

Case Report

Diagnosis of Intestinal Tuberculosis by Tissue Xpert MTB/Rif in an HIV-1 Infected Patient

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Summary

Introduction

HIV infection has changed the epidemiology of tuberculosis and is now considered the main risk factor for extra-pulmonary involvement. In recent years, molecular techniques have emerged as promising diagnostic tools. Only Xpert MTB/Rif assay has been advocated by the WHO for diagnosis of extra-pulmonary TB. Scarce data is available on its utility in gastrointestinal tuberculosis. We report a case of an HIV patient with intestinal tuberculosis diagnosed by Xpert MTB/Rif.

Description

A 31 year-old HIV patient presented with diarrhea, fever, diffuse abdominal pain and weight loss in the last 30 days. Bilateral crackles and right lower quadrant pain were found on physical exam. His CD4 count was 172 cells/ml and his HIV-1 viral load was 2026 copies/ml. Bilateral alveolar infiltrates were seen on the chest X-ray. Ultrasound showed enlarged peritoneal lymph nodes and thickening of the ileocecal region. Smear and Xpert MTB/Rif from sputum were negative. Patient underwent a colonoscopy which showed irregular congested ulcers in ileum and sigmoid. Xpert MTB/Rif from biopsy was positive for TB. Standard four drug therapy was started and patient improved significantly.

Discussion

The majority of experience with Xpert MTB/Rif has been on sputum samples, but it has also been used in a variety of fluid and tissue samples. It has a sensitivity of 89% and specificity of 74% for the diagnosis of extra-pulmonary tuberculosis. To our knowledge, there are no reports on the use of Xpert MTB/Rif in intestinal biopsies. Our case is a precedent for the use of this assay in the future.

Abstract

TB (tuberculosis) and HIV (human immunodeficiency virus) infection remain as major public health problems globally. HIV infection has changed the epidemiology of tuberculosis and is now considered the main risk factor for extra-pulmonary TB. In recent years, molecular techniques have emerged as promising tools for rapid and accurate diagnosis of TB. However, scarce data is available on its utility in gastrointestinal tuberculosis. We report a case of one HIV patient with gastrointestinal symptoms of one month duration. Colonoscopy showed irregular congested ulcers in ileum and sigmoid. Xpert MTB/Rif from biopsy was positive for

TB. Standard four drug therapy was started immediately and patient improved significantly. To our knowledge, there are no published reports on the use of Xpert MTB/Rif in intestinal biopsies. Further studies are needed to characterize the sensitivity and specificity of this assay for the diagnosis of gastrointestinal tuberculosis.

Introduction

TB remains a major public health problem with significant morbidity and mortality globally. The World Health Organization (WHO) estimated that 9.6 million new TB cases occurred in 2014 worldwide [1]. The advent of the HIV pandemic has changed the epidemiology of tuberculosis. HIV infection is now considered the main risk factor for extra-pulmonary involvement in tuberculosis [2].

Extra-pulmonary tuberculosis comprises a wide spectrum of disease affecting all parts of the body. Commonly affected sites include lymph nodes, pleura, urogenital tract, bones and joints, meninges, etc. The intestinal tract is the sixth most common extra-pulmonary site of presentation and represents 2-5% of the cases reported in developing countries [1].

Clinical presentation of intestinal TB is nonspecific and varies from systemic symptoms (i.e. Fever and weight loss) to focal gastrointestinal manifestations (i.e. diarrhea and abdominal pain). In addition, intestinal TB may mimic many other conditions such as inflammatory bowel disease and malignancy [3].

The diagnosis remains challenging, mainly because extra-pulmonary tuberculosis is paucibacillary and standard diagnostic techniques such as Ziehl-Neelsen staining usually fail to identify active cases. Culture is the gold standard; however it is time consuming and lacks sensitivity [4]. Histopathology demonstrating

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caseating epithelioid cell granulomas is highly suggestive of TB but identification of *Mycobacterium tuberculosis* bacilli is still necessary for a definitive diagnosis [5].

Despite the limitation of microbiologic methods and non-conclusive histopathology, health care providers still rely on these methods to diagnose intestinal tuberculosis which pose the risk of an inadequate and delayed treatment.

In recent years, molecular techniques based on nucleic acid amplification have emerged as promising tools for rapid and accurate diagnosis of TB due to their high sensitivity and specificity. Of the many molecular tests available nowadays, only the Xpert MTB/Rif assay has been advocated by the WHO for its use on extra-pulmonary TB [6]. While this technique can be applied to virtually any body fluid/tissue, scarce data is available on the utility of this assay in gastrointestinal tissue samples.

We report a unique case of an HIV-infected subject in whom the diagnosis of intestinal tuberculosis was accomplished by means of Xpert MTB/Rif on a bowel tissue sample obtained by colonoscopy.

Case Report

A 31-year-old HIV-1 infected Peruvian male with history of oropharyngeal candidiasis and wasting syndrome presented to the clinic with complains of watery diarrhea, fever, diffuse abdominal pain and 3 kg (kilogram) weight loss in the last 30 days. He was on antiretroviral therapy with tenofovir disoproxil fumarate/emtricitabine, atazanavir and ritonavir for the last 9 months. On physical exam the patient was febrile and looked chronically ill. Lung examination showed bilateral crackles. Abdomen was soft but tender to palpation in the right lower quadrant. No lymphadenopathy or visceromegaly was detected.

Laboratory data revealed a CD4 count of 172 cells/ml, which dropped from 406 cells/ml four months ago. His HIV-1 viral load was 2026 copies/ml. His CBC (complete blood count) showed hemoglobin of 10.2 g/dl (deciliter) and thrombocytosis of 753,000/mm³ (Cubic millimeter). Liver function tests were within normal limits. Chest X-ray revealed diffuse alveolar infiltrates bilaterally (Figure 1). The abdominal ultrasound showed small calcifications in the liver and spleen, multiple lymph nodes in the abdominal cavity, and bowel wall thickening at the ileocecal region. Acid fast bacilli (AFB) smears



Figure 1: Postero-anterior chest X-ray showing diffuse alveolar infiltrates bilaterally

from 3 non-induced sputum samples were negative. One sputum sample was also sent for Xpert MTB/Rif assay and was reported as negative. Given the predominance of intestinal symptoms, the patient underwent a colonoscopy. Two irregularly shaped ulcers with congested borders of 12 mm in diameter were found in the ileum (Figure 2A), and a 10 mm ulcer with congestive appearance was found in the sigmoid (Figure 2B). Additionally, small white plaques of 5 mm in diameter were found in the rectum and sigmoid. Biopsies from ileum and colon were obtained and specimens were sent for AFB smear, Xpert MTB/Rif assay and AFB culture. For the Xpert assay, a 2:1 volume sample reagent buffer was added to very small pieces of biopsy specimens. The tissue was crushed manually and homogenized with a shaker. It was then transferred to the Xpert test cartridge. The cartridge was inserted into the Xpert device, and the automatically generated result was read after 2 hours. *Mycobacterium tuberculosis* without rifampin resistance was detected by this method. Ziehl-Neelsen staining identified AFB and histopathology disclosed chronic ulcerative ileitis and colitis with formation of suppurative epithelioid granulomas highly suggestive

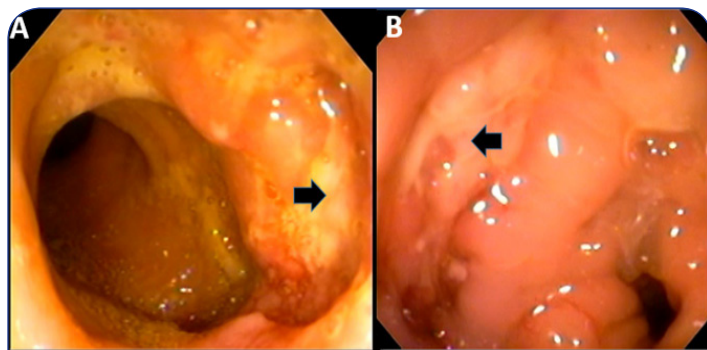


Figure 2: Intestinal mucosa findings seen on colonoscopy
A) Irregular ulcer with congestive borders of 12 mm in diameter in ileum
B) 10 mm ulcer with congestive appearance in sigmoid

of intestinal tuberculosis (Figure 3). One month later, culture from intestinal biopsy was reported as positive for *Mycobacterium tuberculosis* susceptible to isoniazid, rifampin, pyrazinamide and ethambutol but resistant to streptomycin. Sputum culture was also positive for TB with the same susceptibility pattern. Patient was

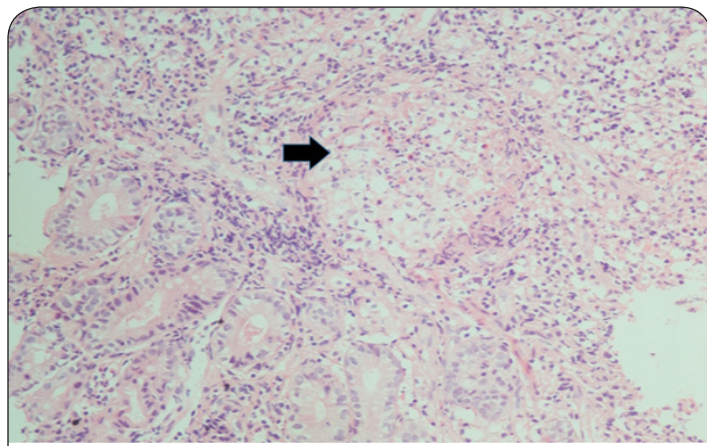


Figure 3: Histopathological examination of ileum biopsy showing a suppurative epithelioid granuloma (H&E x200)

started on standard four drug therapy with isoniazid, rifampin, pyrazinamide and ethambutol immediately after Xpert MTB/Rif result was disclosed. After 2 months ethambutol and pyrazinamide were stopped and daily isoniazid and rifampin were continued for 7 additional months. In terms of antiretroviral therapy, he continued with tenofovir disoproxil fumarate/emtricitabine but atazanavir and ritonavir were switched to raltegravir to avoid drug interaction with rifampin. The patient improved significantly over the following months with complete resolution of his symptoms at the end of anti-TB therapy.

Discussion

In subjects with extra-pulmonary tuberculosis, the rapid identification of *Mycobacterium tuberculosis* in body fluids/tissues allows prompt initiation of specific therapy. Molecular assays have emerged as promising tools for rapid and accurate diagnosis of extra-pulmonary tuberculosis. Of the many molecular techniques currently available, Xpert MTB/Rif assay is unique as it has integrated sample processing and simplified testing. It incorporates a real time polymerase chain reaction (PCR) based on the molecular beacon technology. Xpert MTB/Rif detects *M. tuberculosis* as well as Rifampicin resistance-conferring mutations directly from clinical specimens and provides results in 2 hours. Studies have shown that rifampicin resistance serves a surrogate marker for multidrug resistant tuberculosis [7]. This is very important in developing countries such as Peru, where the rates of multidrug resistant tuberculosis can reach up to 5.3% [8]. The early identification of these cases has proved to be crucial for an optimal and successful treatment regimen.

While the majority of experience with Xpert MTB/Rif has been on sputum samples, it has also been used in a variety of fluid and tissue samples. In fact, it is the only commercial nucleic acid test recommended by WHO guidelines for diagnosis of extra pulmonary tuberculosis. According to the study conducted in South India by Suzana et al.; the Xpert MTB/Rif assay has a pooled sensitivity of 89% and specificity of 74% for the diagnosis of extra pulmonary tuberculosis when compared to culture. In regards to tissue biopsy samples, the sensitivity and specificity were 85.7% and 83.3% respectively [9].

Another important study that assessed the diagnostic accuracy of the Xpert assay in non-respiratory samples was the systematic review conducted by Maynard-Smith et al. Among the 23 studies included in this review that compared Xpert against culture, the sensitivity was very heterogeneous with a median sensitivity of 0.83 (IQR, 0.68-0.94) whereas specificities were typically very high (median, 0.98; IQR, 0.98-1.00). Pooled summary estimates of sensitivity varied substantially between sample types, being the highest for lymph node tissue (96%) and tissue samples of all types (88%) and the lowest for pleural fluid (34%) [10]. These results were consistent with a recent meta-analysis published by E. Penz et al, which reported a low pooled sensitivity of Xpert in pleural fluid (37%) and high in lymph node samples (87%) [11]. These studies determined the diagnostic accuracy of Xpert in extra-pulmonary samples when compared to culture; however,

the sensitivity and specificity of this assay differs when compared against a composite reference standard (clinical features, culture, radiology and histology). In this regard Denking et al. found a pooled sensitivity of 81.2 % in lymph node tissues and 21% in pleural fluid when Xpert was compared with a composite reference standard, which are lower than the results obtained against cultures. However, the pooled specificity of this assay across different sample types remained high (>98.7%) when compared against a composite reference standard [12].

Another important fact to highlight in our case is the co-infection with HIV-1. There is paucity of data on the use of Xpert to diagnose extra-pulmonary TB in this population. One study conducted by Gerardo Alvarez-Uria et al. in India compared the performance of light-emitting diode (LED) auramine fluorescent microscopy and the Xpert assay for the diagnosis of tuberculosis in HIV infected patients. They found that Xpert outperformed LED fluorescent microscopy in all types of specimens, especially in cerebrospinal fluid where the number of positive results increased 11 times with the use of Xpert. Unfortunately, this study did not include any tissue samples [13].

Molecular methods have also been used for the diagnosis of gastrointestinal tuberculosis. A study done by B.Y. Fei et al. in China found that fluorescent quantitative PCR had a sensitivity and specificity of 55.2% and 94.4% when applied to intestinal tissue samples [14]. Other studies that have used different PCR techniques found higher diagnostic accuracy. For instance, a report published by Kusum Sharma et al. showed a sensitivity of 90% and specificity of 100% when multiplex PCR was performed in ileocecal biopsy samples [15]. The literature is very limited on the use of Xpert in gastrointestinal tuberculosis. Only one study has evaluated this assay in peritoneal tuberculosis via omental biopsies and found a very low sensitivity (19%) and a specificity of 100% [16]. To our knowledge, there are no reports on the use of Xpert MTB/Rif in intestinal biopsies. There is a clear need of research in this area.

One of the major strengths of Xpert MTB/Rif assay is its turnaround time with 2 hour results compared to a median of 1 day for smear microscopy, 2 days for other PCR techniques, 14 days for liquid culture and 24 days for solid media [17]. In our patient, this molecular assay allowed a definite diagnosis in 2 hours after obtaining intestinal biopsies which led to an immediate initiation of treatment. We believe that the rapid availability of an accurate diagnosis by this method could reduce mortality and morbidity in this form of extra-pulmonary TB. This case adds to the growing evidence of the utility of this assay for diagnosis of TB on non-respiratory samples. Further studies are needed to characterize the sensitivity and specificity of the assay for the diagnosis of gastrointestinal tuberculosis.

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