

https://dspace.org/

Impact of HIV-1 co-infection on tuberculosis and value of CD4+ lymphocyte counts and concurrent antigen testing in interpretation of tuberculin reactions in hospitalized children with tuberculosis in South Africa

Madhi, Shabir Ahmed

2014-05-20
http://hdl.handle.net10539/14686

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

IMPACT OF HIV-1 CO-INFECTION ON TUBERCULOSIS AND VALUE OF CD4+ LYMPHOCYTE COUNTS AND CONCURRENT ANTIGEN TESTING IN INTERPRETATION OF TUBERCULIN REACTIONS IN HOSPITALIZED CHILDREN WITH TUBERCULOSIS IN SOUTH AFRICA.

Shabir Ahmed Madhi

A research report submitted to the Faculty of Health Sciences, University of the Wiwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Paediatrics

Johannesburg, 1999

DECLARATION

I, Shabir Ahmed Madhi, declare that this research report is my own work. It is being submitted for the degree of Master 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.



.....

Shabir A Madhi

1st day of Febuary, 1999.

Dedication

Dedicated to a true friend,

Fatima Solomon

Publications and presentations arising from this study

Presentations:

1. Oral presentation: Madhi SA, Huebner RE, Scherman G, Gray GE. Correlation of CD4+ lymphocyte counts and companion antigen ("CMI Multitest®") testing in the interpretation of tuberculin skin test in HIV-1 infected and uninfected children with tuberculosis. International Conference on AIDS and STDs in Africa Cote d'Ivoire, November 1997.

2. Poster presentation: Madhi SA. Impact of HIV-1 infection on children with tuberculosis. International Conference on AIDS and STDs in Africa. Cote d'Ivoire, November 1997.

Publications:

1. Madhi SA, Gray GE, Huebner RE, Scherman G, McKinnon D, Pettifor J. Correlation between CD4+ lymphocyte counts, concurrent antigen testing (using the "CMI Multitest®") and tuberculin skin testing in HIV-1 infected and uninfected children with tuberculosis. Pediatr Infect Dis J. (1999-In Press)

2. Madhi SA, Doedens L, Nduc T, Wesley D, Cooper PA. Impact of HIV-1 co-infection on tuberculosis in hospitalized children in South Africa. (Submitted)

Abstract:

There are few reports on the impact of HIV-1 infection on tuberculosis in children. Microbiologic diagnosis of tuberculosis is difficult and much reliance is placed on the tuberculin skin test, as part of a scoring system, in diagnosing tuberculosis in children. A prospective study, enrolling 168 patients with clinical tuberculosis, was performed between July 1996 and January 1997 at the teaching hospitals attached to the Department of Paediatrics and Child Health, University of the Witwatersrand.

Forty-two percent of children with tuberculosis were HIV-1 infected. Extra-pulmonary tuberculosis was diagnosed more frequently in HIV uninfected children. Progressive pulmonary tuberculosis, based on radiographic findings, and mortality was higher in HIV-1 infected children with tuberculosis. HIV-1 infected children with pulmonary tuberculosis showed marked hyporeactivity to tuberculin skin testing. Both CD4+ lymphocyte counts and concurrent delayed type hypersensitivity testing, using the "CMI Multitest®", offered little value in interpreting the tuberculin skin test in HIV-1 infected children with tuberculosis.

The findings of the study suggest that aggressive microbiologic investigations coupled with a low threshold of clinical suspicion is essential in diagnosing tuberculosis in children, especially in HIV infected children.

Acknowledgements:

Grants from the Perinatal HIV Unit, Iris Ellen Hodges Cardio-Vascular Research Grant-University of the Witwatersrand and the Department of Pediatrics and Child Health- Johannesburg hospital.

Assisting colleagues for patient recruitment: Drs T Aduc, L Doedens, D McKinnon, K Petersen and D Wesley.

Dr R E Huebner, for assisting with data analysis and her valuable comments.

Prof PA Cooper for his supervisory role.

Dr E Joseph, paediatric radiologist, for reading of chest radiographs

The nurses at the participating hospitals, without whose dedication, this study would not have been possible.

TABLE OF CONTENTS

	Page
DECLARATION	ii
DEDICATION	iii
PUBLICATIONS AND PRESENTATIONS	iv
ABSTRACT	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES	x
LIST OF TABLES	xi
NOMENCLATURE	xii
PREFACE	xiii
1.0 INTRODUCTION	
1.1 The tuberculosis epidemic	1
1.2 CD4+ lymphocyte counts and "companion" delayed type hypersensitivity testing	2
1.3 Objectives	3
1.3.1 Primary objectives	
1.3.2 Secondary objectives	
2 MATERIALS AND METHODS	
2.1 Study site and sample	5

2.2 Ethics and consent 5

2.3 Patient recruitment 5

2.3.1 HIV-1 testing 6

2.3.2 Microbiological testing 7

2.3.3 Tuberculin skin testing 7

2.3.4 Radiographic investigations 8

2.3.5 Other investigations 9

2.3.6 Categorisation of patients with tuberculosis 9

2.3.7 Nutritional status 10

"CMI Multitest®" 10

2.3.8 CD4+ lymphocyte counts 11

2.4 Statistical analysis 11

3.0 RESULTS 12

3.1 Impact of HIV-1 co-infection in children with tuberculosis

3.1.1 Demographic and HIV results 12

3.1.1.1 HIV results

3.1.1.2 Age, gender and nutritional status

3.1.2 Microscopy and culture of *mycobacterium tuberculosis* 13

3.1.3 Clinical characteristics 14

3.1.4 Tuberculin skin test 15

3.1.5 Chest radiographs 17

3.1.6 Extrapulmonary tuberculosis 19

3.2 CD4+ lymphocyte counts and companion antigen testing ("CMI Multitest®") in the interpretation of tuberculin skin tests. 20

3.2.1 CD4+ lymphocyte count 21

3.2.2 CMI Multitest 24

4.0 DISCUSSION 27

4.1 Prevalence of HIV-1 co-infection 27

4.2 Microbiologic diagnosis 27

4.3 Tuberculin skin testing 29

4.4 Clinical differences in presentation 30

4.5 Extra-pulmonary tuberculosis 32

4.6 CD4+ lymphocyte counts and truberculin skin testing 33

4.7 Concurrent antigen testing and tuberculin skin testing 35

5.0 STUDY LIMITATIONS 37

6.0 CONCLUSIONS 39

REFERENCES 41

3.2 CD4+ lymphocyte counts and companion antigen testing ("CMI Multitest®") in the interpretation of tuberculin skin tests. 20

3.2.1 CD4+ lymphocyte count 21

3.2.2 CMI Multitest 24

4.0 DISCUSSION 27

4.1 Prevalence of HIV-1 co-infection 27

4.2 Microbiologic diagnosis 27

4.3 Tuberculin skin testing 29

4.4 Clinical differences in presentation 30

4.5 Extra-pulmonary tuberculosis 32

4.6 CD4+ lymphocyte counts and tuberculin skin testing 33

4.7 Concurrent antigen testing and tuberculin skin testing 35

5.0 STUDY LIMITATIONS 37

6.0 CONCLUSIONS 39

REFERENCES 41

LIST OF FIGURES

Figure	Page
1. Tuberculin skin test reactions in children with pulmonary tuberculosis.	16
2. Delayed type hypersensitivity reactions to "CMI Multitest®" antigens in HIV infected and HIV uninfected children.	25

LIST OF TABLES

Table		Page
1	Clinical symptoms between HIV-1 infected and uninfected children with pulmonary tuberculosis.	14
2	Reactivity to tuberculin skin testing in HIV infected and uninfected children.	17
3	Radiographic features of children with pulmonary tuberculosis.	18
4	Site of involvement in patients with tuberculosis.	20
5	Differences in median CD4+ lymphocyte counts (25th-75th quartiles) between HIV infected and uninfected children with tuberculosis.	21
6	Association between CD4+ lymphocyte categories and nutritional status of HIV infected and uninfected children.	22
7	Correlation between CD4+ lymphocyte categorisation and tuberculin skin test (TST) reactions in HIV infected and uninfected children.	24

NOMENCLATURE

BCG-Bacillus- Calmette-Guérin

CMI- cell mediated immunity

CT scan- computerized tomographic scan

CXR- chest radiograph

DTH - Delayed type hypersensitivity

EPTB- Extra-pulmonary tuberculosis

HIV – Human immunodeficiency virus type-1

MTB- M*ycobacterium tuberculosis*

PTB- Pulmonary tuberculosis

TB – Tuberculosis

TST- Tuberculin skin test

PREFACE

South Africa has one of the highest incidences of HIV infection and tuberculosis in the world. In children, a microbiologic diagnosis of tuberculosis remains difficult because of difficulties in obtaining suitable specimens for investigation. Much reliance is placed on clinical scoring systems to assist in making the diagnosis of tuberculosis in children. The tuberculin skin test is integral to most of these scoring systems. HIV-1 infection is widely recognised to impair all arms of the immune system, including cell-mediated immunity, resulting in increased anergy to antigens on skin-based delayed type hypersensitivity reactions. The exact impact of HIV-1 co-infection on the clinical presentation and the value of CD4+ lymphocyte counts and/or concurrent antigen testing in interpreting tuberculin reactions have not been fully analysed in children. This study was performed to gain a greater understanding of the above-mentioned issues.

1.0 INTRODUCTION

1.1 The tuberculosis epidemic

Globally, it is estimated that one-third of the world's population is infected by *Mycobacterium tuberculosis* (MTB)[1], with the highest burden of disease being in sub-Saharan Africa and South East Asia[2]. *Mycobacterium tuberculosis* is also recognised as being the leading cause of death from any single pathogen[2]. In 1989, tuberculosis accounted for a total of 13 million deaths globally, including 450 000 deaths in children under 15 years of age[3]. The impact of tuberculosis (TB) on mortality has been further aggravated by the human immunodeficiency type-1 (HIV) epidemic.

South Africa has one of the worst epidemics of TB in the world. Based on notifications of TB, the incidence of tuberculosis in 1994 was estimated to be 311/ 100 000[4]. These incidence figures for TB were in the early stage of the present HIV epidemic. The Global program on Aids estimated that by mid-1994 over 16 million adults and one million children were infected by HIV[2]. The rate of HIV infection in South Africa, like most of other regions in sub-Saharan African remains uncontrolled[5].

In the greater Johannesburg region, the prevalence of HIV infection amongst pregnant women attending ante-natal clinics was estimated at 16% [1997, SAIMR-unpublished surveillance data] and the vertical transmission rate of HIV to newborns at this site was estimated to be 18-30% [Gray GE,

unpublished data]. In 1997, 30% of all paediatric admissions at Chris Hani-Baragwanath hospital were HIV infected [Meyers T, unpublished data].

It was estimated that by mid 1997 3.8 million of the 5.6 million people co-infected by HIV and TB were in sub-Saharan Africa[2]. In South Africa, the prevalence of HIV co-infection amongst patients with tuberculosis was estimated to be 18.9% in adults[4]. In 1992-1994, one study in Kwazulu-Natal, South Africa, found 11% of children with tuberculosis to be HIV co-infected[6]. Statistics on tuberculosis in children are problematic as limitations in culturing MTB from children, make an accurate assessment of this problem difficult. As a result, there are few studies analysing the impact of HIV infection on tuberculosis in children.

1.2 CD4+ lymphocyte counts and "companion" delayed type hypersensitivity testing in the interpretation of the tuberculin skin test.

Tuberculin skin testing (TST) is widely used for screening of infection by *Mycobacterium tuberculosis*. It also serves as an important adjunct in most scoring systems[7,8,9] for the diagnosis of tuberculosis in children. Reaction to intradermal injection of tuberculin in patients infected with MTB, *Mycobacteria sp.* and/or vaccinated with BCG, represents a cell mediated immune reaction to this antigen. The cellular elements involved in cell mediated immunity include CD4+ lymphocytes, CD8+ lymphocytes, neutrophils and macrophages[10]. The value of the CD4+ lymphocyte count in determining the

immunologic capacity of the host is controversial as it forms only part of the immune system and does not provide a functional assay of the cell-mediated immune system.

There are numerous factors that cause hyporeactivity to tuberculin in children with TB. Included amongst these are malnutrition, post-measles illness, post-measles vaccination and acute co-existing illnesses[10]. One of the important risk factors for anergy to TST in children with tuberculosis is co-infection with HIV[11,12,13,14].

There are few studies in children that have assessed the value of concurrent antigen skin testing or the correlation between $CD4^+$ lymphocyte counts[11] and TST in HIV infected children. Delayed type hypersensitivity reactions may be a better index of the functional capacity of the cell mediated-immune (CMI) system[15,16]. Until recently, it was recommended to that TST be coupled with at least two other concurrent antigen skin tests, to which most people in a healthy population would be expected to be sensitised[13]. Candida and mumps antigens and tetanus toxoid are frequently used as "companion" antigens to assess for CMI[13].

1.3 Objectives:

The objectives of this study were:

1.3.1 Primary objectives:

1. Define the prevalence of HIV infection in hospitalised children diagnosed

as having tuberculosis.

2. Evaluate the usefulness of concurrent delayed type hypersensitivity skin testing, using the "CMI Multitest®", in the interpretation of the TST.
3. Study the correlation between CD4+ lymphocyte counts and TST.

1.3.2 Secondary objectives:

1. Analyse for differences in the clinical presentation between HIV infected and uninfected children with tuberculosis.
2. Define the patterns of reactivity to TST in HIV infected and uninfected children.
3. Examine the immunologic categorisation of HIV infected children, using CD4+ lymphocyte counts, presenting with TB.

2.0 MATERIALS AND METHODS

2.1 Study site and sample

The study was conducted between August 1996 and January 1997 at the three hospitals attached to the Department of Paediatrics and Child Health of the University of the Witwatersrand. Children between two months and 12 years of age with suspected tuberculosis were referred to one of a team of six paediatric registrars for evaluation. The escort of the child was interviewed for basic demographic information and a clinical history was obtained.

2.2 Ethics and consent

Ethics clearance for the study was obtained from the Committee for Research on Human Subjects, University of the Witwatersrand (Ethics clearance number: M960821). Consent for inclusion into the study- including informed consent for HIV testing where this was not performed by the attending physician, and "CMI Multitest®"[17] application- was obtained from the parent/guardian of the patients. All other investigations performed were part of the routine tests done on children with tuberculosis.

2.3 Patient Recruitment

Initial enrolment was based on the presence of clinical criteria proposed by Ghidey et al[8] and later modified by Migliori et al[9]. The limitations of these and other scoring systems[7] are well recognised; however, they remain the only available tools to conduct prospective studies on TB in children.

Children were enrolled into the study if they fulfilled at least two of the following six criteria:

1. a positive tuberculin skin test, defined as:
   ≥5mm in immunocompromised children
   ≥10mm in immunocompetent children;
2. an adult contact with active TB and/or an adult who had received treatment within the previous 6 months;
3. a chest radiograph (CXR) suggestive of TB;
4. symptoms and signs suggestive of TB;
5. positive Auramine "O" stain on gastric washings or sputum and/or;
6. histology suggestive of TB.

.

2.3.1 HIV testing:

HIV testing was performed following counselling of the parent either by the attending doctor where HIV infection/AIDS was clinically suspected or by an investigator. Children were screened for HIV infection using the third-generation HIV ELISA test [Axsym® system, HIV1/HIV2; Abbot]. Positive results were confirmed with another third generation HIV ELISA test [HIV1/2 ELISA, Murex*]. Children under 15 months of age with a clinical diagnosis of TB who tested positive on the ELISA test, but who were assessed as being clinically asymptomatic for HIV infection, were further evaluated by HIV-1 DNA PCR[18].

2.3.2 Microbiological testing

Children under five years of age, with suspect PTB had early morning gastric washings performed following an overnight fast. Gastric-washings were performed by inserting a nasogastric tube into the stomach and by aspirating the contents of the stomach. Failure to aspirate any gastric fluid was followed by instilling normal-saline into the stomach and repeating the aspirate. The washings were placed into a sterile container and transported to the laboratory. Specimens were not treated with 10% sodium carbonate during the study period. The assistance of a physiotherapist was utilised to obtain sputum in older children. The staff were requested to send at least three specimens, obtained on three different days.

The Auramine "O" technique was used to stain for acid fast bacilli.
Culture for MTB was performed using the Bactec 460TB® (Becton Dickenson laboratories, Maryland, USA) technique and by plating onto Lowenstein-Jensen medium. TB PCR using the insertion sequence IS 6110 as a probe was performed on growth detected by the Bactec system.

2.3.3 Tuberculin skin testing:

Any child in whom the diagnosis of TB was considered was referred to one of the registrars for TST. Participating registrars were briefed as to the method for performing and recording the TST. The standard WHO PPD (2TU of RT23 Copenhagen containing 0.04 ug of PPD of MTB; 0.01 chinosol and 0.005% Tween 80) was used. The TST was performed on either of the forearms using

the Mantoux method[19] and read at 48-72 hours by one of the study investigators. The transverse diameter of the induration was measured by palpation and using a transparent ruler[19].

2.3.4 Radiographic investigations:

2.3.4.1Chest radiographs (CXR):

A CXR was considered to be suggestive of TB if there were any of the following features: hilar lymphadenopathy, miliary pattern (<2mm interstitial infiltrates), cavitation/breakdown of lung parenchyma. In the presence of a positive contact and/or significantly reactive TST and/or positive Auramine "O" stain – any lung infiltrate was considered as evidence of TB. Initial evaluation of the CXR was performed by one of the investigators and the attending paediatrician. Following completion of the study, all available radiographs were read independently by a paediatric radiologist and a paediatrician using a standard reporting form. The opinion of another paediatrician was obtained where there was discordance between the initial two readers. A single radiograph could manifest with more than one feature.

2.3.4.2 Computerised tomographic (CT) scans:

A paediatric neurologist read all CT scans of the brain for the diagnosis of tuberculous meningitis or tuberculoma. Interpretation of all other imaging was based upon the report of the attending radiologist, i.e. CT scan/sonar of the abdomen and bone radiographs. To minimise over/under-reporting of

tuberculosis none of the above were aware of the child's participation in the study.

2.3.5 Other investigations:

The attending paediatrician/s decided which children to investigate for extra-pulmonary (EPTB) based on clinical suspicion. Fine needle aspiration of lymph nodes, biopsy of tissue sites, bone marrow aspirates, lumbar puncture for CSF analysis, lymph node biopsy, liver biopsy and laporotomy for suspected abdominal TB were performed at the discretion of the attending paediatrician.

2.3.6 Categorisation of patients with tuberculosis:

Children were categorised as either having EPTB or PTB. Further categorisation of patients was done based upon the evidence for TB. Patients were classified as follows:

1. Clinical tuberculosis:

   Children on whom the diagnosis of tuberculosis was based on the presence of at least two of the first four criteria described in section 2.3; but in whom there was no microbiologic and/or histologic evidence of tuberculosis.

2. Probable tuberculosis:

   A positive Auramine "O" stain on any specimen submitted for microscopy and/or histology suggestive of tuberculosis in the presence of other clinical features suggestive of TB.

3. Culture-confirmed tuberculosis:

Culture of MTB from any site.

Patients with only PTB were grouped for analysis as either having definite tuberculosis (including those with probable PTB and culture-confirmed cases) or clinical tuberculosis. Patients with EPTB were considered as having definite TB in the presence of a suggestive histology and/or culture of MTB from any site.

2.3.7 Nutritional status

Nutritional status was categorised based on the Wellcome classification for nutrition[20].

In an effort to define the value of CD4+ lymphocyte counts and concurrent skin antigen testing, using the "CMI Multitest®", children enrolled into the study also had CD4+ lymphocyte counts and the "CMI Multitest®" performed when a clinical diagnosis of TB was made.

2.3.8 "CMI Multitest®"

The "CMI Multitest®" (a multipuncture test with the following panel of antigens: tetanus toxoid, diphtheria toxoid, Streptococcus (group C), tuberculin (old), Candida (albicans), trichophyton (mentogrophytes) and Proteus (mirabilis) and glycerine (negative control) was used to assess delayed type hypersensitivity[17]. The test was administered immediately following the diagnosis of tuberculosis and read 48-72 hours later by one of

the six registrars. The test was applied to the forearm or the antero-lateral aspect of the thigh in younger children. The average of the transverse and vertical diameter for each of the antigens was recorded. Patients were categorised as being anergic if they failed to mount a reaction of ≥2mm to any of the six antigens being evaluated. Reactivity to the tuberculin antigen included in the "CMI Multitest" was ignored for the purposes of this study.

2.3.9 CD4+ lymphocyte counts

Peripheral blood was collected in EDTA anticoagulant within 48 hours of the clinical diagnosis of tuberculosis. Whole blood analysis was performed using an Epics XL Flow Cytometer (Coulter Corporation, Hialeah, FL) with a multiloader and the Coulter Multi-Q-Prep system[21].

The Centers for Disease Control (CDC) criteria were used in the immunologic categorisation of patients based on CD4+ lymphocyte counts, using the lower value of either the absolute CD4+ lymphocyte count or percentage CD4+ lymphocyte count, for the appropriate age group[22].

2.4 Statistical analysis

Analysis was performed using Epi Info (Centers for Disease Control and Prevention. USA. Epi Info, version 6.04-July 1996).

Odd ratios and their 95% confidence intervals (CI) were calculated by contingency tables. Categorical variables were performed using the chi-square test. The Fishers exact test was used when any cell had an expected value of less than five observations. Continuous variables were

analysed using the unpaired student t-test. A p value of <0.05 was considered as being statistically significant.

3.0 RESULTS

3.1 Impact of HIV co-infection in children with tuberculosis:

3.1.1 Demographic and HIV results:

3.1.1.1 HIV results

HIV results were available for 161 of 169 patients enrolled in the study. HIV testing was refused for eight children. These children have been excluded from further analysis in this report. Of these eight children, one child had clinical features suggestive of HIV infection and the other seven were clinically asymptomatic for HIV infection. Three of the latter seven patients had EPTB. A total of eight patients had HIV PCR test performed. Of these, seven were positive and one was negative. The latter child was included in the HIV uninfected group.

Of the 161 children included in the analysis, 42.2% were HIV infected. HIV infection was found in 48.9% of patients with PTB and in 4.2% (1/24) of children with EPTB.

3.1.1.2 Age, gender and nutritional status.

Overall, HIV uninfected children were older than HIV infected children (median ages of 20 and 12.5 months respectively, p=0.045). However, there was no difference in the median age of children with PTB between HIV uninfected (14.5 months) and HIV infected (12.0 months) children, p=0.384.

In HIV uninfected children, those with EPTB (median age 32.0 months) were older than those with PTB only (median age 14,5 months), p=0.004. The only HIV infected child with EPTB was 72 months old. Sixty-four percent of children were males in the HIV infected and uninfected groups.

HIV infected children with PTB were more likely to be malnourished compared to HIV uninfected children (p=0.012). The proportion of children with kwashiorkor, marasmus and underweight-for-age were 13.4%, 25.4% and 37.3% in HIV infected children and 14.3%, 11.4% and 25.7% in HIV uninfected children respectively. There was no difference in the nutritional status of HIV uninfected children with PTB compared to children with EPTB (p=0.086).

3.1.2 Microscopy and culture for *Mycobacterium tuberculosis*

Of the 137 children with PTB, three samples were sent in 101 patients; two samples in 26 patients and one sample in four patients. Six patients (four HIV infected and two HIV uninfected) did not have any gastric washing/sputum sent for analysis. In eight patients Auramine "O" stain, but no culture, was performed (four HIV infected and four HIV uninfected).

There was no difference in the frequency of microbiologic results (smear and/or culture) between HIV infected and uninfected children with PTB. In children where samples were sent for culture, *Mycobacterium tuberculosis* was isolated from 35.6% of HIV infected and 26.9% of HIV uninfected

patients (p= 0.28). MTB was cultured from pleural fluid of one HIV uninfected child. Microscopy for a fast bacilli was positive in 17.5% of HIV infected and 16.2% of HIV uninfected children (p=0.84).

Of all those diagnosed with PTB, a clinical diagnosis was made in 58.2% of HIV infected and 62.9% of HIV uninfected children. Probable PTB was diagnosed in 10.4% and 12.8% and culture-confirmed PTB was diagnosed in a total of 31.3% and 24.3% of HIV infected and uninfected children respectively. There was no difference in case categorisation between HIV infected and uninfected children (p=0.701).

3.1.3 Clinical characteristics

There were no differences in the presenting clinical symptoms between HIV infected and uninfected children except for history of weight loss (Table 1).

Table 1: Clinical symptoms between HIV infected (HIV Pos) and uninfected (HIV Neg) children with pulmonary tuberculosis.

	HIV Pos N=67 (%)	HIV Neg N=70 (%)	P=
Cough > 28 days	35 (52.2)	27 (38.6)	0.109
Fever > 14 days	49 (73.1)	43 (61.4)	0.146
Night sweats > 14	19 (28.4)	20 (28.6)	0.978
Loss of weight > 28 days	41 (61.2)	25 (35.7)	0.002
Measles vaccine/illness*	6 (9.0)	5 (7.1)	0.697
Adult TB contact	25 (37.3)	26 (37.1)	0.983
BCG immunisation	65 (97.0)	67 (95.7)	1.0

*Measles vaccination or illness occurring in the previous one month.

Despite receiving BCG vaccination only 48.9% of HIV infected children had BCG scar formation compared to 80.2% of HIV uninfected children (p=0.0001). Clubbing was also more commonly described in HIV infected children (p=0.015). Thirty percent (4/13) of the HIV infected children and 50% (2/4) of HIV uninfected children with clubbing had culture-proven tuberculosis.

HIV infected children also tended to have a poorer outcome than HIV uninfected children. A total of 13.4% (9/67) of HIV infected children and 1.5% of HIV uninfected children diagnosed with tuberculosis died during the course of the study, based on hospital follow-up of patients (p=0.03). In HIV infected children, mortality did not differ between children with a clinical diagnosis of TB compared to cases of definite tuberculosis (p=0.70).

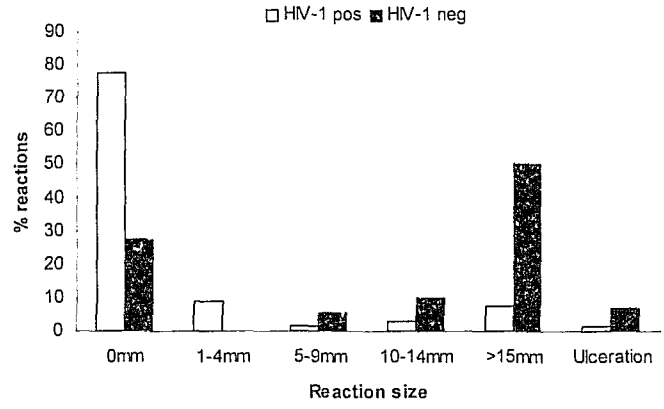
3.1.4 Tuberculin skin test (TST)

Reactivity to TST did not differ between patients with a definite and clinical diagnosis of pulmonary tuberculosis in HIV infected (p=0.58) and HIV uninfected (p=0.166) children. Neither was there a difference in the spectrum of TST reactivity in HIV uninfected children with EPTB compared to those with PTB only (p=0.198).

In children with PTB, 77.6% (52/67) of HIV infected and 27.1% (19/70) of HIV uninfected children with PTB showed no reaction to TST, p<0.0001(figure 1).

Only 13.4% (9/67) of HIV infected children had a reaction of ≥5 mm to TST, of which 88,8% (8/9) had reactions ≥10mm.

Figure1: Tuberculin skin test reactions in children with pulmonary tuberculosis.



HIV infected children were significantly less likely than uninfected children to develop skin reaction sizes to tuberculin at any cut-off level. For example, table 2 shows that only 13.2% of HIV infected children had TST ≥5mm compared to 76.3% of HIV uninfected children, $p<0.0001$.

Reactivity to TST was also lower in HIV infected children regardless of which cut- off level (≥5mm in HIV infected vs. ≥10mm in HIV uninfected) was used ($p<0.0001$ O.R. 0.06, 95%CI 0.02-0.15).

Table 2: Reactivity to tuberculin skin testing in HIV infected and uninfected children with tuberculosis.

	HIV infected N=68 (%)	HIV uninfected N=93 (%)	OR,95%CI
No reaction	53 (77.9)	21 (22.5)	12.11 (5.38-27.74)
≥5mm	9 (13.2)	71 (76.3)	0.05 (0.02-0.12)
≥10mm	8 (11.7)	66 (71.0)	0.05 (0.02-0.14)

P<0.0001 for all strata

HIV uninfected patients with kwashiorkor had significantly lower rates of reactions of ≥10mm to TST than children with normal weight (p=0.002) or children that were underweight-for-age (p=0.005). The rates of TST reactions of ≥10mm in HIV uninfected children were 76.5%, 77.8%, 62.5% and 20% for children with normal weight, underweight-for-age, marasmus and kwashiorkor respectively. In HIV infected children, there were no significant differences in reactions of ≥5mm to tuberculin between different nutritional categories of patients. The rates of reactions of ≥5mm were 23%, 16%, 11.8% and 0% for patients with normal weight, underweight-for-age, marasmus and kwashiorkor respectively.

3.1.5 Chest radiographs (CXR)

A total of 103 chest radiographs (39 HIV infected and 59 HIV uninfected) were available at the end of the study for review by a paediatric radiologist.

The remaining radiographs were sent with the patients to the TB clinics for follow-up. Five (5.4%) of the radiographs were considered to be of a substandard quality to be reported on. These radiographs were excluded from subsequent analysis.

There was no difference in the chest radiographic presentation between clinical and definite cases of PTB in HIV infected and uninfected children. Alveolar consolidation was the most common feature in HIV infected and uninfected children. Pulmonary cavitation and miliary pattern on CXR in patients with PTB occurred more frequently in HIV infected children (Table 3).

Table 3: Radiographic features of children with pulmonary tuberculosis

	HIV infected No=39 (%)	HIV uninfected No=59 (%)	P=
Dense non-lobar consolidation	26 (66.7)	28 (47.5)	0.53
Lobar consolidation	10 (25.6)	10 (16.9)	0.29
Bronchopneumonic changes	5 (12.8)	13 (22.0)	0.25
Interstitial infiltrate	3 (7.7)	3 (5.1)	0.67
Hilar lypmhadenopathy	17 (43.6)	29 (49.2)	0.59
Atelectasis	10 (25.6)	13 (22.0)	0.68
Miliary pattern	4 (10.3)	0	0.019
Effusion	9 (23.1)	9 (15.2)	0.33
Cavitation	12 (30.8)	4 (6.8)	0.001
Endobronchial tuberculosis	3 (7.7)	1 (1.7)	0.29

3.1.6 Extra-pulmonary tuberculosis (EPTB)

Almost all cases (23/24) of EPTB occurred in HIV uninfected children ($p<0.0001$). In HIV uninfected children, patients with EPTB tended to be older than those with PTB only (median age of 32.0 months compared to 14.5 months respectively, $p=0.004$). The only HIV infected child with EPTB (abdominal tuberculosis) was 72 months old. Twelve of the 24 patients with EPTB had a tissue biopsy suggestive of tuberculosis. Five of the 12 also had MTB isolated from gastric washings. A total of 42.1% (8/19) patients with EPTB, who had gastric washings sent, cultured MTB. MTB was isolated in 3 additional patients from a site other than gastric washings (one from a liver biopsy, one from CSF and one from a bone marrow aspirate).

A definite diagnosis of tuberculosis was made in 47.8% of HIV uninfected patients with EPTB and an additional 26.1% (6/23) had a tissue biopsy specimen suggestive of EPTB. The diagnosis of tuberculoma was made on CT scan in three children and tuberculosis meningitis was diagnosed based on CSF findings and a suggestive CT scan in a further three children, in one of whom MTB was isolated from cerebro-spinal fluid. The diagnosis of abdominal tuberculosis in the HIV infected child was based on clinical presentation, CT scan of the abdomen that showed large mesenteric lymph nodes and tissue biopsy showed a suggestive histology. Amongst HIV uninfected children the reticulo-endothelial system was the most common site of extra-pulmonary tuberculosis (Table 4)

Table 4: Site of involvement in patients with tuberculosis

	HIV-1 infected N (%)	HIV uninfected N (%)
Only PTB	67 (99)	70 (75)
Extrapulmonary TB	1 (1)	23 (25)
Liver/Spleen	0	6
Abdomen	1	5
Tuberculoma	0	4
TB meningitis	0	3
Extrathoracic lymph nodes	0	3
Bone (including mastoid)	0	2

.

3.2 CD4+ lymphocyte counts and companion antigen testing "CMI Multitest®" in the interpretation of tuberculin skin tests.

Of the 161 patients with HIV results, 130 patients had CD4+ lymphocyte counts performed and 113 patients were tested for delayed-type hypersensitivity (DTH) reactions using the "CMI Multitest®". Reasons for patients not having tests performed included misplacement of the specimen for CD4+ lymphocytes and refusal on the part of caregivers for further test to be performed on their children. In addition, the "CMI Multitest®" was only obtainable after the study had begun. The eight children who did not have a definite result for HIV were excluded from subsequent analysis.

Microbiologic and/or histologic evidence of tuberculosis was present in 30.7%

(16/52) of HIV infected and 34.6% (27/78) of HIV uninfected children who had CD4+ lymphocytes performed, p=0.65.

3.2.1 CD4+ lymphocyte count

There was no difference in CD4+ lymphocyte categorisation between children with microbiologic and/or histology confirmed tuberculosis compared with those with a clinical diagnosis (HIV infected, p=0.185 and HIV uninfected, p= 0.307). The median CD4+ lymphocyte counts stratified by age is shown in table 5. The median CD4+ lymphocyte counts were severely decreased in HIV infected children and normal in HIV uninfected children for all age-groups.

Table 5: Differences in median CD4+ lymphocyte counts (25th-75th quartiles) between HIV infected and HIV uninfected children with tuberculosis.

	N.	HIV infected		N.	HIV uninfected	P=
All ages	52	660 (224-1274)		78	1523 (1063-2520)	<0.0001
2-12 months	25	965 (436-1530)		26	2148 (1446-2930)	<0.0001
13-60 months	21	643 (326-1108)		32	1581 (1238-2441)	<0.0001
> 5 years	6	148 (15-285)		20	1071 (825-1404)	<0.0001

Nutritional status did not appear to influence the CD4+ lymphocyte count categorisation in HIV infected or uninfected children (Table 6).

Table 6: Association between CD4+ lymphocyte categories and nutritional status of HIV infected and uninfected patients.

	HIV infected CD4+ lymphocyte count				HIV uninfected CD4+ lymphocyte count		
	Normal % (N)	Mod. ↓ % (N)	Severe↓ % (N)		Normal % (N)	Mod. ↓ % (N)	Severe ↓ % (N)
Normal nutrition	23.1 (3)	30.7 (4)	46.2 (6)		81.1(30)	13.5 (5)	5.4 (2)
Underweight-for- age	20.0 (4)	20.0 (4)	60.0(12)		91.3(21)	4.3 (1)	4.3 (1)
Kwashiorkor	16.7 (1)	33.3 (2)	50.0 (3)		84.6(11)	7.7 (1)	7.7 (1)
Marasmic	0 (0)	61.5 (8)	38.5 (5)		80.0 (4)	20.0 (1)	0 (0)
All patients	**15.4 (8)**	**34.6 (18)**	**50.0 (26)**		**84.6 (66)**	**10.3 (8)**	**5.1 (4)**

Legend: Mod ↓=Moderately decreased CD4 counts; Severe ↓=Severely decreased CD4 counts

Fifty percent (26/52) of HIV infected children had severely decreased CD4+ lymphocyte counts and 84.6% (66/78) of HIV uninfected children had normal CD4+ lymphocyte counts. Ninety-three percent (28/30) of HIV uninfected

children with normal CD4+ lymphocyte counts and normal nutritional status had a TST reaction of ≥ 10mm compared to 27.3% (3/11) of patients with kwashiorkor and normal CD4+ lymphocyte counts ($p<0.001$). Sixty-six percent of the latter group of children did not mount any response to TST. There was no such difference in reaction sizes between HIV uninfected patients with marasmus ($p=0.32$) or underweight-for-age ($p=1.0$) compared to children of normal nutritional status. One of the two HIV uninfected children with a normal CD4+ lymphocyte counts and normal nutritional status but hyporeactivity to TST had a recent history of measles.

In well nourished HIV infected children, 66% (2/3) with normal CD4+ lymphocyte counts showed no reaction to TST. Since the overall reactivity to TST was low in HIV infected children, independent of nutritional status, the association between nutrition and CD4+ lymphocytes on TST reactions could not be analysed in this group. One of the four HIV infected children with normal CD4+ lymphocyte count and no reaction to TST had kwashiorkor. There were no other obvious immunosupressive conditions, excluding HIV infection and TB, to account for the hyporeactivity to TST in the remaining patients with normal CD4+ lymphocyte counts in HIV infected and uninfected children.

Differences in TST reactions of ≥10 mm between HIV infected and uninfected children of different immunological categories are shown in table 7. HIV uninfected children tended to have greater reactions to TST in all

immunological categories.

Table 7: Correlation between CD4+ lymphocyte categorisation and tuberculin skin test (TST) reactions in HIV infected and uninfected children.

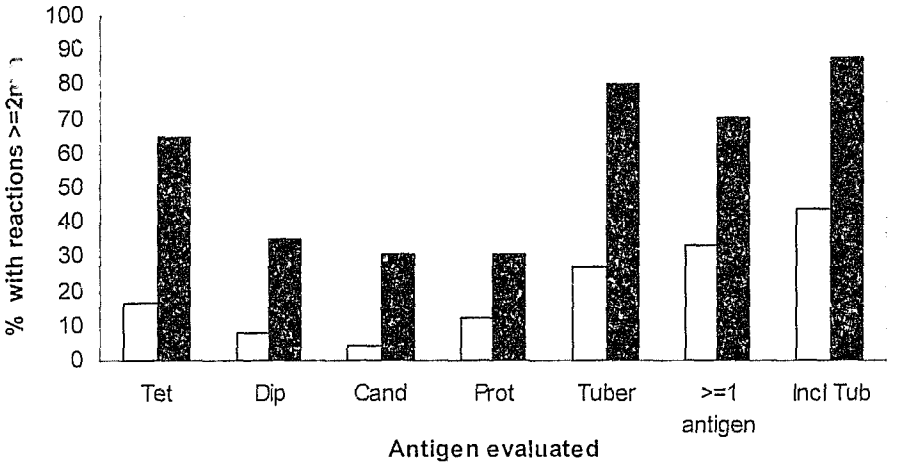
	CD4+ lymphocyte counts			
	Normal	Moderately decreased	Severely decreased	P=
HIV infected TST ≥10mm	50% (4/ 8)	11.1% (2/18)	11.5% (2/26)	0.012
HIV uninfected TST ≥10mm	80.3% (53/66)	75.0% (6/ 8)	50% (2/ 4)	0.35
P=	0.075	0.002	0.075	

In HIV infected children, using the cut-point of ≥5 mm, tuberculin reactions tended to be more frequent (p=0.051) in children with normal compared to children with moderately decreased CD4+ lymphocyte counts and were more frequent than in those with severely decreased CD4+ lymphocyte counts (p=0.037).

3.2.2 CMI Multitest®

In HIV uninfected children, reactivity was greatest to tetanus and diphtheria toxoids (65.0 and 35.4 % respectively), followed by proteus and candida (30.8 % each). HIV infected children had less reactions of ≥2mm to all of the above antigens ($p<0.05$). Reactivity to trichophyton and Streptococcus (group C) were poor (<5%) in both groups of children (Figure 2).

Figure 2: Delayed type hypersensitivity reactions to the "CMI Multitest®" antigens in HIV infected (clear bars) and uninfected (shaded bars) children.



X axis: Tet=tetanus toxoid, Dip=diptheria toxoid Cand=candida; Prot=proteus; Tuber=tuberculin, ≥1 antigen=reaction of ≥2mm to any of the antigens –excluding tuberculin antigen; Incl tub= reaction of ≥2 mm to at least one antigens including

P<005 for reactions to candida and p oteus. P<0.0001 for all other shown observations

One-third (16/48) of HIV infected and 70.1% (46/65) HIV uninfected children mounted a reaction to one of the antigens being analysed (p<0.0001). This increased to 43.8% (21/48) in HIV infected and 87.7% (57/65) in HIV uninfected children, if reaction to tuberculin in the "CMI Multitest®" was included in the analysis (p<0.0001). The odds of HIV infected children testing "anergic" to the evaluated antigens using the "CMI Multitest®" was significantly higher than in HIV uninfected children (OR 4.84, 95%CI 2.02-11 72; p<0.0001).

Anergy to the evaluated antigens did not differ between patients with microbiologic evidence of tuberculosis compared to those with a clinical diagnosis of tuberculosis in HIV infected (p=0.47) and uninfected (p= 0.50) children. In HIV infected and HIV uninfected children there was no difference in reactions (of ≥5mm and ≥10mm, respectively) to TST between children who tested "anergic" and those who tested as having an adequate DTH response to "CMI Multitest®" (p=0.14 and p=0.60 respectively).

The positive predictive value of an intact DTH response to the "CMI Multitest®" in predicting TST reactivity of ≥10mm was 55.% in HIV infected and 76.5% in HIV uninfected children. The negative predictive value was 71.8% and 29.2% at the above-mentioned cut-point in HIV infected and uninfected children respectively.

4.0 DISCUSSION

4.1 Prevalence of HIV co-infection

The prevalence of HIV co-infection in our patients (42.2%) is in the middle of the range of that reported from other areas in Africa, 11-68.6%[6,23,24] and higher than that which is estimated for adults in South Africa[4]. This probably reflects the inherently increased risk of developing disease following infection by MTB in younger children[2] coupled with the immunocompromised state due to co-infection with HIV[2]. It is estimated that 5% of children born in the study area are HIV infected (Gray GE-unpublished data). Against this background, the impact of HIV co-infection on children developing tuberculosis is obvious. South Africa is likely to experience a further rise in HIV-associated tuberculosis, such as that seen in Zambia[23], as the HIV epidemic remains uncontrolled.

The greatest burden of disease appears to be in children under 24 months of age in HIV infected and uninfected children. This is in keeping with observations that the greatest risk of TB infection progressing to disease is in children under 5 years of age and within 2 years of being infected[25]. The early presentation of children with TB at our site reflects the high incidence of tuberculosis in South Africa[4] and consequently the high risk of being infected early in life.

4.2 Microbiologic diagnosis

The culture of MTB from gastric washings in our patients was similar between HIV infected and HIV uninfected children, 35.6% and 26.5 % respectively. The yield from gastric washings in HIV infected children was higher than that demonstrated in Cote d'Ivoire[24] and similar to the yield obtained from other studies in HIV uninfected children[26,27,28]. The trend towards a higher yield of MTB form gastric washings in HIV infected children may have been a consequence of the increase in progressive pulmonary tuberculosis.

A number of cases (3 HIV infected and 7 HIV uninfected) with a positive Auramine "O" stain on gastric washings did not yield MTB on culture. Possible reasons for this include, delay in processing, inadequate storage of the specimen and death of the organism before being plated for culture. An additional reason for this could be that the organisms identified were other saprophytic acid-fast bacilli colonising the stomach. Since these patients required at least one other criteria for inclusion into the study, we think that they were more likely to be true cases of tuberculosis. The problems mentioned may have been lessened if specimens were treated immediately with sodium-carbonate following collection and more detail was spent on the transport and the storage of the specimens[29].

Despite these problems, the yield of MTB from gastric washings in HIV infected children was significantly higher than reactions of ≥5mm to tuberculin skin test (p=0.003). In HIV uninfected children, significant TST reactions were more common than culture of MTB from gastric washings (p=<0.0001).

4.3 Tuberculin skin testing

It is estimated that approximately 10-25% of patients with tuberculosis will not react to TST[26,27,28,30], and this may reach 50% in critically ill patients with tuberculosis[10]. The recommendation for the interpretation of TST in HIV infected patients was to use a lower cut-off value (≥5mm) as being suggestive of tuberculosis[13,31]. In our cohort of patients, 78% of HIV infected children did not mount any response to TST. Eighty-nine percent of HIV infected patients mounting a response of ≥ 5 mm tended to have induration sizes of ≥10mm. This suggest that based on present guidelines[13,31], lowering the cut-off level to be considered as significant in HIV infected patients does not improve the sensitivity of the TST in diagnosing tuberculosis. An additional 8.8% of patients had some reaction to the TST (≥2mm). If any reaction to TST were considered as significant in HIV infected children, this would only increase the yield of "significant" reactions to 22.1%.

The data also show that HIV infected children are 16 fold less likely to have a "significant" reaction to tuberculin compared to HIV uninfected children. It appears that TST, whilst still useful in HIV uninfected children, is of limited use in assisting in diagnosing tuberculosis in HIV infected children.

These results support data from other studies. In one study, no cases of culture confirmed cases of tuberculosis in children with AIDS had any reaction to TST[11]. The data from other studies suggest the reactivity to TST of ≥10mm

vary between HIV infected and uninfected children between 0-38% and 70% respectively[6,11,24,32]. As in our study, others have shown that lowering the cut-off level of TST to be considered as significant in HIV infected children does not appear to increase the number of significant reactions appreciably[24].

The above data suggest caution, even when using a reading of ≥5 mm as a cut-off, in using TST as a screening test when suspecting tuberculosis in HIV infected children. The threshold of clinical suspicion in HIV infected children needs to be low and they probably should be started on TB treatment earlier rather than later. The rapid progression of HIV infection in African children[33] may in part be explained by this dual infection with tuberculosis going by undiagnosed. The sinister interaction between tuberculosis and HIV infection results in infection by *Mycobacterium tuberculosis* progressing to disease 4-8 fold more common in HIV infected adults[34]. Furthermore, infection by MTB appears to hasten the course of HIV infection by activation of the immune system[34].

4.4 Clinical differences in presentation

Apart from signs and symptoms that are familiar to HIV infection and tuberculosis (i.e. weight loss, underweight-for-age) the clinical presentation of HIV infected children did not differ to those in uninfected children. HIV infected children in this study did not have increase contact with possible infectious adult TB cases. Since we did not have a control group, the general rate of exposure of HIV infected children to adult cases of tuberculosis cannot

be commented on as suggested in the study from Cote' d'Ivoire[24].

When comparing clinical signs in our study population, HIV infected children had a higher rate of clubbing and less BCG scar presence. The former may be explained due to an increase in complicated progressive pulmonary tuberculosis, including cavitation, and chronic lung disease in HIV infected children. Forty-five percent of HIV infected children were diagnosed as being HIV positive prior to their admission at which the diagnosis of tuberculosis was made. Undiagnosed lymphocytic interstitial pneumonitis may be a confounding factor, however the children in this study are younger than those in whom the diagnosis of LIP is usually made at the study site (Dr K Simmank-unpublished data).

Although BCG does not correlate with TST conversion[35,36], the lower incidence of a BCG scar observed in this study is intriguing. Explanations for this observation include failure to mount an immune response to BCG vaccination at birth, possibly due to an already compromised immune system in children infected in-utero or peri-natally by HIV. Other studies have not shown any difference in BCG scar presence[6,24]. Failure to develop BCG scars following immunisation in HIV infected children may suggest early impairment of the cell mediated immune system. Further evaluation of this finding is warranted.

The mortality data from our study was limited to the duration of the study

period. The mortality rate was significantly higher in HIV infected (13.4%) children as shown in other studies that had a more structured follow-up for mortality[23,24]. The cause of death may not have been directly related to tuberculosis as most patients died following readmission for acute lower respiratory tract infections.

Unlike the study in Cote d'Ivoire[24], our study suggests that cavitation and miliary-pattern on CXR occurs more commonly in HIV infected children. The increased number of cytolytic CD8+ lymphocyte cells and impaired monocyte function, coupled with a decreased number and dysfunctional CD4+ lymphocyte cells in HIV infected patients[37], may explain the progressive pulmonary tuberculosis in HIV infected children despite most cases presumably being primary rather than reactivation of latent MTB.

4.5 Extra-pulmonary tuberculosis

Significantly, HIV uninfected children with EPTB were older than those diagnosed with PTB only. This may relate to the clinical expression of EPTB being mainly dependent on the host immune response and tissue destruction occurring over a period of time. Unlike other studies[24], the diagnosis of EPTB was uncommon in HIV infected children. A possible explanations for this include may be that, due to an impaired host response to MTB, despite miliary spread having occurred, HIV infected children do not have an overt clinical expression of their illness compared to HIV uninfected children. In addition 50% of HIV infected children die within the first 2 years of life at our

site (Gray GE-unpublished data). The small group of HIV infected children surviving beyond 2 years of age will steadily increase with time, and we could possibly face an increase in the number of HIV infected children diagnosed with EPTB. The older age of HIV uninfected patients with EPTB and the fact that the majority of EPTB in adult studies are diagnosed on blood cultures[36] supports this hypothesis. In addition to the above, the tendency is to investigate HIV infected children less aggressively. This may bias the case detection of EPTB in HIV infected children. Thirty-nine percent of EPTB in HIV uninfected children involved extra-thoracic lymph nodes and/or the liver and spleen. Involvement of the latter sites in HIV infected children would have possibly been attributed to HIV infection and therefore would not have been investigated.

4.6 CD4+ lymphocyte counts and tuberculin skin testing

The immunologic categorisation of HIV infected children is based on the CD4+ lymphocyte count [22]. The limitations of using CD4+ lymphocyte counts as a marker of immunocompetency in HIV infected children includes the large range for normal values[9,39] and that CD4+ lymphocyte counts do not reflect on the functional capacity and subset fraction of these cells[40]. In our study, the mean CD4+ lymphocyte count was significantly lower in all age groups of patients that were HIV infected. The finding that HIV infected children with normal and moderately decreased CD4 counts had lower reactivity to TST suggest that the CD4+ lymphocyte cells are dysfunctional and/or other factors involved in reactivity to TST are impaired in HIV infected patients.[37,41] The

potential role of serum suppressor factors[42], tuberculin antigen excess[43], genetic factors[44], impaired monocyte function[45,46] and sequestration of sensitised T lymphocytes[47] have all been implicated in explaining the absence of reaction to tuberculin in patients with tuberculosis. Malnutrition, particularly kwashiorkor, was responsible for a high percentage of hyporeactivity to TST in HIV uninfected children with normal CD4+ lymphocyte counts in our study[48].

The data suggest that CD4+ lymphocyte counts are of limited value in the interpretation of TST in HIV infected children. Although HIV infected children with normal CD4+ lymphocyte counts are more likely to have TST reactions of ≥ 5mm, only 15% of HIV infected children with tuberculosis had normal CD4+ lymphocyte counts at diagnosis, and of these 50% had a reaction of ≥ 5mm. In HIV infected adults, a small percentage of individuals with CD4+ lymphocyte counts of <200 cells/ul will mount a response to TST; however anergy to tuberculin appears to be greatest in HIV infected adults with a CD4+ lymphocyte count of < 200 cells/ul[14]. In our study, 11.5% of children with severely decreased CD4+ lymphocyte counts had TST reactions of ≥ 5mm.

Further limitations of using CD4+ lymphocyte counts in predicting reactivity to TST is that tuberculosis causes a decrease in CD4+ lymphocyte count as well as impairs CD4+ lymphocyte function independently of other diseases[49,50,51]. Improvement of CD4+ lymphocyte counts and function have been described

in HIV infected and uninfected patients following the initiation of anti-tuberculosis therapy[50,52]. In our study, 8.6% and 4.3% of HIV uninfected patients had moderately and severely decreased CD4+ lymphocyte counts respectively. Also, as mentioned, CD4+ lymphocyte cells are only one of many cellular elements involved in cell mediated immunity as measured by delayed type hypersensitivity[10,53]. This poor correlation between CD4+ lymphocyte cell counts and reactivity to tuberculin has been demonstrated previously in children and adults[14,54].

4.7 Concurrent antigen testing and tuberculin skin testing

DTH testing offers a potential advantage over CD4+ lymphocyte counts in that it provides a measure of the functional capacity of the immune system. Aside from an intact CMI system, reactivity to antigens also depend upon sensitisation of the individual to a particular antigen, the type and quantity of antigen used and local skin factors[55]. Absence of reaction does not necessarily imply an impaired CMI system.

Until 1997, the CDC recommended that TST be interpreted in conjunction with skin testing using at least 2 other common antigens to which the patient had possibly been sensitised[13]. There are no other studies evaluating the usefulness of concurrent antigen testing in interpreting the TST in children with tuberculosis. The most commonly used antigens in assessing in vivo cell mediated immunity are candida antigen and tetanus toxoid. The latter has the advantage of almost certain priming of the host to this antigen by previous

exposure due to immunization[56,57]. The majority of immunised children have a · ,eous delayed type hypersensitivity reaction to tetanus toxoid[57,58]. However, there are conflicting results regarding the value of tetanus toxoid as a suitable antigen in assessing delayed type hypersensitivity[59]. In young children, delayed type hypersensitivity reaction to candida antigen ap, ars to be less compared to tetanus and diphtheria toxoids[60.61].

In an effort to expose children to a multiplicity of potential recall DTH antigens, the "CMI Multitest®" was used to evaluate the DTH and for purposes of correlation to the TST. This test has previously been evaluated as being a reliable substitute for conventional intradermal methods of evaluating DTH in children[61]. In one study, 93% of infants in the USA reacted to at least one of the antigens[61]. In our study, 70.1% of HIV uninfected and 33.3% of HIV infected children had a reaction $\geq$ 2mm to at least 1 of the 6 antigens in the test. Reactivity to tetanus toxoid was most common in HIV infected and uninfected children. The low reactivity to trichophyton and Streptococcus (group C) has been demonstrated in healthy children[61]. The overall pattern of reactivity to antigens was similar to that found in healthy American children, except that reaction to tuberculin antigen was greater in our patients. The lower reactivity of HIV uninfected children in our study to antigens as a whole may be due to the presence of tuberculosis and possibly related to geographic and national differences in reactivity to recall antigens[17,55]. In our study, no adjustments were made for the interpretation of the "CMI Multitest®" in HIV infected children as we were aiming to assess the

correlation of anergy testing with TST reactivity.

The higher "anergy" rate in HIV infected children was not surprising. The poor positive predictive value of the "CMI Multitest®" in HIV infected and HIV uninfected children supports the revised recommendations of the CDC, not to use companion antigen testing when performing TST, even in our setting[60] The paradox that some patients with advanced HIV disease may respond to delayed-type hypersensitivity testing[63] coupled with the possibility of selective anergy to PPD[64,65] further limits the role of concurrent antigen testing in predicting reactivity to the TST.

5.0 STUDY LIMITATIONS

The limitations of this study include that cases were referred by primary-health-care centers and that cases of tuberculosis diagnosed and treated on an out-patient basis were not included. Since most of the cases diagnosed in the latter setting are based on TST (≥10mm reaction) and chest X-rays, the latter group of patients are most likely to be HIV uninfected. This might therefore over-estimate the true prevalence of HIV infection in all children with tuberculosis. Lack of a standard approach to investigating children for EPTB may explain the relatively low occurrence of EPTB in HIV infected children.

Difficulty in conducting prospective studies regarding tuberculosis may have biased the selection of patients included in the study. In addition, lack of suitable scoring systems for diagnosing tuberculosis in HIV infected children

may have resulted in incorrect diagnoses of tuberculosis being made in this group. However the group were highly likely to have had tuberculosis considering the number of patients who had a positive microbiological diagnosis, in keeping with other studies.

6.0 CONCLUSIONS

There is a high prevalence of HIV co-infection in children with tuberculosis. Although the clinical features suggestive of tuberculosis are similar between HIV infected and uninfected children, the former group of patients appear to have more progressive PTB. This is reflected in the higher occurrence of miliary pattern and cavitation on chest radiographs. In addition, HIV infected children with TB have a higher mortality rate.

The data presented suggest that current cut-off values, for TST, used to assist in the diagnosis of tuberculosis in HIV infected children may greatly under-diagnose tuberculosis in this highly susceptible group of patients. The sinister interaction of tuberculosis and HIV infection may in part explain the rapid progression of AIDS in African children[33]. Scoring systems currently proposed to help in diagnosing tuberculosis in children[7,66], make little adaptation for HIV infected children. Despite the limitations of gastric washings in making the diagnosis of tuberculosis, the results suggest this to be a more useful test than TST in making the diagnosis of tuberculosis in HIV infected children. Therefore, clinical suspicion coupled with appropriate microbiological investigation is vital in making the diagnosis of TB in HIV infected children.

Neither, CD4+ lymphocyte counts or concurrent antigen testing was of value in the interpretation of TST reactivity in HIV infected children. The results support the revised guidelines, not to perform concurrent antigen testing,

when performing the TST in HIV infected children suspected of having TB[62].

TST needs to be replaced by more aggressive investigating of HIV infected patients, including gastric washings, when the diagnosis of tuberculosis is suspected. In addition, studies evaluating tissue biopsies (lymph nodes, liver and bone marrow) and blood cultures for MTB in children need to be considered. In the meantime, we recommend that in areas with a high prevalence of tuberculosis, the threshold for starting TB treatment in HIV infected children should be low.

REFERENCES:

1. Kochi A. The global tuberculosis situation and the new control strategy of the World Health Organization. Tuber Lung Dis 1991; 72:1-6.

2. Raviglione MC, Snider DE, Kochi A. Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. JAMA 1995;273:220-226.

3. World Health Organization. Childhood tuberculosis and BCG vaccine: EPI update supplement. Geneva, Switzerland: World Health Organization; 1989.

4. Weyer K, Fourie PB. Report: Assessment of the tuberculosis epidemic in South Africa. Historical perspective and critical information. Tuberculosis control programme review task group. 1996.

5. Mertens TE, Low Beer D. HIV and AIDS: where is the epidemic going? Bull World Health Organ 1996;4:121-129.

6. Jeena PM, Mitha T, Bamber A, Wesley A, Coutsoudis A, Coovadia HM. Effect of human immunodeficiency virus on tuberculosis in children. Tuber Lung Dis 1996;77:437-443.

7. WHO. TB/HIV- A clinical manual. 1996; 61-67.

8. Ghidey Y, Habte D :Tuberculosis in Childhood: an analysis of 412 cases. Ethiop Med J 1983; 21:161-167.

9. Migliori G B, Borghesi A, Rossanigo P, et al. Proposal for an improved score method for the diagnosis of pulmonary tuberculosis in childhood in developing countries. Tuber Lung Dis 1992; 73: 145-149.

10. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. Clin Infect Dis 1993;17:968-75.

11. Nachman SA, Navaie-Waliser M. Tuberculin skin testing in a paediatric population exposed to human immunodeficiencey virus. Pediatr Infect Dis J 1996,15:1041-1042.

12. Graham NMH, Nelson KE, Solomon L, et al. Prevalence of tuberculin positivity and skin test anergy in HIV-1 seropositive and seronegative intravenous drug users. JAMA 1992;267:369-73.

13. Centers for Disease Control. Purified protein derivative (PPD)- tuberculin anergy and HIV infection: guidelines for anergy testing and management of anergic persons at risk of tuberculosis. MMWR 1991;40:27-33.

14. Huebner RE, Schein MF, Hall CA. Barnes SA. Delayed-type hypersensitivity anergy in Human Immunodeficiency Virus-Infected persons screened for infection with *Mycobacterium tuberculosis*. Clin Infect Dis 1994;19:26-32.

15. Colebunders RL, Lebughe I, Nzila N, et al. Cutaneous delayed-type hypersensitivity in patients with human immunodeficiency virus infection in Zaire. J Acquir Immune Defic Syndr Hum Retrovirol 1989;2:576-8.

16. Markowitz N, Hansen NI, Wilcosky TC, et al. Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. Ann Intern Med 19xx;119:185-93.

17. Kniker WT, Anderson CT, Roumiantzeff M. The multi-test system: A standardized approach to evaluation of delayed hypersensitivity and cell-mediated immunity. Ann of Allergy 1979;43:73-79.

18. Lyons S, Bowers ET, McGillivray GM, Blackburn N, Gray GE. Evaluation of the MUREX ICE HIV-1.0.2 capture immunoassay for early identification of HIV-1 seroverting infants in a developing country. Clin Diag Virol 1997;8:1-8.

19. Arnadottir T, Rieder HL, Tre'bucq A, Waaler HT. Guidelines for conducting tuberculin skin test surveys in high prevalence countries. Tuber Lung Dis

1996;77(Suppl 1):1-19.

20. Anonymous. Classification of infantile malnutrition. Lancet 1970;2:302-3.

21. Sherman GG, Galpin JS, Patel JM, et al. CD4+ T cell enumeration in HIV infection with limited resources. J Immunol Methods 1998;222:209-217.

22. Centres for Disease Control and Prevention. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. MMWR 1994; 43:1-19.

23. Luo C, Chintu C, Raviglione M, Diwan V, duPont HL, Zumla A. Human immunodeficiency virus-1 infection in Zambian children with TB: Changing seroprevalence and evaluation of a Thioacetazone-free regimen. Tuber Lung Dis 1994;75:110-115.

24. Mukadi YD, Wiktor SZ, Coulibaly IM, et al. Impact of HIV infection on the development, clinical presentation and outcome of tuberculosis among children in Cote d'Ivoire. AIDS 1997;11:1151-8.

25. Miller FJW, Seale RME, Taylor MD. Tuberculosis in children. Boston: Little, Brown. 1963.

26. Starke JR, Taylor-Watts KT. Tuberculosis in the pediatric population of

Houston, Texas. Pediatrics 1989;84:28-34.

27. Starke JR. modern approach to the diagnosis and treatment of tuberculosis in children. Pediatr Clin North Am 1988;35:441-464.

28. Snider DE, Riedor HL, Combs D, Bloch AB, Hayden CH, Smith MHD. Tuberculosis in children. Pediatr infect Dis J 1988;7:271-278.

29. Hanna BA. Diagnosis of tuberculosis by microbiologic techniques. In: Rom WN, Garay S. Tuberculosis. New York: Little, Brown and Company 1996;11:149-152.

30. Sbarbaro JA. Skin testing in the diagnosis of tuberculosis. Semin Respir Infect 1986;1:234-238.

31. Committee on infectious diseases. Update on tuberculosis skin testing of children. Pediatrics 1996;97:282-284.

32. Chan SP, Birnbaum J, Rao M, Steiner P. Clinical manifestation and outcome of tuberculosis in children with acquired immunodeficiency syndrome. Pediatr Infect Dis J 1996;15:443-7.

33. Lepage P, Spira R, Kalibala S, et al. Care of numan imunodeficiency virus-infected children in developing countries. International Working

Group on Mother-to-Child Transmission of HIV. Pediatr Infect Dis J 1998;17:581-586.

34. Murray JF. Tuberculosis and human immunodeficiency virus infection during the 1990's. Bull Int Union Tuberc Lung Dis 1991;66:21-25.

35. Fine PEM, Sterne JAC, Pönnighaus JM, Rees RJW. Delayed-type hypersensitivity, mycobacterial vaccines and protective immunity. Lancet 1994;344:1245-1249.

36. Hart PDA, Sutherland I, Thomas J. The immunity conferred by effective BCG and vole bacillus vaccines, in relation to individual variations in tuberculin sensitivity and to technical variations in the vaccines. Tubercle 1967;48:201-210.

37. Lane HC, Depper JM, Greene WC, Whalen G, Waldmann TA, Fauci AS. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. N Engl J Med 1985;313:79-84.

38. Barnes PF, Bloch AB, Davidson PT, Snider Jr. DE. Tuberculosis in patients with human immunodeficieny virus infection N Engl J Med 1991;324:1644-1650.

39. Erkeller-Yuksel FM, Deneys V, Yuksel B, et al. Age-related changes in

human blood lymphocyte subpopulations. J Pediatr 1992;120:216-222.

40. Zhang M, Gong J, Iyer DV, et al. T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. J Clin Invest 1994;94:2435-2442.

41. Rose RM. Immunology of the lung in HIV infection: the pathophysiologic basis for the development of tuberculosis in the AIDS setting. Bull Int Union Tuber Lung Dis 1991;66:15-20.

42. Wieczorek Z. Characterisation of lymphocyte E rosette inhibitory factor in the sera from tuberculosis patients. Arch Immunol Ther Exp 1977;25:63-68.

43. Edwards D, Kirkpatrick CH. The immunology of mycobacterial disease. Am Rev Respir Dis 1986;134:1062-1071.

44. Buckley CE. HLA B7 associated tuberculin hyporesponsiveness in BCG treated melanoma patients. Monogr Allergy 1977;II:97-105.

45. Waxman J, Lockshin M. In vitro and in vivo cellular immunity in anergic miliary tuberculosis. Am Rev Respir Dis 1973;107:661-664.

46. Campbell PB. Defective leukotaxis in monocytes from patients with

pulmonary tuberculosis. J Infect Dis 1979;139:409-417.

47. Rook GAW, Carswell JW, Stanford JL. Preliminary evidence for the trapping of antigen specific lymphocytes in the lymphoid tissue of anergic tuberculosis patients. Clin Exp Immunol 1976;26:129-132.

48. Jayalakshmi VT, Gopalan C. Nutrition and tuberculosis. I. An epidemiological study. Indian J Med Res 1958;46:87-92.

49. Swanson Beck J, Potts RC, Kardjito T, Grange JM. T4 lymphopenia in patients with active pulmonary tuberculosis. Clin Exp Immunol 1985;60:49-54.

50. Singhal M, Banavalikar JN, Sharma S, Saha K. Peripheral blood T lymphocyte subpopulations in patients with tuberculosis and the effect of chemotherapy. Tubercle 1989;70:171-178.

51. Katz P, Goldstein RA, Fanci AS. Immunoregulation in infection caused by *M. tuberculosis*: the presence of suppressor monocytes and the alteration of subpopulations of T lymphocytes. J Infect Dis 1979;140:12-21.

52. Martin DJ, Sim JG, Sole GJ, et al. CD4+ lymphocyte count in African patients co-infected with HIV and tuberculosis. J Acquir Immune Defic Syndr Hum Retrovirol 1995;8:386-391.

53. Maher J, Kelly P, Hughes P, Clancy L. Skin anergy and tuberculosis. Resp Med 1992;86:481-484.

54. Nachman SA, Navaie-Waliser M. Tuberculin skin testing in a pediatric population exposed to human immunodeficiency virus. Pediatr Infect Dis J 1996;15:1041-1042.

55. Frazer IH, Collins EJ, Fox JS, Jones B, Oliphant RC, Mackay IR. Assessment of delayed-type hypersensitivity in man: A comparison of the "Multitest" and conventional intradermal injection of six antigens. Clin Immunol and Immunopath 1985;35:182-190.

56. Whittingham S, Feery B, Mackay IR. Use of tetanus toxoid for testing cell-mediated immunity. Aust N Z J Med 1982;12:511-514.

57. Johnson C, Walls RS, Ruwoldt A. Delayed hypersensitivity to tetanus toxoid in man: in vivo and in vitro studies. Pathology 1983,15:369-372.

58. Facktor M, Bernstein R, Fireman P. hypersensitivity to tetanus toxoid. J Allergy Clin Immunol 1973;52:1-12.

59. Delafuente JC, Eisenberg JD, Hoelzer DR, Raymond SG. Tetanus toxoid as an antigen for delayed cutaneous hypersensitivity. JAMA

1983;249:3209-3212.

60. Steele R, Suttle D, LeMaster P, et al. Screening for cell mediated immunity in children. Am J Dis Child 1976;130:1218-1221.

61. Kniker WT, Lesourd BM, McBride JL, Corriel RN. Cell-mediated immunity assessed by multitest CMI skin testing in infants and preschool children. Am J Dis Child 1985;139:840-845.

62. Centre for Disease Control. Anergy skin testing and preventive therapy for HIV-infected persons: Revised recommendations. MMWR 1997,46:1-10.

63. Chin DP, Oamond D, Page-Shafer K, et al. Reliability of anergy skin testing in persons HIV infection. Am J Respir Crit Care Med. 1996;153:1982-4.

64. Nash DR, Douglas JE. Anergy in active pulmonary tuberculosis: a comparison between positive and negative reactors and an evaluation of 5 TU and 250 TU skin test doses. Chest 1980;77:32-7.

65. Rooney JJ, Crocco JA, Kramer, et al. Further observations on tuberculin reactions in tuberculosis. Am J Med 1976; 60:517-522.

66. Fourie PB, Becker PJ, Festenstein F, et al. Procedures for developing a

simple scoring method based on unsophisticated criteria for screening children for tuberculosis. Int J Tuberc Lung Dis 1998;2:116-123.

Author Madhi S A
Name of thesis Impact On Hiv-1 Co-Infection On Tuberculosis And Value Of Cd4+ Lymphocyte And Concurrent Antigen Testing In Interpretation Of Tuberculin Reactions In Hospitalized Children With Tuberculosis In South Africa Madhi S A 1999

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.