Aetiology and presentation of childhood pleural infections in the post-pneumococcal conjugate vaccine era in South Africa

by

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DECLARATIONS

I, *…Lauren Golden…,* hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.
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FORMAT OF THESIS, CONTRIBUTIONS AND ACKNOWLEDGMENTS

Format of Thesis

This thesis is presented in the Publication-ready Manuscript format. It has been submitted to the African Journal of Thoracic and Critical Care Medicine and has been accepted for publication. The manuscript was written according to the journal specifications as per instructions to authors – please see attached under appendices.

Dissertation, authors and contributions

This dissertation is derived from work started and data collected in a larger Pleural Effusion Aetiology study, with the Principal Investigator (PI) Marco Zampoli, who is also the supervisor for this MMED. The Pleural Effusion Aetiology study enrolled children admitted with pleural effusion or empyema at Red Cross War Memorial Children's Hospital into the study between December 2016 and December 2019. This thesis was undertaken to fulfill some of the objectives of the original study, and for the purposes of this MMED we analysed a subset of the data to meet the objectives of this thesis.

Authors listed in manuscript and contributions

Assoc Prof Marco Zampoli – PI on original Pleural effusion aetiology study, Supervisor on MMED thesis, protocol development of Pleural Effusion study, guidance in protocol development on MMED thesis and writeup

Dr S Chaya – Co-supervisor on MMED, assisted in protocol development of MMED thesis, guidance on and review of thesis writeup

Assoc Prof D Gray, Assoc Prof A Vanker, Prof H Zar – assisted in protocol development of Pleural Effusion study, assisted with data collection and recruitment, review of manuscript

Dr S Chaya, Dr A Ayuk A, Dr S Kwarteng Owusu, Dr D Marangu, Dr N Affendi, Dr A Lakhan - assistance in protocol development of Pleural effusion study, patient recruitment to Pleural Effusion study, data collection, review of manuscript

Dr K Reichmuth - Statistical analysis, contribution to final writeup and review of manuscript

Mrs A Visagie – Project management on Pleural Effusion study, administration and logistics for Pleural effusion study

Contribution of MMED candidate Dr L Golden:

Concept and Protocol: Conduction of literature review for subject matter related to dissertation objectives, protocol development for MMED thesis, setting of objectives of the study and applications to departmental research committee and Department of UCT Ethics committees done.
Funding Application: Application for and securing of funding from Paediatric research grant for MMED.
Data: Collection of missing data from Pleural Effusion study data, sourcing of incidence data from Information management unit as required for study. Data entry of all data collected by Pleural Effusion study into RedCap electronic database was done by myself. Extraction of data needed for MMED thesis, cleaning and preparation of data for statistical analysis.
Writeup Analysed data collated into tables and figures for manuscript purposes. Full writeup of manuscript as first author, and submission to chosen journal as per author guidelines, and corresponding author for journal review. Preparation of Publication ready manuscript for MMED thesis submission.

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Thank you to my Lord Jesus who gives me strength and grace every day to walk in his plans for my life.

ABBREVIATIONS

SA	South Africa
PE	Pleural effusion
TB	Mycobacterium tuberculosis
IQR	Interquartile range
PCV	Pneumococcal conjugate vaccine 13 valent
PCR	Polymerase chain reaction
LMIC	Low-and-middle-income countries
RCCH	Red Cross War Memorial Children's Hospital
WFA	Weight-for-age
ICU	Intensive care unit
FBC	Full blood count
WCC	White cell count
LDH	Lactate dehydrogenase
CRP	C-reactive protein
PCT	Procalcitonin
HIV	Human immunodeficiency virus
ADA	Adenosine deaminase
TST	Tuberculin skin test
SD	Standard deviations
IQR	Interquartile range
WAZ	Weight-for-age z-score

ABSTRACT

Complications of respiratory infections include pleural effusion (PE), associated with a high morbidity. Differentiating between PE caused by bacterial infections and *Mycobacterium tuberculosis* (TB) in endemic areas is difficult in children, impacting treatment. We investigated the aetiology of PE and features distinguishing TB from bacterial PE in children.

Methods In this prospective study, children with PE admitted to a tertiary hospital in Cape Town from December 2016 to December 2019 were enrolled. Clinical information and routine laboratory investigations were compared between children with bacterial, TB or unclassified PE, categorised according to study definitions.

Results A total of 91 patients were included; their median age was 31 months (IQR 11.8–102.1). Aetiology was bacterial in 37 (40%), TB in 36 (39%) and unclassified in 18 (20%) patients. *Staphylococcus aureus* was the most common bacterial isolate, confirmed in 24/37 (65%) patients; and *Streptococcus pneumoniae* confirmed in only 3/37 patients (8%). TB was microbiologically confirmed in 12/36 (33%) patients. Patients with TB were older (median age 91.6 vs 11.8 months, $p<0.001$), with more weight loss (28/36 (77.8%) vs 12/37 (32%) patients, $p<0.001$), and longer cough duration (10 vs 4 days, $p<0.001$) than those with bacterial PE. In contrast, the latter had significantly higher median values: serum C-reactive protein (250 vs 122 mg/L, $p<0.001$), procalcitonin (11 vs 0.5 mg/L, $p<0.001$) pleural fluid lactate dehydrogenase (7280 vs 544 U/L, $p<0.001$), and adenosine deaminase levels (162 vs 48 U/L, $p<0.001$) and lower glucose levels (1.3 vs 4 mmol/L, $p<0.001$).

Conclusion Post-PCV, *S. aureus* is the dominant cause of PE in children using traditional culture methods, while TB remains a common cause of PE in our setting. Useful clinical and laboratory differences between TB and bacterial PE were identified, but the cause of PE in 20% of children was underdetermined. Molecular testing of pleural fluid for respiratory pathogens may be useful in such children.

MANUSCRIPT IN PUBLICATION – READY FORMAT

Aetiology and presentation of childhood pleural infections in the post-pneumococcal conjugate vaccine era in South Africa

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ABSTRACT

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BACKGROUND

Acute respiratory infections are a leading cause of childhood mortality globally, [1, 2] and the second highest cause (10%) of mortality in South African (SA) children younger than 5 years.[3] Pleural effusions (PE) are recognized complications of pneumonia, resulting in increased hospital stay, morbidity and mortality.[4] A causative organism is often not identified using routine culture techniques, with up to 60% of cases having no bacteriological identification. [5,6] When a causative organism is found, the most common pathogens are *Staphylococcus aureus* and *Streptococcus pyogenes*. Following the introduction of the 13-valent pneumococcal conjugate vaccine (PCV), there has been an overall decrease in invasive pneumococcal disease and complicated pneumococcal pneumonia, including PE.[7-9] Molecular polymerase chain reaction (PCR) techniques targeting common respiratory pathogens increase the detection rates of respiratory pathogens, specifically *S. pneumoniae*, by up to three times more than culture.[5,7,8] However, these tests are not yet routinely available in low- and middle-income countries (LMIC) thus limiting the interpretation of studies reporting PE aetiology using culture-only techniques.

Mycobacterium tuberculosis is another leading cause of pneumonia in SA and other LMIC, with SA reporting one of the highest incidences of tuberculosis (TB) globally: 520 cases/100 000 population, with children making up 7% of new cases.[10] A serous, straw-coloured PE is a common manifestation of TB in adults and children, TB is and a common cause of PE in TB-endemic settings. Tuberculous empyema, however, is uncommon but reported to be the third most common cause of empyema in SA, occurring in 10–14% of empyema cases. [7,8] The diagnosis of TB is challenging in children due to the paucibacillary nature of the disease and difficulty in obtaining suitable samples. Despite advances in the diagnosis of TB, culture remains the gold standard in children, which may result in missed or delayed diagnosis. Molecular techniques such as Xpert MTB/RIF® and Xpert MTB/RIF-Ultra® on respiratory samples are more sensitive than microscopy in identifying *M. tuberculosis* and have improved early diagnosis of pulmonary TB in adults and children, [11-14] however, the sensitivity of Xpert MTB/RIF® on pleural fluid is low, reported to be 50–55%. [13,15-18] Children with PE in SA and other LMIC are therefore often commenced on empiric TB treatment without confirmation of the disease, as described in a SA study where 43% of children with PE were empirically initiated on TB treatment.[9]

With limited microbiological or laboratory confirmation of aetiology, clinicians in SA and similar LMIC settings rely on a combination of clinical, biochemical, and radiological features to determine the aetiology of PE in children. Our study aimed to investigate the incidence and aetiology of infective PE in children using routine microbiological methods, and to compare clinical and laboratory characteristics and outcomes of PE caused by bacterial infections and TB.

METHODOLOGY

Study design, setting and recruitment

This prospective, descriptive observational study was conducted at Red Cross War Memorial Children's Hospital (RCCH), a tertiary referral hospital in Cape Town, South Africa. Children younger than 13 years admitted with PE from December 2016 to December 2019 were enrolled in the study if they had PE identified clinically and radiographically. Patients with PE due to trauma or following any surgical procedure; transudative PE due to cardiac, renal or liver disease, or other non-infective aetiologies; PE associated with nosocomial infection; or those where informed written consent or assent was not obtained were excluded from the study. Written informed consent from parents and assent (in children older than 7 years) were obtained in all patients. Pleural fluid samples were collected by pleural space aspiration or pleural drainage performed using an aseptic technique.

Data Collection Methods

A data collection form was used by investigators for all patients at enrolment in the study. Data was entered into and managed using REDCap® electronic data capture tools hosted at the University of Cape Town.

Clinical data and interventions

Demographic data, clinical symptoms and signs at presentation, PCV immunisation status, and World Health Organisation referenced weight-for-age (WFA) z-scores were collected. The following interventions and outcome date were recorded: type and duration in situ of a chest drain, if needed; the use of fibrinolysis; the need for surgical drainage; the duration of hospitalisation; the need for ICU admission, and mortality data.

Investigations

A chest radiograph was performed on all patients at admission, and chest ultrasound was requested at the discretion of the treating clinicians.

Routine laboratory investigations were performed on blood and pleural fluid samples. Haematology and biochemical blood investigations included: a full blood count (FBC), white cell (WCC) differential count, serum lactate dehydrogenase (LDH), total protein, albumin, C-reactive protein (CRP) and procalcitonin (PCT). Human immunodeficiency virus (HIV) testing was done in all patients according to SA national guidelines. HIV antibody testing was performed in all patients in whom HIV status was not known, and in HIV-unexposed patients. HIV PCR testing was performed in HIV-exposed children under the age of 18 months, and antibody testing in HIV-exposed children aged 18months and older. Pleural fluid sample investigations included: macroscopic appearance (purulent or turbid, straw coloured, blood stained or bloody), glucose, total protein, LDH, adenosine deaminase (ADA). Cytology and cell count was performed where available. Microbiological tests included blood culture and pleural fluid bacterial and mycobacterial cultures using standard culture techniques. All patients had a tuberculin skin test (TST) and at least two induced sputum samples collected for Xpert MTB/RIF® or Xpert MTB/RIF -Ultra® and mycobacterial culture.

Aetiological definitions

The aetiology of PE was categorised as confirmed or unconfirmed bacterial, TB, or unclassified, according to the following study-defined clinical definitions:

Confirmed TB:[19] microbiological confirmation of TB by positive Xpert MTB/RIF® or Xpert MTB/RIF -Ultra® or mycobacterial culture positive for *M. tuberculosis* from induced sputum and/or pleural fluid.

Unconfirmed TB:[19] microbiological confirmation of TB was not obtained and at least two of the following were present: persistent cough >2 weeks, weight loss or WFA <2 standard deviations (SD) below the mean z-score, household TB contact exposure, or positive TST result (defined as a TST reading at 48-72hrs of ≥10mm in HIV negative patients, or ≥5mm in HIV positive patients).

Confirmed bacterial: patients with pleural effusion who had microbiologically confirmed (pleural fluid or blood culture) bacterial infection with clinically significant organisms.

Unconfirmed bacterial: absence of culture confirmation on blood and/or pleural fluid but supporting laboratory evidence of bacterial infection of pleural fluid, i.e., pus or purulent pleural fluid on macroscopic inspection, neutrophil predominance of pleural fluid, and absence of clinical features of TB as per above definitions.
Unclassified: patients who did not meet the definitions for TB or bacterial infection.

Data Analysis

The annual incidence rate of bacterial PE per 1000 lower respiratory tract infection admissions at the hospital from 2017 to 2019 was calculated by extracting data from the hospital electronic ICD 10-coded admissions database for all recorded lower respiratory tract infections that were not classified as TB as the denominator, and all enrolled PE cases not classified in the TB group as the numerator. This formula was selected for comparison to previous incidence data at our hospital and thus excluded TB cases.[7]

Data were analysed using Stata Statistical Software: Release 13 (2013. College Station, TX: StataCorp LP). The mean with SD and median with interquartile range (IQR) were used to describe normally and non-normally distributed continuous data, respectively. Means were compared between aetiological groups using one-way ANOVA; the Bonferroni correction for multiple comparisons was applied. Medians were compared between aetiological groups using the Kruskal Wallis test; two-sample Wilcoxon rank sum tests were used to identify differences between each pair of groups. Categorical data were compared between aetiological groups using Chi-squared or Fisher's exact tests, as appropriate. A p-value <0.05 was considered statistically significant.

Ethical considerations

The study, as well as the pre-existing parent study obtained ethics approval from the Human Research Ethics Committee of the University of Cape Town [HREC REF 6.61/2019 and HREC REF 254/2016, respectively]. Written informed consent and assent (for participants older than 7 years of age) was obtained in all study participants.

RESULTS

Study population and aetiology classification

Of the 117 children screened, 26 were excluded for reasons relating to PE due to non-infectious causes (8 patients), lack of consent (6 patients), pleural sample collection not done (6 patients), residence in a remote geographical location (3 patients), and nosocomial infection with PE (3 patients). Of the 91 enrolled patients, median age was 31 months (IQR 11.8-102.1), 56 (62%) were male, 10 (15%) were HIV exposed, uninfected and 4 (4%) were HIV infected. Demographic characteristics and clinical features of enrolled patients are displayed in Table 1. Aetiology (confirmed and unconfirmed) was classified as bacterial in 37 (41%), TB in 36 (40%) and unclassified in 18 (20%) patients. Fig.1 shows a detailed breakdown of aetiological categories.

Cough was the commonest presenting symptom, reported in just under 90% of patients, followed by shortness of breath and fever in just under two-thirds of patients. The mean WFA z-score was -0.195 (SD 1.510). Compared to the bacterial group, patients with TB were older (median age 91.6

TABLE 1. DEMOGRAPHIC AND CLINICAL DATA

	Presented as	**Bacterial**	**TB**	**Unclassified**	**Total**	***p-value**
	n	**37**	**36**	**18**	**91**	
Demographics						
Age (months)	median (IQR†)	11.8 (7.2–25.5)	91.6 (49.8–125.6)	30.7 (19.6–100.6)	31.3 (11.8–102.1)	<0.001
Sex male	n (%)	23 (62.2)	21 (58.3)	12 (66.7)	56 (61.5)	0.843
HIV exposed	n (%)	9 (24.3)	3 (8.3)	2 (11.1)	14 (15.4)	0.142
HIV infected	n (%)	1 (2.7)	1 (2.8)	2 (11.1)	4 (4.4)	0.321
Clinical features						
Cough	n (%)	29 (78.4)	26 (72.2)	16 (88.9)	16 (88.9)	0.377
Cough duration (days)	median (IQR)	4 (3–7)	10 (4–21)	5 (3–7)	5 (3–10)	0.012
Shortness of breath	n (%)	28 (75.7)	21 (58.3)	12 (66.7)	12 (66.7)	0.289
TB exposure in past 6 months	n (%)	2 (5.4)	10 (28.6)	1 (5.6)	1 (5.56)	0.01
Weight loss	n (%)	12 (32.4)	28 (77.8)	3 (16.7)	43 (47.3)	<0.001
Chest pain	n (%)	7 (18.9)	22 (61.1)	5 (27.8)	34 (37.4)	<0.001
Antibiotics in the last 7 days	n (%)	20 (54.0)	16 (44.4)	14 (77.8)	50 (55.0)	0.067
WAZ‡	mean (SD§)	0.166 (1.539)	–0.815 (1.361)	0.015 (1.444)	–0.195 (1.510)	0.049
WAZ <-2SD	n (%)	2 (5.4)	4 (11.1)	1 (5.6)	7 (7.7)	0.126
Pyrexia >38°C	n (%)	25 (67.6)	23 (63.9)	11 (61.1)	59 (64.8)	0.885
Pneumothorax	n (%)	7 (18.9)	1 (2.86)	3 (16.7)	11 (12.2)	0.071

**measures comparison across all 3 groups; †Interquartile Range; ‡Weight for age z-Score; §standard deviation*

vs 11.8 months, p<0.001), more reported weight loss (28/36 (78%) vs 12/37 (32%), p <0.001) and chest pain (22/26 (61%) vs 7/37 (19%), p<0.001), and had a longer cough duration (10 vs 4 days, p= 0.01) (Table 1).

HIV testing was performed in all patients: 77/91 (84.6%) were HIV negative, 10/91 (11%) were currently exposed but were HIV uninfected (11%), and 4 patients (4.4%) were infected with HIV.

Aetiology

The incidence of bacterial PE during the study period was 5.4 (95% CI, 4.0, 7.1) per 1000 hospitalised pneumonia cases, with the lowest rates of PE in 2018 (2.6 per 1000 pneumonia hospitalisations), and the highest in 2017 (7.9 per 1000 pneumonia hospitalisations); Table 2. Among the bacterial group, 14/31(45%) blood and 25/37 (68%) pleural fluid samples were culture positive. *Staphylococcus aureus* was the most common bacterial isolate, confirmed in 24/37 (65%) of patients; all isolates were susceptible to cloxacillin. Other organisms isolated were *Streptococcus pneumoniae* (n=3/37, 8%), *Streptococcus pyogenes* (n= 2/37, 5%), *Streptococcus constellatus species* (n=1) and anaerobes (n=2, 5%), one of the anaerobes as a co-infection with TB. (Figure. 2).

Table 2. Incidence of pleural effusion and empyema due to bacterial and unclassified infections, 2017-2019

Years	PE cases enrolled	Total cases of Pneumonia admissions	PE incidence per 1000 Pneumonia admissions (95% CI)
2017	21	2675	7.9 (4.9, 12.)
2018	9	3487	2.6 (1.2, 4.9)
2019	21	3355	6.3 (3.9, 9.6)
Total	51	9517	5.4 (4.0, 7.1)

Overall, 39/91 (42%) participants had completed vaccination with three doses of PCV, and a further 12 patients were appropriately vaccinated for their age – total 51/91 (56%). Of the bacterial group, 20/37 (54%) had complete PCV vaccination, and a total of 31/37 (83%) had vaccination complete for age. Two of the three confirmed *S. pneumoniae* cases were fully vaccinated for PCV and another appropriately vaccinated with two doses for their age.

Among the TB group, microbiological confirmation by Xpert MTB/RIF® and Xpert MTB/RIF -Ultra® or TB culture was possible in 12/36 (33%) patients. Pleural fluid was Xpert MTB/RIF® or Xpert MTB/RIF -Ultra® positive in 5 (14%), and TB culture positive in 8 (22%) of the TB cases. The TST result was positive in 24/36 (67%), 2/18 (11%), and 0 of TB, unclassified, and bacterial PE cases, respectively. One case had confirmed TB and bacterial co-infection.

Laboratory investigations

Table 3 describes selected laboratory findings across the bacterial, TB, and unclassified groups. Statistically significant differences were observed in median values of laboratory indices between bacterial and TB groups: WCC, neutrophil count, CRP, PCT, LDH and ADA were higher in the bacterial group compared to the TB group; glucose was lower and total protein was similar. Overall, clinical and laboratory findings of the unclassified group fell between those of the TB and bacterial groups.

Interventions and Outcomes

Overall, 80% (n=74) of patients had a chest drain inserted, which included 100% (n=37) of the bacterial group. The median duration of chest drain use was 5 days (IQR 3.5–6), and 49% (n=45) received intrapleural fibrinolysis, including 6 (17%) in the TB group. Bacterial infection was associated with higher rates of intensive care unit admission (19%, n=7) and surgical drainage (27%, n=10) compared to the TB and unclassified groups. The median length of hospital stay was 8.5 days (IQR 5–13) and was higher in the bacterial and unclassified groups. There was one death in the unclassified group - the patient was a 20 month old boy, HIV unexposed, who had a negative TST and was fully vaccinated with PCV. He died due to acute respiratory distress syndrome. Table 4 provides further detailed results of interventions and outcomes.

TABLE 3. COMPARISON OF SELECTED LABORATORY FINDINGS BACTERIAL, TB AND UNCLASSIFIED GROUPS

	Presented as	Bacterial	TB	Unclassified	Total	p-value
	n	**37**	**36**	**18**	**91**	
Blood Investigations						
WCC * ($\times 10^9$/L)	median (IQR†)	16.6 (10.6 – 27.7)	10.3 (7.9–13.1)	14.4 (5.0–21.7)	12.0 (8.1–23.5)	0.005
CRP ‡ (mg/L)	median (IQR)	250.5 (188.5–305)	122 (75–156)	125.5 (54–236)	171 (102–257)	<0.001
PCT § (mg/L)	median (IQR)	11.6 (3.4–29.4)	0.46 (0.24–1.43)	4.8 (0.17–14.31)	2.7 (0.39–12.73)	<0.001
Pleural Fluid Tests						
Protein (g/L)	median (IQR)	46.5 (37–51)	55 (51–58)	55 (47–60)	51.5 (42–57)	0.007
LDH ¶ (U/L)	median (IQR)	7280 (3433–16844)	544 (386–820)	1326 (358.5–951)	1071 (473–855)	<0.001
Glucose (mmol/L)	median (IQR)	1.25 (0.6–3.15)	4 (3–5)	5.05 (4–5.6)	3.5 (1.7–4.7)	<0.001
ADA || (U/L)	median (IQR)	116.2 (59.2–275)	47.1 (41.3–58.2)	47.6 (36.8–79.4)	56.1 (44.2–106.4)	<0.001
Neutrophils ($\times 10^9$/L)	median (IQR)	1340 (605–3280)	27 (5.5–120)	260 (106–400)	133 (11–530)	<0.001
Lymphocytes ($\times 10^9$/L)	median (IQR)	460 (115–720)	832 (419–1522)	400 (155–800)	664 (224–1185)	0.142
Cytology	n samples	31	35	16	82	
Reactive Lymphocytes	Yes, n (%)	11 (29.7)	23 (63.9)	7 (38.9)	41 (45.0)	0.044
Neutrophils Scattered	Yes, n (%)	16 (43.2)	8 (22.2)	6 (33.3)	30 (33.0)	0.054

** White Cell Count; † Interquartile Range; ‡ C-reactive protein; § procalcitonin; ¶ lactate dehydrogenase; ||adenosine deaminase*

TABLE 4. OUTCOMES DATA

	Presented As	Bacterial	TB	Unclassified	Total	p-value
	n	**37**	**36**	**18**	**91**	
Chest Drain	yes, n (%)	37 (100)	21 (58.3)	16 (88.9)	74 (81.3)	<0.001
Chest Drain type	pigtail:hard drain:unknown	30:5:2	18:0:3	10:3:3	58:8:8	0.129
Duration of Chest Drain	median (IQR*)	6 (5–11.5)	4 (3–6)	4 (3–6)	5 (3.5–6)	0.003
Duration Fever (Days)	median (IQR)	3 (1–5)	0.5 (0–2.5)	1 (1–4)	1 (0–4)	0.055
Length of Hosp Stay	median (IQR)	10.5 (8.5–17)	6 (3–8)	9 (5–13)	8.5 (5–13)	<0.001
Fibrinolysis	yes, n (%)	29 (78.4)	6 (17.1)	9 (50)	44 (48.9)	0.001
ICU† Admission	yes, n (%)	7 (18.9)	0	3 (16.7)	10 (11.0)	0.013
Surgical Drainage	yes, n (%)	10 (27)	1 (2.8)	1 (5.56)	12 (13.2)	0.007
Bronchopleural Fistula	yes, n (%)	2 (5.6)	1 (2.8)	0	3 (3.3)	0.801
Death	yes, n (%)	0	0	1	1 (1.1)	

**Interquartile Range; †Intensive care unit*

DISCUSSION

Incidence and aetiology

Our study documented the incidence and aetiology of PE in SA in the post-PCV era. The overall incidence of 5.4 per 1000 pneumonia admissions of PE due to bacterial infections was similar to a previous study from the same centre in 2015. In that study the incidence declined from 10.4 to 4.2 cases per 1000 pneumonia admissions after PCV was introduced, and our study thus confirms the sustained effect of PCV in preventing pneumococcal-associated PE.[7]

We found that most (80%) PE in children could be classified as either bacterial infection or TB, occurring with equal frequency (40% each). *S. aureus* was the most common bacterial pathogen isolated on blood and pleural fluid cultures (24/37 [65%] of all bacterial cases), with low rates of *S. pneumoniae*, which was isolated in only 3 patients. This is consistent with findings from other studies using traditional culture-based methods for diagnosis in our setting. [7-9] Of the 91 patients enrolled, complete vaccination with three doses of PCV was documented in only 42% of study participants, and only 54% of bacterial PE cases. This number increased when we accounted for the lower age of patients in the bacterial group (median age 11 months, IQR 7–25) , as 31/37 (83%) of the bacterial group had PCV vaccination complete for age - the third PCV dose is given at 9 months of age in the SA immunization programme.[20] Although we documented a sustained decline in bacterial PE cases in our centre, the true incidence of *S. pneumoniae* infections may be underestimated in the absence of molecular testing, which is more sensitive than culture for detecting bacterial pathogens.[8,21] Two of three *S. pneumoniae* isolates in this study occurred in patients who were fully vaccinated with PCV. The presence of non-vaccine related *S. pneumoniae* serotype disease in this cohort could not be excluded. All *S. aureus* isolates in our study were sensitive to cloxacillin. This is reassuring and consistent with findings in previous studies in this setting [8] and affirms that widespread community-acquired methicillin-resistant *S. aureus* is not common in our setting. These bacterial aetiology findings have important implications for clinicians, highlighting the need to ensure that *S. aureus* treatment is adequately covered in the empiric antibiotic management of PE in our setting. This finding also supports the growing body of evidence which suggests that *S. aureus* empyema is increasing following access to PCV, which may represent an unintended negative consequence of access to PCV. [5-6,24] Our study found only one case of *S. pyogenes*, and thus did not support findings elsewhere of selection of this organism in the era of access to PCV. [5-6]

The high percentage of PE due to TB in our study reflects the high incidence of TB in the population.[10] It is also consistent with other paediatric studies in our setting. One such study described an unexpected finding of 14% confirmed TB cases in a paediatric empyema study where suspected TB cases were actively excluded at enrolment,[7] and another showed that 43% of empyema study cases were empirically treated for TB based on clinical features.[9] This highlights the need for judicious screening for TB in all patients with PE, and the diagnostic challenge of excluding TB in such children. Xpert MTB/RIF® and Xpert MTB/RIF -Ultra® was positive in 5 (14%) pleural specimens, and TB culture detected TB in 8 (22%) cases. This confirms the low sensitivity of Xpert MTB/RIF® in pleural fluid as reported by Scott et al in a large study on the diagnostic performance of Xpert MTB/RIF® on extrapulmonary specimens.[15] One patient was co-infected with TB and bacterial PE. This finding was lower than previously reported [7,9] in PE and pneumonia studies from SA.[22] However, molecular-based testing for bacterial pathogens may reveal more TB-bacterial co-infected patients.

Using our study definitions, we categorised the aetiology of PE as bacterial or TB in 80% of patients. However, 20% of patients did not fulfil the criteria for either category and were categorised as unclassified. The unclassified group had clinical and laboratory characteristics in between those of the TB and bacterial group, with a greater proportion of patients receiving antibiotics prior to presentation (78%) compared to the other groups (TB group 44% and bacterial group 54%). Although not statistically significant, this trend in increased pre- admission antibiotic treatment suggests that the unclassified group could represent partially treated, culture-negative bacterial PE. Alternatively, the unclassified group may represent TB-bacterial co-infected patients. In practice, this unclassified group will therefore derive most benefit from using routine molecular diagnosis to identify a bacterial pathogen in culture-negative pleural fluid samples to guide appropriate antibiotic treatment, and simultaneously avoid unnecessary TB treatment. Clinical and laboratory comparisons of the bacterial and TB groups in this study revealed features that could be helpful in differentiating the two groups. Older age, chronic symptoms and history of TB exposure were more common in children from the TB group; and CRP, PCT, pleural LDH and ADA levels were significantly higher in the bacterial group compared to other groups. These patterns are thus helpful in practice if the diagnosis is still uncertain during management of PE

cases. This study also highlights that in up to 20% of cases where the aetiology is unclassifiable the role of these clinical and laboratory features is limited.

Interventions and outcomes

As expected, patients with bacterial PE needed more interventions (chest drains, fibrinolysis, surgical drainage) compared to those with TB PE. Pre-emptive fibrinolytic therapy is the preferred protocol at RCCH for patients with bacterial PE as it decreases hospital stay, has similar efficacy to video-assisted thoracoscopic surgery, and decreases the need for surgical drainage. [8,23] The fact that 6 TB patients received fibrinolysis highlights the difficulty of distinguishing TB and bacterial infection at the time of presentation. The mean length of stay in our study was 8.5 days (IQR 5–13) which is lower than in similar SA studies. [7,9] Further findings supporting the hypothesis of the unclassified group representing partially treated bacterial infections is the higher frequency of interventions i.e., chest drain (89%), fibrinolysis (50%) and longer length of hospital stay which approaches that of the bacterial group, rather than that of the TB group.

Limitations

A limitation of the study is that the molecular based PCR testing on pleural fluid specimens was not undertaken; future molecular testing of these specimens is planned once funding becomes available; and may reveal that some of the unconfirmed TB and bacterial cases have been misclassified by clinical criteria. Another limitation is that the majority of bacterial PE cases were culture-confirmed, but only half of the TB group had microbiological confirmation of TB. However, comparison of clinical and laboratory data between groups is consistent with a clinical diagnosis of TB. Confirmation of PCV vaccination data was limited by unavailable immunization records at the time of hospital admission.

CONCLUSION

The most important finding of this study was that there has been a sustained reduction in the incidence of PE among hospitalised pneumonia cases in children in SA subsequent to the introduction of PCV into the vaccination schedule. S. aureus is the most important bacterial cause of PE in this setting, and empiric therapy for PE should target this pathogen. TB is an important cause of PE in our setting, diagnosed with equivalent frequency to bacterial-associated PE. We

found clinical and laboratory features that are practically helpful in distinguishing TB from bacterial causes of PE in most cases, but in up to a 20% of children presenting with PE their use in establishing a diagnosis is limited. Molecular testing in routine care would be anticipated to assist in guiding treatment of children where the aetiology of PE is unclassifiable using clinical and routine laboratory investigations.

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TABLE 1. DEMOGRAPHIC AND CLINICAL DATA						
	Presented as	**Bacterial**	**TB**	**Unclassified**	**Total**	***p-value**
	n	**37**	**36**	**18**	**91**	
Demographics						
Age (months)	median (IQR†)	11.8 (7.2–25.5)	91.6 (49.8–125.6)	30.7 (19.6–100.6)	31.3 (11.8–102.1)	<0.001
Sex male	n (%)	23 (62.2)	21 (58.3)	12 (66.7)	56 (61.5)	0.843
HIV exposed	n (%)	9 (24.3)	3 (8.3)	2 (11.1)	14 (15.4)	0.142
HIV infected	n (%)	1 (2.7)	1 (2.8)	2 (11.1)	4 (4.4)	0.321
Clinical features						
Cough	n (%)	29 (78.4)	26 (72.2)	16 (88.9)	16 (88.9)	0.377
Cough duration (days)	median (IQR)	4 (3–7)	10 (4–21)	5 (3–7)	5 (3–10)	0.012
Shortness of breath	n (%)	28 (75.7)	21 (58.3)	12 (66.7)	12 (66.7)	0.289
TB exposure in past 6 months	n (%)	2 (5.4)	10 (28.6)	1 (5.6)	1 (5.56)	0.01
Weight loss	n (%)	12 (32.4)	28 (77.8)	3 (16.7)	43 (47.3)	<0.001
Chest pain	n (%)	7 (18.9)	22 (61.1)	5 (27.8)	34 (37.4)	<0.001
Antibiotics in the last 7 days	n (%)	20 (54.0)	16 (44.4)	14 (77.8)	50 (55.0)	0.067
WAZ‡	mean (SD§)	0.166 (1.539)	–0.815 (1.361)	0.015 (1.444)	–0.195 (1.510)	0.049
WAZ <-2SD	n (%)	2 (5.4)	4 (11.1)	1 (5.6)	7 (7.7)	0.126
Pyrexia >38°C	n (%)	25 (67.6)	23 (63.9)	11 (61.1)	59 (64.8)	0.885
Pneumothorax	n (%)	7 (18.9)	1 (2.86)	3 (16.7)	11 (12.2)	0.071

**measures comparison across all 3 groups; †Interquartile Range; ‡Weight for age z-Score; §standard deviation*

Table 2. Incidence of pleural effusion and empyema due to bacterial and unclassified infections, 2017-2019

Years	PE cases enrolled	Total cases of Pneumonia admissions	PE incidence per 1000 Pneumonia admissions (95% CI)
2017	21	2675	7.9 (4.9, 12.)
2018	9	3487	2.6 (1.2, 4.9)
2019	21	3355	6.3 (3.9, 9.6)
Total	51	9517	5.4 (4.0, 7.1)

TABLE 3. COMPARISON OF SELECTED LABORATORY FINDINGS BACTERIAL, TB AND UNCLASSIFIED GROUPS

	Presented as	Bacterial	TB	Unclassified	Total	p-value
	n	**37**	**36**	**18**	**91**	
Blood Investigations						
WCC * ($\times 10^9$/L)	median (IQR†)	16.6 (10.6 – 27.7)	10.3 (7.9–13.1)	14.4 (5.0–21.7)	12.0 (8.1–23.5)	0.005
CRP ‡ (mg/L)	median (IQR)	250.5 (188.5–305)	122 (75–156)	125.5 (54–236)	171 (102–257)	<0.001
PCT § (mg/L)	median (IQR)	11.6 (3.4–29.4)	0.46 (0.24–1.43)	4.8 (0.17–14.31)	2.7 (0.39–12.73)	<0.001
Pleural Fluid Tests						
Protein (g/L)	median (IQR)	46.5 (37–51)	55 (51–58)	55 (47–60)	51.5 (42–57)	0.007
LDH ¶ (U/L)	median (IQR)	7280 (3433–16844)	544 (386–820)	1326 (358.5–951)	1071 (473–855)	<0.001
Glucose (mmol/L)	median (IQR)	1.25 (0.6–3.15)	4 (3–5)	5.05 (4–5.6)	3.5 (1.7–4.7)	<0.001
ADA || (U/L)	median (IQR)	116.2 (59.2–275)	47.1 (41.3–58.2)	47.6 (36.8–79.4)	56.1 (44.2–106.4)	<0.001
Neutrophils ($\times 10^9$/L)	median (IQR)	1340 (605–3280)	27 (5.5–120)	260 (106–400)	133 (11–530)	<0.001
Lymphocytes ($\times 10^9$/L)	median (IQR)	460 (115–720)	832 (419–1522)	400 (155–800)	664 (224–1185)	0.142
Cytology	n samples	31	35	16	82	
Reactive Lymphocytes	Yes, n (%)	11 (29.7)	23 (63.9)	7 (38.9)	41 (45.0)	0.044
Neutrophils Scattered	Yes, n (%)	16 (43.2)	8 (22.2)	6 (33.3)	30 (33.0)	0.054

** White Cell Count; † Interquartile Range; ‡ C-reactive protein; § procalcitonin; ¶ lactate dehydrogenase; ||adenosine deaminase*

TABLE 4. OUTCOMES DATA

	Presented As	Bacterial	TB	Unclassified	Total	p-value
	n	**37**	**36**	**18**	**91**	
Chest Drain	yes, n (%)	37 (100)	21 (58.3)	16 (88.9)	74 (81.3)	<0.001
Chest Drain type	pigtail:hard drain:unknown	30:5:2	18:0:3	10:3:3	58:8:8	0.129
Duration of Chest Drain	median (IQR*)	6 (5–11.5)	4 (3–6)	4 (3–6)	5 (3.5–6)	0.003
Duration Fever (Days)	median (IQR)	3 (1–5)	0.5 (0–2.5)	1 (1–4)	1 (0–4)	0.055
Length of Hosp Stay	median (IQR)	10.5 (8.5–17)	6 (3–8)	9 (5–13)	8.5 (5–13)	<0.001
Fibrinolysis	yes, n (%)	29 (78.4)	6 (17.1)	9 (50)	44 (48.9)	0.001
ICU† Admission	yes, n (%)	7 (18.9)	0	3 (16.7)	10 (11.0)	0.013
Surgical Drainage	yes, n (%)	10 (27)	1 (2.8)	1 (5.56)	12 (13.2)	0.007
Bronchopleural Fistula	yes, n (%)	2 (5.6)	1 (2.8)	0	3 (3.3)	0.801
Death	yes, n (%)	0	0	1	1 (1.1)	

**Interquartile Range; †Intensive care unit*

LIST OF FIGURES

FIGURE 1.

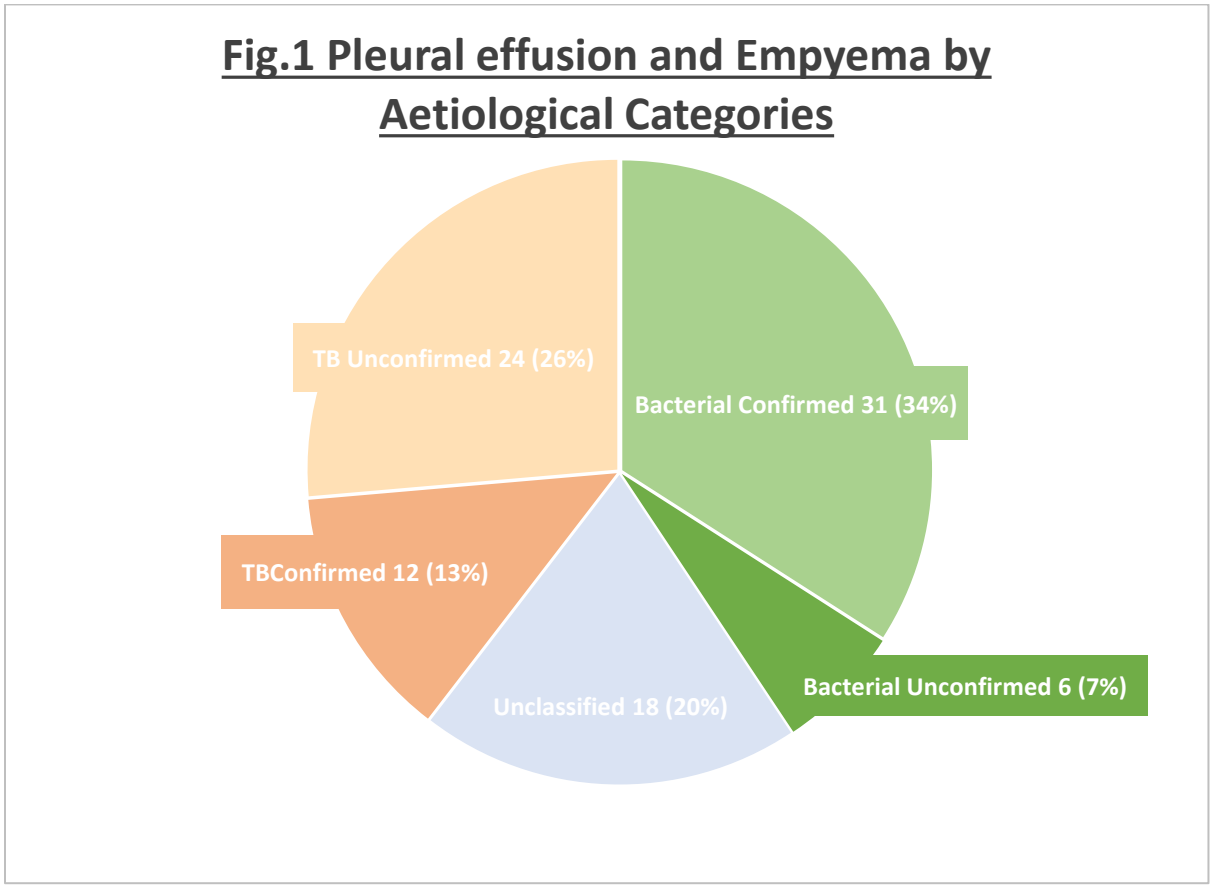


FIGURE 2.

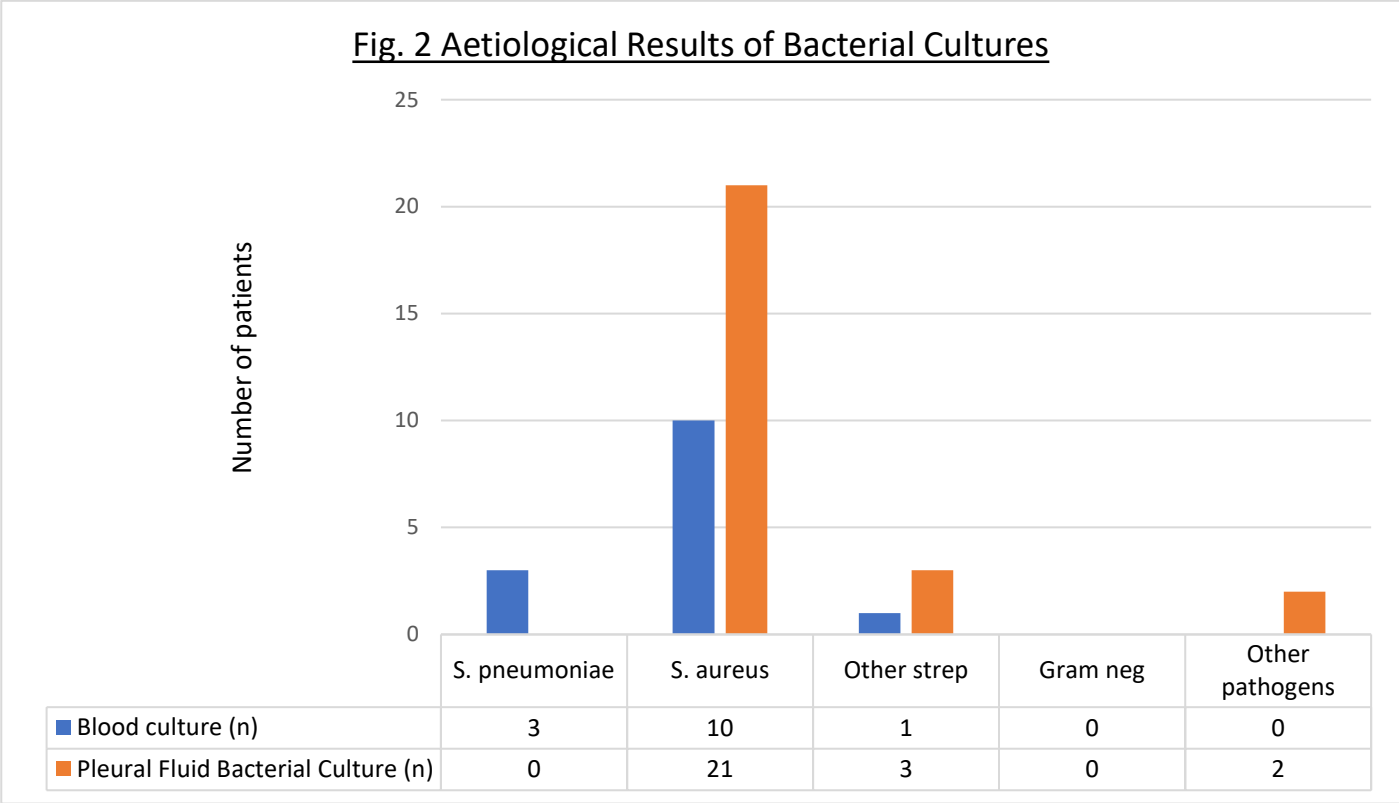


Fig. 2 Aetiological Results of Bacterial Cultures
Number of patients
25
20
15
10
5
0
S. pneumoniae
S. aureus
Other strep
Gram neg
Other pathogens
Blood culture (n) 3 10 1 0 0
Pleural Fluid Bacterial Culture (n) 0 21 3 0 2

APPENDICES

APPENDIX 1. DATA CAPTURE SHEET

Data capture sheet: Molecular diagnosis of pleural effusions in children

Name, Surname: (hospital sticker):

Study ID (Study ID sticker)

Patient co-enrolled on NIH TB study ? Y/N

NIH TB study PID

Date of admission:_____

Study eligibility/screened: Date: _____

Included: Y/N

Reasons for exclusion: trauma / post-surgery / transudate / nosocomial infection / no consent

Other reason: _____

Clinical

-Symptom duration: Y/N Duration_____

-Cough Y/N Duration_____

-Shortness of Breath Y/N Duration_____

-TB exposure in past 6 months: Y /N

-Weight loss: Y / N

-Chest pain: Y / N

-Antibiotics in past 7 days: Y / N Duration_____Name_____

-Weight (Kg): _____ WAZ: _____

-Pyrexia > 38ºC: Y / N

-RR (bpm): _____ bpm

-Mediastinal shift: Y / N

-Pneumothorax Y / N

-Underlying medical condition Y / N: Describe: _____

-Immunizations : Y/N/unknown

>Influenza vaccine Y/N Date_____

>PCV 7/13 (PCV 7 introduced April 2009) (PCV 13 introduced May 2011)
PCV doses:

1 Date_____; Batch number_____
2 Date_____; Batch number_____
3 Date_____; Batch number_____

Blood investigations at presentation

-WCC: _____Neutrophil %_____ Lymphocyte % _____ monocyte %_____Bands%_____

-CRP:_____ PCT:_____

-HIV exposed: Y / N HIV infected: Y / N CD4: %_____

-Serum LDH:_____ TP: _____ albumin: _____

Pleural fluid examination

-Pleural tap done : Y / N Dry tap: Y / N ; pus swab only Y / N
-Pleural fluid macroscopic appearance:
Pus or turbid Y / N
Straw coloured Y / N
Blood stained/bloody Y / N
Other_____

Pleral Fluid dipstix:

-leucocytes : Y /N ___ + : protein Y / N:_____+ ; Blood: Y / N:_____+ PH: _____ glucose: __

Pleural Fluid laboratory results

-Lab Total protein:_____

-LDH: -_____

-Glucose:_____

-ADA:_____

<u>Cytospin</u>: Y/Not done/unsuitable **<u>Micro cell count</u>: Y/ Not done/ unsuitable**

Neutrophil count: _____% Polys: _____cells/uL

Lymphocyte count: _____% Lymphs: _____ cells/uL

Other Cells (macrophages, histiocytes, monocytes): _____% Erythros: _____ cells/uL

Cytology: (print report) Y /not done / unsuitable

_____
_____
_____
_____
_____

Microbiology

<u>Blood culture</u> Pos / Neg / Not done

-*S. pneumoniae* Y / N MIC to penicillin: _____

-*S. aureus* Y / N Sensitive clox: Y / N

-Other Strep Y /N Describe: _____

-Gram neg: Y / N Describe: _____

-Other pathogens Y/N Describe _____

<u>Pleural fluid:</u> Swab only for MCS collected: Y / N Swab only for PCR (study) Y/ N Before alteplase Y /N

-Microscopy: gram stain: Pos / Neg/ Not done Gram pos organism: Y / N Gram neg organisms: Y / N

-Bacterial culture: Pos / Neg

-*S. pneumoniae* Y / N MIC to penicillin: _____

-*S. aureus* Y / N Sensitive to clox: Y / N

-Other Strep Y /N _____

-Gram neg: Y / N _____

-Other Y/N _____

TB investigations

-Mantoux : Pos/Neg If positive _____mm

-Sputum GXpert: Pos/Neg Sensitive to Rifampicin Y / N

-Sputum TB Culture :Pos/Neg

-Pleural fluid GXpert :Pos/Neg

-Pleural fluid TB culture:Pos/Neg

Viral investigations

-Respiratory virus panel on NPA : Pos/Neg / Not done

- RSV / flu A / flu B/ Paraflu / adeno / metapneumo/ bocavirus / coronavirus / rhinovirus/ enterovirus

Clinical course and outcomes

-Chest drain Y / N date ICD in:_____ Hard drain / pigtail (FG size_____)

-Duration (total) of chest drain (days) : _____

-Duration of fever > 38 ° (days) : _____

-Fibrinolysis: Y / N How many doses: _____

-Length of hospital admission days: _____ Date of discharge: _____

-ICU admission: Y / N

-Surgical drainage: Y / N Date : _____

-Bronchopleural fistula Y / N

-Other complications? Y /N : _____

-Death Y / N Date: _____

-Discharge weight _____

Lung function at discharge:

Spirometry (> 4 years)

Pre BD: FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

Post BD:FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

-FOT (>3years):

	Test Average	SD	CV%
R5(cm H20.s/L)			
R11 (cm H20.s/L)			
R13(cm H20.s/L)			
RS17(cm H20.s/L)			
RS19(cm H20.s/L)			
X5 (cm H20.s/L)			
X11 (cm H20.s/L)			
X13(cm H20.s/L)			
X17 (cm H20.s/L)			
X19(cm H20.s/L)			
AX(cm H20/L)			
Fres(Hz)			
VT (L)			

Follow up (3 months): Date of visit: _____

Weight: _____ Length: _____

RR(bpm): _____bpm

Symptoms

-Cough Y/N Duration_____

-Shortness of Breath Y/N Duration_____

- Weight loss: Y / N

-TB contacts/exposure Y/N

-Chest pain: Y / N

-Pyrexia > 38ºC: Y / N

-Chest XRAY _____

Spirometry in children > 4 years:

Pre BD: FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

Post BD:FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

-FOT:

	Test Average	SD	CV%
R5(cm H20.s/L)			
R11 (cm H20.s/L)			
R13(cm H20.s/L)			
RS17(cm H20.s/L)			
RS19(cm H20.s/L)			
X5 (cm H20.s/L)			
X11 (cm H20.s/L)			
X13(cm H20.s/L)			
X17 (cm H20.s/L)			
X19(cm H20.s/L)			
AX(cm H20/L)			
Fres(Hz)			
VT (L)			

Follow-up 6 months: Date of visit: _____

-Weight: _____ Length: _____

- RR(bpm)_____

Symptoms

-Cough Y/N Duration_____

-Shortness of Breath Y/N Duration_____

- Weight loss: Y / N

-TB contacts/exposure Y/N

-Chest pain: Y / N

-Pyrexia > 38ºC: Y / N

Spirometry in children > 4 years:

Pre BD: FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

Post BD:FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

-FOT:

	Test Average	SD	CV%
R5(cm H20.s/L)			
R11 (cm H20.s/L)			
R13(cm H20.s/L)			
RS17(cm H20.s/L)			
RS19(cm H20.s/L)			
X5 (cm H20.s/L)			
X11 (cm H20.s/L)			
X13(cm H20.s/L)			
X17 (cm H20.s/L)			
X19(cm H20.s/L)			
AX(cm H20/L)			
Fres(Hz)			
VT (L)			

Follow up 12 months: Date of visit: _____

-Weight: _____ Length: _____

-RR (bpm)_____

Symptoms

-Cough Y/N Duration_____

-Shortness of Breath Y/N Duration_____

- Weight loss: Y / N

-TB contacts/exposure Y/N

-Chest pain: Y / N

-Pyrexia > 38ºC: Y / N

Spirometry in children > 4 years:

Pre BD: FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

Post BD:FEV1 : ____L _____% pred FVC _____L _____% pred FEV1/FVC: _____% MMEF _____%

-FOT:

	Test Average	SD	CV%
R5(cm H20.s/L)			
R11 (cm H20.s/L)			
R13(cm H20.s/L)			
RS17(cm H20.s/L)			
RS19(cm H20.s/L)			
X5 (cm H20.s/L)			
X11 (cm H20.s/L)			
X13(cm H20.s/L)			
X17 (cm H20.s/L)			
X19(cm H20.s/L)			
AX(cm H20/L)			
Fres(Hz)			
VT (L)			

APPENDIX 2: CONSENT FORM

PARENTAL CONSENT FOR PARTCIPATION IN RESEARCH PROJECT

PROJECT TITLE: Molecular diagnosis of pleural infection in children

Dear Parent/guardian

Thank you for considering our request to include your child _____in this research project which is being conducted at Red Cross Children's Hospital and the University of Cape Town. This information sheet will explain what this research is about and why we feel it is necessary to do this research. Participation in this research is voluntary and you may withdraw your child from this project at any time. Your child will receive the best possible care regardless of whether he/she is included or not. Please read through this form carefully and feel free to ask any questions. Permission to conduct this study has been acquired by the Research Ethics Committee of the University of Cape Town.

What is the purpose of this study?

Your child is being considered for inclusion in this research as he/she has a fluid collection in the chest which is pressing on the lung. This fluid collection is called a pleural effusion. Pleural effusions are usually caused by infections like bacteria or tuberculosis (TB) and can be serious. In order to know which infection it is, doctors usually collect a sample of this fluid and send it away for tests. It is important to know the exact cause so that the correct treatment can be prescribed. Many times, however, the exact cause is not established using usual laboratory tests so doctors prescribe the best treatment they think will work. Sometimes doctors cannot tell if the infection is TB or other germs. This uncertainty may result in unnecessary treatment which may be harmful or unpleasant. This research will try to determine the exact cause of your child's pleural effusion using more advanced laboratory tests on the fluid and sputum ("phlegm") samples which will be collected from your child. Another purpose of the study is to understand whether or not any permanent lung damage is caused by this condition.

What procedure will be done on my child and the collected samples?

- Because the fluid in your child's chest is pressing on the lung or making your child very ill, doctors must collect a sample of this fluid for routine testing regardless of your decision to participate in this research. Collecting this fluid is be done by either sucking the fluid through the ribcage using a needle and syringe or inserting a thin tube into the chest to drain the fluid out if there is a lot of fluid. The chest tube is left in place for several days until the fluid has stopped draining. In addition to the routine tests and medical procedures your child will need, extra 10 ml of the fluid will be collected and stored to do the special tests of this study at a later time. The results of this special test will not be known immediately to you or your doctors.
- Sputum collection: germs causing this sickness in your child usually originate from the nose and throat before spreading to the lungs. For this reason we will also collect a sample of sputum (spit or phlegm) and mucous from the nose. In order to maximise the chances of getting a good quality sputum sample from your child (especially if they are he/she is young) we plan to

nebulise a little salt water (hypertonic saline) beforehand which helps your child to cough and produce more phlegm. To see a video on this go to: http://samumsf.org/blog/portfolio-item/sputum-induction-procedure/

- The fluid and sputum samples will be subjected to routine lab tests available to us which try to identify which germ(s) is making your child sick. These tests are for bacteria and tuberculosis (TB).
- In addition, a sample of pleural fluid, phlegm and nasal mucous will be stored in the lab so that testing can be done at a later time as these tests are not routinely available at the moment.
- Lung function tests: these tests require your child to blow or breathe into a machine that measure how "strong" and healthy the lungs are. These will be done when your child is discharged from hospital and at check-up visits after discharge. Check-up visits are planned for 3, 6 and 12 months after he/she is discharged from hospital.

What risks or discomforts are involved?

There are minimal additional risks to your child over and above those which are associated with the routine care and procedures doctors will do in order to treat your child's condition. Inserting a needle or chest tube into the chest is unfortunately necessary and can be painful and stressful for your child. Doctors will however give your child strong medication and sedatives so that he/she will not feel any pain or discomfort during and after the procedure. Collecting the sputum and nose mucous samples may be a little uncomfortable as it requires us to pass a small tube through nose to suck up the mucous into a container. This can sometimes cause a little irritation in the nose and throat. We will not need to collect more samples from your child if they are also recruited on a separate TB study- we will in this case use the same samples. Lung function tests only require your child to blow or breathe into a machine and cause no discomfort.

The 3 month check-up after discharge is a usual requirement for all children with your child's condition. The subsequent check-ups at 6 and 12 months after discharge are additional for which you will receive financial compensation of R200 for each visit by the study organisers.

What are the possible benefits to my child?

Performing the research tests on the extra fluid taken from your child may reveal the exact cause of your child's illness. This in turn will result in improving our understanding of what causes this illness will in future improve the care of children with the same condition. If the results show that the new test identifies infections not otherwise detected by current methods, then this knowledge may change the way doctors in future perform tests on children with the same condition. We also hope to learn whether or not any permanent lung damage is present after 12 months of the illness.

Confidentiality

The information and medical details relating to your child and his/her illness will be collected in a confidential way and saved in a secure place. Only doctors or medical personnel directly involved in this research will have access to your child's information. Results of all tests that are performed, including the research tests will be made available to your doctors. The findings of this research may be presented

or discussed at medical meetings or published in medical journals. The identity of child will however never be publicly revealed.

Declaration and signed consent

I have read and understood this form and my questions have been answered. I hereby give consent to for my child to participate in this study and that the pleural fluid sample is stored in the laboratory for further testing at a later stage.

Signed: _____ Date: _____

Parent/legal guardian name: _____

Person obtaining consent:	Witness to consent process
Name _____	_____
Role in the study: _____	_____
Signature: _____	_____
Date: _____	_____

Additional information or questions regarding this study can be obtained by contacting

Dr M Zampoli
Department of Paediatrics and Child Heath
Room 419, ICH Building, Red Cross Children's Hospital
Tel: 021 6585309
Email: m.zampoli@uct.ac.za

Or

The Human Research Ethics Committee
University of Cape Town
Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory
Tel: 021 4066626
Email: shuretta.thomas@uct.ac.za

APPENDIX 3. ASSENT FORM

ASSENT FOR PARTCIPATION IN RESEARCH PROJECT (7 YEARS OR OLDER)

PROJECT TITLE: Molecular diagnosis of pleural infection in children

Dear _____

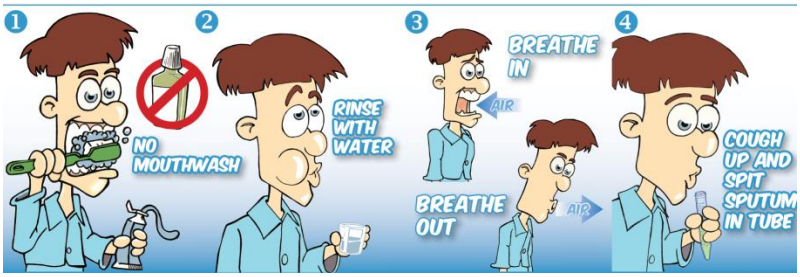
Thank you for considering our request to take part in this research project at Red Cross Children's Hospital. This information sheet will explain what this research is about and why we are doing this research. You are free to say yes or no and it won't make any difference to what treatment you get.

What is the purpose of this study?

You are sick with fluid in your chest which is pressing on your lung. This fluid most likely is due to a germ which has entered your body and made you sick. There are many different types of germs that do this. The best way to find out is by doing tests on the fluid in your chest. Most of the time, however, the usual tests do not give any definite answer and so doctors must often guess what germs are making you sick. This project is looking at whether a new kind of test done on the fluid will help doctors found out more about which germs are making you sick.

What will happen if you agree to take part in this project ?

Because the fluid in your chest is pressing on the lung, doctors will need to remove the fluid by either sucking it through a syringe or putting a small tube in your lung to let it the fluid run out. This procedure is part of your treatment whether you decide to join this research or not. The chest tube is left in place for several days until the fluid has gone. This sounds scary but don't worry. The doctors will give you strong medicine so that you don't feel any pain and sleep through it. The doctors will collect a little extra fluid and store it in the lab to do the new type of test at a later stage. Doctors/nurses will also ask you to spit some phlegm into a bottle and they will suck a little mucous from your nose.



To see a video on this go to: http://samumsf.org/blog/portfolio-item/sputum-induction-procedure/

The doctors would also like to check how long it takes for your lungs to heal from this infection. In order to know this you will be asked to come for a few check-ups for up to 1 year after you leave hospital. At these check-ups the doctors/nurses will ask you to blow into special machines which measure how strong your lungs are.

What are the possible benefits if I agree to join the research?

Performing the research tests on the extra fluid taken may reveal the exact cause of your illness. This in turn will help us learn more about the sickness you have and therefore help children like you in future.

Confidentiality

The information we will record about you and your illness will be kept confidential. Only doctors or medical personnel directly involved in this research will have access to your information. The findings of this research may be presented or discussed at medical meetings or published in medical journals. Your identity will however never be publicly revealed.

Declaration and signed consent

I have read and understood this form and my questions have been answered. I hereby agree to participate in this project and for the doctors to collect and share information they learn about my sickness.

Signed: _____ Date: _____

Person obtaining consent:	*Witness to assent process*
Name _____	_____
Role in the study: _____	_____
Signature: _____	_____
Date: _____	_____

Additional information or questions regarding this study can be obtained by contacting

Dr M Zampoli
Department of Paediatrics and Child Heath
Room 419, ICH Building, Red Cross Children's Hospital
Tel: 021 6585309
Email: m.zampoli@uct.ac.za

Or

The Human Research Ethics Committee
University of Cape Town
Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory
Tel: 021 4066626
Email: shuretta.thomas@uct.ac.za

APPENDIX 4. ETHICS APPROVAL LETTER

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

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: olivia.langenhoven@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

03 October 2019

HREC REF: 661/2019

Dr Marco Zampoli
Department of Paediatrics
Room 419, 5th Floor
ICH Building
Red Cross War Memorial Children Hospital

Dear Dr Zampoli

PROJECT TITLE: A DESCRIPTIVE STUDY COMPARING CLINICAL AND LABORATORY FINDINGS IN TB PLEURAL EFFUSION VS BACTERIAL PLEURAL EFFUSIONS IN CHILDREN AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL (SUB-STUDY - 254/2016) (MMED DEGREE - DR LAUREN GOLDEN)

Thank you for submitting your new study to the Faculty of Health Sciences Human Research Ethics Committee for review.

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 October 2020.

Please remove the email address shuretta.thomas@uct.ac.za and add marc.blockman@uct.ac.za to the Informed consent documents.

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.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Lauren Golden will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC 661/2019

APPENDIX 5. JOURNAL SUBMISSION INSTRUCTIONS

Instructions to Authors of Chosen Journal:
African Journal of Thoracic and Critical Care Medicine

Author Guidelines

Accessed at:
http://www.ajtccm.org.za/index.php/sarj/about/submissions#authorGuidelines

Author Guidelines

First time authors, click here: http://ajtccm.org.za/public/sup/intro.pdf

To submit a manuscript, please proceed to the *AJTCCM* Editorial Manager website:
www.editorialmanager.com/sarj

Author Guidelines

Please take the time to familiarise yourself with the policies and processes below. If you still have any questions, please do not hesitate to ask our editorial staff (tel.: +27 (0)21 532 1281, email: submissions@hmpg.co.za).

Authorship

Named authors must consent to publication. Authorship should be based on: (*i*) substantial contribution to conceptualisation, design, analysis and interpretation of data; (*ii*) drafting or critical revision of important scientific content; or (*iii*) approval of the version to be published. These conditions must all be met for an individual to be included as an author (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org)

Contributors who meet fewer than all 4 of the above criteria for authorship should not be listed as authors, but they should be acknowledged.

If authors' names are added or deleted after submission of an article, or the order of the names is changed, all authors must agree to this in writing.

Please note that co-authors will be requested to verify their contribution upon submission. Non-verification may lead to delays in the processing of submissions.

Author contributions should be listed/described in the manuscript.

Conflicts of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. The existence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest.

If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Authors: Conflict of interest statements are published alongside the content published. Please download and submit the conflict of interest form available here: http://www.icmje.org/conflicts-of-interest/

The authors should indicate how they managed any conflicts of interest with their initial submission. If the Editors determine that a conflict may have influenced any part of a manuscript, the author(s) will be given an opportunity to respond and if necessary to submit additional information indicating how they balanced the conflict.

Declaration of funding sources

All sources of funding are to be declared and will be published at the end of the text

The authors must also describe in their Acknowledgement or Contributions section at the end of the text, the role of the study sponsor(s), if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. If the funding source had no such involvement, the authors should state this

Reviewers are requested to indicate and describe any conflict of interest when they complete/submit their review via our manuscript tracking system.

Research ethics committee approval

Authors must provide evidence of Research Ethics Committee approval of the research where relevant. Ensure the correct, full ethics committee name and reference number is included in the manuscript.

If the study was carried out using data from provincial healthcare facilities, or required active data collection through facility visits or staff interviews, approval should be sought from the relevant provincial authorities. For South African authors, please refer to the guidelines for submission to the National Health Research Database. Research involving human subjects must be conducted according to the principles outlined in the Declaration of Helsinki. Please refer to the National Department of Health's guideline on Ethics in Health research: principles, processes and structures to ensure that the appropriate requirements for conducting research have been met, and that the HPCSA's General Ethical Guidelines for Health Researchers have been adhered to.

Clinical trials

As per the recommendations published by the International Committee of Medical Journal Editors (ICMJE), clinical trial research is any research that assigns individuals to an intervention, with or without a concurrent comparison/control group to study the cause-and-effect relationship between the intervention and health outcomes. All clinical trials should be registered with the appropriate national clinical trial registry (or any international primary register, if relevant), and the trial registration number should be cited at the end of the abstract. All clinical trial reports must also contain a data sharing statement as per the recommendations of the ICMJE. Statements are to indicate:

- whether individual deidentified participant data will be shared;
- what data in particular will be shared; whether additional, related documents will be available;
- when the data will become available and for how long; by what access criteria data will be shared

Please see the ICJME announcement for further details and illustrative examples of data sharing statements: ICMJE Data Sharing Statements for Clinical Trials

Since 1st December 2005, all clinical trials conducted in South Africa have been required to be registered in the South African National Clinical Trials Register. The AJTCCM therefore requires that clinical trials be registered in the relevant public trials registry at or before the time of first patient enrollment as a condition for publication. The trial registry name and registration number must be included in the manuscript.

CONSORT Statement

All papers that describe clinical trials must adhere to the principles outlined in the CONSORT Statement which provides an evidence-based approach to improve the quality of reports of clinical trials. The CONSORT Flow Diagram showing the patients available for the study, those included, and the number at each stage of the study should also be included and the CONSORT Checklist completed and submitted with the manuscript.

Research with animals

When animals are used as subjects, institutional approval of the protocol is necessary and authors should include a statement in the Methods indicating that investigators complied with the relevant national or intetnational guidelines administered by the author's governmental regulatory body. When no formal ethics review process is available, authors must state that humane care was provided in animal experiments, in accordance with stated relevant guidelines.

Protection of rights to privacy

Patient Consent

All studies that involve patients or volunteers require approval from a research ethics commitee and informed consent from all participants. The signed consent forms should be retained by the author but a statement indicating that informed consent has been obtained should be included in the article.

Information that would enable identification of individual patients should not be published in written descriptions, photographs, radiographs and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) has given informed written consent for publication and distribution. We further recommend that the published article is disseminated not only to the involved researchers but also to the patients/participants from whom the data was drawn. Refer to Protection of Research Participants.

Other individuals

Any individual who is identifiable in an image must provide written agreement that the image may be used in that context in the *AJTCCM.*

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Previously published images

If an image/figure has been previously published, permission to reproduce or alter it must be obtained by the authors from the original publisher and the figure legend must give full credit to the original source. This credit should be accompanied by a letter indicating that permission to reproduce the image has been granted to the author/s. This letter should be uploaded as a supplementary file during submission.

Privacy statement

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Ethnic/race classification

Use of racial or ethnicity classifications in research is fraught with problems. If you choose to use a research design that involves classification of participants based on race or ethnicity, or discuss issues with reference to such classifications, please ensure that you include a detailed rationale for doing so, ensure that the categories you describe are carefully defined, and that socioeconomic, cultural and lifestyle variables that may underlie perceived racial disparities are appropriately controlled for. Please also clearly specify whether race or ethnicity is classified as reported by the patient (self-identifying) or as perceived by the investigators. Please note thatit is not appropriate to use self-reported or investigator-assigned racial or ethnic categories for genetic studies.

Manuscript preparation

Preparing an article for anonymous review

To ensure a fair and unbiased review process, all submissions are to include an anonymised version of the manuscript. The exceptions to this requirement are Correspondence, Book reviews and Obituary submissions.

Submitting a manuscript that needs additional blinding can slow down your review process, so please be sure to follow these simple guidelines as much as possible:

An anonymous version should not contain any author, affiliation or particular institutional details that will enable identification.

Please remove title page, acknowledgements, contact details, funding grants to a named person, and any running headers of author names.

Mask self-citations by referring to your own work in third person.

General article format/layout

Submitted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction prior to being sent for review, which will delay publication.
General:
Manuscripts must be written in UK English (this includes spelling).
The manuscript must be in Microsoft Word document format. Text must be 1.5 line spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
Pages and lines should be numbered consecutively.
Please make your article concise, even if it is below the word limit.
Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

IMAGES/PHOTOGRAPHS

Acceptable file types

The image file should be submitted as a high resolution jpeg or tiff Important: Images embedded in a Word document are not acceptable.

Resolution

Images must have a minimum resolution of 300 dpi (dots per inch).

Screenshots and images from the internet

Screenshots and images from the internet are usually only 72 dpi – this is the average resolution that computer screens use – therefore images downloaded from the internet are almost always too small to use for print even though they might look fine on screen.

Author Quick check
If the actual size of the file is:
• less than 500 kb - not great for print
• 500kb - 1000 kb (1 mb) - better
• greater than 1000 kb (i mb) - ideal
The image sent has to be the original i.e. the very first image created.
If it was taken on a camera/cell phone, then that image has to be sent directly from the device's image gallery.
Not a screenshot of the image or via a secondary app (Word, Whatsapp) or uploaded to a website.

Cameras (cell phones) should be set to the highest possible image size

GRAPHS/FIGURES
Acceptable file types
All graphs and figures should be submitted as PDF files

Genetic nomenclature

AJTCCM is a medical journal covering all aspects of respiratory health, therefore for articles involving genetics, it is the responsibility of authors to apply the following:
- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.
- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.
** NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.
- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'
- Use the latest approved gene or protein symbol as appropriate:
Human Gene Mapping Workshop (HGMW): genetic notations and symbols
HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Each paper should have a clear rationale, logical study aims, sufficiently detailed methods, and well supported conclusions. It is advisable to clearly state the hypothesis or aim of the work in the introduction section. The discussion and abstract conclusions should be clearly stated and should be backed up by the data presented in the manuscript. The study outcomes or metrics used to inform the conclusions should be clearly stated and outlined.

Research
Guideline word limit: 3 000 words (excluding abstract and bibliography)
Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Where appropriate, sample size calculations should be included to demonstrate that the study is not underpowered. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

May include up to 6 illustrations or tables.
A max of 20 – 25 references

Structured abstract
This should be no more than 250words, with the following recommended headings:
Background: why the study is being done and how it relates to other published work.
Objectives: what the study intends to find out
Methods: must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
Results: first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
Conclusion: must be supported by the data, include recommendations for further study/actions.
Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors. It should be able to be intelligible to the reader without referral to the main body of the article.
Do not include any references in the abstracts.
Click Here for an example of a good abstract.

Case reports/Scientific letters/Short reports

These include side effects of drugs and brief or negative research findings.

Guideline word limit: 1500 words
Abstract: unstructured, of about 100-150 words
May include only one illustration or table
A maximum of 6 references

Editorials
Guideline word limit: 1 000 words
These opinion or comment articles are usually commissioned but we are happy to consider and peer review unsolicited editorials. Editorials should be accessible and interesting to readers without specialist knowledge of the subject under discussion and should have an element of topicality (why is a comment on this issue relevant now?) There should be a clear message to the piece, supported by evidence.
Please make clear the type of evidence that supports each key statement, e.g.:
expert opinion
personal clinical experience
observational studies
trials
systematic reviews.

Review articles
Contributors are encouraged to write to the Editor about possible papers to be considered for review, and where appropriate a review outline will be submitted to experts in the field for consideration before a full review is commissioned. It is expected that an author or authors have substantial experience and track record in the field that the review is about.

*Guideline word limit: 3 500 words (*unless an alternative word limit has been arranged with the Chief Editor)
Please ensure that your article includes:
Abstract: unstructured, of about 100-150 words, explaining the review and why it is important
Methods: Outline the sources and selection methods, including search strategy and keywords used for identifying references from online bibliographic databases. Discuss the quality of evidence.
When writing: clarify the evidence you used for key statements and the strength of the evidence. Do not present statements or opinions without such evidence, or if you have to, say that there is little or no evidence and that this is opinion. Avoid specialist jargon and abbreviations, and provide advice specific to southern Africa.
Personal details: Please supply your qualifications, position and affiliations and MP number (used for CPD points); address, telephone number and fax number, and your e-mail address; and a short personal profile (50 words) and a few words about your current fields of interest.

Contributors are encouraged to include tables and figures in their reviews to keep to the maximum word count.

Guidelines
Must be endorsed by an appropriate body prior to consideration and all conflicts of interest expressed.
A structured abstract not exceeding 250 words
Recommended sub-headings: Background, Recommendations, Conclusion is required.
Sections and sub-sections must be numbered consecutively (e.g. 1. Introduction; 1.1 Definitions; 2. etc.) and summarised in a Table of Contents.
References, appendices, figures and tables must be kept to a minimum.

Correspondence (Letters to the Editor)
Guideline word limit: 400 words
Letters to the editor should relate either to a paper or article published by the AJTCCM or to a topical issue of particular relevance to the journal's readership
May include only one illustration or table
Must include a correspondence address.

Obituaries
Guideline word limit: 400 words
Should be offered within the first year of the practitioner's death, and may be accompanied by a photograph.

Illustrations/photos/scans

If illustrations submitted have been published elsewhere, the author(s) should provide evidence of consent to republication obtained from the copyright holder.
Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
All images must be of high enough resolution/quality for print.
All illustrations (graphs, diagrams, charts, etc.) must be in PDF form.

Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

Tables should be constructed carefully and simply for intelligible data representation.
Unnecessarily complicated tables are strongly discouraged.
Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author.
Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
Number each table in Arabic numerals (Table 1, Table 2, etc.) consecutively as they are referred to in the text.
Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
Ensure each table has a concise title and column headings, and include units where necessary.
Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

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Rather:
Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:
Combine into one column, *n* (%):

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Rather:
Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. If reference manager software is used, the reference list and citations in text are to be unformatted to plain text before submitting..*
Authors must verify references from original sources.

Citations should be inserted in the text as superscript numbers between square brackets, e.g.
These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
Volume and issue numbers should be given.
First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
Wherever possible, references must be accompanied by a digital object identifier (DOI) link).
Authors are encouraged to use the DOI lookup service offered by CrossRef:
On the Crossref homepage, paste the article title into the 'Metadata search' box.
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Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:
Journal references: Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx.doi.org/10.1000/hgjr.182
Book references: Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
Chapter/section in a book: Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
Internet references: World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
Legal references
Government Gazettes:
National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.
In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.
Provincial Gazettes:
Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.
Acts:
South Africa. National Health Act No. 61 of 2003.
Regulations to an Act:
South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).
Bills:
South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.
Green/white papers:
South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

Case law:
Rex v Jopp and Another 1949 (4) SA 11 (N)
Rex v Jopp and Another: Name of the parties concerned
1949: Date of decision (or when the case was heard)
(4): Volume number
SA: SA Law Reports
11: Page or section number
(N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.
NOTE: no . after the v
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Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.

From submission to acceptance

Submission and peer-review

To submit an article:
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Manuscript
Any supplementary files: figures, datasets, patient consent form, permissions for published images, etc.
Once the submission has been successfully processed on Editorial Manager, it will undergo a technical check by the Editorial Office before it will be assigned to an editor who will handle the review process. If the author guidelines have not been appropriately followed, the manuscript may be sent back to the author for correcting.

Peer Review Process
All manuscripts are reviewed initially by the Editor-in-Chief and only those that meet the scientific and editorial standards of the journal, and fit within the aims and scope of the journal, will be sent for external peer review. Each manuscript is reviewed by either one or two reviewers selected on the basis of their expertise in the field. A double blind review process is followed at AJTCCM.
Authors are expected to receive feedback from reviewers and an editorial decision within approximately 6 weeks of submission. The time period of the entire review process may vary however depending upon the quality of the manuscript submitted, reviewers' responses and the time taken by the authors to submit the revised manuscript.
Manuscripts from review may be accepted, rejected or returned to the author for revision or resubmission for review. Authors will be directed to submit revised manuscripts within two months of receiving the editor's decision, and are requested to submit a point by point response

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Production process

The following process should usually take between 4 - 6 weeks:
An accepted manuscript is passed to a Managing Editor to assign to a copyeditor (CE).
The CE copyedits in Word, working on house style, format, spelling/grammar/punctuation, sense and consistency, and preparation for typesetting.
If the CE has an author queries, he/she will contact the corresponding author and send them the copyedited Word doc, asking them to solve the queries by means of track changes or comment boxes.
The authors are typically asked to respond within 1-3 days. Any comments/changes must be clearly indicated e.g. by means of track changes. Do not work in the original manuscript - work in the copyedited file sent to you and make your changes clear.
The CE will finalise the article and then it will be typeset.
Once typeset, the CE will send a PDF of the file to the authors to complete their final check, while simultaneously sending to the 2nd-eye proofreader.
The authors are typically asked to complete their final check and sign-off within 1-2 days. No major additional changes can be accommodated at this point.
The CE implements the authors' and proofreader's mark-ups, finalises the file, and prepares it for the upcoming issue.

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Errata and retractions

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As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.
Named authors consent to publication and meet the requirements of authorship as set out by the journal.
The submission has not been previously published, nor is it before another journal for consideration.
The text complies with the stylistic and bibliographic requirements in **Author Guidelines**.
The manuscript is in Microsoft Word or RTF document format. The text is single-spaced, in 12-point Times New Roman font, and contains no unnecessary formatting.
Illustrations/figures are high resolution/quality (not compressed) and in an acceptable format (jpeg or pdf). These must be submitted individually as 'supplementary files' (not solely embedded in the manuscript).
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Where possible, references are accompanied by a digital object identifier (DOI).

An abstract has been included where applicable.
The research was approved by a Research Ethics Committee (if applicable)
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