Assessment of airway compression on chest radiographs in children with pulmonary tuberculosis

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A research report submitted to the Faculty of Health Sciences, University of the Cape Town, in partial fulfilment of the requirements for the degree of Master of Medicine in Diagnostic Radiology, MMed (Rad D)

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This is dedicated to my supportive husband.

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Declaration

I, Lisel Verena Richter-Joubert, declare that this research report is my own work. It is being submitted for the degree of MMed (RadD) at the University of Cape Town.

The article has been published in the journal of Pediatric Radiology (DOI 10.1007/s00247-017-3887-9) and has been presented as an oral presentation at the annual congress of the European Society of Paediatric Radiology in May 2017 held in Switzerland as part of and after the registration of the master's degree mentioned above. It has not been submitted before for any degree or examination at this or any other University.

Signed by candidate

Dr Lisel Richter-Joubert

On this 10st day of July 2017

Abstract

Study rationale:

Diagnosis of pulmonary tuberculosis (PTB) in children relies heavily on chest radiography as sputum samples are difficult to obtain and only yield positive results in 30-74% of children treated for PTB. However, radiological signs between lower respiratory tract infections (LRTI) and PTB overlap considerably and there is a wide inter-observer agreement in the detection of lymphadenopathy, considered the hallmark of PTB. Small pliable paediatric airways are easily compressed by enlarged lymph nodes. Unlike lymph nodes, however, the lucent airways contrast against the surrounding mediastinal structures on radiographs, thus airway compression may serve as a more objective criterion for diagnosing PTB. Many studies have reviewed the radiographic features of PTB in children but few included airway compression or used a control group and none have evaluated inter-observer agreement.

Objective:

To investigate frequency and inter-observer agreement of airway compression on chest radiographs in children with PTB compared to those with another LRTI.

Methods:

Chest radiographs of children admitted to Red Cross War Memorial Children's Hospital with suspected PTB were read by two readers according to a standardised format and a 3rd when there was disagreement. Radiographs of children with definite PTB were compared to those with another LRTI. Frequency and location of airway compression were evaluated. Findings were correlated with human immunodeficiency virus (HIV) infection and age. Inter-observer agreement was assessed using kappa statistic.

Results:

Radiographs of 505 children (median age 25.9 months [IQR 14.3-62.2]) were reviewed; 97/505 (19%) children were HIV-infected. Airway compression occurred in 54/188 (28.7%) definite PTB cases versus 24/317 (7.6 %) of other LRTI cases (OR 4.9; 95%CI 2.9–8.3). The left main bronchus was most affected in 51/493 (10.3%). A higher frequency of airway compression occurred in infants at 22/101 (21.8%) compared

to 56/404 (13.9%) in older children (OR 1.7; 95%CI 1.00–3.00). No association between airway compression and HIV infection was found. Inter-observer agreement ranged from none to fair (kappa of 0.0-0.4).

Discussion:

The overall frequency of airway compression in definite PTB is compatible with reports in the literature. Although airway compression used alone is not a specific sign, if seen on radiographs, there is a strong correlation with PTB compared to other LRTI with infants at higher risk due to their smaller airways. Contradictory to other studies, our study showed the left main bronchus to be affected twice more commonly than the bronchus intermedius in both age groups. This is thought to be due to different patient selection. Confirming reports in the literature, no significant association between airway compression and HIV status was found. A disappointing finding was the poor inter-observer agreement. Contributing aspects include the lack of standardised criteria in the definition of airway compression and suboptimal visualisation of the airways on standard chest radiographs due to patient, technical and post processing factors.

Conclusion:

There is a strong association between airway compression on chest radiographs and definite PTB, particularly in infants, irrespective of HIV status. However, its clinical use as an objective criterion in the diagnosis of PTB is limited by poor inter-observer agreement.

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Please note:

Tables and figures are embedded within the literature review and article for ease of reference.

Referencing style and text formatting required by the journal was used throughout the dissertation for consistency.

Abbreviations

BI: bronchus intermedius

CT: computerised tomography

CXR: chest radiograph

ELISA test: enzyme-linked immunosorbent assay test

HIV: human immunodeficiency virus

IQR: interquartile range

LBTB: lymphobronchial tuberculosis

LMB: left main bronchus

LRTI: lower respiratory tract infection

OR: odds ratio

PCR test: polymerase chain reaction test

PTB: pulmonary tuberculosis

RMB: right main bronchus

TST: tuberculin skin test

WHO: World Health Organization

95% CI: 95% confidence interval

Chapter 1: Introduction and Literature Review

The diagnosis of pulmonary tuberculosis (PTB) in children can be challenging [1, 2]. Samples for testing are often not obtained from children and those that are, despite using the best diagnostic tests available, still yield low results [3–5]. The gold standard for diagnosis, microscopy and culture of induced sputum or gastric aspirates, only yields positive results in around 30-50% of children treated for PTB [4, 6]. The newest diagnostic tool, Xpert, identified 74% of culture positive cases in a study conducted in hospitalised children in Cape Town [7]; a second study reported that Xpert was positive in approximately 65% of ambulatory children with culture positive disease [2]. Thus diagnosis of PTB in children still relies heavily on epidemiological, clinical and radiological findings [4, 5].

The chest radiograph (CXR) remains an important investigation, which is readily available, provides diagnostic information and may show associated complications. However, interpretation of CXR may be highly variable as it is a 2-dimensional grey scale modality where lymph nodes are difficult to discern from the other overlapping vascular and mediastinal structures. Hence, poor inter-observer agreement for the detection of hilar lymphadenopathy, a cardinal radiological sign for diagnosis of PTB, has been reported [3, 8, 9]. Well defined, diagnostic features on CXR are needed for childhood PTB [10]. Previous work using computed tomography (CT) has shown a 30% prevalence of airway compression due to lymphadenopathy in children with suspected PTB [11] and a 100% prevalence of airway compression in children with a diagnosis of PTB and symptoms of airway compression [12]. The lucent airways contrast against the surrounding mediastinal structures on plain radiography, thus using airway compression as a surrogate for the presence of enlarged lymph nodes may therefore serve as a more accurate diagnostic feature in children.

The aim of this study was to investigate the frequency and inter-observer agreement of airway compression on chest radiographs in children diagnosed with PTB compared to those diagnosed with lower respiratory tract infection (LRTI) from another cause, thereby determining its utility as an objective diagnostic sign for pulmonary TB.

Due to the increased risk of PTB in HIV-infection and young children [4], additional aims were to correlate these findings with the child's HIV status and age, expecting that a higher prevalence of airway compression may occur in these groups due to immature or suppressed immune systems that may not contain disease.

1.1. Global tuberculosis epidemiology

One of the World Health Organization's goals is to "eliminate TB as a public health problem by 2050" [1]. While some countries (Americas, South East Asia and Western Pacific) have reached the WHO goals for 2015, many of the high burden countries (22 countries in Africa, Eastern Mediterranean and European regions) still have much to achieve in this regard [1, 13, 14]. In 2012 alone 8.6 million people contracted TB [1] and this increased to 10.4 million in 2015 of which 1 million were children under 15 years of age [15]. However, this is likely to be an underestimate as childhood TB is paucibacillary and often smear negative [5, 7]. Current estimates are that children comprise 10-20% of the TB caseload in high burden countries [16]. The addition of HIV co-infection increases the burden with HIV being "the strongest risk factor to develop active TB" [17]. The prevalence of HIV infection in children with TB, in moderate to high burden countries, is estimated to range from 10-60% [18]. The mortality of HIV negative children rose from 74 000 in 2012 (8% of total TB deaths) [1] to 169 000 (13%) in 2015 [15], while the mortality of HIV-infected children with TB stood at 41 000 (10%) in 2015 [15].

1.2. South Africa

Approximately 26% of global TB cases reside in Africa [15]. While the largest number of cases reside in Asia (61%), of which India and China comprise 45% [15], South Africa has one of the highest incidence rates per capita of TB disease, estimated at 1000 per 100 000 people for both 2012 and 2013 [1, 13] with slight improvement to 834 per 100 000 in 2014 and 2015 [14, 15]. TB also ranked the overall number one cause of death in South Africa, while it ranked 4th in children aged between 1-14 years causing approximately 3.6% of childhood deaths in 2015 [19].

1.2.1. Western Cape

In 2015 the Western Cape had the fourth highest incidence of TB in South Africa at 739 per 100 000 [20]. In the same year TB was found to be the 5th commonest cause of death in the province for all ages at 5.3% and 9th commonest at 1.7 % for those ages between 1-14 years [19].

HIV and TB often co-exist. Walters et all in 2008 at Tygerberg Hospital found that 48% of HIV positive children starting anti-retroviral treatment had TB [21], with an estimated incidence of 23 per 100 child-years [18]. A study of children admitted to Brooklyn Hospital for Chest Diseases in Cape Town reported that 31% of children with TB were HIV-infected [22], with a mortality of 14% in HIV infected children [22], while a study based in Durban reported a mortality of 18% from TB in HIV infected children aged younger than 12 years [23]. Therefore, children, especially those co-infected with HIV, are a vulnerable group and are at risk for developing more severe and disseminated disease [4, 24]. TB remains a substantial cause of childhood morbidity and mortality, especially in the context of the current HIV pandemic.

1. 3. Diagnosis of TB in Children

The diagnosis of PTB is challenging and multifaceted. A combination of clinical symptoms, contact history, tuberculin skin testing (TST), laboratory microscopy, Xpert and culture results and radiological findings are used to make the diagnosis [24–27]. However, as childhood TB is paucibacillary [5, 7, 27] and false negative tuberculin skin tests may occur in HIV-infected children or in severely malnourished children [28, 29], the diagnosis relies heavily on radiological features [24, 30]. While "lymphobronchial TB is deemed the 'hallmark' of radiological diagnosis" [12], occurring almost exclusively in children [9, 12], the radiological diagnosis of PTB in children remains challenging as many radiological features of PTB overlap with those of other lower respiratory infections.

The spectrum of radiographic features of PTB in children includes consolidation, mediastinal and hilar lymphadenopathy, nodular infiltrates, miliary disease, pleural effusion, hyperinflation, bronchial narrowing or atelectasis [12, 24, 31] with cavities usually seen in older children [27, 32]. In comparison, the radiographic features of LRTI in children are alveolar and interstitial infiltrates, consolidation, hyperinflation, adenopathy or pleural effusion [33, 34].

Further, there is a poor inter-observer agreement even amongst radiologists when interpreting radiographs for the presence or absence of lymphadenopathy [3, 8, 9]. There is thus a need to find objective features to distinguish PTB from LRTI due to other causes.

Lymphobronchial TB (LBTB) is a known entity in childhood PTB [12]. In LBTB, lymph nodes compress the small and pliable paediatric airways, causing consolidation, collapse and, as a result of the ball and valve

effect of endobronchial TB, air trapping [12]. Airway compression may thus represent an objective feature distinguishing PTB from other LRTI in children.

Many studies have shown that computed tomography (CT) is more sensitive for detecting lymphadenopathy and bronchial narrowing [24] compared to chest radiographs; however CT is not always widely available, especially in the 'high burdened countries', and exposes a child to a high radiation dose [24]. Diagnosis of PTB, thus still rests on chest radiography findings.

1.4. Literature Review

A review of the literature was conducted using the database Pubmed with the keyword combinations of "pulmonary or lymphobronchial tuberculosis", "children", "airway or bronchial", "obstruction or compression", "HIV" and "radiology or radiographs", limited to English articles for the period of January 1990 to December 2016. Searches further included scanning bibliographies of included articles. Seventeen studies were identified that investigated radiographic features of PTB in children in case series or cohort studies; all confirmed that airspace infiltration and lymphadenopathy are the most common radiographic findings (see table 1.1); only 8 of these, however, investigated the prevalence of airway compression.

Some studies highlighted the increased prevalence of lymphadenopathy in children younger than 5 years [24, 25, 35–38] as well as the increased risk of severe and disseminated disease in the younger age group [39, 40]. A North American study by Leung et al. reviewed radiographic features of PTB amongst 191 children younger than 16 years, and reported mediastinal and hilar lymphadenopathy as the commonest features in children under 3 years [35]. Similar results were reported in a Ugandan study by Kisembo et al., in which 72% of children under 4 years had lymphadenopathy compared to 23% of those older than 4 years [36]. Neither study specifically evaluated for airway compression, nor were any HIV-infected children included (table 1.1).

Ten studies compared radiological features of HIV infected with HIV negative children with PTB [22, 25, 37–44]; two studies also included comparison to children of unknown HIV status (table 1.1) [23, 25]. While there is a higher prevalence of TB in HIV infected children [37, 42], nine studies concluded that there was no difference in the radiological findings between HIV infected and HIV negative children [22, 25, 38–44] with

one study from Thailand by Srinakarin et al. reporting that lymphadenopathy in HIV infected children was coupled with a higher prevalence of pulmonary infiltrates compared to HIV negative children [43]. A South African study by Marais et al. deemed the sample size of 25 HIV infected children too small to make definitive conclusion [37] and only two studies by Schaaf et al. and Garcia-Basteiro et al. evaluated airway compression in HIV-infected children with PTB, reporting a similar prevalence between the HIV infected and HIV negative groups (table 1.1) [25, 38].

Eight studies evaluated airway compression as part of a general review of radiographic features in PTB [24, 25, 31, 37, 38, 45–47]; 2 included infant categories [24, 45]. The overall prevalence ranged from 5 % [38] to 38% [31]. Kim et al in South Korea studied children less than 1 year and Schaaf et al. case reviewed children aged less than 3 months hospitalised in Cape Town; all children were culture positive for TB [24, 45]. While Kim et al. used radiologists to read both the chest radiographs and subsequent CT scans and Schaaf et al.'s radiographs were all read by paediatricians, both studies found airway compression to be a common finding in addition to lymphadenopathy and pulmonary infiltrates (table 1.1 and table 1.2) [24, 45]. Kim et al. reported airway compression on CXR in 16% of cases, increasing to 65% on CT scan, as it is a more sensitive modality [24]. Schaaf et al. found airway compression on CXR in 56% of children age less than 3 months, associated most commonly with hyperinflation of the right middle and lower lobes [45], which suggests that the bronchus intermedius is the most commonly affected site of airway compression. This has been supported by some CT studies (table 1.2) [12, 44]. Both studies are limited by small sample sizes of 25 [24] and 38 [45] patients each and thus were inadequately powered. A larger cohort study by Schaaf et al. in Cape Town of 596 children aged less than 13 years with culture confirmed TB, further supported these findings [25]. Of 303 children aged younger than 3 years with PTB [25], 28.8% had airway compression compared to 4.3% of children older than 3 years [25]. Additionally, of those with airway compression 52.6% were aged younger than a year [25]. In comparison, recruiting from the same geographical area, Marais et al. found airway compression to occur in only 6.5% of children aged less than 3 years, with an overall prevalence of 8.8% in those less than 13 years [37]. This discrepancy may result from differences in patient selection or severity of illness.

An important limitation of most studies is the absence of a control group. Only 3 case control studies [38, 46, 48] compared radiological features of those diagnosed with PTB to those diagnosed with LRTI but only

two of these by Garcia-Basteiro et al. and Houwert et al. assessed for airway compression and were again limited by very small sample sizes of 43 (13 culture positive) and 28 (10 culture positive) cases in the TB groups respectively [38, 46] (table 1.1). Based on the evidence, it is therefore not possible to conclude if airway compression is related solely to age due to small compliant airways or due to the type of disease (TB or LRTI of other aetiology).

More recent studies have specifically investigated airway compression using CT and bronchoscopy (table 1.2). In addition to confirming that younger children were more affected by airway compression, these studies also investigated the most common locations of airway compression [11, 12, 44]. In a case series of a 100 children with suspected PTB in South Africa, Andronikou et al. found a 30% prevalence of airway compression on CT [11]. Of these 21 % were of the LMB, followed by 14% of the RMB [11]. This differs from the findings by Lucas et al. and Goussard et al. who only included symptomatic patients and thus biased to children with more severe disease [12, 44]. Both these studies found the bronchus intermedius to be most affected, followed by the LMB [12, 44].

Thus few studies have evaluated for airway compression in PTB and only two studies with small sample sizes by Madhi et al. and Peng et al. had control groups [40, 48], while none specifically evaluated for inter-observer agreement.

In conclusion, the published studies confirm that lymphadenopathy and airway compression are common features of childhood PTB, especially in younger children. No specific association with HIV-infection and compression has been reported. However patient selection bias, small sample sizes and few non-PTB control groups, limit the interpretation of these studies. The aim of this study was thus to investigate the frequency and inter-observer agreement of airway compression on chest radiographs for diagnosis of PTB in children compared to other LRTI's as well as its association to age and HIV infection, thereby determining its utility as an objective diagnostic radiographic sign for PTB in children.

Table 1.1 Review of chest radiographic findings in 17 studies of paediatric PTB **(continued on page 18)**

	Garcia-Basteiro et al. 2015* [38]		Srinakarin et al. 2012 [43]		Peng et al. 2011* [48]		Schaaf et al. 2007 [25]			Marais et al. 2006 [37]	Kim et al. 2006 [24]	Theart et al. 2005 [31]	Soeters et al. 2005 [22]			Iriso et al. 2005 [41]	
Categories	**Def & Prob TB**	**Non-TB**	**HIV +**	**HIV -**	**TB**	**CAP**	**HIV +**	**HIV -**	**?**				**HIV +**	**HIV -**	**?**	**HIV +**	**HIV -**
Sample size	**43**	**723**	**52**	**41**	**26**	**20**	**126**	**274**	**155**	**439**	**25**	**206**	**43**	**95**	**100**	**62**	**64**
Lymph. (%)	17	1.2	33	32	65	0	56	52	48	48	72	52	42	40	36	78	58
Consol. (%)	65	17.8	10	12			67	47	47	21	80		58	41	35	60	55
Patchy/nod infiltrate (%)	2	0	75	73	35	100	10	10	10	1			9	6	4	36	30
AW Comp (%)	5	0.6					14	20	17	8	16	38					

*All studies included only patients with PTB except for Garcia-Basteiro et al., Peng et al. and Houwert et al. who included a non-PTB group.

CAP = community acquired pneumonia

Lymph. = hilar and mediastinal lymphadenopathy

Consol. = includes alveolar, lobar and segmental consolidation

Patchy/nod infiltrate = includes bronchopneumonia and reticulonodular infiltrates

AW Comp. = airway compression

Def. TB = confirmed TB by culture or Xpert

Prob. TB = probable TB according to clinical criteria (positive TST, positive contact, suggestive CXR and response to anti-TB treatment)

Table 1.1 Review of chest radiographic findings in 17 studies of paediatric PTB (continued from page 17)

	De Villiers et al. 2004 [47]	Palme et al. 2002 [39]		Kisembo et al. 2001 [36]	Madhi et al. 2000 [40]		Houwert et al. 1998* [46]			Mukadi et al. 1997 [42]		Schaaf et al. 1993 [45]	Leung et al. 1992 [35]
Categories		**HIV +**	**HIV -**		**HIV +**	**HIV -**	**Def TB**	**Prob TB**	**CAP**	**HIV +**	**HIV -**		
Sample size	**61**	**58**	**459**	**80**	**39**	**59**	**10**	**18**	**117**	**22**	**96**	**38**	**191**
Lymph. (%)	28	17	25	59	44	49	60	44	13	41	77	89	92
Consol. (%)					67	48	60	44	30	14	23	52	69
Patchy/ nod infiltrate (%)	28	76	56	91	21	27	10	2	37	82	72	19	
AW Comp (%)	22						0	11	0			56	

*All studies included only patients with PTB except for Garcia-Basteiro et al., Peng et al. and Houwert et al. who included a non-PTB group.

CAP = community acquired pneumonia

Lymph. = hilar and mediastinal lymphadenopathy

Consol. = includes alveolar, lobar and segmental consolidation

Patchy/nod infiltrate = includes bronchopneumonia and reticulonodular infiltrate

AW Comp. = airway compression

Def. TB = confirmed TB by culture or Xpert

Prob. TB = probable TB according to clinical criteria (positive TST, positive contact, suggestive CXR and response to anti-TB treatment)

Table 1. 2 Review of CT and bronchoscopy (*) findings from 5 studies reporting airway compression in children with PTB

	Kim/Moon et al 1997[49] **N= 41**	Andronikou et al 2004 [11] **N= 100**	Kim et al 2006 [24] **N= 17**	Lucas et al 2012 [12] **N= 98 (**)**	Goussard et al 2013 [44] **N= 250 (**)** *Bronchoscopy
Airway compression:	37	29	65	100	100 (53 bilateral)
Trachea				63	57
Carina					77
LMB		21		64	62
LULB				5	12
LLLB				10	4
RMB		14		20	13
BI		8		75	72
RULB					35
RMLB					20
RLLB				27	20

All numbers are expressed as percentages (%)

N = Sample size

** = Pre-selected with airway compression symptoms

LMB = left main bronchus, **LULB** = left upper lobe bronchus, **LLLB** = left lower lobe bronchus, **RMB** = right main bronchus, **BI** = bronchus intermedius, **RULB** = right upper lobe bronchus,

RMLB = right middle lobe bronchus, **RLLB** = right lower lobe bronchus

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2.1. Title page

Assessment of airway compression on chest radiographs in children with pulmonary tuberculosis

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2.1.2. Abstract

Background:

Diagnosis of pulmonary tuberculosis (PTB) in children relies on chest radiography, however wide inter-observer agreement in detecting lymphadenopathy, the hallmark of PTB, exists. Since small, pliable paediatric airways are easily compressed by enlarged lymph nodes, detection of airway compression may be a more objective criterion for diagnosing PTB.

Objective:

To investigate the frequency and inter-observer agreement of airway compression on chest radiographs in children with PTB compared to those with another lower respiratory tract infection (LRTI).

Material and methods:

Chest radiographs of children admitted to Red Cross War Memorial Children's Hospital with suspected PTB were read by two readers according to a standardised format and a 3rd when there was disagreement. Radiographs of children with definite PTB were compared to those with another LRTI. Frequency and location of radiographic airway compression were evaluated and correlated to HIV status and age. Inter-observer agreement was assessed using kappa statistic.

Results:

Radiographs of 505 children (median age 25.9 months [IQR 14.3-62.2]) were reviewed; 97/505 (19%) children were HIV-infected. Airway compression occurred in 54/188 (28.7%) definite PTB cases versus 24/317 (7.6 %) of other LRTI cases (OR 4.9; 95%CI 2.9–8.3). The left main bronchus was most affected (51/493 [10.3%]). Infants had a higher frequency of airway compression (22/101 [21.8%]) compared to older children (56/404 [13.9%]; OR 1.7; 95%CI 1.00–3.00). No association between airway compression and HIV infection was found. Inter-observer agreement ranged from none to fair (kappa of 0.0-0.4).

Conclusion:

There is a strong association between airway compression on chest radiographs and confirmed PTB, particularly in infants, irrespective of HIV status. However, poor inter-observer agreement limits clinical utility.

Keywords:

Airway compression - pulmonary tuberculosis - lymphobronchial tuberculosis - children - chest radiographs – inter-observer agreement.

2.2. Introduction

The diagnosis of pulmonary tuberculosis (PTB) in children is challenging [1, 2]. Samples for testing are often not obtained from children and those that are, despite using the best diagnostic tests available, still have a low yield [3–5]. The gold standard for diagnosis, culture or Xpert (molecular test that detects the DNA of *M. tuberculosis*) of induced sputum or gastric aspirates, only yields positive results in around a 30-74% of children treated for pulmonary tuberculosis [4, 6–8]. Thus diagnosis of pulmonary tuberculosis in children still relies heavily on epidemiological, clinical and radiological findings [4, 5].

The chest radiograph (CXR) remains an important investigation, which is readily available and provides diagnostic information, however the radiological signs between lower respiratory tract infections and pulmonary tuberculosis overlap considerably. Lymphobronchial tuberculosis (LBTB) is a known entity in childhood tuberculosis [9] yet the chest radiograph is a 2-dimensional grey scale modality where lymph nodes are difficult to discern from the other overlapping vascular and mediastinal structures. Not surprisingly poor inter-observer agreement for detection of hilar adenopathy on chest radiographs, a cardinal radiological sign for diagnosis of pulmonary tuberculosis in children, has been reported [3, 10, 11], highlighting the need for objective diagnostic features.

Many studies have shown that CT (computed tomography) is more sensitive for detecting lymphadenopathy compared to chest radiographs, however CT is not always widely available, especially in the 'high burdened countries'[12]. Diagnosis of pulmonary tuberculosis, thus, still rests heavily on radiographic findings.

Since paediatric airways are small and pliable, particularly in infants, compression by enlarged lymph nodes is expected to be prevalent. Unlike lymph nodes, the lucent airways contrast against the surrounding mediastinal structures on radiographs. Thus airway compression may serve as a more objective criterion,

enhancing the radiographic diagnostic capability for pulmonary tuberculosis and prompting initiation of anti-TB treatment as well as oral steroids to prevent worsening symptoms in children with HIV or malnourishment who are at high risk to develop immune reconstitution syndrome; as is standard practice at our institution, which is located in a tuberculosis endemic area.

The aim of this study was, therefore, to investigate the frequency, location of and inter-observer agreement regarding the presence of airway compression on chest radiographs in children with confirmed pulmonary tuberculosis and those with lower respiratory tract infections (LRTI) from another cause. The association with age and HIV (human immunodeficiency virus) infection was also assessed.

2. 3. Materials and Methods

2.3.1. Study design

This is a secondary analysis of data collected in a larger prospective study of children enrolled in a study of novel diagnostics for pulmonary tuberculosis ("Diagnosis of tuberculosis in HIV infected children – development of microbiological and immunological strategies" with University of Cape Town Faculty of Health Science ethics committee reference 045/2008) [7]. Inclusion criteria for this larger study comprised children aged less than 15 years who presented with symptoms and signs of suspected pulmonary tuberculosis: "cough or difficulty breathing with one of the following 1) positive household tuberculosis contact within the past 3 months, 2) weight loss or failure to gain weight within the past 3 months, 3) a positive TST (tuberculin skin test), defined as 5 mm or more measured transverse skin indurations in HIV-infected patients or 10 mm or more in HIV-negative patients or 4) a chest radiograph "suggestive" of pulmonary tuberculosis" [7].

All children had an intensive work up for microbiological confirmation of tuberculosis including repeated induced sputum specimens for liquid culture and Xpert. Chest radiographs were performed as part of the diagnostic work up at the time of enrolment. Children were excluded if they had received tuberculosis prophylaxis or were already on treatment for more than 72 hrs, were unable to attend follow up appointments such as those not residing in Cape Town, from whom an adequate sputum sample could not be obtained and where informed consent had not been acquired.

All children had their HIV status tested if this was not already known. HIV infection was defined by two positive ELISA's (enzyme-linked immunosorbent assays) in children older than 18 months or a positive HIV PCR (polymerase chain reaction) test for younger children.

2.3.2. Study population

Selected from the database described above, the sample for this study therefore comprised chest radiographs of children aged less than 15 years, admitted to the Red Cross War Memorial Children's Hospital in Cape Town for suspected pulmonary tuberculosis from 1st February 2009 to 31st December 2013. Further, children were only included if they had a diagnosis of definite pulmonary tuberculosis (defined by one or more cultures or Xpert positive for *M. tuberculosis* on induced sputum) or if they had other non-tuberculosis lower respiratory tract infection (children who presented with respiratory signs but were subsequently not clinically considered to have tuberculosis, had negative cultures and Xpert, were not treated for tuberculosis and had clinical improvement at 1 and 3-month follow-up visits). This was designed to exclude all 'possible tuberculosis' cases. Chest radiographs that were not available or missing were also excluded from this study.

2.3.3. Methods

Baseline chest radiographs were obtained by standardised paediatric protocols between 1st February 2009 and 31st December 2013 in digital format. Radiographs were extracted from the picture archiving and communication system (PACS) and converted to JPEG (Joint Photographic Experts Group) for ease of accessibility to readers not stationed at the hospital. This format allowed for windowing and zooming capabilities. Readers were sent the images on flash disc or shared via 'Dropbox'.

Those radiographs meeting the above inclusion and exclusion criteria were interpreted by two readers (a paediatrician with more than 40 years of experience in childhood tuberculosis and a paediatric radiologist with 19 years of experience), blinded to the initial presenting symptoms of the patients, final diagnosis and to each other's findings using a standardised reporting form.

Data for each read radiograph was evaluated for the presence of airway compression and location thereof (trachea, right main bronchus, bronchus intermedius and left main bronchus). Where there was disagreement

in either the presence or location of airway compression between the two expert readers, the chest radiograph was read by a third reader (a paediatric radiologist with 3 years of experience). Number and choice of readers was limited by resources and availability. The majority findings (minimum of two out of three) were taken as the final result. The standardised reporting tick sheet provided 4 options regarding airway compression: present (yes), absent (no), maybe or airway not visible. 'Maybe' answers (total of 178/1209) were designated a 'yes' in the final analysis since in the clinical setting, doubtful cases would be overcalled to err on the side of caution. This, however, still left 3 options, so despite a 3rd reader for majority agreement, discordant results still occurred where all 3 readers ticked different options (Y/ N/ not visible). The degree of compression or displacement was not assessed. In cases where the distal trachea and main bronchi were not discernible, this was generally considered to be technical and assessed as 'not visible'.

Findings by the individual readers as well as the final results were quantified and correlated to the diagnosis of definite pulmonary tuberculosis as a gold standard. The findings were then categorised into age groups of ≤ 1 year and > 1 year, and further correlated with the child's HIV status.

2.3.4. Statistical analysis

The statistical software program STATA 14 (STATA Corporation, College Station, TX USA) was used for the analysis of the data and calculation of the sample size for this study. Based on published literature that 30% of children classified as definite tuberculosis would have airway compression [13, 14] compared to an estimated 15% of the children classified as not tuberculosis, 163 children were required in each group to have 90% power to detect a 15% difference at the 0.05 level of significance. Descriptive statistics were used to summarise radiological findings of airway compression and demographic variables. Median and interquartile ranges were calculated for non-normally distributed continuous variables and categorical variables were expressed as frequencies and percentages. Discordant results between the 3 readers for the final assessment of airway compression (yes/ no/ not visible) were not included in the final analysis. Statistical tests included chi-square test (adjusted if number less than 5) to compare proportions and Kruskal-Wallis to compare medians. Logistic regression was used to explore univariate associations of radiological findings of airway compression and demographic characteristics between children classified as definite pulmonary tuberculosis to other lower respiratory tract infection; multivariate analysis was used to explore the association of

radiological findings of airway compression adjusted for the possible effect of demographic characteristics of the child (gender, age and HIV infection). An interaction between age and radiological findings of airway compression was explored. The Cohen's kappa statistic was used to determine inter-observer agreement between the expert reviewers; a kappa score of 0-0.2 was considered slight; 0.2-0.4 fair; 0.4-0.6 moderate; 0.6-0.8 as substantial and 0.8-1.0 almost perfect [15]. Statistical tests were two-sided at $\alpha = 0.05$.

2.3.5. Ethics

The study of novel diagnostics for tuberculosis was approved by the Faculty of Health Science ethics committee, University of Cape Town (Reference number 045/2008). Separate ethics clearance was obtained for this study (Reference number 815/2014). No additional imaging was performed for the purposes of the study and thus poses no additional risk to the patient, falling within the guidelines of the 1964 Helskini declaration and its later amendments. Informed consent including for radiological imaging was obtained for the original larger study from a parent or legal guardian of the participating children as well as assent from children aged older than 7 years. Patients were anonymised by random encryption, the code of which was kept by the co-author and supervisors.

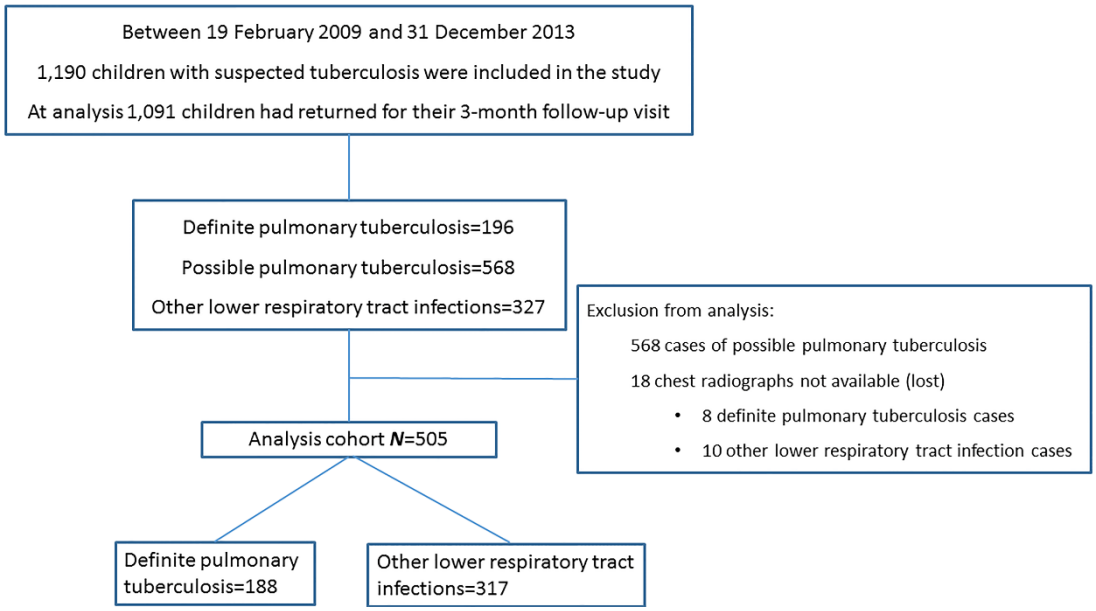
2.4. Results

2.4.1. Baseline characteristics

Of 1190 enrolled in the larger tuberculosis diagnostic study 1091 returned for follow up visits, of which 586 were excluded. These comprised 568 'possible tuberculosis' cases and 18 lost chest radiographs (see figure 2.1). Thus a total of 505 chest radiographs form the study population, consisting of 188 (37.2%) children with definite pulmonary tuberculosis and 317 (62.8%) diagnosed with other lower respiratory tract infections. The median age was 25.9 months (interquartile range 14.3-62.2). There were 273 boys (54.0%) with the proportion of males similar in the two groups; 102/188 (54.2%) in the definite tuberculosis group and 171/317 (53.9%) in the other lower respiratory tract infection group (OR (odds ratio) 0.99; 95%CI (confidence interval) 0.69-1.41). One hundred and one children were one-year-old or less with fewer in the definite pulmonary tuberculosis group than the other lower respiratory tract infection group; 23/188 (12.2%) versus 78/317 (24.6%) respectively (OR 0.43; 95%CI 0.26 – 0.71). There were 97/505 (19.2%) HIV-infected

children, 407/505 (80.6%) uninfected with 1 child whose HIV status was unknown. The proportion of HIV infected children was similar between the two groups; 39/188 (20.7%) in the definite pulmonary tuberculosis and 58/317 (18.3%) in the other lower respiratory tract infection group (OR 1.16; 95%CI 0.74-1.83).

Figure 2.1 Flow diagram indicating derivation of study population from the sample populations



2.4.2. Airway compression by location and reviewer

Radiographic airway compression at 4 locations was assessed by each reviewer (table 2.1). 'Not visible' results were not included in the frequency calculations. In addition, discordant results (Y/N/not visible) for each location (3 for tracheal, 7 for right main bronchus, 10 for bronchus intermedius and 12 for left main bronchus) were not included in the respective final majority agreement calculations. Overall there was airway compression by majority decision in at a least one location in 78/ 505 chest radiographs (15.4%). In 35/505 (6.9%) there was multifocal airway compression. The airway most commonly affected was the left main bronchus, followed by bronchus intermedius (table 2.1).

Table 2.1 Summary of findings for airway compression location by expert reviewer

	Reviewer 1 **N = 505**	**Reviewer 2** **N = 505**	**Reviewer 3** **N = 199**	**Majority agreement**[a] **N = 505**
Tracheal compression	47/505 (9.3%)	35/493[b] (7.1%) not visible: 12	19/199 (9.5%)	22/502[a] (4.4%) discordant: 3
Right main bronchus compression	51/503[b] (10.1%) not visible: 2	21/479[b] (4.4%) not visible: 26	29/197[b] (14.7%) not visible: 2	16/498[a] (3.2%) discordant: 7
Bronchus intermedius compression	32/503[b] (6.4 %) not visible: 2	52/471[b] (11.0%) not visible: 34	35/195[b] (17.9%) not visible: 4	27/495[a] (5.5%) discordant: 10
Left main bronchus compression	60/503[b] (11.9 %) not visible: 2	79/458[b] (17.2%) not visible: 47	46/198[b] (23.2%) not visible: 1	51/493[a] (10.3%) discordant:12
Airway compression at any location	84/505 (16.6%)	107/505 (21.2%)	69/199 (34.7%)	78/505 (15.4%)

N = total number of CXR's reviewed

[a] At least two/three reviewers agreed, if all three reviewers had a different result e.g. Y/N/not visible (discordant), this result was not included in the respective calculations. The difference between the denominator and 'N' equals the number of CXRs with discordant results.

[b] Difference between the denominator and 'N' equals the number of 'not visible' airways, which were not included in the respective calculations.

2.4.3. Correlation of airway compression with pulmonary tuberculosis

Radiographic airway compression was significantly more common in children with definite pulmonary tuberculosis compared to those with other lower respiratory tract infection for all locations with a frequency of 54/188 (28.7%) versus 24/317 (7.6%) respectively (see table 2.2 and figure 2.2). For overall airway compression at any location, there is approximately a 5 fold odds of having definite pulmonary tuberculosis and an almost 8 fold odds of having pulmonary tuberculosis when airway compression is seen at the left main bronchus (see table 2.2 and figure 2.3). Of the 35/505 chest radiographs with majority airway in more than one location 25/188 (13.3%) had definitive pulmonary tuberculosis and 10/317 (3.2%) had other lower respiratory tract infections (p-value <0.001).

Table 2.2 Univariate analysis of the association of airway compression comparing definite PTB to the other LRTI group

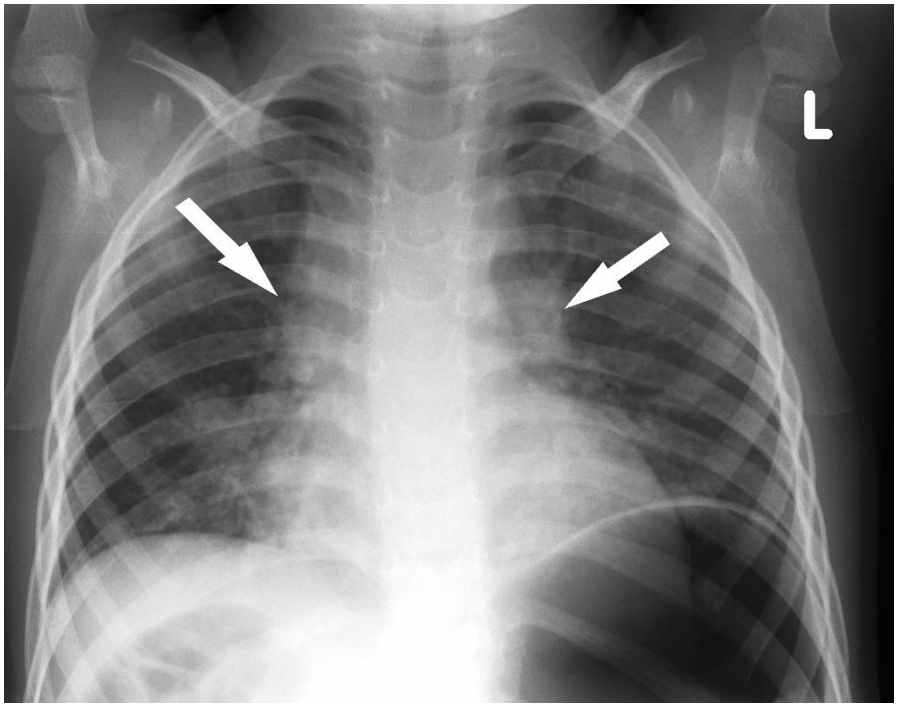
	Total **N = 505**	**Definite PTB** **N = 188**	**Other LRTI** **N= 317**	**OR (95% CI)**
Majority tracheal compression[a]	22/502[a] (4.4%)	14/188 (7.4%)	8/317 (2.5%)	3.1 (1.3-7.6)
Majority right main bronchus compression[a]	16/498[a] (3.2%)	10/188 (5.3%)	6/317 (1.9%)	2.9 (1.1 – 8.3)
Majority bronchus intermedius compression[a]	27/495[a] (5.5%)	19/188 (10.1%)	8/317 (2.5%)	4.4 (1.9-10.3)
Majority left main bronchus compression[a]	51/493[a] (10.3%)	40/188 (21.3%)	11/317 (3.5%)	7.6 (3.8-15.3)
Overall majority airway compression at any location[c]	78/505 (15.4%)	54/188 (28.7%)	24/317 (7.6%)	4.9 (2.9 – 8.3)

N = total number of CXR's reviewed

[a] At least two/three reviewers agreed, if all three reviewers had a different result e.g. Y/N/not visible, this result was not included in the respective calculations. The difference between the denominator and 'N' equals the number of CXRs with discordant results

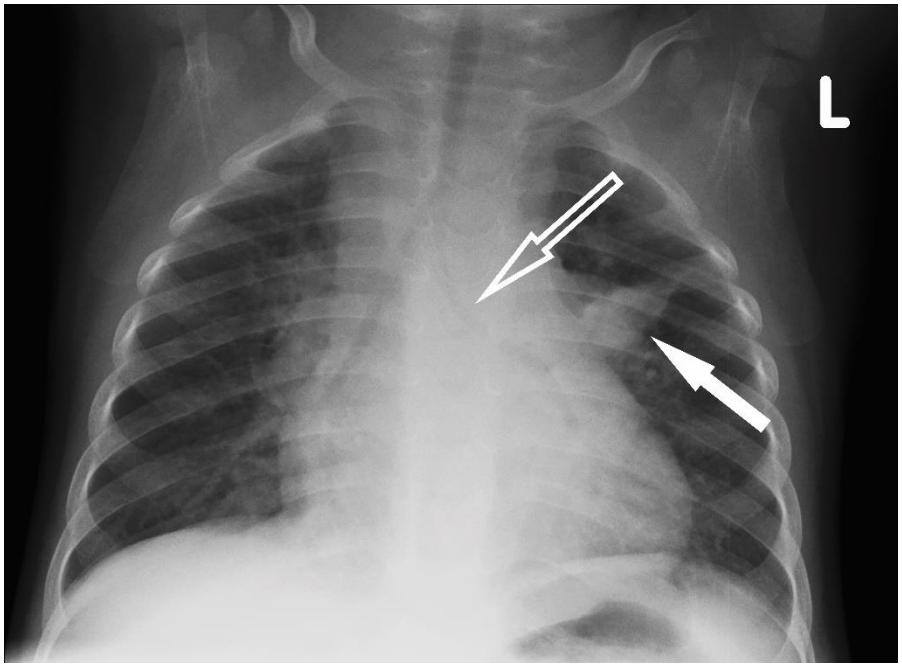
[c]Multiple sites on a single radiograph were counted as 1.

Figure 2.2 Lower respiratory tract infection with no airway compression



Three-year-old male patient; HIV negative. Mediastinal contour is suspicious for lymphadenopathy (arrows) but there was no airway compression present on majority agreement. Patient was subsequently diagnosed with LRTI of another cause.

Figure 2.3 Pulmonary tuberculosis with airway compression



Eighteen-month-old male patient diagnosed with PTB; HIV negative. Majority agreement of airway compression at LMB (open arrow) indicative of lymphadenopathy. Left upper lobe opacity is in keeping with a Ghon focus (solid arrow).

2.4.4. Correlation of airway compression with age

Radiographic airway compression was significantly more common in infants compared to older children with approximately double the risk of developing airway compression in the former (table 2.3). The left main bronchus was the most common location in both age groups, followed by the bronchus intermedius (table 2.3).

Table 2.3 Univariate analysis of the association of airway compression to age

	Total **N = 505**	**≤ 1 year** **N = 101**	**>1 year** **N = 404**	**OR (95% CI)**
Majority tracheal compression[a]	22/502[a] (4.4%) discordant: 3	4/101 (4.0%)	18/401[a] (4.5%) discordant: 3	0.88 (0.20 – 2.65)
Majority right main bronchus compression[a]	16/498[a] (3.2%) discordant: 7	7/99[a] (7.1%) discordant: 2	9/399[a] (2.3%) discordant: 5	3.3 (1.20 – 9.08)
Majority bronchus intermedius compression[a]	27/495[a] (5.5%) discordant: 10	9/98[a] (9.2%) discordant: 3	18/397[a] (4.5%) discordant: 7	2.13 (0.93 – 4.90)
Majority left main bronchus compression[a]	51/493[a] (10.3%) discordant: 12	18/101 (17.8%)	33/393[a] (8.4%) discordant: 7	2.36 (1.27 – 4.39)
Overall majority airway compression at any location[d]	78/505 (15.4%)	22/101 (21.8%)	56/404 (13.9%)	1.7 (1.00 – 3.00)

N = total number of CXR's reviewed

[a] At least two/three reviewers agreed, if all three reviewers had a different result e.g. Y/N/not visible, this result was not included in the respective calculations. The difference between the denominator and 'N' equals the number of CXRs with discordant results

[d] Multiple sites on a single radiograph were counted as 1.

2.4.5. Correlation of airway compression with HIV Infection

There was no association with HIV infection and radiographic airway compression at any location with almost equal frequencies in both groups; 39/188 (20.7%) in the definite pulmonary tuberculosis group compared to 58/317 (18.3%) in the other lower respiratory tract infection group (OR 1.08; 95%CI 0.70-1.75).

2.4.6. Multivariate analysis

By including an interaction term and thus adjusting for the effect of age, HIV status and gender, the multivariate analysis showed a strong association between radiographic airway compression and definite pulmonary tuberculosis with 54/188 (28.7%) of children in the definite pulmonary tuberculosis group compared to 24/317 (7.6%) in the other lower respiratory tract infection group, OR 6.02 (95% CI 3.45-10.51; p-value <0.001) (table 2.4).

Table 2.4 Multivariate analysis of the association of airway compression and child characteristics comparing definite PTB to other LRTI

	Total **N = 504[e]**	**Definite PTB** **N = 188**	**Other LRTI** **N= 316**	**OR (95% CI)**	**P value**
Gender: male	273 (54.2%)	102 (54.3%)	171 (54.1%)	1.09 (0.74 – 1.61)	0.653
HIV infected	97 (19.2%)	39 (20.7%)	58 (18.4%)	1.08 (0.70 – 1.75)	0.747
Age ≤ 1 year	101 (20.0%)	23 (12.2%)	78 (24.7%)	0.33 (0.19 – 0.57)	<0.001
Overall majority airway compression at any location	78 (15.5%)	54 (28.7%)	24 (7.6%)	6.02 (3.45– 10.51)	<0.001

[e] One unknown HIV status excluded from the total 505 CXRs.

2.4.7 Inter-observer agreement

Inter-observer agreement amongst the three readers was poor and very variable. Reader 1 and 3 (paediatrician and paediatric radiologist) had the poorest agreement (none) regarding bronchus intermedius compression while reader 2 and 3 (both paediatric radiologists) had the best agreement at 0.40 (fair [15]) regarding left main bronchus compression. Yet reader 3 only reviewed 199 chest radiographs compared to 505, and thus cannot be equally weighted as the other two readers. The best agreement between the first 2 readers was 0.29 at the left main bronchus, whilst the tracheal, right main bronchus and bronchus intermedius compression scored poorly at 0.08, 0.13 and 0.11 respectively. This poor inter-rater reliability also resulted in the discordant majority agreement (Y/N/ not visible) as illustrated in table 2.1.

2.5. Discussion

Our findings show that there is a strong correlation between the presence of airway compression on chest radiographs and a definite diagnosis of pulmonary tuberculosis in children, even after adjusting for age. The infant group had the highest prevalence of radiographic airway compression, while the left main bronchus was most commonly involved across all age groups. HIV co-infection did not alter the radiological picture. However, inter-observer agreement was poor, which limits the use of airway compression as an objective criterion for the diagnosis of pulmonary tuberculosis in children on standard radiographs.

The overall frequency of airway compression in definite pulmonary tuberculosis was found to be 28.7%, compatible with reports in the literature [13, 12, 16–18, 14]. Although airway compression used alone is not a specific sign, if seen on radiographs, there is a strong correlation with pulmonary tuberculosis (5-6 fold likelihood) compared to other lower respiratory tract infection , thus should raise the suspicion for pulmonary tuberculosis in an endemic setting, thereby prompting the initiation of treatment much like the identification of lymphadenopathy would. This finding supports other studies reporting no airway compression in their sample of non- tuberculosis cases although these studies only included children with mild disease who were just hospitalised overnight [19] or seen at a research clinic [20].

As supported by the literature, due to the smaller airways with less cartilage combined with immature immunity [9, 21, 22], the infant group was found to be at higher risk (double) of developing radiographic

airway compression compared to older children. These findings are compatible with previous reports in the literature where prevalence ranges from 16% in infants [12] to 29% in children aged less than 3 years [16].

Previous studies reported the bronchus intermedius to be the site most affected by airway compression [9, 23]. This has been attributed to it being a 3rd order bronchus with a narrower diameter and mostly affected by the 'nutcracker phenomenon' caused by enlarged right hilar and subcarinal lymph nodes in pulmonary tuberculosis [9, 24, 21, 23]. These studies, however, used CT in the evaluation of airway compression, scoring the degree of narrowing, and only selected patients already symptomatic of airway compromise [9, 23]. This may explain the contradictory findings of our study, which showed the left main bronchus to be affected approximately twice more common than the bronchus intermedius in both age groups. Supportive of our findings, Andronikou et al. postulated that the left main bronchus' anatomical configuration is longer and narrower compared to the right main bronchus placing it more at risk for compression [13]. As our study included a wider range of age and disease severity, our findings may be more representative of the general spectrum of disease, while the smaller calibre bronchus intermedius may be more affected in those symptomatic of airway compromise.

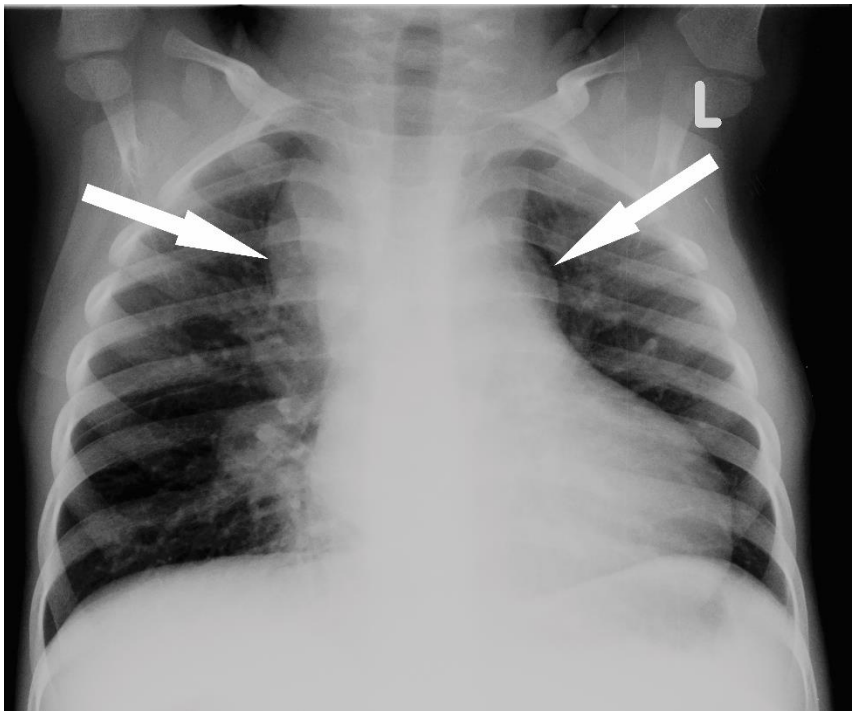
The WHO (World Health Organization) reports the impaired immunity caused by HIV as one of the highest risk factors to develop pulmonary tuberculosis [25]. However, consistent with published literature [8, 16, 20, 26–30, 23], our study also showed no significant difference in the frequency of airway compression between HIV infected and uninfected children. This may be due to the variable and wide degree of disease severity as well as anti-retroviral treatment, which was not assessed.

A disappointing finding was the poor inter-observer agreement amongst the 3 expert readers. Our 3rd reader reviewed less than half the radiographs compared to the first 2 readers and thus cannot be equally weighted, however the results highlight the variability of inter-observer agreement. Although our best inter-observer agreement, located at the left main bronchus, is compatible with the previously reported 30% agreement of radiographic interpretation of hilar adenopathy [10], the fact remains that the inter-observer agreement for radiographs remains low. It should be noted that kappa statistic accuracy decreases if the prevalence of the investigated entity (airway compression) is less than 50%, which would have contributed to the low agreement in this study. However, other concomitant factors are the lack of standardised criteria in the

definition of airway compression both in the literature and in the core study from which the data was derived, as well as suboptimal visualisation of the airways on standard chest radiographs (see figure 2.4). Factors affecting the latter include poor image quality, due to various patient and technical considerations not assessed in this study, but also inherent suboptimal resolution of the airways on standard chest radiography. Use of personalised computers instead of reporting stations and the post-processing of radiographs, i.e. conversion of the radiographs into JPEG would have also resulted in further image degradation despite windowing and zooming capability, however better quality images were unfortunately not available for logistic reasons.

High kilovolt radiographs were previously used to improve visualisation of the airways compared to standard techniques but these have largely been abandoned due to increased cost and lack of improved sensitivity [18]. Another study using the narrow beam, low dose digital radiography scanner [Lodox Systems (Pty) Ltd] showed improved visibility of the airways as well as inter-observer agreement [31] but this radiographic modality is currently only available at selected tertiary centres in South Africa. Unfortunately, in resource limited settings, the widespread installation of these specialised scanners is currently not a feasible solution.

Figure 2.4 Discordant inter-observer agreement



Two-year-old male patient, HIV negative but diagnosed with PTB. Mediastinal lymphadenopathy is evident (arrows), however airways were poorly visualised on this frontal radiograph with discordant results (Y/N/not visible) at all locations, highlighting the lack of objectivity.

2.5.1. Strengths, limitations and recommendations

Strengths of our study included the large sample size with varied ages, different HIV status and use of a confirmed pulmonary tuberculosis group as well as a control group of other lower respiratory tract infection. However our study has several limitations in addition to those already alluded to in the discussion:

Our study was limited to children admitted to hospital, so our findings may not be representative of children with less severe disease presenting to clinics.

In addition the designation of 'maybe' results to 'yes', 178 out of the 488 'yes" results (36%), did skew final numbers, however after the majority agreement analysis, this was not ultimately statistically significant.

As part of the larger study of novel diagnostic for pulmonary tuberculosis from which data for this study was derived ("Diagnosis of tuberculosis in HIV infected children – development of microbiological and immunological strategies" [7]) readers reported the whole chest radiograph, thus the evaluation of airway compression was not done in isolation. Hence the secondary features (collapse and air trapping) may have biased the readers to diagnose airway compression, however this reflects everyday clinical reporting practice.

The lack of the gold standard (CT) to confirm airway compression precluded the assessment of chest radiograph reporting accuracy and thus potentially skewing the overall statistical results. Similarly, due to the known variable radiographic inter-observer agreement and lack of CT validation, airway compression could not be compared to the radiographic presence of lymphadenopathy for the diagnosis of pulmonary tuberculosis. However it would not have been ethically acceptable to expose the children to additional radiation for this purpose.

The lack of standardised criteria with regards to the assessment of airway compression and poor visualisation of the airways contributed to poor inter-observer agreement. Ideally all 3 expert reviewers should have been paediatric radiologists but lack of resources and unavailability precluded this. Also the evaluation of intra-observer variability may have been of value but the readers were unavailable for this to be assessed.

Radiographic quality assessment of airway visualisation on chest radiographs was also not reviewed, thus the extent this influenced the findings, in other word where airway compression was not recognised due to poor quality images, could not be determined.

Future research with standardised criteria for airway compression, making use of standardised severity scores [12], and including assessment of intra-observer agreement are recommended. Comparison of Lodox scanners to CT in the evaluation of airway compression may prove the low dose technique valuable in the routine work-up of pulmonary tuberculosis while further research into the use of mediastinal ultrasound for the identification of lymph nodes is ongoing [32].

2.6. Conclusion

There is a significant association between airway compression on chest radiographs and definite pulmonary tuberculosis compared to other lower respiratory tract infections, particularly in the infant age group, irrespective of HIV status. Left main bronchus was the commonest site involved. However, the use of this as an objective criterion for diagnosis of pulmonary tuberculosis in children is limited by poor inter-observer agreement.

2.7. Conflict of Interest:

The authors declare that they have no conflict of interest.

2.8. Acknowledgements:

We thank the clinical and data study staff at Red Cross Children's War Memorial Hospital, the microbiology laboratory team, the radiology department and expert team, and the children and their caregivers for participating in this study. The core study and database from which this study was derived was funded by the National Institutes of Health, USA (R01HD058971), the National Health Laboratory Services Research Trust, the Medical Research Council of South Africa and the National Research Foundation South Africa.

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Appendix A-1: Ethics Clearance Certificate

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • **Facsimile** [021] 406 6411
Email: Sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

01 December 2014

HREC/REF: 815/2014

Prof H Zar
Department of Paediatrics & Child Health
5th floor
ICH Building
Red Cross Children's Hospital
Rondebosch

Dear Prof Zar

Project Title: ASSESSMENT OF AIRWAY COMPRESSION ON RADIOGRAPHS IN CHILDREN WITH PULMONARY TUBERCULOSIS (MMeD-candidate-L-Richter Joubert)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for approval.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 30 December 2015.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student:- Lisel Richter-Joubert is also involved in this project.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.

Hrec/ref:815/2014

Appendix A-2: Extension of ethical approval for 2016

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☐ Approved	Annual progress report	Approved until/next renewal date	30.12.2016
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	11/11/2015

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	10/11/2015		
HREC REF Number	815/2014	Current Ethics Approval was granted until	30/12/2015
Protocol title	Assessment of airway compression on radiographs in children with pulmonary tuberculosis		
Protocol number (if applicable)	N/A		
Are there any sub-studies linked to this study?		☐ Yes ✓No: This is a sub-study to a larger project: 045/2008	
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof Heather Zar		
Department / Office Internal Mail Address	heather.zar@uct.ac.za		

1.1 Does this protocol receive US Federal funding?	☐ Yes	✓No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	☐ No
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget	☐ Yes	☐ No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix A-3: Extension of ethical approval for 2017

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/12/2017
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	17/11/2016

Comments to PI from the HREC

HUMAN RESEARCH ETHICS COMMITTEE
16 NOV 2016
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	16/11/2016		
HREC REF Number	815/2014	Current Ethics Approval was granted until	30/12/2016
Protocol title	Assessment of airway compression on radiographs in children with pulmonary tuberculosis		
Protocol number (if applicable)	N/A		
Are there any sub-studies linked to this study?	☐ Yes ✓No: This is a sub-study to a larger project: 045/2008		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof Heather Zar		
Department / Office Internal Mail Address	heather.zar@uct.acza		

1.1 Does this protocol receive US Federal funding?	☐ Yes	✓ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	☐ No
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	☐ No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix B: Tick sheet

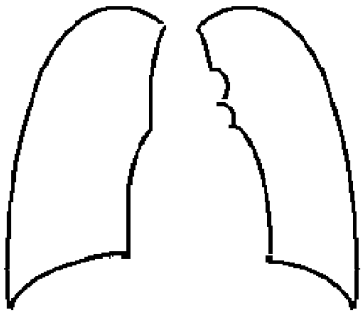
CXR CRF Baseline vs1
26-aug-2010

CXR results form Baseline (enrolment)

10. Chest x-ray date

11. Is the chest x-ray Normal (go to end) Abnormal (go to x) | N | A

Indicate the distribution of abnormalities

12. 

Coder/Reporter:	Name:												
PARENCHYMA	Lung area involved (tick applicable boxes)												
Alveolar (consolidaton)	Y	N	RUL	RML	RLL	LUL	LING	LLL			Segm	Lobar
Ghon focus	Y	N	RUL	RML	RLL	LUL	LING	LLL			Segm	Lobar
Expansile	Y	N	RUL	RML	RLL	LUL	LING	LLL			Segm	Lobar
Cavity (number, size, thickness)	Y	N	RUL	RML	RLL	LUL	LING	LLL				
Nodular infiltr (>2mm)	Y	N	Peri-hilar			Peripheral					Right	Left
Miliary infiltr (up to 2mm)	Y	N	Peri-hilar			Peripheral					Right	Left
Volume loss	Y	N	RUL	RML	RLL	LUL	LING	LLL			Segm	Lobar
Hyperinflation	Y	N	RUL	RML	RLL	LUL	LING	LLL			Segm	Lobar
Calcification (lung)	Y	N	RUL	RML	RLL	LUL	LING	LLL			Segm	Lobar
Peri-hilar streakiness	Y	N	Right	Left								
AIRWAY COMPRESSION AND OR TRACHEAL DISPLACEMENT												
Right paratraceal			y		n		maybe		Not visible			
Right main bronchus			y		n		maybe		Not visible			
Right bronchus intermedius			y		n		maybe		Not visible			
Left main bronchus			y		n		maybe		Not visible			
NODES												
Peri –hilar (P-H)	Y	N	R	L	SC	U						
Paratracheal (PT)	Y	N	R	L	U							
Calcification (nodes)	Y	N	P-H	PT								
PLEURA												
Effusion	Y	N	R	L	B-L	Sm	Lge		Loculated	Y	N	

HEART								
Enlargement	Y	N	U		Pericardial Effusion	Y	N	U

Appendix C: Consent form- English version 6.9 05 April 2013

Increasing prevention and treatment of TB through development of a rapid, sensitive and affordable biological marker and improved microbiologic and immunologic methods for diagnosis of TB in HIV positive and negative children

PATIENT INFORMATION AND CONSENT FORM

Principal investigators:

Professor Heather Zar, Professor Mark Nicol

You and your child are requested to participate in a medical research study that is being done at Red Cross Children's Hospital and New Somerset Hospital. The study is sponsored by the National Institute of Health in America. It has been approved by the Human Research Ethics Committee of the Health Sciences Faculty of the University of Cape Town and will be done according to strict ethical guidelines and principles. The following information describes the study and your child's role as a participant. Please read this carefully and feel free to ask any questions.

Background

The doctors looking after your child suspect that he or she may have tuberculosis (TB). Tuberculosis is a very common infection in South Africa and can cause bad chest infections or serious illness elsewhere in the body especially in children. Tuberculosis can be an especially serious illness in children with HIV. There are tests that are usually done to help find out whether your child has TB but these tests do not often find TB in children. There are new tests that may be better than the usual tests. The aim of this study is to use new tests to help to find out whether TB is the cause of your child's chest problem. If he/she is shown to have TB, then the best available treatment for your child's TB will be given. In addition, we wish to try and find out more about whether there are any specific

circumstances that may have predisposed your child to getting TB or that may influence how effective treatment may be.

Why is this study being done?

This study is being done to find out whether any of the new TB tests can help to confirm if your child has TB. Your child will have the usual tests for TB. In addition, we will do some new tests to see whether these can help to confirm if your child has TB or not. Lastly we will ask you to fill out a questionnaire with questions about your living conditions, the stresses in your life, your own illnesses (if any) and what you think about TB.

What will happen to you if you agree to allow your child to join the study?

Your child will have the usual tests for TB. These include a TB skin test (Mantoux) and three induced sputums where we take mucous from the lungs and a chest Xray. If your child takes part in the study, then additional new tests to check for TB will be done. These tests are:

1. A sample of mucus from your child's nose will be taken thrice to look for the germ that causes TB. The sample may also be checked for other germs.
2. A sample of mucus from your child's chest (sputum) will be taken thrice to look for the germ that causes TB. The sample may also be checked for other germs.
3. A urine test will be taken to look for the germ that causes TB
4. A stool test will be taken to look for the germs that causes TB
5. A swab from your child's nose to look for other germs
6. A small amount of blood (about 1teaspoon) will be taken for other tests to measure how your child's body responds to the TB germ.
7. If your child has TB outside the lungs another sample (for example spinal fluid or water from around the lung) may be checked for the TB germ and the body's response to the germ.
8. In addition, we will do a scan at your child's bedside, to look for TB inside your child's body. This is a quick test for finding TB outside of your child's lungs, for example TB in other organs of the child's body. This test is not painful and lasts only a few minutes.
9. All the samples will be sent to the laboratory to check for the TB germ in the usual way. In addition, new and better methods to find the TB germ will be used. Some of the blood samples will be sent to London, Sussex and Singapore for further diagnostic tests related to TB.

10. You will be asked to fill out a questionnaire with questions about your living conditions, the stresses in your life, your own illnesses (if any) and what you think about TB; A TB treatment survey will be used to ask for questions about your child's TB treatment

If your child's HIV status is not known then he or she will be tested for HIV, as TB can be very serious in children with HIV and may need to be treated for longer and with different medicines. This is routine practice at the hospital. If your child is found to have HIV, then he or she will be referred for treatment of HIV and antiretroviral therapy if needed.

What are the advantages of my child being in the study?

The results of the new tests may help to find out if your child has TB and also what the best treatment for this is. This will help to give your child the best treatment to help him or her recover. If the results of the study are useful, then this may help to develop a better way of finding out if other children have TB as well.

Will the study hurt or make your child feel bad?

Your child will experience discomfort from the needle when blood is taken. Where possible this blood test will be done at the same time as other blood tests and using an anaesthetic cream to dull the pain from the needle. Only a small amount of blood will be taken. Your child may also experience minor discomfort when the mucous is taken from his/her nose and throat. Occasionally this can cause some bleeding from the nose, but this is not serious and usually stops by itself or by applying pressure to the nose.

How long will my child be in the study?

Your child may be followed up 3 times once he/she is discharged from hospital. These visits will take place 1, 3 and 6 months after your child has left hospital. The purpose of these visits is to monitor your child's progress (even if they are not on TB treatment). A blood

samples (about 1 teaspoon of blood) may be taken at these visits for repeat TB tests. Another chest X-ray will also be done at the 6 month visit if your child has been on TB treatment.

Will I be charged for the study?

No, there is no cost to you.

Will I receive any payment?

We will reimburse you for your travel costs when you come for your child's check up at 1, 3 and 6 months.

Does your child have to be in the study?

You may choose for your child to be in this study or not. If you choose not to be in the study then your child will get treatment as usual. You may choose to fill out the questionnaire or not – your decision will not in any way affect the treatment your child receives.

What do I do if I have any questions?

For questions about this study, you can ask the study staff Dr Lindy Bateman (021)6585515 or contact Prof Heather Zar, Tel: 021-6585111. For questions about your rights as a study participant call the Research Ethics Committee, University of Cape Town, Tel: 021-4066492.

Confidentiality

All information that you provide will be considered confidential, and no mention of your child's name or any other identifying information will appear on the stored samples or in any publication in connection with this study. No persons other than the health care workers overseeing your child's care and the study nurses and doctors will have access to any information that identifies your child personally.

You may also choose not to participate in this study and you may refuse to participate at any time. This will not in any way affect the care that your child will receive. You do not have to explain why you do not wish to participate.

Storage of samples

If any of the blood, sputum or nasal samples sample my child has provided for this research project is unused or leftover when the project is completed; this might include analysing or testing DNA

 I give my permission for my child's samples to be stored and used in future research of any type which has been properly approved

 I give permission for my child's samples to be stored and used in future research but only for research on TUBERCULOSIS

 I give permission for my child's samples to be stored and used in future research except for research about _____.

OR I wish my child's samples to be destroyed immediately.

AND

 I want my child's identity to be removed from my child's samples.

 I want my child's identity to be kept with my child's samples.]

I have read and understood this form. My questions have been answered. I agree to allow my child to participate in this study.

I, _____, the parent/ legal guardian of

_____ agree to allow her/him to participate in this study.

I agree/ do not agree (mark with cross as appropriate) to complete the questionnaire.

Signed: _____ Witness: _____

Date: _____ Date: _____

Person obtaining consent:

Name: _____

Role in Study: _____

Signature: _____

Date: _____

If the parent/guardian is unable to read or write the entire counselling process must me observed by an independent witness who can then confirm the procedure once the parent/guardian has given consent.

Fingerprint of parent/guardian

Witness

I confirm that I am independent of the study and that I witnessed the entire enrolment counselling process in the home language of the parent/guardian.

Name: _____

Signature: _____

Date: _____

Appendix D: Journal of Pediatric Radiology instructions to authors

Springer

Medicine - Radiology | Pediatric Radiology - incl. option to publish open access

Radiology

Pediatric Radiology

Managing Editors: P. Strouse; Ø.E. Olsen

ISSN: 0301-0449 (print version)
ISSN: 1432-1998 (electronic version)

Instructions for Authors

GENERAL INFORMATION

Please note that the journal does not offer pre-evaluation. Therefore please directly submit your manuscript to EditorialManager at the link below. The Editors will then contact you.

EditorialManager

It is the Corresponding Author's responsibility to ensure that he/she has the correct authors' names, affiliations, addresses and author sequence when the final corrected proofs are submitted. Please keep in mind that corrections are no longer possible after online first publication. All additional corrections need the approval of the Managing Editors and would result in the publication of an erratum that will be hyperlinked to the article.

Important Information Regarding Radiation Dosimetry

In order to adhere to the ALARA concept, authors should not submit manuscripts that describe techniques that have used inappropriately high radiation exposures for children. Furthermore, when CT has been used, the text should include the CTDI (as a single value when there is one exam or as a range in multiple exams) in manuscript submissions. This will provide significant information for appropriate dosimetry.

Original article

This is the most important type of article because it provides new information based on original research. An original report is new because of the imaging findings in a disease or syndrome; it is new because of unique interventional processes; it is new because it expresses new manifestations or complications or follow-up of a disease or disorder. Original reports can be prospective or retrospective. They can be clinical or basic research. This type of article must not exceed 18 double–spaced typed pages excluding tables and pictures.

Format:

Structured Abstract which should be divided into the following sections:

1) Background – reason for study
2) Objective – give hypothesis being tested
3) Materials and methods – brief but specific to number of subjects, how collected, and whatwas done
4) Results – the findings of the study with statistical significance
5) Conclusion

Body of paper:

Introduction: Briefly describe the objective of the investigation and explain why it is important.

Materials and methods: Describe the research plan, the materials (or subjects), and the methods used, in that order.

Explain in detail how disease was confirmed and how subjectivity in observations was controlled.

Results: Present results in a clear, logical sequence. If tables are used, do not duplicate tabular data in text, but do describe important trends and points.

Discussion:

Describe the limitations of the research plan, materials (or subjects), and methods, considering both the objective and the outcome of the study. When results differ from those of previous investigators, explain the discrepancy.

Conclusion:

In one or two sentences, present the message to be remembered when all else is forgotten. Describe the conclusion of the study, based solely on the data provided in the body of the report. Conclusions must relate directly to the objective of the paper as defined in the title and first paragraph of the report. Do not use abbreviations. Do not use reference citations.

Editorial

Brief article (6 or fewer double spaced typed pages) stating the author's personal opinion on a contentious or timely topic. Minimum illustrations. Author will review articles to align his/her viewpoint.

Format:

No abstract
Sections divided by topic headings

Technical innovation

A short explanation of a certain method or procedure, alteration of a method, or new equipment of interest to radiologists. Limited to 6 double-spaced typed pages. References limited to 8.

Format:

Abstract in paragraph form of less than 125 words
A brief, one–paragraph introduction giving the general background Body of

report:

Introduction with general background.

Description of new technical innovation.

Discussion.

Case report

Short discussion of a single case with unique features not previously described. A case report must be unique by imaging findings, a unique manifestation of a disease or disorder or by making unique use of imaging to reveal a disease or disorder. Images of a second case may supplement either the discussion or the illustration of findings, but a single case must remain the concentration. Limited to 6 double-spaced typed pages. References limited to 8. Authors limited to 5 who are affiliated with the institution that managed the case.

Format:

Abstract in paragraph form (<125 words) and includes:

1) Reason to report

2) What was unique

3) Ramification of this reportBody of report:

Introduction – is a brief paragraph giving general background and specific interest of the case.

Case report – Stress should be on the radiologic aspects; clinical information must be limited to that which provides a background for the radiologist.

Discussion – Concise and focuses on the specific message and significance of radiologic methods. A review of the literature is not appropriate.

Since we receive many case reports, we will attempt to publish those accepted as rapidly as possible. However, priority in getting to publication will be given to original articles and review articles.

Review

Scholarly examination of recent developments on a certain topic as reported in the literature. No new information is described but personal experiences may be expressed.

Reviews are not encyclopedic like a chapter in a textbook; rather, they include only the highlights. Limited to 20 double–space typed pages.

Format:

Abstract in paragraph form introducing scope of paper. Body
of report:

Introduction – background and scope Headings –

used to organize text

Pictorial essay

This is a teaching exercise with the message in the figures and their legends. Text is no more than 9 double–spaced typed pages, and there may be as many as 30 figure parts; however, no new information is included. The value of the paper turns on the quality of the illustrations as well as the timeliness and utility of the message.

Format:

Abstract in paragraph form defining the goals of the essay. Body:

Introduction

Headings – used to organize text

Clinical image

Clinical images are no longer accepted

Letter to the Editor and Reply

Letters to the editor and replies should offer objective analysis of published articles. Letters may also discuss matters of general interest to pediatric radiologists. Material being submitted or published elsewhere should not be repeated in letters.

Format:

Double-spaced on non-letterhead paper, with a salutation of ‘‘Dear Editor’’. The title included on the letter should be short and relevant. The title for a reply is simply ‘‘Reply.’’ Do not use abbreviations in the title, letter, or reply.

Summary of Format for Articles

Types of articles	Maximum pages* (words)	Abstract
Original article	18 (4,500)	Structured
Editorial (Opinion/Commentary)	6 (1,500)	None
Technical innovation	6 (1,500)	Paragraph
Case report	6 (1,500)	Paragraph
Review	20 (5,000)	Paragraph
Pictorial essay	9 (2,250)	Paragraph
Letters to the Editor	2 (500)	None

*Each page double–spaced is approximately 250 words. Total pages include references but not pictures.

EDITORIAL PROCEDURE

Double-blind peer review

This journal follows a double-blind reviewing procedure. Authors are therefore requested to submit:

A blinded manuscript without any author names and affiliations in the text or on the title page. Self-identifying citations and references in the article text should be avoided.

A separate title page, containing title, all author names, affiliations, and the contact information of the corresponding author. Any acknowledgements, disclosures, or funding information should also be included on this page.

MANUSCRIPT SUBMISSION

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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The title page should include:

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A concise and informative title
The affiliation(s) and address(es) of the author(s)
The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

TEXT

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.

- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (zip, 182 kB)

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Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

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Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

SCIENTIFIC STYLE

Please always use internationally accepted signs and symbols for units (SI units).

REFERENCES

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

The entries in the list should be numbered consecutively.

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-0080955-8

  Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

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ISSN.org LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of intext citations and reference list.

EndNote style (zip, 2 kB)

Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer’s LaTeX macro package.

SPECIFIC REMARKS

References with correct punctuation can be found in EndNoteX1 (Windows 2000 SP3, XP [SP2] and Vista) (Mac OS X).

TABLES

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.

- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

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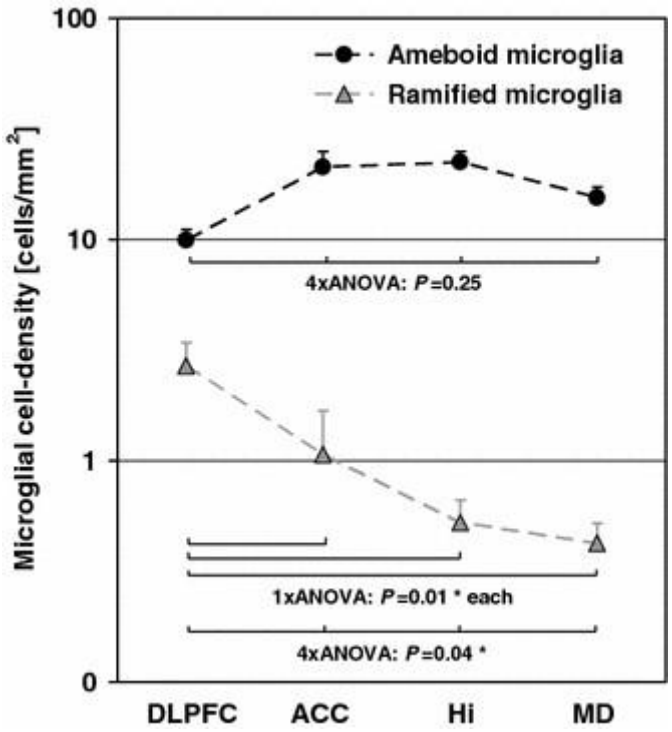
Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

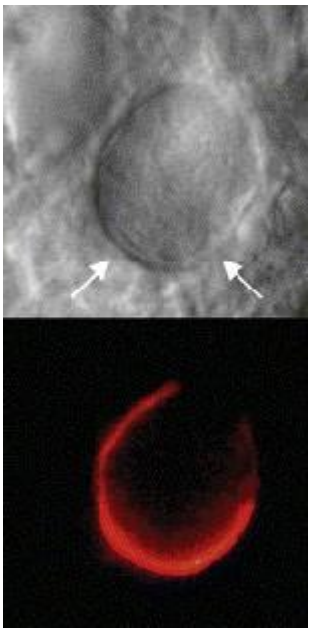
Line Art



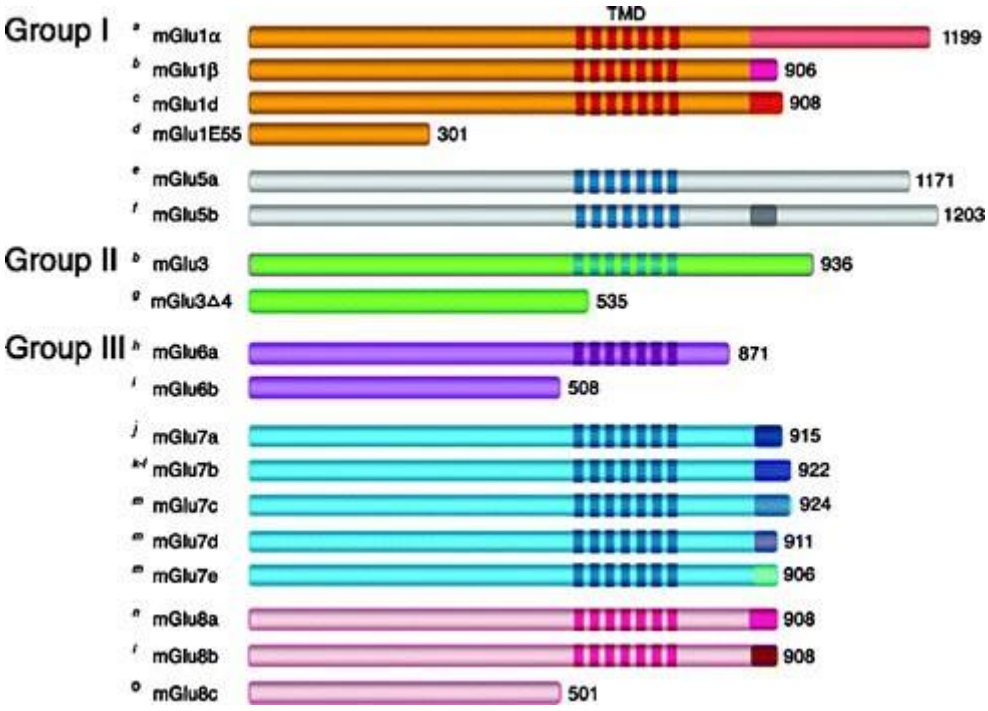
- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

Definition: Photographs, drawings, or paintings with fine shading, etc.
If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
Halftones should have a minimum resolution of 300 dpi.



Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
Combination artwork should have a minimum resolution of 600 dpi.

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- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

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If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,

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- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
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- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
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- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

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Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

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