Note: This is Online Appendix 1 of Enimil AK, Nuttall AJ, Centner CM, Beylis N, Eley BS. Xpert MTB/RIF Ultra and mycobacterial culture in routine clinical care at a paediatric hospital. S Afr J Infect Dis. 2022;37(1), a398. https://doi.org/10.4102/sajid.v37i1.398

Appendix 1

Respiratory specimen collection

Respiratory specimens were collected by standardised induced sputum, gastric lavage, expectoration, tracheal aspirate and bronchoalveolar lavage (BAL) methods.

Induced sputum specimen collection: After a 2-3-hour fast, the patient was pretreated with inhaled salbutamol, followed by sputum induction with 5mls of 5% sterile saline. A sterile mucus extractor of catheter size 6 or 7 was used to collect a sputum specimen. [1]

Gastric lavage specimen collection: The child underwent early morning gastric lavage after an overnight fast of at least 4 hours. Before the child arose, a nasogastric tube (NGT) was passed, and the gastric contents aspirated. If the aspirate volume was less than 20 mL, 20 mL of normal saline was inserted down the NGT, left for 2–3 minutes, then aspirated. Additional 5–10 mL volumes of normal saline were inserted and aspirated until a minimum of 20 mL of aspirate was obtained. [2]

Expectorated sputum specimen collection: For an older child who was able to expectorate, a sputum specimen was collected after deep, spontaneous coughing. [3]

Tracheal aspirate collection: Before the procedure, the patient was preoxygenated for 2 minutes, then disconnected from the ventilator. The tracheal aspirate was obtained by inserting an appropriately sized suction catheter through the endotracheal tube and suctioning for a maximum of 5 seconds. The tracheal aspirate was collected into a mucus trap. After specimen collection, the patient was reconnected to the ventilator. [4]

Bronchoalveolar lavage (BAL) collection: This was done either as a completely blind procedure or under direct vision. Blind BAL was done in the intensive care unit (ICU) by inserting a 10 ml aliquot of warm, sterile saline through the endotracheal tube, followed by suctioning with an appropriately sized suction catheter into a mucus trap. Bronchoalveolar lavage under direct vision was done by a pulmonologist or a pulmonology trainee in theatre under general anaesthetic during bronchoscopy. Once the area of the lung was located, 10

ml of warm saline was inserted through the flexible bronchoscope channel. The saline was then suctioned into a specimen container using the suction channel of the flexible scope. [5]

Microbiological procedures

All microbiology testing was conducted at the NHLS microbiology laboratory, Groote Schuur Hospital, Cape Town, South Africa. Respiratory specimens were processed and analysed according to the laboratory's standard operating procedures and/or relevant manufacturers' instructions. Each specimen was processed for both Ultra and microbiological culture.

In brief, specimens were decontaminated by the addition of N-Acetyl-L-Cysteine-NaOH solution to achieve a final NaOH concentration of 1.5%. After 20 minutes, an equal volume of phosphate buffer was added, followed by centrifugation at 3000xg for 15 minutes. The supernatant was then decanted to leave 1 ml of sediment, which was split; 500 µl was inoculated into an Ultra (Cepheid, Sunnyvale, CA) cartridge. Molecular semi-quantitation of MTBc load by Ultra was categorized as trace, very low, low, medium, high, and very high. The remaining 500 µl of sediment was resuspended in 500 µl phosphate buffer and inoculated into BD BBL Mycobacteria Growth Indicator Tubes (MGIT). The MGITs had been prepared with 800 µl PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) antibiotic mix (Becton Dickinson, Franklin Lakes, NJ). MGITs were incubated at 37ºC in the BD BACTEC MGIT 960 Mycobacteria Culture System (Becton Dickinson) and continuously monitored for 42 days or until positive for growth of acid-fast bacilli that was identified using the Ziehl Neelsen stain. The presence of MTBc was confirmed by GenoType MTBDRplus lineprobe assay (Hain Lifescience GmbH, Nehren, Germany), which also detects rifampicin and isoniazid-resistance associated mutations in MTBc. Species identification of non-tuberculous mycobacteria was not routinely performed.

Study definitions

Confirmed TB (cTB) was defined as microbiological confirmation of MTBc by either culture or Ultra on at least 1 respiratory specimen. [6] Unconfirmed TB (uTB) was diagnosed if the attending clinician treated a child for TB based on the presence of suggestive symptoms or signs of TB, chest radiograph consistent with TB, a close TB contact and/or a positive tuberculin skin test but without microbiological confirmation.

Pulmonary tuberculosis (PTB) is any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or tracheobronchial tree.

Extrapulmonary TB (EPTB) is any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs. Tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constituted EPTB. [7]

HIV infection:

- for a child < 18 months old: a positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test on an independent blood sample.
- for a child >18 months old: two positive serological test results (HIV rapid test or HIV ELISA) or a positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test. [8]

Moderate or severe underweight for age (UWFA) were defined as weight-for-age z score (WAZ) <−2 standard deviations (SD) below the median WHO growth reference standards. [9]

Haemoglobin (Hb) cutoff of < 11 g/dl was defined as anaemia and mean corpuscular volume (MCV) < 70 fL was defined as microcytosis. [10] Microcytic anaemia was defined as Hb <11 g/dl and MCV < 70 fL. [11]

Tuberculosis treatment regimens for uncomplicated, complicated, and central nervous system (CNS) TB as described in the South African childhood Tuberculosis guidelines were used to treat the patients included in this study. [3]

Liver-friendly regimen: An alternative TB treatment regimen containing non-hepatotoxic drugs such as ethambutol, amikacin, levofloxacin and/or linezolid. [12]

Tuberculosis contact history refers to household or close exposure of a child to an individual with PTB. [7]

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