

**Dr Seyram Michelline Wordui**

**Aetiology of pleural effusions diagnosed by routine culture and molecular techniques in children living in a high tuberculosis-endemic setting**

**University of Cape Town**

**Supervisor: Prof Marco Zampoli**

**Master of Philosophy (MPhil) in Paediatric Pulmonology**

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## **Declaration Page**

I hereby declare that this research is my personal work and has not been submitted for any diplomas or degrees in the past. This work has not been published, or submitted for publication, prior to my registration for the afore mentioned degree.

Signed by candidate

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Dr Seyram Michelline Wordui

26/11/2023

## **Abstract**

**BACKGROUND:** Confirming aetiology of pleural effusion in children may be difficult in tuberculosis (TB)-endemic settings. We investigated the role of polymerase chain reaction (PCR) and routine biochemical tests in discriminating pleural effusion caused by bacteria from other aetiologies.

**METHODS:** This is a post-hoc analysis of a cross-sectional study among children with pleural effusion in a tertiary hospital in South Africa, incorporating data from PCR testing of stored pleural fluid. Aetiological classification was defined by microbiological confirmation.

**RESULTS:** Ninety-one children were enrolled, median age 31 months (IQR 12-102). Aetiology of pleural effusion was 40 % (n=36/91) bacteria, 11% (n=10/91) TB, 3% (n=3/91) viruses, 11% (n=10/91) polymicrobial and 35% (n=32/91) had no pathogen identified. The commonest pathogen was *Staphylococcus aureus* (n=27/91; 30%) with similar yields on culture and PCR, followed by *Streptococcus pneumoniae* (n=12/91; 13%), detected more commonly by PCR. PCR reduced the number of children with unconfirmed aetiologies from 48 to 32. Characteristics of children with no pathogen most resembled those with TB. Pleural fluid lactate dehydrogenase  $\geq 1716$  U/L best discriminated bacterial pleural effusion from other aetiologies (sensitivity of 86%; specificity 95%).

**CONCLUSION:** PCR improved detection of pathogens and reduced number of children with unconfirmed aetiologies in presumed exudative pleural effusion.

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I am grateful to the African Paediatric Fellowship Programme for the opportunity to do a supernumerary fellowship at Red Cross War Memorial Children's Hospital and enrol in this MPhil programme at University of Cape Town, and the children and families who participated in this study.

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## **List of Abbreviations**

1. ADA - Adenine deaminase
2. AUC - Area under the Curve
3. CRP – C-reactive protein
4. FBC -Full blood count
5. HIV -Human immunodeficiency virus
6. IQR - Interquartile range
7. LDH - Lactate dehydrogenase
8. LR+ - Positive likelihood ratio
9. LR- - Negative likelihood ratio
10. PCR - Polymerase chain reaction
11. PCV - Pneumococcal conjugate vaccine
12. PE - Pleural effusion
13. RCWMCH – Red Cross War Memorial Children’s Hospital
14. ROC - Receiver operating characteristics
15. TB - Tuberculosis

## Chapter 1: Introduction

### 1.1 Context

Lower respiratory tract infections rank second in disease burden among children younger than 10 years.<sup>1</sup> Sub-Saharan Africa bears a major brunt, even in high vaccination rate settings such as South Africa.<sup>2</sup>

Parapneumonic pleural effusion (PE) is the most common complication of community acquired pneumonia.<sup>3</sup> PE evolves through an exudative stage also called a simple pleural effusion, a fibrinopurulent stage where bacteria invade the pleural fluid and start to multiply, leading to frank pus called empyema, and an organising stage. The fibrinopurulent and organising stages are called complicated PEs as their management requires tube thoracostomy and sometimes surgery to free trapped lungs<sup>4,5</sup> When treated appropriately, PE has an excellent prognosis. However, some patients have pulmonary function abnormalities years later.<sup>6,7</sup>

Guidelines recommend management of parapneumonic PE with empiric antibiotics until identification of aetiologic agents.<sup>8,9</sup> The causative organism for childhood parapneumonic PE in most settings is *Streptococcus pneumoniae* or *Staphylococcus aureus*.<sup>10-12</sup> However, blood and pleural fluid cultures take several days and have low yields.<sup>13,14</sup> This is further compounded by use of antibiotics before children present to hospital, a situation common in our setting.<sup>14,15</sup> Adding molecular testing of pleural fluid enhances yield, and has a shorter turn-around time, therefore potentially improving care with targeted antimicrobial management.<sup>13,14</sup>

In a TB-endemic setting like South Africa, it may be difficult to distinguish between PE caused by TB and bacteria.<sup>14,16</sup> Because the yield of microbiological tests for pleural TB in children is poor, clinical and biochemical parameters are often relied on for diagnosis.<sup>17,18</sup>

Some viruses such as adenovirus cause pleural effusions among children.<sup>19</sup> However, viral testing in childhood PE is not routine.

Golden *et al* prospectively studied 91 children with parapneumonic PE, in Cape Town, South Africa, using routine culture of blood and pleural fluid and TB tests, of which 47% had confirmed aetiologies.<sup>15</sup> The number of children without confirmed aetiologies potentially leads to inappropriate antibiotic use and overdiagnosis of TB. We thus sought to investigate whether incorporating molecular testing of stored pleural fluid from the Golden *et al* study

cohort would improve diagnostic yield. We further investigated which biochemical parameters best discriminate between PE caused by bacteria and other aetiologies to guide initial therapeutic decisions.

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## 1.2 Ethical Considerations

This study adheres to the principles stated in the Declaration of Helsinki (2013). The study obtained approval from the Human Research Ethics committee of the University of Cape Town, HREC REF 607/2022. Informed consent and assent were obtained as part of the original study. In this secondary analysis, only anonymised data was retrieved and analysed. There was no patient contact.

## 1.3 Author guidelines for the journal for which paper has been formatted.

This paper was formatted for the International Journal of Tuberculosis and Lung Disease. This is a leading journal with an impact Factor of 4. Its subject matter is respiratory diseases, including tuberculosis. It allows for 2500 words excluding abstract and tables and figures. This peer-reviewed journal was chosen because of the subject matter of the dissertation which is differentiating between infective causes of pleural effusion among children. The instructions for authors is appended.

## Chapter 2: Publication-ready manuscript

### Aetiology of Pleural Effusions Diagnosed by Routine Culture and Molecular Techniques in Children Living in a High Tuberculosis-Endemic Setting

Seyram Wordui,<sup>1,2</sup> Adelaide Masu,<sup>1</sup> Lauren Golden,<sup>1</sup> Shaakira Chaya,<sup>1,3</sup> Kirsten Reichmuth,<sup>1</sup> Ane Visagie,<sup>1,3</sup> Adaeze Ayuk,<sup>4</sup> Sandra Kwarteng Owusu,<sup>1,5</sup> Diana Marangu,<sup>1,6</sup> Noor Affendi,<sup>1,7</sup> Aruna Lakhan,<sup>1</sup> Diane Gray,<sup>1</sup> Aneesa Vanker,<sup>1</sup> Heather Zar,<sup>1,3</sup> Marco Zampoli<sup>1</sup>

1. Department of Paediatrics and Child Health, University of Cape Town and Red Cross War Memorial Children's Hospital, Cape Town, South Africa

2. Department of Child Health, Korle Bu Teaching Hospital, Accra, Ghana

3. MRC Unit for Child and Adolescent Health, University of Cape Town, South Africa

4. University Teaching Hospital, Enugu, Nigeria

5. Department of Child Health, Komfo Anokye Teaching Hospital, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

6. Department of Paediatrics and Child Health, Kenyatta National Hospital, University of Nairobi, Kenya

7. Department of Paediatrics, Hospital Sultanah Nur Zahirah, Terengganu, Malaysia

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**Corresponding author:** Dr Seyram Wordui

Address: Department of Paediatrics and Child Health  
5<sup>th</sup> floor ICH Building  
Red Cross War Memorial Children's Hospital  
Klipfontein Road, Rondebosch Cape Town, 7700  
South Africa  
Email: swordui@gmail.com

## Summary

**BACKGROUND:** Confirming aetiology of pleural effusion in children may be difficult in tuberculosis (TB)-endemic settings. We investigated the role of polymerase chain reaction (PCR) and routine biochemical tests in discriminating pleural effusion caused by bacteria from other aetiologies.

**METHODS:** This is a post-hoc analysis of a cross-sectional study among children with pleural effusion in a tertiary hospital in South Africa, incorporating data from PCR testing of stored pleural fluid. Aetiological classification was defined by microbiological confirmation.

**RESULTS:** Ninety-one children were enrolled, median age 31 months (IQR 12-102).

Aetiology of pleural effusion was 40 % (n=36/91) bacteria, 11% (n=10/91) TB, 3% (n=3/91) viruses, 11% (n=10/91) polymicrobial and 35% (n=32/91) had no pathogen identified. The commonest pathogen was *Staphylococcus aureus* (n=27/91; 30%) with similar yields on culture and PCR, followed by *Streptococcus pneumoniae* (n=12/91; 13%), detected more commonly by PCR. PCR reduced the number of children with unconfirmed aetiologies from 48 to 32. Characteristics of children with no pathogen most resembled those with TB. Pleural fluid lactate dehydrogenase  $\geq 1716$  U/L best discriminated bacterial pleural effusion from other aetiologies (sensitivity of 86%; specificity 95%).

**CONCLUSION:** PCR improved detection of pathogens and reduced number of children with unconfirmed aetiologies in presumed exudative pleural effusion.

Lower respiratory tract infections rank second in disease burden among children younger than 10 years.<sup>1</sup> Sub-Saharan Africa bears a major brunt, even in high vaccination rate settings such as South Africa.<sup>2</sup>

Parapneumonic pleural effusion (PE) is the most common complication of community acquired pneumonia.<sup>3</sup> PE evolves through an exudative stage also called a simple PE, a fibrinopurulent stage where bacteria invade the pleural fluid and start to multiply, leading to frank pus called empyema, and an organising stage. The fibrinopurulent and organising stages are called complicated PEs as their management requires tube thoracostomy and sometimes surgery to free trapped lungs<sup>4,5</sup> When treated appropriately, PE has an excellent prognosis. However, some patients have pulmonary function abnormalities years later.<sup>6,7</sup>

Guidelines recommend management of parapneumonic PE with empiric antibiotics until identification of aetiologic agents.<sup>8,9</sup> This in most settings is *Streptococcus pneumoniae* or *Staphylococcus aureus*.<sup>10-12</sup> However, blood and pleural fluid cultures take several days and have low yields.<sup>13,14</sup> This is further compounded by use of antibiotics before children present to hospital, a situation common in our setting.<sup>14,15</sup> Adding molecular testing of pleural fluid enhances yield, and has a shorter turn-around time, therefore potentially improving care with targeted antimicrobial management.<sup>13,14</sup>

In a TB-endemic setting like South Africa, it may be difficult to distinguish between PE caused by TB and bacteria.<sup>14,16</sup> Because the yield of microbiological tests for pleural TB in children is poor, clinical and biochemical parameters are often relied on for diagnosis.<sup>17,18</sup>

Some viruses such as adenovirus cause PE among children.<sup>19</sup> However, viral testing in childhood PE is not routine.

Golden *et al* prospectively studied 91 children with parapneumonic PE, in Cape Town, South Africa, using routine culture of blood and pleural fluid, and TB tests of which 47% had confirmed aetiologies.<sup>15</sup> The number of children without confirmed aetiologies potentially leads to inappropriate antibiotic use and overdiagnosis of TB. We thus sought to investigate whether incorporating molecular testing of stored pleural fluid from the Golden *et al* study cohort would improve diagnostic yield. We further investigated which biochemical parameters best discriminate between PE caused by bacteria and other aetiologies to guide initial therapeutic decisions.

## **METHODOLOGY**

### *Study design and population*

This is a post-hoc analysis of a cross-sectional, descriptive observational study conducted at a tertiary hospital in Cape Town, South Africa. South Africa's immunisation schedule includes since 2011 a 13-valent pneumococcal conjugate vaccine. Children 0 to 13 years admitted with PE from December 2016 to December 2019 were enrolled in the initial study.<sup>15</sup>

Inclusion criteria was PE identified clinically and radiographically. Patients who had PE associated with nosocomial infections (new-onset PE after at least 48 hours of hospital admission); transudative PE from cardiac, renal, liver disease, or non-infective causes; and PE following trauma or surgery were excluded. Patients were also excluded if no written informed consent or assent was obtained. Pleural space was aspirated or drained with an intercostal drain under aseptic conditions in the initial study. Portions of pleural fluid from the initial drainage or aspirate were transferred into nucleic acid preservation medium (Primestore®, Longhorn Vaccines and Diagnostics, San Antonio, Texas, USA) and stored at -80 degrees Celsius until the current study.

### *Clinical data collection*

Clinical and laboratory data was collected using a data collection form and entered into a REDCap® electronic data platform hosted at University of Cape Town. Clinical data included demographics, pneumococcal vaccination records, weight, clinical and radiographic examination findings at presentation.

### *Routine laboratory investigations*

Blood investigations included a full blood count (FBC), with differential white cell counts, C-reactive protein (CRP), procalcitonin (PCT), serum lactate dehydrogenase (LDH), total protein, albumin, blood culture and human immunodeficiency virus (HIV) antibody or polymerase chain reaction (PCR) tests. Pleural fluid examination included macroscopic appearance, glucose, total protein, LDH and adenosine deaminase (ADA), cytology and cell count, bacterial and TB cultures, as well as Xpert MTB/RIF® or Xpert MTB/RIF-Ultra®. Patients also had a Mantoux test and at least two induced sputum samples collected for Xpert MTB/RIF® or Xpert MTB/RIF-Ultra® and TB culture. A positive Mantoux test was a reading at 48-72 hours of at least 10 mm in HIV-uninfected children, and at least 5 mm in HIV-infected children.

### *Molecular analysis of stored pleural fluid samples*

Nucleic acid was extracted from pleural fluid using mechanical lysis on a tissueLyser LT® (Qiagen, Germany) followed by extraction on the automated QIA Symphony® platform using the virus/bacteria mini kit (Qiagen, Germany). Quantitative, multiplex real time PCR (qPCR) testing was done with FTDRsp33® (FastTrack Diagnostics, Esch-sur-Alzette, Luxembourg, Germany). This kit detects the following microbes: Influenza A, Influenza B, Rhinovirus, Coronavirus NL63, 229E, OC43, HKU1, Parainfluenza 1, 2, 3, 4, Human metapneumovirus A/B, Bocavirus, *Mycoplasma pneumoniae*, Respiratory syncytial virus A and B, Adenovirus, Enterovirus, Parechovirus, *Chlamydia pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, Cytomegalovirus, Influenza C, *Pneumocystis jirovecii*, *Haemophilus influenzae* type B, *Bordetella* spp. (except *Bordetella parapertussis*), *Moraxella catarrhalis*, *Klebsiella pneumoniae*, *Legionella* spp., and *Salmonella* spp. Strict cycles of quantification cutoff values ranging from 32 to 36 cycles were implemented to determine a positive result.

### *Aetiological classification and definitions*

1. Bacterial: positive culture (blood and/or pleural fluid) and/or positive PCR for bacteria, including samples with two or more confirmed bacterial pathogens, without co-infection with a virus or TB.
2. TB: microbiologically confirmed TB on positive Xpert MTB/RIF® or Xpert MTB/RIF-Ultra® or positive TB culture from induced sputum and/or pleural fluid without any co-infections with viruses or bacteria.
3. Polymicrobial: two or more microbiologically confirmed (culture and/or PCR and/or Xpert MTB/RIF® or Xpert MTB/RIF-Ultra®) pathogens, including bacteria-TB, bacteria-virus, or TB-virus co-infections.
4. Viral: positive PCR of virus on pleural fluid, without co-infection with bacteria and/or TB.
5. No pathogen: no pathogen (bacteria, TB or virus) confirmed by culture, PCR or Xpert MTB/RIF® or Xpert MTB/RIF-Ultra®.

### *Data Analysis*

Data was analysed using STATA (version 10.0, Statcorp, Texas, USA). Continuous variables were expressed as means  $\pm$  standard deviations if normally distributed, or medians and interquartile ranges (IQR), if not normally distributed. The Mann-Whitney U test and

Kruskal-Wallis test were used for comparing non-normally distributed measures and t-test for normally distributed measures. Categorical variables were described using frequencies and proportions and compared with the  $\chi^2$  test. P value of  $< 0.05$  was taken as statistically significant. Underweight-for-age was defined as weight-for-age less than -2 standard deviations (z-scores) below the mean, calculated in STATA.

Receiver operating characteristic (ROC) curves were generated for serum (WCC, CRP, PCT) and pleural fluid (LDH, polymorphs, lymphocytes, glucose, protein, and ADA) biomarkers for predicting their performance in detecting bacterial infections. For this ROC analysis, any sample with confirmed bacteria was classified as bacterial, including polymicrobial infections. Values that classified most samples correctly were chosen as optimal cut-offs. Where there were two optimal cut-offs, the one with the higher specificity was selected.

### *Ethical considerations*

The study obtained approval from the Human Research Ethics Committee of University of Cape Town (HREC REF 607/2022). Informed consent and assent were obtained in the original study.

## **RESULTS**

### *Demographic and clinical characteristics*

Demographic and clinical characteristics of the cohort are presented in table 1. Ninety-one children were enrolled, with a median age of 31 months (IQR 12-102) and 61.5% (n=56/91) were males; 15% (n=14/91) were HIV-exposed but uninfected and 4% (n=4/91) were HIV-positive. Forty-eight percent (n=43/90) had self-reported, or clinician observed weight loss, with 16% (n=14/90) being underweight for age. Forty three percent (n=39/91) had received all doses of PCV-13 and 14% (n=13/90) reported exposure to a household TB contact in the preceding six months. Forty-four percent (n=34/78) reported chest pain and 12% (n=11/90) had pneumothorax. Slightly over half (n=50/91;55%) received antibiotics in the 7 days prior to enrolment into the study.

Differences were observed in age, weight loss, chest pain and Mantoux positivity across the aetiological groups, but only chest pain and Mantoux positivity showed statistical significance (table 1). The bacterial group was younger, median age 13.3 months (IQR 7.6-30.9), followed by the polymicrobial group with a median age of 16.4 months (IQR 11.3-31.2), while the no pathogen group had a median age of 85.8 months (IQR 40.7-117.5). The TB group was oldest, median age 112.8 months (IQR 53.7-145.3). Weight loss was

commonest in the TB group (n=7/10; 70%), followed by the no pathogen group (n=20/32; 62%) and least in the bacterial (n=12/35;34%) and polymicrobial (n=3/10;30%) groups. Chest pain was more common among those who had TB (n=7/10; 70%) and with no pathogen (n=18/32; 56%) compared to those with bacterial infection (n=8/28; 29%), and polymicrobial infections (n=1/5; 20%), table 1. Of those who had a Mantoux test done, none with bacterial infection (n=0/16), half (n=4/8; 50%) with TB and two thirds (n=21/31; 68%) with no pathogen had a positive Mantoux.

### *Aetiology of pleural effusions*

Aetiology of PE was as follows: 40 % (n=36/91) bacteria only, 11% (n=10/91) TB only, 3% (n=3/91) viruses only, 11% (n=10/91) polymicrobial and 35% (n=32/91) had no pathogen identified, table 1. Fifty-one bacterial isolates were confirmed. The commonest pathogen was *S. aureus* (30%; n=27/91) with similar yield on culture (26%; n=24/91) and PCR (23%; n=21/91), table 2. Eleven children had discordant culture and PCR results for *S. aureus*; 4 were PCR positive but culture negative, while 7 were culture positive but PCR negative. The second commonest pathogen was *S. pneumoniae*, detected more commonly by PCR (13%; n=12/91) than culture (3%; n=3/91), table 2; with all culture-positive cases detected by PCR. Overall, 13% (n=12/91) had TB, two of which were TB-bacterial co-infections. *M. catarrhalis*, *H. influenzae* spp., *M. pneumoniae* and viruses were detected only by PCR, whereas anaerobes and other *Streptococci* spp. were detected only by culture. Detected viruses were adenovirus (n=6), rhinovirus (n=3) and bocavirus (n=2). PCR testing increased the overall yield of individual pathogens from 45 to 75. Nineteen additional patients, who previously had no pathogen detected, were diagnosed with single or multiple organisms when PCR testing was added to culture. Details of co-infections in the polymicrobial group are displayed in table 3.

### *Blood and pleural fluid biomarkers*

Blood WCC, CRP and PCT, pleural fluid LDH and polymorphs were significantly higher in the bacterial group compared to the TB group, while pleural fluid protein and glucose were significantly higher in the TB group compared to the bacterial group, table 4. The polymicrobial group had lower WCC and CRP but higher PCT compared to the bacterial group. It also had higher pleural fluid LDH and polymorphs, and similar pleural fluid protein compared to the bacterial group.

Except for Mantoux positivity, post-hoc analysis comparing the no pathogen and TB groups showed similar blood and pleural fluid biomarker profiles ( $p > 0.05$ ).

### *Receiver operating characteristics*

Pleural fluid LDH  $\geq 1716$  U/L performed best at discriminating bacterial PE from other causes (sensitivity 85.7%, specificity 95%; AUC 1.0, figure 1), followed by PCT  $\geq 1$  mg/L (sensitivity 92.7%, specificity 68.6%; AUC 0.9), and CRP  $\geq 166$  mg/L (sensitivity 82.2%, specificity 79.5%; AUC 0.8), supplementary table S1.

## **DISCUSSION**

This study employed molecular techniques to diagnose the aetiology of PE among children in a TB-endemic setting where distinguishing bacterial infections from TB may be clinically difficult. By re-analysing data of a historical cohort of children with PE with additional molecular testing of stored pleural fluid, we demonstrated an improved diagnostic yield of pathogens. Furthermore, we provide optimal cut-off values of widely used blood and pleural fluid biomarkers that best discriminate childhood bacterial from non-bacterial PE, which may be helpful for clinicians in high TB-endemic settings.

The commonest cause of PE in our cohort was *S. aureus*, consistent with previous studies from South Africa and elsewhere.<sup>14,20–22</sup> Compared to the pre-PCV-13 period, the proportion of pleural effusion caused by *S. aureus* has increased while that of *S. pneumoniae* has decreased.<sup>14</sup> This is in keeping with the changing epidemiology of pleural effusion documented in other settings in the post-PCV era.<sup>13</sup> Thus, the current recommendation for first-line broad-spectrum antibiotics for children presenting with pleural effusion in our setting, is appropriate, as it is effective against *S. aureus* and *S. pneumoniae*, as well as uncommon organisms like *H. influenzae* and *M. catarrhalis*.<sup>23</sup>

The increased detection of *S. pneumoniae* by PCR found in our cohort has been previously documented.<sup>10,13,24</sup> Adding PCR testing did not improve the yield of *S. aureus* in our study. However, there were some children in whom *S. aureus* was detected only by PCR. The improved detection of pathogens by PCR testing has the potential to reduce the number of children presumptively treated for TB. Therefore, PCR testing is complementary to traditional culture in the diagnosis of PE.

Our study confirms the well-documented finding that the confirmation of TB in children is difficult, even on respiratory samples, and despite molecular testing.<sup>25–28</sup> Tuberculous pleural

effusion may result from a hypersensitivity reaction to tuberculous antigens in the pleural space when a subpleural caseous focus ruptures into the pleura, resulting in a lymphocyte-predominant paucibacillary exudate, and low rates of microbiological confirmation.<sup>18,29</sup> This likely explains the large proportion of children in our cohort with no pathogen detected, but with similar clinical and biochemical profiles to TB patients. Additionally, we used both Xpert MTB/RIF® and Xpert MTB/RIF-Ultra® in our study, as our laboratory switched to Xpert MTB/RIF-Ultra® midway through the study period. This may also have contributed to a lower detection rate than if we had used Xpert MTB/RIF-Ultra® throughout the study.<sup>28</sup> Our finding of PE associated with polymicrobial infections, most commonly bacterial-viral co-infections, provides new insights into the aetiology of pleural infections. Polymicrobial infections may impact on duration of therapy as well as choice of definitive antimicrobials. We demonstrated that pleural fluid LDH, a widely available inexpensive test, best discriminated bacteria from other infections, which we propose were mostly TB. Ghoor *et al* made a similar observation in a study among South African children, although in that study, not all the TB cases were microbiologically confirmed.<sup>20</sup> Likewise, Lunelli *et al* also found that pleural fluid LDH levels were relatively low among children with TB PE.<sup>30</sup> Therefore, pleural fluid LDH can be used as a guide in deciding whether to discontinue or continue empiric antibiotics and TB treatment with greater confidence when no aetiology is confirmed. In contrast to adults, pleural fluid ADA was not helpful in differentiating bacterial infections and TB and should not be routinely performed in children with PE.

Although confirmation of TB was uncommon, we speculate that most children with no pathogen identified probably had TB based on the similar clinical and biochemical characteristics.

FTDResp33® detects a limited number of respiratory pathogens as compared to other molecular techniques such as broad-range 16srRNA PCR.<sup>31</sup> This may have underestimated the spectrum of bacterial infections in this cohort. Additionally, the number of TB cases may have been underestimated by Xpert MTB/RIF® because of its lower sensitivity compared to Xpert MTB/RIF-Ultra®.<sup>32</sup> Another limitation is that this is a single centre study with a small number of participants in a high TB-endemic setting. The generalisability of our findings may not be applicable to settings with low TB prevalence or different childhood immunisation schedules. Furthermore, being a post-hoc analysis, this study could not explore the immediate impact of PCR testing on management.

## **CONCLUSION**

PCR improved detection of pathogens and reduced number of children with unconfirmed aetiologies in presumed exudative PE.

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*Conflicts of Interest:* none

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Table 1: Demographic and clinical characteristics of study population stratified by aetiology groups.

	Bacteria N=36	Tuberculosis N=10	virus N=3	Polymicrobial N=10	No pathogen N=32	Total N=91	<sup>2</sup> P value
Age in months median (IQR <sup>1</sup> )	13.3 (7.6-30.9)	112.8 (53.7-145.3)	23.7(19.6-31.3)	16.4(11.3-31.2)	85.8(40.7-117.5)	91/91 (100)	0.167
Male n (%)	20/36 (55.6)	4/10 (40.0)	3/3 (100.0)	8/10 (80.0)	21/32 (65.5)	56/91 (61.5)	0.196
HIV-exposed n (%)	8/36 (22.2)	1/10 (10.0)	0/3	2/10 (20.0)	3/32 (9.4)	14/91 (15.4)	0.539
HIV-infected n (%)	2/36 (5.6)	1/10 (10.0)	0/3 (0.0)	0/10(0.0)	1/32 (3.1)	4/91 (4.4)	0.812
Cough duration in days median (IQR)	4 (2-7)	7 (4-14)	7 (1-11)	6 (4-30)	6.5 (4-14)	70/91 (77)	0.167
TB exposure in past 6 months n (%)	3/36 (8.3)	2/9 (22.2)	0/3(0.0)	1/10 (10.0)	7/32 (21.9)	13/91 (14.4)	0.450
Mantoux positive n (%)	0/16(0.0)	4/8(50.0)	0/3(0.0)	1/6 (16.7)	21/31 (67.7)	26/64 (40.6)	<0.001
Weight loss n (%)	12/35 (34.3)	7/10 (70.0)	1/3 (33.3)	3/10 (30.0)	20/32 (62.5)	43/90(47.8)	0.065
Chest pain n (%)	8/28 (28.5)	7/10 (70.0)	0/3(0.0)	1/5 (20.0)	18/32 (56.3)	34/78 (43.6)	0.027
Antibiotics in the last 7 days n (%)	21/36 (58.3)	3/10 (30.0)	3/3 (100.0)	4/10 (40.0)	19 /32 (59.4)	50/91 (55.0)	0.718
Underweight-for age n (%)	4/36 (11.1)	3/10 (30.0)	1 /2(50.0)	0	6/32 (18.6)	14/90 (15.6)	0.197
Pneumothorax n (%)	7/36 (19.4)	0/10(0.0)	0/3(0.0)	1/10 (10.0)	3/31 (9.7)	11/90 (12.2)	0.435

<sup>1</sup> IQR: interquartile range; <sup>2</sup>P value for distribution across all categories

Table 2: Pathogen-specific diagnostic yield of routine culture (blood and pleural fluid), TB tests and FTDRsp33® multiplex PCR of pleural fluid.

Pathogen	Blood culture	Pleural fluid culture	Pleural fluid PCR	Sputum Xpert MTB/RIF® or Xpert MTB/RIF-Ultra®	Pleural fluid Xpert MTB/RIF® or Xpert MTB/RIF-Ultra®	Sputum TB culture	Pleural Fluid TB culture	Total
<i>S. aureus</i>	9	21	21					27
<i>S. pneumoniae</i>	3	0	12					12
Other streptococci spp	0	3						3
<i>M. catarrhalis</i>	0	0	4					4
<i>H. Influenzae</i> spp	0	0	2					2
<i>M. pneumoniae</i>	0	0	1					1
Anaerobes	2	2	0					2
Any virus			11					11
<i>M. Tuberculosis</i>				6	4	6	2	

Table 3: Polymicrobial combinations of pathogens causing pleural effusion.

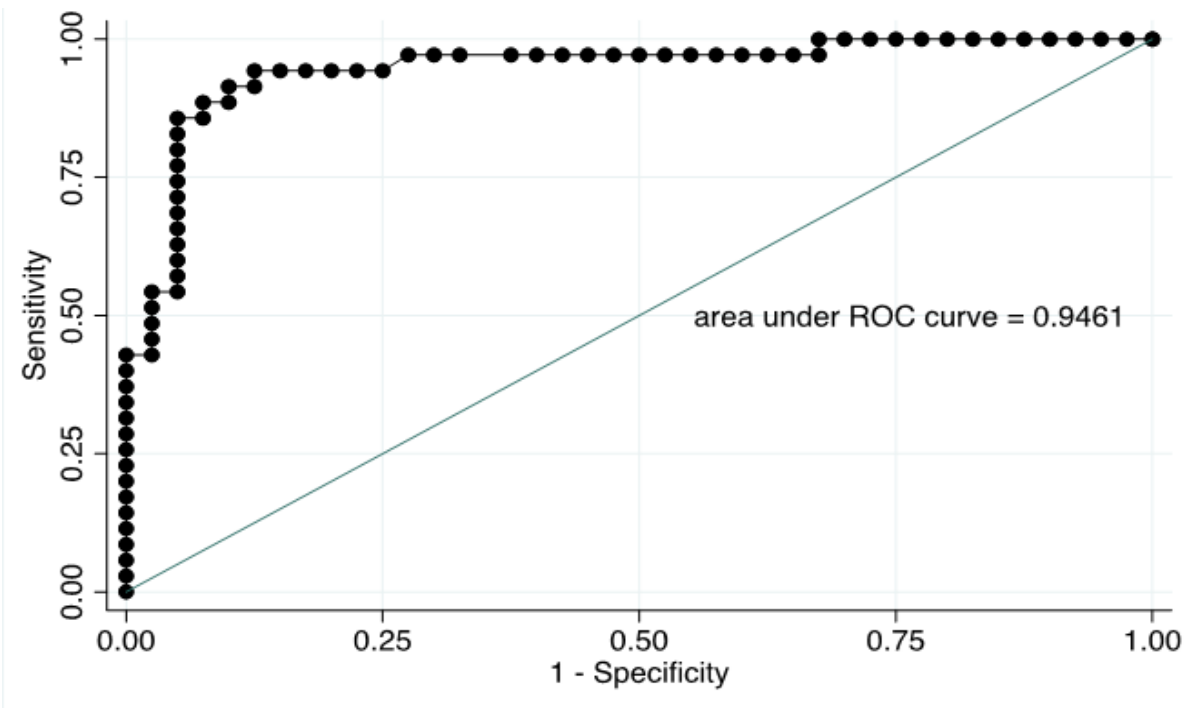
Pathogen combination	Frequency
<i>Staphylococcus aureus</i> +anaerobes + bocavirus	1
<i>Staphylococcus aureus</i> +bocavirus	1
<i>Staphylococcus aureus</i> +adenovirus	2
<i>Staphylococcus aureus</i> + <i>Moraxella catarrhalis</i> + rhinovirus	1
<i>Staphylococcus aureus</i> + <i>Moraxella catarrhalis</i> + <i>Streptococcus pyogenes</i> + rhinovirus	1
<i>Streptococcus pneumoniae</i> + <i>Haemophilus influenzae</i>	1
<i>Streptococcus pneumoniae</i> + <i>Moraxella catarrhalis</i> + adenovirus + rhinovirus	1
<i>Moraxella catarrhalis</i> + <i>Mycobacterium tuberculosis</i>	1
Anaerobes + <i>Mycobacterium</i> <i>tuberculosis</i>	1

Table 4: Comparison of selected laboratory findings stratified by aetiology categories, excluding children with only viruses isolated (n=3); data are presented as median values and inter-quartile ranges (IQR).

	Bacteria N=36	TB N=10	Polymicrobial N=10	No pathogen N=32	Total N=88	<sup>1</sup> P value
Blood investigations						
WCC <sup>2</sup> ( $\times 10^9$ /L)	22.3(10.8-29.7)	10.6 (8.7-14.0)	12. 8 (6.7-23.6)	10.3 (7.9-13.8)	88	0.002
CRP <sup>3</sup> (mg/L)	257.0 (174-297)	141.0 (89-156)	188.0 (166-302)	118.0 (74-164)	86	< 0.001
PCT <sup>4</sup> (mg/L)	10.5 (4.0-24.9)	0.7 (0.2-1.5)	12.2 (1.1-27.1)	0.3 (0.2-0.7)	73	< 0.001
Pleural fluid investigations						
Protein (g/L)	47.0 (37.0-51.5)	53.5 (49.5-57.0)	46.5 (42.5-50.5)	57.0 (53.0-59.0)	74	< 0.001
LDH <sup>5</sup> (U/L)	5855.0 (2170.0-15319.0)	721.0 (497.5-908.5)	8507.0 (2429.5-25274.0)	471.0 (302.0-753.0)	73	< 0.001
Glucose (mmol/L)	1.9 (0.6-3.5)	4.6(2.9-6.4)	0.7 (0.6-2.4)	4.2 (3.1-5.0)	71	< 0.001
ADA <sup>6</sup> (U/L)	148.1 (56.9-311.6)	50.4 (47.1-61.0)	86.0 (46.1-116.2)	46.0 (35.8-56.1)	69	< 0.001
Polymorphs ( $\times 10^9$ /L)	605.0 (260.0-3280.0)	65.0 (10.0-160.0)	1405.0 (20.0-2790.0)	39.5 (4.5-253.0)	46	0.013
Lymphocytes ( $\times 10^9$ /L)	640.0(105.0-1040.0)	1760.0 (450.0-2320.0)	642.5(115.0-1170.0)	659.0 (224.0-1095.0)	46	0.549

<sup>1</sup>P value is across all categories <sup>2</sup>WCC: White cell count; <sup>3</sup>CRP: C-reactive protein; <sup>4</sup>PCT: procalcitonin; <sup>5</sup>LDH: lactate dehydrogenase; <sup>6</sup>ADA: adenosine deaminase.

Figure 1: Receiver operating characteristic (ROC) curve showing performance of pleural fluid lactate dehydrogenase for detecting bacterial pleural effusion.



Supplementary Table S1: The performance of various biochemical parameters for detecting bacterial pleural effusion and their optimal cut-offs.

Parameter	Observation	AUC <sup>2</sup>	Standard Error	95% Confidence Interval	Proposed Cut-off, ≥	Correctly Classified, %	Sensitivity	Specificity	<sup>3</sup> LR+	<sup>4</sup> LR-
WCC <sup>1</sup>	91	0.7	0.1	0.6-0.8	15.9×10 <sup>9</sup> /L	71.4	60.9	82.2	3.4	0.5
Blood neutrophils	86	0.6	0.1	0.5-0.7	74.7×10 <sup>9</sup> /L	60.5	27.9	93.0	4.0	0.8
Blood CRP <sup>5</sup>	89	0.8	0.0	0.8-0.9	166 mg/L	80.9	82.2	79.6	4.0	0.2
Blood PCT <sup>6</sup>	76	0.9	0.1	0.8-0.9	1 mg/L	81.6	92.7	68.6	3.0	0.1
Blood lymphocytes	86	0.4	0.1	0.2-0.5	60×10 <sup>9</sup> /L	51.2	4.7	97.7	2.0	1.0
Serum LDH <sup>7</sup>	84	0.7	0.1	0.6-0.8	439 U/L	67.9	47.6	88.1	4.0	0.6
Pleural fluid LDH <sup>7</sup>	75	1.0	0.0	0.9-1.0	1716 U/L	90.7	85.7	95.0	17.1	0.2
Pleural fluid ADA <sup>8</sup>	71	0.8	0.1	0.7-0.9	99.9 U/L	80.3	58.1	97.5	23.2	0.4
Pleural fluid lymph	48	0.4	0.1	0.2-0.6	3680×10 <sup>9</sup> /L	72.9	0.0	100.0		1.0
Pleural fluid polymorphs	48	0.8	0.1	0.6-0.9	1340×10 <sup>9</sup> /L	79.2	38.5	94.3	6.7	0.7
Pleural fluid protein	76	0.2	0.1	0.1-0.3	83 g/L	52.6	0.0	100.0		1.0
Pleural fluid glucose	73	0.2	0.1	0.1-0.3	10 mmol/L	54.8	2.9	100.0		1.0

<sup>1</sup>WCC: white cell count; <sup>2</sup>AUC: area under the curve; <sup>3</sup>LR+: positive likelihood ratio; <sup>4</sup>LR-: negative likelihood ratio; <sup>5</sup>CRP: C-reactive protein;

<sup>6</sup>PCT: procalcitonin; <sup>7</sup>LDH; lactate dehydrogenase; <sup>8</sup>ADA: adenosine deaminase

# The International Journal of Tuberculosis and Lung Disease

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**Results:** Present the results in logical sequence, referencing figures and tables (see information below on submitting figures and tables). For complex tables only highlight the most important results.

**Discussion:** Bring the reader back to your initial aims, objective or hypothesis, showing how this study has improved our understanding of the topic. **Conclusions:** optional, but if used, please briefly highlight the single most significant aspect of this study.

**Reviews** (State of the Art, Viewpoints, Guidelines) are aimed to inform and educate readers and should stimulate debate around clinical and scientific topics. The IJTLD also welcomes suggestions for review articles on different aspects of TB and across the breadth of respiratory medicine. Authors proposing an unsolicited review should explain in a cover letter why the topic is timely and relevant and include a maximum of 5 examples of their own recent published work supporting their expertise in this field.

Submitted reviews should not overlap with recently published ones on similar topics. Review articles should represent the state of the art in their specific field. The literature review should be up-to-date. Systematic review methods are encouraged but are not mandatory. The IJTLD strongly encourages the use of new imaginative figures and of a pivotal figure to summarise key concepts or conclusions of the review.

Review articles (see Table above) should not exceed 3,500 words (excluding abstract, references, tables and figure captions) and should have a structured summary of 200 words, up to 70 references and 5 moderate-sized tables/figures.

**Minireviews** are focused, expert reviews on cutting-edge issues. One-page proposals will be considered, and will be judged on 1) the scientific importance and novelty of the subject matter, 2) its relevance to the readership of the Journal, and 3) the expertise of the proposed authors. Minireviews are expected to draw conclusions and make recommendations that are based on the evidence presented.

Text up to 2,000 words, a structured summary of 200 words, 3–4 moderate-sized tables/figures and up to 40 references.

## **Forum: Letters and Correspondence**

**Letters** include research letters, case studies and other forms of short communication to the Editor. Research letters are preliminary studies or short reports presented in the shorter format of a Letter to the Editor. Case studies are considered only if they contain original and innovative material, ideally discussing cases in the form of a mini-review of the available literature on the topic. Patient consent should be provided (or, in the case of death, the consent of a relative). Letters do not include an abstract or text headings and start 'Dear Editor,...'. They should not exceed 1,100 words (excluding references, tables and figure captions) and should have no more than 15 references. One figure or one table is mandatory.

**Correspondence** is designed to discuss relevant articles, guidelines, documents or other topical matters recently published in the IJTL, or other journals or media. Correspondence in response to an article published in the IJTL should be submitted within 3 months of the publication date of the original article. All Correspondence is sent to the authors of the original article for a reply and these exchanges are prioritised for publication in the next available issue of the *Journal*. Correspondence does not include an abstract or text headings and starts 'Dear Editor, ...'. They should not exceed 600 words (excluding references, tables and figure captions) and should have no more than 10 references. One figure or one table is highly recommended.

### **Editorials**

Editorials are usually invited by the Editorial Board to allow experts to concisely discuss the findings of an Original Article (sharing their perspective on how the publication advances the field and highlighting the need for specific further research). Unsolicited Editorials are also of interest and can highlight a key initiative or paradigm shift. Editorials do not include an abstract and are between 500–1,400 words (excluding references, tables and figure captions) and should have no more than 25 references and 1-2 figures/tables.

Papers that do not conform to these guidelines will either be rejected, or returned to the authors for revision prior to peer review.

### **FORMATTING**

Authors should submit a single Word document (.doc or .docx) – this document should include the title page, abstract text, references, tables and figures with legends. For ease of peer review, the article should have 1.5 or double spacing and continuous line numbering.

**Title page:** This should contain:

- 1) a concise, informative title of not more than 110 characters and spaces, without abbreviations
- 2) the names and affiliations of all contributing authors, clearly indicating who is linked to each institution
- 3) a running head of not more than 45 characters and spaces
- 4) a word count of the summary, a word count of the text, number of references, tables and figures
- 5) 3-5 keywords that do not appear in the title
- 6) the name, full address and contact details of the corresponding author.

**ACKNOWLEDGEMENTS:** Acknowledge only those people who have made substantial contributions to the study, with their consent. All sources of support in the form of grants, author contributions and all conflicts of interest should also be mentioned.

**REFERENCES:** The accuracy of references is the responsibility of the author. Please use superscript numbers in the text, and they must be numbered in the order in which they are cited. References that are cited more than once retain the same number for each citation. The references list at the end of an article should be arranged in numerical order.

*References to an article:* should include the names of the authors, followed by their initials. List all authors when three or fewer - see the example below:

Gordon JB, Bennett AM. Tuberculosis in reindeer. Scand Rev Respir Dis 1978; 96 (Suppl): 217-219.

When there are more than three authors, list only the first author and add 'et al.'

*References to a piece of work:* (book/monograph) should include the names of the authors, the title of the piece of work, the ISSN number of the publication, the name of the Editor, the place and year of publication, the number of the volume and the first and last page numbers.

*References to a chapter in a book:* should include the names of the authors, the title of the chapter with the word "In" preceding the reference of the work e.g.  
Girling DJ. The chemotherapy of tuberculosis. In: Ratledge C, Stanford J, Grange JM, eds. Biology of the mycobacteria. London, UK: Academic Press, 1989: pp 285-323.

*Electronic references* should be given only when an original citation is unavailable; please provide as much information as possible, including html address.

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Tables and figures should be self-explanatory and easily understood as a standalone element. Numbering of tables/figures corresponds to where they are first cited in the text. All abbreviations included in the title or in the Table/Figure, even if explained in the text of the article, should be expanded in a footnote to be understandable without referring to the text.

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Explanations of data should be included in the legend and linked to the respective element by a number (1, 2, 3 etc). Tables should be treated as a standalone item, so references should be included in their entirety in the legend and not added to the Reference list at the end of the article. Please note that the number and size of the tables need to be accommodated within the pages allocated for each type of article.

*Examples of table sizes:*

Small table with 4-5 columns and 4-5 rows = **1/4 page** in a typeset article

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Large table with 6-10 columns and 12-16 rows = **1 full page** in a typeset article

If there is the need to refer to very large datasets, the excess material can be included as Supplementary Data (please note charges below). The figures and tables in Supplementary Data should be numbered as Figure S1, Table S1 etc (to avoid confusion over labelling of the figures and tables in the main body of the article). Alternatively, the data can be hosted via a service such as Figshare (<https://figshare.com>) with a link embedded in the text.

**Figures:** These should be referred to consecutively in the text. They can be inserted into the Word document at the end of the References or uploaded separately as image files (.jpg, .ppt, .gif, .tif or .bmp). A brief explanatory legend should be provided for every figure to ensure it can be understood as a standalone item.

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