Supplementary Appendix

This Appendix has been provided by the authors to give readers additional information about their work.

Supplement to: P Orikiriza, J Smith, B Ssekyanzi et al. Accuracy of two non-sputum based-approaches for diagnosis of tuberculosis in highly vulnerable children: Xpert MTB/RIF from stool and urine AlereLAM

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1. Additionnal details of the method

1.1 Eligibility criteria

Inclusion criteria

A/ Children younger than 2 years or HIV infected or with severe malnutrition, AND

- A clinical suspicion of tuberculosis defined by the presence of at least 2 of the following signs:
    - Persistent (>2 weeks), unremitting or unexplained cough
    - Persistent (>1week) or unexplained fever (>38°C)
    - Severe malnutrition defined by either weight for height ≤-3 Z score, MUAC<115mm or nutritional oedema (this criterion is not valid in HIV children with severe malnutrition without HIV infection or age < 2 years)
    - Persistent (>2weeks) lethargy or reduced playfulness reported by the care giver (this criterion is not valid in children with severe malnutrition because most severely malnourished children are expected to present lethargy or reduced playfulness)
    - TB contact history or contact history with a person presenting chronic cough defined by a constant cough for at least 1 month, within the past 2 years

OR

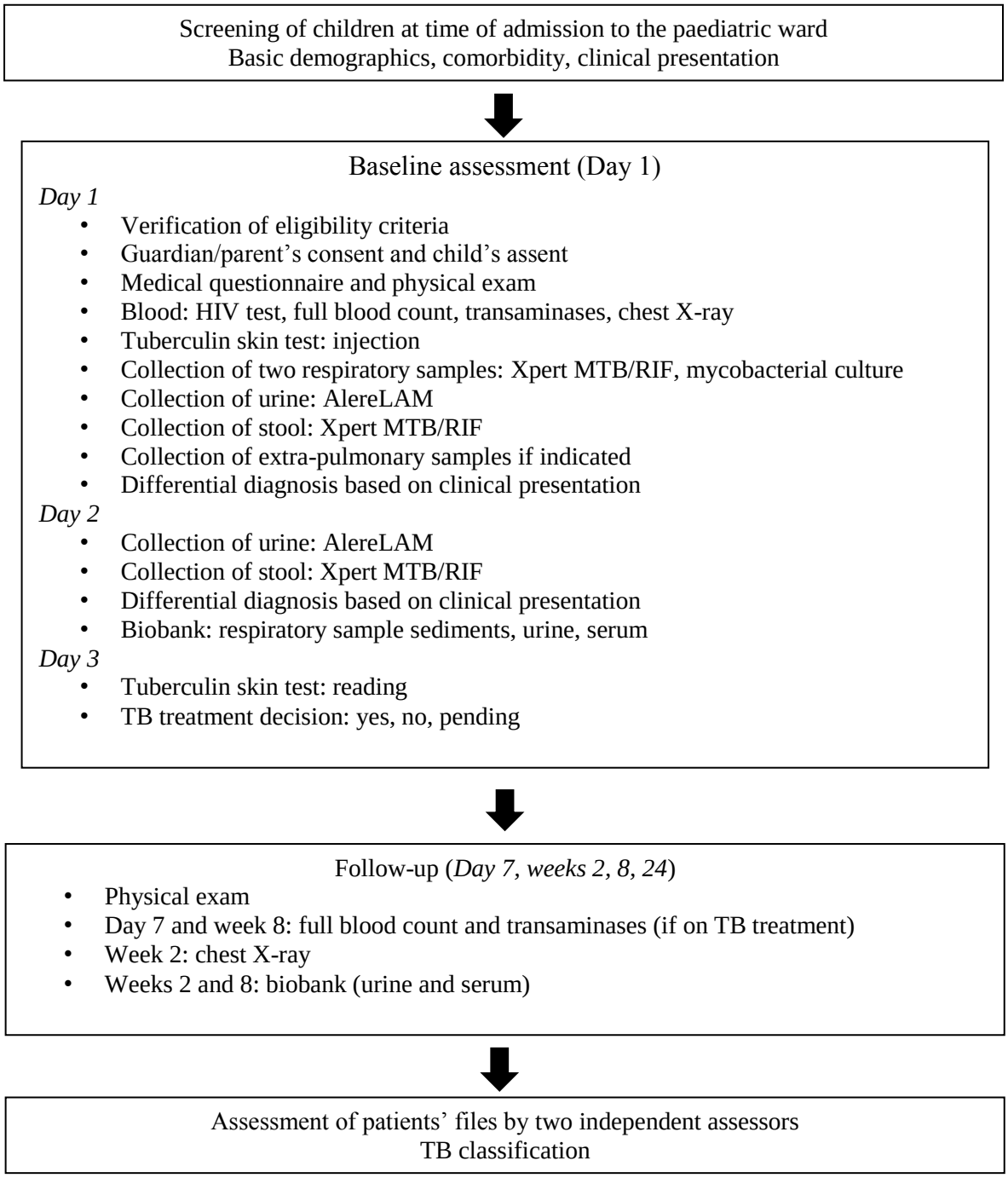
- Any sign suggestive of TB meningitis or disseminated/miliary

B/ Informed consent signed by parent or legal guardian and child's informed assent when feasible according to age and clinical conditions

Exclusion criteria

- Children on anti-tuberculosis treatment

1.2 Figure S1: Scheme of study design



1.3 Laboratory procedures

1.3.1 Respiratory sample collection procedures

NPA was collected by inserting a suction catheter through the nostril into the oropharynx to stimulate a cough reflex with aspiration of secretions into a specimen container. A minimum of 2ml of IS was obtained by nebulisation with 5ml of salbutamol and 5ml of 5% hypertonic saline for 20 minutes then nasopharyngeal suction. For GA, a nasogastric tube was inserted first thing in the morning before feeding or after at least four hours of fasting. At least 2 ml of gastric contents was obtained and transferred into a sterile container. If the aspirate was less than 2 ml, 5 ml of normal saline was administered, left for 2-3 min, and then re-aspirated. The gastric aspirate was neutralized using an equal volume of 4% sodium bicarbonate solution.

1.3.2 Stool sample processing procedures

Approximately 0.5g of the sample was homogenized with saline solution, vortexed for 10 seconds and left to stand for 5 minutes. After settling, 5 ml of supernatant was transferred into a 50 ml centrifuge tube where an equal volume of 3% NaOH solution was added at 1.5% final concentration, vortexed lightly and left to stand for 20 minutes followed by neutralization as described for respiratory samples. An additional decontamination step was introduced to reduce and the risk of PCR inhibitors and the final sediment was homogenised in 2.5ml of PBS before Xpert MTB/RIF using manufacturer's instructions for sediments

1.3.3 Culture of blood samples

Two blood samples were inoculated in Bactec Myco/F Lytic (Becton and Dickinson, New Jersey, USA) Mycobacteria bottles used in the 9240 MGIT instrument for mycobacterial culture. ZN microscopy and blood agar were used to verify culture purity and MPT 64 (SD Bioline)-Rapid Diagnostic Test to confirm MTB. Two other blood samples were inoculated into aerobic Bactec blood culture bottles and placed in the automated Bactec 9240 (Becton and Dickinson, New Jersey, USA) instrument for a minimum of 5 days. All positive culture bottles were sub-cultured

onto appropriate media, depending on the type of bacteria seen in the gram staining technique and drug susceptibility testing performed using disk diffusion method and, when appropriate, E-tests for the minimum inhibitory concentrations.

1.3.4 Pneumocystis jiroveci infection test

In children with clinical suspicion of *Pneumocystis jiroveci* infection (HIV infected or < 2 years old with acute hypoxic pneumonia), NPA was also tested withdirect qualitative immunofluorescence microscopy test (Bio rad, Hercules, USA) with monoclonal antibody immunofluorescent stain based on manufacturer's instructions. The reading was made by two different laboratory trained staff and a third reader in case of discrepancy.

1.3.5 HIV testing

HIV testing used determine® HIV rapid testing followed by StatPak®, in case of positive result, and finally Unigold® as tie breaker where there was discordance according to national guidelines. Children below 18 months, with a positive rapid test were confirmed using PCR DNA method. CD4 cell count using the BD FACS Count™ system was retrieved from the HIV clinic patient's files.