BURDEN OF HIV INFECTION AND HIV-ASSOCIATED MORBIDITY IN ZIMBABWEAN ADOLESCENTS

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I, Rashida Abbas Ferrand, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

My Role:

I designed the questionnaires for each of the studies and the study protocols, with input from Liz Corbett, Frances Cowan and Lisa Langhaug. The laboratory Standard Operating Procedures were written by Praise Musvaire and Doreen Maranda. I trained the research assistants (RA) and the study clinicians (Drs). Barbra Whande and Manuel Singano (RAs) recruited patients into the hospital study; Arnold Mafukidze and Nick Mangeya (Drs) performed clinical examinations and followed up the patients during their hospital stay under my supervision. Barbra Whande and Lucia Munaiwa (RAs) recruited participants into the primary care study; John Matsekete (clinical officer) performed clinical examinations and applied the IMAAI algorithm, under my supervision. I cleaned the data and conducted data analyses for the hospital and primary care studies, with statistical advice from Natasha Larke. I developed the algorithm under the guidance of Helen Weiss. I designed the primary care qualitative study, with input from Liz Corbett. The interview guides were written jointly by Caroline Trigg (who runs a research company called Target) and myself, with input from Webster Mavhu. Caroline and her team conducted the in-depth interviews and the focus group discussions and translated the transcripts from Shona to English. Caroline did the data analysis including developing a coding frame, with input into thematic coding by myself.

Abstract

This thesis concerns the clinical epidemiology of HIV infection in Zimbabwean adolescents. Without treatment, there is a very high risk of death in the early years of life in HIV-infected infants. However, in recent years increasing numbers of adolescents have been presenting to health care services with symptomatic HIV infection and with features suggesting longstanding disease. Population-based surveys in Southern Africa have shown HIV prevalence rates among older children and adolescents to be much higher than would be anticipated if HIV-infants were not surviving early childhood.

The burden and spectrum of HIV-associated morbidity among adolescents was investigated with two studies at secondary and primary care level, respectively. The main finding was of an extremely high prevalence of HIV infection at both levels of the health system, with HIV infection being the single most common cause of hospital admission and death among adolescents. Mother-to-child transmission was the most likely source of HIV infection in the majority, suggesting a substantial epidemic of older survivors of vertical HIV infection. Other countries with severe HIV epidemics may be experiencing a similar trend as their HIV epidemics mature.

The lack of awareness of the possibility of survival to older childhood and adolescence with maternally-acquired, untreated HIV infection results in many missed opportunities for diagnosis, with HIV infection frequently not diagnosed until presentation with a severe HIV-related illness. The median CD4 count in

HIV-infected adolescents in primary care was 350cells/μl compared to a median CD4 count of 151cells/μl among hospitalised adolescents, suggesting that HIV testing in primary care identifies HIV-infected adolescents at an earlier stage of infection. Provider-initiated HIV testing and counselling in primary care was highly acceptable to adolescents and guardians.

Provision of care has been adversely affected by under-appreciation of the numbers of surviving adolescents living with HIV, and the special needs of this age-group have not been distinguished from those of younger children. Young people who have acquired HIV perinatally are stigmatised by society who assume they must have acquired it through "bad" behaviour themselves, since it is not widely appreciated that long-term survival following vertical infection is possible. Immediate priorities are earlier diagnosis of HIV infection and improved management of HIV-infected adolescents. Possible areas of intervention are discussed in the final chapter. Similar studies are needed in neighbouring countries to investigate the generalisability of these findings.

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Acronyms

AIDS	Acquired immune deficiency syndrome
ANC	Antenatal care
APC	Acute primary care
ART	Antiretroviral therapy
BSI	Bloodstream infection
CD4	CD4+ lymphocyte
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CMV	Cytomegalovirus
CrAg	Cryptococcal antigen
CSF	Cerebrospinal fluid
CT	Counselling and testing
CXR	Chest radiograph
EBV	Epstein Barr virus
ELISA	Enzyme-linked immunosorbent assay
ENT	Ear, nose and throat
FEV1	Forced expiratory volume in 1 second
FiO_2	Fraction of inspired oxygen
GI	Gastrointestinal
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papilloma virus
HrCT	High resolution computed tomography scan
HSV-2	Herpes simplex virus-2
IMAAI	Integrated Management of Adult and Adolescent Illness
IGF-1	Insulin-like growth factor-1
IQR	Inter-quartile range
LIP	Lymphoid interstitial pneumonitis
LJ	Lowenstein-Jensen media
LR	Likelihood ratio
LTS	Long-term survivors
LV	Left ventricular
MTCT	Mother-to-child transmission
MUAC	Mid-upper arm circumference
NCHS	National Center for Health Statistics
NNT	Number needed to test
NPV	Negative predictive value
NYHA	New York Heart Association
OR	Odds ratio
PCP	Pneumocystis pneumonia

PE	Progressive encephalopathy
PEFR	Peak expiratory flow rate
PITC	Provider-initiated HIV testing and counselling
PMTCT	Prevention of mother-to-child transmission
PPV	Positive predictive value
ROC	Receiver operator characteristics
SIV	Simian immunodeficiency virus
SF	Shortening fraction
STI	Sexually transmitted infection
TB	Tuberculosis
TNF	Tumour necrosis factor
UNAIDS	United Nations Joint Programme on HIV/AIDS
WHO	World Health Organisation

Preface

I'd like to thank everyone who has helped me with the research and the writing of this thesis. My supervisor, Liz Corbett, has supported me in every sense of the word since I met her. She has always encouraged me to persevere and aim high and I feel privileged to have had the opportunity to learn with her. My second supervisor, Diana Gibb, filled many holes in my knowledge, helped me ask the right questions and introduced me to the world of paediatrics.

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1 Introduction

1.1 Background

The HIV pandemic has been established for nearly thirty years and approximately 33 million people worldwide were estimated to be living with HIV infection in 2008.[1] HIV has had a dramatic demographic impact in countries with generalised epidemics, where adult mortality has doubled or trebled, life expectancy has fallen to levels typical of the 1950s and up to one in four children have become orphaned as a result of parents dying of HIV/AIDS.[2-3] Southern Africa is the worst affected region, with HIV prevalence rates exceeding 15% in most countries.[1] Adult HIV prevalence has been at these extremely high rates for the last 10 to 15 years, with even higher rates among pregnant women. Without interventions, the risk of mother-to-child transmission (MTCT) of HIV is 25-35%, and thus the adult epidemic has been accompanied by a regional epidemic of vertically-acquired HIV, with substantial impact on child mortality.[1 4]

Unlike adults, where median time from HIV infection to development of AIDS is about 10 years, the natural history of HIV in infants is dominated by a very high risk of rapid progression and the probability of survival has been reported from prospective studies of vertically HIV-infected babies to be about 50% by age two years in African cohorts.[5-7] Survival to older childhood without antiretroviral therapy (ART) among HIV-infected infants was thus considered exceptional.

Exact survival probabilities for infants infected by HIV through MTCT are ill-defined beyond five years of age as there are no direct cohort data, and in developed countries the natural history of HIV has been modified by availability of ART. Emerging epidemiological data, however, suggests that up to a third of HIV-infected infants do not have rapidly progressive disease, and median survival in this subgroup of slow progressors is thought to be at least eight years and potentially up to sixteen years.[8-9] As such, overall survival to adolescence for all vertically infected infants is likely to be in the range of 10 to 20% of those infected. Thus an emerging epidemic of HIV-infected long-term survivors (LTS) of vertical infection is anticipated in countries with severe longstanding HIV epidemics during the coming decade, with profound implications for adolescent health.

Population-based surveys in Southern Africa that have included older children have consistently reported high HIV prevalence rates of between 2-6% among older children and young adolescents.[10-13] Of note, young women are at disproportionately higher risk of HIV infection through sexual transmission than young men, but these surveys showed no difference in HIV prevalence by sex in this age-group, implying a non-sexual route of HIV acquisition.[14] Studies from other countries in the region confirm that considerable numbers of HIV-infected older children and adolescents with features suggestive of long-standing HIV infection are presenting for care.[15-16]

Diagnosis of HIV infection is a prerequisite for accessing care and treatment. Global coverage of HIV testing and counselling remains low, despite improved

access to voluntary counselling and testing (CT) services. With increased availability of ART internationally, there has been a shift from client-initiated to provider-initiated approaches to HIV testing. World Health Organisation (WHO) guidelines advise health care providers in countries with generalised HIV epidemics to offer HIV testing to anyone attending any health facility as a standard part of health care (provider-initiated testing and counselling).[17] Children, however, face additional barriers in accessing HIV diagnosis. In children younger than 18 months, diagnosis of HIV requires expensive virological tests which are not always readily available in many parts of the world where the risk of HIV in infancy is highest.[18] In adolescents, access to HIV diagnosis is difficult because of paucity of diagnostic services aimed at this age-group and the perception that only sexually active adolescents are at risk of HIV infection.

1.2 Project starting points

The observation that adolescents were increasingly presenting to health care services with symptomatic HIV infection and features of longstanding disease, was the initial observation that led to the current work.[15] The findings from population-based surveys in Southern Africa, which showed higher than anticipated HIV prevalence rates among adolescents, raised the possibility that long-term survival of children following MTCT might be a phenomenon of greater public health significance than had previously been appreciated.[10-13] The identification of a population with slow progressing infection would provide the potential to advance the understanding of the mechanisms involved

in the control of HIV infection through studies of the immunological and virological aspects of HIV infection.

Zimbabwe has the 5th highest adult HIV prevalence in the world and less than half of HIV-infected pregnant women access interventions for prevention of MTCT, so that vertical infection continues to occur.[1] [18] Zimbabwe has experienced an otherwise comparable HIV epidemic, but with much earlier onset than neighbouring countries; HIV prevalence rates peaked at 29% as far back as 1997. ART for prevention of mother-to-child transmission (PMTCT) started to become available only around 2004.[19] Thus Zimbabwe is likely to already have a relatively advanced epidemic of adolescent LTS.

HIV diagnosis and treatment initiatives in Southern Africa have mainly been aimed at adults, and more recently at young children. Adolescents have been a priority target group for prevention efforts, but there has been little provision of HIV testing or treatment services for this age-group. Delay in diagnosis may compromise treatment outcomes and contribute to unwitting onward HIV transmission. Identification of strategies to facilitate HIV testing and entry into care in adolescents will help focus attention on the needs of this age-group that have so far not been considered.

1.3 Aims and objectives

The overall aims were to investigate the clinical epidemiology of HIV infection among adolescents in Zimbabwe, a country with a long-standing, severe HIV

epidemic, and to explore how earlier diagnosis and entry into HIV care in this age-group can best be achieved.

The specific objectives were to investigate:

1. the prevalence of HIV infection among adolescents presenting acutely at different levels of health care services
2. the relative importance of HIV infection as a cause of morbidity and mortality among adolescents
3. the likely modes of HIV acquisition among adolescents
4. the most common clinical consequences of late diagnosis of vertically-acquired HIV infection
5. the uptake and acceptability of provider-initiated testing and counselling among adolescents in primary health care
6. an approach to creating a diagnostic algorithm for HIV infection and evaluating its potential performance in low and high HIV prevalence settings

1.4 Outline of thesis

In Chapter 1, the starting point that led up to the project, the aims and objectives of the research and the plan of the thesis are outlined. In Chapter 2, a review of published literature relating to the epidemiology, natural history and clinical aspects of HIV infection in adults and children is presented. Studies relating to long term survival in children following vertical HIV infection and morbidity in HIV-infected older children in the pre-ART era are also reviewed.

Methods that are common to more than one study are described in Chapter 3. The methods specific to individual studies are described in the respective chapters in which the study is described.

Chapters 4 and 5 refer to a study conducted in the two public hospitals of Harare. In Chapter 4, the prevalence of HIV infection and the causes of admission among 301 hospitalised adolescents are reported. In Chapter 5, the likely mode of HIV acquisition among these hospitalised adolescents is explored.

Chapters 6 to 8 refer to studies conducted in two primary health care polyclinics in Harare among 594 adolescents, 506 attending for acute primary care (APC) and 88 attending for antenatal care (ANC). In Chapter 6 the burden of undiagnosed HIV infection among adolescents presenting acutely for primary care is reported and the mode of HIV transmission is further explored by comparing prevalence of characteristics consistent with vertical transmission among APC attendees with sexually active adolescents (i.e. adolescents attending for ANC). The perception of risk of vertically-acquired HIV infection and the acceptability of provider-initiated HIV testing among adolescents and their guardians was investigated through a qualitative study and is reported in Chapter 7. In Chapter 8 a simple algorithm using clinical and demographic data that could be used by health care workers at primary care level to identify adolescents at risk of being HIV-infected in low and high HIV prevalence settings is described.

The main conclusions, recommendations for further research and potential intervention strategies are discussed in Chapter 9.

2 Literature review

2.1 Human Immunodeficiency Virus infection

2.1.1 Origin of the HIV pandemic

HIV is a retrovirus that primarily infects monocytes and CD4+ T-helper lymphocytes (CD4 cells), resulting in a lifelong infection characterised by progressive immunodeficiency and the occurrence of opportunistic infections.[20] The HIV epidemic was identified in 1981 in the United States when cases of unusual tumours and opportunistic infections (Kaposi's sarcoma and *Pneumocystis* pneumonia [PCP]) in previously well homosexual men were recognised as being due to a new form of acquired immune deficiency syndrome or AIDS, associated with a selective depletion of CD4 cells. Epidemiological investigations indicated that the disease was sexually transmitted and could also be acquired from administration of infected blood products and exposure to blood-contaminated needles.[21-22] HIV was identified as being the cause of AIDS in 1983, and the development of a serological test to identify HIV infection in 1984 was followed by the discovery that AIDS was not only a western disease but also occurring in the heterosexual population of central African countries.[23] In Kinshasa, Democratic Republic of Congo (DRC), the annual number of cases of Kaposi's sarcoma diagnosed in a large public sector hospital tripled from 1970 to 1984, and epidemic increases in the wasting syndrome (termed "Slim disease"), a sentinel marker of AIDS, were noted in the late 1970s in Uganda and DRC.[24-25] HIV is now recognised to have originated in Africa, which remains the worst affected continent.

HIV arose through cross-species transmission of the related simian immunodeficiency virus (SIV), which is widespread in old world non-human primates.[26] The virus was probably first introduced to human in the 1950s, and the hypothesis that Central Africa was the origin of the HIV epidemic is consistent with the retrospective identification of an HIV positive man living in Kinshasa in 1959.[27] The existence of two distinct HIV species, HIV-1 and HIV-2, was recognised in 1985. Molecular phylogeny studies show that HIV-1 evolved from a strain of SIV in a particular sub-species of chimpanzees (*Pan troglodytes troglodytes*), with each of the three groups of HIV-1, M, N and O, arising from geographically distinct chimpanzee populations.[28-29] HIV-2 infection is most commonly found in West Africa and originated from a separate monkey species, the sooty mangabey (*Cercocebus atys*).[26] More recently, a new HIV-1 variant called Group P, distinct from the three main groups of HIV-1 and thought to be derived from SIV in gorillas, has been identified, further supporting the hypothesis that zoonotic SIV transmission to man occurs periodically in this part of Africa.[30] Simian studies have confirmed that, as in HIV, both sexual (horizontal) and perinatal (mother-to-child) transmission are the main modes of transmission of SIV.[31]

The ability of HIV to evolve rapidly as a result of error-prone transcription and high rates of mutation per replicative cycle and the selection pressure by the human population with their own HLA-restricted cellular immune response, has resulted in exceptional genetic diversity and led to selection of viral adaptations to facilitate human-to-human transmission over a relatively short period of time.[32] Notably, Group M HIV-1, which has given rise to the global

pandemic, has numerous clades or subtypes A-K as well as circulating recombinant forms, and has relatively high person-to-person infectivity. [33-34] The resulting pandemic has so far infected an estimated 60 million people and caused over 25 million deaths.

2.1.2 Pathogenesis of HIV infection

Primary infection via the mucosal or parenteral route results in rapid viral replication which initially overwhelms the body's immune response resulting in extremely high viral loads in blood. Viral replication is partially controlled to a quasi-steady lower "viral set-point".[35] The level of the set-point is predictive of the rate of subsequent disease progression and response to treatment with higher levels having a worse prognosis.[36] The picture in the blood is however, a poor reflection of the much higher activity in lymphoid tissue, with the gut-associated lymphoid tissue being the most active site of HIV replication.[37-38] HIV replication results in a substantial depletion of CD4 cells in lymphoid tissue.[39] The immune response to HIV is multifaceted: cytotoxic (CD8) T-lymphocytes play an important role in controlling infection but are unable to eliminate the infection;[40] the humoral response allows infected individuals to be identified with antibody detection methods and also plays a role in controlling primary infection.[41-42]

The replication of HIV involves an enzyme common to all retroviruses called reverse transcriptase, that catalyses the synthesis of a DNA copy of the viral RNA. Being an RNA virus with a reverse transcription step, HIV is subject to a high mutation rate during replication. Mutants that are recognised with a low

affinity by the immune response have a replication advantage and selectively replace the initial infecting strain. Eventually the capacity for immune regeneration is exhausted and immune function against other pathogens becomes increasingly compromised as a consequence of CD4 cell depletion.[40] Without the intervention of antiretroviral drugs, progressive immunosuppression leads to an increasing frequency of opportunistic infections, ultimately resulting in death.[20]

HIV infection however, is characterised not only by immune suppression but also by chronic immune activation.[43] Infection is accompanied by persistent CD8 T-lymphocyte stimulation and increased pro-inflammatory cytokine production and lymphocyte turnover, leading to accelerated immunological senescence.[44-45] Long-term disease can result in fibrosis and scarring of supporting lymphoid tissue which is unable to adequately generate CD4 cells despite viral suppression.[46] Chronic stimulation of the immune system may be a response to ongoing release of HIV antigens from the latent pool of infected cells and/or to bacterial antigens from the gut that are able to translocate as a result of damage to mucosal lymphoid tissue during early infection.[43 47] Even in individuals where HIV levels in plasma are "optimally" suppressed by ART, persistent immune activation contributes to residual immune dysfunction and may be a factor in the pathogenesis of non-AIDS disease such as cardiovascular or lung disease.[48]

2.1.3 HIV Epidemiology in Africa

HIV-related illnesses are projected to continue as leading global causes of premature mortality in the next two decades. Sub-Saharan Africa is by far the

worst affected region in the world; 70% of global incident HIV infections and deaths attributable to HIV worldwide occurred in this region in 2008.[1]

Unlike industrialised countries, the majority of Africans living with HIV are women, and HIV prevalence is typically much higher among young women than young men. Age-trends suggest very high HIV incidence in the late teens and early 20s for women, with peak incidence in men occurring in the late 20s and early 30s. These differences are likely to reflect younger age at sexual debut for women and their greater exposure to older partners who have had longer to acquire HIV infection themselves. There may also be greater biological susceptibility to acquiring HIV following exposure to an infected partner among women.[14] The prevalence of HIV among women in child-bearing age reaches more than 15% in some parts of Southern Africa, with major implications for mother-to-child HIV transmission.[1]

The region's epidemics vary significantly in scale: Southern Africa is disproportionately affected with adult HIV prevalence rates of 15% or more in most countries, in contrast to less severe epidemics in Western Africa and the Sahel countries.[49] In Eastern and Central Africa HIV prevalence is intermediate, typically in the 5-10% range. Studies addressing the question of why the HIV epidemic is so much worse in Southern Africa have identified sub-regional differences in the following critical aspects:

i) A greater prevalence of concurrent partnerships in Southern Africa than in East and West Africa which allows multiple partners to be

infected, particularly during the period of high infectiousness that characterises acute HIV infection, when HIV is introduced into any given sexual network;[1 50]

ii) A much higher prevalence of a second sexually transmitted infection (Herpes Simplex Virus-2 [HSV-2]) than in West Africa that greatly increases individual susceptibility to acquiring HIV infection;[51-52]

iii) A lower prevalence of male circumcision in Southern Africa. Male circumcision has been shown to be highly protective of HIV transmission to men and, when widely practised, may limit the magnitude of the HIV epidemic by indirectly protecting women too.[53-54]

Following introduction of HIV into a previously unexposed population, there is first a period of rising prevalence with relatively little morbidity and mortality, as most infected individuals have been relatively recently infected. This is followed by a phase of rising morbidity and mortality, typically accompanied by stabilisation of HIV prevalence rates, with the proportion of infections among people in stable low-risk partnerships increasing. Eventually, HIV prevalence begins to decline, with falling prevalence now being reported from most of the worst affected countries in sub-Saharan Africa.[1]

Both behavioural change and rising mortality among infected persons as the HIV epidemic matures are likely to have contributed to true declines in HIV prevalence in some countries.[55] However, there have also been a number of fundamental changes to the methodologies used for producing national

estimates, contributing to apparent declines especially in the highest HIV prevalence countries. The major changes have been greater use of population-based national HIV prevalence surveys with less reliance on antenatal surveys, upward revision of the assumed median survival from HIV infection to death in untreated individuals from 8 to 11 years and a downward adjustment (×0.8) from antenatal clinic HIV prevalence estimates when these are used to extrapolate HIV prevalence in the general population in urban areas, based on studies that have shown a consistent trend towards overestimation of HIV in the general population without this adjustment.[56-57] Expanded surveillance systems have yielded more representative data and national population-based HIV prevalence surveys, adjusted for non-response and other biases, have shown considerably lower HIV prevalence rates than extrapolations obtained from sentinel site surveillance.[58]

Since 2003, there has been an unprecedented increase in access to HIV treatment in resource-limited settings, which is helping to drive a decline in HIV-associated mortality. It is likely that the effect of improved access to treatment will be an increase in HIV prevalence rates in Africa, as has occurred in high-income countries where ART has been available for much longer periods.[59]

2.2 Paediatric HIV infection

Since its identification in 1981, HIV has spread rapidly through sub-Saharan Africa mainly through heterosexual transmission. HIV prevalence rates have

been particularly high among pregnant women and the adult HIV epidemic has thus been followed by an HIV epidemic among children, acquired predominantly through MTCT. The global epidemiology of paediatric HIV mirrors that of women: 90% of paediatric infections occur in sub-Saharan Africa and the improvements in child health outcomes observed in the 1970s and 1980s were reversed during the 1990s with child mortality rates being one third to two thirds higher than they would have been in the absence of HIV/AIDS.[60]

2.2.1 Mother-to-child (vertical) HIV transmission

MTCT can occur *in utero*, during delivery and postnatally through breast-feeding. Without interventions, the rate of MTCT ranges from 25-35%, with differences in rates between populations due to the additional risk through breast-feeding.[4 61] Transmission during late pregnancy and the intrapartum period contributes relatively more to the overall rate of vertical transmission than the early intrauterine period.[61-62] The risk of MTCT is increased by high maternal plasma HIV viral load, advanced immunosuppression in the mother, vaginal delivery and conditions during labour that increase the risk of the infant coming into contact with contaminated fluids in the maternal genital tract (prolonged labour, premature rupture of membranes).[63]

Breastfeeding approximately doubles the risk of vertical HIV transmission in populations where breast-feeding is the norm compared with areas where breastfeeding is uncommon.[63-64] Transmission can occur at any point during the breastfeeding period, although there is evidence that this is highest in the

first three months postpartum.[65-66] It is not possible to confidently distinguish perinatal from early breast-feeding acquisition of HIV, but after the first three months the risk of transmission appears to be relatively constant throughout the breast-feeding period so that the longer the duration of breast-feeding the greater the risk of late postnatal transmission, with a cumulative transmission risk of 12-16% at 24 months.[65-66] Risk factors associated with an increased risk of breast milk transmission include sub-clinical and clinical mastitis, high viral load in breast milk and seroconversion of the mother during breastfeeding, reflecting increased infectiousness during early HIV infection.[67-68] Mixed feeding (adding other foods or fluids to the infant's diet in addition to breast milk) carries an increased risk compared to exclusive breast-feeding, possibly because exposure to pathogens in non-sterile foods and fluids results in intestinal inflammation and increased intestinal epithelial permeability.[69-72]

2.2.2 Prevention of mother-to-child HIV transmission

Recommended interventions to reduce the risk of MTCT include ART during pregnancy, labour and in the early neonatal period, elective Caesarean section delivery in women with detectable viraemia, and avoidance of breast-feeding. The rapid widespread implementation of these interventions in industrialised countries has reduced MTCT rates to 2% or less.[73]

The first trial to demonstrate the efficacy of ART in reducing mother-to-child HIV transmission was the ACTG 076 randomised, placebo-controlled trial in 1994 which showed a 67% reduction in MTCT with a regimen of antepartum and intrapartum zidovudine for the mother and six weeks of zidovudine for the

infant.[74] Initially it was suggested that ART during pregnancy was too complex and costly for low-resource settings, and possibly also more toxic due to higher prevalence of nutritional deficiencies and anaemia. Alternative strategies for prevention of mother-to-child HIV transmission (PMTCT) with shorter course, inexpensive single ART drug regimens were subsequently developed.[75-76] A single dose of nevirapine for the mother during labour and for the newborn in the first 48 hours decreased transmission by nearly 50% and was recommended as a PMTCT strategy for resource-limited settings.[77] However, development of resistance to nevirapine is a major concern with the use of monotherapy, as this may compromise the subsequent maternal and infant response to nevirapine-based ART.[78-80] Guidelines have also recommended exclusive breastfeeding in resource-limited settings as use of breast milk substitutes was not culturally acceptable or affordable, and mortality risk from replacement feeding exceeds the risk of MTCT through breast-feeding, due to lack of access to safe water and poor hygiene.[77 81-82] Using simple ART regimens and/or exclusive breastfeeding, it is possible to reduce MTCT rates to about 12% at 6 weeks of age.[61 75-76]

2.2.3 Challenges in controlling the paediatric HIV epidemic

HIV prevalence rates are disproportionately high among young women of reproductive age and there is a high incidence of HIV seroconversion during pregnancy and the postpartum period.[83] In one study in South Africa HIV incidence was 10.7% per 100 pregnant patient years in women who had tested HIV-negative earlier in pregnancy.[84] There is also an unmet need for family

planning, which results in high rates of poorly planned and unintended pregnancies.[85]

Despite well-evidenced biomedical interventions to reduce the risk of MTCT, there are an estimated 1000 new paediatric infections daily, the vast majority acquired through MTCT in sub-Saharan Africa.[1] Unlike the rapid widespread implementation from the mid-1990s in developed countries, implementation and scaling up of PMTCT programs in sub-Saharan Africa has been hampered by inadequate funding, weak health systems, and socio-cultural and institutional barriers. There is a relatively low HIV testing rate during pregnancy in sub-Saharan Africa and only 45% of pregnant women and 30% of exposed infants received ART for PMTCT in 2008.[18]

Although trends are now changing towards greater effort to provide more holistic care, PMTCT was initially conceptualised and rolled out as a simple intervention, accomplished during one antenatal visit. In high-resource settings Highly Active ART (HAART) has gradually become the mainstay for PMTCT, but in Africa attention remained focused on finding short-course, cheap, easy regimens. ART was delivered as an intervention to reduce MTCT transmission risk, funded and supported by HIV prevention rather than treatment programmes that often failed to concurrently identify and treat women who met ART eligibility criteria from the perspective of their own health.[86] HIV testing in the antenatal setting has not been routinely linked to CD4 cell count testing, unlike other HIV testing programs. PMTCT effectively ends at delivery and there has been poor linkage between PMTCT programs and maternal and

child health services. This has resulted in limited attention being paid to infant follow-up and postnatal interventions such as early infant diagnostic testing and cotrimoxazole prophylaxis.[87]

There remains an ongoing risk of transmission through breast-feeding, with most HIV-infected women breastfeeding their infants for prolonged periods of time.[88-89] Clinical trials clearly indicate the efficacy of ART prophylaxis during breastfeeding in reducing the risk of MTCT and guidelines are currently being modified to provide ART for the infant during the breastfeeding period, as well as lowering the CD4 count threshold of providing ART for pregnant women from 200 to 350cells/µl, as national ART programmes expand.[90-92]

2.3 Natural history of HIV infection

2.3.1 Adults

The first clinical signs and symptoms of HIV infection occur at the time of the initial immune response to HIV infection ("seroconversion illness"), which has variable severity but usually consists of a self-limiting infectious mononucleosis-like syndrome, with symptoms starting about six weeks after the primary infection. Primary HIV infection is followed by a variable period of clinical latency, usually lasting several years, during which most individuals remain well despite sustained viral replication at a high rate, a rapid turnover of CD4 cells and continued inflammation.[20] During this stage the CD4 count declines at a variable, but usually slow and steady, rate of approximately 40-60cells/µl per year. Immune function becomes progressively compromised as a

consequence of CD4 cell depletion, leading to an increasing frequency of opportunistic infections.[20]

Most studies have reported similar HIV disease progression rates in Africa to those reported in developed countries before the use of ART, with a median time from seroconversion to AIDS of about ten years.[5 93-94] However, survival after diagnosis appears to be shorter in African countries and this may be partly due to more advanced immunodeficiency at diagnosis of HIV infection in Africa.[95-96] The risk of death is highest in those individuals with a CD4 count of less than 200cells/μl, with a steep rise in risk among those with a CD4 count of less than 50cells/μl.[95 97]

The spectrum of infections during the course of HIV infection varies with the degree of immunosuppression. In the early stages, increased susceptibility to infection is limited to high grade pathogens such as *Mycobacterium tuberculosis*, whereas individuals with advanced immunosuppression (particularly when the CD4 count drops below 200cells/μl) are at risk of life-threatening disease following infection with low grade pathogens such as *Pneumocystis jirovecii*. The extent of exposure to potential pathogens also influences the pattern of HIV-associated disease, so that the incidence and relative frequency of the common opportunistic pathogens differs markedly between different geographical regions and also by age (as discussed below).[98-101] Thus, the main causes of HIV-associated morbidity and mortality should ideally be investigated for each distinct region and subpopulation in which prophylaxis against opportunistic infections is being considered.

The two most frequent opportunistic infections of developed countries, PCP and Kaposi's sarcoma, are less prevalent among African hospitalised patients.[98 102] Clinical and autopsy studies show that the commonest causes of HIV-associated mortality in Africans are tuberculosis (TB), wasting syndrome, pneumonia, cryptococcal meningitis, and bloodstream infections (BSI).[94 103-108] The most frequent isolates in patients with BSI are *M.tuberculosis, Streptococcus pneumoniae*, *Salmonella spp* and *Cryptococcus neoformans*.[105 109-111] Minor mucocutaneous problems, respiratory tract infections, severe bacterial infections, chronic fever and chronic diarrhoea are common before patients develop AIDS.[94 112] However, prevalence of specific conditions are affected by referral patterns, the study population (outpatients versus hospitalised patients), diagnostic methods used and the diseases reported.[98]

2.3.2 Infants and children

Infants who acquire HIV infection from their mothers develop immunodeficiency much faster than adults. In Europe and the US, approximately 20-25% of HIV-infected children progressed to AIDS or died in infancy in the pre-ART era while the median incubation period in adults is about 10 years, with only a minority developing AIDS in the first three years after infection.[113-114] This may be explained by the immaturity of the immune system at the time of HIV acquisition in infants (as discussed in section 2.4.1). In general, an early onset of HIV-related conditions is associated with an increased risk of death.[115-116] The most frequent AIDS-defining conditions are PCP which has a peak incidence between three and six months of age, cytomegalovirus (CMV) infection, and progressive encephalopathy (PE)

associated with loss of brain growth, developmental delay and motor abnormalities.[117-122] HIV-infected infants are exposed to primary infection with opportunistic organisms, which differs from the reactivation of such infections that occurs in adults. These manifestations may also be a result of the newborn macrophage system being particularly susceptible to HIV infection (and to other pathogens), leading to dissemination to the central nervous system and to other sites such as the lung, when infected monocytes migrate as tissue macrophages.[123-124]

Failure to thrive, oral candidiasis, hepatosplenomegaly, unexplained persistent fever and diarrhoea are also common findings.[116 119 125-130] HIV-infected children are less likely than adults to develop opportunistic infections such as toxoplasmosis, TB, cryptococcosis, and histoplasmosis but more likely to develop serious bacterial infections.[119 131] Pneumonia, meningitis and septicaemia are especially common, tend to recur and have higher rates of treatment failure.[132] The main causative agents are similar to the main causes of infectious disease in HIV-uninfected children namely *Streptococcus pneumoniae, Salmonella spp* and *Haemophilus influenzae* but morbidity and case-fatality rates are higher.[132] Malnutrition and hospitalisations for serious bacterial infections predict mortality independently of immunosuppression.[16]

The overall rate of disease progression in HIV-infected infants has been thought to be more rapid in Africa. Table 2.1 summarises studies that investigated mortality rates in children by infant and maternal HIV status. As discussed above, in European and American cohorts approximately 25% of HIV-infected

infants progress to AIDS or death in the first years of life, while median survival is about two years in most African cohorts.[7] This may be due to more intense exposure to pathogens such as malaria and enteric pathogens, and a background of undernutrition. Outside these relatively small observational studies there is a lack of data on mortality rates of HIV-infected children at population level. A major constraint has been the difficulty in confirming HIV status among infants, as passively acquired maternal anti-HIV antibodies persist for up to eighteen months, precluding the use of antibody testing methods. Thus, prevalence estimates of HIV infection in babies have been obtained indirectly from estimates of adult HIV prevalence, using assumptions relating to fertility and HIV transmission rates and mortality in women of childbearing age.[133] Maternal death is an additional independent risk factor for child mortality, and there is evidence that maternal HIV infection also impacts on child mortality regardless of the HIV status of the child.[134-136] This is likely to be due to a combination of poor care given by a sick mother, poverty due to parental illness, increased exposure to infections from immunocompromised parents, and also the potential for subtle immune dysfunction in HIV-exposed but uninfected infants that may arise from suboptimal maternal imprinting of the neonatal immune system.

Table 2.1 Mortality rates in HIV-infected children (adapted from Little et al[137])

Study location and year	First author	Study design	N	Median follow-up	Age at mortality estimation	Child Mortality (%)			Comments
						M+ I+	M+ I-	M- I-	
USA, 1996[138]	Barnhart	Cohort	2148	Not stated	4 years	16%	ND	ND	
Europe, 1997[113]	Blanche	Cohort	392	37.4 months	6 years	26%	ND	ND	Mainly untreated
South Africa, 1998[126]	Bobat	Cohort	48	28.5 months (mean)	12 months 24 months	27.1% 35.4%	ND	ND	
Haiti, 1999[127]	Jean	Cohort	169	15 months	15 months	64%	ND	6%	Routine immunisation; No PMTCT
Malawi, 1999[139]	Taha	Observational	702	16.8 months	12 months 24 months	10.4% 33.9%	ND 4.6%	3.6% 3.6%	Routine immunisation; Follow-up after first year of life
Rwanda, 1999[140]	Spira	Cohort	401	27.4 months (M+ I+) 51.5 months (M+ I-) 51.4 months (M- I-)	12 months 24 months 5 years	26% 45% 62%	ND ND 4%	ND ND 4%	Routine immunisation
Europe, 2001[141]	Gray	Cohort	55	Not stated	12 months 5 years 10 years	26% 40% 46%	ND	ND	Untreated arm
Kenya, 2001[142]	Mbori-Ngacha	Intervention (formula feeding)	197	15 months	24 months	46%	8.1%	ND	Breast-fed arm
West Africa, 2001[143]	Dabis	Intervention (Zidovudine)	205	6 months	12 months 18 months	47.5% 51%	ND 7.8%	ND ND	Placebo arm. Mostly breast fed
Sub-Saharan Africa, 2004[7]	Newell	Meta-analysis of seven trials	3468	15.5 months	12 months 24 months	35.2% 52.5%	4.9% 7.6%	ND ND	Interventions: vitamin A, birth canal cleansing, peripartum ART only
Uganda, 2006[135]	Brahmbhatt	Observational	4604	Not stated	12 months 18 months 24 months	30.9% 45.2% 54.1%	9.9% 13.1% 16.6%	9.1% 11.3% 12.8%	

M+ = mother HIV+ve; M- = mother HIV-ve; I+ = infant HIV+ve; I- = infant HIV-ve; ND = Not determined

2.4 Long-term survival following untreated vertical HIV infection

Two patterns of disease progression in infants, rapid and slow progression, have been described which in part reflect timing of HIV infection. Approximately 20-30% of infected infants are rapid progressors and mortality approaches 100% by two years of age in this group. In the remainder, progression is much slower, with a substantial minority expected to survive childhood even without diagnosis and treatment.[118 122 130 144-148]

2.4.1 Timing of HIV infection relative to birth and course of infection

Several lines of evidence suggest that timing of infection is critical in influencing the subsequent course of disease, with *in utero* or intrapartum HIV transmission having a worse prognosis than later postnatal transmission. The peak viral load and the viral set-point are significantly higher, a poor prognostic sign, in infants infected during birth or the first few weeks of life (early infection) than in those infected after 3 months of age.[149-153] Furthermore, epidemiological studies have consistently shown at least a two-fold higher risk of progression to AIDS or death by 12 months of age in those with early infection compared to those infected later, even after controlling for later time of infection and duration of follow-up while infected.[7 154-158]

A number of congenital and perinatal infections, such as CMV, *Toxoplasma gondii* and rubella virus, result in more severe disease when these agents are acquired early during gestation. It is well established through experimental studies in animals that the timing of exposure to pathogens and antigens

relative to birth can have a critical impact on the nature and effectiveness of the subsequent immune response, with infections *in utero* and the first few weeks of life tending to elicit a suboptimal and/or dysfunctional immune response. For example, domestic cats have a naturally occurring retrovirus (Feline Immunodeficiency Virus [FIV]) that, like HIV, can be transmitted vertically *in utero*, intrapartum or through breastmilk.[159] Depending on the timing of infection, cats can either develop a chronic infection characterised by high level viraemia, and a high burden of morbidity and mortality from viral-associated cancers and a syndrome of degenerative and infectious disease, or a self-limiting acute infection that can occasionally be fatal but is more typically accompanied by an effective immune response that leads to near-complete eradication with undetectable viral loads and a strong and persistent cytotoxic T-cell response.[160-162]

The immune system of the foetus is adapted towards self-tolerance in order to avoid destructive autoimmune responses.[163] Although T-cells capable of activation are detectable in the foetus as early as 20 weeks of pregnancy, there is polarization of T-cells towards a dominance of T-helper cell-2 (Th-2) and regulatory T-cell (T-reg) responses which suppress a broad spectrum of immune responses.[164-167] The neonatal immune system is antigenically inexperienced and characterised by a profound T-cell maturational defect with ineffective cytokine production and cytotoxic T-cell responses, slower interaction with B-cells and delayed rejection of foreign antigens.[147 163 168-169] Fundamental changes to the immune system occur in the first few weeks of life (e.g. down-regulation of T-reg and Th-2 responses in favour of Th-1 responses

which are required to control HIV infection) as development of the immune system switches from one adapted towards self-tolerance to one predominantly adapted towards mounting effective innate and acquired responses to external pathogens.[163] Maturation of the immune system starts from birth onwards in response to environmental pathogens and continues particularly during the first two years of life.[170] As such, it is not surprising that timing of infection has a major prognostic significance for HIV infection, with HIV-infected neonates, who are immunologically relatively less mature, likely to be at higher risk of rapid progression than infants who are infected later.[168]

There are several other possible factors besides timing of infection that may influence the course of infection in infants, such as genetic susceptibility, characteristics of the infecting virus, gestational age and maternal health status.[171-173] However, unlike timing of infection, the association of these factors with disease progression has been less consistently shown in epidemiological studies. Investigation into the genetics of long-term survival in adults has so far shown relatively little beyond confirming that HLA polymorphism plays a major role in the chances of mounting a successful response to HIV infection.[32]

2.4.2 Early epidemiological data

Although accounting for only about 2% of vertical transmission globally, data on long-term survival comes mainly from cohorts in industrialised countries. Data from prospective cohort studies in industrialised countries with follow-up beyond five years of age in the pre-ART era also supports the existence of two

distinct prognostic groups: "fast" and "slow" progressors. These data showed that rates of AIDS/death in the pre-ART era are at their maximum in the first two years of life but decline thereafter among survivors of this early period, such that at least a third of vertically-infected children are alive by 13 years of age.[118 130 138 141 174-177] Population surveillance data confirmed this and showed an *overall* median survival of at least 8 years in untreated HIV-infected infants.[178] Slow progressors run a more chronic course and have a variable pattern of disease progression with some developing clinical signs and immunological alterations after two to three years of age, while other remain alive and symptom-free for a decade or more.[138 141 147 179-181]

Data from a five-year prospective natural history study of HIV-infected children from Rwanda was the first study to support the possibility of two patterns of disease progression in Africa.[140] The cumulative incidence of AIDS was 28% at two years and 35% at five years, with the risk of death being 5.1 times higher in those who were HIV-infected in the first three months of life than in those infected later. However, the study was based on small numbers with large losses to follow up.

2.4.3 Epidemiological data from Africa

In Africa, there have been no natural history cohort studies assessing disease progression beyond five years of age, and so the epidemiology of HIV in older African HIV-infected children remains unclear. Based on extrapolation from the high mortality rates observed during the early years of life among HIV-infected

infants, the widely-held perception has been that without ART survival to older childhood in Africa was likely to be infrequent.

Recent national population-based surveys in Southern Africa, however, have consistently reported high HIV prevalence rates of between 1-5% among older children (Table 2.2). Up to the age of 14 years there was no difference in HIV prevalence by gender. However, after this age, HIV prevalence was disproportionately higher in females than in males, reflecting the higher risk of HIV infection through sexual transmission in younger women compared to men.[14] In a survey of public health facilities in South Africa in 2005, HIV prevalence was 15% in older children (aged 2 to 9 years) attending health-care services for any reason with investigations supporting vertical transmission as the source of infection for the majority of children.[182] Almost all HIV-infected children had HIV-infected mothers and the major, albeit rare, risk factor for mothers having an HIV-infected child if HIV-negative themselves was a history of the child having been breast-fed by another woman. Studies from other countries in the region show that considerable numbers of older HIV-infected children are presenting for care.[15 183] Among 514 HIV-infected and ART-naive children aged 1-14 years recruited into the CHAP trial in Zambia during 2001-2003, 38% were aged 10 years or older.[16] However, studies have discounted the possibility of long-term survival as an explanation for the observed HIV prevalence rates in older children and it is notable that older children have not until recently, been included in most national population-based HIV prevalence surveys.[184]

Table 2.2 HIV prevalence among children in Southern Africa

Location and year	Sample and participation rate (%)	Age (years)	N	Prevalence (%)	Comments
Chimanimani District, Zimbabwe 2005[11]	Household survey; 68%-74% participation (by age, 2-24 years)	2-5 6-8 9-11 12-14 15-18	330 189 170 601 301	2.7 5.8 1.3 3.0 5.3	Dried blood spots; no difference in HIV prevalence by sex
South Africa 2004[13]	Household survey; 65% participation (all ages)	2-9 10-14 15-18	1,377 973 945	6.2 4.7 5.0	Oral mucosal transudate for HIV testing; no difference in HIV prevalence by sex
South Africa 2005[185]	Household survey; 55% participation for children 2-14 years	2-4 5-9 10-13 15-19	729 1,341 1,745 2,154	5.1 4.4 1.7 5.9	Dried blood spots for HIV testing; no difference in HIV prevalence by sex below 15 years of age
South Africa 2008[186]	Household survey; 59% participation for children 2-14 years; 68% for 15-24yrs	2-14 15-24	5809 5344	2.5 8.7	Dried blood spots for HIV testing; no difference in HIV prevalence by sex below 15 years of age
Free State, South Africa 2002[187]	Health facilities: 57 primary clinics, 25 hospitals; 99% participation	2-5 6-9	2,737 1,347	14.9 14.6	Rapid test kits with confirmation by ELISA; No difference in HIV prevalence by sex; Hospital prevalence: 21.5%; Primary care facilities prevalence: 13.7%
Botswana 2004[10]	National Household AIDS indicator survey; 61% participation (all ages)	1.5-4 5-9 10-14 15-19	Not stated	6.3 6.0 3.9 6.6	Oral mucosal transudate for HIV testing; No difference in HIV prevalence by sex below 15 years of age
Serowe and Kweneng District, Botswana 2005[188]	Household survey; participation not stated	2-11 12-14 15-24	351 110 302	6.0 5.5 12.3	HIV test not specified; HIV prevalence not stratified by gender
Swaziland 2006[12]	Demographic and Health Survey; 85-90% participation (by age, 2-19 years)	2-4 5-9 10-14 15-19	820 1,367 1,402 2,438	5.1 4.2 2.6 5.8	Dried blood spots for HIV testing; no difference in HIV prevalence by sex below 10 years of age

Older children are at risk of acquiring HIV through sexual transmission (i.e. sexual abuse or early sexual debut). However, while not discounting the high rates of sexual abuse reported from African countries, penetrative forced sex during childhood occurs at a much lower frequency at a population level than

exposure to HIV at birth and in infancy in this region, with the possible exception of South Africa.[189-191] The use of unsterilised needles or other skin-piercing instruments for medical or ritual purposes has potential for HIV transmission. Although studies have shown an association between hypothesized parenteral risk factors such as scarification and injections, the potential relevance of these factors need to take into account cultural factors. Patients in Africa often express a strong preference for parenteral rather than oral therapy and a belief that parenteral medication is more effective than oral medication. Thus, causality between hypothesized parenteral risk factors and HIV is difficult to establish.[23] The lack of association between HIV seropositivity and childhood vaccinations probably reflects the wider use of properly sterilised injection equipment in immunisation programs.[192] Furthermore, while practices for collecting and transfusing blood vary throughout the continent, routine screening of blood products has been established for well over a decade in most countries. Investigation in Uganda among more than 5000 children aged 0-12 years showed no evidence for post-natal transmission other than through breastmilk.[193]

As discussed above, infants infected postnatally from breast milk appear to have a better prognosis than those infected perinatally or *in utero*.[7 154-155] In industrialised countries HIV-positive mothers almost all formula-feed their babies, while in Africa most mothers breastfeed their babies even if they know themselves to be HIV-infected. Furthermore, unlike in industrialised countries, scale-up of ART for children began relatively recently and coverage remains limited.[18] The African paediatric epidemic may thus have a natural history

quite unlike that in developed countries, with a higher proportion of untreated slow progressors who have acquired their HIV post-natally.

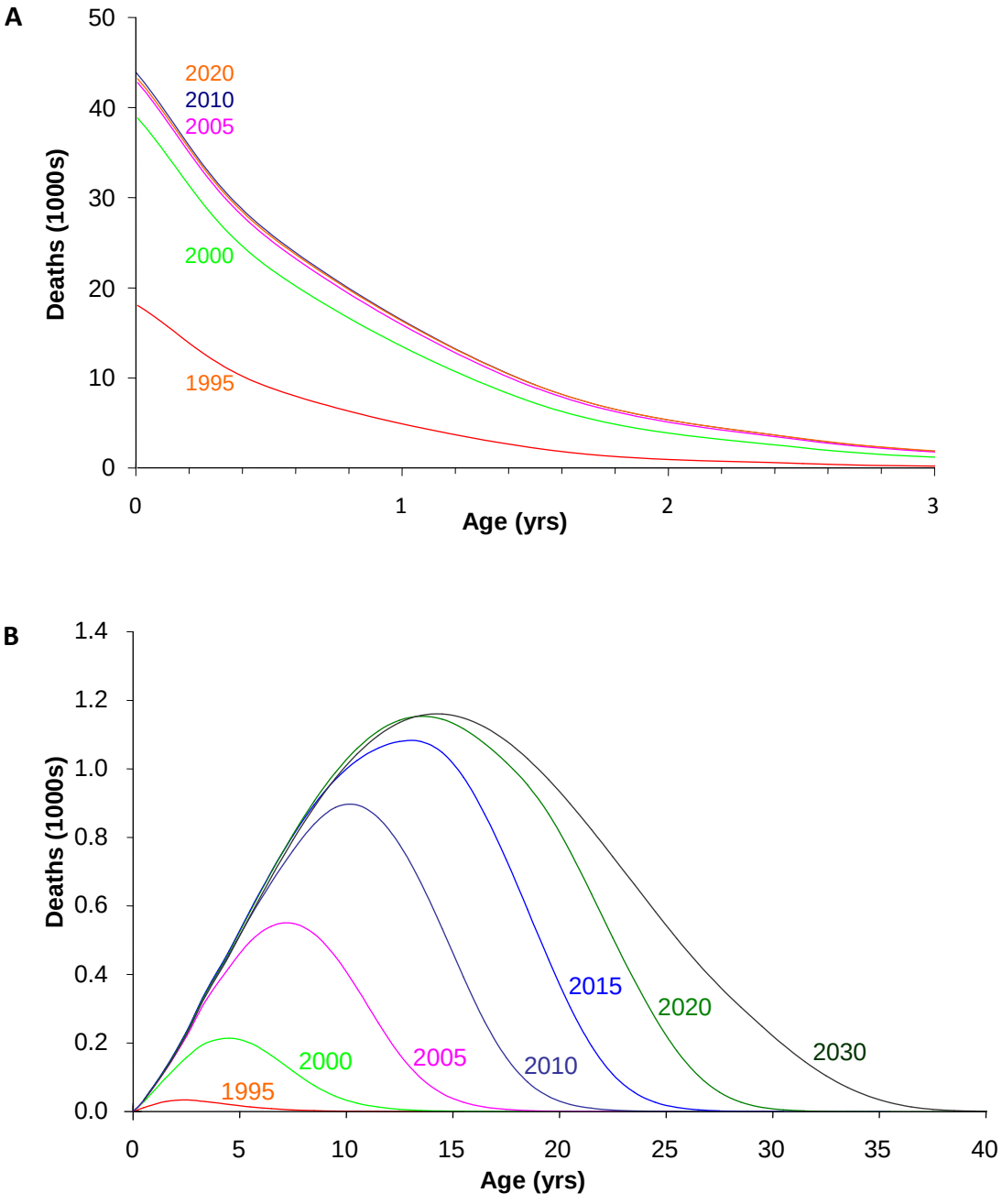
2.4.4 Current understanding

Survival estimates among vertically infected infants in Africa have been successively revised upwards in recent years as evidence for better prospects of long-term survival emerge.[9] A pooled cohort analysis estimated that at least 13% of such children will survive to 10 years and that the available data are most compatible with about one third being slow progressors whose median survival is at least 8 years with no upper limit to this estimate possible from the existing data.[8] A survival model that combined the estimate of a third of vertically-infected infants being slow progressors with an assumption that median survival among this group will approximate that observed in children infected horizontally in Western Europe (mostly haemophiliacs who received infected blood products early on in the HIV pandemic) provided the most recent UNAIDS estimate that 17% of HIV-infected African infants infected at birth would survive to age 15 years.[9 114] Using similar assumptions (i.e. median survival of 16 years among slow progressors), a substantial epidemic of older vertically-infected children is predicted in countries with early and severe heterosexual epidemics.[194]

The main limitation of these projections is that the median survival is extrapolated from that of children from developed countries infected through parenteral transmission, whose survival may well be quite different from those of vertically-infected slow-progressors in Africa. However, the projections are

compatible with observed epidemiological data, suggesting that the model estimates provide a reasonably close approximation of the true natural history.[194]

Figure 2.1 Estimated number of HIV-associated deaths per year by time and age among vertically-infected children who are A) fast-progressors and B) slow-progressors in South Africa, assuming no PMTCT or ART. ***Courtesy of Dr Brian Williams***



The dynamics of the epidemic of slow progressors are such that numbers of vertically-infected adolescents build up slowly after the onset of the adult epidemic in any given country, reflecting the time needed for infants to reach older childhood. However, because of this same time-lag, the epidemic of slow-progressors will continue to grow for a decade or more after the adult epidemic declines or effective PMTCT has been put in place.[194] Furthermore, mortality and morbidity among slow progressors is relatively spread-out in time compared to that of fast-progressors, occurring over a much wider age-range, in contrast to the more immediately obvious and visible epidemic of infant deaths among fast-progressors, as shown in Figure 2.1. These dynamics may partly explain why international guidelines for HIV care provision have been focused on infants and younger children.[184 195-196]

2.5 HIV-associated morbidity in older children

Although the spectrum of HIV-associated morbidity is a continuum with infections predominating in all age-groups, HIV results in certain manifestations particular to children. These manifestations occur as a consequence of the damaging effects of HIV infection on the body during a period of physiological development as well as during a period when the immune system is not fully mature. Additionally, timing of persistent viral infections such as Epstein Barr virus (EBV), CMV and Human papilloma virus (HPV) in relation to timing of HIV infection may also be important in pathogenesis of chronic complications. Unlike in adults, infections with viruses such as CMV, EBV and HPV are usually acquired after acquisition of HIV infection and onset of immune dysfunction,

resulting in increased likelihood of development of virus-mediated pathology. Once established, these conditions result in complications in older children which may not be fully reversible with ART.

The data on effects of longstanding HIV infection in children, however, are very limited. Most studies that have hitherto been carried out on chronic conditions have been largely cross-sectional in nature, often lacked controls and enrolled small numbers of children mainly from developed countries, many of whom had received prior ART.

2.5.1 Chronic lung disease

Lung disease is the commonest cause of morbidity and mortality in HIV-infected children with an estimated 80% of HIV-infected children presenting to health care with a respiratory problem.[197] As children get older, they have an increased risk of developing HIV-associated chronic lung disease. In one cohort from USA chronic lung disease was present in 58% of HIV-infected children who died.[198] The range of conditions includes chronic infections, a condition seen almost exclusively in HIV-infected children known as lymphoid interstitial pneumonitis (LIP) and bronchiectasis.[199-201] The spectrum appears to be similar in industrialised countries as in resource-limited countries with the exception of TB being more common in resource-limited settings.[202]

In children, malnutrition and the immaturity of the immune system results in a tendency to develop frequent respiratory infections and HIV infection further increases the risk of pneumonia.[132 203] The severity and protracted course of

infective episodes in HIV-infected children favour the progression of pulmonary infections to chronic lung disease. Viral infections, such as influenza A and B, parainfluenza 1-3 and adenovirus, occur more frequently, persist for longer and the outcome is poorer, which may be because co-existing bacterial pneumonia is more common in HIV-infected children.[204] The burden of hospitalization for viral-associated pneumonia is 2-8 times greater in HIV-infected children and can be lead to development of a destructive immunopathological condition, bronchiolitis obliterans.[201] In high HIV-prevalence settings, TB is an important cause of chronic respiratory disease. Studies have estimated that HIV infection increases the risk of TB disease by a factor of 20.[205] The increased risk of TB among HIV-positive children is mainly attributable to immunosuppression, but also to the fact that HIV-infected children are more likely to be exposed to TB within their families.[206] TB can be complicated by fibrosis, chronic atelectasis and bronchiectasis. The inherent difficulty in confirming TB in children in resource-limited settings, compounded by reduced sensitivity and specificity of clinico-radiological diagnostic criteria from concurrent HIV, means that HIV-infected children with chronic lung disease are frequently diagnosed as having smear-negative pulmonary TB and given multiple courses of anti-TB treatment based on clinical symptoms of chronic cough and failure to thrive and non-specific radiological abnormalities.[200]

The prevalence of LIP was 20-30% in the developed world in the pre-ART era and similar prevalence rates have been observed in clinical, bronchoscopy and necropsy studies in African children.[207-209] LIP is characterised by lymphocytic and plasma cell infiltration of the inter-alveolar and inter-lobar septa and

hyperplasia of pulmonary lymphoid tissue. Pathogenesis is most likely due to EBV, which may initiate an abnormal lymphoproliferative response in the context of prior immune dysfunction.[210-211] There is also infiltration of other organs, manifest as generalised lymphadenopathy, bilateral non-tender parotid enlargement and enlargement of the liver and spleen. The diagnosis is supported by a chest radiographic pattern of diffuse reticulonodular infiltrates, which may be difficult to distinguish from pulmonary or miliary TB and often results in incorrect utilisation of prolonged and repeated courses of anti-TB therapy in HIV-infected children with chronic respiratory symptoms particularly in settings where TB is endemic.[200]

Of note, LIP was associated with a relatively slow rate of disease progression in HIV-infected children in the pre-ART era.[140] Thus LIP may be over-represented among children with slow-progressing HIV infection. Although LIP has an improved survival compared with other AIDS indicator diseases, it is associated with considerable respiratory morbidity; the incidence of recurrent episodes of lower respiratory tract infections is double that of HIV-infected children without LIP, and there is a high risk of pulmonary fibrosis, cystic lung disease, failure of the lungs to grow, and bronchiectasis.[207 212]

Bronchiectasis, an abnormal and permanent dilatation of the bronchi, occurs as a late sequel of infection or as a result of healing of lung tissue with fibrosis. It is a disease-entity in its own right, causing increased susceptibility to lower respiratory tract infections regardless of age or aetiology. As discussed above, HIV infection in childhood is a strong risk factor for bronchiectasis, and leads to

a further increased risk of recurrent bacterial pneumonia and to chronic respiratory symptoms.[213] In one series of HIV-infected children with chronic respiratory symptoms, the prevalence of bronchiectasis was about 15%.[213] As in other systemic causes of diffuse bronchiectasis, such as hypogammaglobulinaemia, cystic fibrosis and ciliary disorders, failure to manage the respiratory condition aggressively leads to a vicious cycle of superinfections that accelerate the rate of bronchial wall destruction and pulmonary function decline, leading to progressive respiratory failure. Over time, involvement of the pulmonary blood circulation results in pulmonary hypertension and right heart failure (cor pulmonale). This is associated with secondary growth failure, wasting, and a very high mortality.[212 214] Although preventable, there are a limited range of treatment options for established chronic lung disease, and so children with this complication of HIV infection are likely to have reduced quality of life and life expectancy even when started on ART.

2.5.2 Cardiac disease

Although cardiac disease in the context of HIV infection is well-recognised, most studies have been done in adults and the risk in children is generally under-appreciated. Cardiac disease as an underlying cause of mortality shows a significant age-related trend in children; the risk of dying from cardiac disease is virtually nil in infancy, but cardiac abnormalities were directly responsible for death in 12% of HIV-infected young American children with this figure increasing to 25% in children aged over ten years.[198 215]

Estimates of the incidence of cardiac disease in HIV-infected children vary widely depending on the methods of ascertainment. Subclinical abnormalities are commonly detected on electrocardiography[i] or echocardiography[ii]. These abnormalities develop early in life and are persistent.[216-217] The main limitation of these early studies has been the cross-sectional design and lack of controls, making it difficult to ascertain the clinical significance of these abnormalities. The most commonly reported abnormality is left ventricular (LV) dysfunction, indicated by a low shortening fraction (SF).[216] SF is a measurement made on echocardiography and represents a summation of factors that affect LV function including contractility, pre-load and after load. LV dysfunction can be due to decreased contractility, dilatation and impaired relaxation due to hypertrophy of the muscular wall of the LV. Asymptomatic LV dysfunction may ultimately progress to dilated cardiomyopathy, with hypotensive hypoperfusion of the systemic circulation due to pump failure.

In the P2C2 cohort, a natural history study of cardiopulmonary complications of vertically-acquired HIV infection in USA, between 5-8% of children had a low baseline SF, and the cumulative incidence of LV dysfunction after five years was 28%.[218-220] The risk of cardiac disease increased as the level of immunosuppression increased.[221] The cause of myocardial damage in HIV infection is likely to be multi-factorial, with infections as well as direct immunological effects of HIV infection playing a role. The virus itself has been detected in myocardial cells as well as pericardial fluid in HIV-infected

[i] A non-invasive recording of the electrical activity over time of the heart captured by skin electrodes

[ii] A non-invasive ultrasound technique that provides two-dimensional images of the heart

children.[222] Possible mechanisms include direct mononuclear immune cell activation, cytokine effects or immunopathology that may be potentiated by co-infections.

The prevalence of cardiac abnormalities among HIV-infected children in resource-poor settings appears to be even higher than in industrialised countries for reasons that are as yet unclear. In a cross-sectional study in Uganda, where mean age was 6.8 yrs, 17% of children in care had left ventricular systolic dysfunction, a finding similar to that of a study of hospitalised children in South Africa.[223-224] The symptoms of cardiac disease are non-specific and may often be attributed to non-cardiac pathologies particularly pulmonary infections.[223 225] Thus cardiac abnormalities are probably under-diagnosed and may have an impact on morbidity and mortality as children grow older. Early LV dysfunction can only be diagnosed by echocardiography and the skills needed limit the availability of echocardiography and so preclude diagnosis in most HIV-infected children.

2.5.3 Growth failure

Growth failure, defined by a growth rate that is below the appropriate growth velocity for age, is reported in up to 30-80% of HIV-infected children.[226-227] The exact prevalence is difficult to ascertain due to the different case definitions which have been used for investigative and surveillance purposes. Growth failure is distinguished from weight loss or wasting which is primarily due to inadequate calorie intake. Disturbances in growth patterns, although very variable, become apparent by 3-4 months of age, persist and increase with

time.[226 228] In one retrospective study, 42% of vertically-infected children over five years old had a growth velocity of less than the 5th centile, an indication of severe growth failure, and growth faltering in terms of both height and weight was observed for at least up to 10 years of age in the European Collaborative Study as well as in a cohort of Ugandan children.[141 183 229] HIV-infected children experience nearly proportional declines in both height and weight such that normal "height-for-weight" is maintained, and wasting may become apparent only as children develop more advanced immune deficiency.[229] Similar stunting, with normal height-for-weight, is also reported in HIV-positive haemophiliac boys infected through contaminated blood products.[230]

Growth can be exacerbated by a variety of nutritional, endocrine and immunological disorders. The pattern of concurrent impairment of weight and height suggests that other mechanisms underlie HIV-related growth failure other than nutrition, as weight tends to fall off before height in conditions of protein energy malnutrition. Also, in contrast to children with protein energy malnutrition, increasing nutritional intake in children with HIV-associated growth failure improves weight but has no effect on linear growth/height velocity or lean body implying that it cannot be solely explained by inadequate nutrition.[231] Furthermore, the alteration in body composition in HIV-infected children with growth failure includes disproportionate decreases of fat-free or lean body mass, a characteristic pattern that is distinct from that in children with nutritionally based stunting, but similar to the lipodystrophic pattern of weight loss and redistribution observed in adults with HIV infection.[232-233] There is no consistent relationship between Vitamin A or other micronutrient

deficiencies and growth failure, or between gastrointestinal (GI) disease and malabsorption and growth.[234-237] However, these associations were investigated using cross-sectional studies which did not assess risk of poor growth in the future. Various neuroendocrine abnormalities that have the potential to affect growth have been identified, including abnormalities in the thyroid axis and adrenal axis and elevated glucagon levels, although no single endocrine abnormality is consistently encountered in HIV-infected children with growth retardation.[229] Primary growth hormone deficiency is found only occasionally and insulin-like growth factor-1 (IGF-1), the main determinant of linear growth velocity after infancy, is variably reduced, although reduced tissue sensitivity to IGF-1 may be an important factor.[238-239]

Virological response to ART is highly correlated with rates of growth in children, with ART regimens that result in optimal suppression of HIV viral load having the best growth responses.[240] This suggests that HIV itself may contribute to the pathogenesis of growth impairment. Linear growth retardation is a major complication of several childhood inflammatory diseases. For example, Crohn's disease, characterised by transmural inflammation of the GI tract, severely inhibits growth in about a third of affected children with a significant proportion becoming short adults.[241-242] Although growth failure in Crohn's disease used to be attributed chiefly to undernutrition, there is increasing evidence that the inflammatory process itself may directly inhibit growth through cytokines such as IL-6 that are induced by intestinal inflammation and that suppress IGF-1.[243-244] The GI tract is one of the most important sites of HIV replication and a central element in the immune

dysregulation accompanying HIV infection.[245] Hence, growth retardation in HIV infection may also be related to the pro-inflammatory milieu, as has been observed in Crohn's disease.[246] Malnutrition, increased metabolic requirements and an adverse social environment are likely to exacerbate faltering growth and thus the degree of growth impairment may be more marked in resource-limited settings.[247]

Growth is not only related to height and weight gain. HIV infection is also accompanied by skeletal abnormalities, including decreased bone mineral content and bone mineral density.[248-249] This is a result of both decreased bone accrual as well as increased bone turnover during growth. Childhood is a critical period for bone mineral acquisition and a compromised bone mass level poses a risk of development of osteoporosis and its complications in the future. The pathogenesis of abnormal bone metabolism is not clear and is probably multifactorial related to both nutritional deficiency e.g. Vitamin D, and HIV-related processes.[250] Osteogenic cells are targets of HIV infection, and cytokines such as IL-6 and tumour necrosis factor (TNF) activate osteoclasts and are over-expressed in HIV infection.[251-254] ART, particularly tenofovir, may contribute further to bone loss.[255]

HIV infection also interferes with sexual maturation and pubertal delay is common among children entering adolescence.[256-257] Among haemophiliac boys pubertal delay is associated with diminished androgen production and subsequent growth hormone secretion, but little is known about the association between HIV infection and endocrine dysfunction in vertically-infected

children.[230 258-259] Some studies suggest that age at onset of puberty is not related to the clinical or immunological status, a finding also observed in other childhood chronic conditions such as cystic fibrosis, juvenile chronic arthritis and inflammatory bowel disease.[256 260-261] However, other studies show that degree of HIV-related immune suppression is associated with delayed initiation of pubertal development.[257]

Delay in pubertal development is likely to influence final adult height and cause significant psychological morbidity and possibly contribute to social stigma of HIV infection. The final extent of catch-up growth and pubertal development is likely to be age-dependent, with less good outcomes reported when ART is started in older children and adolescents.[262-263] Furthermore, growth responses to ART in resource-limited settings are blunted compared to those in industrialised countries, potentially reflecting higher background malnutrition and infection rates.[263]

2.5.4 Encephalopathy

PE, characterised by a stepwise or continuous deterioration, has been well documented in HIV-infected infants and young children. Older children who experience an early AIDS-defining illness are also at risk of impaired cognitive functioning, even after controlling for environmental variables, but the pattern of impairment is usually consistent with a static encephalopathy with neuro-developmental deficits being fixed and non-progressive.[264-265] It is not clear why one subgroup of HIV-infected infants develops a more severe PE whereas another subgroup does not, but it has been suggested that this may be related to

the timing of central nervous system HIV -- infection with early infection of the developing brain *in utero* being associated with a more aggressive course --, the accompanying viral load and genetic vulnerability.[266-267] The mechanism of brain impairment due to HIV is not well understood, but proposed mechanisms include direct neuronal injury, macrophage destruction resulting in neurotoxicity, co-infection by other agents, and integration of the virus into the central nervous system cell lines.[268]

In HIV-infected children who survive to older childhood without major illness, overall cognitive function appears to be within the normal range, although studies vary significantly with respect to methods and often lack controls, making comparisons of findings difficult.[183] However, even in seemingly asymptomatic older children there are deficits that are not easily identified by routine testing. These include deficits in fine motor function, memory, perceptual performance, quantitative abilities and mental processing and language abilities.[269-272] These skills are critical as children approach adulthood particularly with respect to adherence to medication, education and career planning and risk behaviours as well as to overall quality of life.

In older children, psychiatric morbidity, which is rare in younger children, is more frequent. There is a significantly higher incidence of psychiatric hospitalization among HIV-infected older children and adolescents compared with the general paediatric population in industrialised countries, the most common reasons for hospitalization being depression and behavioural disorders.[273] However, it is often difficult to determine whether aetiology is

attributable to organic or psychosocial factors. There are few data from resource-limited settings where exposure to HIV occurs in the context of different environmental factors which may result in a different risk profile for cognitive, neuropsychiatric and behavioural problems.

2.6 Diagnosis and Treatment of HIV Infection

2.6.1 HIV diagnosis

HIV counselling and testing (CT) is a prerequisite for access to care and measures to reduce risk of onward transmission, such as PMTCT. For adults and children older than 18months, diagnosis of HIV infection relies on detecting antibodies to HIV in blood. HIV testing has become more convenient in recent years with the availability of rapid tests. These do not require invasive procedures, specialised equipment or laboratory technicians and the result is available within an hour. Because of the passive transplacental transfer of maternal HIV antibodies to the infant, newborn infants and children younger than eighteen months test positive for the presence of HIV antibodies even in the absence of true infection. Therefore, definitive diagnosis of HIV infection requires the use of HIV-specific RNA or DNA nucleic acid tests to detect the virus itself, instead of the inexpensive and readily available serological assays used in older children and adults.[274]

There are two methods of CT provision: client-initiated and provider-initiated. The former corresponds to what is usually referred to as voluntary CT or VCT and requires individuals to actively seek testing and counselling at a facility that

offers these services. Until a few years ago, most countries used a client-initiated approach to provide HIV testing through stand-alone facilities, facilities integrated in health settings, mobile services and community-based settings. Evidence has also shown that providing tests in convenient locations, such as workplaces and home-based testing, contributes to increased uptake of HIV testing.[275-276] Provider-initiated HIV testing and counselling (PITC) requires health care providers to initiate and offer CT. With widespread availability of treatment for HIV infection, there has been a notable shift in recent years from client-initiated to provider-initiated HIV testing which has much lower costs per HIV-positive person tested than alternative strategies.[277-278] International guidelines recommend routine PITC to all people seen in all health facilities in generalized epidemics as part of access to universal testing and care, and in selected health facilities used by populations that may be at special risk of HIV in low-level and concentrated epidemics.[17]

Although the availability and uptake of CT has continued to increase, under-diagnosis of HIV infection remains a significant issue globally. Up to 60% of individuals remain undiagnosed globally, with wide differences across countries and across at-risk population groups.[18] Uptake of HIV testing is limited by fear, stigma, underestimation of risk, negative reactions to disclosure, and limited access to treatment services.[279] Health system factors, including lack of human resources and adequate training for health care providers, create obstacles to effective PITC implementation. In children younger than 18 months, virological tests required for HIV diagnosis are expensive and complex to perform, and not available in many parts of the world where the risk of HIV

in infancy is highest. Late diagnosis prevents the timely initiation of ART, resulting in higher early mortality after starting treatment, and poorer outcomes.

2.6.2 Treatment of HIV infection

The goal of HIV treatment is to suppress HIV replication and to prevent or reverse HIV-related symptoms and immunosuppression. Treatment is life-long as current drugs are not curative. Following the licensing of zidovudine as an antiretroviral drug in 1987, the difficulties in treating HIV were demonstrated in the coming years as drug resistance rapidly developed to mono- and to dual therapy. Development of HAART, a combination of three drugs that resulted in sustained suppression of viral replication, in 1996, led to marked reductions in HIV-associated morbidity and mortality in industrialised countries.[280] However, adherence to treatment is critical to durable treatment success and sub-optimal adherence results in "breakthrough" viral replication and development of drug-resistant mutant virus strains. Furthermore, prolonged use of ART is associated with dyslipidaemia, insulin resistance and disturbances in bone metabolism. Co-morbidities such as diabetes and cardiovascular disease are now more common causes of morbidity and mortality than opportunistic infections among HIV-infected adults in industrialised countries, where HAART has been available for over a decade.[281]

During the past ten years there has been an unprecedented drive to scale up access to treatment for people living with HIV infection in low and middle-income countries as a public health emergency. Following the 2001 United

Nations General Assembly Special Session on HIV/AIDS (UNGASS) Declaration of Commitment to control the spread of HIV, the WHO launched the "3 by 5" initiative in 2003 to provide ART to three million people by 2005. Subsequently, at the United Nations General Assembly High-Level Meeting on AIDS in 2006, countries committed to work towards universal access to comprehensive HIV prevention and treatment by 2010. Political commitments have been backed by international initiatives such as US President's Emergency Plan for AIDS Relief (PEPFAR) and The Global Fund to fight AIDS, TB and Malaria (GFATM) and other bilateral, national and nongovernmental or private funding sources to support global scale-up of ART. Access to ART has continued to expand rapidly with an estimated coverage of ART in low and middle-income countries of 40% in 2008, a marked improvement but still leaving a large treatment gap.[18]

Although high levels of adherence to treatment and virological suppression have been achieved in treatment programmes, early mortality following ART initiation are several fold higher among patients in resource-limited settings compared to that of patients in high-income settings, even after adjusting for baseline immunodeficiency.[282] Up to a quarter of patients in sub-Saharan Africa die in the first year of ART, contributing to substantial losses in overall patient retention.[283] Although several factors contribute to mortality, the key issue is presentation with advanced HIV disease, and the early deaths reflect the spectrum of causes of death prior to ART initiation such as TB, sepsis, cryptococcal meningitis and HIV wasting syndrome.[283]

Treatment of children lagged behind that of adults because of difficulties in getting appropriate drug formulations and palatable drugs, and determining practicable criteria for starting HIV treatment.[284] Like adults, infants show a strong immunological response to treatment, although they have a higher risk of persistent viraemia, possibly reflecting difficulties of giving medicines consistently and at the required doses to young children. [285-287] The risk of developing drug resistance is a major concern in children who will hopefully be taking treatment for a greater proportion of their lives than adults. As access to ART improves and prolonged ART use becomes more common, morbidity from a similar range of treatment-related non-infectious conditions as adults may become a particular concern for children, who will be taking ART during the critical years of physiological development.[288]

2.7 Adolescent Health

2.7.1 Context of adolescence

Adolescence (from Latin: *adolescere* meaning "to grow up") is a transitional stage of physical, mental and psychological development generally occurring between the onset of puberty and maturity. The supposed start and end of the period of adolescence varies in different countries and cultures and hence it is difficult to rigidly define the time frame in which adolescence occur and the age at which an individual is considered to be chronologically and legally mature. The World Health Organization defines adolescents as young people between the ages of ten and eighteen years and this is the definition used in the thesis.

Adolescents currently constitute the largest age group in the world, making up approximately a fifth of the world population, with 85% living in resource-limited settings. Adolescence is a period of life during which a large proportion of key life-course events are experienced including completing school, attaining puberty, becoming sexually active and becoming economically productive. This can be a personally and socially problematic period: an age group too old to be entirely dependent but with few skills and little experience in navigating an adult world. Adolescence is universally recognised as the link in the life cycle between childhood and adulthood, and most societies mark this stage through rites associated with prospective adult roles such as reproduction, responsibility, occupation and more autonomy.[289] This transitional period from childhood to adulthood involves multiple interactions between biological and socio-cultural influences. The physical and psychosocial changes that occur during adolescence reveal the impact of generational and early-childhood factors on development and predict potential threats to health and well-being in adulthood.[289]

2.7.2 Morbidity in adolescence

In developed countries the major causes of morbidity among adolescents have behavioural aetiologies, such as substance and alcohol abuse, self-harm and interpersonal violence and risky sexual behaviour.[290] In hospital, chronic diseases, trauma, oncology and mental disorders account for the majority of admissions.[291] In Africa, research on adolescent morbidity has been limited and focused on reproductive health, reflecting the high incidence of STIs including HIV, and obstetric problems among young people.[292-293] A South African study

in the pre-HIV era found that the disease profile on the paediatric wards differed markedly by age, with the most frequent diagnoses in young children being infectious while chronic diseases and mental health problems were the commonest causes of admission among adolescents.[294]

2.7.3 HIV and adolescent health

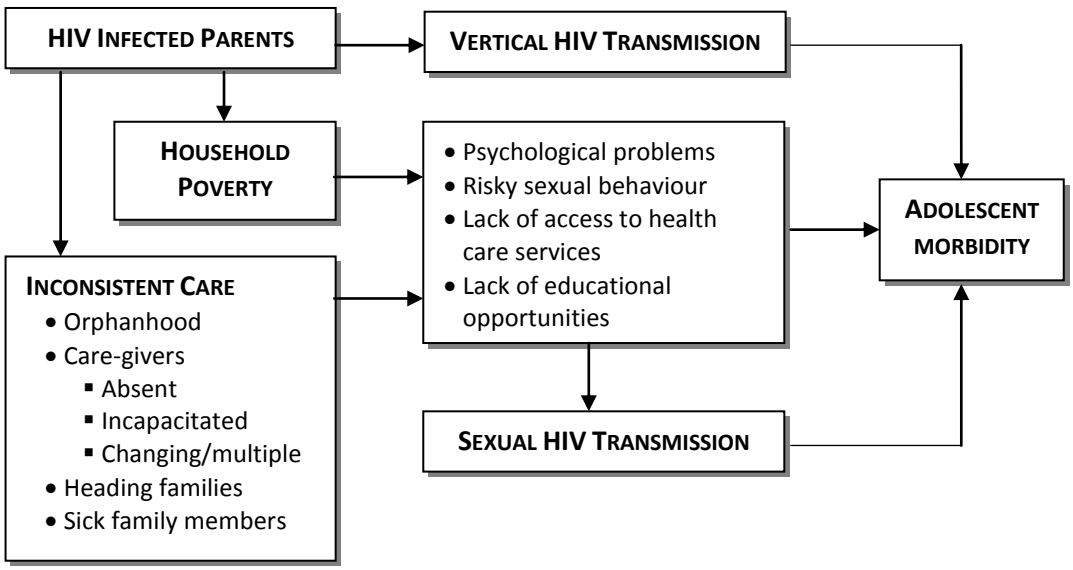
Adolescents are highly susceptible to adverse consequences of HIV infection through parental death and impoverishment (Figure 2.2). The orphanhood epidemic in Africa, which has accompanied the HIV epidemic, has "aged" along with the HIV epidemic, and as a result over 50% of AIDS orphans are adolescents. Adolescent HIV orphans have a higher likelihood of being HIV-infected themselves through maternal transmission, and are more likely to suffer from psychological problems, to drop out of school and to experience forced sex and engage in risky sexual behaviour, resulting in disproportionate risk of acquiring sexually transmitted HIV infection. [3 295-298]

The recognition that young people are at high risk of HIV transmission has resulted in this age group being a priority target for HIV prevention efforts for many years. However, less attention has been paid to care of adolescents who are already HIV-infected. In industrialized countries, an increasing proportion of vertically-infected children survive into adolescence as a result of ART, and a similar trend is expected in resource-limited settings as infant diagnosis and ART become increasingly available.[299] Furthermore, clinicians from countries with severe HIV epidemics report the presentation of HIV-infected adolescents with features of longstanding infection and estimates of survival from infancy to

adolescence following HIV infection have been successively revised upwards in recent years, as discussed in Section 2.4.

Whilst the epidemiology of sexually-acquired HIV infection among 15-24 yr olds is well-described, there are few data on the burden and disease patterns of vertically-acquired HIV infection in older children and adolescents, especially in sub-Saharan Africa. The proportion of children requiring HIV care who are adolescents is expected to increase considerably due to a combination of large numbers of LTS reaching adolescence with undiagnosed or recently diagnosed HIV and, in future, growing numbers of children who have been taking ART from infancy. Thus, the need for appropriate care services for HIV-infected adolescents will become an increasingly important part of HIV care in Africa.[300]

Figure 2.2 Potential ways in which the HIV epidemic may affect adolescent health



2.7.4 Health needs of HIV-infected adolescents

As well as interfering with the normal physiological and psychosocial development that occurs during this period, HIV infection places additional stressors on the life of an adolescent. During childhood, caregivers are often heavily involved in managing their child's illness. As children reach adolescence, expectations for them to take increasing responsibility for themselves emerge. Whilst often deferred in childhood (and not plausible in infants), disclosure of HIV status is crucial during adolescence as individuals approach cognitive maturity and puberty. Young adults have to make decisions about sexual relationships and their treatment and plan for the future, and these decisions can only be made with an accurate understanding of the nature of their illness.[301] Although the benefits seem considerable, disclosure of HIV status is typically delayed because of lack of skills in discussing HIV with minors and emotional unpreparedness of primary care-givers.[302-303] Reluctance of parents and caregivers to disclose HIV status is often due to fear of discrimination and stigma toward both the adolescent and the family as a whole, the concern that their child will not be able to deal with their HIV infection, the inherent disclosure of the mother's HIV status to their child, and possibly because of the painful feelings of guilt at having infected their child.[304-305]

Over and above HIV-related issues that are common to any age-group, adolescents are likely to face "recurrent and cumulative" psychological distress including illness and death of parents and siblings, the fear of being "abnormal" and confronting mortality and an uncertain future.[297 306-307] There are also

issues with schooling such as absenteeism due to illness or clinic appointments, disclosure of HIV status to school officials, and possible stigmatisation from classmates and teachers.[308] Many live in poor households with HIV infection often perpetuating the cycle of poverty. The emotional effects of coping with HIV infection are severe, with high rates of psychiatric morbidity among HIV-infected adolescents in industrialised countries, although it is likely that this is due to a combination of the neuropathological effects of HIV infection and environmental factors.[309] These factors affect treatment adherence and undermine success of ART.[310-311] With sexual maturity comes the anxiety about relationships, and the risks associated with being sexually active i.e. unwanted pregnancies, acquisition of STIs, the risk of rejection if they disclose their HIV status to their sexual partner, and the risk of onward transmission of HIV.[312]

2.7.5 Health Services for adolescents

While many paediatricians see young children as their "core business", a high proportion of time is spent looking after adolescents.[313] In some developed countries adolescent medicine exists as a separate speciality in recognition of the fact that this age-group has differing, and often complex, health needs from those of younger children or adults. In Africa, the health needs of adolescents are addressed mostly at primary care level and dedicated health care services for young people are the exception rather than the rule. Transition from paediatric to adult care occurs early, typically between ages of 8 to 12 years, and adolescents are variably managed by paediatricians and adult physicians, with little emphasis on their specific health needs.

The particular context of adolescence impacts on utilisation and delivery of HIV services and on adherence. Access to HIV diagnosis and care is likely to be particularly difficult in adolescence because of financial dependence, orphanhood and paucity of CT services aimed at this age-group. Uptake of HIV testing is low among adolescents, with even fewer engaging with care.[185 314] Without appropriately tailored services, young people are likely to fall through the cracks of paediatric or adult-orientated HIV care.

2.8 Zimbabwe

Zimbabwe is a land-locked country bordering South Africa, Mozambique, Zambia and Botswana, with Harare being the capital city. It is divided into 8 provinces and the population in 2006 was approximately 12 million with 55% of the population aged below 20 years and 22% being aged between 10 and 19 years.[315] The economy has more than halved over the past decade with GDP/capita now around $400. This has had a considerable negative impact on the education sector, with declining school enrolment rates, and on all aspects of the health sector. Mortality rates were among the lowest in the region prior to the HIV/AIDS epidemic but have since increased and the life expectancy at birth in 2002 was 45 years.[316]

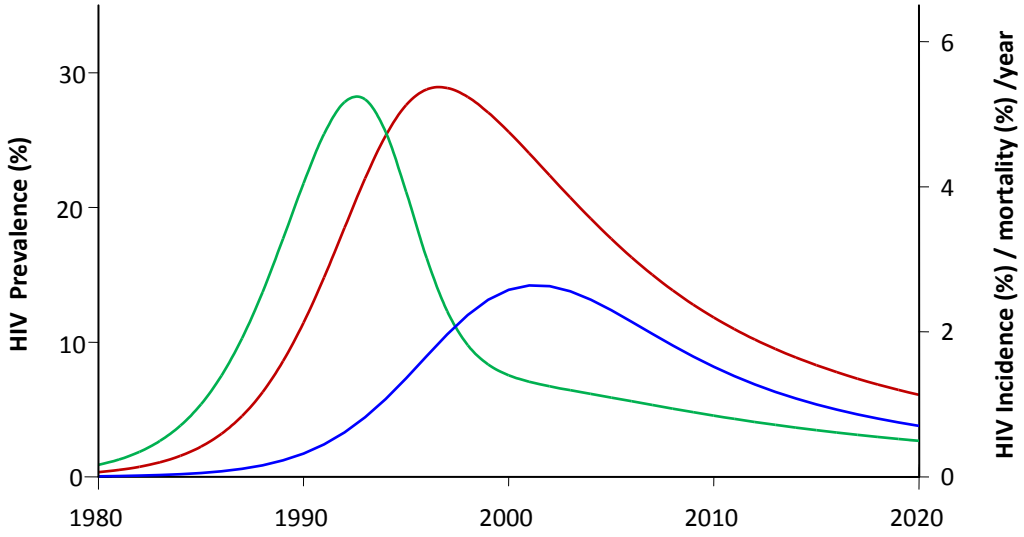
2.8.1 HIV in Zimbabwe

Zimbabwe has a wealth of published and unpublished HIV data. HIV sentinel surveillance in ANC has been ongoing since 1989. Two population based sero-

surveys were conducted in the last six years, with behavioural data collected in both surveys.[317-318]

Zimbabwe has experienced an otherwise comparable HIV epidemic of earlier onset than neighbouring countries. There is relatively little inter-province and urban-rural difference with respect to adult HIV prevalence rates.[318] HIV prevalence fell substantially in Zimbabwe over the past decade from 29.3% in 1997 to 15.6% in 2007 (Figure 2.3).

Figure 2.3 Adult (15 to 49 years) HIV prevalence (red), HIV incidence (green) and HIV-related mortality (blue) rates in Zimbabwe
Courtesy of Dr Brian Williams



Although it may be expected that prevalence would decline in a mature HIV epidemic such as that in Zimbabwe due to increasing AIDS-related mortality and a fall in incidence as a result of saturation of infection within high-risk groups, these trends are partly attributable to changes in sexual behaviour leading to reduced incidence rates.[319] Firstly the decline in prevalence was

observed among young women among whom AIDS-related mortality is low. Secondly, declines in prevalence were observed in longitudinal studies where trends could not be generated through shifting biases in fertility or representativeness of antenatal clinic surveillance estimates, and HIV incidence rates have declined in the same period. Thirdly, national and regional surveys indicated reductions in sexual risk behaviour, especially men forming fewer casual sexual partnerships and visiting commercial sex workers less frequently and sustained levels of condom use.[320] The extent of recent international migration from Zimbabwe is substantial albeit difficult to quantify.[55] Available data show that selective migration of HIV-infected individuals is unlikely to have contributed significantly to the decline in HIV prevalence.[319]

Zimbabwe was one of the few sub-Saharan African countries to implement interventions aimed at controlling the spread of the HIV epidemic, including scaling up of condom distribution programs, treatment services for STIs and HIV screening of donated blood, early during the HIV epidemic. Zimbabwe has maintained a strong blood safety record despite recent economic difficulties, and thus parenteral transmission is unlikely to contribute significantly to the spread of HIV infection.

Although HIV incidence has declined in Zimbabwe, it still has one of the highest prevalence rates in the world.[19] Given the early onset of the HIV epidemic, Zimbabwe is likely to have a relatively advanced epidemic of untreated adolescent LTS. To obtain projections of HIV burden among older children, we modelled demographic, HIV prevalence, MTCT and child survival: 3.4% of 10

year olds were estimated to be living with HIV acquired through MTCT in 2007.[194] The age-profile of HIV-associated mortality is evolving towards a point where HIV deaths among vertically-infected adolescents are likely to outnumber those among infants within a few years.[194] As is typical for the region, however, routine HIV programme data are reported for only 3 age categories: 0 to 4, 5 to 14 and 15 to 49 years, providing no clear age-profile of HIV infection in adolescents.

Although testing of pregnant women was introduced in 2001, only about 40% of HIV-positive pregnant women were receiving ART prophylaxis for PMTCT in 2008, so that vertical transmission continues to occur.[18] Nearly one in three children are orphaned, the majority aged over 10 years, and about 120,000 children are estimated to be HIV-infected.[321] ART started to become available in the public sector only around 2004 and in 2008, 36% of children in need of ART in Zimbabwe were receiving it.[18] ART is usually initiated in hospital HIV services, with on-going care decentralised to local primary care services where possible.

2.9 Summary

The severe adult HIV epidemic in Southern Africa has been followed by an epidemic of vertically-acquired HIV among children. There is increasing recognition that a substantial minority of HIV-infected infants have a slow course of HIV disease and thus can survive to adolescence and beyond even without prior treatment. As the HIV epidemic has matured, the first

presentation of vertically acquired HIV in older childhood and adolescence has been increasingly observed, and a major epidemic of previously untreated vertically-infected adolescents is now anticipated in high HIV burden countries. The early onset of the HIV epidemic in Zimbabwe combined with the relatively late availability of PMTCT and ART provides an opportunity to investigate the clinical epidemiology of HIV infection among adolescents, and to contribute to understanding about the contribution of HIV to morbidity among adolescents, who have hitherto been poorly represented in studies.

3 Methods

The individual studies described in the following chapters relied on a common approach to investigate HIV prevalence, mode of HIV acquisition and morbidity among adolescents. The methods used that are common to more than one study and specific procedural issues arising as a consequence of conducting research on adolescents are outlined below rather than in individual chapters.

3.1 Clinical methods

3.1.1 Anthropometric assessment

The anthropometric assessment included measurement of height, weight, mid-upper arm circumference (MUAC) and stage of pubertal development. MUAC was measured to the nearest millimetre, weight was measured to the nearest 0.1kg and height was measured to the nearest 0.1cm. Height was measured using a stadiometer, with the participant standing upright (a point on the upper and lower back against the stadiometer), feet placed in the tracing on the stadiometer base and with socks and shoes removed. Weight was measured using a digital scale which was calibrated before each measurement. Participants were weighed with their clothes on but any heavy clothing, such as coats and jumpers, socks and shoes removed.

Growth was assessed by using a standardized age- and sex-specific growth reference to calculate height-for-age *Z*-scores (HAZ), weight-for-age *Z*-scores (WAZ) and body-mass-index-for-age *Z*-scores (BMIZ). *Z*-scores of less than −2 for height- and weight-for-age were considered to represent stunting and

wasting, respectively. Each z-score expresses the anthropometric value as standard deviations below (-ve) or above (+ve) the mean of the reference growth curve.

The z-score scale is linear and hence summary statistics such as a mean, standard deviation and standard error can be computed from z-score values. These were compared with a reference set that has an expected mean z-score of 0 and a standard deviation of 1.0 for all normalised growth indices. Although it would have been ideal to use the 2006 WHO global child growth standards as reference, these were only available for children up to the age of five years. Hence, British growth references were used instead, as these are more recent than the alternative NCHS/CDC references.[322] Also, the NCHS/CDC references are based on examination of American children where the mean of the weight distribution is higher than that in British children and may be even less suitable for comparison with African populations. WHO growth curves for school children and adolescents have, more recently, become available, but these are also derived from the 1977 NCHS growth references for the 5-19 year age-group which have been merged with the WHO Child Growth Standards (0-5 years), and weight-for-age standards for children aged over 10 years are not available.[323]

Pubertal development was evaluated using Tanner's Staging, based on breast size and shape, and pubic hair growth and on size of external genitalia and pubic hair growth in boys.[324] Tanner Puberty Stage 1 or 2 in those aged over 13

years was considered to represent pubertal delay. Onset of menarche in girls was also documented.

3.1.2 Classification of clinical conditions

Pre-set diagnostic algorithms were used to define the cause of admission to hospital and any underlying chronic conditions, or reason for attendance to primary care. These were adapted from previous studies of HIV-associated morbidity and from case definitions used in the 2005 WHO HIV Staging Classification.[105 108 325] Among hospital admissions, case definitions of clinical conditions were defined as "definite", "probable" or "possible" and were based on a combination of specific symptoms, clinical signs and investigations (Appendix A). Diagnoses of chronic conditions that had been made in the past following specialist investigations and were documented in the case notes, were also recorded. At primary care level, the classification of the presenting complaint was broadly adapted from the WHO Integrated Management of Adult and Adolescent Illness (IMAAI) guidelines. IMAAI comprises a series of guidelines for syndromic classification and management at primary care level for use in high HIV prevalence settings.[326] The 2005 Adult WHO Classification was used to stage HIV infection.[325] The WHO Performance Scale was used to assess functional state:

Stage 1 - Asymptomatic, normal activity

Stage 2 - Symptomatic, normal activity

Stage 3 - Bedridden, <50% of the day during the last month

Stage 4 - Bedridden, >50% of the day during the last month

3.2 Laboratory methods

3.2.1 HIV testing and CD4 counts

Unreported (anonymised) HIV testing was carried out for study purposes and diagnostic HIV testing was offered at the same time with pre- and post-test counselling, to all participants.

Unreported HIV tests were run using a dedicated HIV test number, and results were recorded on a form that used the dedicated HIV test number only and had no other personal identifiers or the Study ID number. HIV test numbers and results were entered into a dedicated database, containing no other data except for the date of the test, the type of test kit used and the test results. The file that linked HIV test numbers and individual study ID numbers was maintained in a separate database. A STATA computer program was written that allowed files containing individual study data to be merged to HIV test results by linking study ID numbers and HIV test numbers while simultaneously dropping all personal identifiers, so that HIV test data could be used without compromising individual confidentiality. Numbers of participants were sufficiently large so that the potential for individual identification on the basis of results alone was not possible.

For participants who wished to know their results and accepted diagnostic testing, HIV tests were carried out according to national testing guidelines. Two rapid HIV antibody tests (Abbott Determine and SD Bioline) were run in parallel using the participant's Study ID number, and discordant or indeterminate

results were resolved using an ELISA assay (Vironostika). An ELISA test was required for 0.4% of samples.

CD4+ lymphocyte (CD4) counts were determined by flow cytometry (CyFlow® Counter: Partec). CD4 count blood samples were processed within six hours of venepuncture.

3.2.2 Herpes simplex virus-2 serology

HSV-2 is a sexually transmitted infection (STI), highly prevalent in Africa.[327] HSV-2 is synergistic for HIV acquisition and is a good biological marker of sexually-acquired HIV.[52 328] The initial infection is followed by seroconversion 4-6 weeks after infection, and antibodies persist for life.

HSV-2 antibodies were detected using a type-specific ELISA assay (HerpeSelect, Focus diagnostics, Cypress, USA) that detects antibodies to the HSV-2 glycoprotein G2 antigen. This test was chosen because it has a high sensitivity (98%), particularly for detecting early seroconversion, compared to the other widely used G2 ELISA assay, (Kalon).[329-330] The incidence of HSV-2 is highest among young people and in the population studied for this project, many participants would have been infected recently. Thus, the assay with the shortest time to detecting antibodies following seroconversion was used (median time to detection: HerpeSelect 21 days *vs.* Kalon 120 days, $p<0.001$).[330] HerpeSelect appears to have a lower specificity than Kalon in African populations, although a study showed 100% concordance with the Western blot using sera from a Zimbabwean population.[331-332] Specificity is increased when a

higher cut-off of 3.5 for positivity is used, instead of the 1.5 cut-off recommended by the manufacturer. However, given that HSV-2 serology was used for exploring mode of HIV transmission and given that Harare has low rates of the infectious conditions such as malaria and intestinal and blood stream parasitic diseases that can cause false-positive serological reactions, the manufacturer's recommended cut-off was used to maximise sensitivity.[331 333]

3.3 Consent and disclosure procedures

Participants who were aged 16 years old and above were presumed competent to give consent. Where neurocognitive impairment was suspected, consent was sought from the guardian to participate in the study. Those under the age of 16 years were considered minors and consent to participate in the study and to undergo HIV testing was sought from the guardian and assent sought from the participants. However, emancipated minors i.e. participants under the age of 16 years who were married or had children could give consent independently. If there was no designated guardian, consent was sought from two physicians responsible for the clinical care of the participant. In primary care, eligible minors who were not accompanied by their guardian were followed up at home to get consent from a guardian. If there was disagreement between the guardian and adolescent about participating in the study or undergoing diagnostic HIV testing, both were counselled until consensus was reached.

A flyer containing brief information about the study in the local language, Shona, was given to eligible adolescents and their guardians, before inviting them to take part in the study. A more detailed information sheet was given to

those who agreed to participate. Written consent (in Shona) was obtained from participants and their guardians. For those participants and/or guardians who were unable to read, consent was obtained verbally in the presence of a witness.

Participants were encouraged to have a guardian present when HIV test results were given but the participant could refuse permission to have their test result disclosed to the guardian. In reality, refusal to have test results disclosed to a guardian did not occur. The participant's HIV status was not disclosed to any health care worker without the consent of the participant. In cases where participants had tested HIV-positive prior to the study but did not know their HIV status, guardians were encouraged to disclose the HIV status to the participant and this was done in all cases with the counselling and support of the study nurses.

3.4 Ethical approval

Ethical approval to conduct studies was obtained from the London School of Hygiene and Tropical Medicine Ethics Committee, the Medical Research Council of Zimbabwe, the Joint Research Ethics Committee of the University of Zimbabwe and the Biomedical Research and Training Institute Institutional Review Board. For the studies conducted among hospitalised adolescents, ethics approval was also obtained from the respective hospital ethics committees.

3.5 Data management

Data were entered into a Microsoft Access database using EPI-Info 2002, which was used for data storage and manipulation. Data were analysed using STATA 10.0 (STATA Corporation, Texas, USA). Data analysis methods for each study are described in the relevant chapters.

4 The burden of HIV infection and spectrum of HIV-associated morbidity in acute hospital admissions among adolescents

4.1 Introduction

As discussed in Chapter 2, symptomatic HIV in older children and adolescents has increasingly been observed in clinical practice in African countries.[15 183 334] These observations, along with the relatively high HIV prevalence in this age-group in recent national surveys in Southern Africa, raises the potential for HIV to be an important cause of ill-health and mortality among adolescents. The spectrum of HIV-related disease varies considerably with age, but adolescents have been poorly represented in clinical studies of HIV infection, and thus distinctive features of HIV-associated morbidity in this age-group may be missed.

This chapter presents data from a cross-sectional hospital-based study assessing the burden and clinical manifestations of HIV in hospitalised adolescents in Harare. This design was chosen as the initial study for this thesis because similar studies have shown high HIV prevalence in hospitalised patients even when the corresponding prevalence rates of HIV infection in the general population were relatively low, thus providing good statistical power at a manageable sample size for the estimated population prevalence of adolescent long term survivors of MTCT of 2 to 4% in Zimbabwe.[105-106] Previous hospital studies have also provided important data on the main age-specific causes of HIV-related illnesses and deaths, stimulating further research on the specific disease entities as well as contributing to the development of

interventions aimed at prevention of opportunistic infections. Furthermore the existence of previous adult and child studies of a similar design from several African countries provided a good basis for identifying the specific distinguishing features of morbidity among adolescents.[105 335]

4.2 Methods

4.2.1 Participant recruitment and assessment

Harare Central Hospital and Parirenyatwa Hospital are the two public sector hospitals in Harare and cater to two-thirds of Harare's population, the remaining one-third using private health care facilities. The cost of hospitalisation in public sector facilities is partially subsidised by the government. Malaria transmission does not occur in Harare. Between September 2007 and April 2008, patients admitted to either hospital were enrolled consecutively the following day. Recruitment was limited to weekdays and to a maximum of five patients per site per day in order to maintain a manageable flow of clinical and laboratory work.

Patients aged between 10 and 18 years admitted with any acute medical or surgical condition, including trauma, were eligible. Patients were excluded if moribund, requiring intensive care admission, or admitted for obstetric or elective reasons, or previously enrolled into the study. Obstetric admissions were excluded because pregnancy is a physiological state, albeit one that carries a high risk to health, and also because pregnant adolescents are unrepresentative in many respects for e.g. being all female, sexually mature and

sexually active and with a disproportionately high risk of horizontally-acquired HIV infection.

Standardised assessment included social and clinical history, height, weight, and Tanner puberty staging, and laboratory investigations (full blood count, PITC, and CD4 count for HIV-infected participants). Onward referral for HIV care services was made for all testing HIV-positive. Where diagnostic HIV testing was declined, participants and their guardians were asked to consent to unreported (anonymised) HIV-testing for study purposes. In addition, all participants with febrile, wasting, or respiratory illness had a standardised infectious screen (blood culture, blood films for malaria, cryptococcal antigen (CrAg) testing, two sputum specimens, and chest radiography). If PCP was suspected, patients underwent sputum induction with nebulised hypertonic saline.

Otherwise investigations followed standard hospital guidelines including lumbar puncture for suspected meningitis and cerebral computed tomography (CT) for focal neurological signs or suspected encephalopathy. Participants were followed for the duration of their hospital stay.

4.2.2 Case definitions

Pre-set diagnostic algorithms defined the definitive or presumptive cause of admission and any underlying chronic conditions (see Appendix A). The Adult WHO Classification was used to stage HIV infection.[325]

4.2.3 Laboratory methods

The Myco/F Lytic (MFL, Becton Dickinson) system, which can detect bacteraemia, fungaemia and mycobacteraemia, was used for blood culture.[336] Blood cultures were inspected daily for seven days and then once weekly for eight weeks or until growth was observed, using a handheld UV Woods lamp (Figure 4.1). The sensor at the bottom of each bottle fluoresces in the presence of UV light if there is a decrease in oxygen concentration, reflecting microbial growth. Media was then withdrawn, and investigated using microscopy and culture. Bacterial growth was identified through Gram staining and culture at 37°C on conventional media (blood, chocolate and MacConkey agar), with biochemical tests for confirmation (Table 4.1). Fungal and mycobacterial growth was investigated as described below.

Figure 4.1 Fluorescence of a blood culture bottle under a UV Woods lamp.

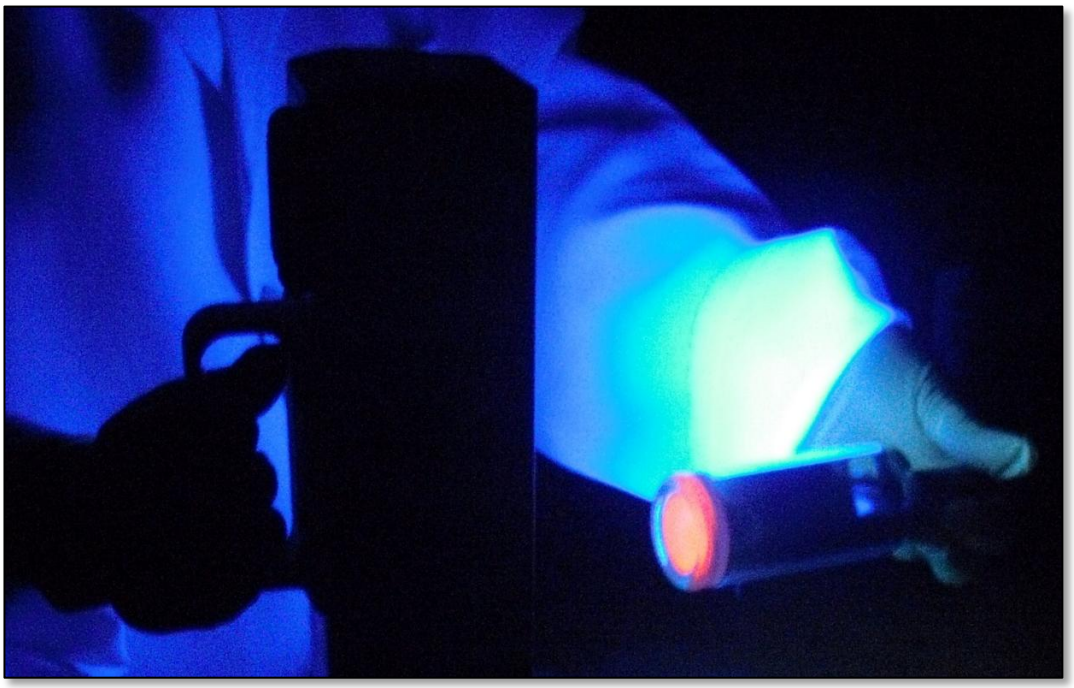


Table 4.1 Tests used for confirmation of the identity of cultured bacterial and fungal pathogens

Appearance on culture	Suspected organism on culture	Identification Test
Gram +ve cocci (clusters)	Staphylococcus spp	• Catalase, coagulase and DNAase test: to distinguish between *Staph aureus* and coagulase-negative Staphylococcus
Gram +ve cocci (chains)	Streptococcus spp	• *(Haemolysis pattern on culture agar: α vs. β vs. none)* Optochin test to distinguish between *Strep pneumonia* and *Strep viridians*
Gram -ve bacilli	Coliforms	• Carbohydrate utilisation to distinguish between lactose fermenting (LFC) vs. non-lactose fermenting coliforms • Indole, Kligler Iron tube (carbohydrate utilisation, gas production, H_2S production) to distinguish between Salmonella spp, *Escherichia.coli*, Enterobacter spp
Gram -ve bacilli	Pseudomonas spp	• Oxidase test
Gram -ve bacilli	Salmonella spp	• Serological testing (O & H)
Gram +ve yeast		• Urease test to identify Cryptococcus spp • Germ tube test to identify *Candida albicans*

Concentrated decontaminated sputum specimens and positive blood cultures were examined under fluorescent microscopy (Auramine-O) and cultured for mycobacteria (Lowenstein-Jensen media [LJ]). Species identification used MBP64 lateral flow antigen capture tests (Capillia), microscopic cording, and culture morphology and growth characteristics at 37 deg C (one LJ slope and one PNB-containing LJ slope), room temperature and 45 deg C. Cerebrospinal fluid (CSF) and positive blood cultures were investigated for Cryptococcus using India ink contrast staining, culture on Sabouraud media, and CrAg detection. CrAg was detected using latex agglutination (IMMY, Alpha Laboratories), with agglutination at a serum dilution at 1:8 being considered positive. Induced sputum was stained with Grocott's silver stain. Thick blood films for malaria were examined under a microscope after Giemsa staining.

4.2.4 Sample size calculations

We estimated that between 2 to 4% of all Zimbabwean 10 year olds were likely to be HIV-infected LTS based on a forward projection from a demographic model of the Zimbabwean general population that included UNAIDS estimates of the magnitude and time course of the adult HIV epidemic, the risk of mother to child transmission, and an estimated 36% likelihood of survival to adolescence with untreated maternally acquired HIV.[194] Studies estimating the relative rate of all-cause hospitalisation and death by HIV status in other age-groups have given incidence rate ratios in the region of 15 to 25 leading us to project a likely HIV prevalence in acute adolescent admissions of 25% to 50%.[105 335] A sample size of 310 was chosen to detect an approximate 5% confidence interval (CI) around the estimated HIV prevalence at a 95% confidence level. This would provide 80-90% power to detect differences between the HIV-infected and HIV-negative group for the considered exposures (orphanhood, stunting, pubertal delay, previous TB) with 95% confidence, in the considered range of HIV prevalence (Table 4.2).

Table 4.2 Sample sizes required to detect an association between HIV status and the considered exposure at the given power. The table gives the expected range of frequency of exposures

Overall HIV prevalence	Prevalence of exposure		Sample size	Power
	HIV+ve	HIV-ve		
25%	50%	25%	265	90%
	30%	10%	293	90%
	15%	2%	294	80%
	20%	5%	297	80%
50%	50%	30%	303	90%
	30%	15%	309	80%
	15%	2%	254	90%
	20%	5%	261	90%

4.2.5 Data analysis

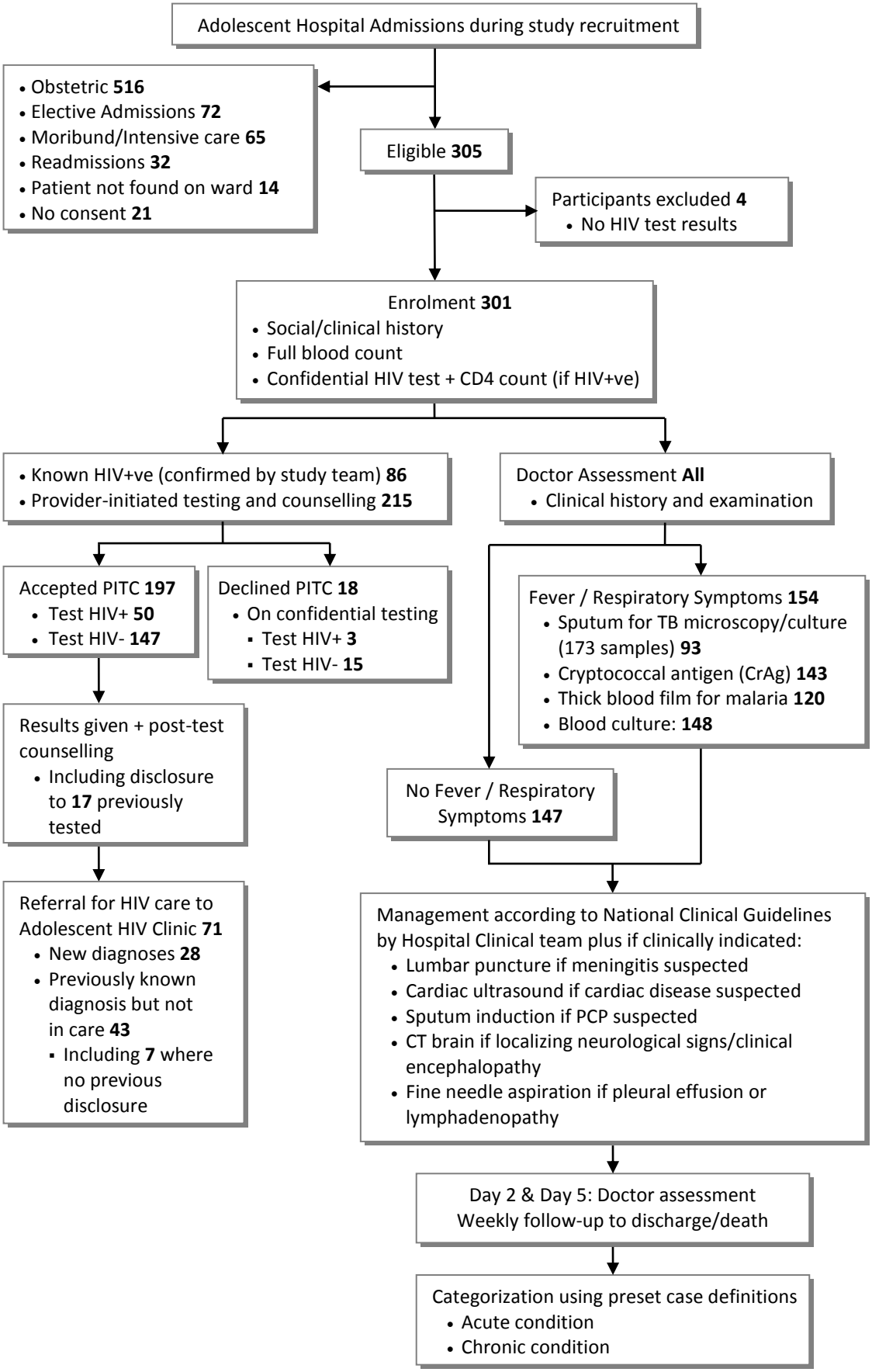
Continuous variables were compared using Student's *t* test for normally distributed variables and Mann Whitney U test for variables not normally distributed. Categorical variables were compared using the chi-squared (χ^2) or Fisher's exact test as appropriate.

Multivariate logistic regression was used for analysis of risk factors for mortality using a conceptual framework.[337] Potential risk factors were considered in two groups: socio-demographic (sex, age, orphanhood, difficulty raising clinic fees, TB or illness in a household member, food shortage, type of care-giver) and clinical (stage of HIV, previous TB, ART status, stunting, wasting, pubertal delay, chronic disease, anaemia, poor performance scores, self-perceived poor health, broad diagnosis category). An initial model retained all socio-demographic factors that reached statistical significance at $p < 0.05$ on multivariate adjustment for confounding with other variables in this subgroup. Independent risk factors from the clinical variables were then assessed by adding each one into the socio-demographic multivariate model. The multivariate model was developed further by excluding single factors sequentially (starting from the least significant) until all remaining factors were statistically significant.

4.3 Results

Of 1,025 total adolescent hospital admissions, 340 were eligible. 301 participants were recruited, with a refusal rate of 6% (Figure 4.2).

Figure 4.2 Study recruitment procedure



4.3.1 Socio-demographic and clinical characteristics

Table 4.3 Baseline demographic, clinical and growth characteristics of adolescents admitted to hospital (n = 301 unless specified otherwise)

Characteristic	No. (%) of Participants		*p*-Value
	HIV+ve N = 139	HIV-ve N = 162	
Demographic			
Age (years)			
<12	41 (29)	47 (29)	0.025
12- 15	69 (50)	60 (37)	
>15	29 (21)	55 (34)	
Female	65 (47)	65 (40)	0.25
Orphan			
Maternal or double	88 (63)	25 (15)	<0.001
Paternal	24 (17)	34 (21)	0.41
Married	1 (1)	8 (5)	0.041
Currently attending school	97 (70)	116 (72)	0.73
Previously tested for HIV	103 (74)	20 (12)	<0.001
HIV status already known by participant	86 (62)	20 (12)	<0.001
Clinical			
Previous TB treatment	61 (44)	3 (2)	<0.001
Self-rated general health "fair" or "poor"	120 (86)	49 (30)	<0.001
Poor performance scores (WHO scale 3/4)[a]	64 (46)	20 (12)	<0.001
Growth and sexual development			
Weight-for-age $z < -2$ ($n = 266$)[b]	88 (72)	29 (20)	<0.001
Height-for-age $z < -2$ ($n = 263$)[c]	63 (52)	32 (23)	<0.001
Body mass index $z < -2$ ($n = 263$)[c]	74 (73)	28 (27)	<0.001
Median MUAC, mm (IQR) ($n = 296$) [d]	172.5 (147-190.5)	204.5 (180-231)	<0.001
Pubertal delay	20 (14)	4 (2)	<0.001
Occurrence of menarche (females only)	18 (28)	34 (52)	0.005

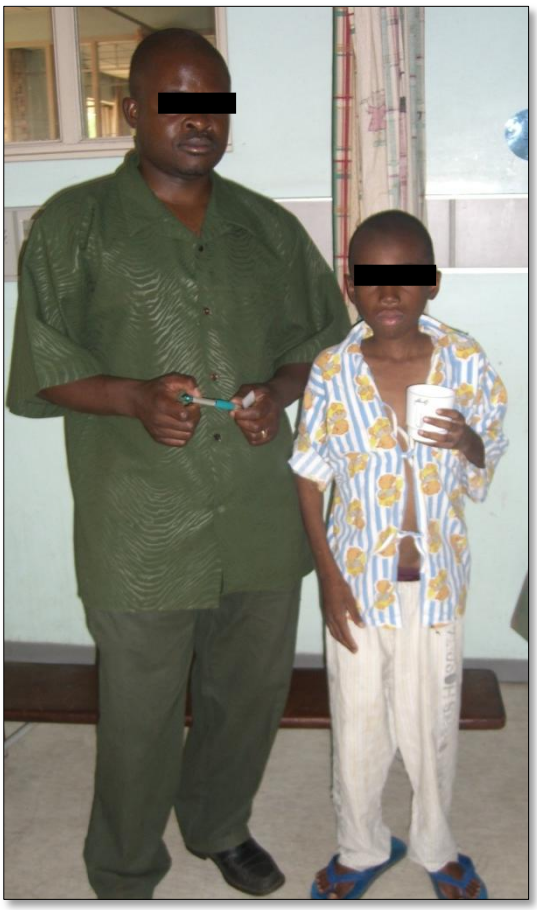
[a] Scale 3: bedridden, <50% of the day during the last month; scale 4: bedridden, >50% of the day during the last month.

[b] Data missing for 35 patients due to inability to stand: 20 too ill, 8 fractured lower limb, 4 chronic disability, 3 pathology in lower limb (2 joint infection/1 soft tissue infection).

[c] Data missing for 38 patients: 35 as for [b] plus 2 unable to stand upright and 1 acute confusion.

[d] Data reported as median, not as percentage of patients

Figure 4.3 Stunting (Height-for-age z-score -3.54) in a 14-year old HIV-infected male participant shown next to the study research assistant



Participants and their guardians consented to having their pictures taken and the images being used for scholarly contributions.

4.3.2 Burden of HIV infection

The prevalence of HIV was extremely high, but within our anticipated range, at 46%. The median age at diagnosis of HIV infection was 12 years (IQR 11-14). Of the 139 participants who were HIV-positive, 86 (62%) had tested HIV-positive prior to admission and knew their HIV status. Fifty participants tested positive following PITC; of these, 17 had tested HIV-positive previously but had not been told of their HIV infection. The median age of the participants who had previously tested HIV-positive but were unaware of their diagnosis was 13

years (IQR: 12-15 years). All guardians, however, agreed to disclosure to the participants with assistance of study counsellors. Only 18 (6%) participants declined PITC, of whom three were HIV-positive on unreported HIV testing and are likely to remain unaware of their HIV infection.

Of the 103 HIV-infected participants who had tested HIV-positive prior to this admission (with or without their knowledge), the median age at testing was 12 years (IQR 10-14) and 88 (85%) had tested within two years of this admission. Ninety four (91%) participants cited either chronic ill-health and/or hospitalisation with an HIV-related illness as a reason for HIV testing. Of the 103 participants, 78 (75%) were taking cotrimoxazole (9 of whom were unaware of their HIV status), and 44 (43%) were taking ART (3 of whom were unaware of their HIV status) for a median duration of 121 days.

4.3.3 Stage of HIV Infection

HIV-infected participants were profoundly immunosuppressed at presentation: 115 (83%) participants had WHO stage 3 or 4 disease and the overall median CD4 count was 151cells/µl (IQR 57-328). Interestingly, there was no significant difference in CD4 count by ART status ($p<0.59$): this may reflect the short length of time that patients had been on ART.

4.3.4 Causes of hospitalisation

The most frequent diagnosis among HIV-infected participants was infection with TB, pneumonia, cryptococcosis, and septicaemia being the most common

diagnoses (Table 4.4). Among HIV-negative participants, the commonest cause of admission was trauma, followed by acute exacerbations of chronic medical conditions, predominantly cardiac (Table 4.4). The median duration of stay in hospital for HIV-infected participants was 9 days (IQR 6-16) and for HIV-negative participants 7 days (IQR 4-18).

4.3.4.1 Disease-specific microbiological findings

113 (81%) and 41 (25%) of HIV-infected and HIV-negative admissions ($p < 0.001$) met criteria for the standardised infectious screen, of whom 20 (18%) and two (5%) had positive blood cultures, respectively. The most frequently identified pathogens in HIV-infected participants were non-typhoidal *Salmonella* species (7 patients) and *M.tuberculosis* (4 patients).

Cryptococcus spp were identified in blood culture in three participants, in cerebrospinal fluid (CSF) culture in five patients, and seven participants had a positive serum CrAg only.

There were 27 TB diagnoses of which 13 were pulmonary TB: six were sputum smear- positive, one was culture-positive only, and six were radiological diagnoses (smear- and culture-negative pulmonary disease with failure to respond to broad-spectrum antibiotics, but response to TB treatment at 1 month). *M.tuberculosis* was identified in blood culture in five participants, and the remaining nine had TB meningitis (n=2), pleural TB (n=3), TB arthritis (n=1), pericardial TB (n=1), military TB (n=1) and intra-abdominal TB (n=1).

Table 4.4 Causes of admission among adolescents admitted to hospital

Cause of Admission (Up to Four Causes Allowed)	No. (%) of Participants		*p*-Value
	HIV+ve n = 139	HIV-ve n = 162	
Infection	96 (69)	30 (19)	<0.001[a]
Mycobacterial disease			
Any mycobacterial disease	25 (18)	3 (2)	-
TB	24	3	-
Mycobacterium avium-intracellulare disease	1	0	-
Bacterial infection			
Any bacterial infection	65 (47)	20 (12)	-
Acute pneumonia	24	1	-
Bronchitis	9	2	-
Meningitis	9	2	-
Soft tissue or bone/joint infection	2	5	-
Enteritis	5	3	-
Urinary tract infection	1	1	-
Ear, Nose and Throat infection	0	2	-
Sexually transmitted infection	1	1	-
Septicaemia	12	1	-
Organ abscess[a]	2	2	-
Fungal Infection			
Any fungal infection	35 (25)	0 (0)	-
Cryptococcosis	15	0	-
Oesophageal candidiasis	21	0	-
PCP	1	0	-
Other infection			
Any other infection	8 (6)	10 (6)	-
Malaria	2	5	-
Viral	3	3	-
Other[b]	3	2	-
HIV wasting syndrome	15 (11)	-	-
Traumatic injury	4 (3)	53 (33)	<0.001
Road-traffic accident	0	19	-
Assault	1	4	-
Accident in the home	3	30	-
Complications of previous trauma	1	6	-
Overdose/other psychiatric disorder	1 (0.7)	18 (11)	<0.001
Acute surgical	2 (1.4)	15 (9)	0.004
Acute medical non-infectious	53 (48)	52 (38)	0.080
Stroke	4	0	-
Cardiac failure	9	12	-
Exacerbation of chronic condition other than cardiac/respiratory	1	16	-
Malignancy	8	6	-
Severe anaemia (Hb < 7g/dl)	34	14	-
Drug toxicity[c]	4	0	-
Malnutrition	3	1	-
Other[d]	4	12	-

Data in shaded bars indicate numbers and percentages of participants with ≥1 diagnosis in that category

[a] HIV+ve: empyema 2; HIV-ve: intracranial abscess 2

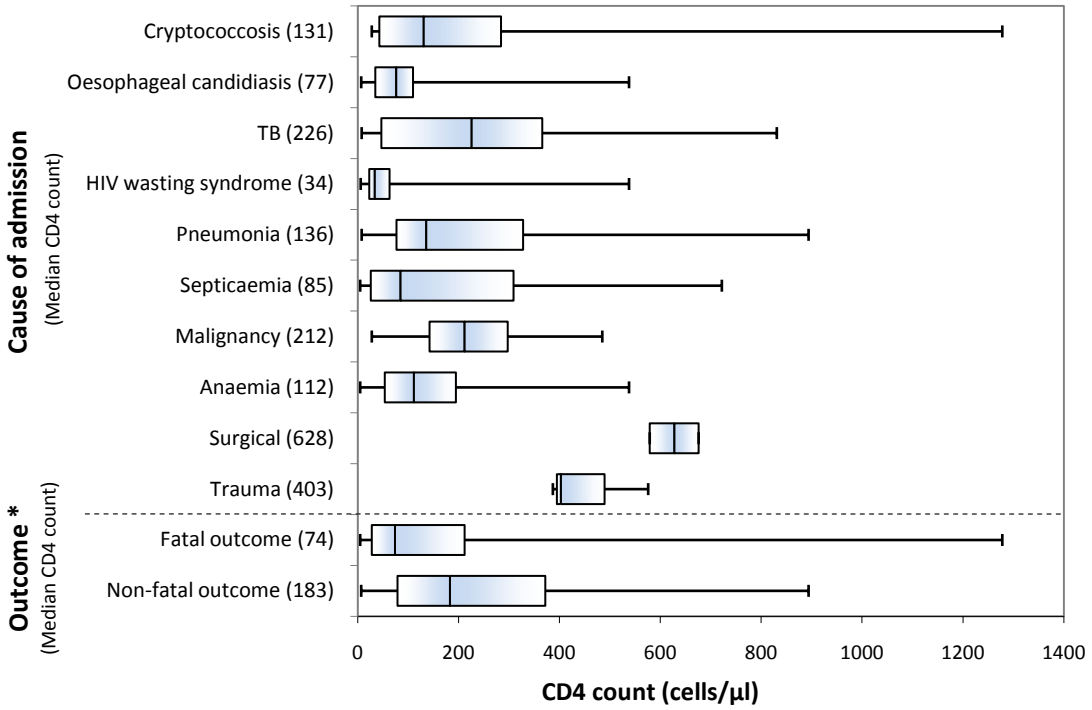
[b] HIV+ve: scabies 2; self-limiting fever 1; HIV-ve: rheumatic fever 1; self-limiting fever 1

[c] Stevens-Johnson syndrome due to cotrimoxazole 2; hepatitis due to nevirapine 1; lactic acidosis due to stavudine 1

[d] HIV+ve: anal fissures 1; renal condition 2; deep vein thrombosis 1; HIV-ve: renal condition 5; dysfunctional uterine bleeding 3; gastritis 2; hepatitis 1; pellagra 1

4.3.4.2 CD4+ counts by diagnostic group and outcome

Figure 4.4 Box-and-whisker plot showing median and range of CD4+ T lymphocyte counts in HIV-positive adolescents (regardless of ART status), according to cause and outcome of admission



Lower and upper line bars show the minimum and maximum CD4+ T lymphocyte counts and the lower, middle and upper points on the solid bars denote the 25th, median and 75% centile respectively. Median values are also shown in parentheses after admission categories. Values are based on CD4 T-lymphocyte counts recorded at admission for 10 participants with HIV wasting syndrome (67%), 16 participants with oesophageal candidiasis (76%), 11 participants with septicaemia (92%), 30 participants with anaemia (88%), 12 participants with cryptococcosis (80%), 21 participants with pneumonia (88%), 7 participants with malignancy (88%), 23 participants with TB (96%), 3 participants with trauma (75%) and 2 participants with surgical conditions (100%); and 23 participants with fatal outcomes (72%) and 102 participants with non-fatal outcomes (95%).

*p-value <0.003 for difference in median CD4 count in those with fatal *vs.* non-fatal outcome

4.3.5 Chronic clinical conditions

84 (28%) participants had underlying chronic medical conditions other than HIV (26% in HIV-infected versus 29% in HIV-negative, $p < 0.56$). In addition, 70% of HIV-infected participants had chronic skin complaints, although these

were rarely responsible for the admission. Admission as a result of acute exacerbation of a chronic condition accounted for 26 (19%) and 44 (27%) admissions in HIV-infected and HIV-negative participants, respectively (p <0.082). Chronic lung disease and cardiac disease were the most common serious HIV-related complications (Table 4.5). In HIV-negative participants, rheumatic heart disease was the most common chronic condition.

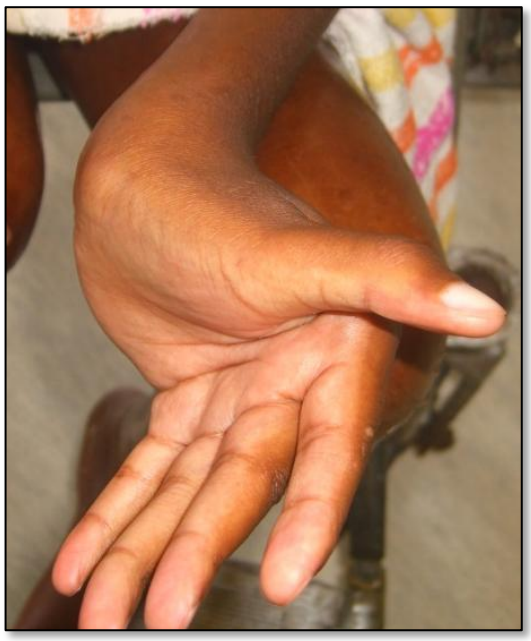
Table 4.5 Chronic conditions among adolescents admitted to hospital

Chronic Condition	No. (%) of Participants		*p*-Value
	HIV+ve N = 139	HIV-ve N = 162	
Chronic lung disease	17 (12)	0 (0)	<0.001
Cardiac disease	9 (6)	12 (7)	0.82
Rheumatic heart disease	0	9	-
Cor-pulmonale	9	0	-
Dilated cardiomyopathy	1	1	-
Other	0	2	-
Diabetes	0 (0)	7 (4)	0.016
Epilepsy	0 (0)	3 (2)	0.25
Asthma	0 (0)	4 (2)	0.13
Chronic skin disease	97 (70)	11 (7)	<0.001
Other chronic	23 (17)	25 (15)	0.63
Neurological	7	2	-
Malignancy[a]	8	6	-
Haematological	1	3	-
Chronic infection/inflammation	1	5	-
Blindness	4	0	-
Polyarthritis	1	2	-
Congenital	1	7	-

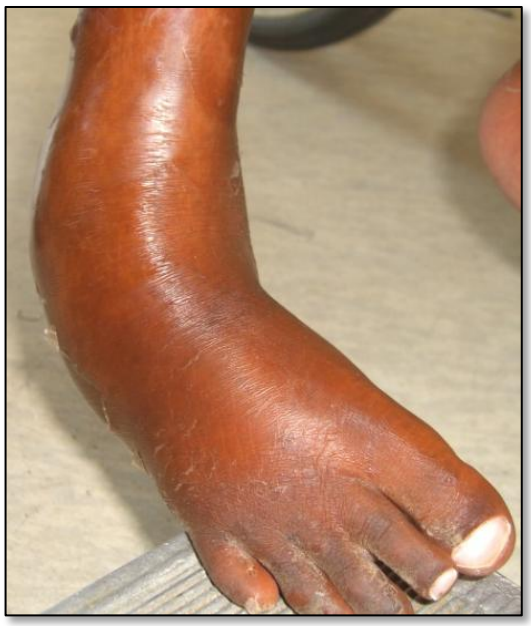
[a] HIV-ve: 4, Kaposi's Sarcoma; 2, Non-Hodgkins's lymphoma; 1, osteogenic sarcoma; 1, cholangiocarcinoma. HIV-negative: 3, haematological malignancy; 2, intracranial tumours; 1, myxoid neurofibroma

Figure 4.5 Chronic complications of HIV infection. A1 and A2) Burnt-out deforming seronegative arthritis in a 12 year old B) Chronic uveitis (complicated by corneal deposits in R eye and corneal perforation in the L eye) in a 15 year old

A1



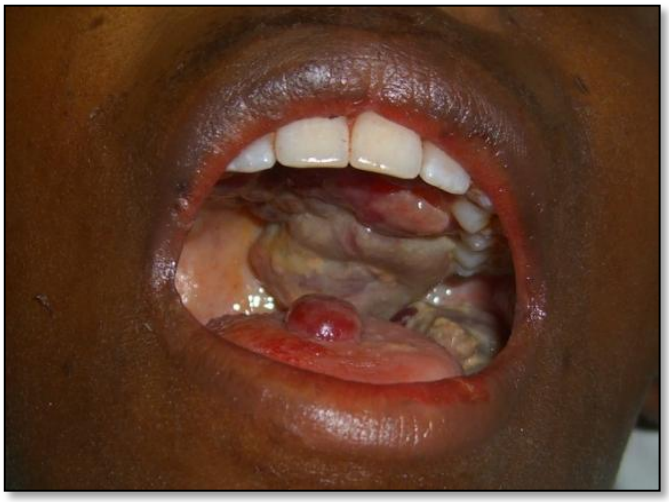
A2



B

Figure 4.6 Disseminated Kaposi Sarcoma in HIV-infected participants. A) Palatal and lingual disease B) Skin and lymphatic disease C) Lung disease

A



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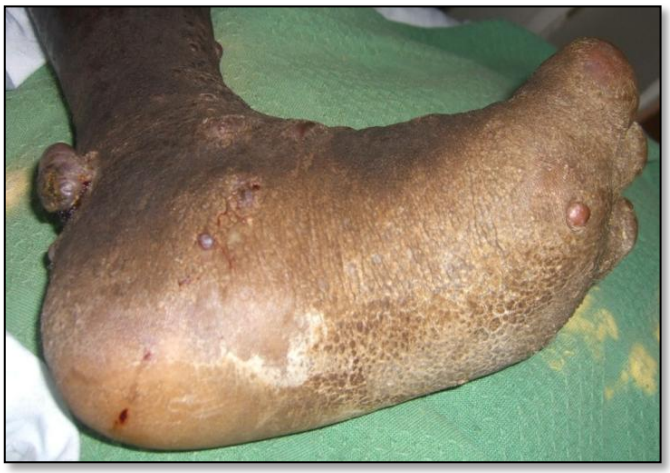


C

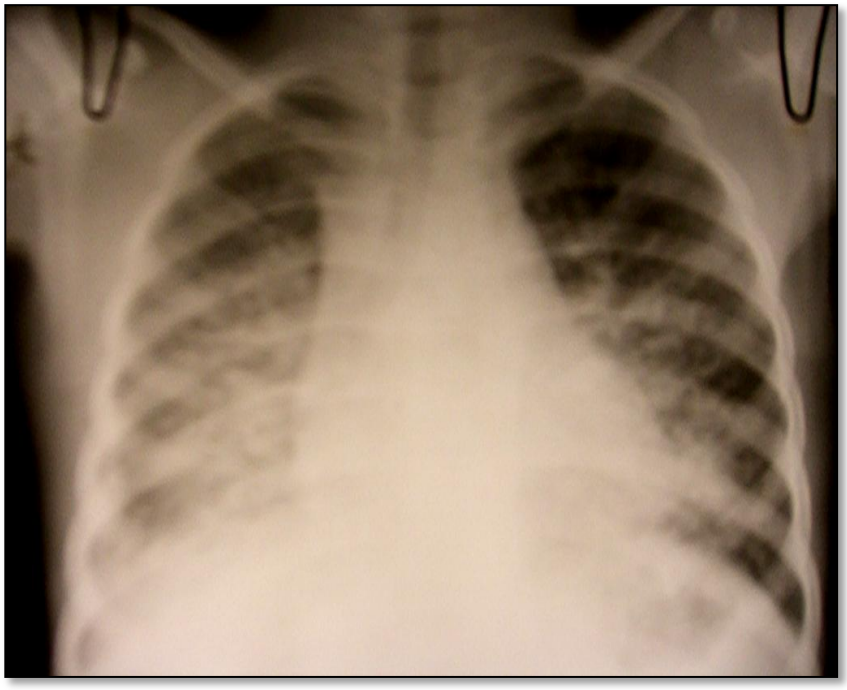
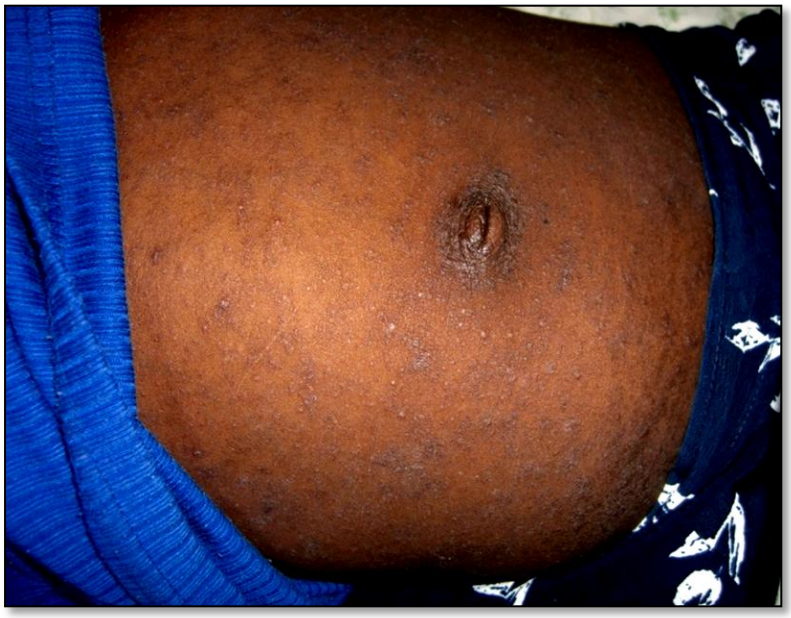
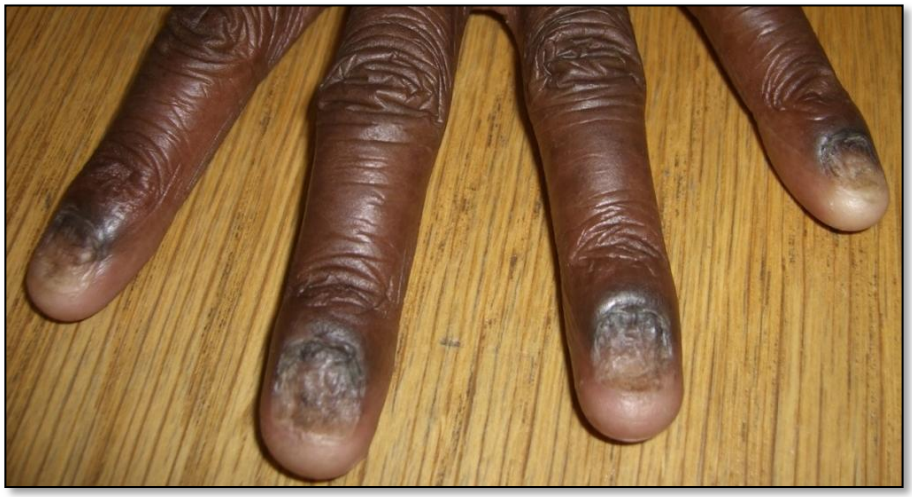


Figure 4.7 Chronic skin disease in HIV-infected participants. A) Widespread papular pruritic eruption (PPE) B) Onycholysis secondary to fungal nail infection C) Molluscum-contagiosum

A



B



C

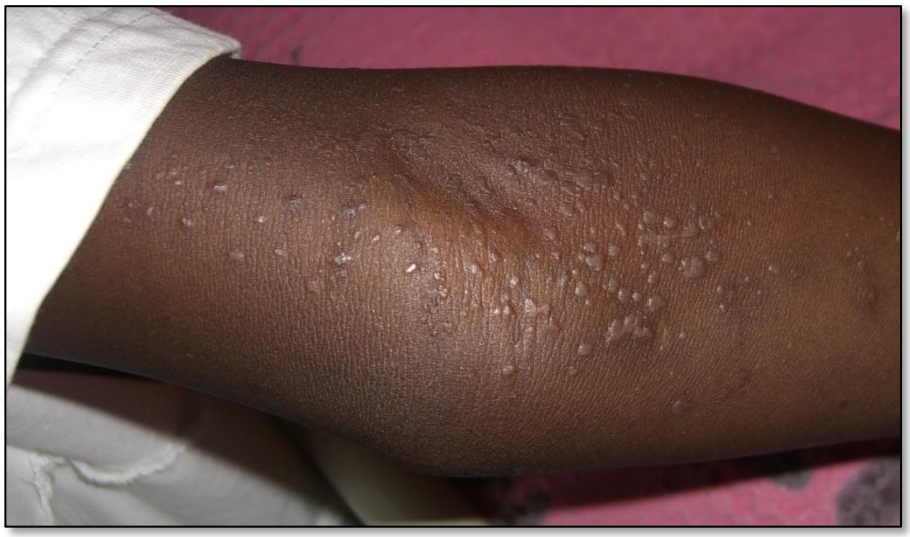
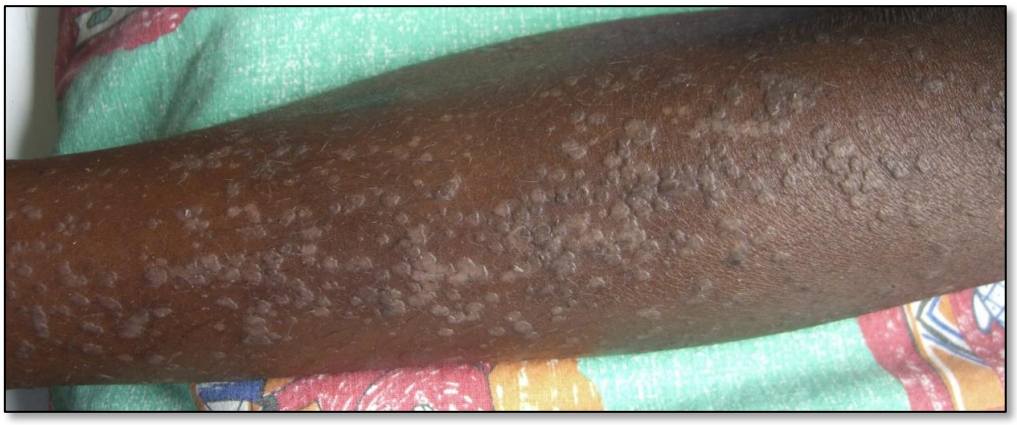


Figure 4.8 Human papilloma virus infection in HIV-infected participants. A1) and A2) Verruca plana B) Verruca vulgaris

A1



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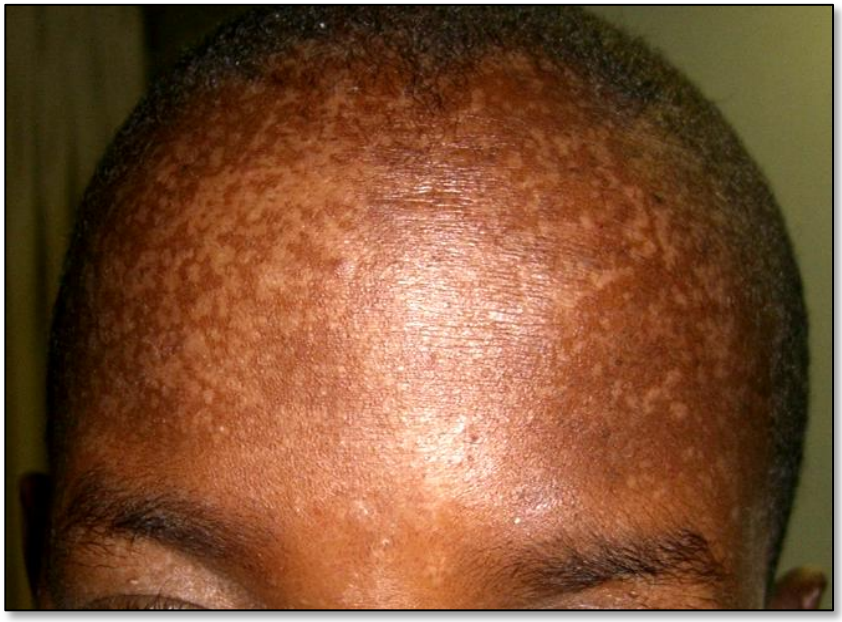

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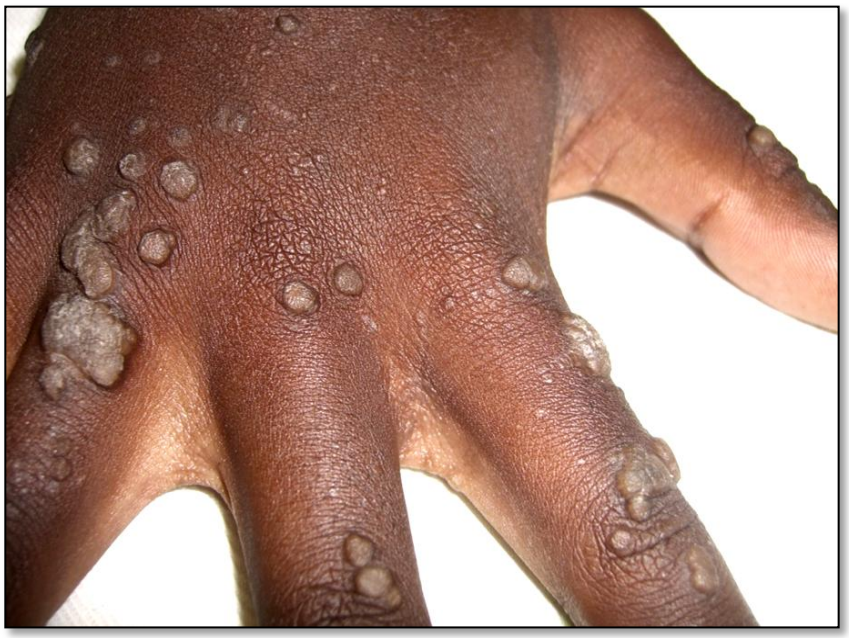


4.3.6 Causes of and risk factors for death

32/139 (23%) HIV-infected participants died in hospital compared with 11/162 (7%) HIV-negative participants (sex- and age-adjusted odds ratio [OR] for death for HIV-infected patients 3.7, 95% CI 1.7-7.8, $p < 0.001$). The median time to death was 12 days (IQR 4-21) in HIV-infected and 10 days (IQR 6-20) in HIV-negative participants.

Death was significantly associated with low CD4+ T lymphocyte count in HIV-infected patients (74 versus 183 cells/μl, $p < 0.003$). The highest case-fatality rates among HIV-infected participants were from HIV wasting syndrome (53%), any malignancy (50%), and drug toxicity (50%), and among HIV-negative participants the highest case-fatality rates were for malignancy (83%).

Table 4.6 Causes of death among adolescents admitted to hospital

Cause of Death	No. (%) of Deaths		No. Died/Total (Case Fatality Rate)
	HIV+ve (N = 32)	HIV-ve (N = 11)	
TB	4 (13)	1 (9)	5/27 (19)
Pneumonia	3 (9)	1 (9)	4/25 (16)
Meningitis	2 (6)	1 (9)	3/11 (27)
Bloodstream infection	2 (6)	0	2/13 (15)
Other infection[a]	1 (3)	1 (9)	2/11 (18)
Cryptococcosis	6 (19)	0	6/15 (40)
HIV wasting syndrome	8 (25)	0	8/15 (53)
Drug toxicity	2 (6)	0	2/4 (50)
Malignancy	4 (13)	5 (46)	9/14 (64)
Cardiac failure	0	2 (18)	2/21 (10)

[a] HIV-ve = empyema; HIV-ve = osteomyelitis

Table 4.7 Risk factors for death among adolescents admitted to hospital (n = 301 unless specified)

Variable	No. Died/Total (%)	Univariate OR (95% CI)	*p*-Value	Final Multivariate OR[c] (95% CI)	*p*-Value
Sociodemographic					
Age (years)					
> 15	5/84 (6)	1.0		1.0	
12 to 15	27/129 (21)	4.2 (1.5-11.4)	0.005	2.5 (0.8-7.4)	0.11
<12 y	11/88 (13)	2.3 (0.7-6.8)	0.15	2.0 (0.6-7.0)	0.25
Primary caregiver					
Biological parent	12/126 (10)	1.0		1.0	
Not biological parent	31/175 (18)	2.0 (1.0-4.2)	0.05	1.3 (0.5-2.9)	0.60
Clinical[a]					
HIV WHO stage[b]					
HIV-negative	11/162 (7)	1.0	-	1.0	-
HIV-infected and stage 1/2	2/24 (8)	1.2 (0.3-5.7)	0.85	1.0 (0.2-5.7)	0.99
HIV-infected and stage 3	5/36 (14)	1.6 (0.5-5.1)	0.42	1.6 (0.5-5.4)	0.49
HIV-infected and stage 4	25/79 (32)	5.1 (2.3-11.4)	<0.001	2.8 (1.1-7.1)	0.03
Previously treated for TB					
Yes	25/237 (11)	1.0	-	1.0	-
No	18/64 (28)	2.4 (1.2-4.9)	0.02	1.3 (0.6-3.2)	0.53
Poor performance score (WHO scale 3/4)					
No	12/217 (6)	1.0	-	1.0	-
Yes	31/84 (37)	8.2 (3.9-17.3)	<0.001	6.9 (3.0-15.8)	<0.001
Self-perceived poor health					
No	8/132 (6)	1.0	-	1.0	-
Yes	35/169 (21)	3.0 (1.3-6.9)	0.01	0.4 (0.1-1.5)	0.19
Pubertal delay					
No	34/276 (12)	1.0	-	1.0	-
Yes	9/25 (36)	3.1 (1.2-8.1)	0.021	4.0 (1.4-11.6)	0.01
Stunting (*n* = 263)					
No	11/168 (7)	1.0	-	1.0	-
Yes	17/95 (18)	3.1 (1.3-7.3)	0.01	1.2 (0.5-3.2)	0.69
Wasting (*n* = 266)					
No	6/149 (4)	1.0	-	1.0	-
Yes	23/117 (20)	5.2 (2.0-13.7)	0.001	2.1 (0.6-6.9)	0.23
Chronic disease (other than skin disease)					
No	22/21 (10)	1.0	-	1.0	-
Yes	21/84 (25)	2.7 (1.3-5.3)	0.005	2.8 (1.3-6.0)	<0.001
Haemoglobin (*n* = 282)					
>11g/dl	12/124 (10)	1.0	-	1.0	-
7.0-10.9 g/dl	13/110 (12)	1.0 (0.4-2.4)	0.97	0.4 (0.1-1.1)	0.07
<7.0 g/dl	14/48 (29)	3.6 (1.5-8.7)	0.005	0.8 (0.3-2.5)	0.72

[a] Univariate ORs adjusted for significant sociodemographic variables (age group and type of primary caregiver)

[b] Adult WHO staging system

[c] Adjusted for HIV stage, chronic disease, pubertal delay, and performance score

Of the considered socio-demographic risk factors, younger age and having a primary care-giver who was not the parent were associated with an increased risk of death (Table 4.7). After adjusting for these factors, advanced HIV disease, severe anaemia, TB treatment in the past, poor self-rated health, a chronic disease (except chronic skin disease), pubertal delay (Tanner puberty stage 1/2 in those aged 14 years or above), poor performance scores, wasting, and stunting were all associated with increased risk of death. In the final multivariate model, WHO stage 4 HIV infection (OR 2.8, 95% CI 1.1-7.1; $p < 0.032$), chronic disease (OR 2.8, 95% CI 1.3-6.0; $p < 0.009$), pubertal delay (OR 4.0, 95% C.I 1.4-11.6; $p < 0.011$), and poor performance scores (OR 6.9, 95% C.I 3.0-15.8; $p < 0.001$) remained independently associated with increased risk of death.

4.4 Discussion

4.4.1 HIV burden and HIV-associated morbidity

The main finding of this study high burden was that HIV infection is the single most common cause of acute hospital admission and in-hospital death. Nearly one in two adolescents admitted to hospital were HIV-infected, the majority with profound immunosuppression; the median CD4 count among untreated HIV-infected patients (141 cells/μl) was similar to that reported in other studies of hospitalised African adults in the pre-ART era as was the spectrum of HIV-associated infections.[105 338] However, adolescents had an additional and heavy burden of chronic complications such as growth failure, lung disease and cardiac disease, typically reported in HIV-infected children. In about a fifth of

HIV-infected patients, hospital admission was precipitated by an exacerbation of the underlying chronic condition. An underlying chronic disease (excluding skin disease) was independently associated with an increased risk of in-hospital death.

4.4.1.1 Chronic lung disease

More than 10% of HIV-infected patients met the case-definition for chronic lung disease and of these, 53% had end-stage complications of secondary right heart failure or cor pulmonale. The prevalence of chronic lung disease was felt to have been underestimated as a conservative case definition was used and it was difficult to distinguish underlying chronic lung disease in patients who were presenting with acute-on-chronic complications due to lack of pre-admission chest radiographs.

In order to investigate this possibility further I, therefore, carried out a cross-sectional survey for chronic lung disease in stable HIV-infected adolescents drawn systematically from two adolescent HIV care clinics in Harare. The results are summarised in Appendix B and confirm the impression that underlying chronic lung disease is highly prevalent in HIV-infected adolescent LTS and is one of the main threats to survival among adolescents on ART.

4.4.1.2 Skin disease

Skin disease, although incidental to the need for admission in only a small minority of cases, was an extremely common manifestation of HIV infection,

and should be a strong indicator for diagnostic HIV testing. Although not commonly life-threatening, skin disease is associated with considerable psychological morbidity.[339] In communities with a high burden of HIV, social isolation is compounded by the strong association of skin disease with HIV and subsequent stigmatization.[340]

4.4.2 Delay in diagnosis of HIV infection

One of the major findings was that diagnosis of HIV infection was frequently delayed in this age group until presentation with advanced HIV disease. A substantial minority had not been diagnosed with HIV before hospitalisation, and most others reported relatively recent diagnosis following a prolonged history of recurrent infections and chronic ill-health. The beneficial effects of early HIV diagnosis and ART on reducing the risk of opportunistic infections and reducing mortality are well-recognised in children.[341] In addition to irreversible chronic complications such as those observed in the study, older age at diagnosis and delay in starting ART may potentially result in suboptimal immune response and blunted catch-up growth and pubertal development.[262-263 342]

The findings of the study strongly support implementation of PITC for all patients in this age group attending health facilities, and stimulated the study at primary care level described in Chapter 6, as our data suggest that by the time LTS are seen at hospital level they are already critically ill and have a high burden of irreversible complications, notably so for lung disease. The high

uptake of HIV testing in this study indicates that PITC is acceptable to adolescents and their guardians at least in a hospital setting.

Reluctance of guardians to disclose the true nature of the underlying illness was also apparent in that a substantial minority of the HIV-infected participants had not been told their HIV test results and some participants were even taking cotrimoxazole or ART without knowledge of their diagnosis. Non-disclosure is associated with anxiety, reduced likelihood of accepting medical care and exclusion from social support.[303] Clear advice to health professionals to not only offer PITC but also to assist guardians with disclosure may improve timely diagnosis and adherence to subsequent ART.[343]

4.4.3 Other implications of the study findings

The high prevalence of stunting, pubertal delay and other established complications discussed above, as well as the history of chronic ill-health (which prompted HIV testing in the majority of cases) are indicative of longstanding HIV infection, and consistent with MTCT being the major route of infection (as explored further in Chapter 5).

The widely held perception until recently has been that, due to the high risk of rapid progression in HIV-infected infants, survival to late childhood and adolescence with untreated HIV was unusual. Thus, adolescents and older children have been considered to be at low risk of long-standing HIV infection, which has resulted in failure to prioritise this age-group in HIV testing and care

programmes. The findings of this study, however, suggest that long-term survival following MTCT is now a major cause of morbidity among adolescents in Zimbabwe and likely to be so in other Southern African countries.[194] Given the potential implications of the study findings, the mode of HIV acquisition among study participants was explored and the results are presented in Chapter 5.

4.5 Limitations of the study and generalisability

The study was hospital-based and therefore focused on sicker patients and more severe HIV-associated complications. One of the study sites was based at a referral hospital, which may have resulted in a higher proportion of specialist diagnoses such as cancer.

Both hospitals had ART clinics, but only nine (6%) of the HIV-infected participants were admitted from these: instead the vast majority of our participants (87%) were referred directly from primary health care clinics or through hospital casualty departments and there was a high participation rate (94% of eligible subjects). The only local alternatives to these two hospitals are private facilities. The results are, therefore, likely to be representative of the pattern of acute severe morbidity and mortality in Harare.

Additional studies in Zimbabwe and the region that lend support to these findings being generalisable to the rest of Zimbabwe and most likely the Southern African region are that a high proportion of 0 to 19 year olds

attending HIV care clinics throughout Zimbabwe (ranging from 25% to 56% in the eight provinces of Zimbabwe) are in the 10 to 19 year-old age-group, and that similar (although delayed onset) epidemics of LTS were projected for South Africa .[194 344]

5 Mode of transmission of HIV infection among hospitalised adolescents: an exploratory study

5.1 Introduction

As discussed in Chapters 2 and 4, HIV with advanced immunosuppression and characteristics suggesting long-standing infection, potentially dating back to infancy, now features as a prominent cause of morbidity in African older children and adolescents, having been rarely encountered in the early years of the HIV epidemic.[23] This suggests either long-term survival from maternal transmission during infancy which would not have been obvious early on during the HIV epidemic given the intrinsic time lag between HIV infection and reaching adolescence. Alternatively, HIV transmission by sexual or parenteral routes is more common during childhood than has been anticipated. This Chapter aims to summarise the evidence for and against each of these alternatives as being the predominant mode of transmission in the HIV-infected adolescent participants from the study described in Chapter 4.

Investigating the likely mode of transmission was important because natural history cohort data from which to directly estimate survival probabilities for HIV-infected children growing up in resource-limited settings are limited to the first few years of life. As such, indirect approaches have instead been used to estimate survival probabilities beyond the age of 5 years. More recent estimates have assumed two distinct subgroups of infants: the majority (fast progressor group) assumed to have exponential mortality with a median survival of less than one year, and the slow progressors assumed to have a

much longer median survival in the range of 8 to 16 years.[8-9 194] These have superseded earlier models based on extrapolation of infant survival, resulting in underestimation of the probability of survival beyond 5 years of age. Similarly, there are no good data on the frequency of sexual or parenteral exposure to HIV at a young age.

When the HIV epidemic was initially identified, the routes of HIV transmission were established through epidemiological investigations. Unlike in North America and Europe, in early studies African AIDS patients rarely reported a history of homosexual activity or of intravenous drug use. Several lines of epidemiological evidence supported heterosexual transmission in Africa. These included demographic factors (1:1 male to female ratio among cases, younger age and single marital status for female cases) and sexual factors (higher number of sexual partners and sexual exposures and association with STIs in cases).[23] The association of HIV seropositivity with blood transfusions and number of medical injections particularly in children who had HIV seronegative mothers also identified a parenteral route of HIV transmission.[192] Similar data helped discount the role of arthropods in HIV transmission; the lack of evidence for increased exposure to HIV infection among non-spousal household contacts of AIDS suggested that insect transmission did not occur over short distances and there was a very low prevalence in children between 1 and 15 years of age in Kinshasa, where there is a high incidence of malaria in childhood.[23 192]

Table 5.1 Comparison of features expected from different modes of HIV transmission in adolescents

Demographic feature or risk factor	Long-term survival following mother-to-child transmission	Parenteral	Sexual	
			Pre-pubertal	Post-pubertal
Sex ratio-F:M	Equal	Equal	F>M	F>>M
Family history	Suggestive of HIV-affected family: parental/ sibling ill-health or death (except rare instances of surrogate infant breast-feeding)	No direct link to family health	No direct link to family health	No direct link to family health
Exposure to parenteral routes of HIV transmission[a]	Low	High	Low	Low
Concurrent STIs	Low risk (below healthy peers)	Low risk (same as healthy peers)	High risk (above healthy peers)	High risk (above healthy peers)
HBV/HCV prevalence	Background prevalence	High	High	High
HSV-2 prevalence	Very low	Very Low	High	High
Presentation with Acute seroconversion illness	No	Yes	Yes	Yes
Growth Failure	High prevalence of stunting	Variable, according to age at infection	Variable, according to age at infection	None
Sexual maturity	Delayed puberty	Variable delay in pubertal development, according to age at infection	Variable delay in pubertal development, according to age at infection	No delay in pubertal development
Chronic complications of childhood HIV infection	Potentially high prevalence of disorders linked to slow progression in children (LIP) and/or favourable HLA genotype (HLA-27 and sero-negative arthritis)	Variable risk, according to age at infection	Variable risk, according to age at infection	No

[a] blood transfusions, scarification, unsafe injections

We used a similar approach described above to explore the likely mode of transmission among hospitalised HIV-infected adolescents. The putative association of demographic, clinical, parenteral and sexual factors with different modes of transmission is summarised in Table 5.1. The prevalence of these factors were compared in HIV-infected and uninfected participants to explore the likely mode of transmission. Although we anticipated that it would not be possible to categorise the mode of transmission in any given individual with complete confidence, we hypothesised that associated factors may be sufficiently characteristic at group level to allow identification of the predominant mode of transmission with reasonable certainty.

5.2 Methods

The participant selection process is described in the previous Chapter (Section 4.2.1).

5.2.1 Exploring route of HIV acquisition

A self-completion questionnaire (with no personal identifiers except a study number) investigating sexual behaviour was given to participants aged 12 years and older. The questionnaire was an abbreviated version of one developed, piloted and used in the interim survey of a cluster randomized trial evaluating a community-based, multi-component HIV and reproductive health intervention among rural Zimbabwean youth (Frances Cowan, Lisa Langhaug, Webster Mavhu: *Regai Dzive Shiri* Project).[345] HSV-2 serology was used as a biological marker for sexual activity.[328]

A standardised questionnaire was administered to the participant to ascertain history of personal illness and pregnancy, knowledge of HIV, perceived risks of personal HIV infection, history of possible parenteral exposure to HIV and reasons for previous HIV testing. Responses indirectly indicating previous sexual exposure (e.g. previous pregnancy, marriage, and previous testing due to sexual risk or pregnancy) were compared with data obtained from the sexual behaviour questionnaires for inconsistency in reporting sexual behaviour.

A detailed family clinical history was recorded for each participant through interview with the participant and the guardian. This included vital status, age at and cause of death, HIV status and history of TB and prolonged ill-health in biological parents and natural siblings (defined as those with the same biological mother as participants).

5.2.2 Data analysis

The prevalence of factors suggestive of the considered routes of HIV transmission (parenteral, sexual, and vertical) was compared between HIV-infected and uninfected participants using the chi-squared (χ^2) or Fisher's exact test as appropriate.

5.3 Results

Data on personal and family clinical history, sexual history and parenteral HIV risk factors is summarised in Table 5.2. Unlike other studies which show a much higher incidence of HIV infection among females than males in young people, there was no difference in HIV prevalence by gender, and HIV-infected participants were

significantly less likely to be married or have experienced sexual debut. Four (1.3%) participants tested HSV-2 positive, of whom two were HIV-positive and with a history of previous sexual exposure. All partners of married participants were alive but their HIV status was not known.

HIV-infected participants were more likely to be maternal, but not paternal orphans and to give a history of death of natural siblings. Additionally, a higher proportion of HIV-infected participants reported HIV or TB as the cause of death of their parents and HIV infection in their parents compared to HIV-negative participants. HIV-infected participants themselves, were more likely to have had multiple hospital admissions and frequent clinic visits, suggesting chronic ill-health.

There was no association between HIV status and potential parenteral risk factors for HIV infection. It is notable that nearly all participants (96%) were breastfed by their biological mother; three participants were also breastfed by a non-biological mother.

Table 5.2 Factors expected to vary by route of HIV transmission among the study population (139 HIV+ve & 162 HIV-ve participants)

Factor	No. (%) *unless otherwise specified*		*p*-value	No. of missing responses	
	HIV+ve	HIV-ve		HIV+ve	HIV-ve
Female	65 (47)	65 (40)	0.25	0	0
Past Clinical history					
>3 clinic visits/year in ≥3 of past 5yrs	42 (31)	11 (7)	<0.001	2	1
≥2 previous hospital admissions	39 (29)	21 (13)	<0.001	6	1
Cause of 1st ever hospitalisation					
Never hospitalised	51 (37)	105 (65)			
Infection/malnutrition/TB	60 (44)	14 (9)		2	0
Non-HIV related	15 (11)	36 (22)			
Other[a]	11 (8)	7 (4)			
Median (IQR) age of 1st ever hospitalisation	8.5 (2-11)	8 (3-11)	0.51		
Family history					
Orphan					
Maternal	31 (22)	8 (5)	<0.001	1	0
Paternal	24 (17)	34 (21)	0.41	3	4
Double	57 (41)	17 (10)	<0.001		
Cause of death in mothers (n=113)					
HIV-related/TB/pneumonia	35 (40)	10 (11)	<0.029	43	13
Non-infection related	5 (20)	7 (28)			
Cause of death in fathers (n=132)*			<0.051		
HIV-related/TB/pneumonia	35 (43)	10 (20)		34	31
Non-infection related	12 (15)	10 (20)			
Mother HIV-infected*	35 (25)	7 (4)	<0.001	101	137
Father HIV-infected*	27 (19)	4 (3)	<0.001	108	144
Siblings HIV-infected[a]	9 (4)	0 (0)	<0.001	196	367
No. of natural siblings who had died[b]	26 (12)	15 (4)	<0.001	1	0
Sexual history					
HSV-2 seropositive	2 (1)	2 (1)	0.88	0	0
Married	1 (1)	8 (5)	0.041	0	0
Self-reported sexual debut (n=150)[c]	6 (9)	19 (22)	0.032	72	79
Self-reported forced sexual debut (n=149)[d]	5 (8)	4 (5)	0.46	73	79
Parenteral risk factors					
Received injections at rural health clinic	20 (14)	31 (19)	0.27	0	0
Received injections from traditional healer	1 (0.7)	0 (0)	0.46	0	0
Scarification	3 (2)	11 (7)	0.10	0	0
One or more surgical procedures	12 (9)	33 (20)	0.004	0	0
Median (IQR) number of injections	10 (7-15)	8 (5-11)	0.61	0	0
Received at least one blood transfusion	15 (11)	10 (6)	0.15	0	0

[a] Cardiac failure 5; arthritis 2; stroke 1; acute flaccid paralysis 1; no cause given 9

[b] Total 627 siblings: HIV-ve participants 225 siblings; HIV-negative participants 407 siblings

[c] Total 150 participants: 67 HIV+ve; 83 HIV-ve; questionnaire not completed by 151 participants: 88 under 12 y, 63 too ill.

[d] Total 149 participants: 66 HIV+ve; 83 HIV-ve; questionnaire not completed by 151 participants: 88 under 12 y, 63 too ill, 1 no response given

* significant difference in missing responses in HIV+ve *vs.* HIV-ve

5.4 Discussion

5.4.1 Mode of HIV transmission

Table 5.3 summarises the evidence for each of the considered modes of HIV transmission from the data shown in Table 5.2, and supports MTCT as the predominant route of HIV acquisition in hospitalised HIV-infected adolescents. Low rates of self-reported sexual debut, and a much lower prevalence of HSV-2 infection than would be anticipated for sexually-acquired HIV in Southern Africans were observed in the study.[328] Although HSV-2 seropositivity does not establish an individual's source of infection, HSV-2 infection is a highly prevalent sexually-acquired infection in Southern Africans that significantly increases risk of HIV acquisition.[52 327 346] As such, it serves here as an independent marker of sexually-acquired HIV that can be used to corroborate the self-reported data concerning likely mode of transmission that was collected from participants. These findings, along with the negative association with marriage (given that marriage at young age increases risk of HIV for young African girls[14]), and the equal sex distribution of HIV infection in an age-group where females are at much higher risk of being HIV-infected through sexual exposure, makes recent sexual transmission very unlikely to be the major route.[14 347]

The strong association of HIV infection with chronic ill-health and with maternal and sibling (but not paternal) orphanhood or parental and sibling HIV infection are all consistent with long-term survival following MTCT as the source of HIV infection in most study participants. Of note, there was no association between HIV infection and possible risks factors for HIV acquisition through parenteral exposure. Zimbabwe is one of the few African countries to have had strong

policies to prevent parenteral transmission very early on in the course of the HIV epidemic and has an unusually good safety record for preventing percutaneous HIV-infection.[348-349]

Table 5.3 Evidence for and against considered modes of HIV transmission in HIV-infected adolescent participants

Observation expected to vary by mode of transmission	LTS following MTCT	Parenteral	Sexual: pre or post puberty		Comment
			Pre	Post	
Growth	+++	+	+	No	Severe stunting suggests young age at infection
F=M	+++	+++	No	No	Equal F:M ratio inconsistent with sexual transmission
Sexual maturity	+++	+	+++	No	Sexual immaturity suggests pre-pubertal infection
Concurrent STI	+++	+++	No	No	HSV-2 higher in HIV-ve than in HIV-infected
History of blood transfusion	+++	No	+++	+++	Guardians/participants uncommonly report parenteral risk
Scarification/unsafe injections	+++	No	+++	+++	
HBV (blood transmissible)	ND	ND	ND	ND	Not ascertained (Infant HBV vaccination available since 1996)
Lack of acute seroconversion illness	+++	+	No	No	None diagnosed
Family history	+++	No	No	No	Frequent maternal and sibling death/ known HIV infection
Childhood clinical history	+++	+	+	No	Frequent childhood hospitalisation and TB treatment
Chronic complications of early childhood (not adult) HIV infection*	+++	+	+	No	LIP and planar EV-associated warts highly prevalent

Observation consistency with postulated mode of HIV transmission: +++=highly consistent; +=consistent direction, but not extent, of finding; No=inconsistent; ND=not done

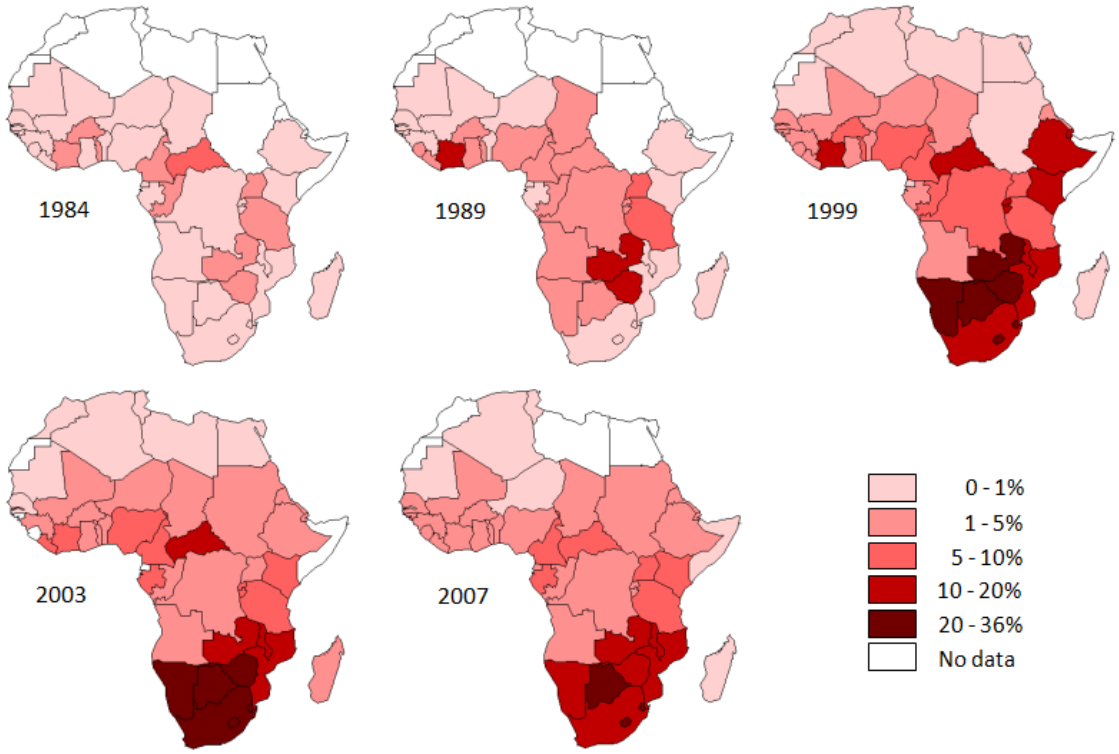
*Only occurs when HIV precedes infection with a second virus usually acquired in early childhood: LIP=lymphoid interstitial pneumonitis following EBV infection; EV=epidermodysplasia verruciformis (EV-associated planar warts following HPV infection)

5.4.2 Implications of study findings

If, as strongly suggested by this study, long-term survival following MTCT is the main cause if HIV infection in older childhood, then this raises the possibility that

survival with HIV infection from infancy is more likely than previously anticipated from earlier studies showing high mortality rates in early life among HIV-infected infants.[7] These findings are supported by population-based HIV prevalence surveys in Southern Africa (summarised in Chapter 2, Table 2.2) that have consistently reported HIV prevalence rates of 1-5% among older children, much higher than would be anticipated given the high HIV-associated mortality observed in the first two years of life.

Figure 5.1 Estimated HIV prevalence rate in 15-49 year olds in African countries (Source UNAIDS[350-353])



If MTCT is indeed the predominant mode of transmission, then HIV-infection reflecting long-term survival from the paediatric HIV epidemic in the 1990s, is now a major cause of adolescent morbidity and mortality in Harare. These findings support epidemiological model findings that predict an emerging epidemic of LTS

of MTCT in Zimbabwe.[194] Zimbabwe is unusual in having had high HIV prevalence rates from the late 1980s with a subsequent decline in HIV incidence (Figure 5.1) and so may be a few years ahead of other countries in the region that have had otherwise comparable but later onset HIV epidemics, with respect to the subsequent epidemic of HIV-infected adolescent LTS.[19]

The magnitude of the epidemic of LTS may be different in other low income countries in Africa because Harare has unusual features resulting in low competing causes of mortality in childhood. These include a) lack of malaria and b) a public service provision and town planning that have been unusually well implemented until the past few years, resulting in a low risk of enteric illnesses.[354] Hence, vertically-infected infants are more likely to survive childhood to reach adolescence.

5.5 Limitations of the study

The study was exploratory in nature and was not able to confirm source of HIV infection at individual level. There were three main sources of bias: Firstly, data on sources of parenteral exposure may not adequately capture the risk of transmission through this route, and data relied on recall by participants and their guardians; Secondly, the proportion of parents with unknown HIV status was significantly higher for HIV-negative than HIV-infected participants. The higher proportion of HIV infection in parents of HIV-infected participants may be a consequence of more parents of HIV-infected participants having previously tested. Similarly, a large proportion of siblings had not been HIV tested although in this case there was no difference in the proportions of siblings with unknown HIV

status by HIV status of the participant. Thirdly, sexual behaviour data was only available from half of the of the study population, the remainder being too ill or too young to complete the questionnaire. There is, however, no reason to believe that those who did not complete the questionnaire were at higher risk of sexual transmission than participants who completed the questionnaire. Participants may also have underreported sexual debut, and one (HIV-negative participant) who was HSV-2 serology positive denied being sexually active. However, the overall low HSV-2 serology rates support low rates of sexual activity. Production of antibodies is suppressed with advanced immunosuppression which may have resulted in underestimation of HSV-2 prevalence in HIV-infected immunosuppressed participants. However, this has not been demonstrated in other studies where the same assay was used.[355] Other potential limitations of the HSV-2 assay are discussed in Chapter 3 (Section 3.2.2).

6 Undiagnosed HIV infection among adolescents seeking primary health care in Zimbabwe

6.1 Introduction

The study detailed in Chapter 4 showed that hospitalised adolescents in Harare had a high burden of HIV frequently accompanied by one or more chronic complications typically described for untreated paediatric HIV infection. Inpatient mortality was extremely high, and although most cases were known to be HIV-infected by the time of admission, the diagnosis had often been made only recently but following a prolonged history of frequent minor illnesses managed at primary care level. It was thus postulated that there would be a higher proportion of undiagnosed HIV-infection and presentation with less advanced disease at primary care level compared to secondary care settings. If this were the case, then any diagnostic intervention would be best placed at primary care level, providing multiple opportunities to diagnose HIV among LTS of vertical HIV transmission before the development of life-threatening illnesses. Furthermore, there was a large gap between estimates of numbers of adolescent LTS in Zimbabwe in 2008 (43,357) and the numbers of adolescents in HIV care nationally (10,500), suggesting either a high burden of undiagnosed HIV in this age-group or alternatively that the projected numbers of vertically-infected adolescents had been over-estimated.[194 344]

The aims of the study detailed in this Chapter were to extend investigation of the prevalence, severity, and clinical manifestations of HIV-infection to primary care level. This study also provided the opportunity to further explore the mode of

transmission, and to investigate the acceptability of PITC among adolescents attending primary health care services in Harare, Zimbabwe.

6.2 Methods

6.2.1 Study population

Participants were recruited from Epworth and Mabvuku Clinics, two primary care polyclinics in the high-density suburbs of Harare, Zimbabwe, with catchment populations of about 120,000 and 60,000 people respectively. Primary care clinics are run by nurses and services offered by primary care polyclinics include acute care as well as ANC. ANC attendees, but not all acute clinical care attendees, are routinely offered PITC. Single-dose nevirapine to pregnant HIV-infected mothers is offered for PMTCT.

Individuals aged between 10 and 18 years attending the primary care polyclinics for any reason were enrolled consecutively on weekdays over a six-month period in 2009. ANC attendees were included in the study as a control group to investigate the association of factors suggestive of long-term survival following vertical transmission (discussed in Chapter 5) among APC attendees. The associations between these factors and HIV infection were compared in the ANC attendees (where HIV infection was acquired through sexual transmission) and among participants attending for APC.

Clinic attendees were not eligible if they were too ill to take part (defined as those who required urgent hospitalisation), had been previously enrolled in the study, or were aged below 16 years and not accompanied by a guardian.

6.2.2 Provider-initiated HIV testing and counselling

All participants were asked to consent to providing blood for unreported (anonymised) HIV testing for study purposes, and were offered PITC, either through study personnel (APC attendees) or as part of the PMTCT programme (ANC attendees). Group pre-test counselling and individual post-test counselling was given as part of the PITC process. The group counselling lasted for 30 minutes and basic facts about HIV/AIDS including modes of transmission, benefits of testing and the effects of HIV on the body were discussed. The testing process was explained and participants were given the opportunity to ask questions. Participants who declined HIV testing following group pre-test counselling were offered individual counselling. HIV-positive participants were started on cotrimoxazole, and were referred for HIV care to adolescent clinics at one of the two central hospitals in Harare, where CD4 counts were performed.

6.2.3 Mode of HIV transmission

HIV-infected APC attendees were given a self-administered questionnaire and asked to choose the most likely source of their HIV-infection from the following options: born with it/from mother; from injections or blood transfusion; from boyfriend/girlfriend or husband/wife; or from an unwanted sexual encounter. Study nurses assessed mode of transmission based on a case-definition derived from the hospital study (longstanding chronic, ill-health, sibling and maternal death, growth failure, no history of blood transfusions and sexual debut), but were not blinded to participants' responses. HSV-2 positivity was used as a biological marker of sexually-acquired HIV infection. [328]

6.2.4 Clinical assessment

All participants had MUAC, height, and weight measured and Tanner pubertal staging to assess growth.[324] Pre-set diagnostic algorithms were adapted from the WHO IMAAI to broadly classify the presenting complaint.[326]

6.2.5 Sample size calculations

A sample size of 250 was required to detect an approximate 5% CI around the estimated HIV prevalence of 15% at a 95% confidence level, and an assumption of a 10% participation refusal rate. The 15% estimate was based on the observed HIV prevalence among 2-9 year olds attending primary health care facilities in South Africa.[182] The estimated frequency of the exposure variables are shown in Table 6.1 (extrapolated from the results of the study described in Chapter 4). The chosen sample size provided 80-90% power to detect ORs of around 3.0 or higher between the considered risk factors and HIV infection, with a CI of 95%.

Table 6.1 Sample sizes required to detect a bivariate association between the considered exposure variable and HIV infection, with the given power, with a confidence level of 95%. The table gives the expected approximate prevalence of the considered variables

Variable	Prevalence of variable in HIV-ve	Power 80%			Power 90%		
		OR 2.5	OR 3.0	OR 5.0	OR 2.5	OR 3.0	OR 5.0
Previous TB/skin complaints	5%	972	630	246	1320	858	336
Maternal orphan	15%	414	282	120	558	372	162
Stunting	20%	354	240	108	474	318	144
Poor self-rated health	30%	306	216	108	402	282	138

The sample size was, however, doubled as the dataset was used to design an algorithm for identifying HIV infection among adolescents in primary care

(described in Chapter 8) which required the dataset to be randomly split into two. An ethics amendment was obtained to be able to recruit 500 participants.

6.2.6 Data analysis

A χ^2 or Fisher's exact test was used as a test for association between categorical variables, a Student's *t*-test for normally distributed variables and a Mann Whitney U test for variables not normally distributed. A p-value <0.05 was considered statistically significant.

6.3 Results

6.3.1 Demographic and clinical characteristics

Of 626 clinic attendees, 12 were not accompanied by a guardian, 7 were too ill to be recruited and 13 refused consent; the remaining 594 adolescent primary care attendees were recruited into the study, of whom 506 (85%) were APC attendees and 88 (15%) were ANC attendees. Nearly half of all participants were orphaned and 35% were not attending school (Table 6.2). ANC attendees were more likely than APC attendees to be married (85% *vs.* 5%, p<0.001) and were older (median age 17 *vs*. 14 years, p<0.001).

6.3.2 HIV prevalence

The overall HIV prevalence was 15%. APC attendees had a significantly higher HIV prevalence than ANC attendees (6%, p<0.007). Unlike hospitalised HIV-infected adolescents, where less than a third tested for the first time, 70 of the 86 HIV-infected APC participants (81%) were newly diagnosed by the study team. Newly diagnosed HIV-infected APC participants were less immunosuppressed than those

who tested for the first time during hospital admission (median CD4 count 329 *vs.* 204 cells/μl). Four newly diagnosed HIV-infected participants died before registering for HIV care.

Table 6.2 Baseline characteristics of participants by HIV status (n=594)

Characteristic	No. (%) of participants					
	APC attendees (N=506)			ANC attendees (N=88)		
	HIV+ve (n=86)	HIV-ve (n=420)	*p*-value	HIV+ve (n=5)	HIV-ve (n=83)	*p*-value
Median Age (IQR)	14 (11-16)	14 (11-16)	0.63	16 (15-17)	17 (16-18)	0.09
Female	48 (56%)	211 (50%)	0.35	5 (100%)	83 (100%)	-
Clinic site						
Epworth	35 (41%)	181 (43%)	0.69	5 (100%)	74 (89%)	0.44
Mabvuku	51 (59%)	239 (57%)		0 (0%)	9 (11%)	
Orphanhood Status						
Maternal orphan	15 (17%)	36 (8%)	0.013	0 (0%)	6 (7%)	0.53
Paternal orphan	21(24%)	84 (20%)	0.39	0 (0%)	18 (22%)	0.24
Double orphan	29 (34%)	56 (13%)	0.001	2 (40%)	13 (16%)	0.16
Attending School	62 (72%)	327 (78%)	0.23	0 (0%)	0 (0%)	-
Married	7 (8%)	18 (4%)	0.17	3 (60%)	72 (87%)	0.16
Biological parent as primary caregiver	34 (40%)	264 (63%)	0.001	0 (0%)	15 (18%)	0.58
Median (IQR) MUAC (mm)	202 (177-225)	219 (195-247)	0.001	250 (242-255)	243 (234-261)	0.94
Pubertal Delay	15 (17%)	27 (6%)	0.001	0 (0%)	0 (0%)	-
Median (IQR) Height-for age z-score	-1.7 (-2.63 - -0.76)	-0·71 (-1·42- -0.01)	0.001	-0.75 (-0.97- -0.11)	-0.77 (-1.3- -0.13)	0.98
Stunting	34 (40%)	51 (12%)	0.001	1 (20%)	7 (8%)	0.39
Median (IQR) Weight-for age z-score	-2.03 (-3.13 - -1.14)	-0.90 (-1.64- -0.27)	0.001	-0.2 (-0.64- 0.63)	0.08 (-0.43- 0.63)	0.55
Wasting	43 (50%)	63 (15%)	0.001	0 (0%)	0 (0%)	-

6.3.3 Presenting complaints

The most common presenting complaints in APC patients were diarrhoea, ear, nose and throat (ENT) infection and skin infection: HIV prevalence in patients presenting with these complaints ranged between 20 and 24% (Table 6.3), but was above that in routine ANC attendees (5%) for all categories except malaria.

Participants presenting with possible TB or an STI had the highest HIV prevalence (50% and 40%, respectively), with those presenting with malaria (5%), chronic lung or heart conditions (7%), trauma (8%) and urinary tract infection (8%) having the lowest risk of underlying HIV infection. HIV-infected participants were more likely to present with infectious conditions than HIV-negative participants (82% *vs*. 67%, p<0.001).

Table 6.3 Syndromic classification of the presenting complaints in 506 adolescents attending APC services and 88 ANC attendees

Cause of attendance (up to 3 causes allowed)	Total	HIV prevalence (%)
Acute primary care attendees (N=506)		
Diarrhoea/ Dysentery	106 (21%)	20
Ear, Nose and Throat infection	99 (20%)	22
Skin infection	65 (13%)	23
Lower Respiratory Tract infection	45 (9%)	24
Headache	45 (9%)	16
Other non-infectious cause[a]	44 (9%)	16
Urinary tract infection	29 (6%)	7
Possible TB	26 (5%)	50
Trauma[b]	38 (8%)	8
Non specific abdominal pain	24 (5%)	8
Malaria	22 (4%)	5
Other infection[c]	22 (4%)	23
Surgical problem	22 (4%)	9
Sexually transmitted infection	20 (4%)	40
Chronic lung or heart problem, including asthma	14 (3%)	7
More than one presenting complaint	117 (23%)	30
Antenatal care attendees (N=88)		
Routine antenatal care visit	79 (90%)	5
Sexually Transmitted infection	5 (6%)	20
Other infections[d]	4 (5%)	100

[a] HIV+ve: allergic conjunctivitis 3; suspected pregnancy 2; visual impairment 1; follow-up visit 1; HIV-ve: allergic conjunctivitis 14; visual impairment 3; suspected pregnancy 3; gynaecological problem 3; requesting HIV test 2; epilepsy 2; arthritis 1; drug allergy 1; diabetes mellitus 1; epistaxis 1; follow-up visit 1; growth on eye 1, limb pain and general weakness (no cause identified) 4

[b] Includes wound infection following trauma in 3 participants

[c] HIV+ve: oral candidiasis 3; hepatitis 1; dental abscess 1; HIV-ve: hepatitis 9, dental abscess 2; gingivitis 2; eye infection 2; mastitis 1; worm infestation 1

[d] Skin infection 2; possible TB 1; urinary tract infection 1

6.3.4 Mode of HIV acquisition

As shown in Table 6.2, findings were very similar to those presented for hospitalised patients in Chapter 5: age and sex did not differ by HIV status in APC attendees. HIV-infected APC attendees were significantly more likely to be maternal or double, but not paternal orphans, than their HIV-negative counterparts and to be stunted. These associations were not observed for ANC participants, although numbers were small in this group. HIV-infected APC attendees were also more likely to have pubertal delay than HIV-negative counterparts.

Of the 86 HIV-infected APC participants, 69 (80%) selected vertical transmission as the most likely source of their infection, 4 (5%) chose injections or blood transfusion and 13 (15%) chose sexual transmission. There was a high concordance between participant and nurses' assessment of likely mode of HIV transmission (Table 6.4). However, nurses were not blinded to participants' responses, which could partly explain the high concordance observed.

Table 6.4 Comparison of self-rated and nurse-rated assessment of most likely mode of HIV acquisition among APC HIV-infected participants (n=86)

		Nurses' assessment		
		Mother-to-child	Sexual	Parenteral
Self-rated assessment	Mother-to-child	69	0	0
	Sexual	1	12	0
	Parenteral	2	1	1

95.4% inter-rater agreement; κ score = 0.85

HSV-2 prevalence among ANC attendees was significantly higher than among APC attendees (14% *vs.* 4%, p<0.002). Being an ANC attendee, regardless of HIV status,

was associated with an increased odds of being HSV-2 positive (OR 3·6, p<0.001). Among APC participants, there was an association between HIV and HSV-2 for HIV-infected participants who considered themselves likely to have been sexually infected with HIV (24·8, p<0.001, but not in those who considered themselves vertically infected (OR 0.40, p<0.38) (Table 6.5). The median CD4 count was 305 (IQR 174-480) cells/μl in the vertically-infected group.

Table 6.5 Risk of being HSV-2 positive among participants attending acute APC services and ANC services, according to HIV status and self-reported most likely source of HIV infection

	No. HSV-2+ve (%)	OR for HSV-2 positivity (95% C.I)	*p*-value
HIV-ve APC	14/420 (3%)	1.00 (Ref)	-
HIV-ve ANC	10/83 (12%)	3.97 (1.7-9.3)	0.001
HIV+ve ANC	2/5 (40%)	19.33 (3.0-125)	0.002
Sexually-infected HIV+ve APC	6/13 (46%)	24.85 (7.43-83.6)	0.001
Non-sexual mode of HIV infection: HIV+ve APC[a]	1/73 (1%)	0.40 (0.05-3.1)	0.38

[a] 4=parenteral and 69=vertical

6.4 Discussion

6.4.1 Undiagnosed HIV infection in adolescents

The main finding of this study was the substantial burden of previously undiagnosed HIV infection across a wide range of presenting complaints among adolescents attending APC services in Harare. In contrast, the prevalence of HIV infection among adolescents attending routine ANC services was only 6%: similar to the national ANC surveillance estimate for 15-19 year olds (6.8% in 2009), and consistent with declining HIV incidence in Zimbabwe.[1 320 356]

In common with hospitalised adolescents, HIV-infected APC attendees had a high prevalence of features suggesting longstanding infection, such as pubertal delay and stunting and little to suggest sexual transmission as the predominant cause. There was a strong association of HIV with maternal and double, but not paternal, orphanhood suggesting mother-to-child HIV transmission. The high prevalence of HSV-2 in acutely unwell HIV-positive adolescents who reported having probable sexually-acquired HIV, and positive association between HIV and HSV-2 infections in ANC attendees contrasts with the very low prevalence of HSV-2 (below that of HIV-negative participants) in acutely unwell adolescents who selected vertical or parenteral transmission as their most likely source of HIV infection. If maternal transmission is indeed the predominant source of HIV among the acutely unwell adolescents in this study, then the main implications are that there is a high burden of undiagnosed LTS in Zimbabwe, and that routine testing of this age-group at primary care level is strongly indicated.

6.4.2 Provider-initiated HIV testing and counselling in primary care

Few studies have focused on the spectrum of morbidity related to undiagnosed HIV presenting at primary care level, and none have focused specifically on adolescents. As in other studies, HIV infected individuals were significantly more likely to present with possible TB or STI, and were also more likely to have multiple complaints.[357-358] However, HIV prevalence was high across the entire spectrum of common presenting complaints, suggesting that universal PITC should be adopted, rather than targeting specific clinical presentations.

In contrast to hospitalised adolescents, most HIV-infected adolescents at this level of the health system were previously undiagnosed, and their median CD4 count was relatively high (329 cells/μl). Studies have shown that HIV-infected adults commonly consult primary care with HIV-related symptoms prior to their eventual diagnosis, and children consult primary care services with a greater frequency than adults.[359] Thus, implementing PITC at primary care level is likely to have a much greater impact on reducing diagnostic delay and, if linked to prompt entry into HIV care, improving long term prognosis than a similar intervention at hospital level. The acceptability of PITC was very high (97%) in this study, with both adolescents and their guardians supporting routinely offered HIV testing, as has been shown for younger children in South Africa.[360] Thus, PITC at primary care level is likely to be a particularly effective strategy in promoting earlier HIV diagnosis in this age-group.

6.4.3 Implications of study findings

Evidence from Zimbabwe suggests that there are increasing numbers of LTS of MTCT reaching adolescence, the majority of whom are not yet in HIV care.[194 344] This is likely to be generalisable to the region. The current study adds to the data presented in previous chapters and existing literature by demonstrating a high burden of undiagnosed HIV infection with features suggesting MTCT as the predominant source of infection in adolescents presenting to APC services.

In contrast to our findings in hospitalised patients, however, the results of this study raise the hope of being able to diagnose LTS who still have a good prognosis if provided with HIV care through a simple facility-based intervention. Most of the

newly diagnosed patients had high CD4 counts and relatively minor intercurrent illnesses. This makes a strong argument for routine implementation of diagnostic HIV testing for older children and adolescents attending primary care services, regardless of the presenting complaint. Frontline service providers also need to be made aware that the epidemiology of HIV in older children and adolescents is changing, and that the main risk of infection in this group appears to be MTCT, which has implications for affected adolescents, and their guardians and siblings.

6.5 Limitations of the study

Assessment of the likely mode of HIV infection was through a brief questionnaire asking participants to report their likely mode of HIV acquisition. Participants' perception of personal risk of being HIV-infected may have been influenced by the information obtained during pre-test counselling, particularly regarding vertical HIV infection. However, the very low prevalence of HSV-2 in participants selecting non-sexual transmission concurs with data obtained through self-report. The nurses' assessment may also have been influenced by participants' responses, although they were asked to provide a reason for their selected choice of mode of transmission based on a case definition. The proportion of HIV-infected adolescents who were newly diagnosed may have been overestimated as those with known HIV infection may preferentially present to their HIV care clinic with complaints. However, this is unlikely to have a significant effect given the relatively small numbers of older children in HIV care.

7 Perception of risk of vertically-acquired HIV infection and acceptability of PITC among adolescents in primary health care

7.1 Introduction

International guidelines recommend routine PITC to all people seen in health facilities in generalized epidemics as part of universal access to HIV testing and care, and countries with severe HIV epidemics, such as Zimbabwe, are increasingly moving towards provision of PITC in all health facilities.[17] The results described in the previous chapter showed a substantial burden of undiagnosed HIV infection among adolescents attending primary health care services.

Despite the clear benefits of PITC, a number of considerations around HIV testing in adolescents can act as barriers to implementation of PITC in this age-group. Firstly, HIV testing of older children has not been emphasized in HIV programs and there may be lack of awareness of the burden of HIV in this age-group among health care workers. Secondly, HIV testing of under 16 year olds requires consent of a legal guardian: a potential barrier that may be compounded by changing or informal guardianship due to parental death.[295 361] The ability of children to get diagnosed is thus likely to be influenced by guardians' awareness of risk, and their willingness to initiate the process of HIV CT. Thirdly, advice to test a child for HIV infection may not be acceptable to guardians as it carries with it the implication that the child has either been infected vertically (implying that the mother and potentially the father and other siblings are HIV-positive), or horizontally, which then raises the question of sexual transmission. Guardians may also fear stigmatization of the child and other HIV-infected family members if they are

known to be HIV-infected, or may fear that the child or other family members may themselves be unable to cope with the knowledge and consequences of being HIV-positive.

This chapter presents the results of 3 sub-studies that were nested into the study investigating HIV-related morbidity among adolescent primary clinic attendees (Chapter 6). These were focused around the themes of

- exploring acceptability of PITC to adolescent APC attendees and their guardians, given the sensitivities outlined above
- mode of transmission and knowledge, attitudes and perceptions of the risks of adolescent HIV infection in the context of MTCT
- Entry into HIV care and acceptability of family HIV CT following PITC at primary care level

These topics were chosen to clarify and understand issues that arose from the hospital-based study described in Chapter 4, in particular the reasons for delay in diagnosis and access to care and awareness of risk of HIV infection in this age-group.

7.2 Methods

7.2.1 Study population

The participant selection process is described in detail in the previous chapter (Section 6.2.1). Consecutive patients aged between 10 and 18 years attending for acute primary care (but not the ANC clinics) were invited to take part in the study. All participants were offered an HIV test following group pre-testing counselling.

Participants and guardians (where available) were asked to complete a confidential questionnaire designed to elicit information on acceptability of the CT process and reasons for consenting to or declining HIV testing, including the perceived advantages of HIV testing and factors in counseling that may have influenced the decision whether to undergo HIV testing. The questionnaires were completed before HIV test results were known.

HIV-infected participants were referred to one of two HIV care clinics in central Harare, and were provided with a three months follow-up appointment with the primary care study team. The follow-up was intended to allow any problems with entry into HIV care to be identified and rectified, and also to provide an opportunity for HIV CT of the participants' relatives.

7.2.2 In-depth interviews with participants and guardians

In-depth interviews were used to explore the acceptability of HIV testing including the perception of risk of HIV infection among adolescents, and the benefits and disadvantages of HIV testing. Interview participants were sampled purposively from the participants of the parent study to include HIV-negative and HIV-positive participants, but with deliberate over-sampling of HIV-positive participants; participants who had declined and accepted diagnostic testing, respectively; and participants from both study sites. Those who had tested HIV-positive prior to participation in the study were not interviewed. Interviews were conducted in the participants' homes one week after HIV testing had been offered. A professional social science consultant was engaged to facilitate this study. Participants were interviewed on their own by an experienced professional field worker of the same

sex as the interviewee and in the local language, Shona. The interviews lasted between 30 to 45 minutes and were recorded and transcribed verbatim.

7.2.3 Data analysis

Questionnaires and interview transcripts were translated into English for data processing and analysis. Qualitative data was subjected to thematic analysis, whereby common themes were identified and topical codes developed and applied to transcripts. For quantitative data, a chi-squared test (χ^2) or Fisher's exact test was used as a test for association between categorical variables, a Students' *t* test for normally distributed variables.

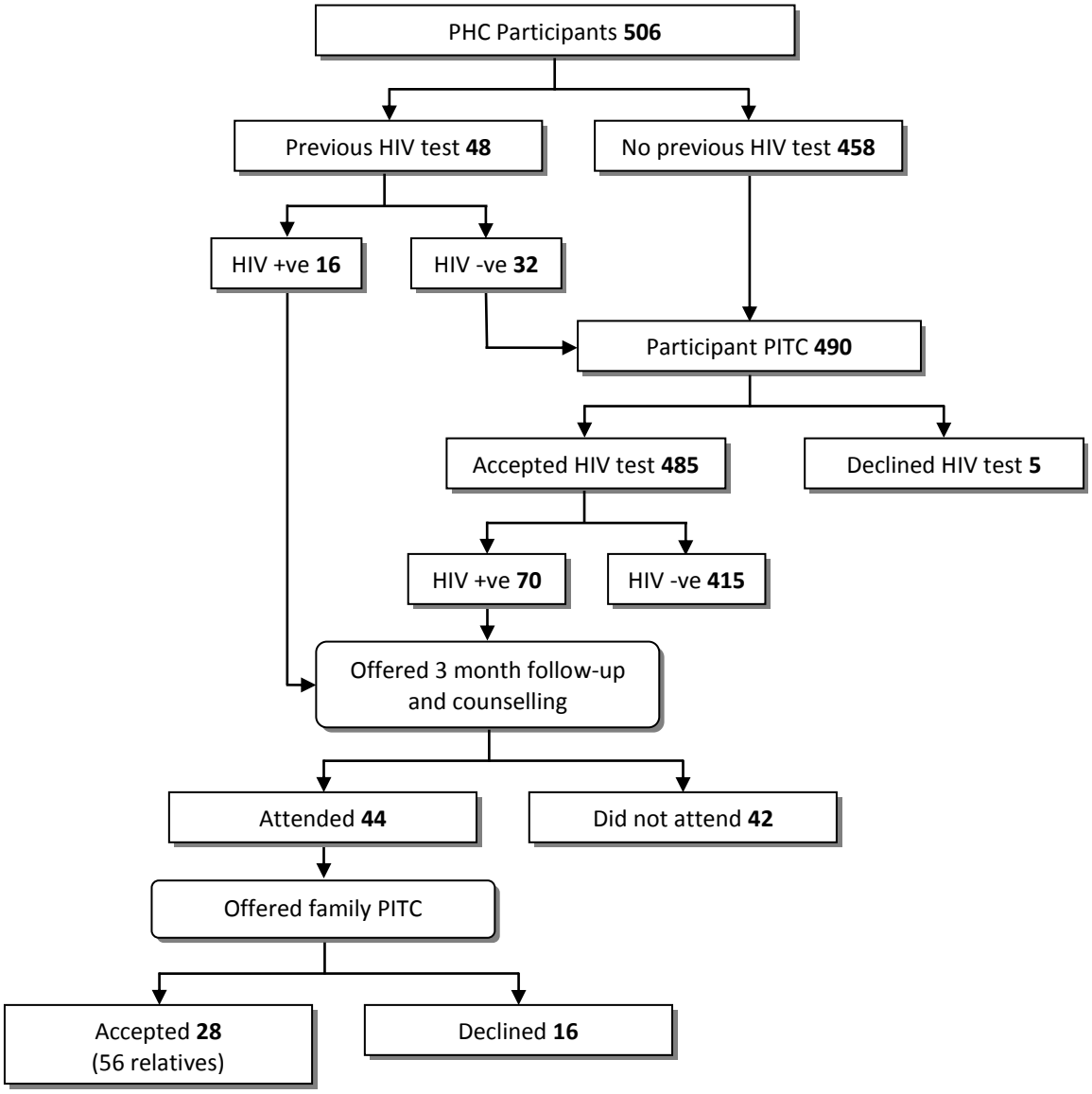
7.3 Results

The number of participants in the parent and sub-studies is shown in Figure 7.1. 71 interviews were conducted: 30 with participants who tested HIV-positive, 29 with guardians of participants who tested HIV-positive, 7 with participants who had tested HIV-negative, 4 with participants who declined HIV testing and 1 with a guardian who declined consent for their child to be tested. The median age of participants interviewed was 14 years (IQR: 11-16years) and 22 (54%) were female, similar to the age and sex of participants in the parent study from which the sample was drawn. The guardians interviewed were mother (n=9), aunt (n=6), sibling (n=6), grandmother (n=3), uncle (n=2), father (n=1), stepmother (n=1) and niece (n=1).

7.3.1 Acceptability of PITC among adolescents and families

Of the 506 PHC attendees, 48 (9%) had previously had an HIV test of whom 16 had tested HIV-positive. PITC was offered to all 490 participants who were not already known to be HIV-infected (Figure 7.1). Only five (1%) participants declined HIV testing, and 70 (14%) participants tested HIV-positive. OF the 32 participants who had previously tested HIV-negative, one tested positive during the study.

Figure 7.1 Uptake of PITC among adolescents and families of HIV-infected adolescents



Of the 86 HIV-infected participants, 44 (51%) attended three months later for family-based counselling and had all registered for care. Of the remaining 42 participants, 21 (24%) relocated from the area, 6 (1%) died and 15 declined further follow-up. It was not possible to trace whether these participants were attending for HIV care. Of the 44 participants who attended, 56 family members of 28 HIV-infected participants underwent HIV testing: 7/16 siblings, 6/7 mothers, 3/3 fathers, 2/2 partners and 6/28 other relatives tested HIV-positive (overall HIV prevalence 43%). The one participant with a mother who was confirmed to be HIV-negative stated that the most likely route of transmission was through the bleeding sores of his HIV-infected cousin in early childhood.

The main reasons for accepting HIV testing among participants and their guardians when they attended for APC related to the general importance of knowing one's HIV status, to investigate the cause of recurrent or chronic illness and being able to access treatment if diagnosed with HIV infection (Table 7.1). HIV-positive adolescents and their guardians were significantly more likely than HIV-negative counterparts to cite recurrent ill-health as the main reason for testing.

The remainder of Section 7.3.1 and Section 7.3.2 refer to data obtained through in-depth interviews.

7.3.1.1 Reasons for declining HIV testing

Of the five participants who declined HIV testing, three were not mentally prepared and feared the test outcome. One participant did not test as he did not wish to test in front of his father who had accompanied him to the clinic, and one participant wanted to test but his mother did not provide consent. The fears

Table 7.1 The main reason cited by participants and their guardians for the participant accepting HIV testing following PITC, by HIV status of the participant

Reason for HIV Testing	Participants' reasons for accepting HIV testing (n=477)*			Guardians' reasons for consenting to have their child tested (n=310)*		
	HIV+ve (%) N=68	HIV-ve (%) N=409	p-value	HIV+ve (%) N=45	HIV-ve (%) N=265	p-value
To know status: peace of mind and to plan for the future	41 (60.3%)	297 (72.6%)	0.10	10 (22.2%)	140 (52.8%)	0.001
Urged to test/ Parents Approve	2 (2.9%)	1 (0.24%)	ND	-	-	-
Recurrent Illness/ Not Well	22 (32.4%)	68 (16.6%)	0.005	24 (53.3%)	73 (27.6%)	0.001
Parent/s HIV-infected/died	0 (0.0%)	1 (0.24%)	ND	6 (13.3%)	13 (4.9%)	0.11
On TB Treatment	-	-	-	0 (0.0%)	1 (0.4%)	ND
Had sex	0 (0.0%)	1 (0.24%)	ND	-	-	-
Re-test**	-	-	-	1 (2.2)	0 (0.0%)	0.001
Sexually abused	0 (0.0%)	1 (0.24%)	ND	0 (0.0%)	2 (0.8%)	ND
Early access to treatment if found to be HIV positive	3 (4.4%)	40 (9.8%)	0.001	4 (8.9%)	36 (13.6%)	0.001

ND-not determined

*Reasons not given by 2 HIV+ve ve adolescents, 6 HIV-ve adolescents, and 3 guardians of HIV-ve adolescents

**Participant tested HIV positive as an infant, participant not aware of status, not in HIV care

expressed by these five participants were not being able to get married if HIV-positive, fear of being beaten at home, fear of dying, fear of other people finding out and needing more food.

> *"I was tested but my mother refused to get the results. I don't know why she was afraid; she didn't explain it to me. After the blood sample was taken and we were called in for the results she said she*

was not prepared to take them and went away. Personally I want to know my HIV status. I know how important it is." (12 year old male who declined HIV testing)

"I wasn't ready for it. I only came to treat him for what was wrong that day. I wasn't going to accept the results; I was scared to hear. No-one can expect something that terrifies you. As a young boy he has never thought of anything that can go wrong. Knowing might not affect him but it will affect me as an adult." (Mother of 12-year old male who declined HIV testing quoted above)

"I was afraid to get tested, not because of any bad thing which I was suspected to have done. I had some fear inside, how will my parents accept it, how am I going to cope with the situation. If only I was above 18 years I could stand for myself but now I am dependent on my parents; how will they accept it? My health would be affected because I would be thinking of that most of the time." (17 year old male who declined HIV testing)

7.3.1.2 Perceived benefits and disadvantages of HIV testing

Regardless of the participants' HIV status or whether the participants had accepted testing, there was strong support for HIV testing for this age-group. There was unanimous agreement among the participants that HIV testing was beneficial, regardless of the test result. Participants identified several benefits to HIV testing, including earlier access to HIV treatment, access to HIV prevention and motivation to stay HIV-negative, and the ability to plan lives around marriage and child-bearing. The main concerns about HIV testing were about adverse psychological reactions such as stress, depression, worry about premature death, withdrawal from society, stigma and the desire to take revenge on their sexual partner that

might accompany testing HIV-positive, or complacency following a negative HIV test leading to an increase in risk-taking behaviours.

> *"You can start thinking too much which is not good, so you keep being sick and die early. Also, people you live with might say - 'you and your disease, we can't live together.' "* (HIV negative female, aged 15 years)

7.3.1.3 Concerns regarding PITC in primary health care

All study participants felt that the information provided during pre-test counselling was comprehensible and adequate to make a decision about HIV testing. There was no concern that PITC put pressure on participants or guardians to accept testing or resulted in loss of confidentiality. At interview, the participants noted that counselling had reinforced messages that motivated them to consider testing. In particular, the realization that vertically-infected children could survive to adolescence was a trigger for some participants to test.

> *"I was surprised to learn about transmission from mother to child during breastfeeding and delivery. This alone prompted me to be tested because I was not aware of this before."* (11 year old HIV negative male)

> *"The most important message was that children are still being detected positive, even when they are 18 years of age. The virus can be in your body for a very long time without any symptoms showing."* (12 year old HIV negative male)

Participants expressed mixed feelings about group versus individual pre-test counselling with some participants expressing a preference for an individual pre-test session rather than a group session.

"Counselling should be done individually because in a group some might be domineering and give all the answers whereas one might not understand." (16 year old HIV negative female)

"Individually is best because whatever is said will be directed at me. If I ask a question, it is about myself and the answers will be directed at me, not the group. In a group, one person can say we have understood and the others will just follow and some will be afraid to ask questions." (11 year old HIV negative male)

The main concern regarding the PITC process was the need for more detailed post-test counselling and psychosocial support. Participants felt more support was needed for those testing HIV-positive, and HIV-negative participants expressed concern that PITC might focus on those who had tested HIV-positive with little opportunity to reinforce primary HIV prevention messages. Additionally, the period when participants waited to be tested and then waited for their results was typically filled with fear, highlighting the need for the testing process to be reassuring and prompt.

7.3.2 Perception of risk of HIV infection in adolescence

7.3.2.1 Prior contemplation of HIV testing

A third of HIV-positive participants interviewed had contemplated being tested before. Seventeen (65%) guardians of HIV-infected participants interviewed had also considered getting their child tested and about half had discussed having their child tested with others, usually another family member. The main reasons mentioned by participants for not having tested previously were lack of money for transport or clinic consultation fees, prevarication, and no one to accompany them.

"I must admit I had thought of it before because I was experiencing stomach pains, my legs were painful and I couldn't walk properly. I hadn't gone before because I just kept putting it off, but not for any reason in particular." (HIV-infected 11 year old male)

"I wanted to go on my own before I became seriously ill but I had no money for transport and to pay at the clinic." (HIV-infected 17 year old male)

Guardians also mentioned these reasons as well as a lack of awareness of testing services and one had been dissuaded by her husband who considered that their son was "*not all that sick".*

"It wasn't common fifteen years ago to test children but he was tested and I knew he was positive. Later on he became strong and fit and it was then difficult to believe he was positive because he was healthy." (Guardian of 15 year old HIV-infected male, not in HIV care)

"Yes, I thought about it but didn't realize that children were being tested for AIDS. I thought only adults were tested. I didn't know of any clinic that was offering the service and thought you could only go to a private doctor if your child was seriously ill." (Guardian of 12 year old, HIV-infected boy)

"Yes, because she had been coughing a lot. But we kept putting it off." (Guardian of 10 year old HIV-infected female)

7.3.2.2 Reasons for suspecting HIV infection

Eight (28%) HIV-infected participants and 19 (66%) guardians of HIV-infected participants had strongly suspected that the HIV test results would be positive. The main reason for suspecting HIV infection among participants and their

guardians was recurrent ill-health, and guardians also volunteered the death or HIV status of the child's parents or siblings and poor growth of the child as reasons for suspecting HIV infection. Over two thirds of guardians (n=23) identified the deaths of parents and siblings or HIV infection in parents as a reason for having their child tested and eight guardians reported 14 sibling deaths of whom two had died after the age of five years.

> *"My father died of TB, my mother is HIV positive and I have been diagnosed with TB."* (18-year old HIV-infected male)
>
> *"He is really stunted in his growth and is forever sick with a runny stomach and severe acne and pimples."* (Guardian of 16 year old HIV-infected male)
>
> *"The way I was being affected by headaches with the whole of my body feeling weak, I suspected it could be HIV. I had noticed that my symptoms were the same as my sister and two brothers who have died."* (17 year old HIV- infected male)
>
> *"My child was born with the virus. I could tell from his health as he was not fit and strong like other children of his age. By the age of two he was not crawling and he was supposed to be running. He was tested for HIV at the age of two and was positive but I didn't believe it. I just ignored it. He has been living with the virus from that time without any treatment and he is now 17."* (Guardian of 17 year old HIV- infected male)
>
> *"I got it from my parents; I was infected while I was still young. I think I got it in 1998 while I was being breastfed by my mother. Yes, I grew up being sick and that's where I think I might have acquired it. I was never given blood so it can't be from a blood transfusion."* (11-year old HIV-infected male)

In addition to the HIV-infected participants' and their guardians' own suspicions regarding their HIV status, twelve guardians (41%) and nine (31%) participants mentioned that others had raised the possibility of HIV infection and suggested HIV testing, which prompted them to consider testing. These suggestions were given by family members to the guardians, although a third of the group noted that friends had also offered such advice. For adolescents, this suggestion had come from a parent (38%), other family members (50%), or a friend (22%) but only one reported receiving this suggestion from a health care worker. The basis of others' suspicions was the poor health state or short height of the adolescent.

> *"Two of his siblings have died - a girl who was 21 months old and a boy of eleven months. I even relocated because of their deaths because I thought people were bewitching me… My sister encouraged me to be tested and now I know they died because of this disease."* (Mother of 11 year old HIV-infected male)

HIV-infected participants and their guardians who had expected a negative result did so because the child was not sexually active, had enjoyed a general good state of health throughout childhood or had illnesses not perceived as typical symptoms of HIV/AIDS.

> *"I had a very positive mind and thought that if I had the virus from birth I would have been dead long back so I was expecting a negative result. I was never sick from childhood which would have made me suspect I could be positive."* (14 year old HIV-infected male)

> *"I thought she would be negative because the symptoms of HIV/AIDS I have heard about are totally different from what she was feeling. She has been complaining of headaches, feeling weak*

and refusing to eat. At one time I thought she was pregnant." (Guardian of 17 year old HIV-infected female)

7.3.2.3 Lay perception of risk of HIV infection in adolescence

Although the majority of HIV-infected adolescents and their guardians felt that the source of HIV infection was probably from the mother, less than half of those interviewed felt that others would believe that this was actually the case. Most interviewees mentioned that HIV was associated with sexual intercourse in people's minds and eleven (38%) guardians said that people did not expect a child infected by its mother to survive early childhood.

> *"The general public lacks knowledge because they think that one can only be infected through sexual intercourse. They don't think of these other means. They say since young ones have not experienced sexual matters, they can't be affected."* (16-year old HIV-infected male)

Guardians commented that most people in their communities do not believe that adolescents are at risk from HIV and that people believe the only infection route for adolescents is through sexual intercourse. Similar views were expressed by those who had tested HIV-negative and those who had opted-out of HIV testing. These adolescents exhibited negative impressions of those at risk of HIV infection and felt they were not at risk of HIV infection, unless they engaged in unprotected sex. About half of the participants did not think it possible that HIV-infected infants could survive to adolescence and most had learnt of long-term survival through the patient information and counselling they had received during the study. Some participants remained sceptical that HIV-infected infants could live

into their teens and remarked that it would be assumed that an HIV positive adolescent would have contracted the virus through sexual intercourse.

> *"It is very difficult for other members of the family to accept that one of the children is positive. They would just think I got it from a sexual partner even if I have not engaged in that. Very few will think of themselves as having infected their children. The knowledge about mother to child transmission is not widespread."* (15 year old HIV negative male)

> *"Some would chase away the child and say -'go and stay where you got the disease' - because many people still think one gets HIV through being mischievous."* (17-year old HIV negative female)

> *"The risk is very low for boys compared to ladies. The ladies become sexually active early. You find four ladies in love with one man. The risk for boys is only if you get a sugar mummy or have used a sharp instrument."* (17 year old male who declined HIV testing)

> *"People who are at risk are those who go to beer halls and pubs - prostitutes. They will be after boyfriends and sleep around with all sorts of men."* (17 year old female who declined HIV testing)

> *"The people who are at risk are prostitutes, promiscuous or adulterous people. Also soldiers, because they work away from home and end up having extra-marital affairs. No other types of people are at risk."* (16 year old female who declined testing)

7.4 Discussion

7.4.1 Feasibility and acceptability of PITC

Contrary to our expectations, the main finding of this study was the very high acceptance rate of PITC in primary care among adolescents. Given the results of

the study of hospitalised adolescents whereby the vast majority of adolescents had tested after presentation with a severe illness, we had anticipated a high refusal rate to PITC. A uniform offer of testing, with an explanation as to why the group as a whole is being targeted appeared to be highly acceptable and there was strong endorsement from adolescents and their guardians that HIV testing for this age-group is beneficial and desirable, regardless of the level of perceived risk of being HIV-infected and potential implications for the participant and other family members.

The substantial burden of undiagnosed HIV infection among adolescents attending primary health care services strongly supports the routine HIV testing in this age-group. HIV testing is a means of promoting HIV prevention in an age-group that is also at high risk of HIV infection through horizontal HIV transmission.[362] Additionally, some family members of HIV-infected adolescents underwent testing as a result of the adolescent testing, with nearly 50% of relatives who tested being HIV-infected. Hence, PITC in children may be an entry point for the whole family to access HIV testing and care. However, even at primary care level, six out of 86 participants died shortly after diagnosis, highlighting the importance of HIV diagnosis as early as possible. The observation that such a high proportion of HIV-infected biological parents were alive is intriguing and study of this group may provide further insight into immune mechanisms involved in control of HIV infection.

Notably, nearly a quarter of HIV-infected participants had relocated from the area within three months of HIV diagnosis. Although this is speculative, HIV-infected

participants may have been sent to the rural areas (where medical consultation fees are lower, and ART is often easier to access than in Harare) or the whole family may have departed following a diagnosis of HIV infection in the adolescent which may have revealed the HIV status of the child's parents. This may also account for the low uptake of HIV testing among family members and highlights the need for interventions to facilitate linkage into care following HIV diagnosis and for family-based interventions to accompany routine HIV testing for children.

7.4.2 Knowledge, attitudes and perceptions of HIV risk in adolescents

Our results show a stark contrast in knowledge and perceptions between HIV-infected and HIV-uninfected participants and their guardians regarding possibility of HIV infection in adolescence acquired through MTCT. The majority of HIV-infected participants and their guardians acknowledged that they had anticipated a positive result, and attributed this predominantly to MTCT because of the combination of a) failure to thrive in the child and b) known or suspected maternal HIV infection or death, and c) deaths among siblings. These spontaneously volunteered reasons for suspecting an adolescent to be a long term survivor of MTCT were the same variables shown to be most strongly associated with underlying HIV infection in our hospital and primary care level data.

In contrast, however, the perception of personal risk of being HIV-infected was low among uninfected and asymptomatic HIV-infected participants. Perceptions of the reaction that HIV-infected adolescents could anticipate were more uniform: participants and guardians from both HIV-positive and HIV-negative groups stated that community members would assume that HIV in an adolescent was sexually-

acquired, and that communities would not believe that the infection had been present from infancy. Stigmatizing attitudes were commonly expressed by HIV-negative participants and their guardians, and were strongly anticipated by HIV-infected participants and their guardians.

7.4.3 Barriers to HIV testing

Although the majority of the HIV-infected adolescents had suspected HIV and had even contemplated HIV testing as had their guardians, they had prevaricated for various reasons, including not knowing where to get testing. Lack of awareness of VCT services or reluctance of health care providers to discuss risk behaviours creates barriers to HIV testing.[363-364] There has been not only a dearth of health information and education about long term survival in HIV-infected children, but indeed the perception that survival of HIV-infected infants beyond the first few years of life is exceptional. These may have contributed to the current unsatisfactory situation of missed opportunities for PITC and family-initiated testing, with important implications for health education and health provider training. Participants reported having received encouragement in the past to test from friends and relatives but not from any health care providers. Without the knowledge of how to access testing and in the face of information that has stressed the low chance of surviving with HIV, families may hesitate to have older children tested.

The study also raises an important possibility that the low awareness of long-term survival from MTCT may have a further major deterrent effect, in that the question of *how* the adolescent became infected then automatically arises. The default

assumption that HIV among adolescents is sexually-acquired means that adolescents automatically anticipate stigma linked to having acquired HIV sexually should they test HIV positive, and this may deter them further from testing. Health care providers may be reluctant to discuss HIV infection in a sexually immature adolescent with guardians if they are themselves unaware that long-term untreated survival is more common than previously thought, as it might mean raising the question of sexual abuse.

7.4.4 Other implications of Study Findings

As well as systematic under-appreciation of the burden of HIV in this age group, adolescents face formidable barriers in accessing HIV diagnosis. Over and above ill-health and loss of parental input due to ill-health and death, lack of health information and education may be contributing to the high risk of prolonged diagnostic delay once signs and symptoms of HIV have developed.[365] The study highlights the need to publicise the growing problem of MTCT to general public, and health providers alike, and provide information on where families with older children who may be at risk of MTCT can seek testing. This may serve the purpose of destigmatising adolescent HIV infection in the broader community, while also enabling health providers to feel less inhibited about offering diagnostic testing. There are operational issues with regards to provision of routine HIV testing in healthcare facilities.[366-368] There is a potential for adolescents identified with HIV infection to be subjected to exploitation from others who assume behavioural HIV acquisition, and adequate counselling will be a critical requirement of PITC scale-up. Within primary healthcare services significant attention will need to be paid to staff training, motivating staff to offer HIV testing routinely, maintaining patient

confidentiality, mechanisms for referral and access to appropriate services for those who test HIV-positive, as well as reduction of stigma and discrimination.

7.5 Limitations of the study

Given the small numbers of those who opted-out of HIV testing in this setting, it was not possible to study possible deterrents to uptake of PITC in adolescents, including the influence of guardians on the likelihood of adolescents considering HIV testing. However, the extremely high uptake demonstrates that there are unlikely to be significant barriers to uptake in this group. Furthermore, there was strong support from guardians who are likely to be an important influence on adolescents considering HIV testing and whose consent is required for adolescents to undergo testing. The uptake of PITC among family members of HIV-negative participants may have been lower than that among families of HIV-infected participants, but was not assessed.

HIV CT was carried out by motivated, well-trained research nurses and testing rates may well be lower under routine conditions. The perceptions and views of health-care workers to PITC were not assessed and the uptake of PITC among family members of HIV-negative participants was not determined. The questionnaires were given after the pre-test discussion and the information given may have influenced participants' responses and acceptability of HIV testing. The interviews were conducted some time after the HIV test which may have introduced some recall bias. Data on sexual risk factors was not collected and sexual abuse as a reason for testing may have been underestimated, as participants and their guardians may have been reluctant to divulge this. However, while not

discounting the high rates of sexual abuse reported from some African countries, the risk of acquiring HIV infection through sexual abuse is limited to penetrative forced sex, which occurs at a low rate during childhood and adolescence in this region, with the possible exception of South Africa.[189-190] Also, the association of HSV-2 infection with self-reported horizontally-acquired but not vertically-acquired HIV, further support the validity of the self-reported mode of HIV acquisition.

8 A primary-care level algorithm for identifying adolescents at risk of HIV infection

8.1 Introduction

As described in previous chapters, HIV prevalence was high among adolescents accessing both hospital and primary acute care services. Moreover, HIV infection was previously undiagnosed in all but a small minority of adolescents accessing primary care services. At both health service levels, characteristic features suggesting long-standing or maternally-acquired HIV infection were noted to be present in most cases, and were spontaneously volunteered by adolescents and guardians (Chapter 7).

In this study, data collected prospectively from primary health care in Harare was used to investigate whether or not a simple screening tool could be constructed to identify adolescents at high risk of undiagnosed HIV infection in the context of populations at high risk of MTCT at the time of their birth. If so, then potential applications would include a) screening of adolescents for priority offer of diagnostic HIV testing in high HIV prevalence settings with mature epidemics but where implementation of routine testing is suboptimal, and b) in low HIV prevalence settings where HIV testing and counselling is not routinely considered, for example among African immigrants in low HIV prevalence settings.

8.2 Methods

8.2.1 Study Population and data collection

The participant selection process and the sample size justification are described in detail in Chapter 6 (Section 6.2.1 and Section 6.2.5). Patients aged 10 to 18 years

attending two primary care polyclinics in Harare for any reason except ANC were offered an HIV test following group pre-test counselling and asked to consent for participation in the study, including an additional HIV test for study purposes, regardless of whether they accepted diagnostic HIV testing. A standardised questionnaire was used to record demographic details, clinical history and reason for clinic attendance.

8.2.2 Data analysis

A random number generator was used to divide the dataset into two: a "train" and "test" dataset, with an equal number of HIV-positive participants in each dataset. The train dataset was used to create and optimise the screening algorithm, which was then evaluated in the test dataset.

8.2.2.1 Algorithm criteria

The algorithm was designed for use at primary care level and thus variables that could be measured by primary health care workers were selected. Candidate variables used to construct the algorithm were defined *a priori,* and were coded as binary variables. The considered criteria were defined as in Table 8.1. Other criteria that were considered included age, sex, educational level, history of TB, self-rated health and history of hospitalisation (at least one night stay in hospital).

8.2.2.2 Construction and optimisation of the algorithm

Logistic regression modelling was used to estimate the univariate OR for the association of each variable with HIV infection. Variables with a p-value <0.1 were included in an initial multivariate model.[369] Variables not independently

statistically significant (i.e. with a p-value >0.05) were excluded from the model, so the final model included only those variables independently associated with HIV.

Table 8.1 Definitions of variables predictive of HIV infection among adolescents

	Definition used in questionnaire
Recurrent upper respiratory tract infections	More than two upper respiratory tract infections (URTI) over a period of at least six months
Recurrent chest infections	More than two chest infections over a period of at least one year
Recurrent diarrhoea	More than three episodes of diarrhoea over a period of at least six months, with at least a week's diarrhoea-free period between each episode
Recurrent skin problems	More than 3 episodes of skin complaints occurring over a period of a year or more
Wasting	Weight-for age z-score <-2
Stunting	Height-for age z-score <-2
Poor Functional ability	Illness affecting ability to function in daily life
Possible TB	Suggestive symptoms: Cough >2 weeks, night sweats, weight loss, fevers
Oral candidiasis	White spots or plaques in the mouth
Possible Sexually transmitted infection	Vaginal/urethral discharge or genital sores

The log of the probability of being HIV-infected (p) was calculated for different combinations of variables V_i from the final multivariable model as follows:

$$\log (p/1\text{-}p) = \text{constant} + \beta_1V_1 + \beta_2V_2 + \beta_3V_3 \ldots\ldots \beta_nV_n$$

where V_i is the binary variable i (coded as 1 if the variable is present and as 0 if the variable is absent) and β_i is the log(OR) associated with variable V_i.

The next step was to select a cut-off value of p, which would discriminate which individuals should be considered as being at higher risk of HIV infection and be referred for an HIV test. Using this cut-off, an algorithm was devised which classified individuals into one of two groups: 'high risk for HIV" and "low risk for

HIV". To choose the optimal cut-off, the sensitivity and specificity of the algorithm against the HIV test result were calculated for a range of cut-offs. The positive predictive value (PPV), negative predictive value (NPV) and the likelihood ratio (LR) of the algorithm using different cut-offs were also calculated.

To increase the sensitivity of the algorithm without compromising specificity, additional variables with very high specificity (>97%) for HIV infection were added as options to the model at the desired cut-off of *p.*

8.2.2.3 Evaluation of the algorithm

The optimised algorithm was applied to the test dataset and sensitivity, specificity, PPV and NPV and LR calculated. The PPV, NPV, and number needed to HIV test to detect one HIV-positive individual were then calculated for varying HIV prevalence levels.

8.3 Results

A total of 506 participants (97% of those eligible) were enrolled during the study period of whom 86 (17%) were HIV-positive. 251 participants were randomly assigned to the train set and 255 to the test dataset. There were 43 HIV-infected participants in each dataset.

8.3.1 Variables associated with HIV infection

Age, sex and pubertal delay were not associated with HIV infection on univariate analysis. Orphanhood, hospitalisation, recurrent skin problems, possible STI and

poor functional ability were independently associated with increased risk of HIV infection in the multivariable analysis, and were included in the algorithm (Table 8.2). Under the multivariable logistic regression model, a cut-off of p=0.12 corresponded to an individual who met more than one of the five criteria in the model being considered as "high risk for HIV", and hence would be offered HIV testing under the proposed algorithm.

Table 8.2 Sensitivity, specificity, crude odds ratio and adjusted odds ratio for variables associated with HIV infection

	Sensitivity (%)	*Specificity (%)*	*Crude OR*	*p-value*	*Adjusted OR**	*p-value*
History of TB	9	97	3.45	0.06	4.37	0.08
Orphan	77	58	4.59	<0.001	3.93	0.002
Ever hospitalised	35	90	4.77	<0.001	4.05	0.003
≤ Primary education level	63	54	2.01	0.04	1.85	0.14
Recurrent URTI	44	82	3.66	<0.001	2.41	0.06
Recurrent chest infections	37	85	3.26	0.001	1.76	0.23
Wasting	35	85	2.95	0.004	1.91	0.17
Stunting	28	86	2.39	0.03	1.64	0.34
Recurrent diarrhoea	53	74	3.20	0.001	1.66	0.25
Recurrent skin problems	51	82	4.69	<0.001	4.07	0.001
Self-rated poor health	65	79	7.16	<0.001	2.44	0.06
Poor Functional ability	51	86	6.47	<0.001	4.82	<0.001
Possible TB	12	97	3.78	0.03	2.96	0.16
Possible sexually transmitted infection	12	96	3.29	0.05	5.35	0.015

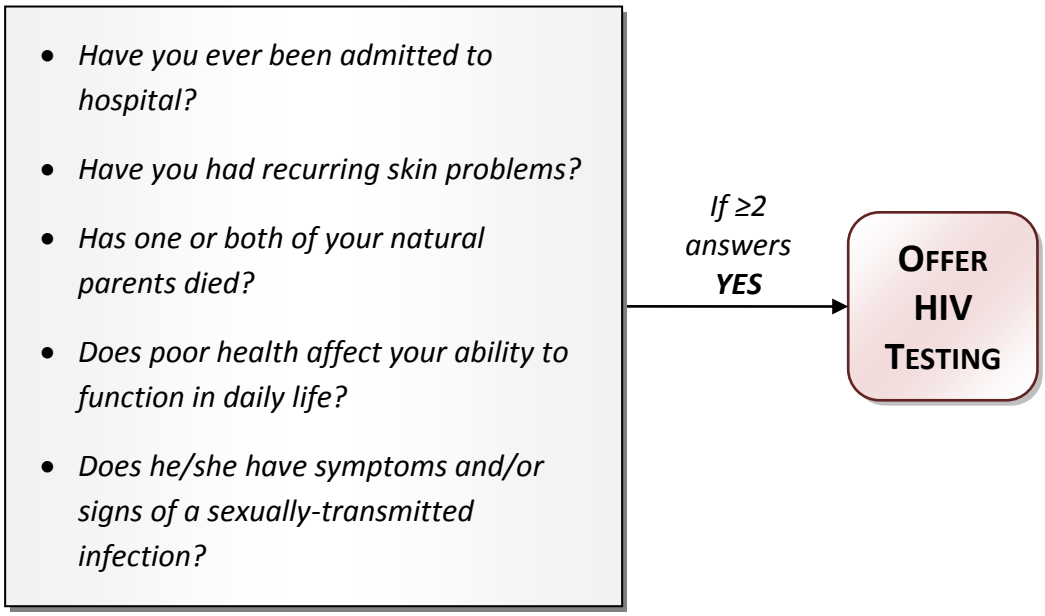
*Adjusted for orphanhood, hospitalisation, chronic skin problems, functional ability and possible STI

8.3.2 Identification of the optimum algorithm

Using a cut-off of p=0.12, the sensitivity and specificity of the algorithm to predict HIV infection in the trainer dataset were 77% and 81% respectively. Lower and higher cut-offs resulted in a substantial drop in specificity and sensitivity

respectively: for example, using a cut-off of p=0.38 (corresponding to >2 criteria in the algorithm being met) resulted in a specificity of 95% but sensitivity dropped to 47%. Thus, other cut-offs were not considered further. High specificity variables (history of TB, history of herpes zoster and presence of oral candidiasis) were tested in various combinations as an option to the original algorithm, but resulted in no significant improvement in sensitivity of the algorithm, and thus the original model was retained as the final algorithm (Figure 8.1).

Figure 8.1 Adolescent "HIV suspect" algorithm



8.3.4 Test data set

Applying the algorithm (with a cut-off of p=0.12) to the test dataset gave a sensitivity of 74.0% (95% CI: 64%-82%) and a specificity of 80% (95% CI:71%-87%) with the algorithm correctly classifying the HIV status of 79% of participants. As an additional check for internal validity of the model, bootstrapping of the dataset was carried out and the sensitivity and specificity on the test dataset after 50,000 iterations were 81% (95% CI: 73-88%) and 77% (95% CI: 68-82%) respectively.

8.3.5 Prediction of HIV Status in low HIV prevalence settings using the test dataset

The algorithm had high estimated NPV in both low and high HIV prevalence settings and would result in an estimated 60% decrease in the number of people needing to test to identify one HIV-infected individual, compared with universal testing (Table 8.3).

Table 8.3 Prediction of HIV status in different HIV prevalence settings

HIV prevalence in acutely unwell adolescents	PPV	NPV	NNT+ algorithm	NNT- algorithm	Reduction in NNT+ compared to universal testing
0.05%	0.1%	100%	754	4380	62%
0.1%	0.3%	100%	377	2189	62%
0.5%	1.3%	99.8%	76	437	62%
1%	2.6%	99.5%	38	218	62%
2%	5.1%	99.1%	19	108	61%

PPV = Positive predictive value; NPV = Negative Predictive Value; NNT+ algorithm = Number needed to test to identify 1 HIV-infected adolescent after application of algorithm; NNT- algorithm = Number needed to test to identify 1 HIV-infected adolescent misclassified by algorithm as not at HIV risk

Using the algorithm in an adolescent clinic population with a low prevalence of HIV infection for e.g. 0.1% underlying risk of HIV infection, the algorithm would identify 377 adolescents for HIV testing for every one true positive (compared to a 1000 adolescents screened to identify one HIV-infected adolescents). However, it is also important to minimise the number of false negative results (i.e. HIV-infected adolescents identified as being low risk): using the algorithm an individual would be falsely classified as "not at risk" for every 2189 adolescents screened.

8.4 Discussion

8.4.1 Utility of a diagnostic algorithm

This study shows that a simple, question-based algorithm can identify underlying HIV infection with reasonable sensitivity and specificity in African adolescent primary care attendees born into a population in which the adult HIV epidemic was at high prevalence at the time of their birth. Existing paediatric algorithms to identify children with HIV infection, such as the IMCI/HIV algorithm, tend to focus on diagnosis of younger children with symptomatic HIV infection, which may not be applicable to older children, and are also less evidence-based.[370] The algorithm had a sensitivity and specificity equal to or better than other tools to identify HIV infection in children, including algorithms based on clinical signs and symptoms and even paediatrician assessment, and requires a very simple assessment that could be administered following minimal training.[371-372] Data used to develop the algorithm was systematically and prospectively collected, avoiding the biases associated with use of retrospective or routine clinical data. Parallel HIV testing with two rapid test kits was used, with discordant results resolved by retesting stored specimens with the original two tests plus an ELISA test and hence, misclassification of HIV status will be minimal.

Zimbabwe now has a rapidly declining HIV epidemic, and the studies described in the earlier chapters have established that vertically-acquired HIV is likely to be responsible for most *symptomatic* HIV infections in this age-group. This may also be true for some neighbouring countries, although the decline in HIV incidence and prevalence has been more pronounced in Zimbabwe than elsewhere in the region.[16 194] Current international recommendations are that HIV testing should be

routinely offered to all attendees in health facilities, aiming to reduce the risk of late presentation with advanced HIV/AIDS and for secondary HIV prevention.[17] However, in practice not all national policies are in line with the current international guidelines and often exclude children, and human resources and other constraints may adversely affect implementation.[18] In settings where universal testing is not routine for this age-group, a risk assessment based on the type of screening algorithm presented here is proposed as a preferable alternative to no offer of testing.

Health care workers in primary care are often reluctant to discuss HIV testing with patients, and this may be particularly true for older children and younger adolescents where considering a diagnosis of HIV will raise uncomfortable questions about the source of their infection. Use of an algorithm may then serve to prompt this process in an age-group that is not well served by alternative testing services.

8.4.2 Application of algorithm in low HIV prevalence settings

Maternally-acquired infection also appears to be the predominant risk-factor in older African children and adolescents who present with previously undiagnosed symptomatic HIV infection having emigrated from high to low HIV prevalence countries, such as the UK.[373-374] For example, there has been a well-publicised death in the UK of a previously undiagnosed 10 year old whose parents had been diagnosed with HIV six years earlier.[375] Undiagnosed HIV should, therefore, be considered in all acutely unwell adolescents from families with known risk factors

for HIV (e.g. immigrants from high HIV prevalence settings or intravenous drug users).

In low HIV prevalence countries, most HIV testing is carried out through free-standing or sexual health services. However, many newly diagnosed HIV-infected individuals report prior consultation in primary care, implying that opportunities for earlier diagnosis are frequently missed.[359 376] Facility-based testing is a highly cost-effective way of identifying HIV-positive individuals even in low HIV-prevalence settings.[377-378] The algorithm has a very high negative predictive value in low prevalence settings and, if validated, may be an attractive alternative to universal testing as, despite a low positive predictive value, it still results in a substantial decrease in numbers of adolescents who would need to be offered HIV testing. An additional advantage may be expansion and normalisation of HIV testing in primary care, a relatively under-utilised resource for provision of HIV testing.[379-380]

8.4.3 Other implications of study findings

The study has evaluated a tool to identify underlying HIV infection among acutely unwell adolescents in whom the predominant risk factor for HIV is maternal transmission. It recognises the changing epidemiology of symptomatic adolescent HIV infection, thus prioritising high risk adolescents and their families for appropriate CT.

As well as identifying individuals at high risk, the algorithm may serve to raise awareness among health providers of the need to consider long-term survival in

acutely unwell older children and adolescents at risk of maternally-acquired HIV infection.

8.5 Limitations of the study

The high prevalence of HIV infection (17%) in the study population, which was drawn from otherwise unselected adolescents attending acute care services at primary level, provided the statistical power needed to develop this type of algorithm in a relatively small sample size. The test-train method provides an internal validation of this approach but gives no insight into external validity. The algorithm may perform differently in populations with a different mix of sexually-acquired, parenterally and vertically-acquired adolescent HIV infection. The positive and negative predictive values of the algorithm will vary by background HIV prevalence and prevalence of variables in the model for e.g. past TB treatment and presentation with an STI may be uncommon in adolescents who have grown up in countries where these infections are well controlled, which may decrease the PPV of the algorithm. However, the sensitivity and specificity of the algorithm will remain unchanged. The algorithm is presented as a promising approach that can be adapted and according to local context, and the performance of the algorithm should be further validated in other settings.

9 Conclusions and recommendations

As noted in Chapter 1, the starting point for this series of studies was the observation of increasing numbers of adolescents presenting to health care services in Harare with symptomatic HIV infection and features suggestive of longstanding disease. The epidemiological studies described in the previous chapters provide strong circumstantial evidence to support our initial hypothesis that this is predominantly due to a substantial epidemic of adolescent LTS of MTCT. The data are consistent from a number of key perspectives:

- Evidence of complications (stunting, pubertal delay, chronic lung disease, treatment for TB and chronic skin disorders) that are consistent with long standing HIV infection from childhood

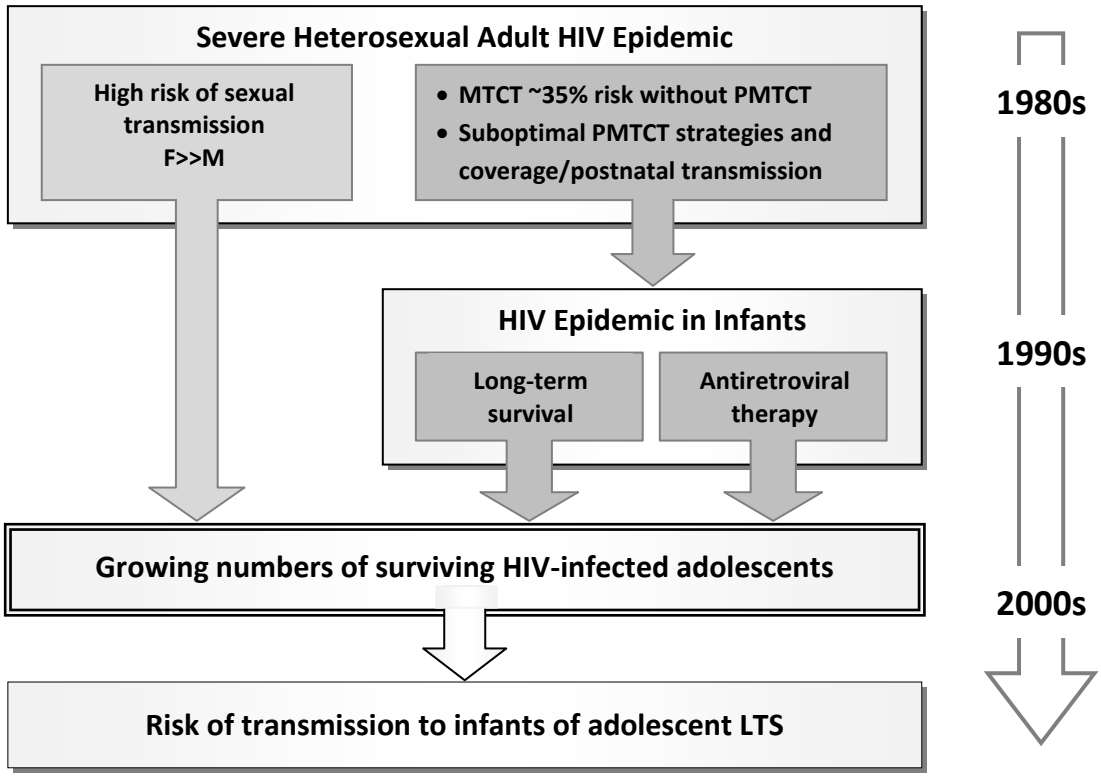
- Lack of any evidence suggesting forced or voluntary sexual debut in all but a small minority of HIV-infected adolescents, including HSV-2 serology, self-reported sexual history (hospital study), self-rated likely mode of transmission (primary care study) and in-depth interviews with HIV-infected participants and their guardians

- Family attributes that are consistent with maternal HIV infection, including maternal death or known HIV infection, a high prevalence of HIV in family members who were tested as a result of HIV-diagnosis in an adolescent, reported sibling deaths, and the responses given by HIV-infected participants and their guardians on in-depth interview

Furthermore, as the risk of parenteral HIV infection is also known to be low in Zimbabwe, the rest of this discussion will consider the clinical and regional research implications, assuming that MTCT is indeed the dominant mode of transmission.[349]

Vertically acquired HIV used to be regarded as an inevitably fatal illness with death in early childhood. However, as HIV epidemics have matured, an epidemic of LTS of vertical transmission has become apparent in Zimbabwe, with substantial numbers of perinatally-infected children who have survived despite lack of treatment presenting with symptomatic HIV in older childhood and adolescence.

Figure 9.1 Evolution of the HIV epidemic in sub-Saharan Africa



9.1 Implications of research findings

The most striking implications follow from the observation that HIV infection is now the predominant cause of serious morbidity among adolescents in Harare. The findings underscore the importance of scaling up PMTCT interventions as well as early infant HIV diagnosis and immediate treatment of HIV infection in infants. These interventions will however, not affect the current cohort of surviving children who have slowly progressing HIV infection that was acquired before the introduction of PMTCT services. Potentially, current single-dose PMTCT interventions may further increase the proportion of vertical HIV infections that are acquired after the first few weeks of life (and thus more likely to be slow-progressing infections), although reducing the overall burden of infected infants.

A key question that arises concerns the generalisability of study findings. This calls for urgent investigation into whether or not there is a similar trend regionally and steps to identify and implement appropriately tailored interventions to facilitate earlier diagnosis and entry into care of HIV-infected children and adolescents, with the aim of reducing the risk of severe immunosuppression and chronic complications. In addition, there is need to consider the special management issues that apply to adolescent LTS.

9.1.1 Generalisability of findings

The median survival of slow progressors plays a critical role in determining the magnitude and duration of the HIV epidemic in older survivors of MTCT in the region but cohort studies to estimate this would require a very long follow-up that would not now be ethical given the increasing availability of ART. However, it may

be possible to obtain indirect evidence of the distribution of survival through much more extensive prevalence surveys than have so far been carried out, through data from health facilities implementing provider-initiated or routine HIV testing and from data on the age distribution of patients entering clinical care with HIV infection.

The magnitude of the epidemic of HIV infection among adolescents may vary between countries depending on the severity and duration of the adult HIV epidemic and the prevalence of competing causes of mortality in childhood e.g. exposure to malaria, enteric illnesses and levels of food insecurity. Hence the impact of HIV on ill-health, morbidity, and mortality in adolescence in other countries in Africa needs further study. As mentioned in Chapter 2, however, the available data concerning the age-distribution of children in care is already known to be similar to that of Harare for the rest of Zimbabwe,[344] and in Lusaka, Zambia,[16] and also in Blantyre, Malawi where 38% of 1,733 paediatric HIV patients drawn from an urban population of less than 700,000 are 10 years or older (unpublished data, Dr B O'Hare).[344]

Given i) the consistency of the available data, ii) the magnitude of the projected epidemic of HIV in older children in Zimbabwe and South Africa (peaks of 3.4% and 3.3% of all 10 years being LTS in 2007 and 2020 respectively), and iii) the high risk of major preventable complications, notably of chronic lung disease, investment in rapid multi-country assessment studies may be already warranted.[194]

9.1.2 Access to HIV testing and care

HIV testing of older children has not been emphasized in HIV programs and health providers and the community lack awareness of the burden of HIV in this age-group. Routine health facility-based testing services may not be early or universal enough to be an acceptable strategy for this age-group.[365] Other models such as youth-friendly testing services, school-linked testing and family-centred testing may engage this group more effectively and should be actively explored.

Similarly, interventions to facilitate access to HIV care for those who test HIV-positive are needed, especially in the light of our findings from primary care level. Cohort studies from Malawi, South Africa and Mozambique showed that ART uptake among eligible individuals was as low as 13 to 56%.[381-383] Outcomes for individuals who don't meet eligibility criteria for ART may be even worse, with only 4% of such individuals retained in pre-ART care in Thyolo, Malawi.[384] Access to care is likely to be even more difficult for adolescents as discussed in Chapter 7. The substantial barriers to accessing ART result in initiation of ART at an advanced stage of immunosuppression, which in turn is associated with significantly worse treatment outcomes.[283 385] A consensus on how care should be optimally delivered is also needed to avoid this age-group falling through the net of adult and paediatric services.

9.1.3 Management of HIV-infected adolescents

HIV interventions to date have mainly targeted younger children who face different challenges from those confronting adolescents.[344] These include several

unaddressed questions around clinical management, adherence and psychosocial and sexual health.

9.1.3.1 Clinical issues

The pathogenesis of chronic complications notably lung disease is not clearly understood and the optimum management strategies particularly in resource-limited settings need to be defined. The other question concerns the use of ART. Currently, adult guidelines are used to decide when adolescents should be initiated on ART. This may be inappropriate for this age-group as ART may also be indicated for prevention and treatment of chronic complications including growth failure, regardless of immunological status. Very little is known about pharmacokinetics and efficacy of different ART formulations in African adolescents.[386] Current international guidelines recommend dosing by weight, with the adult regimen from 25kg. Increasing age, undernourishment and pubertal and growth delay may each affect drug metabolism for e.g. nevirapine levels in African children on fixed dose combinations are reduced in stunted but increased in wasted children.[387-389] Vertically HIV-infected adolescents in Africa are frequently underweight and stunted, and it is unclear whether age or weight should be used to guide ART drug dosage.

Although ART substantially reduces morbidity and mortality in children, irrespective of previous clinical and virological status, the extent of immune restoration may be age-dependent.[390] CD4 count recovery has been shown to be greater in younger children and this may reflect either greater thymic productivity in pre-adolescent children or poorer treatment adherence in older children.[391-392]

The final extent of catch-up growth and pubertal development may be blunted when ART is started at an older age.[262]

9.1.3.2 Adherence

Evidence is emerging that adolescents are at extremely high risk of early treatment failure. Early ART outcomes are much worse in adolescents than in adults, with very low self-reported complete adherence and high rates of detectable viraemia, nevirapine resistance and clinical disease progression.[392-395]

Table 9.1 Potential risk factors for poor adherence in adolescents

Gender	Contentious but women report more time off ART than men[396]
Sexual maturity	Tanner's stage
Disabling Co-morbidity	Chronic lung disease and neurocognitive impairment
Adverse effects of treatment	Real and perceived side-effects[397]
Poverty	Affects clinic attendance and attrition[398]
Dependency & carer issues	Orphanhood, carer ill-health, lack of supervision, guardian neglect[399-400]
Distance from ART clinic	Journey time and travel cost[401]
Disclosure and stigma	Experienced or anticipated HIV stigma[279]
Health beliefs	Disbelief in HIV as a biological entity of disease[402]
Reluctance to miss school	Nondisclosure at school/ teacher's attitudes

Drug resistance as a result of poor adherence or suboptimal dosing of ART has major implications for patients and health systems. Second-line therapy is expensive, less accessible, has poor outcomes, and adds substantially to ART programme costs even when rates of first-line treatment failure are low.

9.1.3.3 Sexual and reproductive health

Accessibility of sexual health services for young people is poor due to reluctance of health care workers to provide such services to this age-group reflecting the

cultural context in which services are delivered, and lack of confidentiality and privacy.[403]

Vertically-infected adolescents are often cared for in paediatric settings where reproductive health issues are not often addressed but sexual activity among this group is more common than many paediatricians assume. In a study of Ugandan HIV-infected adolescents aged 11-21 years, about a quarter of participants reported prior or current sexual exposure and barriers to adopting preventive behaviours included peer pressure, stigma, partners' ignorance, alcohol use and desire to have children.[404] There are several reports of pregnancies in vertically-infected adolescents, with three out of ten pregnancies resulting in abortion in one series.[405-407] Cervical screening is not standard among HIV-infected adolescents although nearly 50% of vertically-infected sexually-active females have persistent cervical cytological abnormalities.[408]

9.1.4 Correlates of slow progression

The identification of a population with slow progressing infection provides the opportunity to study disease pathogenesis and mechanisms involved in control of HIV infection including genetic, virological and immunological determinants of delayed disease progression. This has implications for development of future strategies targeted against HIV such as therapeutic vaccines, particularly against the clades that are prevalent in sub-Saharan Africa.

9.2 Potential interventions and further research

These are summarised in Table 9.2.

Table 9.2 Research priorities and interventions concerning HIV-infected adolescent LTS

Aims	Research objectives and possible interventions
Population-based epidemiology and interventions	
Is the Zimbabwean epidemic of LTS typical of Southern Africa?	• Confirm facility-based findings elsewhere in the region • Investigate competing cause of childhood mortality: malaria, poor sanitation and unclean drinking water as potential disproportionate hazards to survival of HIV-infected children • Prevalence of HIV-infected LTS at community level ▪ School surveys ▪ Include older children and adolescents in Demographic Health Surveys (DHS) *Include questions concerning maternal vital status and whether participants already known to be HIV-infected. Repeated surveillance until prevalence of undiagnosed HIV infection in older children declines*
Can facility-based diagnostic interventions reliably identify infected adolescents & older children?	• Investigate impact of health facility-based PITC on undiagnosed HIV infection • Investigate perceptions of health care workers re. PITC • Investigate feasibility of implementing community-based HIV testing and counselling diagnosis through School Health Programmes using different models e.g. opt-out testing, voluntary CT • Investigate feasibility of school-based HIV care services • Investigate school-based barriers to accessing care
How can adolescent-friendly HIV testing and care interventions be implemented?	Interventions: ▪ Training of health-care workers and teachers ▪ Advocacy and health communication to increase general awareness ▪ Family-based counselling services promoted through community outlets such as schools, churches ▪ Promotion of HIV testing of adolescents e.g. home-based HIV testing, adolescent testing days in primary health clinics

Aims	Research objectives and possible interventions
Biomedical research	
Host and viral attributes of LTS	• Confirming mother-to-child HIV transmission among LTS (Viral genotyping and phylogenetic analysis of HIV from mother, child and sibling samples) • Genetic and immunological correlates of slow progression of HIV infection (e.g. HLA and KIR typing)
HIV care	
How can virological outcomes following ART be improved?	• Investigate interventions to promote adherence: Directly observed therapy, peer-led support, health education, economic enablers, community-based provision of ART, once daily dosing • Pharmacokinetic studies in stunted adolescents to define optimal ART dosing
Complications of HIV infection: Chronic lung and cardiovascular disease; neurocognitive complications	• Significance of non-TB mycobacteria in chronic lung disease • Impact of respiratory infections on decline in lung function • Investigate interventions effective in other forms of bronchiectasis (e.g. cystic fibrosis): i) NSAIDS, ii) inhaled antibiotics iii) oral antibiotics iv) TB preventive therapy v) phosphodiesterase inhibitors for prevention & treatment of pulmonary hypertension • Investigate approaches to improving infection control in the context of chronic cough and heightened susceptibility to *M.tuberculosis* infection • Investigate neurocognitive deficits that may affect executive functioning, contributing to poor adherence and poor learning
Reproductive & sexual health	• Mother and child outcomes in female LTS who have children (potential for high maternal mortality and risk of HIV to child) • Investigate effectiveness of ART in preventing second-generation perinatal HIV transmission given potential for transmission of HIV resistant to one or more classes of ART Interventions: ▪ Cervical screening ▪ Health education ▪ Family planning and syndromic treatment of STIs
Psychosocial health	• Development of interventions to address stigma directed at health providers, families, teachers and HIV-infected adolescents (to address self-stigmatisation) • Interventions focused on providing life-skills to deal with sexual abuse and economic exploitation • Training health-care workers in disclosure practices

9.3 Summary

Service provision has been adversely affected by the under-appreciation of the numbers of surviving older children and adolescents living with HIV in Africa. The need to consider HIV as a cause of ill health in older children has not been emphasized to health providers, and little or no provision has been made for the special needs of this age group. While awaiting further data of the burden of HIV in other countries, there is an urgent need to develop and rapidly implement policies and programmes aimed at providing early diagnosis and improving the care provided to the expanding numbers of older children and adolescents who are growing up with HIV. A model that integrates HIV clinical care with sexual and reproductive health, psychological, educational and social services will aid this previously neglected age-group to prepare for an independent and productive future and help reduce the impact of this devastating epidemic at community and societal level.

Assuming that the high prevalence of older LTS is confirmed through population-based studies specifically addressing this phenomenon, then we can anticipate the need for clinical trials to address some of the unique features that accompany delayed diagnosis of vertically-acquired HIV infection, for example prevention and treatment of chronic lung and cardiac disease.

Postscript

In addition to being a special group with unusual needs, we may also have much to learn from this previously neglected group. Global eradication of HIV infection is unlikely to be an achievable aim, and there are already signs that the political will needed to continue to expand international investment in HIV prevention and treatment programmes in Africa may be waning.[409] The survival to adolescence of a much higher proportion of untreated infants than anticipated pays testament to the powerful forces of natural selection that have shaped our immune systems and maintained the genetic heterogeneity required to cope with previously unencountered pathogens over hundreds of millions of years.[410]

Pathogen-host co-evolution through analogous natural selection processes are thought to have greatly mitigated the pathogenicity of SIV strains in their normal host species.[411] Although we may feel that we should have no need of recourse to natural selection in the fight against HIV/AIDS, neither now in this technological age, nor in the future, African populations have already experienced huge mortality rates and extremely high life-time risks of dying from HIV. The current generation of LTS thus have the unique evolutionary significance of being the first to take the critical step of survival to reproductive age while living with this novel and deadly virus, and for this, as well as for humanitarian reasons, deserve our support, encouragement and investment to live their life to its full potential.

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Appendix A Case Definitions

The following case definitions were used to define acute and chronic conditions among adolescents admitted to hospital.

A.1 Acute conditions

Tuberculosis (TB)

i) More than five colonies of *M.tuberculosis* from any specimen *or*

ii) AFB on tissue biopsy or at least two positive sputum smears *or*

iii) Caseating granulomata on tissue biopsy *or*

iv) response to treatment for one of:

1. pulmonary disease - compatible chest radiograph changes and no response to antibiotics
2. exudative pleural effusion;
3. pericardial effusion;
4. meningitis- CSF lymphocytic pleocytosis, raised protein, low glucose, negative CrAg and fungal culture;
5. intra-abdominal TB- lymphadenopathy on abdominal ultrasonography
6. disseminated disease- febrile illness of more than 2 weeks duration, weight loss, pancytopenia and no response to antibiotics

Bacterial pneumonia

i) Acute onset (≤ 1week) of symptoms plus airspace consolidation on chest radiograph performed at presentation, plus clinical response to antibiotics *or*

ii) Three of:

1. fever
2. cough
3. purulent sputum
4. pleuritic chest pain
5. leucocytosis

iii) Plus evidence of consolidation on examination, plus clinical response to antibiotics.

Lower respiratory tract infection (LRTI)

Same as definition of bacterial pneumonia, but with no chest radiograph changes and/or no consolidation on respiratory examination.

Pneumocystis pneumonia (PCP)

i) Pneumocystis organisms detected in induced sputum *or*

ii) Subacute onset of cough, exertional dyspnoea, elevated respiratory rate at rest (>25/min), diffuse ground-glass or interstitial shadowing, radiological and clinical response to high-dose trimethoprim-sulfamethoxazole.

Bacterial meningitis

Conventional pathogen identified in CSF by microscopy or if CSF leucocytosis (>50cells/mm^3) that is predominantly (>80%) neutrophils, plus clinical response to antibiotics

Cryptococcosis

i) Cryptococcus isolated from blood or CSF *or*

ii) serum or CSF antigen positive at a dilution of greater than 1:8

Septicaemia

Isolation of clinically significant bacterial pathogen from blood culture plus clinical illness compatible with pathogen isolate.

Enteritis

Acute onset (≤ 1 week) of diarrhoea (3 or more loose stools /day).

Malaria

i) Visualisation of malaria parasite on thick blood film *or*

ii) Consistent clinical symptoms, appropriate exposure history plus not other cause of fever found and response to anti-malarial treatment.

Drug Toxicity

i) History of exposure to appropriate drug (≤1 week before onset of symptoms) plus recognised adverse reaction to drug (e.g. zidovudine/anaemia) *or*

ii) Stevens Johnson syndrome or new rash or anaphylaxis plus no other cause for the symptoms.

Oesophageal candidiasis

Oral candidiasis and recent onset of retrosternal chest pain on swallowing, plus response to fluconazole.

HIV Wasting Syndrome

Weight loss or cachexia, with diarrhoea or fever, or both, for at least one month, not known to be due to a condition unrelated to HIV infection, plus negative investigations for TB.

Urinary Tract Infection

≥2 of dysuria, fever, flank tenderness plus no abnormal vaginal or urethral discharge.

Sexually Transmitted Infection

i) (Women) Vaginal discharge and/or lower abdominal tenderness

ii) (Men) Urethral discharge and/or dysuria *or*

iii) genital sore/ vesicle / ulcer.

Kaposi Sarcoma

i) Characteristic gross appearance of erythematous or violaceous plaque-like lesion on skin *or*

ii) mucous membrane *and/or*

iii) characteristic histological appearance of lesion.

Stroke

i) Sudden or rapid onset of focal neurological deficit,

ii) Plus compatible CT appearance of stroke (bleed or infarct and no space occupying lesion) *and* no history of fevers *and* no constitutional symptoms *and* no Chest radiographic features suggesting TB.

Trauma

Injury or later consequences of an injury

A.2 Chronic conditions

Cardiac disease

Clinical finding of cardiac failure and cardiomyopathy, valve lesions or pericardial disease on echocardiography.

Chronic lung disease

i) Radiological appearance of focal scarring, *and/or*

ii) Opacification *and/or*

iii) Cystic changes *and/or*

iv) bronchial wall thickening,

v) Plus negative TB smears & cultures from current episode

vi) Plus two of

1. Clubbing
2. History of recurrent (at least 2) episodes of cough productive of copious amounts of purulent sputum
3. Persistent fine basal crepitations on auscultation
4. Cor pulmonale.

Cor pulmonale

i) Case definition for chronic lung disease met plus

ii) *Either* echocardiographic finding of right ventricular enlargement

iii) *Or* two of the following:-

1. Ascites
2. Hepatomegaly
3. Raised jugular venous pressure
4. Ankle oedema.

HIV-associated encephalopathy

i) HIV infection

ii) CT scan appearance of generalised cortical atrophy and no space occupying lesion or localised infarct,

iii) Plus each of the following:-

1. Two of
    1) Hyperreflexia
    2) Palmo-mental reflex,
    3) Memory loss (e.g. forgets names, places, conversations)
    4) Apathy
    5) Slowness of thinking
2. No other cause found to explain the clinical findings
3. Course of illness over weeks to months.

Diabetes

i) Previous diagnosis of diabetes mellitus made by a physician or blood glucose stix >10mmol/l (for the first time) *or*

ii) Ketones in urine, ≥2+ glucose in urine, shallow and fast breathing, polyuria and excessive thirst.

Bronchial Asthma

i) History of recurrent wheeze *or*

ii) Chest tightness on waking, at night or during exercise

iii) Symptomatic relief achieved through use of salbutamol plus audible wheeze at examination.

Epilepsy

i) Witnessed seizure or fit

ii) Plus diagnosis of epilepsy made by a physician *and*

iii) History of recurrent (at least 2) seizures unprovoked by alcohol/drugs.

Appendix B Chronic lung disease among HIV-infected adolescents with vertically-acquired HIV infection

The study investigating HIV-associated morbidity among hospitalised adolescents, described in Chapter 4 of this thesis, suggested a high burden of chronic lung disease among HIV-infected adolescents, as a complication of longstanding HIV infection. However, pre-admission history of chronic lung problems was not well documented and past chest radiographs were not available, making it difficult to distinguish underlying chronic lung disease in patients who were presenting with acute-on-chronic complications. A prospective study was thus carried out in collaboration with King's College Hospital, London to investigate the burden and clinical features of chronic lung disease among stable vertically-infected adolescents (aged 10 to 18 years) in HIV care. Preliminary results are summarised in this appendix.

B.1 Methods

Consecutive patients attending two outpatient clinics in Harare were recruited with no other inclusion criteria applied. Exclusion criteria were:- horizontally-acquired HIV, acute respiratory disease, taking intensive phase of treatment for smear-positive TB, lung malignancy, pregnancy, sickle cell disease. Mode of HIV acquisition was assessed by a physician, based on history of maternal and sibling HIV/death, history of chronic ill-health, pubertal delay and stunting, report of sexual activity by participant. Participant assessment included the following:

- Anthropometric assessment and standardised respiratory examination
- Oxygen saturation
- Exercise testing (brisk 200m walk)
- Spirometry (spirometric standards were obtained from age, sex and height matched healthy Malawian children[412])
- CD4 lymphocyte count
- Sputum examination for bacterial and mycobacterial culture, if able to expectorate
- Chest radiography (CXR)
- High resolution computed tomography (HrCT) scan (independently reviewed by two thoracic radiologists, blinded to clinical and CXR data)
- Doppler echocardiography independently reviewed by two cardiologists, blinded to clinical and other data

B.2 Results

116 participants were recruited. The mean age was 14 years (S.D 2.62) and 57% were female. The median CD4 count was 384 cells/μl (IQR 180-584), and 69% were taking ART (median duration 20 months, IQR 5-40 months). Only one patient was a smoker. There was a high prevalence of chronic respiratory symptoms and severe restriction of exercise tolerance (Table B1), and spirometry showed common and severe deficit in lung function (Table B1).

Symptoms, signs and spirometric indices were not associated with CD4 count, taking ART or duration of time on ART. 86% of CXRs were abnormal; the two

most common patterns on HrCT suggested obliterative bronchiolitis and bronchiectasis. 13% of patients had pulmonary hypertension on Doppler echocardiography.

Table B1. Clinical features of respiratory disease in participants

Clinical Features	N (%)
Clinical History	
>2 courses of antibiotics for LRTI in past year	48 (41)
Hospitalised for LRTI in past year	19 (16)
Symptoms	
Recurrent cough productive of purulent sputum*	77 (62)
Exceptional chest pain	49 (42)
NYHA Dyspnoea Scale >1	24 (21)
Signs	
Respiratory rate >25/min at rest	33 (28)
Resting oxygen saturation <92% at rest	15 (13)
Oxygen saturation drop of ≥5% on exercise testing (n=72)*	21 (29)
Spirometric	
FEV1 % predicted < 80%	52 (44)
PEFR % predicted < 80%	33 (28)

*Most days in at least 3 months in the year in past two years
** 3 not mobile, 8 O2 sats<92%, 26 RR>25, 7 O2 sats<92% and RR>25)

B.3 Conclusions

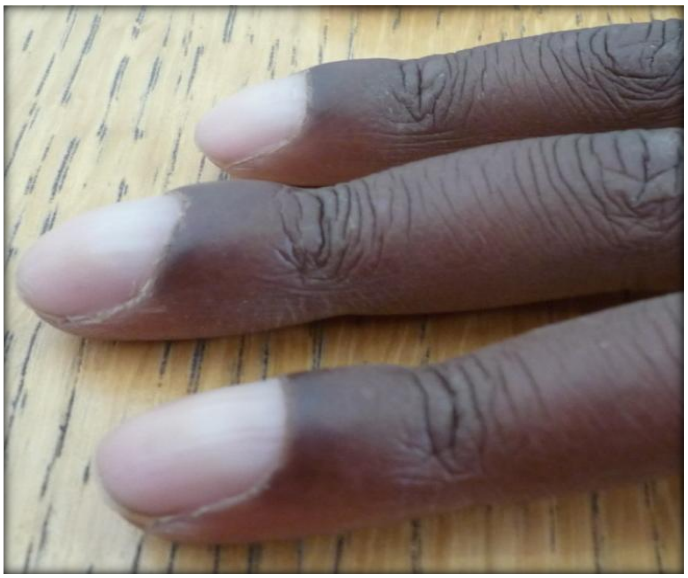
Vertically-infected African adolescents attending for HIV care have an extremely high prevalence of severe and disabling chronic lung disease, with over half of the systematically selected adolescents in this clinic displaying features (resting tachypnoea, resting hypoxia, drop in Fi02 on exercise, and pulmonary hypertension) that have been well documented to carry a poor prognosis of medium term (5 year) survival in cohort studies of chronic lung disease from non-HIV causes.[413] Extending these studies to investigate the prevalence of chronic lung disease in adolescents presenting with HIV at other levels in the health system is thus a matter of urgency, as are studies focused on better

management and prevention of respiratory disease in HIV infected older children and adolescents.

These findings also support the conclusions from the hospital-based study detailed in Chapter 4, namely that current HIV testing and care practice need to be much more proactive and aimed at younger patients in the community or primary care level if severe and irreversible complications from untreated maternally-acquired HIV are to be effectively prevented. Of note, clinical signs and spirometric indices were independent of immunological status or HIV treatment status, suggesting that once established, ART does not significantly reverse the clinical course of lung disease.

Figure B1. A) Finger clubbing in a study participant B) HrCT showing cystic destruction of the lung C) HrCT showing mosaic attenuation of the lung, suggestive of obliterative bronchiolitis D) Flow volume loop in a 13 year old female showing a severe restrictive defect

A



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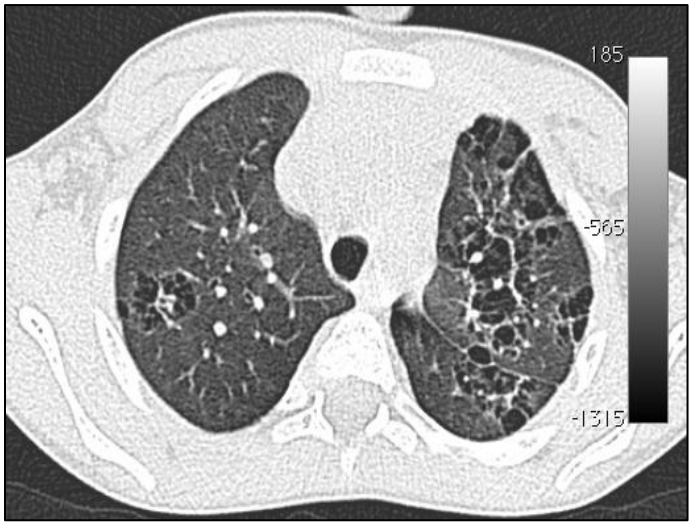
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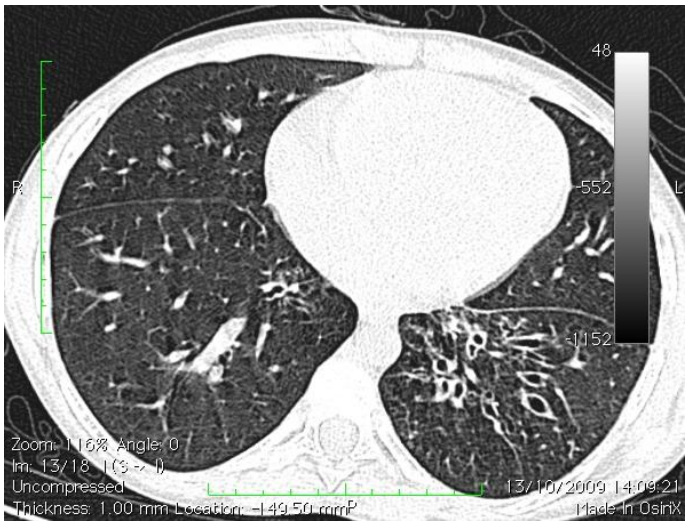
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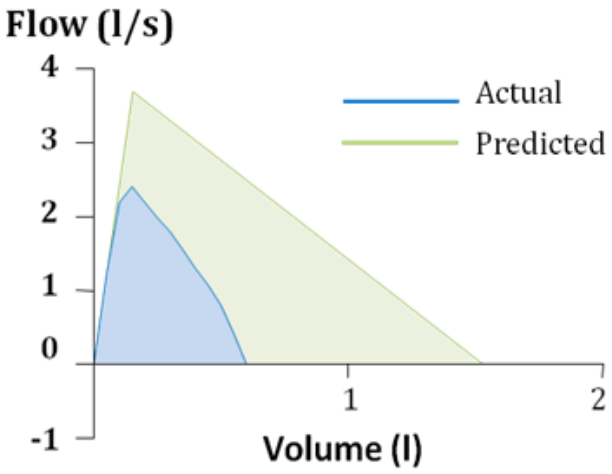
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