Antimycobacterial treatment among children at start of antiretroviral treatment and antimycobacterial treatment after starting antiretroviral treatment among those who started antiretroviral treatment without antimycobacterial treatment at a tertiary antiretroviral paediatric clinic in Johannesburg, South Africa.

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A minithesis submitted in partial fulfillment of the requirements for the degree of Master of Public Health in the School of Public Health, University of the Western Cape.

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Keywords:

TB prevalence, antimycobacterial treatment-free survival, BCG disease, antimycobacterial treatment, antiretroviral therapy, ART/antimycobacterial co-treatment, immune reconstitution, mycobacteria other than TB, HIV-infected children, mycobacterium.

Abstract:

Background: Although clinicians encounter antimycobacterial treatment in Human Immunodeficiency (HIV)-infected children as one of the most common treatments coadministered with antiretroviral treatment (ART), quantitative data on the extent of antimycobacterial treatment among HIV-infected children at the time of commencement of ART and at different times during ART is scarce. The baseline risk factors associated with being on both ART and antimycobacterial treatments are not known and it remains to be elucidated how the different exposure factors impact on the antimycobacterial treatment-free survival of children who begin ART without antimycobacterial treatment.

Objectives: To describe the prevalence of antimycobacterial treatment among children at the time of starting ART and the antimycobacterial treatment-free survival after starting ART.

Design: A retrospective cohort study based on record reviews at the Harriet Shezi children's clinic (HSCC).

Population: HIV-infected children less than fifteen years of age presumed ART naïve started on ART at HSCC.

Analysis: A descriptive analysis of the prevalence of antimycobacterial treatment at time of start of ART was done. Kaplan Meier (KM) survival curves were used to determine the antimycobacterial treatment-free survival and logistic regression was used to analyze the association between baseline factors and future antimycobacterial treatment among children who had no antimycobacterial treatment at time of start of ART.

Results: The prevalence of antimycobacterial treatment at the time of starting ART was 518/1941 (26.7%, 95% confidence interval (CI): 24.7-28.7). Among children who started ART without antimycobacterial treatment, the KM cumulative probability of antiretroviral and antimycobacterial (ART/antimycobacterial) co-treatment in the first 3 months of starting ART was 4.6% (95% CI: 4.1-5.2), in the first 12 months it was 18.1% (95% CI: 17.0-19.2) and in the first 24 months of starting ART it was 24% (95% CI: 21.9-25.1). Survival analysis suggested that children with high baseline

viral load, advanced World Health Organization (WHO) stage of disease, very low normalized weight for age (waz) and very young age (less than one year) at start of ART had significantly reduced antimycobacterial treatment-free survival (log rank $p < 0.05$) in the first two years of starting ART. In the logistic regression model, age less than one year {Odds ratio (OR): 3.7 (95% CI: 2.2-6.0; $p < 0.0001$)} and very low weight for age Z-score (waz < -3) {OR; 2.2 (95% CI: 1.4-3.6; $p = 0.0015$)} were the two critical risk factors independently associated with future antimycobacterial treatment.

Conclusions: Antimycobacterial treatment is extremely common among HIV-infected children at the time of starting ART and early after starting ART and the incremental risk of being on ART/antimycobacterial co-treatment decreases with time on ART. The results emphasize the need for a heightened and careful alertness for mycobacterial events especially among children starting ART with severe malnutrition and those who start ART at age less than one year. The results further suggest that it is probably optimal to start ART in children before their nutritional status has deteriorated severely in the course of the HIV disease so that they get protection against mycobacterial events by early ART.

Declaration

I declare that 'Antimycobacterial treatment among children at start of antiretroviral treatment and antimycobacterial treatment after starting antiretroviral treatment among those who started antiretroviral treatment without antimycobacterial treatment at a tertiary antiretroviral paediatric clinic in Johannesburg, South Africa' is my own work, that it has not been submitted for any degree or examination in any other university and that all sources I have used have been acknowledged by complete references. I also declare no conflicts of interest in the data that I am submitting.

Tamuka Chivonivoni

Signed November 2010

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CHAPTER 1: INTRODUCTION

Background:

ART provided as part of chronic HIV care has been shown in observational studies to reduce the incidence rate of TB among HIV-infected adults in South Africa by about 70 to 90% (Lawn, Badri & Wood, 2005; Maher, Harries & Getahun, 2005; Miranda *et al*. 2007) and among children by about 70% (Martinson *et al.* 2009; Edmonds *et al.* 2009). Despite this, clinicians still encounter TB as the commonest opportunistic infection in HIV-infected persons before starting ART and while they are receiving ART (Lawn, Wilkinson, Lipman & Wood, 2008).

In the early years of ART there was little evidence about the benefits of concurrent administration of ART and antimycobacterial treatment and a significant proportion of physicians treating both conditions were skeptical about giving the treatments together simultaneously. Some treatment guidelines were emphasizing completion of TB treatment before starting ART for those whose CD4 was above 200 cells/µl while on TB treatment or at least completing the intensive phase of TB treatment before ART initiation if the CD4 was between 100 and 200cells/µL and for those who had CD4 count less than 100cells/µL there was no clear consensus and in such cases ART was only introduced as soon as it was practicable (BHIVA, 2005). A retrospective study in the United Kingdom (Dean *et al.* 2002), recommended starting ART early after initiation of TB therapy for patients with advanced HIV disease (CD4 < 100 cells/µl) and to defer ART until the continuation phase of TB therapy (i.e. after 2 months) for patients who are clinically stable (CD4 > 100 cells/µl).

Recent studies have however clearly shown the overwhelming benefits of ART/antimycobacterial co-administration in patients with HIV/TB co-morbidity on mortality (Karim *et al*. 2010; Violari *et al*. 2008; Velasco *et al.* 2009; Varma *et al*. 2009) and morbidity (Edmonds *et al.* 2009; Dean *et al*. 2002). Results from a randomized clinical trial in South Africa (Karim *et al.* 2010) reported significantly reduced mortality among the 425 patients (5.4/100 person years, or 25 patients) in the pooled 'early

integrated arm' (ART within two weeks of TB treatment) and 'delayed integrated arm' (after two months of TB treatment) compared to that among the 213 patients in the 'sequential arm' (ART after completion of TB treatment) (12.1/100 person years, or 27 patients), p <0.005. Similarly, an observational prospective study in Spain reported that starting ART in the first two months of TB treatment was an independent predictor of survival (HR: 0.37, 95% CI 0.17-0.66) when compared to initiation after three months of TB treatment (Velasco *et al.* 2009). Another prospective study in Thailand also demonstrated an increased risk of death the longer ART was delayed (HR 9.0, 95% CI 1.1-73.0) for those for whom ART was delayed compared to those who initiated ART within the first 120 days of TB treatment (Varma *et al.* 2009). In South Africa, it was reported from a large retrospective study, that starting ART within the first 30 days of TB treatment did not increase mortality (HR 1.28, 95% CI 0.78-2.10) compared to 31 to 60 days (HR: 1.08, 95% CI 0.59-1.98), 61-120 days (HR:0.89, 95% CI 0.48-1.63) and >120days (HR: 1.25, 95% CI 0.65-2.43) (Westreich *et al.* 2009). In the United Kingdom it was shown from a retrospective cohort that ART during TB treatment was associated with significant reductions in viral load and AIDS-defining illness (3.5 versus 24.5%; relative risk (RR) = 0.14) and mortality (Dean *et al*. 2002).

Although this may be the case, there are concerns about adherence (Nachega *et al*. 2008), high pill burden, drug interactions (Boulle *et al*. 2008; Jaspan *et al.* 2007) and overlapping toxicity which is seen especially with nevirapine-based ART regimens (Nachega *et al*. 2008; Lawn *et al*. 2008).

Because of the weighted benefits of ART/antimycobacterial cotreatment provided by the new evidence, several national guidelines including the South African (DOH, 2009) have been adopted to start ART as soon as it can be tolerated after starting antimycobacterial treatment and this is usually within two to eight weeks.

Although the HIV and mycobacterial co-infection is a huge public health problem in children in as far as the dual management of the two co-morbidities and the high frequency are concerned, quantitative data on the extent of ART/antimycobacterial co-treatment among children is lacking.

Justification for the study:

This study is done in South Africa where a high number of TB patients are also HIV-infected (WHO report, 2009) and therefore warranting dual therapy for the two conditions. In order to help managers and to plan resource allocation it is important to quantify the needs of HIV-infected children for antimycobacterial treatment at different phases in their encounter with HIV treatment services, including prior to initiating ART and during the early and later phases of ART.

Co-treatment of HIV and mycobacterial infections in children is complex due to some of the reasons mentioned above and requires modification of ART regimens in some cases (Lawn & Wood, 2006) and careful monitoring. For this reason, it is important to determine the extent of the co-treatment so that policy makers can design appropriate algorithms in the area of ART/antimycobacterial co-treatment. The limitations posed by the above mentioned complexities of ART/antimycobacterial co-treatment make the South African public sector paediatric HIV clinician quickly exhaust the only available option for ART among the very young children on antimycobacterial treatment, two nucleoside reverse transcriptase inhibitors (NRTIs) and the one available protease inhibitor (PI) in children, Kaletra (ritonavir boosted lopinavir). It is therefore important to quantify the extent of the problem so that governments can plan drug supplies and bargain with pharmaceutical companies for affordable prices of better drugs for co-treatment of HIV and mycobacteria.

In addition to planning for medicines, knowledge of the extent of the requirements of the dually infected children also puts public health officials and policy makers in a picture to see and possibly appreciate the need for newer and more efficient mycobacterial diagnostic interventions.

HIV clinicians also need to know how much antimycobacterial treatment there is at the start and after starting ART and the risk factors associated with starting antimycobacterial treatment among children on ART so that they can provide quality care to their patients and public health managers can also plan to adopt HIV/TB control measures at community level. Knowledge of the baseline risk factors associated with future mycobacterial events among children on ART can also help physicians to make

rough forecasts of the events to be anticipated for and help them to prepare. It is also very important for physicians to know the critical times to do follow up of high risk children groups so that they are not caught unawares when such events arise.

CHAPTER 2: LITERATURE REVIEW

The millennium development goal target 6.c wishes for the TB incidence to decrease by half by 2015 from baseline level in 1990 but in reality the global incidence of TB has been rising since 1990 from 6.6 million cases to 8.3 million cases in 2000, to 9.24 million cases in 2006 and 9.27 million cases in 2007 (WHO report, 2009). Approximately 31% of these cases were from the Africa region and about 15% of the total 9.27 million cases were HIV-infected (WHO report, 2009). Of the HIV-infected incident TB cases approximately 79% were from the African region (WHO report, 2006; WHO report, 2009). South Africa and Nigeria occupy positions in the top five of the worst TB burdened countries in the world both with 0.46 million cases in 2007 (WHO report, 2009). Of the total incident TB cases annually, it is further estimated that 11% are childhood TB cases (age less than 15 years) of which 75% of the childhood cases occur annually in 22 high-burden countries. It is further estimated that the 22 high burden countries together account for 80% of the world's estimated incident TB cases (WHO, 2006).

South Africa has a high TB case notification rate (CNR). Because of difficulties in diagnosis, especially among children, and record ambiguity, the CNR actually reflects the cases that were given antimycobacterial treatment (for any reason) but not the true incidence of TB disease (Marais *et al*. 2006). The national CNR has risen from 169/100000 population in 1998 to 722/100000 in 2006 (National Department of Health (DOH), 2006). KwaZulu-Natal had the highest rise in CNR from 110/100000 to 1075/100000 followed by Western Cape from 464/100000 to 1030/100000 while the CNR in Gauteng rose from 123/100000 to 500/100000 in the same period (DOH, 2006) and this is attributed mainly to HIV. A Cape Town study in two socioeconomically disadvantaged communities

revealed a high CNR of TB in children less than five years (3588/100000), which was about 3.5 times that in adults and the 0-14 year age group contributed 39% of the total caseload (van Rie *et al*. 1999).

The Joint United Nations HIV/AIDS programme (UNAIDS), (2007), estimated the new annual HIV infections in children less than 15 years globally to be between 390000-420000. In South Africa among children between 2 and 14 years the 2008 prevalence of HIV was 2.5% CI; 1.9-3.5. (South Africa National HIV Prevalence, Incidence, Behavior and Communication Survey, 2008).

TB/HIV Collaboration

The TB/HIV collaboration being implemented by several national programmes is seen as a feasible policy strategy to tackle the serious impairments in the national TB programmes, to improve the quality and continuity of care of patients with HIV/TB co-morbidities and provide joint planning, monitoring and interpretation of progress towards TB and HIV control targets (Maher *et al*, 2005; WHO report, 2008; WHO, 2004). This strategy involves HIV testing and counseling among TB patients and providing interventions including cotrimoxazole prophylaxis (CPT), isoniazid preventive therapy (IPT) and ART to those HIV-infected and eligible. The strategy also involves TB screening, detection, ensuring infection control in health care and congregate settings and treatment of TB and other interventions for HIV related opportunistic infections among those that are found HIV-infected (WHO, 2004).

Influence of HIV on TB and diagnosis of TB in HIV-infected clients

HIV weakens the immune system and promotes the progression of latent to active TB (Mofenson *et al.* 2004; Cohen *et al*. 2006). It promotes the progression of recently acquired TB infection to active disease (Zar *et al*. 2006). HIV increases the rate of TB re-infection (Maartens, 2000) and relapses and increases the rate of transmission in the community (Maher *et al*. 2005). Children with HIV infection are at higher risk of more severe forms of TB (De Cock, Grant & Porter, 1995) and treatment outcomes are worse compared to HIV-uninfected children (Wilkinson and Davies, 1998; Rekha and

Swaminathan, 2007). HIV increases the incidence of TB and in Sub-Saharan Africa, a region with very high HIV rates, the incidence is growing at approximately 4% per annum (Maher *et al*. 2005). Of all the opportunistic co-infections affecting HIV-infected children TB is the most common (Williams and Dye, 2003; Ruxrungtham and Phanuphak, 2001; Swaminathan, 2004). The proportion of smear negative TB and extrapulmonary TB is higher among HIV-infected patients (WHO, 2004) making the diagnosis of TB more difficult compared to that in HIV-uninfected patients.

The clinical presentation of TB as with many other opportunistic infections in the face of immunodeficiency is very variable depending on the level of immunodeficiency (Tantisiriwat & Powderly, 1999; Macdougall, 1999) and this poses serious diagnostic difficulties (Graham & Chaisson, 1993; Pape, 2004; Woldehanna & Volmink, 2004; Maher *et al.* 2005; Keshinro & Ya Diul, 2006). Although TB diagnosis in HIV-infected adults is difficult (Reid and Shah, 2009), authorities generally agree that TB diagnosis in HIV-infected children is even more challenging (DOH, 2000; Newton *et al.* 2008). Some of the reasons for this are the paucibacillary (Chakraborty & Shingadia, 2007) nature of the disease, the fact that children less than 10 years do not easily produce sputum and that there are more cases of disseminated TB (Mofenson *et al.* 2004; Chakraborty & Shingadia, 2007). These and other factors prompted the National Department of Health, South Africa (2000) to develop guidelines for diagnosis of TB in children. Diagnostic work up includes the tuberculin skin test, chest x-ray, history of contact with a suspicious TB case and clinical features that include cough for more than 2 weeks, chest pain and failure to thrive as depicted on the Road to Health Card (RTHC) without alternative explanation, recurrent lower respiratory tract infections not responding to antibiotics, painless matted cervical lymph nodes, more than a single episode of fever without alternative explanation, constitutional symptoms like fever, general body weakness, diarrhea, tachycardia and vomiting.

The WHO is currently evaluating and endorsing several new laboratory ways of diagnosing TB. These include the liquid culture and rapid species identification which provide shorter turn-around times and

are more sensitive than traditional direct smear microscopy and TB culture currently being employed in most high TB burdened developing countries. The molecular line probe assay is for detecting TB bacteria and identifying susceptibility to isoniazid/rifampicin in smear-positive sputum samples. Nucleic acid amplification techniques such as the Gene Xpert have higher sensitivity than sputum smear microscopy and can provide a diagnosis of Mycobacterium tuberculosis and identify resistance to rifampicin and isoniazid in 90 minutes. The Gene Xpert could assist to more rapidly diagnose Mycobacterium tuberculosis complex and differentiate it from nontuberculous mycobacterial infections and might therefore be beneficial to diagnosis of TB in children when sputum samples can be obtained.

BCG disease presents a special challenge to the diagnosis of TB disease in immunocompromised children in that it cannot be distinguished symptomatically from TB disease (Hesseling *et al*. 2003) and this difficulty is worsened by the fact that our laboratories do not routinely perform polymerase chain reaction (PCR) on samples from patients and the new TB diagnostic tools have not been implemented on a wider scale (WHO, 2008) to speciate the different mycobacterial strains. It presents in the following main forms, (1) the localized disease which may present as abscesses, ulceration or fistula at or around the vaccination site (2) as regional disease with ipsilateral axillary lymphadenitis and (3) the disseminated disease where sites beyond the satellite node are involved (Fallo *et al*, 2005; Hesseling *et al*, 2006). In an assessment of baseline factors that influence either the development of BCG complications or not in HIV-infected infants, one study found that only very low CD4 percentage (13.6+/-11) was associated ($p<0.01$) and not the viral load, the disease stage nor the mean follow-up time (Fallo *et al.* 2005).

The position of WHO on BCG is that it should not be given to known HIV-infected children regardless of symptoms and degree of immunocompromise but in children whose HIV status is unknown BCG should be given in all asymptomatic children at birth irrespective of HIV exposure status (WHO, 2007). For programmatic reasons, International Union against Tuberculosis and Lung

Diseases (IUTLD) BCG Working Group supports this revised WHO BCG vaccination policy, in countries highly endemic for TB until all programs are in place for implementing selective deferral of HIV-exposed infants (Hesseling *et al.* 2008). This is unfortunately the practical situation in most developing countries where the burden of HIV and TB are very high, screening for TB in babies is complex, PMTCT coverage is still behind and HIV testing is done around six weeks after birth in the public sector. It therefore means that almost all children are vaccinated before their HIV status is known although this is not the ideal. BCG vaccination coverage at birth in 2008 was around 89% globally and in the Afro region it ranged from 60 to 89% between 1990 and 2008 (WHO, 2009). It protects against severe forms of TB especially TB meningitis and disseminated TB but its efficacy was variable in different studies ranging from 65 to 85% in HIV uninfected children (Trunz, Fine & Dye, 2006). In HIV-infected children the benefits of BCG vaccination is doubted by other evidence. In Argentina, there was no difference in the frequency of TB disease in HIV-infected children among those vaccinated and those who were not (Fallo *et al*. 2005) raising the need for further prospective randomized clinical trials to establish the merits and demerits of BCG at birth policy.

Impact of immune status, ART and previous TB on the incidence of TB:

Retrospective studies have reported reduced incidence (Walters *et al.* 2008; Mhlongo *et al*. 2004; Miranda *et al*. 2007; Martinson *et al.* 2006) and reduced mortality (Walters *et al.* 2008) due to TB among children on ART compared to those who were not.

Mathematical modeling suggested that a big population coverage (greater than 75%) and high adherence levels would be required to effectively reduce TB if ART is started early in HIV disease (Currie *et al.* 2003; Atun *et al.* 2007) and that universal testing with prompt ART initiation would reduce HIV incidence and mortality to less than one case per thousand within ten years of implementation and therefore reduce incidence of TB (Granich *et al.* 2009).

Seyler *et al*. (2005) concluded after multivariate analysis that the only risk factor for TB during ART is previous TB. Some authorities argue that the study was biased as the population composition was too narrow, composed of patients with advanced clinical HIV disease and advanced immunodeficiency which limited the applicability of multivariate modeling for the two important potentially confounding factors in the study, the baseline CD4 count and the baseline clinical stage of the disease (Lawn, Badri & Wood, 2005).

In Cape Town, Lawn, Badri & Wood (2005) concluded that the ongoing risk of TB is dependent upon the nadir CD4 count and the clinical advancement of the HIV disease and past TB was not a risk factor.

Martinson *et al.* (2006) found a lower incidence of TB (1.76 per 100 child years) among children whose viral loads were less than 400 copies per milliliter (ml) while it increased with increasing viral loads and that ART protects children from TB.

Immune reconstitution:

TB disease can occur early after starting ART. It has been shown in both high income and low resource settings that the incidence of TB is highest in the first three months of starting ART (Lawn *et al*. 2008; Chakraborty & Shingadia, 2007; Bonnet *et al.* 2006) and thereafter it falls rapidly and remains steady at lower rates (Lawn *et al*. 2008).

ART improves TB specific immune responses and restores the manifestations of TB that were lost during immunosuppression (Lawn, Badri & Wood, 2005; Lawn, Bekker & Wood, 2005). Immune reconstitution inflammatory syndrome (IRIS) is commonly associated with mycobacterial infections, mainly BCG, Mycobacterium tuberculosis and non-tuberculous mycobacteria (Boulware, Callans & Pahwa, 2008; Smith *et al*. 2009). Two main mechanisms have been speculated to be possible explanations for IRIS in children, one and more important in younger children and infants being that of unmasking of a subclinical opportunistic infection or inflammation in early ART, and two, a

paradoxical response where there is resurgence of clinical manifestations of a pre-ART infection or inflammatory reaction in early ART being more prominent in the older ages (Meintjies & Davies, 2008; Boulware, Callans & Pahwa, 2008). A Thai study reported immune reconstitution syndrome caused by nontuberculous mycobacterial (NTM) infection in 9 of 153 (5.9%) HIV-infected children 2 to 26 weeks after initiation of ART. The clinical syndrome included fever and dyspnea (2 children), fever and abdominal pain (3), subcutaneous nodules or suppurative lymphadenitis (4) and the causative species were Mycobacterium avium (4), Mycobacterium scrofulaceum (3), Mycobacterium kansasii (1) and Mycobacterium simiae (1) (Puthanakit et al. 2006).

ART/antimycobacterial co-treatment:

There is very sparse pharmacokinetic data especially in children to direct the optimal doses of ART/antimycobacterial cotreatments and mostly such therapy is being guided by extrapolation from adult data. A recent South African study limited by the sample size showed that doubling the dose of the Kaletra which is done in adults on rifampicin-based antimycobacterial treatment in ART/antimycobacterial co-treatment achieves sub-therapeautic Kaletra levels if done in children (McIlleron *et al*. 2009). They recommended the addition of extra ritonavir to further boost the Kaletra. Evidence from a South African prospective study has shown that nevirapine-based ART co-administered with rifampicin-based antimycobacterial treatment has inferior virological outcomes compared to efavirenz-based ART and rifampicin-based antimycobacterial treatment (Boulle *et al*. 2008). In Thailand a retrospective study (Manosuthi *et al.* 2008) showed that at 48 weeks subjects who received nevirapine-based ART and rifampicin-based antimycobacterial treatment (n=111) had inferior virological outcomes (viral load less than 50 copies) than efavirenz-based ART and rifampicin-based antimycobacterial treatment (n=77), (77.9% versus 67.9%) and that the rate of ART discontinuation due to side effects was higher among those who received nevirapine-based ART and rifampicin-based antimycobacterial treatment (7.2%) compared to those who received efavirenz-based ART and antimycobacterial treatment (none) although it was not statistically significant (p=0.084). A

retrospective cohort study in Botswana (Shipton *et al.* 2009) reported similar virological outcomes (viral load less than 400 copies) at one year between those who received ART alone, either nevirapine or efavirenz based NNRTI backbone (n=155) and those that received ART and antimycobacterial treatment (n=155) (82% versus 91%, p=0.28) but the trend towards hepatotoxicity was more among the later (9% versus 3%, p=0.05).

Unfortunately, in the South African public sector context, especially in centers other than tertiary, for children under three years of age or less than 10 kilograms (kg) weight, the options for ART regimens is limited to only two NRTIs (lamivudine and stavudine/zidovudine/abacavir) and kaletra (DOH, 2005) compromising the potential of constructing a second line regimen (WHO, 2008). Although recommended conditionally by the WHO for first line use where kaletra use is not feasible because of cold chain requirements and affordability (WHO, 2008), in South Africa nevirapine is generally not given due to concern about the possibility of resistance following the single dose exposure to nevirapine provided to HIV exposed babies during prevention of mother to child transmission (PMTCT) which is now a universal practice in the country. Efavirenz is not given because of lack of evidence of efficacy and toxicity in children less than three years. The major challenge is now to establish the safe and efficacious doses of kaletra and efavirenz during ART/antimycobacterial co-treatment to be used in the very young children now that there is going to be an increased number of infants and young children on ART following new data and recommendations (Violari *et al.* 2008; WHO, 2008).

CHAPTER 3: METHODOLOGY

Aim:

To describe the prevalence of antimycobacterial treatment among HIV-infected children receiving HIV care at the time of starting ART and the antimycobacterial treatment-free survival after ART initiation.

Objectives:

1. To describe the proportion of children receiving antimycobacterial treatment at start of ART.
2. To describe the two year antimycobacterial treatment-free survival of children after commencing ART.
3. To determine whether selected pre-ART factors predict which children will require ART/antimycobacterial co-treatment.

Design and Setting:

This was a retrospective cohort study involving record reviews of all eligible children (see below) started on ART in the HSCC in the period 1st April 2004 to 1st April 2008. Antimycobacterial treatment was examined as the outcome and not TB disease because of the difficulty of diagnosing TB disease in children and the fact that antimycobacterial treatment was not only given for TB disease but also for BCG disease, for mycobacterium avium complex (MAC) and for other mycobacteria other than TB (MOTT) but in the HSCC electronic database that was used, the reason for antimycobacterial treatment was not clearly delineated. If an antimycobacterial treatment warranting condition was suspected, children were initiated onto antimycobacterial treatment (and this was indicated as TB treatment in the database and no mention of the specific medication used was done making it impossible for one to attempt to differentiate the mycobacteria from each other). Although medication such as azithromycin, ciprofloxacin and clarithromycin were used in cases where MAC was isolated, no mention of this treatment was evident in the database and therefore the mention of 'TB treatment' in the database referred to all antimycobacterial treatment (see sample of database in annexure). The same argument applied for BCGosis and BCG IRIS. It was therefore impossible for the author to

differentiate different mycobacteria based on the medications used. The confidence of the clinician that antimycobacterial treatment was needed for a child was taken as confirmation that the condition was indeed present.

The data was entered onto excel database by trained data entry clerks who in turn received paper-based data from the treating physicians. The quality of the data was frequently checked by the data manager who works closely with the resident epidemiologist to double check any data entry queries and to clean the data.

Routine care at HSCC:

HSCC caters for the biggest number of public sector pediatric HIV patients compared to any other childrens' HIV clinic in South Africa. It is located in a tertiary academic centre, Chris Hani Baragwanath hospital (CHBH), in Soweto. It caters for children that come as referrals from the clinics in the Johannesburg metropolis predominantly the west and southern Johannesburg areas, children diagnosed HIV-infected in the main CHBH hospital wards, HIV-infected children referred from private nursing homes and also for children brought directly from the community and then tested HIV-infected. It offers both primary health care services and specialist services. Its staff range from community counselors, dieticians, social workers, psychologists, administrators, data clerks, epidemiologists, nurses, researchers, pharmacists, medical officers and specialist pediatricians and the patients range from infants to adolescents.

Children were generally started on first line ART according to the national ART guidelines of South Africa (DOH, 2005). This consisted of two NRTIs, specifically stavudine (D4T) and lamivudine (3TC), and one NNRTI (efavirenz) in children above the age of three years and weight above 10 kg or two NRTIs (stated above) and the PI, kaletra in children below the age of 3 years or less than 10 kg. If a child was started before the age of 3 years on a kaletra-based regimen there was generally no change in the regimen after they attained the 3 year age or 10 kg cut off point unless there were specific

indications such as poor tolerance or development of side effects. If lipodystrophy or hyperlactatemia occurred, D4T would be replaced with zidovudine (AZT) or abacavir (ABC). If hypercholesterolemia occurred, kaletra would be substituted with efavirenz. Changes within the first line were prompted by events like the development of side effects and intolerance to medication while a total change to second line would happen with virological failure sometimes confirmed by genotypic resistance testing done only on those cases suspected to be harboring resistance based on virological and immunological profiles. Switching to second line therapy was dependent upon the limited ART options for children. For children who needed ART/antimycobacterial co-treatment and were above the age 3 years or 10kg limits stated above, their first line ART regimen was generally not adjusted. For those that were below this cut off, kaletra dosing was adjusted upwards depending on the body surface area. It is worthwhile mentioning that the time period spanned by this study is before emergence of new data showing the inadequate dosing of kaletra in children on ART/Antimycobacterial co-treatment (McIlleron *et al*. 2009).

Before starting ART, each child had to be confirmed HIV-infected by either the enzyme linked immunosorbent assay (ELISA) test for children above 18 months of age or the deoxyribonucleic acid polymerase chain reaction (DNA PCR) test for those below 18 months of age. Baseline assessments were done on each patient before starting ART. These included a thorough history and examination to stage the patient according to the WHO HIV staging system and to exclude active TB disease (DOH, 2000) and any other opportunistic infections. For those patients diagnosed as having TB disease or other mycobacterial disease on either clinical grounds or laboratory confirmation, antimycobacterial treatment would be instituted straight away and ART usually delayed for at least two weeks depending on the level of immunocompromise and the collective physicians' opinion. Baseline assessments on all patients included weight and height measurements, chest x-ray (CXR), CD4 count, viral load (VL), full blood count (FBC) and alanine transferase (ALT). Any other additional tests were done based on specific medical indications.

During the period in question, children with clinically suspected active TB disease or other mycobacterial event were generally not started on ART but taken through a more rigorous series of investigations for TB such as sputum or gastric washings for TB microscopy, TB culture and sensitivity and TB blood culture (TB bactec), tuberculin skin test (TST) and radiological tests. Histological diagnosis was also carried out for cases especially of suspicious TB lymphadenitis and TB abscesses. Diagnosis of TB disease depended upon a confirmation of mycobacterium isolated from some of the above tests or was based on the criteria for diagnosing TB disease in children (DOH, 2000; WHO, 2006) and also the clinicians' suspicion if tests were negative. The later was always done in consultation with the specialist resident paediatrician.

For children below eight years of age whose ailment warranted antimycobacterial therapy, the medications used were for a total duration of 6 months rifampicin, isoniazid and pyrazinamide (ethambutol was added to this intensive phase if children were above eight years of age) for the first two months of intensive phase and rifampicin and isoniazid for the continuation phase of four months. Other antimycobacterial agents that were used were azithromycin, clarithromycin, amikacin and ciprofloxacin for MOTT. These were used for a more prolonged period of at least 18 months guided by the clinical response to treatment and the follow up cultures for mycobacterial isolation.

Children with suspicious BCG disease after starting ART would be given three drug antimycobacterial treatment consisting of rifampicin, isoniazid and ethambutol for six months if the area of induration was greater than fifteen millimeters (mm) on the TST for localized BCG disease or if there was evidence of disseminated BCG disease (BCG-osis). If the TST was less than fifteen mm they would be treated with steroids and investigated for active TB in the ways described above.

Inclusion criteria:

- Children less than 15 years at ART initiation
- Started ART in the period 1st April 2004 to 1st April 2008 (last ART start date)

- Presumed ART naïve at the time of first contact with HSCC

Population and Sample:

Records of all children started on ART at the HSCC programme in the period 1st April 2004 to 1st April 2008 were accessed from the clinic database.

Covariates:

Demographic: Age at start of HAART.

Clinical: Baseline WHO stage of HIV disease, baseline CD4 percentage, baseline normalized weight for age z scores (waz), baseline viral load.

Other variables in the database

Previous TB treatment was left out of the whole data analysis although it was recorded in the database because it was deemed unobjective in this particular study. This was because it was difficult to ascertain the previous basis of diagnosis, whether it was really TB or any other mycobacterial events, whether the child completed treatment or not, and where the child got the treatment from as most of this information was obtained from the child's guardian who was not necessarily the one who was caring for the child before especially in cases of adopted children or those who were coming from children's homes and these constituted a significant number. Although IPT would probably have altered the risk of developing mycobacterial events, it was not emphasized in the HSCC practice at the time in question and practitioners were generally not prescribing it once children were started on ART. Therefore although this variable was included in the database it was left out of this analysis for that reason. Although it is known as a risk factor for TB in children, maternal or close contacts TB status were not included in the HSCC database and in this analysis it was therefore not included among the risk factors. The other variables in the database were deemed irrelevant for the study in

question and these variables are only mentioned and defined in annex 1 below because they are too many to mention here.

The CD4 and viral load bloods were done and recorded at six months intervals and therefore although in some literature (Hermans *et al*. 2010) it has been reported the CD4 and or the viral load at the time of TB was significant risk factor it was impossible for the records to tell the CD4 or viral loads at the time of mycobacterial events in the current analysis and any such analysis would have probably misrepresented the truths. For instance if a child developed a mycobacterial event in the second month from the last recorded CD4 or viral load the next CD4 and viral loads were only done and recorded in the sixth month in the routine HSCC practice.

It was not possible to retrospectively tell the type of mycobacterial event that led to the child receiving antimycobacterial treatment from the HSCC database. Although some of this data was probably obtainable from the physical records made by the physicians, this attempt was not made because of the huge volumes of records that needed to be compiled and analyzed in the limited time of the research.

Ethics and Confidentiality:

Approval for use of data from the ART database of the HSCC for retrospective studies was granted by the University of the Witwatersrand ethics committee as part of 'The International Epidemiological Database to Evaluate AIDS (IeDEA) Collaboration' and from the Ethics Committee of the University of the Western Cape (UWC).

Data analysis:

The SAS software package (SAS version 9.0 (SAS, Cary, North Carolina, USA)) was used to clean and analyze the HSCC database. Period of follow-up commenced at start of ART and ended at the closure of the database on 11th April 2008 for children who did not go on antimycobacterial treatment.

Children exited follow-up at date of first antimycobacterial treatment recorded, database censoring occurred at date of last visit if child was lost to follow-up or transferred or died. Kaplan-Meier (KM) models were used to determine the cumulative probabilities of being on antimycobacterial treatment among children who started ART without antimycobacterial treatment and also to determine associations between selected pretreatment factors and future antimycobacterial treatment. KM curves were plotted for antimycobacterial treatment-free survival in general and then stratified by baseline risk factors. The Log rank test was used for comparison. Antimycobacterial treatment-free survival was defined as the time from start of ART to the date of antimycobacterial treatment or date of last clinic visit if lost to follow-up. Logistic regression modeling was then fitted to determine the independent associations of each covariate with the antimycobacterial treatment using the chi-squired test and the odds ratios as measures of association and risk respectively. A p-value <0.05 was considered statistically significant.

Definitions:

The baseline CD4 percentage was defined as the CD4 percentage measured within ninety days prior to starting ART and if more than one record was found in the database the one closest to ART start date was taken and used in analysis. A similar definition was used for baseline viral load being defined as the viral load measured within ninety days prior to starting ART and if more than one record was found in the database the one closest to ART start date was taken and used in analysis. The baseline WAZ was defined as the WAZ measured within ninety days prior to starting ART and if more than one record was found in the database the one closest to ART start date was taken and used in analysis. The baseline WHO stage was defined as the lowest WHO stage achieved before ART was initiated according to the WHO staging definitions for HIV (with stage 4 being the lowest and stage 1 being he highest in that ascending order). The WHO stages 3 and 4 were combined and defined as advanced disease since the WHO staging system was only ending at stage 3 before latest WHO guidelines

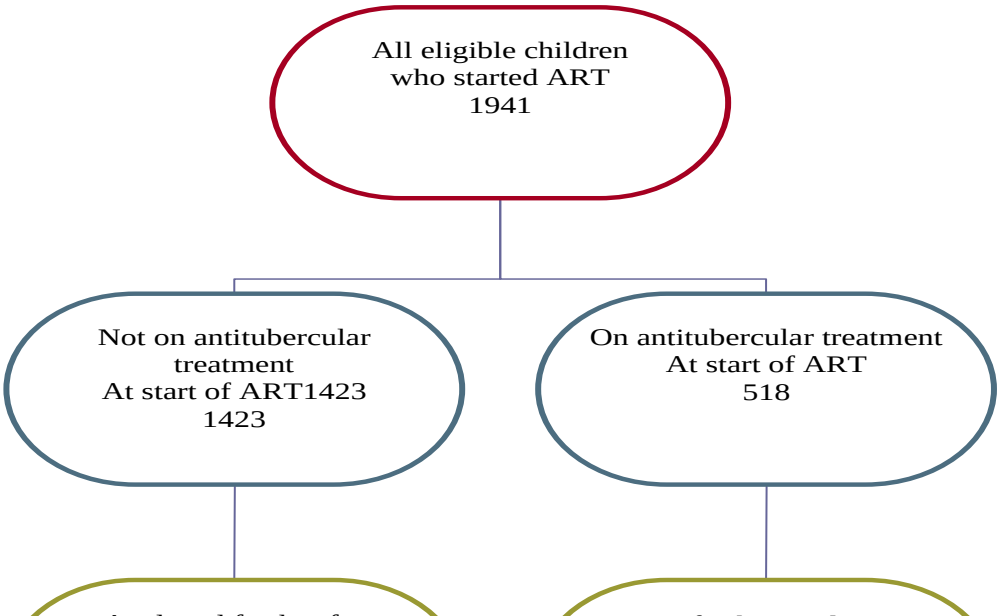
introduced the stage 4 in children and therefore the system divided the cohort (for HSCC the new system was introduced after 1st October 2005 using the draft WHO guidelines).

Age was defined as the age in days at the start of ART. The date of antimycobacterial treatment was estimated as the first date of the mention of antimycobacterial treatment in the database at or after the start of ART. Viral load <400 copies/ml was defined as suppressed, CD4% was categorized into three groups (1) less than 15%, (2) between 15 and 25% and (3) above 25% in accordance with WHO criteria for grading immunosuppression. Waz was categorized using standard clinical definitions of Centers for Disease Control and Prevention (CDC), (2000) of severe (< -3), moderate (between -2 and -3), mild (between -2 and -1) and normal (above -1).

Frequency distributions for each variable were done and examined and obvious errors that occurred in data entry detected. Observations with missing or outlying values were censored during the analysis of the variables where such were encountered. Due to time constraints, no effort was made to counter-examine the electronic database against the actual patient's files but because of the continuous cleaning and updating of the database for the purpose of other research being done it is hoped that the data is generally representative of the truth.

CHAPTER 4: RESULTS

Figure1: Flow diagram for eligibility criteria



A total of 1941 children met the criteria for enrolment. The profile of the whole cohort of children meeting enrollment criteria in terms of their pre-treatment characteristics is as shown Table 1. Among those who met the criteria 1423 (73.3%) were not on antimycobacterial treatment at the time of starting ART and these are the ones that were further analyzed for antimycobacterial treatment-free survival and risk factors for future antimycobacterial treatment. No further analysis was done among those 518 who were enrolled while on antimycobacterial therapy (26.7%).

Table 1: The profile of baseline characteristics:

Characteristic	Category	N (%) frequency
Age (years) %	<1	246 (12.7)
	1-3	424 (21.8)
	3<x<=5	343 (17.7)
	5<x<=15	928 (47.8)
Median age (days) IQR	1728 (754-2771)	1941
Viral load (copies/ml)	<401	35 (1.8)
	401-750 000	1372 (70.7)
	>750 000	371 (19.1)
	Missing	163 (8.4)
median Viral load (copies/ml) IQR	150000 (40000-590000)	1941
Weight for age (Z- scores)	<-3	523 (26.9)
	-3<z<-2	430 (22.2)
	-2<z<-1	528 (27.2)
	>-1	460 (23.7)
Median WAZ (IQR)	-1.97 (-3.1 to -1.0)	1941
CD4 percent %	0-14.9	1320 (68.0)
	15-24.9	414 (21.3)
	>25	105 (5.4)
	Missing	102 (5.3)
Median CD4 % (IQR)	11.2 (6.9-15.7)	1941
WHO stage	1	140 (7.2)
	2	435 (22.4)
	3 & 4	1244 (64.1)
	Missing	122 (6.3)
antitubercular treatment at ART start	Yes	518 (26.7)
	No	1423 (73.3)

By the time children start ART the prevalence of antimycobacterial treatment was 518/1941 {26.7%, (95% CI: 24.7-28.7)}. Most children started ART when they already had advanced HIV disease (64.1% when WHO stage 3 and 4) and (68% when CD4 less than 15%) only 5.4% of children started ART when the CD4 was above 25% (median CD4 11.2%, interquartile range (IQR), 6.9-15.7%). Approximately 26.9% of children started ART when they were severely malnourished (baseline waz less than -3). The median baseline waz was -1.97 (IQR, -3.1 to -1.0). Most of the children that started

ART were older and fell in the age range five to fifteen years (47.8%) while 12.7% were less than one year. The median baseline age in days was 1728 (IQR, 754-2771).

Table 2: KM cumulative probability of antimycobaterial treatment at 3, 12 and 24 months post ART initiation

Time point	Antimycobacterial treatment (%)	95% Confidence interval (%)
Prevalence of antimycobacterial treatment at ART start n/N (%)	518/1941 (26.7)	24.7-28.7
New antimycobacterial treatment post ART start (0-3 months)	4.6	4.1-5.2
New antimycobacterial treatment post ART start (0-12 months)	18.1	17.0-19.2
New antimycobacterial treatment post ART start (0-24 months)	24.0	21.9-25.3

Table 2 shows the prevalence of antimycobacterial treatment at start of ART (26.7%) and the cumulative proportions of children started on antimycobacterial treatment during the first 3, 12 and 24 months after initiating ART (KM analysis). The cumulative probability of ART/antimycobacterial co-treatment among children who started ART without antimycobacterial treatment was 4.6% in the first 3 months after starting ART and increased to 18% at the end of first year and thereafter the increase was gradual to 24% at 2 years.

Table 3: The KM cumulative probability of antimycobacterial treatment (0-3 months) among those without antimycobacterial treatment at ART start stratified by baseline characteristics

Variable	Category	Number at risk	New mycobacterial Rx by 3 mo	Cumul probab of mycobacterial Rx by 3 mo	log rank p-value
Age (years)	<1	153	12	13	
	1 to 3	249	15	9	
	>3 to 5	244	12	7	0.0004
	>5 to 15	683	24	4	
Viral Load (copies/ml)	<400	17	0	0	
	400 to 750000	975	42	6	0.0162
	>750000	228	13	8	
Weight for age (z score)	<-3	299	26	12	
	-3<z<-2	294	11	5	<0.0001
	-2<z<-1	386	17	6	
	>-1	350	9	4	
CD4 (%)	>25	76	3	5	
	15-24.9	321	12	5	0.213
	<15	873	42	7	
WHO stage	Stage 1	123	1	0.8	
	Stage 2	356	9	3	<0.0001
	Stage 3	756	50	10	

With the exception of baseline CD4 (p = 0.213) all other baseline factors were statistically significant risk factors for future antimycobacterial treatment (log rank p < 0.05) among those who started ART without antimycobacterial treatment. The cumulative probability of ART/antimycobacterial co-treatment decreased with increasing age. There were increasing chances of co-treatment among those

who started ART with advanced malnutrition, those with very high viral loads and those who started ART when they had advanced clinical stage of the disease.

Table 4: The KM cumulative probability of antimycobacterial treatment (0-12 months) among those without antimycobacterial treatment at ART start stratified by baseline characteristics

Variable	Category	Number at risk	New mycobacterial Rx by 12 mo	Cumul probab of mycobacterial Rx by 12 mo	log rank p-value
Age (years)	<1	173	70	52	<0.0001
	1 to 3	270	54	25	
	>3 to 5	259	33	14	
	>5 to 15	717	72	12	
Viral Load (copies/ml)	<400	24	4	19	<0.0001
	400 to 750000	1032	133	15	
	>750000	249	70	34	
Weight for age (z score)	<-3	332	99	35	<0.0001
	-3<z<-2	316	50	21	
	-2<z<-1	409	53	15	
	>-1	362	27	8	
CD4 (%)	>25	85	18	30	0.2046
	15-24.9	335	48	18	
	<15	939	152	19	
WHO stage	Stage 1	129	7	6	<0.0001
	Stage 2	367	34	2	
	Stage 3	814	172	26	

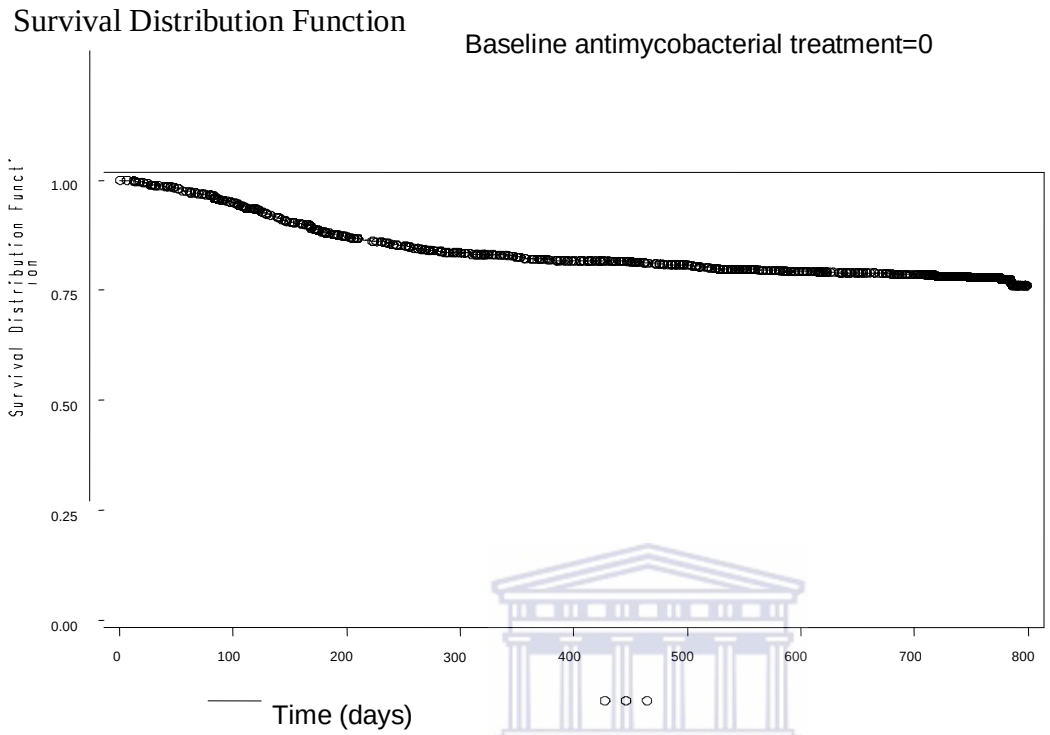
With the exception of baseline CD4 (p = 0.2046) all other baseline factors were statistically significant risk factors for future antimycobacterial treatment (log rank p < 0.0001) among those who started ART without antimycobacterial treatment.

Table 5: The KM cumulative probability of antimycobacterial treatment (0-24 months) among those without antimycobacterial treatment at ART start stratified by baseline characteristics

Variable	Category	Number at risk	New mycobacterial Rx by 24 mo	Cumul probab of mycobacterial Rx by 24 mo	log rank p-value
Age (years)	<1	173	74	54	
	1 to 3	270	62	23	<0.0001
	>3 to 5	260	41	18	
	>5 to 15	718	86	15	
Viral Load (copies/ml)	<400	25	4	18	
	400 to 750000	1033	155	18	<0.0001
	>750000	249	79	37	
Weight for age (z score)	<-3	333	105	36	
	-3<z<-2	316	59	24	<0.0001
	-2<z<-1	410	62	18	
	>-1	362	37	13	
CD4 (%)	>25	85	18	26	
	15-24.9	336	53	20	0.2046
	<15	939	181	22	
WHO stage	Stage 1	129	11	10	
	Stage 2	369	43	13	<0.0001
	Stage 3	814	189	30	

With the exception of baseline CD4 ($p = 0.2137$) all other baseline factors were statistically significant risk factors for future antimycobacterial treatment (log rank $p < 0.0001$) among those who started ART without antimycobacterial treatment.

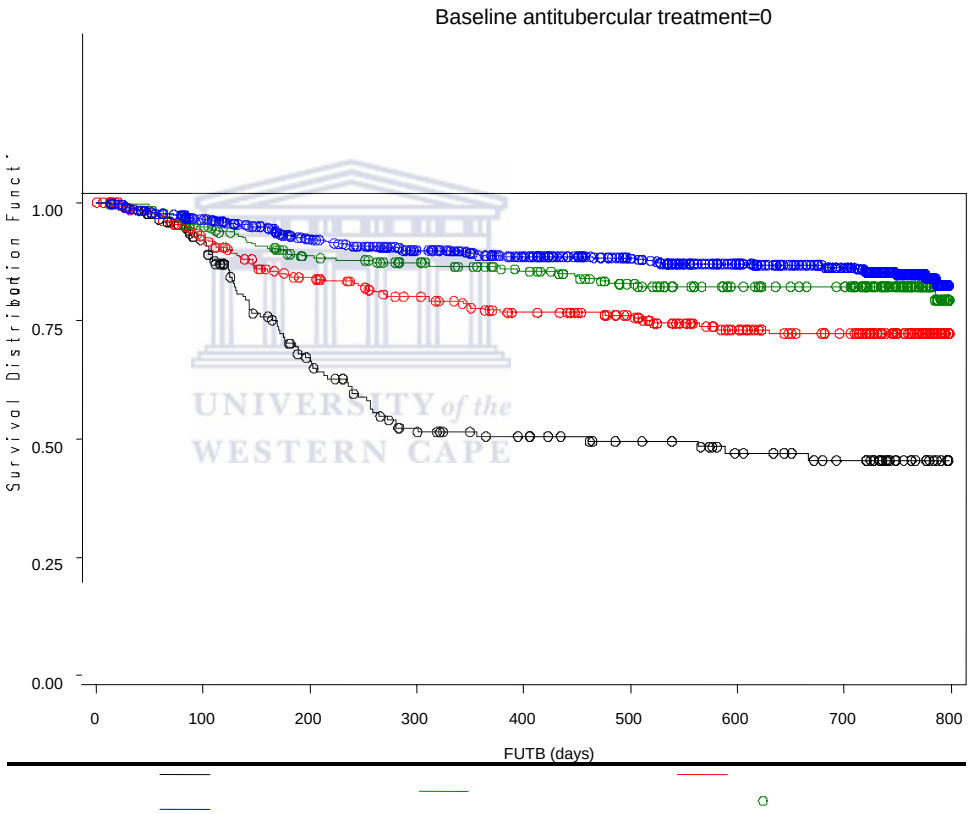
FIGURE 2: 2-year antimycobacterial treatment-free survival function among children initiated ART without antimycobacterial treatment in period 01.04.2004-01.04.2008



Approximately 24% (95% CI: 21.9-25.3) of the children who started ART without antimycobacterial treatment had antimycobacterial treatment in the first 24 months of ART. From the shape of the KM plot above which is quite steep from time zero to about the mid of 300 and 400 days one can deduce that most mycobacterial events occurred within the first 12 months of starting ART. Thereafter the curve flattens out implying much reduced numbers of mycobacterial events.

FIGURE 3: 2-year antitubercular treatment-free survival distribution function among children initiated ART without antitubercular treatment in the period 01.04.2004-01.04.2008 stratified by baseline age categories (P<0.0001)

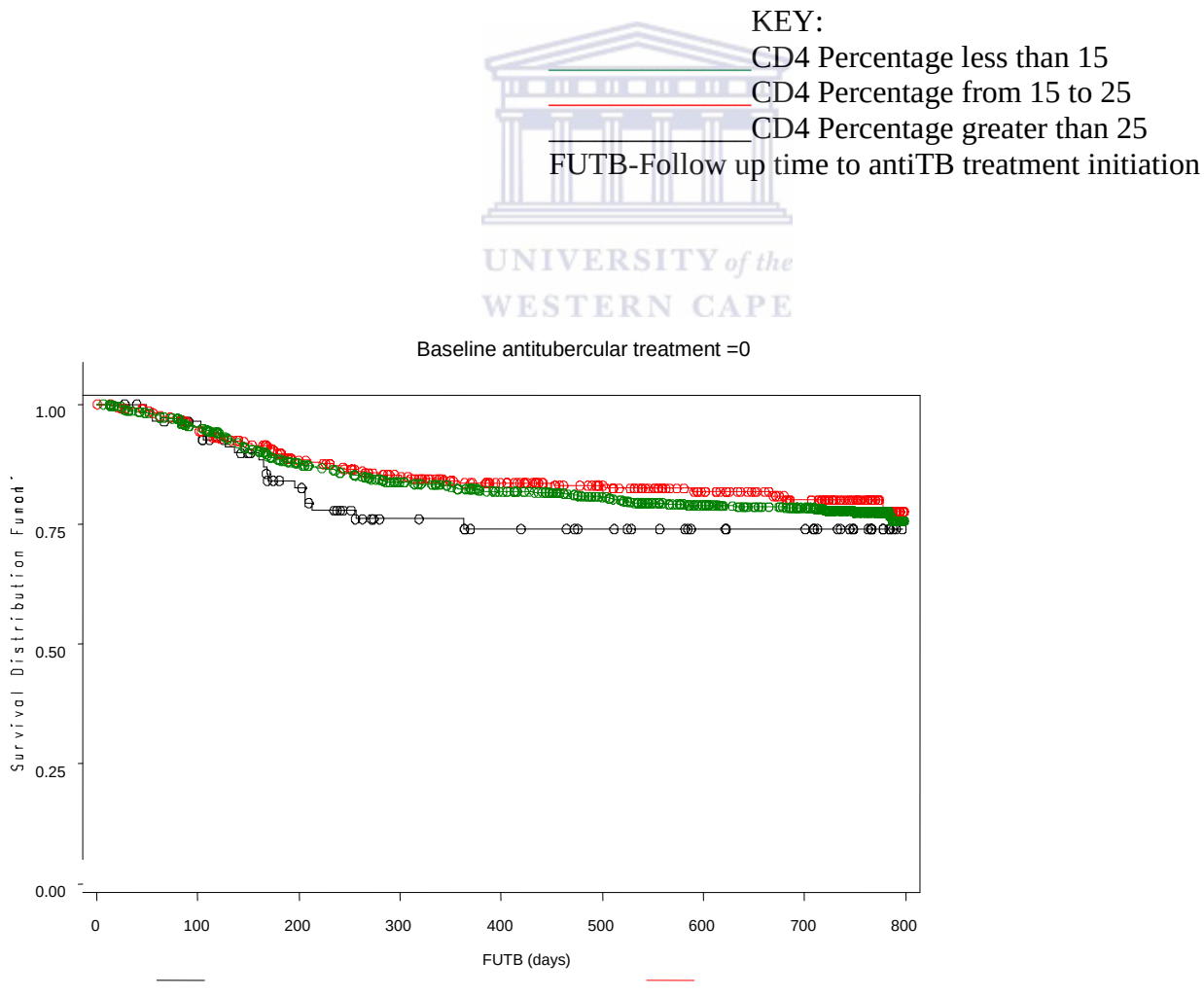
KEY:
_____age < 1 year
_____age 1 to 3 years
_____age >3 to 5 years
_____age >5 to 15 years
FUTB – Follow up time to antiTB treatment initiation



Children less than one year had significantly lower antimycobacterial treatment-free survival of 48% at 12 months and 46% (log rank p < 0.0001) by 24 months. The trend was also seen in the age group one to three years although the antimycobacterial treatment-free survival seemed to be much better than the former. Above the age group one to three years there seemed to be no significant difference

in the antimycobacterial treatment-free survival among the age groups. The KM curve was steepest in the first 12 months of starting ART implying the occurrence of most mycobacterial events happening in that period and thereafter there seemed to be a gradual decrease in the number of events as shown by the flattening of the curve (Figure 3).

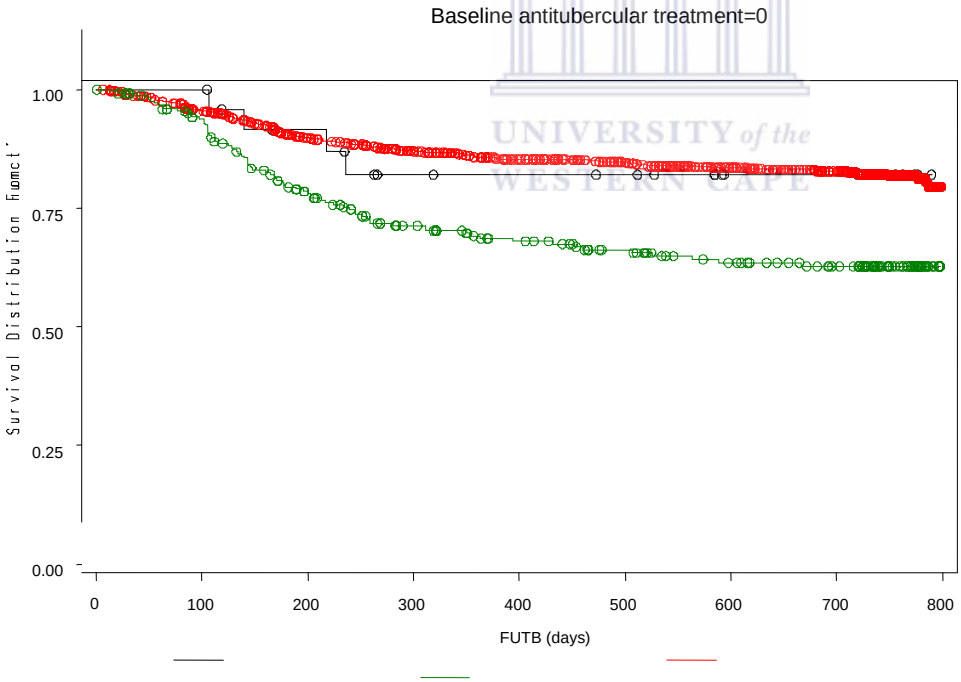
FIGURE 4: 2-year antitubercular treatment-free survival distribution function among children initiated ART without antitubercular treatment in the period 01.04.2004-01.04.2008 stratified by baseline CD4 percentage (P=0.2137)



There was no difference in antimycobacterial treatment-free survival between children whose baseline CD4 percentage was high (> 25%) and those whose CD4 percentage was very low (< 15%) (Log rank p = 0.213) (Figure 4).

FIGURE 5: 2-year antitubercular treatment-free survival distribution function among children initiated ART without antitubercular treatment in the period 01.04.2004-01.04.2008 stratified by baseline viral loads (P<0.0001)

KEY:
_____ Viral load greater than 750000
_____ Viral load from 400 to 750000
_____ Viral load less than 400
FUTB-Follow up time to antiTB treatment initiation



High baseline viral loads (> 750000) were significantly associated with reduced antimycobacterial treatment-free survival (log rank p < 0.0001) compared to those with lower viral loads (Figure 5).

FIGURE 6: 2-year antitubercular treatment-free survival distribution function among children initiated ART without antitubercular treatment in the period 01.04.2004-01.04.2008 at HSCC stratified by baseline weight for age (P<0.0001)

KEY:

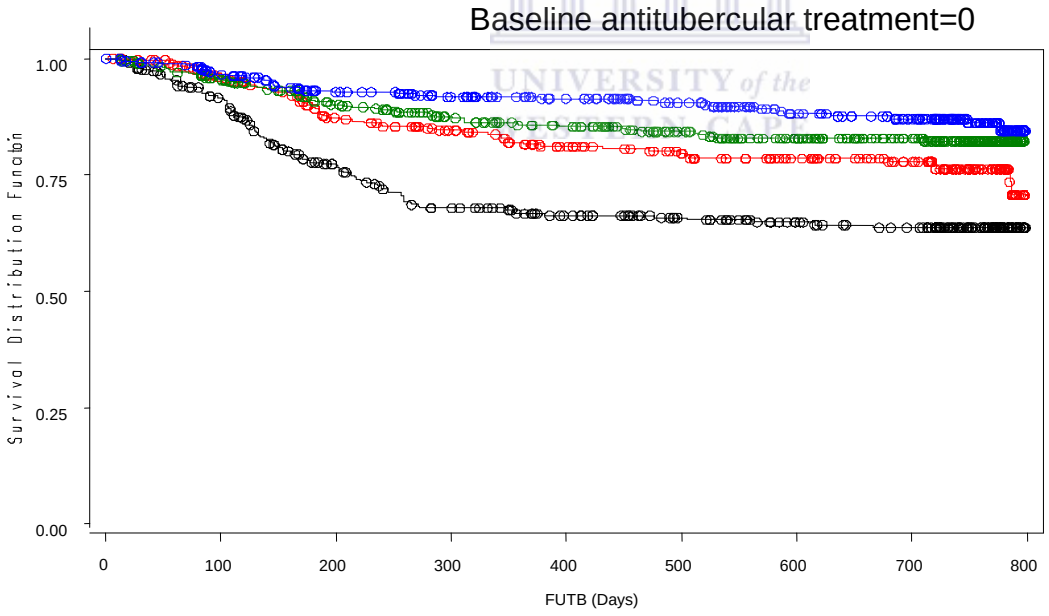
_____Weight for age greater than -1

_____ Weight for age between -2 and -1

_____ Weight for age between -3 and -2

_____ Weight for age less than -3

FUTB-Follow up time to antiTB treatment initiation



Severe malnutrition (waz -3) was significantly associated with reduced antimycobacterial treatment-free survival (log rank p < 0.0001) compared to those who had higher weight for age (Figure 6) with

only slight differences in the antimycobacterial treatment-free survival comparing those with waz in the -3 to -1 range relative to those with waz > -1 (log rank p < 0.0001).

FIGURE 7: 2-year antitubercular treatment-free survival distribution function among children initiated ART without antitubercular treatment in the period 01.04.2004-01.04.2008 stratified by baseline WHO stage of HIV (P<0.0001)

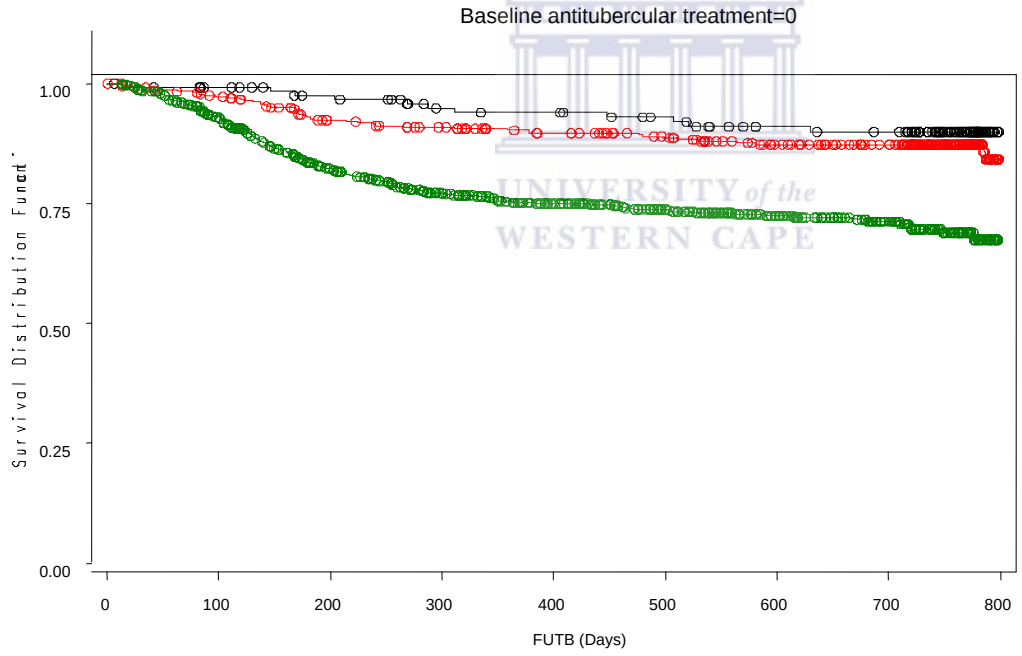
KEY:

_____WHO group 1

_____WHO group 2

_____WHO group 3

FUTB-Follow up time to antiTB treatment initiation



Baseline advanced HIV disease (WHO stage 3 and 4) was significantly associated with a reduced antimycobacterial treatment-free survival compared to earlier disease stages (log rank p < 0.001).

Logistic regression modeling was then used determine the role of baseline characteristics as predictors of antimycobacterial treatment. The Likelihood Ratio Test (LRT) for global null hypothesis (Beta = 0) was statistically significant (P < 0.0001) showing that all the baseline characteristics put together were associated with future antimycobacterial treatment.

Table 6: Results of maximum likelihood and odds ratios estimates for baseline factors and antitubercular treatment at 24 months.

Characteristic	Category	Odds ratio (95 % CI)	P-value
Age (years)	<1	3.665 (2.249-5.972)	<0.0001
	1 to 3	1.899 (1.242-2.905)	0.7394
	>3 to 5	1.545 (0.992-2.406)	0.3175
	>5 to 15	1.0	
Viral Load (copies/ml)	<400	0.476 (0.127-1.777)	0.3592
	400 to 750000	0.759 (0.510-1.129)	0.7891
	>750000	1.0	
Weight for age (z score)	<-3	2.235 (1.389-3.596)	0.0015
	-3<z<-2	1.686 (1.046-2.719)	0.2799
	-2<z<-1	1.191 (0.736-1.926)	0.1562
	>-1	1.0	
CD4 (%)	>25	0.654 (0.350-1.221)	0.5604
	15-24.9	0.616 (0.420-0.902)	0.2260
	<15	1.0	
WHO stage	Stage 1	0.466 (0.238-0.916)	0.2397
	Stage 2	0.486 (0.326-0.725)	0.1641
	Stage 3 & 4	1.0	

From the results (Table 6) age less than one year {Odds ratio (OR): 3.7 (95% CI 2.2-6.0; p < 0.0001)} and very low weight for age (waz < -3) {OR; 2.2 (95% CI 1.4-3.6; p=0.0015)} were the two critical risk factors independently associated with future antimycobacterial treatment. For age above one year,

CD4 above 15%, waz >-2, WHO stage 1 & 2 and viral load below 750 000 the trend was towards protective from antimycobacterial treatment ($p > 0.05$).

CHAPTER 5: DISCUSSION

A previous study from adults in South Africa (Lawn *et al*. 2007) reported the prevalence of antimycobacterial treatment of 160/756 (21%) among patients (median age 33 years) starting ART in a clinic in Gugulethu, South Africa which was close to what was found in this study among children (26.7%).

Data from children about the antimycobacterial treatment-free survival among those started ART without antimycobacterial treatment is scarce. There are no previous reports of cumulative probability of antimycobacterial treatment among children who started ART without antimycobacterial treatment but recent data has shown that the incidence of TB among children decreased with increasing time on ART from 18.9 per 100 person-years in the first 6 months to 5.3 per 100 person-years after 12 months of ART (Edmonds, 2009). Similarly, quite recent data from Uganda among adults showed incident TB to also decrease with time from ART initiation with the incidence rates (95% CI) at 0–3, 3–6, 6–12 and 12–24 months being 11.25 (9.58–13.21), 6.27 (4.99–7.87), 2.47 (1.87–3.36) and 1.02 (0.80–1.31), respectively (Hermans *et al*. 2010). A Senegalese study also reported similarly among adults that over the first 4 years on HAART, the annual incidence rate decreased regularly from 4.5 cases/100 P-Y [95% IC: 2.8 - 7.7] during the first year to 3.5 [1.9 – 6.5] the second year, 1.5 [0.6 - 4.1] the third year and to 0.4/100 P-Y [95% IC: 0.0 – 2.9] the fourth year (Etard *et al*, 2009). The current study is limited in that it cannot report on the incidence density rate of antimycobacterial treatment because of its design which cannot distinguish repeated cases of antimycobacterial treatment exposure while on ART. Besides the limitation of not knowing the level of adherence to ART among children in this cohort there is agreement with previous reports (Bekker *et al*. 2003; Lawn, Badri & Wood, 2005,

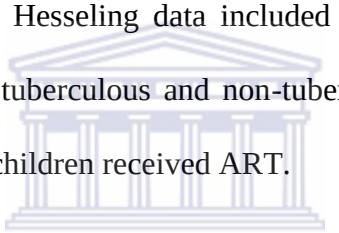
Hermans *et al*. 2010) that showed that the highest incidence of mycobacterial events was in the first year of ART compared to the later years (Figure 2).

CD4 percentage: Higher baseline CD4 above 22% among children less than five years was associated with much better survival in general in a Thai study (Vanprapar *et al.* 2000). Retrospective reports from adults found a high risk of TB associated IRIS in patients with very low baseline CD4 counts (Lawn, Badri & Wood, 2005; Lawn *et al.* 2007; Sirisanthana *et al.* 2004). Similar findings have been reported in recent Ugandan data that found incident TB among adults during ART was independently associated with baseline CD4 count of <50 cells/mm^3 (hazard ratio [HR] 1.84 [1.25–2.70], $P = 0.002$). Etard *et al.* (2009), in a prospective cohort also reported a low baseline CD4 being associated with early incident TB in Senegal. The current data suggests a protective role from antimycobacterial treatment events of higher baseline CD4 (above 15%) compared to below 15% although this was not statistically significant ($p > 0.05$) (Table 6). A prospective study that looked into the incidence of IRIS in Johannesburg reported TB as the commonest form of IRIS and it mainly affected those who started ART with advanced immunosuppression (Murdoch *et al*. 2008). Smith *et al*. 2008, concluded that CD4 percentage lower than 10% was a risk factor (OR 5.84, CI 1.72-19.8) for IRIS (mostly BCG-osis and TB) among young children after starting ART. Another South African study among children reported that IRIS occurred especially among those who were younger (median age 7 vs. 10 months, $P = 0.007$) with a lower CD4 cell percentage (median 13.9 vs. 19.2, $P = 0.009$) at HAART initiation than controls (Smith *et al.* 2009). A prospective study from South Africa also reported similarly that BCG IRIS risk was associated with lower baseline CD4 percentage among infants and most cases were diagnosed soon after initiating ART (Rabie *et al.* 2008). However, there is contradiction with Van Rossum *et al.* (2001) and Seyler *et al.* (2005) whose data suggested that the pre-treatment baseline CD4 was not a critical factor in determining future immune reconstitution and ability to fight diseases. The later study (Seyler *et al*. 2005) was limited in its generalisability by the narrow patient selection base which consisted mainly of patients with advanced HIV disease and therefore mostly low baseline CD4 counts. Among the selected baseline factors that influence either

the development of BCG complications or not in HIV-infected infants, only very low CD4 percentage (13.6+/-11) was associated ($p<0.01$) and not viral load, the disease stage nor the mean follow-up time (Fallo *et al*. 2005). The current study was limited by the small numbers of children in the CD4 category above 25% (only 6.8%). This was however unavoidable since the standard practice at the time emphasized starting ART when CD4 was less than 25% except in clinically advanced disease (DOH, 2005). A previous report from HSCC within the same period showed that MAC was commonest among children who had very low absolute CD4 count of less than 50 (Kleynhans *et al*, 2008). The prevalence of MAC was found to be very low in this particular study (13/2354 or 0.55 over a four year period between 2004 and 2007).

Age group: The current study reports a lower antimycobacterial treatment-free survival among children less than one year of age compared to the age groups above. Smith *et al*. (2008) found age less than 6 months a significant risk factor (OR 4.5, CI 1.73-11.74) for IRIS mostly in the form of BCG-osis and TB disease. Possible reasons for the lower antimycobacterial treatment-free survival in the current study include the contribution of BCG disease, BCG IRIS, the increased difficulty in diagnosing TB disease in the very young children (Chakraborty & Shingadia, 2007; National Department of Health, 2000; Mofenson *et al.* 2004) and the raised index of suspicion generally taken by practitioners when dealing with this age group which likely pushed practitioners to treat many unconfirmed cases as TB disease. The overlapping features of HIV and TB disease especially in the very young children probably resulted in many HIV-infected children being put on antimycobacterial treatment compared to the older children in whom diagnosis of TB is less challenging. The higher rates of antimycobacterial treatment in this age group could also reflect the low immune response associated with infancy especially those whose household contacts had TB disease (infants are almost always in contact with adult caregivers increasing their susceptibility to infections). This was however difficult to assess in this study because of its design and because data on household contacts about TB was not included in the database. The contribution of BCG disease in particular was probably the biggest in this age group if one is to look at evidence from a similarly high TB/HIV burden setting

(Hesseling *et al*. 2009; Smith *et al*. 2009). Data from a multicentre surveillance prospective study among South African children less than one year suggested very high incidence of disseminated BCG disease per 100 000 BCG-vaccinated, HIV-infected infants which were as follows: 778 (95% confidence interval, CI: 361-1319) in 2004 (vertical HIV transmission rate: 10.4%); 1300 (95% CI: 587-2290) in 2005 (transmission rate: 6.1%); and 1013 (95% CI: 377-1895) in 2006 (transmission rate: 5.4%) and the pooled incidence over the study period was 992 (95% CI: 567-1495) per 100 000 (Hesseling *et al*. 2009). The Hesseling data however did not report on the influence of ART among the HIV-infected children since this could have possibly made a difference in the incidence of disseminated BCG disease and a further stratification is therefore another research question before concrete recommendations are made about BCG vaccination in high TB/HIV settings with high ART coverage. It is also possible that the Hesseling data included cases of BCG IRIS (since the only distinguishing tests carried out were tuberculous and non-tuberculous mycobacteria from the BCG Danish strain if indeed some of these children received ART.



It is also possible although unquantifiable in the current data to conclude that some cases of mycobacterial events were due to BCG IRIS and TB IRIS especially considering data from other studies documenting high frequencies of such events in the first few months of ART (Smith *et al.* 2009). A South African study reported that BCG reaction was most common occurring in 24/34 (71%) children, primarily injection site lesions and/or ipsilateral axillary lymphadenitis with abscess (Smith *et al.* 2009). Subclinical TB was also possibly missed when children were started on ART because of diagnostic difficulties and these cases could have become overt TB cases soon after starting ART (unmasking TB) and therefore received antimycobacterial treatment.

In the HSCC the practice was that (due to lack of diagnostic resources) once the practitioners were not sure after investigation proved negative for common opportunistic infections and there was poor response to conventional antibiotics but the clinical features still pointed towards TB disease, antimycobacterial treatment was started. Data on this fact was however not readily quantifiable in this

study because of its design. The contribution of MOTT could also have been a significant factor here. Since in the analysis the date closest to the first record of 'antimycobacterial treatment' for a particular record was the only one used, for most children who were later diagnosed with MOTT microbiologically or from histological specimens, they were initially started on standard antituberculous treatment while being investigated for MOTT and that standard treatment for TB was continued together with that for the MOTT. Kleynhans *et al*, 2008, from the HSCC in the same period of this data analysis reported that 9 out of the 12 cases (75%) that were confirmed as MAC were already on TB treatment by the time MAC was diagnosed.

WHO stage: The current study is suggesting a protective role of the early stages of HIV disease compared to stages 3 and 4 (combined in this case for reasons mentioned in the methods section) although this was not statistically significant ($p > 0.05$). This is also corroborated by some reports from adults that observed higher incidences of TB among patients who started ART with very advanced disease (Lawn, Badri & Wood, 2005; Badri, Wilson & Wood, 2002) compared to earlier WHO stages 1 and 2.

Weight for age: Malnutrition at start of ART in this data was found to be an important risk factor for future antimycobacterial treatment. A South African prospective study among infants reported higher risk of BCG associated IRIS of low weight for age children (waz -1.5 (-2.5—0.5)), p=0,007 compared to children of higher waz at ART initiation (Rabie et al, 2008). A similar conclusion was drawn by another South African study, NEVEREST 2 (Smith *et al*. 2008), that higher risk of IRIS predominantly in the form of BCG-osis and TB were found among young children who were severely malnourished with weight for age z-score less than 2 standard deviations from the mean (OR 4.13, CI 1.53-11.11). It should however be noted that the two studies above reported for children under one year of age. A stratification of waz and age group less than one year was going to be more appropriate in the current study in order for a fair comparison to be drawn with the infant studies mentioned above. Conclusions from these studies could however be a reflection of the behavior of attending

practitioners who probably after failing to get good results from treatment of other HIV related opportunistic infections causing failure to thrive would then prescribe antimycobacterial treatment. They should therefore be regarded cautiously. The WHO scoring system for diagnosis of TB in children is also brought into question (van Rheenen, 2002) here as 26% of the children in the current study started ART when they were already severely malnourished and therefore got a score of '4' and only needed few other parameters to get to '7' and become eligible for TB diagnosis and treatment.

Viral loads: The current study is reporting a protective effect from mycobacterial events of baseline viral loads less than 750 000. There is also corroboration with the data from Smith *et al*. 2008 that showed that baseline viral loads above 750 000 were a risk factor for IRIS (mostly BCG-osis and TB disease) in the first few months of starting ART in the very young children. One can however extrapolate the data to mean an increased survival which agrees with the Thai data (Vanprapar *et al.* 2000) that found a protective role of low viral loads (less than 500 000). However, the current study has a limitation of the small numbers of children with VL<400 and the very few events in this category also limited rational comparability with the rest of the viral load groups. A study that was limited by small sample size (Martinson *et al.* 2006) drew similar conclusions however of a lower incidence of TB among children with low viral loads.

For reasons mentioned in the methods section the study did not analyze the CD4 level, viral load or the adherence to ART at the time children developed mycobacterial events although these have been shown in another study to be important predictors for development of mycobacterial events (Hermans *et al.* 2010). A study in the United Kingdom, a resource-rich low HIV prevalence setting, however showed no association between either the virological level or the immunological status at the time of TB diagnosis (Cohen *et al.* 2008). This later study was limited by the very small sample size comprising only 18 children.

Another parameter that was not considered in this study was the importance of previous TB treatment on the development of new mycobacterial events although this was found in other study to have a

significant impact (Seyler *et al*. 2005). The reason for this omission was that it was difficult with the time given for the study for the researcher to individually visit the records of each patient, and therefore validate the data, to find if indeed they had been treated for TB, whether TB treatment was completed, the adherence to TB treatment, where the TB treatment was provided and how such a diagnosis was made.

The fact that more than a quarter (26.7%) of all children who started ART were already on antimycobacterial treatment (WHO stage 3 and 4 disease) suggests that physicians in the HSCC were probably starting ART too late in the course of HIV disease among children and losing the opportunity of protection against TB that ART (Mhlongo *et al*. 2004; Lawn, Badri & Wood, 2005; Martinson *et al.* 2006; Miranda *et al*. 2007; Walters *et al.* 2008) could have provided had it been given earlier.

However this data seems to suggest that there is a lot of subclinical TB which then manifests as clinical disease after starting ART as the immunity improves as shown by the exponential fall (Fig. 2) in the survival time to antimycobacterial treatment soon after commencing ART.

The study's validity was good because there was careful follow up of all participants and careful recording of patient recruitment and selection, particularly reasons for exclusion. The HSCC database is also continuously cleaned and updated by trained data managers and epidemiologists because of so many studies concurrently using it. The study was reliable because clinicians at HSCC all used the same diagnostic protocol for TB disease, BCG disease and MAC in children and in all cases where one was not sure they consulted each other and also seeked the opinion of the resident paeditrician before offering treatment. Attempts to make the study more generalizable were made through widening the cohort inclusion criteria such as not limiting the CD4 levels, the viral load levels, the nutrition levels and the WHO stage of the disease to specific levels.

Unlike TB incidence studies in resource limited countries, that are hugely limited by unreliable diagnostic tools, atypical clinical presentations, atypical radiological findings and the high number of

smear and culture negatives in HIV-infected children this study precludes these issues by looking at those who received antimycobacterial treatment for any reason.

Limitations: The generalizability of the study was limited since clinicians in other settings might use different diagnostic protocols and a different management approach for TB events such as not giving antimycobacterial treatment in cases of suspicious BCG disease and immune reconstitution related to mycobacteria while others do. The other constraint to generalizability is that HSCC is well funded with highly trained HIV clinicians, a situation that is unusual in most public practices in South Africa and other African countries. For instance not all ART clinics outside tertiary research centres like HSCC measure baseline viral loads pre-ART. Although in South Africa the standard of practice is to measure viral loads at ART initiation in children, in Namibia for example, baseline viral loads are not recommended in the public sector. The findings are therefore a reflection of the HSCC practices in the period in question.

Routinely collected data usually has missing data elements whose randomness can not be guaranteed and the data is generally not clean. Limits of routinely collected data was also through mistakes made at the point of data entry from paper onto the electronic format. This probably biased the findings of this study.

As far as the field for antimycobacterial treatment was concerned the HSCC database for the follow up visits did not contain important variables like the basis of putting the patient on antimycobacterial treatment and date of antimycobacterial treatment commencement. This limited the possibility of calculating the incidence rate of antimycobacterial treatment due to the various reasons for which children received the treatment such as BCG disease and MAC (see above). There was also no record of MOTT or MAC in the database although these cases were seen and treated using antimycobacterial agents (Kleynhans *et al,* 2008). Following on this one can deduce that the data reported from HSCC to the national TB statistics was therefore not a true representation of the actual cases of TB disease since 'TB treatment' was used as a proxy for TB disease in the database. This same argument may

also be applicable to other clinics' settings that report TB data for research purposes and for national statistics. This can result in an overestimation of the statistics of TB disease because antimycobacterial treatment may be started for non-tuberculosis mycobacterial disease and in the very young children in many settings in South Africa antimycobacterial treatment is given for BCG disease and MOTT.

There were losses to follow up due to death, transferring out and defaulting among cohort children. One would reasonably expect that more death happened among children who had a very low CD4 percentage, high viral loads, severely malnourished and very advanced clinical stage of the disease and this probably confounded the results. A study that takes this into account is certainly necessary in future. However because the analysis was done using the KM plots, it is assumed that these losses were censored reasonably accurately.

The use of the KM plots was however limited by the fact that the actual date of start of antimycobacterial treatment was not known and therefore was just estimated using the knowledge of the clinic operations that it was generally within a two week period after starting antimycobacterial treatment that the information was recorded in the database.

Because the study was retrospective unavoidable errors of measurement could have occurred due to different measures being done by different scales and by different people and this was especially so for weight. Laboratory and human errors could also have taken place in the measurements of the CD4 and viral loads and transportation of specimens to the laboratory and also during entry onto the electronic formats.

A potentially great confounder of the study is the fact that the diagnosis of 'antimycobacterial treatment warranting condition' was in addition to the tests mentioned above, dependent on the behavior of the attending physicians at a particular time and this could have changed over time during the cohort.

Another limitation of the use of survival analysis methods was that for those children who were put on antimycobacterial treatment after starting ART one could not ascertain for how many different times a particular child was on antimycobacterial treatment and certainly some children received antimycobacterial treatment more than once during the cohort period. So these children were counted only the first time they received antimycobacterial treatment and not the subsequent times and this probably reduced the extent of ART/antimycobacterial co-treatment which was the outcome of interest.

This study has been limited in scope and excluded cases that were on TB treatment (26%) at the start of ART in order to accommodate the limited academic requirements for which it is primarily intended to serve. It is however appreciated that in order to truly reflect the bigger picture for purposes of inciting action on the part of stakeholders, it should have included this population.

Besides the limitations, this study has shown that there is a high prevalence of antimycobacterial treatment among children at start of ART and that many children go on ART/antimycobacterial cotreatment especially soon after starting ART.

CHAPTER 6: CONCLUSIONS

The prevalence of antimycobacterial treatment at the time of starting ART was 518/1941 (26.7%, 95% CI: 24.7-28.7). Survival analysis suggested that children with high baseline viral load, advanced World Health Organization (WHO) stage of disease, very low normalized weight for age (waz) and very young age (less than one year) at start of ART had significantly reduced antimycobacterial treatment-free survival (log rank $p < 0.05$) in the first two years of starting ART. In the logistic regression model, age less than one year {Odds ratio (OR): 3.7 (95% CI: 2.4-5.9; $p < 0.0001$)} and very low weight for age Z-score (waz < -3) {OR; 2.2 (95% CI: 1.4-3.6; $p = 0.0015$)} were the two critical risk factors independently associated with future antimycobacterial treatment.

The high prevalence of antimycobacterial treatment at ART initiation underlines the importance of stricter screening to detect more mycobacterial events before starting ART. The results emphasize the need for a heightened and careful alertness for mycobacterial events among children after starting ART especially among the very young and those starting ART with severe malnutrition. Knowledge of the estimated antimycobacterial treatment-free survival and incidence helps policymakers in planning limited resources appropriately.

To the HSCC this data prompts inclusion in the database of parameters like date of diagnosis of TB disease, the basis of diagnosis and the type of mycobacterial event that warranted antimycobacterial treatment which were missing at the time of the data recording for this particular study but are clearly important for future research purposes and action. The definition of antimycobacterial treatment in the HCSS database was also not conforming to the standard definition of six months antimycobacterial treatment for standard TB disease and this definitely needs to be urgently reviewed.

To the scientific world the results suggest that we should do more research into better methods of prevention and diagnosis of mycobacterial events among children during HIV care. The high prevalence of ART/antimycobacterial co-treatment especially in children less than one year calls for an urgent need to give priority to research projects that attempt to find safer, more effective drugs for ART/antimycobacterial co-treatment and with low chances of negatively interacting with each other.

Finally, but most importantly, the results of this study provide public health policymakers with a picture of the extent of ART/antimycobacterial co-treatment in children so that they can make informed decisions about the various needs of the children on co-treatment and in the formulation of guidelines for ART/antimycobacterial co-treatment especially among the very young where there are still very limited drug options and where diagnosis of mycobacterial events is especially challenging.

More research into development of more accurate and quick diagnostic instruments for diagnosis of mycobacterial events in children is urgently required.

REFERENCE LIST

1. Atun, R., Lebcir, R., Drobniewski, F., McKee, M., Coker, R. (2007). High Coverage with HAART is Required to Substantially Reduce the Number of Deaths From Tuberculosis: System Dynamics Simulation. *Int J STD AIDS*, 18 (4):267-73.
2. Badri, M., Wilson, D. & Wood, R. (2002). Effect of Highly Active Antiretroviral Therapy on Incidence of Tuberculosis in South Africa: A Cohort Study. *Lancet 359: 2059-2064.*
3. Bekker, L-G., Orrell, C., Reader, L., Matoti, K., Cohen, K., Martell, R., et al (2003). Antiretroviral Therapy in a Community Clinic-Early Lessons From A Pilot Project. *S.Afr Med J. 93: 458-462.*
4. BHIVA Treatment Guidelines of Tuberculosis Infection (2005). *HIV Medicine,* 6 (supplement 2):62-83.
5. Bonnet, M., Pinoges, L., Varaine, F., Oberhauser, B., O'brien, D., Kebede, Y., Hewison, C., Zachariah, R. & Ferradini, L. (2006). Tuberculosis after ART Initiation in HIV-Infected Patients from Five Countries with a High Tuberculosis Burden. [Online], Available: *http://www.epicentre.msf.org/research/publication-research/Bonnet1000. Accessed 11 September 2008.*
6. Boulle, A., Van Cutsem, G., Cohen, K., Hilderbrand, K., Mathee, S., Abrahams, M., Goemaere, E., Coetzee, D., Maartens, G. (2008). Outcomes of Nevirapine- and Efavirenz-Based Antiretroviral Therapy when Coadministered with Rifampicin-Based Antimycobacterial Therapy. *JAMA*, 300 (5):530-539.
7. Boulware, D., Callens, S. & Pahwa, S. (2008). Pediatric HIV Immune Reconstitution Inflammatory Syndrome (IRIS). *Curr Opin HIV AIDS*. 3(4): 461–467.

8. Centres for Disease Control and Prevention (CDC) (2000). The National Centre for Health Statistics in Collaboration with the National Centre of Chronic Disease Prevention and Health Promotion. [Online], Available: www.cdc.gov.growthcharts
9. Chakraborty, R. & Shingadia, D. (2007). Treating Opportunistic Infections In HIV-Infected Children (extended) Guidelines for the Children's HIV Association (CHIVA). *CHIVA Children's HIV Association of UK and Ireland* (1-71).
10. Cohen, T., Lipsitch, M., Walensky, R., Murray, M. (2006). Beneficial and Perverse Effects of Isoniazid Preventive Therapy for Latent Tuberculosis Infection in HIV-Tuberculosis Co-Infected Populations. *PNAS, 103 (18)*: 7042-7047.
11. Cohen, J., Whittaker, E., Walters, S., Lyall, H., Tudor-Williams, G., Kampmann, B. (2008). Presentation, Diagnosis and Management of Tuberculosis in HIV-Infected Children in the UK. *HIV Medicine,* 9 (5): 277-284.
12. Currie, C., Williams, B., Cheng, R., Dye, C. (2003). Tuberculosis Epidemics Driven by HIV: Is Prevention Better than Cure? *AIDS,* 17 (17):2501-8.
13. Dean, L., Edwards, G., Ives, J., Matthews, G., Fox, F., Navaratne, L., Fisher, M., Taylor, P., Miller, R., Taylor, B., de Ruiter, A., Pozniak, L. (2002). Treatment of Tuberculosis in HIV-Infected Persons in the Era of Highly Active Antiretroviral Therapy. *AIDS,* 16 (1): 75-83.
14. De Cock, K., Grant, A., Porter, J. (1995). Preventive Therapy for Tuberculosis in HIV-Infected Persons: International Recommendations, Research, and Practice. *Lancet,* 345: 833–836.
15. Edmonds, A., Lusiama, J., Napravnik, S., Kitetele, F., Van Rie, A. and Behets, F. (2009). Antiretroviral Therapy Reduces Incident Tuberculosis in HIV-Infected **C**hildren**.** *International Journal of Epidemiology, doi:10.1093/ije/dyp208*

16. Etard, J., Diouf, A., De Beaudrap, P., Koivugui, A., Ngom-Guèye, N., Ndiaye, I., Ecochard, R., Sow, P., Delaporte, E. (2009). Short and Long-Term Incidence of Tuberculosis and CD4-Cell Count Dynamic on HAART in Senegal. *Open AIDS Journal*, 3:67-70.

17. Fallo, A., Torrado, L., Sánchez, A., Cerqueiro, C., Schargrodsky, L., López E. (2005). Delayed Complications of Bacillus Calmette-Guérin (BCG) Vaccination in HIV-Infected Children. *IJID*, 9: 96-103.

18. Graham, N. & Chaisson, R. (1993). Tuberculosis and HIV infection: Epidemiology, Pathogenesis and Clinical aspects. *Annals of Allergy,* 71 (5): 421-433.

19. Granich, R., Gilks, C., Dye, C., De Cock, K., Williams, B. (2009). Universal Voluntary HIV Testing with Immediate Antiretroviral Therapy as a Strategy for Elimination of HIV Transmission: A Mathematical Model. Lancet, 373 (9657):48-57.

20. Harries, A. (2006). Editorial: HIV/AIDS and TB. *Tropical Doctor,* 36: 64-67.

21. Hermans, S., Kiragga, A., Schaefer, P., Kambugu, A., Hoepelman, A., Menabe, Y. (2010). Incident Tuberculosis During Antiretroviral Therapy Contributes to Suboptimal Immune Reconstitution in a Large Urban HIV Clinic in Sub-Saharan Africa. PLoS ONE 5 (5): e10527. doi:10.1371/journal.pone.0010527.

22. Hesseling, A., Schaaf, H., Hanekom, W., Beyers, N., Cotton, M., Gie, R., Marais, B., van Helden, P. & Warren, R. (2003). Danish Bacille Calmette-Guérin Vaccine–Induced Disease in Human Immunodeficiency Virus–Infected Children. *Clinical Infectious Diseases,* 37:1226–1233.

23. Hesseling, A., Hussey, G., von Reyn, C., Graham, S., Fine, P., Cotton, M., Gie, R. (2008). Consensus IUATLD Statement on the Revised World Health Organization Recommendations Regarding BCG Vaccination in HIV infected Infants. IUTLD BCG Working group. *38th World Union Conference, Cape Town.*

24. Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva (2007). AIDS Epidemic Update. [ONLINE]: Available, http://www.unaids.org/ Accessed February 2010.

25. Jaspan, H., Boulle, A., Berrisford, A. (2008). Two-Year Outcomes of Children on Non-Nucleoside Reverse Transcriptase Inhibitor and Protease Inhibitor Regimens in a South African Pediatric Antiretroviral Program. *Pediatric Infectious Disease Journal*, 27 (11): 993-998.

26. Karim, S., Naidoo, K., Grobler, A., Padayatchi, N., Baxter, C., Gray, A., Gengiah, T., Nair, G., Bamber, S., Singh, A., Khan, M., Pienaar, J., El-Sadr, W., Friedland, G., Karim, Q. (2010). Timing of Initiation of Antiretroviral Drugs during Tuberculosis Therapy. *N Engl J Med,* 362:697-706.

27. Keshinro, B. & Ya Diul, M. (2006). HIV/TB: Epidemiology, Clinical Features and Diagnosis of Smear-Negative TB. *Tropical Doctor*, 36: 68-71.

28. Kleynhans, L., Palmer, M., Yotebieng, M., Goga, A. & Meyers, T. (2008). Frequency and Clinical Features of MAC Infection in HIV Positive South African Children Attending Harriet Shezi Clinic, Chris Hani Baragwanath Hospital. A Retrospective Analysis. *15th conference on Retroviruses and Opportunistic Infections.*

29. Lawn, S. (2006). Impact of Antituberculosis Treatment on Virological Response to Highly Active Antiretroviral Therapy: Implications for Resource-Limited Settings? *The Journal of Infectious Diseases,* 194:1467–1468.

30. Lawn, S., Badri, M. & Wood, R. (2005). Tuberculosis among HIV-infected patients receiving ART: Long Term Incidence and Risk Factors in a South African Cohort. *AIDS,* 19: 2109-2116.

31. Lawn, S., Bekker, L. & Wood, R. (2005). How Effectively does ART Restore Immune Responses to Mycobacterium Tuberculosis? Implications for Tuberculosis Control. *AIDS,* 9(11): 1113-1124.

32. Lawn, S., Myer, L., Bekker, L-G., Wood, R. (2007). Tuberculosis-Associated Immune Reconstitution Disease: Incidence, Risk Factors and Impact in an Antiretroviral Treatment Service in South Africa. *AIDS,* 21(3):335-341.

33. Lawn, S., Wilkinson, R., Lipman, M. & Wood, R. (2008). Immune Reconstitution and 'Unmasking' of Tuberculosis during Antiretroviral Therapy. *Am J Respir Crit Care Med*, 177:680-685.

34. Maartens, G. (2000). *Tuberculosis in Developing Countries.* [Online], Available http://www.medscape.com/viewarticle/418928. Downloaded: 04/02/ 2007 [2330Hrs].

35. Macdougall, D. (1999). TB & HIV: The Deadly Intersection. *J Int Assoc Physicians AIDS Care,* 5 (5): 20-27.

36. Maher, D., Harries, A. & Getahun, H. (2005). Tuberculosis and HIV interaction in Sub-Saharan Africa: Impact on Patients and Programmes; Implications for Policies. *Tropical Medicine & International Health*, 10 (8): 734-742.

37. Manosuthi, W., Mankatitham, W., Lueangniyomkul, A., Chimsuntorn, S., Sungkanuparph, S. (2008). Standard-Dose Efavirenz Versus Standard-Dose Nevirapine in Antiretroviral Regimens among HIV-1 and Tuberculosis Co-Infected Patients who Received Rifampicin. *HIV Medicine*, 9 (5): 294-9.

38. Marais. B., Hesseling, A., Gie, R., Schaaf, H., Beyers, N. (2006). The burden of Childhood Tuberculosis and the Accuracy of Community-Based Surveillance Data. *Int J Tuberc Lung Dis*. 10(3):259-63.

39. Martinson, N., Moultrieh, H., Barry, G., van Niekerk, R., Coovadia, A., Cotton, M., Violari, A, Gray, G., Chaisson, R., Mclntyre, J., Meyers, T. (2009). HAART and the Risk of Tuberculosis in HIV-Infected South African Children: A Multi-site Retrospective Cohort. *Int.J.Tuberc Lung Dis*, 13(7): 862-867.

40. McIlleron, H. et al. (2009). Double-dose lopinavir/ritonavir provides insufficient lopinavir exposure in children receiving rifampicin-based anti-antimycobacterial treatment. *Sixteenth Conference on Retrovirus and Opportunistic Infections, Montreal, abstract 98*.
41. Meintjies, G. & Davies, M. (2009). Assessing the Contribution of the Immune Reconstitution Inflammatory Syndrome to Mortality in Developing Country Antiretroviral Therapy Programs. *Clin Infect Dis.* 49(6): 973–975.
42. Mhlongo, N., Martinson, N., Violari, A., Newell, K., Chaisson, R., McIntyre, J. & Gray G. (2004). Incidence of HIV associated TB in Adults and Children Treated with ART in Soweto, South Africa. *Int Conf AIDS. 15:* abstract no. MoPeB3202.
43. Miranda, A., Morgan, M., Jamal, L., Laserson, K., Barreira, D., Silva, G., Santos, J., Wells, C., Paine, P. & Garrett, D. Impact of Antiretroviral Therapy on the Incidence of Tuberculosis: The Brazilian Experience, 1995–2001. *PLoS ONE* 2(9).
44. Mofenson, L., Oleske, J., Serchuck, L., Van Dyke, R., Wilfert, C. (2004). Treating Opportunistic Infections among HIV-Exposed and Infected Children. Recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. *MMWR*, 53 (RR14); 1-63.
45. Murdoch, D., Venter, W., Feldman, C., Van Rie, A. (2008). Incidence and Risk Factors for the Immune Reconstitution Inflammatory Syndrome in HIV Patients in South Africa: A Prospective Study. *AIDS* 22 (5):601-610.
46. Nachega, J., Hislop, M., Dowdy, D., Gallant, J., Chaisson, R., Regensberg, L. and Maartens, G. (2008). Efavirenz Versus Nevirapine-Based Initial Treatment of HIV Infection: Clinical and Virological Outcomes in Southern African Adults. *AIDS*. 22 (16):2117-25.
47. National Department of Health, South Africa (2000). HIV/AIDS Policy Guideline Tuberculosis (TB) and HIV/AIDS.

48. National Department of Health (Directorate: Health Systems Research and Epidemiology). (2006). Notification System, Pretoria. [Online], Available: http://www.healthlink.org.za/healthstats/16/data. Accessed May 2009.
49. National Department of Health of South Africa, (2005). Guidelines for the Management of HIV-Infected Children: National department of Health, South Africa; 2005. First edition.
50. National Department of Health of South Africa, (2009). National Tuberculosis Management Guidelines.
51. Newton, S., Brent, A., Anderson, S., Whittaker, E., Kampmann, B. (2008). Paediatric Tuberculosis. *The Lancet Infectious Diseases. Review.* 8 (8): 498-510.
52. Pape, J. (2004). Tuberculosis and HIV in the Caribbean: Approaches to Diagnosis, Treatment and Prophylaxis. *Topics in HIV Medicine,* 12 (5): 144-149.
53. Puthanakit, T., Oberdorfer, P., Ukarapol, N., Akarathum, N., Punjaisee, S., Sirisanthana, T., and Sirisanthana, V. (2006). Immune Reconstitution Syndrome from Nontuberculous Mycobacterial Infection after Initiation of Antiretroviral Therapy in Children with HIV Infection. *Pediatr Infect Dis J.* 25(7): 645–648.
54. Rabie, H., Violari, A., Madhi, S., Gibb, D., Steyn, J., van Niekerk, R., Josipovic, D., Innes, S., Dobbels, E., & Cotton, M. for the CHER team. (2008). Complications of BCG Vaccination in HIV Infected and Uninfected Children; Evidence from the Children with HIV Early Antiretroviral Therapy (CHER) Study. 15th CROI, 2008.
55. Reid M., Shah, S. (2009). Approaches to Tuberculosis Screening and Diagnosis in People with HIV in Resource-Limited Settings. *The Lancet Infectious Diseases. Review.* 9 (3):173-184.
56. Rekha B., Swaminathan S. (2007). Childhood Tuberculosis - Global Epidemiology and the Impact of HIV. *Paediatr Respir Rev*. 8(2):99-106.
57. Ruxrungtham, K. and Phanuphak, P. (2001).Update on HIV/AIDS in Thailand, *J Med Assoc Thai* 84 (Suppl. 1):S1–S17.

58. Seyler, C., Toure, S., Messou, E., Bonard, D., Gabillard, D. & Anglaret, X. (2005) Risk Factors for Active Tuberculosis after Antiretroviral Treatment Initiation in Abidjan. *Am J Respir Crit Care Med*. 172:123-127.

59. Shipton, L., Wester, C., Stock, S., Ndwapi, N., Gaolathe, T., Thior, I., Avalos, A., Moffat, H., Mboya, J., Widenfelt, E., Essex, M., Hughes, M., Shapiro, R. (2009). Safety and Efficacy of Nevirapine- and Efavirenz-Based Antiretroviral Treatment in Adults Treated for TB-HIV Co-Infection in Botswana. *Int J Tuberc Lung Dis*, 13 (3): 360-336.

60. Singh, A., Bairy, I. and Shivananda, P. (2003). Spectrum of Opportunistic Infections in AIDS Cases. *Indian J Med Sci 57:16–21.*

61. Sirisanthana, V., Puthanakit, T., Oberdorfer, A., Akaratham, N., Sirisanthana, T. (2004). Immune Reconstitution Inflammatory Syndrome in Thai HIV-Infected Children after Initiation of Highly Active Antiretroviral Therapy. *Int Conf AIDS.* 15: abstract no. TuPeB4404.

62. Smith, K., Kuhn, L., Coovadia, A. et al (2008). Immune reconstitution inflammatory syndrome in young children initiating ART. *15th CROI, February 2008, Boston, MA. Oral abstract 75. [Online], Available:* http://www.retroconference.org/2008/Abstracts/31092.htm. Accessed 2nd November 2008

63. Smith, K., Kuhn, L., Coovadia, A., Meyers, T., Hu, C., Reitz, C., Barry, G., Strehlau, R., Sherman, G., and Abrams, E. (2009). Immune Reconstitution Inflammatory Syndrome among HIV-Infected South African Infants Initiating Antiretroviral Therapy. *AIDS*, 23(9): 1097–1107.

64. South Africa National HIV Prevalence, Incidence, Behavior and Communication Survey, (2008). ONLINE], Available: http.//www.hsrc.ac.za. Accessed November, 2010.

65. Stop TB Partnership and World Health Organization, (2008). New Laboratory Diagnostic Tools for TB Control. [ONLINE], Available: http.//www.stoptb.org. Accessed August, 2010.

66. Swaminathan, S., Nandini, K., Hanna, L., Somu, N., Narayanan, P., Barnes, P. (2000). T-lymphocyte Subpopulations in Tuberculosis. *Indian Pediatr*; 37: 489-495.

67. Swaminathan, S. (2004). Tuberculosis in HIV-infected children. *Paediatric Respiratory Reviews,* 5 (3):225-230.

68. Tantisiriwat, W. & Powderly. (1999*).* Prophylaxis and Treatment of Opportunistic Infection in Patients on ART. *AIDS Read,* (2): 122-130.

69. Trunz, B., Fine, P., Dye, C. (2006). Effect of BCG Vaccination on Childhood Tuberculous Meningitis and Miliary Tuberculosis Worldwide: A Meta-Analysis and Assessment of Cost-Effectiveness. *Lancet,* 367:1173-1180.

70. van Rheenen, P. (2002). The use of the Paediatric Tuberculosis Score Chart in an HIV-Endemic Area. *Tropical Medicine & International Health*, 7(5):435-441

71. van Rossum, A., Scherpbier, H., van Lochem, E., Pakker, N., Slieker, W., Wolthers, K., Roos, M., Kuijpers, J., Hooijkaas, H., Hartwig, N., Geelen, S., Wolfs, T., Lange, J., Miedema, F. & de Groot, R. (2001). Therapeutic Immune Reconstitution in HIV-1-Infected Children is Independent of their Age and Pretreatment Immune Status. *AIDS*, 15(17):2267-2275.

72. Van Rie, A., Beyers, N., Gie, R., Kunneke, M., Zietsman, L., Donald, P. (1999). Childhood Tuberculosis in an Urban Population in South Africa: Burden and Risk Factor. *Arch Dis Child,* 80 (5):433-437.

73. Vanprapar, N., Chearsakul, S., Chokephaibulkit, K., Phongsamart, W., Lolekha, R. (2000). High CD4+ T-cells Percentage and/or Low Viral Load are Predictors of 1-5 Years Survival in HIV-1 Vertically Infected Thai Children. *J Med Assoc Thai,* 85 Suppl 2:S690-3.

74. Varma, J., Nateniyom, S., Akksilp, S., Mankatittham, W., Sirinak, C., Sattayawuthipong, W., Sirinak, C., Burapat, C., Kittikraisak, W., Monkongdee, P., Cain, K., Wells, C., Tappero, J. (2009). HIV Care and Treatment Factors Associated with Improved Survival During TB Treatment in Thailand: An Observational Study. *BMC Infect Dis*, 9:42.

75. Velasco, M., Castilla, V., Sanz, J., Gaspar, G., Condes, E., Barros, C., et al. (2009). Effect of Simultaneous use of Highly Active Antiretroviral Therapy on Survival of HIV Patients with Tuberculosis. *J Acquir Immune Defic Syndr*, 50 (2): 148-52.

76. Violari, A., Cotton, M., Gibb, D., Babiker, A., Steyn, J., Jean-Phillip, P., McIntyre, J., Team obotCS. *(2007).* Antiretroviral Therapy Initiated Before 12 Weeks of Age Reduces Early Mortality in Young HIV-Infected Infants: Evidence from the Children with HIV Early Antiretroviral Therapy (CHER) Study [Abstract WESS 103]. *4th IAS Conference on HIV Pathogenesis and Treatment; Sydney*.

77. Violari, A., Cotton, M., Gibb, D., Babiker, A., Steyn, J., Jean-Phillip, P., McIntyre, J., Madhi, S. (for the CHER Study Team), (2008). Early Antiretroviral Therapy and Mortality among HIV-Infected Infants. *NEJM, 359:2233-2244.*

78. Walters, E., Cotton, M., Rabie, H., Schaaf, H., Walters, L. & Marais, B. (2008). Clinical Presentation and Outcome of Tuberculosis in Human Immunodeficiency Virus Infected Children on Anti-Retroviral Therapy. *BMC Pediatrics*, 8(1):1471-2431.

79. Westreich, D., MacPhail, P., Van Rie, A., Malope-Kgokong, B., Ive, P., Rubel, D., Boulmé, R., Eron, J., Sanne, I. (2009). Effect of Pulmonary Tuberculosis on Mortality in Patients Receiving HAART. *AIDS,* 23 (6): 707-15.

80. Wilkinson, D., Davies, G. (1998). Pediatric Tuberculosis in Rural South Africa—Value of Directly Observed Therapy. *Journal of Tropical Pediatrics,* 44(5):266-269.

81. Williams, B. and Dye, C. (2003). Antiretroviral Drugs for Tuberculosis Control in the Era of HIV/AIDS. *Science. 301*:1535–1537.

82. Woldehanna, S. & Volmink, J. (2004). Treatment of Latent Tuberculosis Infection in HIV-infected Persons. *The Cochraine Database of Systematic Reviews*, 1: 1-90.

83. World Health Organization (WHO), Geneva (2004). BCG Vaccine. WHO Position Paper. *Weekly Epidemiologic Rec,* 79 (4): 27-38.

84. World Health Organization (WHO), Geneva (2004). Interim Policy on Collaborative TB/HIV Activities. WHO/HTM/TB/2004.330.

85. World Health Organization (WHO), Geneva (2004). TB/HIV: A Clinical Manual. 2nd Edition. 1-210.

86. World Health Organization (WHO), Geneva (2006). Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children. WHO/HTM/TB/2006.371.

87. World Health Organization (WHO), Geneva (2007). WHO Position Papers: Revised BCG Vaccination Guidelines for Infants at Risk for HIV Infection. *Weekly Epidemiological Record*, 21 (193-196).

88. World Health Organization (WHO), Geneva (2008). Report of the WHO Technical Reference Group, Paediatric HIV/ART Care Guidelines Group Meeting. Antiretroviral Therapy for Infants and Children. [ONLINE], Available: *http.//www.who.int/hiv/pub/meetingreports/art*

89. World Health Organization/United Nations Children's Educational Fund (WHO/UNICEF), (2009). Global Immunization 1980-2008, BCG Coverage Estimates. 193 WHO member states. [ONLINE], Available:
hppt://www.who.int/entity/immunization_monitoring/diseases/BCG_coverage.jpg

90. World Health Organization (WHO), Geneva, (2009). Disseminated Bacille Calmette-Guérin Disease in HIV-Infected South African Infants. *Bullettin of The World Health Organization*, 87 (7).

91. Zar, H., Cotton, M., Strauss, S., Karpakis, J., Hussey, G., Schaaf, S., Rabie, H. & Lombard, C. (2006). Effect of INH Prophylaxis on Mortality and Incidence of TB in Children with HIV. A Randomized Controlled Trial. *Student BMJ*, 14:441-484.

Annex 1.

An outline of the variables in the database that were used in analysis

InitialvisitiD: this was the identity number that the clinic would assign to a particular patient on their first visit to the clinic before starting ART.
VisitDateTime: this were the dates that the patient came for consultations either on routine appointments or if there was a medical problem that needed attention by the clinic.
PatientId: This was the identity number that the patient was given on the day ART was commenced. It was then used together with the initialvisitiD to identify and distinguish those on ART and this number was then subsequently used to identify the patient on future visits.
DateOfBirth: This was the date that a particular patient was born.
SexF: This was the gender of the patient female.
SexM: This was the gender of the patient male.
PopulationGroupAfrican: This was the population group of the patient that was of African origin.
TransferredInOnArt: This was to identify those patients that were transferred in on ART from other ART sites to HSCC.
ClinicStartedOnArt: This was to identify those patients that were transferred in on ART from local clinics like Zola clinic and Lilian Ngoyi clinic to HSCC.
DateStartedOnArtPreviousClinic: This was the date ART was started for those patients who were started ART at other clinics before being integrated into the HSCC care.
StartedOnArtInWardsCHB: This was to identify those patients that were transferred in on ART from Chris Hani Baragwanath wards to HSCC.
ArtStartDate: This was the date that ART was started for those who started in HSCC.
BodyWeight: This was the weight on a particular visit date.
Temperature: This was the temperature on a particular visit date.
Height: This was the height on a particular visit date.
CD4Count: This was the CD4 count that was recorded on a particular visit date and this was done six monthly.
ViralLoad: This was the viral load that was recorded on a particular visit date and this was done six monthly.
CD4CountPercentage: This was the CD4 percentage that was recorded on a particular visit date and this was done six monthly.

TBStatusNoTB: This was if the patient had no TB on a particular visit date.
TBStatusPreviousTB: This was an initial visit record to distinguish those who had a history of TB before enrolling in the HSCC.
TBStatusInvestigate: This was for those patients who were presenting with symptoms of TB and were being investigated for TB.
TBStatusMDRTB: This was for those patients that were on treatment for MDR-TB on a particular visit.
TBStatusTBRx: This was for those patients that were on 'TB treatment' on a particular visit date.
TBStatusProphylaxis: This was for patients who were on INH preventive therapy.
TBRxINHStartDate: This was the date that INH preventive therapy was started.
WhoClinicalStage1: This was the clinical stage 1 of HIV disease according to the standard WHO staging system.
WhoClinicalStage2: This was the clinical stage 2 of HIV disease according to the standard WHO staging system.
WhoClinicalStage3: This was the clinical stage 3 of HIV disease according to the standard WHO staging system.
WhoClinicalStage4: This was the clinical stage 4 of HIV disease according to the standard WHO staging system.
WhoClinicalStagCondition2: This was the clinical staging condition 2 as per the WHO staging protocol.
WhoClinicalStagCondition3: This was the clinical staging condition 3 as per the WHO staging protocol.
OtherMeds: This was any other medication that the patient was taking like cotrimoxazole.