# A TIME-SERIES ANALYSIS ON THE IMPACT OF THE ANTIRETROVIRAL TREATMENT PROGRAM ON THE BURDEN OF HOSPITALIZATION FOR CULTURE-CONFIRMED *MYCOBACTERIUM TUBERCULOSIS* IN SOWETAN CHILDREN

**Ziyaad Dangor**

**9901107f**

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# DECLARATION

**I, Ziyaad Dangor declare that this research report is my own work. It is being submitted for the degree of Masters of Medicine in Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.**

**……**

**The ……..day of ……..,2011**

# DEDICATION

**I dedicate this work to my dear father, Yusuf Dangor, who has nurtured me through this academic endeavor. I thank him for his encouragement and continuous support.**

# ABSTRACT

*Introduction*

Highly active antiretroviral treatment (HAART) programs in heavily HIV-TB burdened countries may reduce the risk of TB in children directly by improving the immune system of HIV-infected children; and indirectly by reducing the force of transmission from the adult population. The incidence of childhood TB is a sentinel measure of the control of infectious adult TB cases in the community.

*Objective*

We evaluated the impact that scaling-up of the HAART program in Soweto had on the incidence of hospitalization for culture-confirmed TB in children.

*Methods*

The study was undertaken in Soweto, where the prevalence of HIV was 4-5% in children between 2005 and 2009. The estimated HAART coverage increased from 43% in 2005 to 84% by 2009 in children with HIV/AIDS. Hospitalized cases of culture-confirmed TB in children 3 months to 14 years of age were identified through laboratory and clinical electronic databases.

*Results*

Overall, the incidence (per 100 000) of hospitalization for culture-confirmed TB declined by 58% (95%CI 49.3-65.2) from 2005 (71.4) compared to 2008-9 (30.0); $p<0.0001$. This included a 67% (95%CI 58.5-74.8) reduction in incidence among HIV-infected children from 2005 (1 601) compared to 2008-9 (517; $p<0.0001$).

In addition, a 33% reduction was observed in HIV-uninfected children (incidence 19.3 vs 12.9; p=0.016). Fifty-six percent of TB episodes, across all study periods, occurred in HIV-infected children who were mainly (76%) severely immunocompromised.

*Conclusions*

Up-scaling of the HAART program in South Africa has been associated with decline in the incidence of culture-confirmed TB, more so in HIV-infected than HIV-uninfected children. Severely immunocompromised HIV-infected children, however, need to be identified and targeted with HAART and other strategies to further reduce the burden of TB in this group.

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# ABBREVIATIONS

| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| AZT | Zidovudine |
| CHBH | Chris Hani Baragwanath Hospital |
| ELISA | Enzyme linked immunosorbent assay |
| HAART | Highly active antiretroviral therapy |
| HIV | Human Immunodeficiency Virus |
| HREC | Human Research Ethics Committee |
| MGIT | Mycobacterial Growth In-tube |
| MTB | *Mycobacterium tuberculosis* |
| NHLS | National Health Laboratory Service |
| PCR | Polymerase chain reaction |
| PMTCT | Prevention of mother to child transmission |
| RMPRU | Respiratory and Meningeal Pathogens Research Unit |
| TB | Tuberculosis |
| WHO | World Health Organisation |

# 1.0 INTRODUCTION

## *1.1 Background*

South Africa has one of the highest tuberculosis (TB) and human immunodeficiency virus (HIV) co-infection burdens in Africa, including in children (1-5). HIV increases the risk of progression of Mycobacterium *tuberculosis* (MTB) infection to disease as well as reactivation of latent TB (6). TB impacts negatively on HIV infection by accelerating HIV-viral replication and lowering CD4+ T-lymphocyte counts (6-8).

The diagnosis of TB remains challenging in children. Culture of MTB from patient samples, whilst the most definitive measure of confirmation of TB in children (9), has poor sensitivity (10-30%) for diagnosing TB in children (10,11). Consequently, diagnosing childhood TB is mainly based upon non-specific clinical criteria and often the use of un-validated algorithms (12). This is further confounded in HIV-infected children, particularly if not receiving highly active antiretroviral treatment (HAART), because of the overlap of symptoms and radiological features of TB which are also attributable to HIV/AIDS or other non-TB opportunistic infections (13, 14).

In 2004 the South African government initiated a national roll-out of HAART management for HIV-infected individuals. Certain clinical and immunological criteria needed to be present before instituting treatment. In Gauteng, the overall estimated coverage with HAART in HIV-infected children requiring treatment increased from 21.5% in 2004 to 83.4% in 2009 (15).

*1.2 Literature review*

HAART has been reported to reduce the incidence of initiation of anti-TB treatment or clinical diagnosis of TB in HIV-infected children, compared primarily to historical incidence in the same cohort of children whilst not receiving HAART (16-19). These studies were all conducted in highly endemic African countries and showed reductions of between 50 and 85% in the incidence of TB; however, several limitations were present. Unlike adult studies, where the benchmark to diagnosing TB is sputum positive culture, the paediatric studies evaluated the impact of HAART primarily on cases with a probable or possible diagnosis of TB. A further limitation was that these studies only examined HIV-infected children and are not able to deduce whether the changes observed in incidence may have been temporally related to other factors related to management of HIV-infected children on HAART. The above mentioned studies only focused on children attending HIV clinics and not those admitted to hospital with TB which is the primary focus of this study.

Likewise, a similar effect was observed in the adult studies which demonstrated the reduction in the incidence of TB in patients on HAART as compared to those not on treatment (20-25). Two of these studies looked primarily at the changes in the incidence of TB over time, prior to the implementation of HAART as compared to when HAART became more available in that setting (20,21). In the study conducted by Middelkoop et al. in Cape Town, published in 2011, the case notification rates of TB in the adult population increased between 1997 and 2004 by an average of 187 cases per 100 000/year and peaked at the beginning of 2005. Subsequently the case notification rates of TB declined between 2005 and 2008 by 202 cases per 100 000/year as HAART became more available. The second study conducted by

Tseng et al. in Taiwan published in 2009 noted the increasing incidence of TB prior to the availability of HAART which was in 1997. The incidence of TB/HIV co-infection rose from 1.9% to 3.8% between 1993 and 1998 and decreased from 3.83% to 0.94% between 1998 and 2006.

The aim of this study was to demonstrate the impact that scaling-up of the HAART program in South Africa has had on the incidence of hospitalization for culture-confirmed TB in children.

## *1.3 Hypothesis and Objectives*

Null hypothesis

Increased access to HAART has not reduced the incidence of culture-confirmed tuberculosis in a setting with a high prevalence of paediatric HIV infection and high incidence of childhood TB.

*Primary objective*:

To determine the impact of up-scaling of the antiretroviral treatment program on the incidence of culture-confirmed tuberculosis in HIV-infected and HIV-uninfected children

*Secondary objectives*:

- To determine the in-hospital outcome of children with culture-confirmed TB.
- To determine the impact of the antiretroviral treatment program on the overall burden of tuberculosis (possible, probable and confirmed) in children.

## 2.0 STUDY DESIGN & METHODS

### *2.1 Study Population*

A retrospective, time-series analysis comparing hospitalization incidence of culture-confirmed and all-categories of TB in children across the early (2005), intermediate (2006, 2007) and established (2008, 2009) HAART eras was undertaken at Chris Hani Baragwanath Hospital(CHBH). This hospital serves approximately 1.12 million mainly black urban South Africans, including 120 000 children under five years of age (15). An estimated 90% of individuals in Soweto requiring hospitalization are admitted to CHBH, as less than 10% have medical insurance and CHBH is the only public-hospital in the study setting. All public-based health care, including access to HAART and hospitalization, is provided for free by the state to children.

### *HIV prevalence and management in study population*

The prevalence of HIV in Gauteng was estimated as being 4-5% in children and 18%-19% in adults between 2005 and 2009 (15). In the early HAART era it is estimated that 42.6% of children eligible for HAART were on treatment as compared to the intermediate and established eras where 63.6% and 80.5% respectively were on HAART in this province (15). During the study period, the criteria for initiating patients on HAART included more than two hospital admissions per year or hospitalization for more than four weeks with an HIV related illness; World Health Organization (WHO) stage III/IV disease; CD4+ percentage of <20% in children under 18 months of age or <15% if over 18 months of age. First line HAART included stavudine, lamivudine and lopinavir-ritonavir in children under 3 years of age and less than 10 kilograms in weight ; for those over 3 years of age stavudine, lamivudine and efavirenz was the recommended first line regimen (26).

The prevention of mother to child transmission program (PMTCT) started in South Africa in 2001 as a pilot program in a few selected areas. This initial program which included single doses of nevirapine to the mother during labour and to the newborn following delivery was subsequently rolled out nationally from 2004 onward. In April 2008 the PMTCT program was further modified. This included that mothers with CD4+ counts <350 were eligible for being initiated on HAART, whilst those with CD4+ >350 were provided with zidovudine (AZT) daily during the pregnancy. In addition, single dose nevirapine was provided to the mother during labour as well as to the newborn at birth. Furthermore, the newborn was provided with twice daily zidovudine for 7 to 28 days post-partum, stratified upon whether the mother received more than 4 weeks of AZT or HAART treatment during her pregnancy (27).

HIV diagnosis, as standard-of care in children was based on enzyme-linked immunosorbent assay (ELISA) test in children older than 18 months of age and HIV-1 polymerase chain reaction (PCR) reactivity test in children under 18 months of age. As there is a high prevalence of HIV in this community, hospital staff routinely council parents on HIV testing and request to have their children tested. The majority of known HIV-infected children during the course of this analysis would have received care at one of two paediatric HIV clinics based at CHBH. Criteria for admission to the hospital and investigation for TB of HIV-infected and uninfected children was at the discretion of attending physicians in the hospital admission ward.

*TB Diagnosis in study population*

The standard of care for diagnosing TB in children hospitalized included having a low threshold of performing 2-3 gastric washings or induced-sputum sample collection for

MTB culture in children admitted with pneumonia and those with clinical signs and symptoms of TB. Other specimens submitted for TB culture when indicated may include fine needle aspiration of lymph nodes, tissue specimens from biopsies of organs and cerebrospinal fluid. Childhood TB cases were categorized using the modified WHO case definitions into three groups namely confirmed, probable and possible TB on pre-defined inclusion criteria (28). (Table 1)

**Table 1: Inclusion criteria using World Health Organization (WHO) case definitions for all forms of childhood tuberculosis.**

| ***Confirmed*** | ***Probable*** | ***Possible*** |
|---|---|---|
| *Mycobacterium tuberculosis* cultured from clinical specimens in a patient with compatible clinical features of active tuberculosis | Acid alcohol fast bacilli visualised in clinical specimens, without culture confirmation of disease caused by *Mycobacterium tuberculosis* | All children with a discharge diagnosis of TB (not meeting criteria for confirmed and probable TB), identified through an electronic database of archived discharge summaries and admission registries to the paediatric wards |

Children under three months of age were excluded from the study on the presumption that they may have acquired TB congenitally (29). Other exclusion criteria were if the admission records were not traceable, the TB culture result did not correlate with the admission or if the results were positive for the same patient less than 6 months apart.

Laboratory methods used by the National Health Laboratory Service (NHLS) for culturing MTB from sputum and other body fluid cultures (excluding blood) were processed using the N-acetyl-L-cysteine-NaOH method. From 2004 until 2006, these samples were incubated in the radiometric BACTEC™ 460 TB system (Becton Dickinson, Sparks, Maryland) and from 2006 until 2009 the BACTEC™ MGIT 960™ TB System (Mycobacterium Growth Indicator Tube) (Becton Dickinson, Sparks, Maryland) was used. Blood for MTB culture was inoculated at the bedside into BACTEC™ MycoF/Lytic medium bottles and incubated in the laboratory in the BACTEC™ 9240 blood culture system (Becton Dickinson, Sparks, Maryland). Direct microscopy of non blood specimens was performed with auramine fluorochrome stain with grading according to the WHO/IUATLD system.

*2.2 Study Method*

Microbiological records in an electronic database were obtained from the NHLS, Department of Microbiology. All children under 15 years of age with MTB culture or auramine-smear positive on microscopy were identified. The results were cross referenced with the electronic database of archived discharge summaries and admission registries to the paediatric wards at CHBH occurring from 1st January 2005 until 31st December 2009. These results will be time framed into the early (2005), intermediate (2006,2007) and established (2008,2009) HAART eras. (Figure 1)

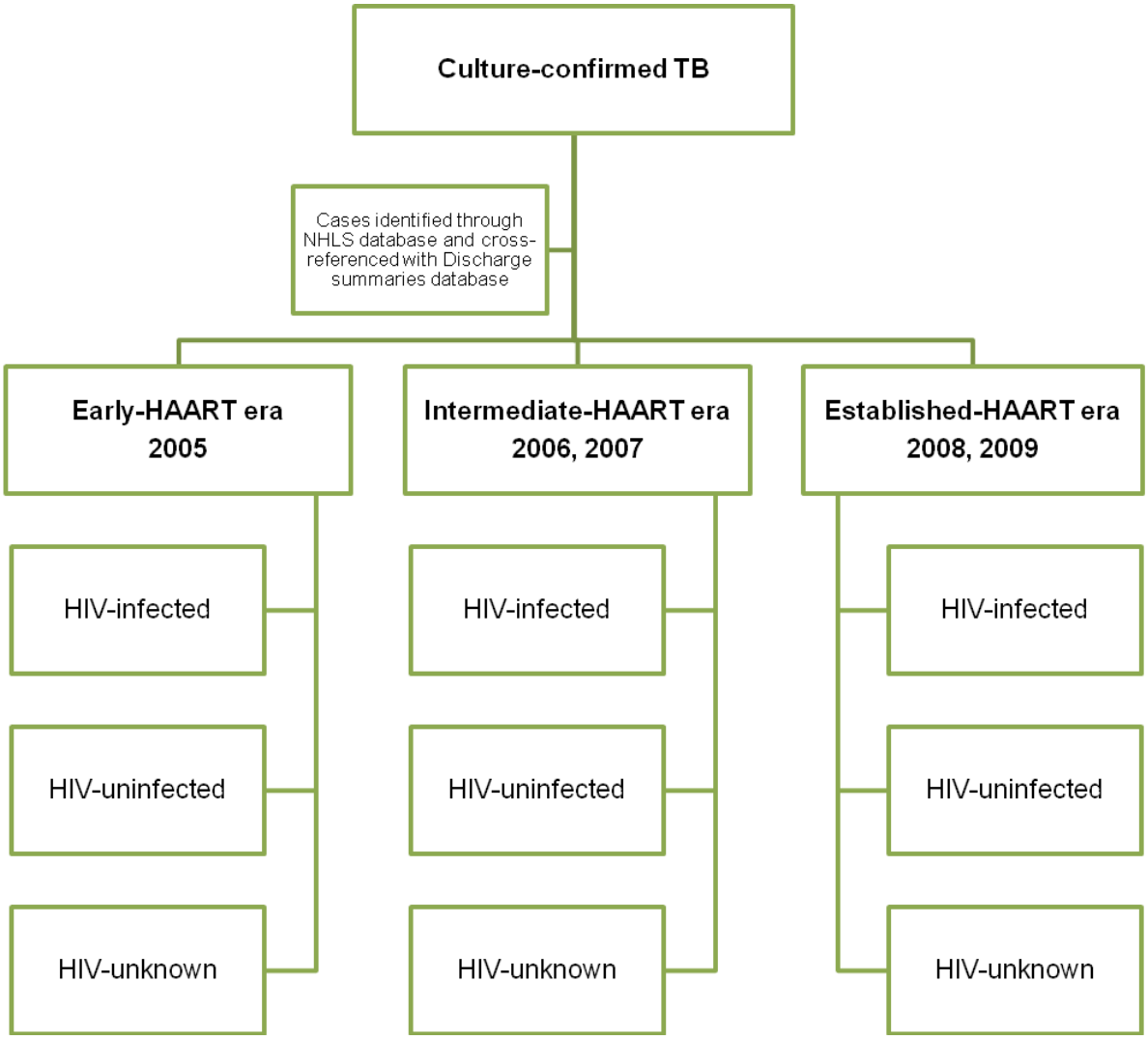


**Figure 1: Schematic representation of study methods used to collect data in children with Culture-confirmed TB**

Children with possible TB were identified through the electronic database of archived discharge summaries as those with any discharge diagnosis of TB not meeting the criteria for confirmed and probable TB. Outcomes of children hospitalized for culture-confirmed TB were also reviewed from the electronic discharge database. Children were also categorized as having pulmonary or extra-pulmonary TB from the available electronic databases and laboratory results. Incidence data were stratified by three age groups; 3-23 months, 2-4yrs and 5-14yrs of age. Laboratory results and clinical

records were analyzed for HIV status and CD4+ lymphocyte counts. CD4+ counts within three months prior to or after the admission were included in the analysis. The WHO levels of immune suppression based on CD4+ percentage/count categorization were used to stage the extent of immunosupresssion (30).

Permission to conduct the study at CHBH was obtained from the Medical Advisory Committee at the hospital. Permission was also obtained from the NHLS for access to electronic data and laboratory records. Ethics approval was granted by the Human Research Ethics Committee (HREC), University of Witwatersrand.

*2.3 Statistical Analysis*

Estimated incidences of TB (culture-confirmed, probable, possible and overall) were calculated for the three HAART eras stratified by age and HIV status to yield stratum specific incidence rates. Incidence risk ratios (IRR) were calculated by comparing group specific incidences for the early, intermediate and established eras of HAART.

The numerators for incidence calculation were the number of TB episodes hospitalized during each era; and the population denominator was based on mid-year population estimates from the Gauteng Department of Health and Social development for region D (Soweto) in Johannesburg as per Statistics South Africa report (31). HIV prevalence in the study population was estimated from the projections of the AIDS and Demographic models developed by the Actuarial Society of South Africa (15). (Table 2)

**Table 2: Population denominators and HIV prevalence by age-group in Soweto, South Africa[1]**

| Period | Year | Age | Total population[1] | HIV prevalence[2] |
|---|---|---|---|---|
| Early HAART | 2005 | <2yrs | 51 350 | 5.00% |
| | | 2 - <5yrs | 70 212 | 5.07% |
| | | 5 - <10yrs | 101 746 | 2.01% |
| | | 10 - <15yrs | 90 561 | 0.07% |
| Intermediate HAART | 2006 | <2yrs | 53 865 | 5.00% |
| | | 2 - <5yrs | 73 743 | 5.08% |
| | | 5 - <10yrs | 106 074 | 2.47% |
| | | 10 - <15yrs | 92 385 | 0.13% |
| Intermediate HAART | 2007 | <2yrs | 52 632 | 5.00% |
| | | 2 - <5yrs | 74 044 | 5.08% |
| | | 5 - <10yrs | 110 501 | 2.94% |
| | | 10 - <15yrs | 96 643 | 0.22% |
| Established HAART | 2008 | <2yrs | 50 930 | 5.00% |
| | | 2 - <5yrs | 74 383 | 5.21% |
| | | 5 - <10yrs | 115 451 | 3.28% |
| | | 10 - <15yrs | 100 757 | 0.36% |
| Established HAART | 2009 | <2yrs | 48 785 | 5.00% |
| | | 2 - <5yrs | 74 788 | 5.42% |
| | | 5 - <10yrs | 120 930 | 3.50% |
| | | 10 - <15yrs | 104 683 | 0.54% |

[1]Population estimates for region D (Soweto) in Johannesburg as per Statistics South Africa (31)

[2]HIV prevalence in the study population was estimated from the projections of the AIDS and Demographic models developed by the Actuarial Society of South Africa (15)

Unadjusted data were quoted in the results. However, to account for those cases with an unknown HIV result, we extrapolated our data to include these cases. The unknown cases were divided into HIV-infected or HIV-uninfected based on the proportional percentage of known results in the two groups. As a further analysis, we extrapolated all the unknown results as HIV-uninfected as the clinician was probably not suspecting HIV in them and hence these patients were not tested.

$\chi^2$ test (or Fisher's exact test) were used to compare the distribution of categorical variables. 95% confidence intervals were reported and *p*-values<0.05 were considered statistically significant. Demographic data and estimates of incidence and risk ratio were analyzed using STATA version11.0 and Epi Info 3.5.1 (CDC, Atlanta, USA).

# 3.0 RESULTS

## *3.1 Culture-confirmed TB*

The number of cases identified with culture-confirmed TB in 2005 was 224, of which 132(60%) were HIV-infected. In 2007, 163 cases were identified and in 2009 the number of cases dropped to 92. Fifty-six percent of TB episodes, across all study periods, occurred in HIV-infected children (Table 3). The median age of presentation was 1year 8 months across the five years. Although the median age was higher in the 2009 year, these values were not statistically significant when comparing them using Chi squared trends. The majority of children with culture- confirmed TB were underweight for age with a median z-score of -2.81(range-7.96 to 2.34). The case fatality rate of children with culture-confirmed TB over the 5 year period was 2.9(95%CI 1.9-4.3). Of the 24 fatalities, 15(63%) were HIV infected. Four cases had extra-pulmonary TB and the median age was 10months (range: 3months to 12 years) (Table 4).

**Table 3: Number of cases hospitalized for TB stratified by HIV status.**

| | Overall TB | | | Confirmed TB | | | Probable TB | | | Possible TB | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TOTAL | PTB | EPTB | TOTAL | PTB | EPTB | TOTAL | PTB | EPTB | TOTAL | PTB | EPTB |
| ***2005*** | ***866*** | ***760*** | ***106*** | ***224*** | ***197*** | ***27*** | ***63*** | ***59*** | ***4*** | ***579*** | ***504*** | ***75*** |
| **HIV-infected** | **543** | 485 | 58 | **132** | 117 | 15 | **37** | 35 | 2 | **374** | 333 | 41 |
| **HIV-uninfected** | **230** | 185 | 45 | **59** | 48 | 11 | **21** | 19 | 2 | **150** | 118 | 32 |
| **HIV-unknown** | **93** | 90 | 3 | **33** | 32 | 1 | **5** | 5 | 0 | **55** | 53 | 2 |
| ***2006*** | ***711*** | ***609*** | ***102*** | ***226*** | ***194*** | ***32*** | ***45*** | ***42*** | ***3*** | ***440*** | ***373*** | ***67*** |
| **HIV-infected** | **421** | 370 | 51 | **119** | 104 | 15 | **22** | 20 | 2 | **280** | 246 | 34 |
| **HIV-uninfected** | **203** | 165 | 38 | **72** | 61 | 11 | **14** | 13 | 1 | **117** | 91 | 26 |
| **HIV-unknown** | **87** | 74 | 13 | **35** | 29 | 6 | **9** | 9 | 0 | **43** | 36 | 7 |
| ***2007*** | ***607*** | ***487*** | ***120*** | ***163*** | ***129*** | ***34*** | ***12*** | ***11*** | ***1*** | ***432*** | ***347*** | ***85*** |
| **HIV-infected** | **360** | 287 | 73 | **94** | 75 | 19 | **7** | 6 | 1 | **259** | 206 | 53 |
| **HIV-uninfected** | **178** | 141 | 37 | **49** | 37 | 12 | **4** | 4 | 0 | **125** | 100 | 25 |
| **HIV-unknown** | **69** | 59 | 10 | **20** | 157 | 3 | **1** | 1 | 0 | **48** | 41 | 7 |
| ***2008*** | ***616*** | ***490*** | ***126*** | ***115*** | ***90*** | ***25*** | ***12*** | ***10*** | ***2*** | ***489*** | ***390*** | ***99*** |
| **HIV-infected** | **350** | 269 | 81 | **60** | 46 | 14 | **5** | 5 | 0 | **285** | 218 | 67 |
| **HIV-uninfected** | **226** | 185 | 41 | **48** | 37 | 11 | **6** | 4 | 2 | **172** | 144 | 28 |
| **HIV-unknown** | **40** | 36 | 4 | **7** | 7 | 0 | **1** | 1 | 0 | **32** | 28 | 4 |
| ***2009*** | ***581*** | ***467*** | ***114*** | ***92*** | ***69*** | ***23*** | ***22*** | ***19*** | ***3*** | ***467*** | ***379*** | ***88*** |
| **HIV-infected** | **324** | 253 | 71 | **53** | 39 | 14 | **8** | 7 | 1 | **263** | 207 | 56 |
| **HIV-uninfected** | **234** | 193 | 41 | **38** | 29 | 9 | **11** | 9 | 2 | **185** | 155 | 30 |
| **HIV-unknown** | **23** | 21 | 2 | **1** | 1 | 0 | **3** | 3 | 0 | **19** | 17 | 2 |

**Table 4: Demographic data of children hospitalized with culture-confirmed TB**

| | 2005 | | 2006 | | 2007 | | 2008 | | 2009 | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Total no. of cases** | **224** | | **226** | | **163** | | **115** | | **92** | |
| **Age** | | | | | | | | | | |
| <2yrs | **130** | 58% | **130** | 58% | **85** | 52% | **60** | 52% | **35** | 38% |
| 2-4yrs | **30** | 13% | **41** | 18% | **26** | 16% | **18** | 16% | **19** | 21% |
| 5-14yrs | **64** | 29% | **55** | 24% | **52** | 32% | **37** | 32% | **38** | 41% |
| mean(years) | **3.25** | | **3.08** | | **3.83** | | **3.75** | | **5yr** | |
| median(years) | **1.25** | | **1.25** | | **1.83** | | **1.75** | | **2.75** | |
| range | **3m-13yrs** | | **3m-14.25yrs** | | **3m-14yrs** | | **3m-13.25yrs** | | **3m-13.83yrs** | |
| **Gender** | | | | | | | | | | |
| M | **115** | 51% | **125** | 55% | **92** | 56% | **61** | 53% | **55** | 60% |
| F | **109** | 49% | **101** | 45% | **71** | 44% | **54** | 47% | **37** | 40% |
| **Weight for Age(<10yrs)** | | | | | | | | | | |
| Mean Z-scores | **-2.702** | | **-2.847** | | **-2.737** | | **-3.048** | | **-2.743** | |
| Median Z-scores | **-2.64** | | **-2.72** | | **-2.65** | | **-3.28** | | **-2.84** | |
| Range | **-7.08 - 2.34** | | **-7.96 - 0.75** | | **-7.01 - 1.84** | | **-7.57 - 1.78** | | **-6.35 - 2.31** | |
| **Outcomes** | | | | | | | | | | |
| Discharged | **157** | | **208** | | **149** | | **105** | | **89** | |
| unknown | **61** | | **13** | | **10** | | **3** | | **1** | |
| Demised | **6** | | **5** | | **4** | | **7** | | **2** | |
| Case fatality rate | **2.7[1.0-5.7]** | | **2.2[0.7-5.1]** | | **2.5[0.7-6.2]** | | **6.1[2.5-12.1]** | | **2.2[0.3-7.6]** | |

*3.2 Trends in incidence of culture-confirmed TB*

The incidence (per 100 000) of culture-confirmed TB declined by 58% (95%CI 49.3-65.2) from the early(2005)HAART era (71.4) compared to the established(2008-9)HAART era (30.0); p<0.0001. This included a 67% (95%CI 58.5-74.8) reduction in incidence among HIV-infected children from the early(2005)HAART era (1 601) as opposed to the established(2008-9)HAART era (517; p<0.0001). In addition, a 33% reduction was observed in HIV-uninfected children (incidence 19.3 vs 12.9; p=0.016). The incidence of culture-confirmed TB extrapolated to include all the HIV unknown results as HIV-uninfected demonstrated a reduction of 53.3% (95%CI 37.8-65.0; p<0.0001) (Table 5).

**Table 5: Incidence of culture-confirmed TB for the 3 study periods in children 3 months to 14yrs**

| | Early-HAART<br>Incidence of TB[1]<br>[95%CI]<br>(N of TB cases) | Intermediate-HAART<br>Incidence of TB[1]<br>[95%CI]<br>(N of TB cases) | Established-HAART<br>Incidence of TB[1]<br>[95%CI]<br>(N of TB cases) | IRR[2] | p=[3] | %reduction[4] |
|---|---|---|---|---|---|---|
| **Confirmed TB** | 71.4[62.3-81.4]<br>(224) | 59.0[53.2-65.1]<br>(389) | 30.0[26.0-34.3]<br>(207) | 0.42[0.35-0.51] | <0.0001 | 58.0[49.3-65.2] |
| HIV-infected | 1601.4[1341.5-1896.2]<br>(132) | 1119.2[974.6-1279.0]<br>(213) | 517.2[426.4-621.5]<br>(113) | 0.32[0.25-0.41] | <0.0001 | 67.7[58.5-74.8] |
| HIV-uninfected | 19.3[14.7-24.9]<br>(59) | 18.9[15.7-22.6]<br>(121) | 12.9[10.3-15.9]<br>(86) | 0.67[0.48-0.93] | 0.0155 | 33.4[7.2-52.2] |
| HIV-infected EX [5] | 1880.4[1598.2-2197.3]<br>(155) | 1303.1[1146.9-1474.5]<br>(248) | 540.1[447.2-646.4]<br>(118) | 0.29[0.23-0.36] | <0.0001 | 71.3[63.6-77.4] |
| HIV-uninfected EX [6] | 22.6[17.6-28.6]<br>(69) | 22.0[18.5-26.0]<br>(141) | 13.3[10.7-16.4]<br>(89) | 0.59[0.43-0.81] | 0.0009 | 41.1[19.3-57.0] |
| HIV-uninfected EX[7] | 30.1[24.3-36.9]<br>(92) | 27.5[23.6-31.8]<br>(176) | 14.1[11.4-17.2]<br>(94) | 0.47[0.35-0.62] | <0.0001 | 53.3[37.8-65.0] |

[1] number of cases per 100 000
[2] incidence risk ratios-Established vs Early HAART era
[3] $\chi^2$ test or Fischer test
[4] %reduction between Early and Established HAART era
[5] incidence of TB in HIV-infected extrapolated proportionally to include HIV-unknown
[6] incidence of TB in HIV-uninfected extrapolated proportionally to include HIV-unknown
[7] incidence of TB in HIV-uninfected extrapolated to include all cases with HIV-unknown

*3.3 Trends in incidence of culture-confirmed TB stratified by age*

Stratified by age, the incidence of culture-confirmed TB in the 3 to 23 month age group decreased (62%; 95%CI 51.0-71.1) across the time periods; from 253.2 per 100 000 in the early era to 95.3 per 100 000 in the established HAART era; p<0.0001 (Figure 1).

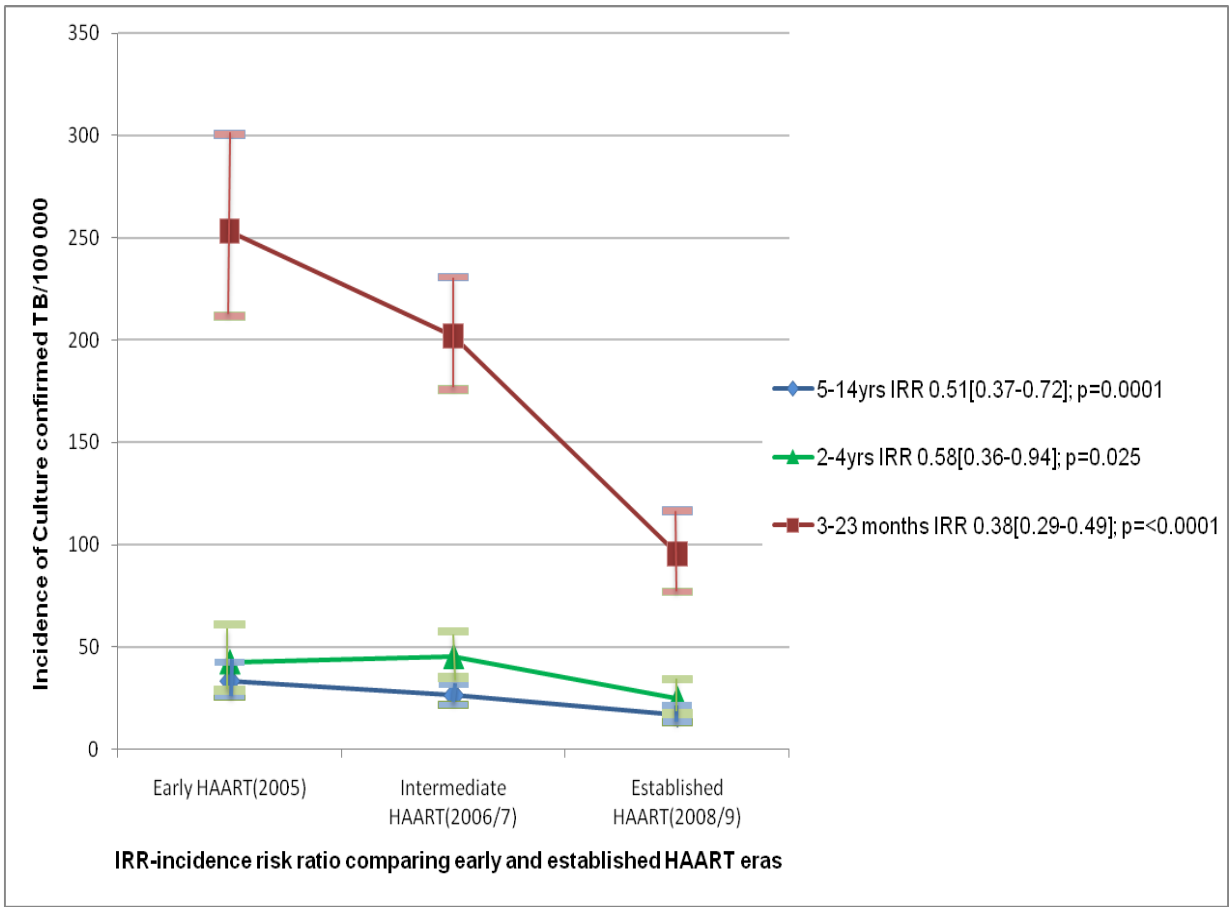


**Figure 2: Incidence of culture-confirmed TB for the 3 study periods stratified by age**

The reduction was more evident in the HIV-infected children, 3 to 23 months, among whom the incidence decreased from 2803.7 in the early-HAART era to 782.2 in the established HAART era, 72.1% reduction (95%CI 58.9-81.1); p<0.0001. The incidence of culture-confirmed TB in HIV-infected children in the 2-4year and 5-14 year age groups also indicated a 65.0%(95%CI 29.8-82.6) and 66.2% (95% CI 50.0-77.2) reduction respectively when comparing the early and established HAART eras (Figure 2).

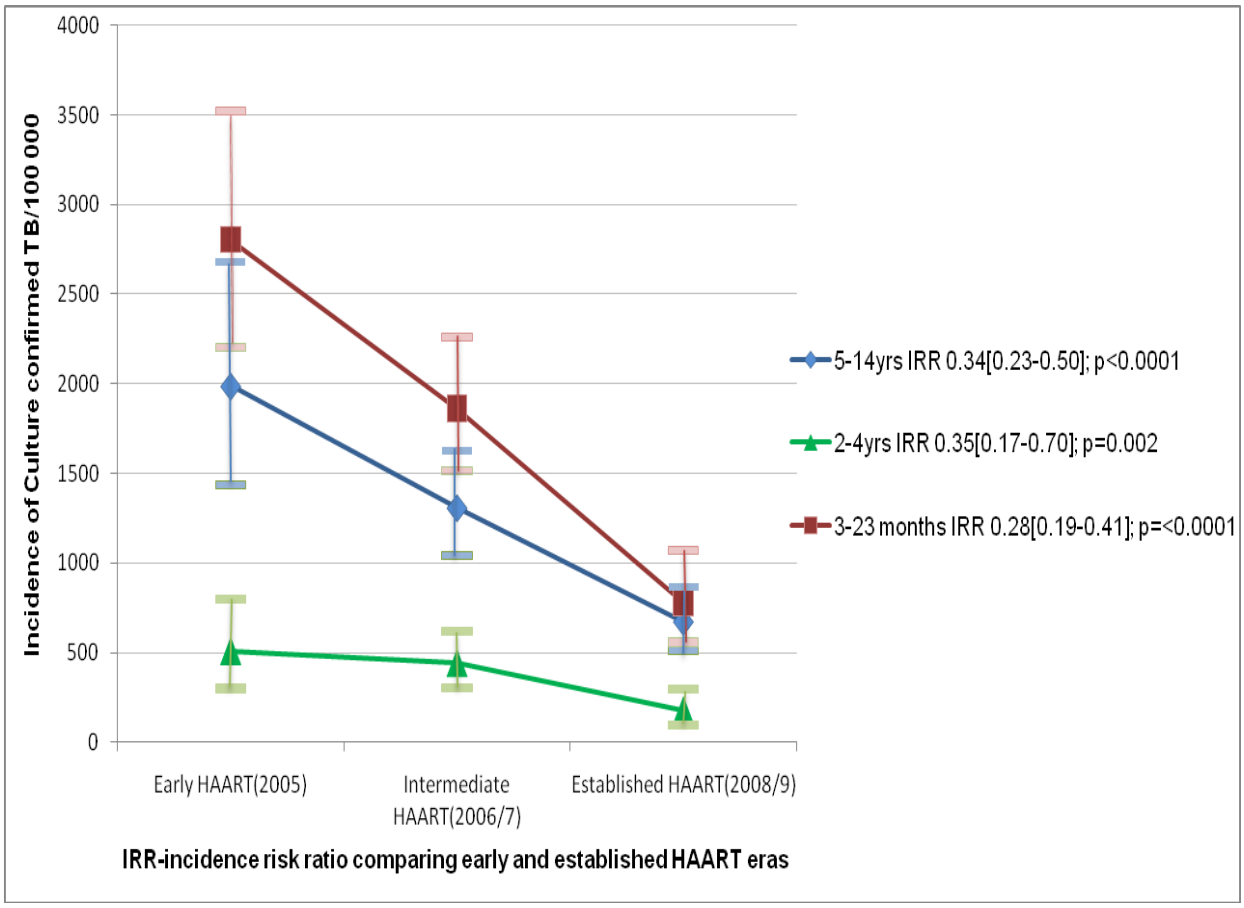


**Figure 3: Incidence of culture-confirmed TB in HIV-infected children for the 3 study periods stratified by age**

There was no statistically significant reduction in HIV-uninfected children in the 2-4 year and 5-14 year age groups but a reduction of 35.9% (95%CI 33.8-57.5; p=0.032) was observed in children 3 to 23 months of age (Figure 3).

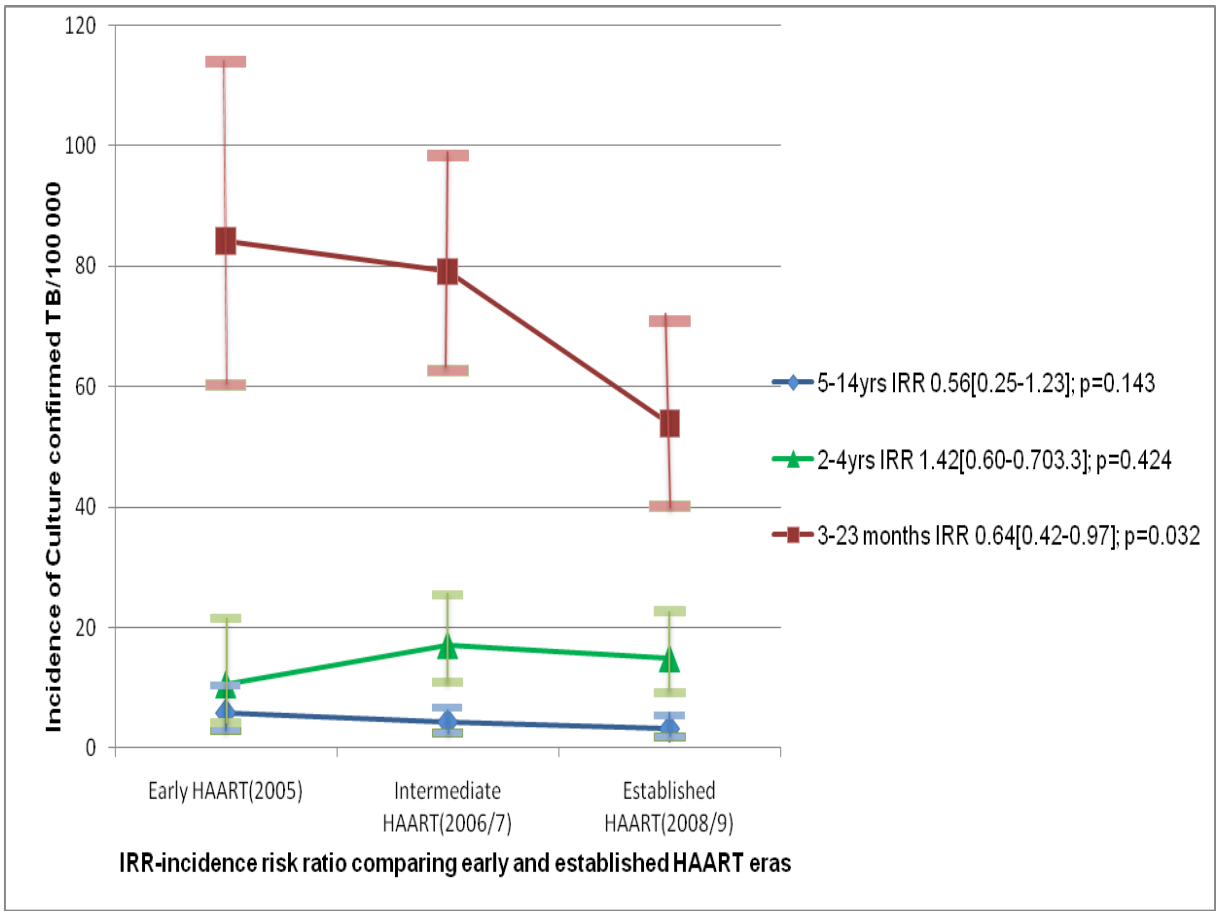


**Figure 4: Incidence of culture-confirmed TB in HIV-uninfected children for the 3 study periods stratified by age**

*3.4 Immunological classification for established HIV infection in children hospitalized for culture-confirmed TB*

More than two thirds of HIV infected children with culture-confirmed TB had a CD4 count done within three months of being diagnosed TB culture positive. Seventy-six percent of these cases across all three study periods occurred in HIV-infected children who were categorized as severely immunocompromised (Table 6).

**Table 6: WHO immunological classification for established HIV infection in children hospitalized for culture-confirmed TB**

| Year | TB cases | HIV-infected | *HIV infected with CD4* | Immunological Classification[1] Not significant[2] | Mild[3] | Advanced[4] | Severe[5] |
|---|---|---|---|---|---|---|---|
| 2005 | **224** | **132** | ***91*** | 9 | 4 | 7 | 71 (78%) |
| 2006 | **226** | **119** | ***83*** | 8 | 2 | 8 | 65 (78%) |
| 2007 | **164** | **94** | ***66*** | 6 | 3 | 7 | 50 (76%) |
| 2008 | **115** | **60** | ***46*** | 3 | 2 | 5 | 36 (78%) |
| 2009 | **92** | **53** | ***51*** | 5 | 2 | 2 | 42 (82%) |

**Note: WHO immunological classification[1] for established HIV infection(CD4% or absolute number per mm3)**

| | Not significant[2] | Mild[3] | Advanced[4] | Severe[5] |
|---|---|---|---|---|
| **<11 months** | >35% | 30-35% | 25-29% | <25% |
| **12-35 months** | >30% | 25-30% | 20-24% | <20% |
| **36-59 months** | >25% | 20-25% | 15-19% | <15% |
| **>5yrs** | >500 | 350-499 | 200-349 | <200/<15% |

*3.5 Trends in incidence of Overall TB*

The incidence of overall TB (culture-confirmed, probable and possible) declined from 275.9 per 100 000 in the early HAART era to 173.3 per 100 000 in the established HAART era; p<0.0001. Among HIV-infected children, the incidence of overall TB declined by 53.2% (95%CI 47.7-58.1) in the early HAART era (6587.4) compared to established HAART era (3084.8) p<0.0001). No significant reduction was noted in HIV-uninfected children (Table 7).

**Table 7: Incidence of overall TB for the 3 study periods in children 3 months to 14yrs**

| | **Early-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **Intermediate-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **Established-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **IRR[2]** | **p=[3]** | **%reduction[4]** |
|---|---|---|---|---|---|---|
| **Overall TB** | 275.9[257.9-294.9] (866) | 199.7[189.1-211.0] (1318) | 173.3[163.6-183.4] (1197) | 0.63[0.58-0.69] | <0.0001 | 37.2[31.5-42.4] |
| HIV-infected | 6587.4[6061.3-7144.5] (543) | 4103.8[3826.4-4395.4] (781) | 3084.8[2859.5-3322.7] (674) | 0.47[0.42-0.52] | <0.0001 | 53.2[47.7-58.1] |
| HIV-uninfected | 75.3[65.9-85.6] (230) | 59.4[53.6-65.7] (381) | 68.8[62.6-75.4] (460) | 0.91[0.78-1.07] | 0.2644 | 8.6[-7.1-22.0] |
| HIV-infected EX [5] | 7376.0[6820.8-7961.5] (608) | 4655.6[4360.6-4964.5] (886) | 3254.2[3022.8-3498.0] (711) | 0.44[0.40-0.49] | <0.0001 | 55.9[51.0-60.3] |
| HIV-uninfected EX [6] | 84.4[74.4-95.4] (258) | 67.4[61.2-74.1] (432) | 72.7[66.4-79.4] (486) | 0.86[0.74-1.00] | 0.0513 | 13.9[-0.1-26.0] |

[1] number of cases per 100 000
[2] incidence risk ratios-Established vs Early HAART era
[3] $\chi^2$ test or Fischer test
[4] %reduction between Early and Established HAART era
[5] incidence of TB in HIV-infected extrapolated proportionally to include HIV-unknown
[6] incidence of TB in HIV-uninfected extrapolated proportionally to include HIV-unknown

*3.6 Trends in incidence of probable TB*

A 75% (95%CI 62.8-83.8) reduction was demonstrated for probable TB between the early (20.1 per 100 000) and the established (4.9 per 100 000) HAART era; p<0.0001. There was an 86% (95%CI 75.1-93) reduction in incidence among HIV-infected children from early HAART era (448.9) compared to the established HAART era (59.5); p<0.0001. In addition, a 63% (95%CI 29.9-80.5) reduction was observed in HIV-uninfected children (incidence 6.9 vs 2.5; p=0.015) (Table 8).

**Table 8: Incidence of probable TB for the 3 study periods in children 3 months to 14yrs**

| | **Early-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **Intermediate-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **Established-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **IRR[2]** | **p=[3]** | **%reduction[4]** |
|---|---|---|---|---|---|---|
| **Probable TB** | 20.1[15.4-25.7] (63) | 8.6[6.5-11.2] (57) | 4.9[3.4-6.9] (34) | 0.25[0.16-0.37] | <0.0001 | 75.5[62.8-83.8] |
| HIV-infected | 448.9[316.2-618.2] (37) | 152.4[102.1-218.8] (29) | 59.5[31.7-101.7] (13) | 0.13[0.07-0.25] | <0.0001 | 86.7[75.1-93.0] |
| HIV-uninfected | 6.9[4.3-10.5] (21) | 2.8[1.7-4.4] (18) | 2.5[1.5-4.1] (17) | 0.37[0.20-0.70] | 0.0015 | 63.0[29.9-80.5] |
| HIV-infected EX [5] | 485.3[346.9-660.2] (40) | 189.2[132.5-261.8] (36) | 64.1[35.0-107.5] (14) | 0.13[0.07-24.2] | <0.0001 | 86.8[75.7-92.8] |
| HIV-uninfected EX [6] | 7.5[4.8-15.7] (23) | 3.3[2.0-5.0] (21) | 3.0[1.8-4.6] (20) | 0.40[0.22-0.72] | 0.0018 | 60.3[27.7-78.2] |

[1] number of cases per 100 000
[2] incidence risk ratios-Established vs Early HAART era
[3] $\chi^2$ test or Fischer test
[4] %reduction between Early and Established HAART era
[5] incidence of TB in HIV-infected extrapolated proportionally to include HIV-unknown
[6] incidence of TB in HIV-uninfected extrapolated proportionally to include HIV-unknown

*3.7 Trends in incidence of possible TB*

Possible TB had minimally declined from 579 cases in 2005 to 467 cases in 2009. The incidence of possible TB had not significantly changed when comparing the 2006/7 and 2008/9 periods but did show a decline of 25.0% (95%CI 16.8-32.3) when comparing the early (184.5 per 100 000) and established (138.4 per 100 000) HAART eras; $p<0.0001$ (Table 9).

**Table 9: Incidence of possible TB for the 3 study periods in children 3 months to 14yrs**

| | **Early-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **Intermediate-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **Established-HAART** Incidence of TB[1] [95%CI] (N of TB cases) | **IRR[2]** | **p=[3]** | **%reduction[4]** |
|---|---|---|---|---|---|---|
| **Possible TB** | 184.5[169.8-200.1] (579) | 132.1[123.5-141.2] (872) | 138.4[129.8-147.5] (956) | 0.75[0.68-0.83] | <0.0001 | 25.0[16.8-32.3] |
| HIV-infected | 4537.2[4098.0-5008.9] (374) | 2832.2[2601.1-3077.9] (539) | 2508.1[2304.8-2724.2] (548) | 0.55[0.49-0.63] | <0.0001 | 44.7[37.1-54.4] |
| HIV-uninfected | 49.1[41.5-57.6] (150) | 37.8[33.2-42.8] (242) | 53.4[48.0-59.2] (357) | 1.09[0.90-1.32] | 0.3883 | Nil |
| HIV-infected EX [5] | 5010.3[4549.3-5503.5] (413) | 3158.0[2914.1-3416.7] (601) | 2650.0[2441.1-2871.7] (579) | 0.53[0.47-0.60] | <0.0001 | 47.1[40.1-53.2] |
| HIV-uninfected EX [6] | 54.3[46.4-63.2] (166) | 42.3[37.4-47.6] (271) | 56.4[50.8-62.4] (377) | 1.04[0.86-1.25] | 0.6907 | Nil |

[1] number of cases per 100 000
[2] incidence risk ratios-Established vs Early HAART era
[3] $\chi^2$ test or Fischer test
[4] %reduction between Early and Established HAART era
[5] incidence of TB in HIV-infected extrapolated proportionally to include HIV-unknown
[6] incidence of TB in HIV-uninfected extrapolated proportionally to include HIV-unknown

## 4.0 DISCUSSION

The introduction of HAART into the public health program in a South African setting with a high co-burden of HIV-TB in children has been temporally associated with a drastic decline in hospitalization for culture-confirmed TB among both HIV-infected and less so, HIV-uninfected children. In particular, whilst HAART treatment of HIV-infected children may have accounted for the reduction in emperic initiation of anti-TB or clinical diagnosis of TB in other pediatric studies (16-19), the observation of a decline in microbiologic confirmed TB in our study provides the most definitive evaluation on the impact of up-scaling of the HAART program on childhood TB in a country with a high burden of TB and HIV. In addition, the decline of culture-confirmed TB in young HIV-uninfected children observed in our study, may be due to reduced pressure of TB transmission within the community, possibly resulting from the up-scaling of the HAART program in HIV-infected adults in the same community as demonstrated in a study by Middelkoop et al.(20) in Cape Town between 2005 and 2008 (TB incidence; 6 513 to 4 714 per 100 000).

Although HIV-infected children may also have benefited from a reduced load of infectious cases of TB because of treatment of HIV-infected adults with HAART, the greater magnitude of decline observed in HIV-infected compared to HIV-uninfected children indicates the added benefit of adequately treating these children with HAART. Nevertheless, despite the decline in incidence of TB in HIV-infected children, the burden of culture-confirmed TB in our study remained 40 (95%CI 30.4-53.2) fold greater compared to HIV-uninfected children in 2008-9. In comparison, a study conducted in Cape Town in 2008 by Hesseling et al.(32),

demonstrated the incidence of culture-confirmed TB between 2004 and 2006 in HIV-infected and uninfected infants as 1 596 per 100 000 and 65.9 per 100 000 respectively. Her study reported the relative risk of culture-confirmed TB in HIV-infected as 24.2 (95%CI 17-34) as compared to HIV-uninfected infants.

The magnitude of burden of disease is clearly in children under 2 years of age. The most dramatic decline in incidence was evident in this group in both HIV-infected and HIV-uninfected children. Data available from the PCV study conducted by Moore et al.(33) in Soweto between 1998 and 2005 described the incidence of culture-confirmed TB as 321.4(95%CI 247.5-410.4) in children under 2 years of age over that period. The incidence of culture-confirmed TB in HIV-infected children was reported as 3 183.2 (95%CI 2284.3-4318.4) in children under 2 years of age. Similarly, our data in children under 2 years of age demonstrated an incidence of culture-confirmed TB as 253.2 (95%CI 211.6-300.5) and in HIV-infected of 2 803.7 (95%CI 2200.1-3518.0) per 100 000 in 2005; hence we can appreciate the reduction in the incidence of culture-confirmed TB as the coverage of HAART increased.

When extrapolating the numbers of unknown HIV results proportionally into both HIV-infected or HIV-uninfected groups, we demonstrated a 4% (67 vs 71) greater reduction in the incidence of culture-confirmed TB in the HIV-infected group comparing the early and established HAART eras (Table 5). This highlights that we may have marginally underestimated the effect that HAART has had on the incidence of culture-confirmed TB in HIV-infected children.

As a further analysis, we extrapolated all the unknown HIV results as HIV-uninfected based on the likelihood that these children had no clinical evidence of HIV and were thus not tested. This indicated a reduction of 53.3% (95%CI 37.8-65.0) in HIV-uninfected children with culture-confirmed TB when comparing the early and established HAART era (Table 5). Thus the unadjusted reduction in the HIV-uninfected may have been an underestimate of the true impact of reduced transmission of TB in the community.

Furthermore, our data reflects that of the HIV-infected children hospitalized for culture-confirmed TB, seventy six percent had severe immunosuppression. This compares to a study conducted in Cape Town in 2008 by Hesseling et al.(32) which demonstrated a mean CD4+ of 10.7% in HIV-infected children, corroborating our findings that much of the residual burden of TB as occurring in severely immuncompromised children and highlighting the gaps in the HAART program during our study period.

Most paediatric studies demonstrating the decline in the incidence of TB compared patients who were not on HAART versus those on HAART. The diagnosis of TB was made primarily on clinical diagnosis, rather than microbiologic confirmed cases and may have over-estimated the effect of HAART on the burden of TB in HIV-infected children (16-19). The only study to have reported an association of HAART and reduction in culture-confirmed TB among HIV-infected children was by Martinson et al. (16). His study confirmed TB in 23% of the cases but only showed a reduction in the incidence of TB in this confirmed group of 3.8/100py to 2.7/100py as compared to all cases of TB where the incidence was reduced

from 21.1/100py to 6.4/100py. On the other hand, a prospective study conducted by Frigati et al. (34) from 2003 to 2007 in Cape Town demonstrated that HAART reduced TB risk by 0.32 (95% CI 0.07-1.55) in HIV-infected children. However, this result was statistically not significant.

Identifying TB bacilli by microscopy is uncommon in children and this is evident by the low numbers of cases in our sample population (35). Despite this, we noted a significant reduction in the incidence of only smear-positive cases of TB (20.1 to 4.9 per 100 000; $p<0.0001$). Clinically diagnosed TB had only declined by 25%(95%CI 16.8-32.3) over the time period of the study. This highlights the difficulties clinicians continue to face when diagnosing TB in children and many a time over-diagnosing TB in HIV-infected children.

The number of cases with culture-confirmed extra-pulmonary TB remained fairly consistent over the study period. Evidence suggests that extra-pulmonary TB is not disproportionately increased in HIV-infected children (32,36). In our study, we also noted no significant changes in the cases of extra-pulmonary TB as HAART became more accessible. However, it should be noted that our study was not powered to make this conclusion.

The first limitation to this study was that we used a cohort of only hospitalized patients with TB and thus we may have underestimated the overall burden of TB in this population. However, it should be noted that until 2010 most if not all HIV-infected children in this region were treated at the two HIV in-hospital clinics and thus would be admitted for diagnostic work up and treatment.

A second limitation is that there may have been changes in clinician behavior in terms of referral, admission and investigating children with suspected TB over the time period of the study. The DOTS program, its successes and failures has not been measured. The health departments' surveillance and identification of source cases may have also had an impact. The absence of information on clinical and immune responses to HAART and treatment failures on individual subjects is a further limitation.

A third limitation is that we have not taken into account whether cases received primary or secondary prophylaxis for TB. However, in a recent study conducted by Madhi et al. in 2011 in South Africa, no benefit of primary isoniazid(INH) prophylaxis in children was demonstrated (37).

A fourth limitation was the change over in the laboratory methods used to culture TB; from the radiometric BACTEC™ 460 TB system used from 2004 to 2006 to the BACTEC™ MGIT 960™ TB System used thereafter. The main reason for this change was a move away from radioactive hazard of the radiometric BACTEC™ 460 system. The BACTEC 460 has been shown to be slightly superior to the MGIT in terms of yield as there is less contamination of specimens (38).

Unfortunately, due to the lack of available data on patient outcomes in our database, we were only able to classify the patient outcomes as discharged, demised or unknown. We did attempt to retrieve more detail on the missing outcomes by reviewing the hospital registries and patient files but those later than 5 years were inaccessible; this explains the high number of unknown outcomes in the year 2005.

Another limitation to this study was that we used population estimates as our denominator for this study region (31). We did not account for any influx of children to this region over the study period. However as a separate analysis using the total number of patients hospitalized as a denominator (39), similar declines were shown.

A further limitation was the coverage of HIV-infected children on HAART which was already estimated at 43% in 2005. Ideally we would have liked to analyzed data prior to 2005, when the coverage with HAART was lower. Regardless, a temporal association between increased coverage of HAART and the incidence of culture confirmed TB is apparent.

## 5.0 CONCLUSION

Up-scaling of the HAART program in South Africa has resulted in a dramatic decline in the incidence of culture-confirmed TB in HIV-infected children, as well as possibly indirectly in HIV-uninfected children. The greatest impact was observed in children under two years and this highlights the need for targeting these children with HAART early in the course of their HIV infection. The indirect effect in HIV-uninfected children may have been attributable to a decreased level of TB transmission in the adult population, however the magnitude of burden of disease in the HIV-infected children remained 40 fold greater at the end of our study period. Severely immune-compromised HIV-infected children need to be targeted with antiretroviral treatment in addition to other strategies to further reduce the burden of TB.

## 6.0 RECOMMENDATIONS

This study provides some long-awaited optimistic news in the battle against HIV and TB. Since 2010 the Department of Health has further improved the antiretroviral treatment program in South Africa. These included changes to the PMTCT program as well as starting all infants on treatment regardless of staging of disease. It will be useful to measure the impact this has had in the next 5 years as we continue the fight against this dual pandemic. A further useful study will be to measure the impact that the introduction of the pneumococcal vaccine has had on the burden of TB in this setting (40).

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