Case Report

Smear and Xpert MTB/RIF Negative Tuberculosis: A Lingering Dilemma in Children and the HIV Infected – Two Case Reports

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Abstract

Confirmatory diagnosis of tuberculosis (TB) in children and human immunodeficiency virus-infected individuals continues to pose a challenge in resource-limited settings. Although the Xpert MTB/RIF assay is sensitive and rapid, its ability to detect *Mycobacterium tuberculosis* is low in smear-negative TB, a compelling situation to resort to empirical therapy based on high index of suspicion. We report two cases clinically suspected to be TB in whom repeated sputum smear microscopy and Xpert MTB/RIF tests were negative, but based on high index of suspicion and the World Health Organization recommended clinical criteria, both were commenced on anti-TB therapy and made remarkable recovery at treatment completion.

Keywords: Acid-fast bacilli smear, children, HIV, microscopy, *Mycobacterium tuberculosis*, Xpert MTB/RIF

Introduction

Of the estimated 10 million new tuberculosis (TB) cases reported in 2017, 1 million involved children.[1,2] The true burden of childhood TB is not known due to challenges of accurate diagnosis and underreporting.[1] However, in 2016, among children aged <15 years in Nigeria, its incidence was approximately 13.8%.[2]

The overlap of clinical features of TB with those of common childhood infections in the tropics, difficulty in specimen collection, low sensitivity of smear microscopy, and poor availability of facilities for specimen culture diagnosis, all contribute to the challenge and delay in diagnosing TB.[3] Although the recent innovation of the Xpert TB/RIF assay technology has led to improvement in case detection among individuals with paucibacillary disease, there are still limitations regarding the validity of this tool.

Case Reports

Case 1

A 7-year-old maternal orphan presented with a 12-month history of recurrent low-grade fever, cough, and drenching night sweats. He also had a 7-month history of recurrent diarrhea and weight loss. He had not received any vaccinations and had a significant history of contact with undiagnosed chronically coughing adults. His mother had died 6 months earlier of an undiagnosed disease characterized by chronic cough, fever, and weight loss. She had received no antenatal care during his pregnancy. A younger sibling had also died in infancy from an illness associated with fever, recurrent diarrhea, and weight loss. His father who was apparently well was a 65-year-old herdsman.

Examination revealed a chronically ill boy who was moderately pale, with generalized peripheral lymphadenopathy. He was severely malnourished with a weight of 10 kg and a height of 99 cm (weight-for-height [WFH] Z-score, <−3). A nontender uniformly distended abdomen with moderate hepatosplenomegaly was the only other significant finding.

A diagnosis of TB/HIV coinfection was made based on HIV antibody tests, confirmed later by enzyme-linked immunosorbent assays (Bios HivEIA-HIV-Ag/Ab, China). Gastric washings on two separate occasions were both acid-fast bacilli (AFB) and Xpert MTB/RIF negative. His Mantoux test

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read 2 mm, while the chest X-ray revealed dense perihilar opacities and blunted costophrenic angles. His packed cell volume (PCV) was 17% while other parameters of the full blood count were within normal limits. Bacteriologic cultures yielded no growth while his CD4 count, CD4%, and viral load were 118 cells/μl, 2%, and 1525,817 copies/ml, respectively.

Based on the strong consideration of the paucibacillary nature of TB disease in HIV-infected children with severe immunosuppression, he was commenced on anti-TB treatment (R + H + Z + E), along with nutritional rehabilitation. He improved remarkably with a 5-kg weight gain over a 4-week period following which he was started on highly active antiretroviral therapy (AZT/3TC/EFV). He was discharged after 8 weeks of hospital stay, and at follow-up 2 months later, he weighed 20 kg.

Case 2

A 2½-year-old son of an artisan presented with a 4-month history of multiple body swellings and recurrent fever, cough, night sweats, and weight loss of 3-month duration. The swellings involved his lower jaws, left clavicle, right elbow, and right ankle. He had received bacillus Calmette–Guérin vaccination at 4 weeks of age and had no known contact with TB cases. There was no family history of sickle cell anemia. His mother had antenatal care with no major illness in pregnancy, and his delivery was uncomplicated.

On examination, he was afebrile, pale with brown silky hair, and generalized lymphadenopathy. He was severely malnourished with a weight of 8 kg and height of 95 cm (WFH z-score, <−3). He had a necrotic ulcer at the sternal edge of the left clavicle which had a foul-smelling purulent discharge and from which extruded the necrotic medial end of the clavicle [Figure 1]. He had discharging sinuses on both sides of his jaw as well as diffuse, tender right elbow and right ankle joint swellings. His respiratory rate was 24/min and he had flat, stony dull percussion notes on the right hemithorax with reduced air entry. A clinical diagnosis of sepsis with right lobar pneumonia complicated by pleural effusion and multifocal osteomyelitis was initially entertained.

His investigation results revealed no AFB and negative Xpert MTB/RIF assay on three gastric washing, pleural aspirate and wound swab samples. His Mantoux test was 2 mm while his chest X-rays showed a right pleural effusion. His full blood count revealed anemia (PCV of 27%) and leukocytosis with a neutrophil predominance. His hemoglobin electrophoresis pattern was AA and antibody tests for HIV were negative. Thoracocentesis yielded 400 ml of exudative pleural fluid, and the necrotic clavicle was surgically extracted. Although his blood culture yielded no growth, *Staphylococcus aureus* was isolated from the discharging sinuses and pleural aspirate, and based on the sensitivity pattern, intravenous clindamycin was commenced along with daily wound dressings. Despite these, however, the discharging sinuses persisted, and the chest tube continued to drain actively by day 21.

Based on the high index of suspicion, TB therapy (R + H + E + Z) was commenced. Clinical response was evident by the 5th week as he stopped coughing, gradually regained appetite, and gained 3 kg in weight within 2 weeks. The chest tube stopped draining by the 6th week and repeat chest X-rays showed marked improvement with lung re-expansion. There was also a gradual healing of the sinuses. The patient was discharged on anti-TB therapy and maintained steady improvement at monthly follow-up visits [Figure 2].

We were limited by the inability to carry out TB culture of the specimens obtained in both cases due to nonavailability of culture facilities in our center.

Discussion

Case I was a slow HIV progressor and likely a missed opportunity for the prevention of mother-to-child transmission (PMTCT) who later acquired TB. His diagnosis was missed despite the mothers' ill health and sibling death resulting in delayed presentation. The suspicion for TB/HIV coinfection at

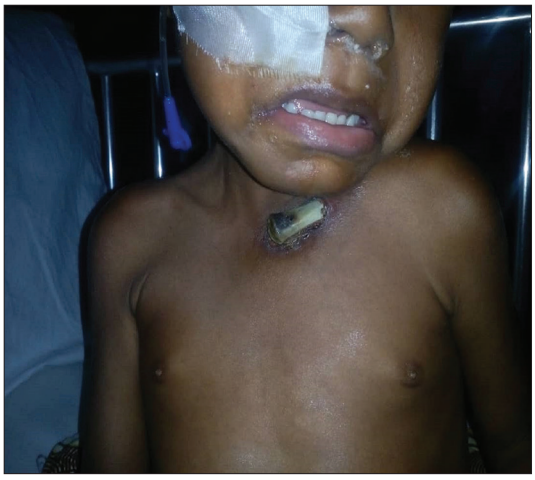

Figure 1: A 2½-year-old boy with extruded necrotic medial end of the left clavicle

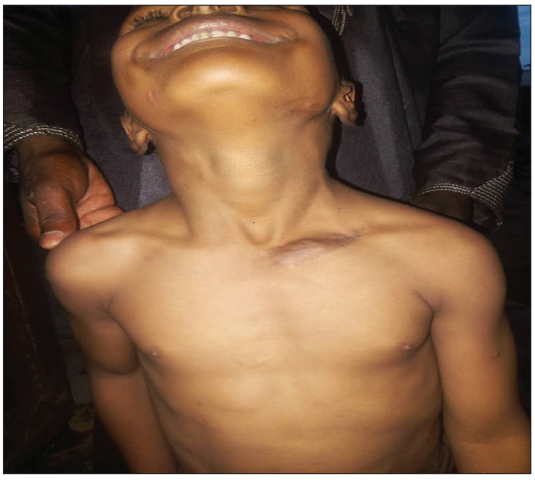

Figure 2: The same child at 5 years of age showing a linear hypertrophied scar over healed sinus site and asymmetry of the chest wall

presentation was very high, given the maternal circumstances as well as the chronicity of the symptoms and examination findings in the child.

The PMTCT program has helped avert HIV infection in about 1.5 million infants between 2000 and 2015.[4] Despite its existence in Nigeria since 2001, coverage was still very low at 32% in 2016.[4,5] HIV is an important risk factor for TB, and several researchers have shown that early commencement of antiretroviral therapy provides protection against TB.[6,7]

Case 2 had features of chronic osteomyelitis with right lobar pneumonia and pleural effusion with poor treatment response. The isolated organism, *S. aureus*, is a recognized cause of osteomyelitis although gram negatives and anaerobes may also be implicating organisms.[8] Antibiotic response was, however, poor as rapid response is often noted within the 1st week of antibiotic therapy.[8] It is probable that this was a pyogenic coinfection following the immunosuppressive effect of TB and highlights how symptoms and signs of diseases in the tropics overlap, thereby making the diagnosis of TB difficult.

Rapid detection of *Mycobacterium tuberculosis* and early initiation of targeted therapy has been shown to result in better outcome.[9] The World Health Organization (WHO) in 2010 rolled out the Xpert MTB/RIF, an automated heminested polymerase chain reaction system which amplifies the rifampin resistance-determining region of the *M. tuberculosis* rpoB gene and simultaneously detects TB and resistance to rifampicin.[10] Policy guideline recommends the Xpert MTB/RIF be used as an initial diagnostic test in individuals suspected of MDR- or HIV-associated TB.[10] However, a negative Xpert MTB/RIF result does not rule out TB.

Steingart *et al*.[11] in a systemic review involving 1936 adult participants in middle- and low- income countries reported a pooled sensitivity of 98% among non-HIV and 86% among individuals with HIV infection. In another meta-analysis of 15 studies involving 4768 respiratory specimens of 3640 children, sensitivity of Xpert MTB/RIF as shown in some studies was 79.9%–98% with higher values recorded for respiratory than non-respiratory samples.[12] These values were, however, much higher than the 68% obtained by Reither *et al*.,[13] in a low-resource, high TB burden multicenter study. Further still, Bacha *et al*.[14] demonstrated no difference in its performance (which was poor at 8% sensitivity) among children who were malnourished or had HIV in Tanzania. They also noted that it performed only half as well as culture. These studies have shown that although the Xpert MTB/RIF improves the yield in smear-positive cases, its sensitivity when used in smear-negative samples is usually seen in children and in those with HIV is still suboptimal.

Ioannidis *et al*.[15] reported an increase in sensitivity from an initial 72.5% for the first test to 85.1% with a second test and 90.2% with a third test among smear-negative samples.[15] This indicates that repeat testing of samples will improve yield, but it also increases the cost of testing by up to 20-fold.[16] The variation of sensitivity from reported studies may be as a result of the presence of nontuberculous mycobacteria[12,17] or variation in resistant strains of *M. tuberculosis* as was demonstrated by Rufai *et al*.[18] in India where a significant number of rifampicin resistance was not detected due to genetic mutations in the P533L region of the E probe in up to half of the cases tested.

A new cartridge, Xpert MTB/RIF Ultra, was recently introduced as a replacement for the current Xpert MTB/RIF assay.[19] It is formatted to overcome the shortcomings of Xpert MTB/RIF, especially the ability to detect rifampicin resistance, especially rpoB C533G mutations.[18] It has a better sensitivity in detecting *M. tuberculosis* in sputum-smear negative persons, extrapulmonary sites, in HIV-positive patients as well as children.[17] While the sensitivity of the Xpert MTB/RIF Ultra to detect the organism has improved significantly, it comes at a cost to its specificity such that more individuals are likely to be falsely diagnosed with TB.[17]

The cases presented in this report have revealed gaps in the TB management guidelines as the national algorithms for TB and HIV for children are silent on the use of cultures and repeat Xpert MTB/RIF tests to further evaluate smear-negative cases and improve diagnostic yield in suspected cases. It also highlights the importance of continued use of clinical acumen to decide when to start anti-TB therapy and the need for new diagnostics that search for "characteristic markers" or "bio signatures" elaborated by the organisms to detect TB in other body samples beside sputum,[20] especially among children who often have poor expectorating ability and a predisposition to disseminated TB.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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