

https://dspace.org/

Association between clinical characteristics and TB investigation results in HIV-infected children treated for TB at a government sector paediatric HIV clinic in Soweto, South Africa

Fairlie, Lee

2014
http://hdl.handle.net/10539/15487

Downloaded from a DSpace repository. https://dspace.org/

Table of Contents

i) Cover sheet
ii) Declaration
iii) Co-Author letter
iv) Acknowledgements
v) Title page
vi) Abstract
vii) Introduction
viii) Methods
ix) Results
x) Discussion
xi) References
xii) Appendix
    A- Letter from the editor IJTLD
    B- Article reprint
    C- Ethics clearance certificate

Association between Clinical Characteristics and TB investigation Results in HIV-infected Children Treated for TB at a Government Sector Paediatric HIV Clinic in Soweto, South Africa

Accepted for publication by International Journal of Tuberculosis and Lung Disease on 24 January 2014 as:

"Microbiological Investigation for tuberculosis among HIV-Infected Children in Soweto South Africa"

Lee Fairlie

0217743F

February 2014

A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the Masters of Medicine in Paediatrics (MMED)

ii) Declaration:

I Lee Fairlie declare that this research report is my own work being submitted to complete the MMED Paediatrics Degree at the University of the Witwatersrand, Johannesburg. It has not previously been submitted for examination purposes at this or any other university. I completed my FCPaeds (SA) qualification as a Specialist Paediatrician at the University of the Witwatersrand in 2005.

Signed 

Date 15/5/2014

iii) Letter by co-authors

3 February 2014

Post Graduate Office
University of the Witwatersrand

Re: Permission from Co-authors to use accepted publication for MMED submission

Dear Sir/Madam

This letter serves to confirm that we give permission for Dr Lee Fairlie, Student number 0217743F to use the publication titled "Microbiological Investigation for Tuberculosis in HIV-Infected Children in Soweto, South Africa" for her MMED Research Report, registered as "Association between Clinical Characteristics and TB investigation Results in HIV-infected Children Treated for TB at a Government Sector Clinic Paediatric HIV Clinic in Soweto, South Africa".

Dr Fairlie is the lead author and has done the majority of work in the following areas: 1) Formulation of the concept phase of the study – including literature review, definition of study aim, protocol submission, planning of data collection strategies. 2) Collection and collation of data for the study / article 3) Interpretation of results and 4) Writing of the journal article.

The signatures below confirm that permission has been granted.

Dr Harry Moultrie (supervisor) _____ Date 03/02/2014

Dr Tammy Meyers (supervisor) Meyers Date 4/2/2014

Mr Evans Muchiri _____ Date 04/02/2014

Dr Natalie Beylis _____ Date 03/02/2014

Please contact Dr Fairlie should any further information be required.

iv) Acknowledgements

I would like to acknowledge the children and their families attending HSCC whose data was used in this study.

I would like to thank my Supervisors Dr Harry Moultrie and Dr Tammy Meyers for their support, mentorship and guidance during the completion of this manuscript.

v) Title Page

Title: Microbiological Investigation for tuberculosis among Human Immunodeficiency Virus-Infected Children in Soweto South Africa

Authors: Fairlie L [1]; Muchiri E[1]; Beylis CN [2]; Meyers T [3]; Moultrie H[1]

Institutions:

1) Wits Reproductive Health and HIV Institute (WRHI), Faculty of Health Sciences, University of the Witwatersrand, South Africa
2) National Health Laboratory Service (NHLS), Mycobacteriology Referral Laboratory & Department of Clinical Microbiology & Infectious Diseases, University of Witwatersrand, South Africa
3) Harriet Shezi Children's Clinic, Chris Hani Baragwanath Hospital, Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand, South Africa

Corresponding author:

Dr Lee Fairlie

Mailing address:

WRHI

Corner Esselen and Klein Streets

Hillbrow

2001

South Africa

Tel: +27 82 780 9997

Fax: +27 11 358 5400

Email: lfairlie@wrhi.ac.za

Author Contributions:

LF, TM and HM conceived of the study. LF designed the study, collected data, drafted the manuscript and coordinated the revisions to the manuscript. NCB collected data and participated in study design and drafting of manuscript. EM and HM performed the statistical analysis and contributed to drafting of the manuscript. All authors read and approved the final manuscript.

Competing Interests and Funding

The authors have no funding or competing interests to declare.

vi) Abstract

Setting

Paediatric HIV Clinic, Harriet Shezi Children's Clinic in an academic hospital, Chris Hani Baragwanath Hospital, Soweto, South Africa.

OBJECTIVE

To describe and compare clinical, immunological and virological characteristics of HIV-infected children co-treated for TB, comparing those investigated microbiologically and those not, with a detailed description of microbiological TB investigation results.

Design

Retrospective cross sectional analysis of HIV-infected children treated for TB aged <15 years between 1 October 2007 and 15 March 2009.

Results

TB treatment was initiated in 616/3358(18%) children attending Harriet Shezi Children's Clinic during the study period. Microbiological TB investigation results were available in 399/616(65%) of children started on TB treatment, with culture-confirmed TB diagnosed in 49/399(12%). Drug susceptibility testing was performed in 29/49(59%), with 5/29(17%) children having isoniazid-resistance, 3 with MDR-TB. Children > 8 years and between 3-8 years were more likely to have culture-confirmed TB compared to < 3 years (AOR 9.4; 95% CI 2.26 – 39.08 and AOR 6.7; 95% CI 1.60 – 27.69 respectively) as were those with CD4 count < 200 cells/mm^3 compared to > 500 cells/mm^3 (AOR 3.95; 95%CI 1.23 – 12.72).

Conclusion

Our study in HIV-infected children showed a high TB case rate, a low rate of definite TB and a high rate of drug-resistant TB according to WHO case definitions. Increased uptake of available TB tests and the availability of new, cheap and easily implementable diagnostic tests for TB remains a priority in high TB/HIV burden settings.

vii) Introduction

Globally, 8.7 million people were newly diagnosed with tuberculosis in 2011, and 6% of the incident cases were estimated to occur in children less than 15 years of age.[1] An estimated 34.0 million [31.6–35.2 million] people in the world were HIV-infected by 2010, of these 3.4 million [3 000 000–3 800 000] were children under the age of 15 years. The paediatric HIV epidemic is largely restricted to lower resource environments, with more than 90% of HIV-infected children living in Sub-Saharan Africa.[2] Tuberculosis (TB) is the most common opportunistic infection in Human Immunodeficiency Virus (HIV)-infected children, and between 24-44% of HIV-infected South African children under 24 months of age attending HIV clinics are on TB treatment at the time of starting combination antiretroviral therapy (cART).[3,4] A Ugandan study reported that 17 % of children and adolescents less than 18 years of age were on TB treatment at the time of starting cART.[5]

In South Africa an estimated 460,000 (410,000-520,000) children are HIV-infected[6], the TB incidence rate is 993/100,000 population [1] and up to 52% of children with culture-confirmed TB are HIV-infected.[7] HIV and TB co-infected children experience high rates of morbidity and mortality, particularly in those who are severely immunocompromised and not yet receiving cART.[8-11] In HIV-infected children the risk of TB disease is up to 4 times greater in those with a CD4 percentage < 15%. [9] A Cape Town based study showed that while INH and ART independently reduced TB risk by 0.22 and 0.32 respectively in HIV-infected children, the combination of INH and ART reduced the risk by 0.11, and in children on ART, INH reduced the risk of TB disease by 0.23. [12] A multi-center study in young children, started on ART early and with only 8% having CDC class B or C disease, INH isoniazid as pre-exposure prophylaxis did not improve disease-free survival among HIV-infected children.[8]

Diagnosing TB in HIV-infected children remains difficult. World Health Organization (WHO) case definitions for TB disease include clinical features and microbiological investigation results, with radiological features more important in cases with negative microbiological results.[13] Case definitions include "Tuberculosis suspect"; "Case of Tuberculosis" and "Definite case of Tuberculosis" which are all described in detail in the "Methods" section. Cases of TB are classified according to the anatomical site of disease; bacteriological results including drug resistance, history of previous TB treatment and the HIV status of the patient.[13] Potential for both over- and under-diagnosing TB in HIV-infected children is high with overlapping clinical features between both diseases further complicated by additional co-existing comorbidities common in this population.[14] There are a number of point scoring systems, diagnostic classifications and diagnostic algorithms developed for use in resource-limited settings, however correlation between these tools is poor to moderate, and wide variability in the frequency of TB diagnosed based on these diagnostic tools, may under-or overestimate the TB burden in children by as much as 82%.[14, 15] Point scoring systems have a low specificity in HIV-infected children, given the overlap of symptoms such as lymphadenopathy and malnutrition and may lead to over-diagnosis of TB in HIV-infected children.[14, 16]In children tuberculin skin test (TST) and chest x-ray (CXR) may have greater diagnostic importance than in adults.[17, 18] However, TST which cannot distinguish TB infection from TB disease may frequently be negative in immunocompromised and malnourished individuals.[19] Wide variability in CXR interpretation between experienced clinicians and poor correlation with culture-confirmed TB suggests CXR is an unreliable adjunct to TB diagnosis in children.[15, 20]

Microbiological confirmation of TB remains the most accurate method of diagnosis, and two Cape Town based studies report successful microbiological TB investigation through gastric washing and induced sputum procedures in primary health clinics.[21, 22] In children Xpert MTB/Rif on 2 specimens detects twice the number of MTB cases compared to smear microscopy and up to 70% of culture positive specimens.[23, 24] Researchers currently recommend

two Xpert MTB/Rif specimens as the first TB investigations in children.[23, 24] Microbiological confirmation of TB either through microscopy, culture and sensitivity testing or through Xpert MTB/Rif requires good quality respiratory specimens and these tests are not performed routinely in most clinical settings because of limited staff capacity, skill shortage, low yield and difficulty in conducting them in small children. The performance of newer diagnostic tests such as commercially approved Interferon Gamma Release Assays (IGRAs) are differentially affected by chronic malnutrition, HIV infection and age and do not differentiate TB infection from disease. Caution in interpreting results of IGRAs in these populations together with expense, limits use in resource limited settings with high HIV prevalence.[25]

Co-treatment with TB therapy and cART in HIV-infected children remains complicated making accurate TB diagnosis important. The drug-drug interactions between rifampicin-based TB treatment and cART, particularly lopinavir/ritonavir which is inadequately boosted may result in reduced virological suppression rates.[4, 26] Polypharmacy with TB treatment and cART may affect adherence and increases the possibility of both ART-associated and TB drug-associated side effects.

Data describing the clinical, immunological and virological associations with TB diagnosis in HIV-infected children are scarce as are reports regarding microbiological TB investigation results. This retrospective cross sectional review sought to describe and compare clinical, immunological and virological characteristics of HIV-infected children co-treated for TB, distinguished by whether microbiological TB investigations were conducted or not, and to describe results of microbiological TB investigations.

viii) Methods

Study design

We conducted a retrospective cross sectional review of microbiological TB investigations performed in HIV-infected children attending Harriet Shezi Children's Clinic (HSCC), who were treated for TB, in Soweto, South Africa between 1 October 2008 and 15 March 2009.

Subjects

HIV-infected children < 15 years of age treated for TB.

Standard of care

The HSCC is a public outpatient paediatric HIV clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, South Africa. HIV diagnosis was according to South African National Department of Health (SANDoH) guidelines with HIV DNA PCR testing in children less than 18 months of age and an HIV ELISA test in those over 18 months of age.[27] TB screening was conducted at initial and subsequent clinic visits using symptomatic enquiry and clinical assessment, with further investigations in those with suspected TB. WHO case definitions were applied including 'TB suspects': defined as those presenting with signs and symptoms suggestive of TB; 'TB case' defined as a definite TB case or where a health care worker has diagnosed TB and has initiated a full course of TB treatment; and 'Definite case' of TB as a confirmed diagnosis of Mycobacterium tuberculosis through culture or a newer method such as molecular line probe assay or in countries where this is not possible, two positive specimens for AFB.[13] Investigations included CXR, TST and microbiological investigations. All microbiological specimens were obtained from the respiratory tract and extrapulmonary sites including blood, pleural fluid and cerebrospinal fluid (CSF) according to clinical indication. Children diagnosed with TB were prescribed a standard 3 drug regimen of rifampicin, isoniazid (INH) and pyrazinamide with ethambutol/ethionamide added as a fourth drug for complicated TB including TB meningitis or disseminated TB.[28]

During the study period, children were initiated on cART if they were symptomatic for HIV according to the following criteria: < 1 year of age with WHO stage II-IV, CD4 count ≤ 1500 cells/mm^3 or 35%; 1-5 years WHO stage III or IV, CD4 count ≤ to 20%; > 5 years WHO stage III or IV, CD4 count ≤ to 200 cells/mm^3 or 15%.[27] Guidelines for TB/HIV co-treatment during the study period recommended starting cART two months after TB initiation or completing TB treatment before starting cART if possible in stable children.[27] Children under 3 years of age received a protease inhibitor-based regimen and children over 3 years an efavirenz-based regimen. At the time of the study stavudine and lamivudine were used as the nucleoside reverse transcriptase inhibitor (NRTI) backbone.

Laboratory investigations

Microbiological investigations for TB included smear microscopy, culture and drug susceptibility testing (DST). Specimens were processed according to WHO guidelines using the NALC-NaOH decontamination process; a 0.5ml portion of the processed, centrifuged pellet was inoculated into MGIT™ (Mycobacterial Growth Indicator Tube) (Becton Dickinson, Maryland, USA) tubes and incubated for a total of 6 weeks. Growth detected in automated MGIT instruments was confirmed as being acid fast bacillus (AFB)-positive by Ziehl Neelsen stained smear. Confirmation of *Mycobacterium tuberculosis* complex (MTBC) was by the GenoType® Mycobacterium CM assay (Hain Lifesciences, Nehren, Germany). DST was only performed on clinician request, guided by the National TB Control Programme Guidelines and was not routine during the study period.[28] Isolates of MTBC were initially tested against INH and rifampicin using the MGIT™960 proportion method (Becton Dickinson, Maryland, USA). If resistance was found against either/ both drugs, the isolate would undergo further DST against streptomycin, ethambutol, ofloxacin, kanamycin and ethionamide using the MGIT proportion method. Definitions for drug-susceptible and drug-resistant TB were as follows: susceptible MTB: isolate susceptible to both INH and rifampicin; INH mono-resistant and rifampicin mono-resistant if resistant to only INH or rifampicin respectively; multi-drug-resistant (MDR)-TB if resistant to both INH and rifampicin and extensively drug-resistant (XDR)-TB if MDR-TB that was also resistant to ofloxacin and kanamycin.

Data Collection

Demographic, clinical and laboratory data at the time of TB treatment initiation, with a 3 month window period, were extracted from three databases. Demographic and HIV related data from the HSCC clinical database; microbiological TB data from the National Health Laboratory Service (NHLS) database; and TB treatment data from the CHBAH TB Care Centre (TBCC) ETR.net database, a SANDoH TB data system. The TBCC and HSCC databases were merged using the hospital unique patient identifier to include only children treated for TB. Microbiological MTB investigation results for children included in the merged dataset of children attending HSCC treated for TB were then imported from the NHLS Mycobacteriology Referral Laboratory dataset. The final data set included all children treated for TB, regardless of whether or not they were investigated microbiologically for TB. Manual file reviews and NHLS database searches were conducted by study staff for missing values.

Data extracted included age (calculated from date of birth), weight for age z-scores (WAZ-scores), height for age z-scores (HAZ-scores), WHO clinical staging, HIV $\log_{10}$ viral load and CD4 count. WAZ-scores and HAZ-scores were generated using WHO reference ranges. Characteristics for children at TB treatment initiation were stratified according to whether children were investigated microbiologically for TB and according to TB investigation results.

Statistical analysis

Statistical analysis and data management were carried out in STATA version 12.0 (STATA Corporation, College Station, TX). Medians and interquartile ranges (IQR) were used to summarize the data. Chi-square tests were used to explore associations between grouped categorical variables of age, anthropometrical measures, CD4 count and percentage, HIV $\log_{10}$ viral load and the outcome of TB investigations. Age was categorized as of < 3, ≥ 3 to $<8$and ≥ 8 years in keeping with differential TB disease risk. [17] The Wilcoxon rank sum test and the chi-squared test were used to compare continuous variables and categorical variables respectively. Univariable and multivariable logistic models were fitted to identify factors associated with a positive TB investigation outcome among HIV-infected children investigated

microbiologically for TB. Multivariate models were developed using step-wise backward elimination using likelihood ratio tests to identify the model with the best fit.

Ethics approval

This study was approved by the University of the Witwatersrand Human Sciences Research Ethics Committee certificate number M110743.

ix) Results

Of the 3358 HIV-infected children who attended the HSCC at least once during the study period, 616 (18%) were co-treated for TB and were included in the analysis. (Figure 1) One hundred and twenty nine children were receiving cART at the time (Table 1). At the initiation of TB treatment the median age was 4.2 years with an interquartile range (IQR) of 1.1 to 8.4 years, 67% of children had WHO clinical stage IV disease, median WAZ-score -2.0 (IQR -1.1 to -3.3) and HAZ-score -2.4 (IQR -1.5 to -3.3). The median CD4 count was 425 cells/mm^3 (IQR 180– 847), median CD4 percentage 14.4% (IQR 8.4 – 21%) and the median log_{10} HIV viral load 4.6 copies/ml (IQR 3.5 – 5.3). A higher than expected proportion amongst those investigated microbiologically for TB were male (p=0.01). There were no other clinically or statistically significant differences in characteristics of children who were and were not investigated microbiologically (Table 1).

Figure 1: Description of HIV-infected children attending HSCC between 1 October 2007 and 15 March 2009 stratified by TB treatment and microbiological investigation

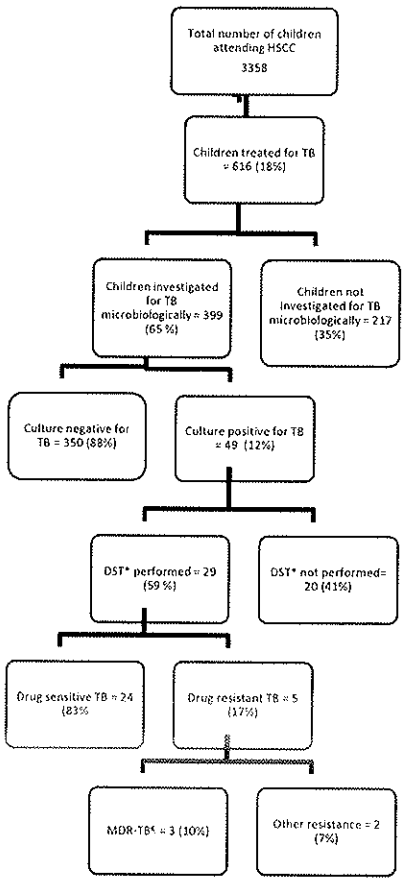


Table 1: Characteristics of children attending the HSCC treated for TB between 1 October 2007 and 15 March 2009

	All children treated for TB (n=616)	Not investigated microbiologically for TB (n=217)	Investigated microbiologically for TB (n=399)	p-value
Male (n, %)	339(55%)	105(48%)	234(58%)	0.01
Age in years (median, IQR)	4.2 (1.1 – 8.4)	4.2 (1.2 – 8.6)	4.2 (1.0 – 8.3)	0.23
CD4% (median, IQR)	14.4 (8.4 – 21.0)	15.2 (10.0 – 22.0)	13.8 (7.9 – 20.3)	0.07
CD4 (median, IQR)	425 (180 – 847)	389 (164 – 747)	445 (200 – 859)	0.33
Log_{10} HIV VL copies/ml (median, IQR)	4.6 (3.5 – 5.3)	4.6 (3.2 – 5.1)	4.6 (3.7 – 5.4)	0.21
WHO stage 4 (%)	67	65	68	0.42
Weight for Age Z-scores (median, IQR),n	-2.0 (-1.1 – -3.3), n=440	--1.9 (-0.9 – -3.2), n=146	-2.1 (-1.1 – -3.4), n=294	0.25
Height for Age Z-scores (median, IQR),n	-2.4 (-1.5 – -3.3), n=521	-2.4 (-1.5 – -3.1), n=176	-2.4 (-1.5 – -3.3), n=345	0.43
Receiving cART	129 (21%)	50 (23%)	79 (20%)	0.35

IQR= Interquartile range

Microbiological investigation was conducted in 399(65%) of the children co-treated for TB (Figure 1). Twenty five children (6%) had specimens which stained positive with the Ziehl Neelsen stain (+AFB). TB was confirmed in 49(12%) children by mycobacterial culture. The median age of children with culture-confirmed TB was 8.2 years (IQR 5.7 – 10.1) compared to 3.3 years (IQR 0.9 – 7.9) in those investigated microbiologically with negative TB culture results (p=<0.001) (Table 2). Children with culture-confirmed TB (definite TB) had a median CD4 count of 157 cells/mm^3(IQR 41 - 299 cells/mm^3) and CD4 percentage of 10% (IQR 3 – 19%) compared to 491 cells/mm^3 (IQR 239 – 924 cells/mm^3) and 14% (IQR 8 – 20%) in children with negative TB culture results (p=0.02 and <0.001 respectively) (Table 2). DST was performed in 29(59%) children with culture-confirmed TB. Drug resistant TB was diagnosed in 5(17%) children, of which 3 were MDR-TB. All three MDR-TB strains were resistant to ethambutol and streptomycin and a third child's MTB isolate was also resistant to ethionamide. Two children were infected with INH mono-resistant MTB. (Figure 1)

Table 2: Characteristics of HIV-infected children co-treated for TB at HSCC from 1 October 2007- 15 March 2009 stratified by microbiological TB investigation results

	TB confirmed on culture (n=49)	Negative TB culture (n=350)	p-value
Male (n, %)	25(51%)	209(60%)	0.25
Age in years (median, IQR)	8.2 (5.7 – 10.1)	3.3 (0.9 – 7.9)	<0.001
CD4% (median, IQR)	9.8 (3.0 – 18.7)	14.4 (8.4 – 20.5)	0.02
CD4 (median, IQR)	157 (41 – 299)	491 (239 – 924)	<0.001
Log_{10} HIV VL copies/ml (median, IQR)	4.6 (3.4 – 5.2)	4.7 (3.7 – 5.4)	0.35
WHO stage 4 (%)	76%	67%	0.22
Weight for Age Z-scores (median, IQR)	-2.1 (-1.2 - -3.3), n=30	-2.1(-1.1 - -3.4), n=264	0.85
Height for Age Z-scores (median, IQR)n	-2.4(-1.4 - -3.2), n=40	-2.5 (-1.5 - -3.3), n=305	0.48

IQR= Interquartile range

Multivariate logistic regression confirmed that confirmation of TB on culture was independently associated with both older age and a lower CD4 count after adjusting for sex, WAZ and HAZ. Children > 8 years and children between 3-8 years were more likely to have culture-confirmed TB compared to those < 3 years (AOR 9.4; 95% CI 2.26 – 39.08 and AOR 6.7; 95% CI 1.60 – 27.69 respectively). (Table 3) Children with a CD4 count < 200 cells/mm^3 had increased odds of culture-confirmed TB compared to those with a CD4 count > 500 cells/mm^3 (AOR 3.95; 95%CI 1.23 – 12.72). (Table 3)

Table 3: Logistic regression models for factors associated with TB confirmation in HIV-infected children co-treated for TB

Characteristic at start of TB treatment *		Unadjusted Odds ratio, 95% CI	Unadjusted p-value	Adjusted Odds Ratio, 95% CI	Adjusted p-values
Sex					
	Male	1		1	
	Female	1.42 (0.72-2.21)	0.25	1.89 (0.70 – 5.10)	0.21
Age					
	<3 years	1		1	
	3-8 years	4.01 (1.49 – 10.79)	<0.001	6.65 (1.60 – 27.69)	0.009
	>8 years	9.39 (3.75 – 23.47)	<0.001	9.40 (2.26 – 39.08)	0.002
CD4 count					
	>=500	1		1	
	200-500	2.69 (1.02 – 7.10)	0.04	0.65 (0.16 – 2.54)	0.53
	<200	6.81 (2.75 – 16.8)	<0.001	3.95 (1.23 – 12.72)	0.02
CD4 percentage					
	>=25	1			
	<25	1.35 (0.46-4.00)	0.59		
Viral load					
	<400	1			
	>=400	0.79 (0.33 - 1.90)	0.60		
WHO Stage					
	Stage 3	1			
	Stage 4	1.51 (0.75– 3.00)	0.24		
On cART at start of TB treatment					
	Yes	1			
	No	0.95 (0.45-2.01)	0.91		
Weight for age Z-score		1.04 (0.84 – 1.29)	0.69	0.62 (0.38-1.02)	0.06
Height for age Z-scores		1.12 (0.93 – 1.35)	0.25	1.78 (1.09 – 2.91)	0.02

*242/399 children investigated for TB microbiologically were included in this model as they had all available data.

x) Discussion

Our study showed a high TB case rate (18%) in HIV-infected children attending a public HIV treatment clinic, with a low microbiological TB investigation rate and low rate of definite TB, confirmed by culture or AFBs in those investigated microbiologically. Similar high TB/HIV co-treatment rates have been described in other Sub-Saharan settings. In Uganda, 17% of HIV-infected children (median age 6.6 years) were co-treated for TB.[5] In a study from four South African hospitals in Johannesburg and Cape Town, 21% of HIV-infected children, (median age 6.3 years) were co-treated for TB. In 23% of this cohort of children a definite TB diagnosis was made (AFB smear *n* = 30; TB culture *n* = 24; or biopsy *n* = 11) although the number with culture-confirmed TB was not described.[29] In South African HIV-infected children who are less than 2 years, TB case rates of 40% by 1 year of follow up on cART have been described.[3] Although only 12% of TB cases in our cohort had culture-confirmed TB, (definite TB cases) of concern was that only 65% of children classified as a TB case were investigated microbiologically. Interestingly, apart from male gender having increased TB microbiological investigations, there was no difference in clinical or immunological characteristics between children for whom microbiological TB investigation results were conducted and those not. Girls were 42% more likely to have TB confirmation than boys. In our study, children investigated microbiologically with culture-confirmed TB were older and more immunologically compromised than those investigated with negative TB culture results. The correlation between immunological suppression and confirmed TB in children is likely due to a number of factors: although we

don't have data regarding source of specimen, 80% of TB specimens processed at NHLS are respiratory in origin and the same is likely in this study. Older children are more likely to expectorate and produce adequate sputum samples. We also expect declining immune function with advancing age and disease progression in HIV-infected children. Adult studies however, have shown lower rates of microbiological TB diagnosis with severe immunodeficiency.[30]

We also found that children who had culture-confirmed TB and underwent DST had high rates of INH-resistant TB (17%) with 10% of children having MDR-TB. These findings are consistent with studies in Cape Town and Johannesburg showing INH-resistant TB in up to 15 % and MDR-TB in 9% of children with culture-confirmed TB.[7, 31] Given these findings it is concerning that more children were not investigated microbiologically for TB prior to commencing TB treatment. TB culture is labour-intensive and time-consuming leading to long turnaround times for results and low sensitivity in children[19] and recent WHO policy updates recommend the use of Xpert MTB/RIF rather than conventional microscopy, culture and DST as the initial diagnostic test in children presumed to have MDR-TB or HIV-associated TB but as yet rollout has been limited in routine clinic settings.[32]Xpert MTB/Rif rely on respiratory specimen collection, which remains the barrier to microbiological investigation in children. Two Cape Town-based studies have shown that it is feasible for gastric washings and induced sputum for microbiological TB testing to be performed in a primary clinic setting and that 2 consecutive day gastric washings may have a similar diagnostic yield to induced sputum, this technique being particularly useful in infants and young children.[21,22] Ongoing training of health care workers in specimen collection techniques is required to ensure that these rapid technologies are increasingly utilized in community settings.

This study has some limitations. Data were collected retrospectively and were dependent on accurate recording by the attending clinician and capture by data capturers. TB treatment data was not accurately collected in the HSCC database, therefore, only children registered for TB treatment at the CHBAH TBCC were included which may have excluded children initiated on TB treatment outside of CHBAH with a possible underestimate in TB cases. Patient name or identifying numbers being incorrectly recorded between the data sets may have resulted in some children being investigated for TB microbiologically but not linked in the NHLS dataset. We were unable to break down the source of microbiological specimens due to data extraction methods at the time but as 80% of TB specimens received by NHLS are pulmonary specimens we assume the same holds for this study. Due to the large cohort, and quality of data within files regarding CXR and TST data was not confirmed in a file review. Studies have found poor correlation between culture-confirmed TB and CXR findings and wide variability in interpretation between clinicians.[20] We did not have sufficiently accurate data to describe TB suspects not treated for TB. As children who entered this cohort were HIV-infected survivors, there is potential for survival bias with left censored data.

Conclusions

Our study showed a high TB case rate in HIV-infected children with a low rate of microbiological TB investigation and microbiological confirmation. Of concern, among those investigated microbiologically, a high rate of drug resistant TB was found. These findings suggest that efforts to increase the use of currently available tests should be encouraged through staff education and training. There is also a need for new simple, cheap, rapid and reliable TB tests to become available particularly in resource limited settings, with a high endemic TB burden.

xi) References

1. World Health Organization. Global Report Tuberculosis 2012; 2012.

2. World Health Organization. Global HIV/AIDS Response. Epidemic update and health sector progress towards universal access. http://wwwunaidsorg/en/media/unaids/contentassets/documents/unaidspublication/2011/20111130_ua_report_enpdf. 2011.

3. Reitz C, Ashraf Coovadia, Ko S, Meyers T, Strehlau R, Sherman G, et al. Initial Response to Protease-Inhibitor-Based Antiretroviral Therapy among Children Less than 2 Years of Age in South Africa: Effect of Cotreatment for Tuberculosis. The Journal of Infectious Diseases. 2010; **201**: 1121-31.

4. Frohoff C, Moodley M, Fairlie L, Coovadia A, Moultrie H, Kuhn L, et al. Treatment outcomes among HIV-infected infants and young children following modifications to protease inhibitor-based therapy due to tuberculosis treatment. PLoS One. 2011; **6**(2).

5. Bakeera-Kitaka S, Conesa-Botella A, Dhabangi A, Maganda A, Kekitiinwa A, Colebunders R, et al. Tuberculosis in human immunodefi ciency virus infected Ugandan children starting on antiretroviral therapy. Int J Tuberc Lung Dis. 2011; **15**(8): 1082-6.

6. UNAIDS. HIV and AIDS Estimates 2011. http://wwwunaidsorg/en/regionscountries/countries/southafrica/. 2011.

7. Fairlie L, Beylis NC, Reubenson G, Moore DP, Madhi SA. High prevalence of childhood multi-drug resistant tuberculosis in Johannesburg, South Africa: a cross sectional study. BMC Infectious Diseases. 2011; **11**(28): 1.

8. Madhi SA, Huebner RE, Doedens L, Aduc T, Wesley D, Cooper PA. HIV-1 co-infection in children hospitalised with tuberculosis in South Africa. Int J Tuberc Lung Dis. 2000; **4**(5): 448-54.

9. Elenga N, Kouakoussui KA, Bonard D, Fassinou P, Anaky M-F, Wemin M-L, et al. Diagnosed Tuberculosis During the Follow-up of a Cohort of Human Immunodeficiency Virus-

Infected Children in Abidjan, Co^te d'Ivoire ANRS 1278 Study. The Pediatric Infectious Disease Journal. 2005; **24**(12): 1077.

10. Hesseling A, Westra A, Werschkull H, Donald P, Beyers N, Hussey G, et al. Outcome of HIV infected children with culture confirmed tuberculosis. Arch Dis Child 2005; **90**.

11. Mukadi YD, Wiktor SZ, Coulibaly I-M, Coulibaly D, Mbenguell A, Folquetll AM, et al. Impact of HIV infection on the development, clinical presentation, and outcome of tuberculosis among children in Abidjan, Côte d'Ivoire. AIDS. 1997; **11**: 1151-8.

12. Frigati LJ, Kranzer K, Cotton MF, Schaaf HS, Lombard CJ, Zar HJ. The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis in children infected with HIV in a high tuberculosis incidence setting. Thorax. 2011; **66**(6).

13. World Health Organization. Treatment of tuberculosis Guidelines Fourth edition. http://WHO/HTM/TB/2009420. 2009.

14. Edwards DJ, Kitetele F, Rie AV. Agreement between clinical scoring systems used for the diagnosis of pediatric tuberculosis in the HIV era. Int J Tuberc Lung Dis 2007; **11**(3): 263-9.

15. Hatherill M, Hanslo M, Hawkridge T, Little F, Lesley Workman, Mahomed H, et al. Structured approaches for the screening and diagnosis of childhood tuberculosis in a high prevalence region of South Africa. Bull World Health Organ. 2010; **88**: 312-20.

16. van Rheenen P. The use of the paediatric tuberculosis score chart in an HIV-endemic area. Tropical Medicine and International Health. 2002; 7 (5): 435-41.

17. B. J. Marais, S. M. Graham, M. F. Cotton, N. Beyers. Diagnostic and Management Challenges for Childhood Tuberculosis in the Era of HIV. The Journal of Infectious Diseases. 2007; **196**: S76-85.

18. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Starke JJ, Enarson DA, Donald PR, Beyers N. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis. 2004 Apr;8(4):392.

19 Eamranond P, Jaramillo E. Tuberculosis in children: reassessing the need for improved diagnosis in global control strategies. Int J Tuberculosis and Lung Disease. 2001; **5**(7): 594-603.

20. Sawry S, Moultrie H, Mahomed N, Rie AV. Diagnostic performance of CXRs for the diagnosis of TB in children initiating HAART. Abstract #175. 12-15 June 2012; Durban, South Africa. www.tbconference.co.za .

21. Hatherill M, Hawkridge T, Zar HJ, Whitelaw A, Tameris M, Workman L, et al. Induced sputum or gastric lavage for community based diagnosis of childhood pulmonary tuberculosis? Arch Dis Child. 2009; **94**: 195-201.

22. Moore HA, Apolles P, Villiers PJTd, Zar HJ. Sputum induction for microbiological diagnosis of childhood pulmonary tuberculosis in a community setting. Int J Tuberc Lung Dis 2011; **15**(9): 1185-90.

23. Nicol MP, Workman L, Isaacs W, Munro J, Black F, Eley B, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. Lancet Infect Dis. 2011; **11**(11): 819-24.

24. Sekadde MP, Wobudeya E, Joloba ML, Ssengooba W, Kisembo H, Bakeera-Kitaka S, et al. Evaluation of the Xpert MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis in Uganda: a cross-sectional diagnostic study. BMC Infectious Diseases. 2013; **13**: 133.

25. Mandalakas AM, Wyk Sv, Kirchner HL, Walzl G, Cotton M, Rabie H, et al. Detecting Tuberculosis Infection in HIV-Infected Children: A Study of Diagnostic Accuracy, Confounding and Interaction. The Pediatric Infectious Disease Journal. 2013; **32**(3): e111-8.

26. McIlleron H, Ren Y, Nuttall J, Fairlie L, Rabie H, Cotton M, et al. Lopinavir exposure is insufficient in children given double doses of lopinavir/ritonavir during rifampicin-based treatment for tuberculosis. Antiviral Therapy. 2011; **16**.

27. South African National Department of Health. Guidelines for the Management of HIV-infected children. 2008.

28. South African National Department of Health. South African National Tuberculosis Guidelines. 2008.

29. Martinson NA, H. Moultrie, Niekerk Rv, Barry G, A. Coovadia, Cotton M, et al. HAART and risk of tuberculosis in HIV-infected South African children: a multi-site retrospective cohort. Int J Tuberc Lung Dis. 2009; **13**(7): 862-7.

30. Kenyon TA, Creek T, Laserson K, Makhoa M, Chimidza N, Mwasekaga M, et al. Risk factors for transmission of Mycobacterium tuberculosis from HIV-infected tuberculosis patients, Botswana. Int J Tuberc Lung Dis 2002; **6**(10): 843-50.

31. Seddon JA, Hesseling AC, Marais BJ, Jordaan A, Victor T, Schaaf HS. The evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa. Int J Tuberc Lung Dis. 2012; **16**(7): 928-33.

32. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosisand rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update.http://wwwstoptborg/wg/gli/assets/documents/WHO%20Policy%20Statement%20on%20Xpert%20MTB-RIF%202013%20pre%20publication%2022102013pdf. 2013.

xii) Appendices

A- Letter from the editor International Journal Tuberculosis and Lung Disease

B- Article reprint

C- Ethics clearance certificate

Appendix A: Letter from the editor

Lee Fairlie

From: onbehalfof+steve.graham+rch.org.au@manuscriptcentral.com on behalf of steve.graham@rch.org.au
Sent: 25 January 2014 12:24 AM
To: Lee Fairlie
Cc: steve.graham@rch.org.au; ijtld@theunion.org; mborgdorff@theunion.org
Subject: IJTLD - Decision on Manuscript ID IJTLD-11-13-0839.R1

24-Jan-2014

Dear Dr. Lee Fairlie

Thank you for sending us the revised version of your article entitled

"Microbiological Investigation for tuberculosis among HIV-Infected Children in Soweto South Africa" (Original Article)

and your reply to the reviewers' comments.

I am pleased to inform you that your manuscript has been accepted for publication in one of the forthcoming issues of the IJTLD, subject to the usual editorial revisions.

Your article will be checked before being prepared for publication, and you will be contacted by the Editorial Office if there are any elements missing.

If you have any queries, please contact the Editorial Office at ijtld@theunion.org. Remember to include the IJTLD number of your accepted manuscript in the subject line of your e-mail.

With kind regards,

Sincerely,

Dr. Stephen Graham
Associate Editor
IJTLD

Please note:
1. On acceptance, an electronic copyright form must be supplied by each listed author. All authors will now be contacted so that they can log on and complete the form on Manuscript Central. No article can be published until a form has been submitted by each author.
2. Articles must comply in terms of length with journal requirements for their submission category, otherwise authors may be asked to make modifications before their article can be published. Authors whose articles are over length (e.g., over 6 pp for original articles) will incur charges of 200€/page.

The IJTLD Instructions to Authors can be viewed on the ScholarOne Manuscripts website (http://mc.manuscriptcentral.com/ijtld) or on the Union website (http://www.theunion.org).

This inbound email has been scanned by the IS Mail Control service.
For more information please visit http://www.is.co.za

Appendix B: Article reprint

Title: Microbiological Investigation for tuberculosis among HIV-Infected Children in Soweto South Africa

Authors: Fairlie L [1]; Muchiri E[1]; Beylis CN [2]; Meyers T [3]; Moultrie H[1]

Institutions:

1) Wits Reproductive Health and HIV Institute (WRHI), Faculty of Health Sciences, University of the Witwatersrand, South Africa

2) National Health Laboratory Service (NHLS), Mycobacteriology Referral Laboratory & Department of Clinical Microbiology & Infectious Diseases, University of Witwatersrand, South Africa

3) Harriet Shezi Children's Clinic, Chris Hani Baragwanath Hospital, Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand, South Africa

SUMMARY

SETTING

Paediatric HIV Clinic in an academic hospital, Soweto, South Africa.

OBJECTIVE

Describe and compare clinical, immunological and virological characteristics of HIV-infected children co-treated for TB, comparing those investigated microbiologically and those not, with description of microbiological TB investigation results.

DESIGN

Retrospective analysis of TB/HIV-infected children aged <15 years between 1 October 2007 and 15 March 2009.

RESULTS

TB treatment was initiated in 616/3358(18%) children during the study period. Microbiological TB investigation results were available in 399/616(65%) children, culture-confirmed TB diagnosed in 49/399(12%). Drug susceptibility testing was performed in 29/49(59%), with 5/29(17%) children having isoniazid-resistance, 3 with MDR-TB. Children > 8 years and between 3-8 years were more likely to have culture-confirmed TB compared to < 3 years (AOR 9.4; 95% CI 2.26 – 39.08 and AOR 6.7; 95% CI 1.60 – 27.69 respectively) as were those with CD4 count < 200 cells/mm^3 compared to > 500 cells/mm^3 (AOR 3.95; 95%CI 1.23 – 12.72).

CONCLUSION

Our study in HIV-infected children showed a high TB case rate, a low rate of definite TB and a high rate of drug-resistant TB according to WHO case definitions. Increased uptake of available TB tests and availability of new diagnostic tests remains a priority in high TB/HIV burden settings.

INTRODUCTION

Tuberculosis(TB) is the most common opportunistic infection in Human Immunodeficiency Virus (HIV)-infected children, with up to 40% receiving TB treatment at the time of commencing combination antiretroviral therapy (cART).[1] In South Africa an estimated 460,000(410,000-520,000) children are HIV-infected [2], the TB incidence rate is 993/100,000 population [3] and up to 52% of children with culture-confirmed TB are HIV-infected. [4] In HIV-infected children the risk of TB disease isup to 4 times greater in those with a CD4 percentage < 15%. [5] Potential for both over- and under-diagnosing TB in HIV-infected children is high with overlapping clinical features between both diseases further complicated by additional co-existing comorbidities common in this population. [6]

World Health Organization(WHO) case definitions for TB disease include clinical features and microbiological investigation results, with radiological features more important in cases with negative microbiological results. [7] In children tuberculin skin test(TST) and chest x-ray(CXR)may assume greater diagnostic importance than in adults. [8, 9] However, TST which cannot distinguish TB infection from TB disease may frequently be negative in immunocompromised and malnourished individuals. [10] Wide variability in CXR interpretation between experienced clinicians and poor correlation with culture-confirmed TB suggests CXR is an unreliable adjunct to TB diagnosis in children. [11, 12] Microbiological confirmation of TB remains the most accurate method of diagnosis, and two Cape Town based studies report successful microbiological TB investigation through gastric washing and induced sputum procedures in primary health clinics. [13, 14] These tests are not performed routinely in most clinical settings because of limited staff capacity, skill shortage, low yield and difficulty in conducting them in small children.

Co-treatment with TB therapy and cART in HIV-infected children remains complicated making accurate TB diagnosis important. The drug-drug interactions between rifampicin-based TB treatment and cART,

66 particularly lopinavir/ritonavir which is inadequately boosted may result in reduced virological
67 suppression rates. [1, 15] Polypharmacy with TB treatment and cART may affect adherence and increases
68 the possibility of both ART-associated and TB drug-associated side effects.

69 Data describing the clinical, immunological and virological associations with TB diagnosis in HIV-infected
70 children are scarce as are reports regarding microbiological TB investigation resultsThis retrospective
71 cross sectional review sought to describe and compare clinical, immunological and virological
72 characteristics of HIV-infected children co-treated for TB, distinguished by whether microbiological TB
73 investigations were conducted or not, and to describe results of microbiological TB investigations.

74 METHODS

75 *Study design*

76 We conducted a retrospective cross sectional review of microbiological TB investigations performed in
77 HIV-infected children attending Harriet Shezi Children's Clinic (HSCC), who were treated for TB, in
78 Soweto, South Africa between 1 October 2008 and 15 March 2009.

79 *Subjects*

80 HIV-infected children < 15 years of age treated for TB.

81 *Standard of care*

82 The HSCC is a public outpatient pediatric HIV clinic at Chris Hani Baragwanath Academic Hospital
83 (CHBAH) in Soweto, South Africa. HIV diagnosis was according to South African National Department of
84 Health (SANDoH) guidelines. [16] TB screening was conducted at initial and subsequent clinic visits using
85 symptomatic enquiry and clinical assessment, with further investigations in suspected TB. WHO case
86 definitions were applied including 'TB suspects': defined as those presenting with signs and symptoms

suggestive of TB; 'TB case' defined as a definite TB case or where a health care worker has diagnosed TB and has initiated a full course of TB treatment; and 'Definite case' of TB as a confirmed diagnosis of Mycobacterium tuberculosis through culture or a newer method such as molecular line probe assay or in countries where this is not possible, two positive specimens for AFB. [7] Investigations included CXR, TST and microbiological investigations. All microbiological specimens were obtained from the respiratory tract and extrapulmonary sites including blood, pleural fluid and cerebrospinal fluid (CSF) according to clinical indication. Children diagnosed with TB were prescribed a standard 3 drug regimen of rifampicin, isoniazid (INH) and pyrazinamide with ethambutol/ethionamide added as a fourth drug for complicated TB including TB meningitis or disseminated TB. [17] CART initiation was according to SANDoH guidelines at the time which recommended starting cART two months after TB initiation or completing TB treatment before starting cART if possible in stable children. [16]

Laboratory investigations

Microbiological investigations for TB included smear microscopy, culture and drug susceptibility testing (DST). Specimens were processed according to WHO guidelines using the NALC-NaOH decontamination process; a 0.5ml portion of the processed, centrifuged pellet was inoculated into MGIT™ (Mycobacterial Growth Indicator Tube) (Becton Dickinson, Maryland, USA) tubes and incubated for a total of 6 weeks. Growth detected in automated MGIT instruments was confirmed as being acid fast bacillus (AFB)-positive by Ziehl Neelsen stained smear. Confirmation of *Mycobacterium tuberculosis* complex (MTBC) was by the GenoType® Mycobacterium CM assay (Hain Lifesciences, Nehren, Germany). DST was only performed on clinician request, guided by the National TB Control Programme Guidelines and was not routine during the study period. [17] Isolates of MTBC were initially tested against INH and rifampicin using the MGIT™960 proportion method (Becton Dickinson, Maryland, USA). If resistance was found against either/ both drugs, the isolate would undergo further DST against streptomycin, ethambutol,

ofloxacin, kanamycin and ethionamide using the MGIT proportion method. Definitions for drug-susceptible and drug-resistant TB were as follows: susceptible MTB: isolate susceptible to both INH and rifampicin; INH mono-resistant and rifampicin mono-resistant if resistant to only INH or rifampicin respectively; multi-drug-resistant (MDR)-TB if resistant to both INH and rifampicin and extensively drug-resistant (XDR)-TB if MDR-TB that was also resistant to ofloxacin and kanamycin.

Data Collection

Demographic, clinical and laboratory data at the time of TB treatment initiation, with a 3 month window period, were extracted from three databases. Demographic and HIV related data from the HSCC clinical database; microbiological TB data from the National Health Laboratory Service (NHLS) database; and TB treatment data from the CHBAH TB Care Centre (TBCC) ETR.net database, a SANDoH TB data system. The TBCC and HSCC databases were merged using the hospital unique patient identifier to include only children treated for TB. Microbiological MTB investigation results for children included in the merged dataset of children attending HSCC treated for TB were then imported from the NHLS Mycobacteriology Referral Laboratory dataset. The final data set included all children treated for TB, regardless of whether or not they were investigated microbiologically for TB. Manual file reviews and NHLS database searches were conducted by study staff for missing values.

Data extracted included age (calculated from date of birth), weight for age z-scores (WAZ-scores), height for age z-scores (HAZ-scores), WHO clinical staging, HIV $\log_{10}$ viral load and CD4 count. WAZ-scores and HAZ-scores were generated using WHO reference ranges. Characteristics for children at TB treatment initiation were stratified according to whether children were investigated microbiologically for TB and according to TB investigation results.

Statistical analysis

Statistical analysis and data management were carried out in STATA version 12.0 (STATA Corporation, College Station, TX). Medians and interquartile ranges(IQR) were used to summarize the data. Chi-square tests were used to explore associations between grouped categorical variables of age, anthropometrical measures, CD4 count and percentage, HIV $\log_{10}$ viral load and the outcome of TB investigations. Age was categorized as of < 3, ≥3 to <8and ≥ 8 years in keeping with differential TB disease risk. [8] The Wilcoxon rank sum test and the chi-squared test were used to compare continuous variables and categorical variables respectively. Univariable and multivariable logistic models were fitted to identify factors associated with a positive TB investigation outcome among HIV-infected children investigated microbiologically for TB. Multivariate models were developed using step-wise backward elimination using likelihood ratio tests to identify the model with the best fit.

Ethics approval

This study was approved by the University of the Witwatersrand Human Sciences Research Ethics Committee.

RESULTS

Of the 3358 HIV-infected children who attended the HSCC at least once during the study period, 616 (18%) were co-treated for TB and were included in the analysis. (Figure 1) One hundred and twenty nine children were receiving cART at the time (Table 1). At the initiation of TB treatment the median age was 4.2 years with an interquartile range (IQR) of 1.1 to 8.4 years, 67% of children had WHO clinical stage IV disease, median WAZ-score -2.0 (IQR -1.1 to -3.3) and HAZ-score -2.4 (IQR -1.5 to -3.3). The median CD4 count was 425 cells/mm^3 (IQR 180– 847), median CD4 percentage 14.4% (IQR 8.4 – 21%) and the median $\log_{10}$ HIV viral load 4.6 copies/ml (IQR 3.5 – 5.3). A higher than expected proportion amongst those investigated microbiologically for TB were male (p=0.01). There were no other clinically or statistically

154 significant differences in characteristics of children who were and were not investigated
155 microbiologically (Table 1).

156 Microbiological investigation was conducted in 399(65%) of the children co-treated for TB (Figure 1).
157 Twenty five children (6%) had specimens which stained positive with the Ziehl Neelsen stain (+AFB). TB
158 was confirmed in 49(12%) children by mycobacterial culture. The median age of children with culture-
159 confirmed TB was 8.2 years (IQR 5.7 – 10.1) compared to 3.3 years (IQR 0.9 – 7.9) in those investigated
160 microbiologically with negative TB culture results (p=<0.001) (Table 2). Children with culture-confirmed
161 TB (definite TB) had a median CD4 count of 157 cells/mm^3(IQR 41 - 299 cells/mm^3) and CD4 percentage
162 of 10% (IQR 3 – 19%) compared to 491 cells/mm^3 (IQR 239 – 924 cells/mm^3) and 14% (IQR 8 – 20%) in
163 children with negative TB culture results (p=0.02 and <0.001 respectively) (Table 2). DST was performed
164 in 29(59%) children with culture-confirmed TB. Drug resistant TB was diagnosed in 5(17%) children, of
165 which 3 were MDR-TB. All three MDR-TB strains were resistant to ethambutol and streptomycin and a
166 third child's MTB isolate was also resistant to ethionamide. Two children were infected with INH mono-
167 resistant MTB. (Figure 1)

168 Multivariate logistic regression confirmed that confirmation of TB on culture was independently
169 associated with both older age and a lower CD4 count after adjusting for sex, WAZ and HAZ. Children > 8
170 years and children between 3-8 years were more likely to have culture-confirmed TB compared to those
171 < 3 years (AOR 9.4; 95% CI 2.26 – 39.08 and AOR 6.7; 95% CI 1.60 – 27.69 respectively). Children with a
172 CD4 count < 200 cells/mm^3 had increased odds of culture-confirmed TB compared to those with a CD4
173 count > 500 cells/mm^3 (AOR 3.95; 95%CI 1.23 – 12.72).
174
175 DISCUSSION

176 Our study showed a high TB case rate (18%) in HIV-infected children attending a public HIV treatment
177 clinic, with a low microbiological TB investigation rate and low rate of definite TB, confirmed by culture
178 or AFBs in those investigated microbiologically. Similar high TB/HIV co-treatment rates have been
179 described in other Sub-Saharan settings. In Uganda, 17% of HIV-infected children (median age 6.6 years)
180 were co-treated for TB. [18] In a study from four South African hospitals in Johannesburg and Cape Town,
181 21% of HIV-infected children, (median age 6.3 years) were co-treated for TB. In 23% of children a
182 definite TB diagnosis was made(AFB smear *n* = 30; TB culture *n* = 24; or biopsy *n* = 11) although the
183 number with culture-confirmed TB was not described. [19] In South African HIV-infected children who are
184 less than 2 years, TB case rates of 40% by 1 year of follow up on cART have been described. [20] Although
185 only 12% of TB cases had culture-confirmed TB, (definite TB cases) of concern was that only 65% of
186 children classified as a TB case were investigated microbiologically. Interestingly, apart from male
187 gender having increased TB microbiological investigations, there was no difference in clinical or
188 immunological characteristics between children for whom microbiological TB investigation results were
189 conducted and those not. In our study, children investigated microbiologically with culture-confirmed TB
190 were older and more immunologically compromised than those investigated with negative TB culture
191 results. The correlation between immunological suppression and confirmed TB in children is likely due to
192 a number of factors: although we don't have data regarding source of specimen, 80% of TB specimens
193 processed at NHLS are respiratory in origin and the same is likely in this study. Older children are more
194 likely to expectorate and produce adequate sputum samples. We also expect declining immune function
195 with advancing age and disease progression in HIV-infected children. Adult studies however, have
196 shown lower rates of microbiological TB diagnosis with severe immunodeficiency. [21]

197 We also found that children who had culture-confirmed TB and underwent DST had high rates of INH-
198 resistant TB (17%) with 10% of children having MDR-TB. These findings are consistent with studies in
199 Cape Town and Johannesburg showing INH-resistant TB in up to 15 % and MDR-TB in 9% of children with

culture-confirmed TB [4, 22] Given these findings it is concerning that more children were not investigated microbiologically for TB prior to commencing TB treatment. TB culture is labour-intensive and time-consuming leading to long turnaround times for results and low sensitivity in children [10] and recent WHO policy updates recommend the use of Xpert MTB/RIF rather than conventional microscopy, culture and DST as the initial diagnostic test in children presumed to have MDR-TB or HIV-associated TB but as yet rollout has been limited in routine clinic settings. [23]Xpert MTB/Rif rely on respiratory specimen collection, which remains the barrier to microbiological investigation in children. Two Cape Town-based studies have shown that it is feasible for gastric washings and induced sputum for microbiological TB testing to be performed in a primary clinic setting and that 2 consecutive day gastric washings may have a similar diagnostic yield to induced sputum, this technique being particularly useful in infants and young children. [13, 14] Ongoing training of health care workers in specimen collection techniques is required to ensure that these rapid technologies are increasingly utilized in community settings.

This study has some limitations. Data were collected retrospectively and were dependent on accurate recording by the attending clinician and capture by data capturers. TB treatment data was not accurately collected in the HSCC database, therefore, only children registered for TB treatment at the CHBAH TBCC were included which may have excluded children initiated on TB treatment outside of CHBAH with a possible underestimate in TB cases. Patient name or identifying numbers being incorrectly recorded between the data sets may have resulted in some children being investigated for TB microbiologically but not linked in the NHLS dataset. We were unable to break down the source of microbiological specimens due to data extraction methods at the time but as 80% of TB specimens received by NHLS are pulmonary specimens we assume the same holds for this study. Due to the large cohort, and quality of data within files regarding CXR and TST data was not confirmed in a file review. Studies have found poor correlation between culture-confirmed TB and CXR findings and wide variability

in interpretation between clinicians. [12] We did not have sufficiently accurate data to describe TB suspects not treated for TB.

CONCLUSIONS

Our study showed a high TB case rate in HIV-infected children with a low rate of microbiological TB investigation and microbiological confirmation. Of concern, among those investigated microbiologically, a high rate of drug resistant TB was found. These findings suggest that efforts to increase the use of currently available tests should be encouraged through staff education and training. There is also a need for new simple, cheap, rapid and reliable TB tests to become available particularly in resource limited settings, with a high endemic TB burden.

REFERENCES

1. Frohoff C, Moodley M, Fairlie L, Coovadia A, Moultrie H, Kuhn L, et al. Treatment outcomes among HIV-infected infants and young children following modifications to protease inhibitor-based therapy due to tuberculosis treatment. PLoS One. 2011; **6**(2).
2. UNAIDS. HIV and AIDS Estimates 2011. http://wwwunaidsorg/en/regionscountries/countries/southafrica/. 2011.
3. World Health Organization. Global Report Tuberculosis 2012; 2012.
4. Fairlie L, Beylis NC, Reubenson G, Moore DP, Madhi SA. High prevalence of childhood multi-drug resistant tuberculosis in Johannesburg, South Africa: a cross sectional study. BMC Infectious Diseases. 2011; **11**(28): 1.
5. Elenga N, Kouakoussui KA, Bonard D, Fassinou P, Anaky M-F, Wemin M-L, et al. Diagnosed Tuberculosis During the Follow-up of a Cohort of Human Immunodeficiency Virus-Infected Children in Abidjan, Coˆte d'Ivoire ANRS 1278 Study. The Pediatric Infectious Disease Journal. 2005; **24**(12): 1077.
6. Edwards DJ, Kitetele F, Rie AV. Agreement between clinical scoring systems used for the diagnosis of pediatric tuberculosis in the HIV era. Int J Tuberc Lung Dis 2007; **11**(3): 263-9.
7. World Health Organization. Treatment of tuberculosis Guidelines Fourth edition. http://WHO/HTM/TB/2009420. 2009.
8. B. J. Marais, S. M. Graham, M. F. Cotton, N. Beyers. Diagnostic and Management Challenges for Childhood Tuberculosis in the Era of HIV. The Journal of Infectious Diseases. 2007; **196**: S76-85.
9. BJ M, RP G, HS S, AC H, CC O, JJ S, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. INT J TUBERC LUNG DIS. 2004; **8**(4): 392.
10. Eamranond P, Jaramillo E. Tuberculosis in children: reassessing the need for improved diagnosis in global control strategies. Int J Tuberculosis and Lung Disease. 2001; **5**(7): 594-603.

11. Hatherill M, Hanslo M, Hawkridge T, Little F, Lesley Workman, Mahomed H, et al. Structured approaches for the screening and diagnosis of childhood tuberculosis in a high prevalence region of South Africa. Bull World Health Organ. 2010; **88**: 312-20.
12. Sawry S, Moultrie H, Mahomed N, Rie AV. Diagnostic performance of CXRs for the diagnosis of TB in children initiating HAART. Third TB conference Durban, South Africa,. 2012.
13. Hatherill M, Hawkridge T, Zar HJ, Whitelaw A, Tameris M, Workman L, et al. Induced sputum or gastric lavage for community based diagnosis of childhood pulmonary tuberculosis? Arch Dis Child. 2009; **94**: 195-201.
14. Moore HA, Apolles P, Villiers PJTd, Zar HJ. Sputum induction for microbiological diagnosis of childhood pulmonary tuberculosis in a community setting. Int J Tuberc Lung Dis 2011; **15**(9): 1185-90.
15. McIlleron H, Ren Y, Nuttall J, Fairlie L, Rabie H, Cotton M, et al. Lopinavir exposure is insufficient in children given double doses of lopinavir/ritonavir during rifampicin-based treatment for tuberculosis. Antiviral Therapy. 2011; **16**.
16. South African National Department of Health. Guidelines for the Management of HIV-infected children. 2008.
17. South African National Department of Health. South African National Tuberculosis Guidelines. 2008.
18. Bakeera-Kitaka S, Conesa-Botella A, Dhabangi A, Maganda A, Kekitiinwa A, Colebunders R, et al. Tuberculosis in human immunodefi ciency virus infected Ugandan children starting on antiretroviral therapy. Int J Tuberc Lung Dis. 2011; **15**(8): 1082-6.
19. Martinson NA, H. Moultrie, Niekerk Rv, Barry G, A. Coovadia, Cotton M, et al. HAART and risk of tuberculosis in HIV-infected South African children: a multi-site retrospective cohort. Int J Tuberc Lung Dis. 2009; **13**(7): 862-7.
20. Reitz C, Ashraf Coovadia, Ko S, Meyers T, Strehlau R, Sherman G, et al. Initial Response to Protease-Inhibitor-Based Antiretroviral Therapy among Children Less than 2 Years of Age in South Africa: Effect of Cotreatment for Tuberculosis. The Journal of Infectious Diseases. 2010; **201**: 1121-31.
21. Kenyon TA, Creek T, Laserson K, Makhoa M, Chimidza N, Mwasekaga M, et al. Risk factors for transmission of Mycobacterium tuberculosis from HIV-infected tuberculosis patients, Botswana. Int J Tuberc Lung Dis 2002; **6**(10): 843-50.
22. Seddon JA, Hesseling AC, Marais BJ, Jordaan A, Victor T, Schaaf HS. The evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa. Int J Tuberc Lung Dis. 2012; **16**(7): 928-33.
23. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosisand rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update. . http://wwwstoptborg/wg/gli/assets/documents/WHO%20Policy%20Statement%20on%20Xpert%20MTB-RIF%202013%20pre%20publication%2022102013pdf. 2013.

297 Table 1: Characteristics of children attending the HSCC treated for TB between 1 October 2007 and 15

298 March 2009

	All children treated for TB (n=616)	Not investigated microbiologically for TB (n=217)	Investigated microbiologically for TB (n=399)	p-value
Male (n, %)	339(55%)	105(48%)	234(58%)	0.01
Age in years (median, IQR)	4.2 (1.1 – 8.4)	4.2 (1.2 – 8.6)	4.2 (1.0 – 8.3)	0.23
CD4% (median, IQR)	14.4 (8.4 – 21.0)	15.2 (10.0 – 22.0)	13.8 (7.9 – 20.3)	0.07
CD4 (median, IQR)	425 (180 – 847)	389 (164 – 747)	445 (200 – 859)	0.33
Log_{10} HIV VL copies/ml (median, IQR)	4.6 (3.5 – 5.3)	4.6 (3.2 – 5.1)	4.6 (3.7 – 5.4)	0.21
WHO stage 4 (%)	67	65	68	0.42
Weight for Age Z-scores (median, IQR),n	-2.0 (-1.1 – -3.3), n=440	--1.9 (-0.9 – -3.2), n=146	-2.1 (-1.1 – -3.4), n=294	0.25
Height for Age Z-scores (median, IQR),n	-2.4 (-1.5 – -3.3), n=521	-2.4 (-1.5 – -3.1), n=176	-2.4 (-1.5 – -3.3), n=345	0.43
Receiving cART	129 (21%)	50 (23%)	79 (20%)	0.35

299 IQR= Interquartile range

300

301

Table 2: Characteristics of HIV-infected children co-treated for TB at HSCC from 1 October 2007- 15 March 2009 stratified by microbiological TB investigation results

	TB confirmed on culture (n=49)	**Negative TB culture (n=350)**	**p-value**
Male (n, %)	25(51%)	209(60%)	0.25
Age in years (median, IQR)	8.2 (5.7 – 10.1)	3.3 (0.9 – 7.9)	<0.001
CD4% (median, IQR)	9.8 (3.0 – 18.7)	14.4 (8.4 – 20.5)	0.02
CD4 (median, IQR)	157 (41 – 299)	491 (239 – 924)	<0.001
Log_{10} HIV VL copies/ml (median, IQR)	4.6 (3.4 – 5.2)	4.7 (3.7 – 5.4)	0.35
WHO stage 4 (%)	76%	67%	0.22
Weight for Age Z-scores (median, IQR)	-2.1 (-1.2 - -3.3), n=30	-2.1(-1.1 - -3.4), n=264	0.85
Height for Age Z-scores (median, IQR)n	-2.4(-1.4 - -3.2), n=40	-2.5 (-1.5 - -3.3), n=305	0.48

IQR= Interquartile range

321 Table 3: Logistic regression models for factors associated with TB confirmation in HIV-infected children
322 co-treated for TB

Characteristic at start of TB treatment		Unadjusted Odds ratio, 95% CI	Unadjusted p-value	Adjusted Odds Ratio, 95% CI	Adjusted p-values
Sex					
	Male	1		1	
	Female	1.42 (0.72-2.21)	0.25	1.89 (0.70 – 5.10)	0.21
Age					
	<3 years	1		1	
	3-8 years	4.01 (1.49 – 10.79)	<0.001	6.65 (1.60 – 27.69)	0.009
	>8 years	9.39 (3.75 – 23.47)	<0.001	9.40 (2.26 – 39.08)	0.002
CD4 count					
	>=500	1		1	
	200-500	2.69 (1.02 – 7.10)	0.04	0.65 (0.16 – 2.54)	0.53
	<200	6.81 (2.75 – 16.8)	<0.001	3.95 (1.23 – 12.72)	0.02
CD4 percentage					
	>=25	1			
	<25	1.35 (0.46-4.00)	0.59		
Viral load					
	<400	1			
	>=400	0.79 (0.33 - 1.90)	0.60		
WHO Stage					
	Stage 3	1			
	Stage 4	1.51 (0.75– 3.00)	0.24		
On cART at start of TB treatment					
	Yes	1			
	No	0.95 (0.45-2.01)	0.91		
Weight for age Z-score		1.04 (0.84 – 1.29)	0.69	0.62 (0.38-1.02)	0.06
Height for age Z-scores		1.12 (0.93 – 1.35)	0.25	1.78 (1.09 – 2.91)	0.02

323

324

325

326

327

328 ACKNOWLEDGEMENTS

329 We would like to acknowledge the children and their families attending HSCC. LF, TM and HM
330 conceived of the study. LF designed the study, collected data, drafted the manuscript and coordinated
331 the revisions to the manuscript. NCB collected data and participated in study design and drafting of
332 manuscript. EM performed the statistical analysis and contributed to drafting of the manuscript. All
333 authors read and approved the final manuscript.

334 COMPETING INTERESTS

335 The authors declare that they have no competing interests.

Appendix C: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Lee Fairlie

CLEARANCE CERTIFICATE	**M110743**
PROJECT	Association between Clinical Characteristics and TB Investigation Results in HIV Infected
Children	Treated for TB at a Governement Sector Paediatric
HIV	Clinic in Soweto
INVESTIGATORS	Dr Lee Fairlie.
DEPARTMENT	Harriet Shezi's Childrens Clinic
DATE CONSIDERED	29/07/2011
DECISION OF THE COMMITTEE*	Approed uncoditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 29/07/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr T Meyers

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...