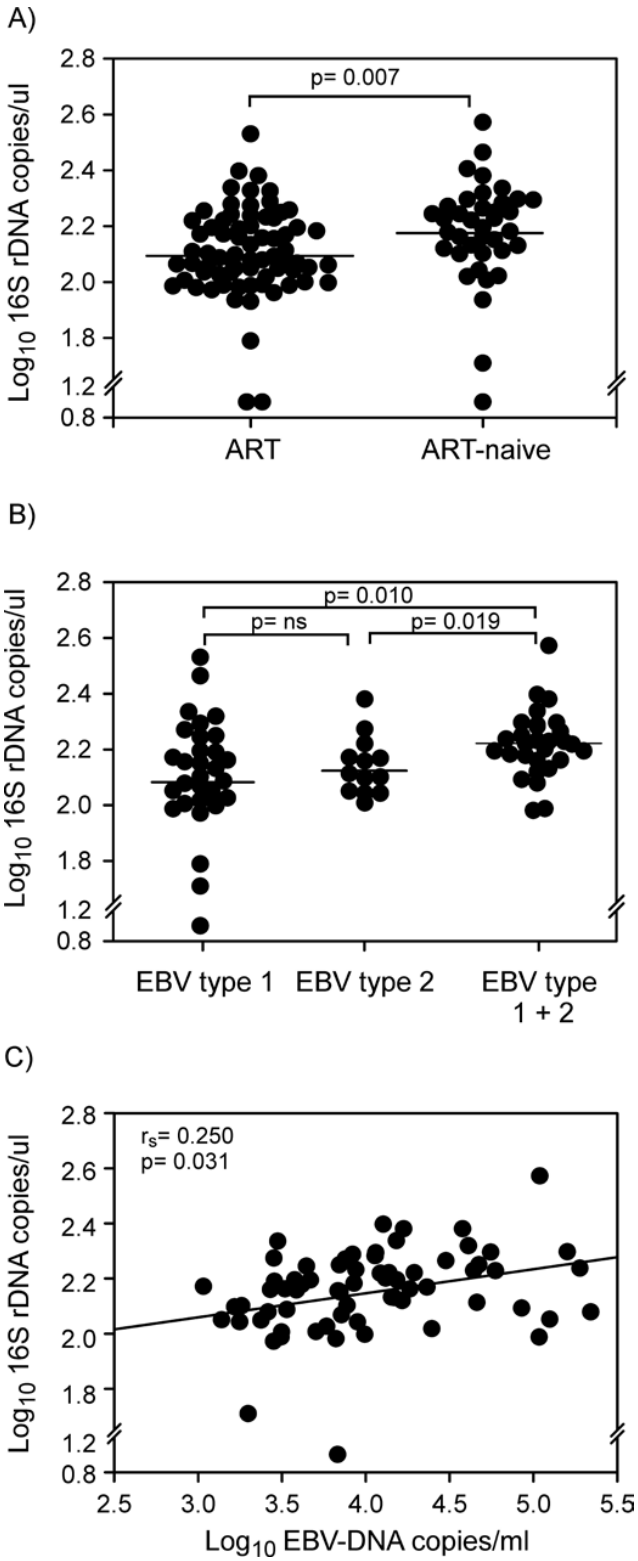

A)
Log10 EBV DNA copies/ml
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
p= 0.0001
p= ns
p= 0.002
EBV type 1
EBV type 2
EBV type 1+2
B)
Log10 EBV DNA copies/ml
p= ns
p= ns
p= 0.021
p= 0.0059
ART
naive
EBV type 1
EBV type 2
EBV type 1+2
EBV total

Figure 2


A)
p= 0.007
Log10 16S rDNA copies/ul
2.8
2.6
2.4
2.2
2.0
1.8
1.2
0.8
ART
ART-naive
B)
p= 0.010
p= ns
p= 0.019
Log10 16S rDNA copies/ul
EBV type 1
EBV type 2
EBV type 1 + 2
C)
rs= 0.250
p= 0.031
Log10 16S rDNA copies/ul
2.5
3.0
3.5
4.0
4.5
5.0
5.5
Log10 EBV-DNA copies/ml

Viral load detection using dried blood spots in a cohort of HIV-1-infected children in Uganda: outcomes and correlations with clinical and immunological criteria for treatment failure

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Keywords: children, antiretroviral therapy, viral load, dried blood spot

Abstract (250 w)

Background: Delays detecting treatment failure (TF) often occur when combined antiretroviral therapy (cART) is monitored using clinical and immunological criteria. We describe viral load (VL) measurement using dried blood spots (DBS) and explore the accuracy of clinical and immunological criteria for TF in a cohort of HIV-1-infected children.

Methods: 949 HIV-infected children followed with monthly clinical visits and 6 monthly CD4 testing at the Home Care Department, Nsambya Hospital (Uganda), were studied. Whole blood was collected on DBS from 204 children for VL testing and HIV-1 RNA was quantified by real-time PCR using ROCHE COBAS TaqMan System. TF was defined using WHO 2010 criteria.

Results: Overall, 104 (F 53) children on cART for >24 months were included in analyses. At DBS collection, median time from cART initiation was 34.19 months (IQR 32.87); median CD4 count and CD4% were 412 (IQR 439) and 11.8% (IQR 10.1). Thirty (28.8%) children had VL<1000 cp/ml, while 46/104 (44.2%) and 28/104 (26.9%) had VL of 1000-5000 and >5000 cp/ml. Thirteen (12.5%) children had TF according to immunological (1/13) and/or clinical criteria (12/13). Of those, 3 clinical failure had VL <1000 cp/ml, 6 had VL 1000-5000 cp/ml and 3 had VL >5000 cp/ml; one immunological failure had VL >5000 cp/ml. Clinical and immunological criteria poorly predicted VL above either 1000 or 5000 cp/ml whole blood thresholds.

Conclusion: High VL can be detected despite absence of clinical and immunological failure. Using DBS for VL monitoring is feasible and should be implemented to detect TF in children living in low-middle income countries.

Introduction (2035 words)

In most low-middle income countries (LMIC) HIV-1-infected children receiving combination antiretroviral treatment (cART) are monitored using clinical and immunological criteria. Large randomized trials didn't show any additional benefit in terms of disease progression and mortality using routine laboratory monitoring, compare to clinical monitoring alone (1, 2). Additionally, ARROW trial reported high virological response in children, at 3.7 years of follow-up (2). Several other evidences reported high rates of virological suppression in children up to 5-6 years after treatment initiation (3), however delays in detecting treatment failure by clinical and immunological monitoring alone were often described, as a result of a very low sensitivity in predicting virological failure (4, 5).

The 2013 WHO Consolidated Guidelines now recommend VL monitoring as the preferred approach to detect treatment failure in LMIC (6). However, an effective implementation of VL monitoring is very challenging due to the high cost of virological assays, lack of adequate laboratory facilities and appropriate specimens handling (6).

Dried blood spots (DBS) for virological monitoring seems to be a very promising tool in LMIC. Filter papers used for DBS collection could be easily and safely shipped and HIV-1 nucleic acids amplification is usually successful after storing DBS for less than 1-2 weeks at room temperature (7), facilitating specimen collection from rural and very remote areas (8). However, several challenges can affect the accuracy and the use of DBS for VL monitoring within routine clinical practice. Variability in amplification rates has been described for several commercial and in-house methods for RNA extraction and amplification (7, 9). In addition, HIV-1 RNA levels extracted from DBS reflect the contribution of plasma plus cell-associated viral nucleic acids, therefore most studies agree that whole blood samples yield higher levels than plasma samples,

particularly at low HIV-1 RNA plasma levels (9-11). Environmental factors like prolonged storage at high temperatures and humidity may also underestimate VL from DBS, compared to plasma (7, 12).

The aim of this study was to evaluate the accuracy of clinical and immunological criteria based on the detection of VL greater than 1000 and 5000 cp/ml whole blood at DBS in a cohort of HIV-1-infected children in Uganda.

Methods

An observational cohort study was set up among HIV-1-infected children enrolled at the Home Care Department (NHC) of St. Raphael of St. Francis Nsambya Hospital in Kampala, Uganda. All children were evaluated monthly for clinical follow-up and self-reported adherence. Laboratory investigations including full blood count, liver function tests, creatinine and CD4 cell count were carried out every 6 months, or more frequently if clinically required. WHO 2006 guidelines (13) were followed for initiation of Cotrimoxazole prophylaxis, cART, and management of opportunistic infections. In the absence of contraindications, cART was prescribed after counselling and written consent from the caregiver.

Quantitative HIV-1-RNA detection was not part of routine examinations, however in 2010 DBS were collected for quantitative HIV-1 RNA detection in HIV-1-positive children enrolled in a parallel study investigating the Epstein-Barr Virus (M.R. Petrara et al, presented at the 20th Conference on Retroviruses and Opportunistic Infections – CROI 2013, Atlanta, GA, US, 3 to 6 March 2013). Whole blood samples were collected in EDTA-containing tubes by trained nurses; 50 µl of EDTA-blood were spotted onto each of the 5 circles of the Protein Saver TM 903 Card

(Whatman GmbH, Hahnestra, Germany) and left to dry overnight at room temperature. DBS were stored in individual zip-lock bags containing a desiccant and sent after a median time of 43 days (IQR 15-63) to the reference laboratory of Viral Oncology Unit, AIDS Reference Centre (Padova, Italy). Roche Cobas taqman internal QS was included to the samples before the QIAGEN extraction. For each sample, RNA was extracted from two 50 µl of DBS (total whole blood volume of 100 µl) using QIAamp Viral RNA Mini Kit (Qiagen) and eluted using 75 µl of the elution buffer heated at 70°C (High Pure RNA Isolation Kit, Roche Diagnostics). Total HIV-1 RNA (cell-associated and plasma) levels were determined using the Amplicor HIV-1 Monitor Test (Roche Diagnostic Systems), with estimated lower limit of detection of 2.23 $\log_{10}$ (170 cp/ml) HIV-1 RNA copies/ml, according to the spot volume and final RNA elution. Results were expressed as HIV-1 RNA copies/ml whole blood.

Clinical and immunological treatment failure were defined using WHO 2010 criteria (immunological failure: CD4 count of ≤200 cells/mm3 or CD4% ≤10% for child of 2-5 years of age and CD4 count of ≤100 cells/mm3 for child >5 years of age; clinical failure: new WHO stage 3-4 event occurred >24 weeks after cART initiation)(14). DBS collected from children who received cART for >24 weeks were included in the analyses. Gender, age, CD4 cell count/percentage, WHO stage, self-reported adherence and antiretroviral regimens at first cART and at DBS collection were assessed, as were median time from cART initiation to DBS collection and from specimen collection to laboratory analysis. Primary analyses included assessment of proportions of children with clinical failure (CF) and/or immunological failure (IF) and with VL <1000, 1000-5000, and >5000 cp/ml. Differences in demographic and clinical characteristics among children falling into these three groups were assessed using the Kruskal-Wallis test for continuous variables and the Chi-square test for categorical variables. The

predictive value of CF and/or IF for VL greater than either 1000 or 5000 cp/ml was assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) statistics from receiver operating characteristic (ROC) curves. All analyses were carried out using STATA 12 (College Station, TX).

Results

DBS were collected from 204 (M 103, F 101) children with median age of 7.70 years, (IQR 5.42-11.26) at specimen collection. 104 (M 51, F 53) out of 204 (51%) children tested for VL received cART for >24 months and were included in this analysis. Baseline characteristics and univariate analysis are included in Table 1.

At cART initiation median age was 6.44 years (IQR 4.05-8.72). The majority (97/104, 93.27%) of children started an non-nucleoside reverse transcriptase inhibitors (NNRTI)-based cART while 7 (6.73%) started a protease inhibitor (PI)-based regimen. At birth, 2 children were exposed to Nevirapine (NVP) for prevention of mother to child transmission (PMTCT), 35 children were not and for 67 children history of PMTCT was unknown.

Among cART-treated children, DBS were collected at a median of 34.19 months (IQR 19.57-52.44) after cART initiation. VL was detected from DBS stored at ambient temperature for a median time of 49 days (IQR 34-64, i.e. time from blood collection in Uganda to DBS shipment to the laboratory in Italy. At the time of specimen collection, 68 (65.4%) children were still receiving the first cART regimen, while 36 (34.6%) substituted at least one drug (1 drug substitution was reported for 27 children, 2 and 3 substitutions occurred in 8 and 1 children, respectively). Overall, 96/104 children (92.31%) were still receiving an NNRTI-based regimens at specimen collection (65.63% of these on first-line), while 8/104 (7.69%) were on PI-based regimens (62.5% on first-line). Drug substitutions most frequently involved NRTI drugs (16

children substituted 3TC-D4T with AZT-3TC and 16 did the reverse) while 9 children substituted EFV with NVP and 4 NVP with EFV. Reasons for drug substitution were reported only for 7/36 children: 4 children developed AZT-related toxicity, in 2 cases the reason was drug stock out and in one drug interactions. Only one child switched from 3TC-D4T-NVP to AZT-3TC-LPV/r because of clinical failure. Poor adherence (defined as assumption of <95% doses received, as per self-report) was reported only for 1 child out of 104 (0.96%), considering the period of time between first cART and DBS collection.

Thirty (28.8%) children had VL<1000 cp/ml after a median time of 41.63 months (IQR 28.52-54.28) from cART initiation, while VL of 1000-5000 and >5000 cp/ml were detected respectively in 46/104 (44.2%) and 28/104 (26.9%) children at median of 31.31 (IQR 15.47-45.60) and 37.72 (IQR 21.16-55.62) months after cART initiation.

At the time of DBS collection 13 (12.5%) children had treatment failure using immunological and/or clinical criteria, 1 with immunological failure and 12 with clinical failure (6/12 without immunological failure and 6/12 with no concomitant CD4 cell data). Of those, 3 clinical failures had VL (DBS) <1000 cp/ml, 6 had VL (DBS) 1000-5000 cp/ml and 3 clinical and 1 immunological failures had VL (DBS) >5000 cp/ml. All these children were receiving an NNRTI-based regimen and 8/12 had cART substitution before DBS collection. None switched from NNRTI-based to PI-based regimen before DBS collection. At univariate analysis neither initial regimen (p= 0.117, Chi-square test) nor cART regimen at the time of specimen collection (p=0.850, Chi-square test) were associated with virological levels .

The sensitivity and specificity of clinical and/or immunological criteria in detecting VF as per WHO 2010 were very low at either 1000 or 5000 cp/ml whole blood VL thresholds (Table 2).

Discussion

Our findings suggest that a high proportion of children may have detectable viral load after at least 24 weeks of cART in the absence of clinical and immunological failure. Despite an observed low rate of clinical and immunological failure, 71.2% children had VL >1000 cp/ml on DBS specimens, 26.9% with VL>5000 cp/ml. The majority (92.3%) of children were receiving an NNRTI-based therapy (mostly were on AZT-3TC-NVP). Even though a single VL determination cannot define virological failure, VL >5000 cp/ml on whole blood was detected in more than a quarter of children.

Our findings are in contrast with data reported by other studies conducted in LMIC, accounting for virological suppression (<400 cp/ml) rates up to 85% at 48 weeks in children on NNRTI-based regimens, even if previously exposed to NVP (3, 15). In our cohort, a single determination of VL makes hard to discriminate between treatment failure and slow but progressive decrease of viral load from a very high initial viremia, particularly for the VL 1000-5000 cp/ml group. However, DBS were collected at a median time of more than 2,5 years (34.19 months, IQR 19.57-52.44) after cART initiation, therefore slow virological response is unlikely (16, 17).

Adherence to cART may have been overestimated, particularly for older children/adolescents at major risk of poor adherence (18), considering that it was self-reported. Furthermore, lack of data on cART resistances makes difficult to give an exhaustive explanation on the virological outcomes observed in the cohort.

As previously mentioned, HIV-1 RNA levels extracted from DBS reflect both plasma viremia and cell-associated viral nucleic acids. Whole blood viremia may reflect cell-associated residual HIV-1 replication occurring in children receiving cART and with undetectable plasma VL (19).

Studies reported high correlations between viral loads assessed using plasma and DBS when plasma VL was greater than 3000 cp/ml (10, 20), therefore in our cohort VL may be slightly overestimated particularly in <1000 and 1000-5000 cp/ml groups. A potential contamination of HIV-1 DNA cannot be excluded. However, considering the number of cells in the DBS sample (21) and the low percentage of HIV-infected CD4+ cells with positive HIV-1 DNA (22-24), the amount of viral DNA detected in our assay should be consistently low.

It should also be noted that the prolonged storage of DBS at room temperature as a results of operational challenges, including lack of an appropriate facility to freeze and store specimens, may have slightly altered the whole blood viremia. The optimal period of keeping DBS at room temperature it has been reported to be 14 days (7), while our specimens were stored at room temperature for a median of 49 days (IQR 34-64).

Lastly, clinical and immunological failure was assessed retrospectively based on routinely collected data, potentially leading to inaccurate classifications.

In our cohort immunological and clinical criteria as per WHO 2010 guidelines poorly predict the presence of a viral load greater than either 1000 cp/ml or 5000 cp/ml (whole blood) from DBS. The low sensitivity and positive predictive values for immunological and/or clinical failure confirm those reported by literature (4) (L. Barlow-Mosha L et al, presented at the 19th International AIDS Conference 2012, Washington, US, 22 to 27 July 2012). This finding further support the WHO recommendations that VL monitoring should be implemented and used to earlier identify cases of treatment failure (6).

This study provides data on virological outcome in a program setting among children on cART routinely monitored by clinical and immunological criteria alone. We confirm that VL

monitoring using DBS is feasible in LMIC. Studies are needed to improve the accuracy of viral load determination from DBS to increase test accuracy and detect early virological failure.

209 **TABLES AND FIGURES**

210

211 **Table 1.** Univariate analysis of 104 cART experienced HIV-positive children with VL <1000,

212 1000-5000, and > 5000 cp/ml whole blood.

	Overall (n=104)	**VL<1000** (n=30, 28.8%)	**VL 1000-5000** (n=46, 44.2%)	**VL>5000** (n=28, 26.9%)	p-value for trends
Male	51 (49.04%)	14 (46.67%)	19 (41.30%)	18 (64.29%)	0.152
At cART initiation					
Age (years)	6.55 (9.21-3.67)	6.44 (4.05-8.72)	6.81 (4.22-9.77)	5.75 (2.70-8.92)	0.534
CD4 cell count	412 (674-235)	527 (245-732)	341 (254-628)	567 (198-574)	0.849
CD4 %	11.8 (18-7.9)	12.1 (9.07-18.59)	9.82 (7.38-17.40)	10.64 (7.62-16.01)	0.685
WHO stage					0.542
I	10 (9.62%)	3 (10.00%)	3 (6.52%)	4 (14.29%)	
II	66 (63.46%)	21 (70.00%)	28 (60.87%)	17 (60.71%)	
III	26 (25.00%)	5 (16.67%)	15 (32.61%)	6 (21.43%)	
IV	2 (1.92%)	1 (3.33%)	0 (0.00%)	1 (3.57%)	
First cART regimen:					0.117
3TC+D4T+EFV	11 (10.58%)	4 (3.85%)	6 (13.04%)	1 (3.57%)	
3TC+d4T+LPV/r	2 (1.92%)	1 (3.33%)	0 (0%)	1 (3.57%)	
3TC+D4T+NVP	20 (19.23%)	6 (20%)	9 (19.57%)	5 (17.86%)	
AZT+3TC+EFV	25 (24.04%)	8 (26.67%)	12 (26.09%)	5 (17.86%)	
AZT+3TC+LPV/r	5 (4.81%)	1 (3.33%)	3 (6.52%)	1 (3.57%)	
AZT+3TC+NVP	41 (39.42%)	10 (33.33%)	16 (34.78%)	15 (53.57%)	
NNRTI-based first regimen	97 (93.27%)	28 (93.33%)	43 (93.48%)	26 (92.86%)	0.117
PI-based first regimen	7 (6.73%)	2 (6.67%)	3 (6.52%)	2 (7.14%)	0.117
cART regimen at VL testing*					0.687
3TC-D4T-EFV	8 (7.69%)	3 (10%)	5 (10.87%)	0 (0%)	
3TC-D4T-LPV/r	1 (0.96%)	0 (0%)	1 (2.17%)	1 (3.57%)	
3TC-D4T-NVP	26 (25.00%)	2 (6.66%)	11 (23.91%)	10 (35.71%)	
AZT-3TC-EFV	23 (22.12%)	9 (30%)	9 (1.96%)	6 (21.43%)	
AZT-3TC-LPV/r	7 (6.73%)	3 (10%)	2 (4.35%)	1 (3.57%)	
AZT-3TC-NVP	39 (37.50%)	13 (43.33%)	18 (39.13%)	10 (35.71%)	
NNRTI-based regimen at VL testing	96 (92.31%)	27 (90.00%)	43 (93.48%)	26 (92.86%)	0.850
PI-based regimen at VL testing	8 (7.69%)	3 (10.00%)	3 (6.52%)	2 (7.14%)	0.850
Median time from cART initiation to DBS collection (months/IQR)	34.19 (19.57-52.44)	41.63 (28.52-54.28)	31.31 (15.47-45.60)	37.72 (21.16-55.62)	0.255

213

* ART regimen at time of VL testing represents first ART regimen for patients with no drug substitutions and most recent ART regimen prior to VL testing for those with substitutions

Table 2. CD4 and clinical failure to predict virological failure for VL>1000 cp/ml and VL>5000 cp/ml whole blood , respectively) in cART experienced children.

	Virological failure (VL>1000 cp/ml) *				
	AUC	**sensitivity**	**specificity**	**PPV**	**NPV**
CD4≤200 or ≤10% for child 2-5 years and CD4 ≤100 for child >5 years and/or clinical failure	0.5176	13.51	90.00	76.92	29.67
	Virological failure (VL >5000 cp/ml) **				
	AUC	**sensitivity**	**specificity**	**PPV**	**NPV**
CD4≤200 or ≤10% for child 2-5 years and CD4 ≤100 for child >5 years and/or clinical failure	0.5122	14.29	88.16	30.77	73.63

* n=30 for VL <1000 cp/ml; n=74 for VL >=1000 cp/ml whole blood

** n=76 for VL <5000 cp/ml; n=28 for VL >5000 cp/ml whole blood

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