International Journal of Infectious Diseases 54 (2017) 36–38

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

Paradoxical results of two automated real-time PCR assays in the diagnosis of pleural tuberculosis

Soraya E. Morales-López [a,b,*], Jayr A. Yepes [b], Irina Anzola [a], Hernán Aponte [c], Claudia R. Llerena-Polo [d]

[a] Laboratorios Nancy Flórez García S.A.S., Valledupar, Colombia
[b] Programa de Microbiología. Universidad Popular del Cesar, Valledupar, Colombia
[c] Hospital Rosario Pumarejo de López, Valledupar, Colombia
[d] Grupo Micobacterias. Instituto Nacional de Salud, Bogotá, Colombia

ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form 9 November 2016
Accepted 11 November 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
pleural tuberculosis
Colombia
Abbott RealTime MTB
Xpert MTB/RIF

ABSTRACT

Tuberculosis (TB) is a major cause of worldwide mortality. We report the case of a non-HIV-infected woman with clinical suspicion of pleural tuberculosis and contradictory results between Xpert® MTB/RIF and Abbott RealTime MTB assays from pleural fluid specimen. Liquid and solid cultures for tuberculosis were performed with negative results. The patient received treatment, and clinical improvement was observed. Both techniques detect *Mycobacterium tuberculosis* complex, but they have different targets and limits of detection. Abbott RealTime MTB results correlated well with the clinical findings of the patient.

1. Introduction

Tuberculosis is a global public health problem. The World Health Organization recommends the use of nucleic acids amplification tests (NAAT) for diagnosis of tuberculosis in developing countries and estimated that between 2000 and 2014 the effective diagnosis and treatment saved 43 million lives.[1,2]

Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) amplifies the *rpoB* gen of *Mycobacterium tuberculosis* (MTB).[3] Abbott RealTime MTB (Abbott, Des Plaines, USA) detects both the insertion sequence 6110 and protein antigen B.[4] We report the case of a woman with clinical suspicion of pleural tuberculosis, culture negative and contradictory results of two automated NAATs.

2. Case report

A 29-year-old woman from Valledupar (Colombia), HIV negative, presented to the pneumology department with a clinical picture of one month of evolving chest pain associated with cough, fever and dyspnea. The patient was treated with prednisolone, without clinical improvement.

In the physical examination she was febrile, with tachypnea and without abnormal respiratory noises in the lower lobe of the right lung. There were no other findings of interest. A history of urinary infection one month ago, treated with ciprofloxacin and cephalexin, was referred.

There was a suspicion of right-sided pneumonia and pleural effusion. Empirical treatment with ampicillin sulbactam and doxycycline was prescribed. Chest ultrasound confirmed right pleural effusion with an estimated volume of 610 mL (Figure 1A), then thoracentesis was performed for treatment and diagnosis.

The citrine-yellow fluid extracted showed lymphocytic predominance and elevated LDH level (928 IU/mL). An aliquot was centrifuged at 3000 *g* for 20 minutes and the pellet was used for Ziehl Neelsen, Gram, Wright and Chinese Ink stains. In all cases the results were negative. Cultures in two Ogawa Kudoh solid media were conducted, and PCR for identification of MTB from pleural fluid was ordered.

The real-time PCR testing was performed according to the protocols suggested by the manufacturers. After 120 minutes, the results "MTB not detected" and "MTB detected" were generated for Xpert® MTB/RIF and Abbott RealTime MTB, respectively.

* Corresponding author. Programa de Microbiología- Universidad Popular del Cesar, Valledupar - Colombia. Laboratorios Nancy Flórez García S.A.S. Carrera 9 N° 7A-42. Tel.: +57 5 5716046 Valledupar- Colombia.
E-mail address: sorayaeugeniam@hotmail.com (S.E. Morales-López).

http://dx.doi.org/10.1016/j.ijid.2016.11.011

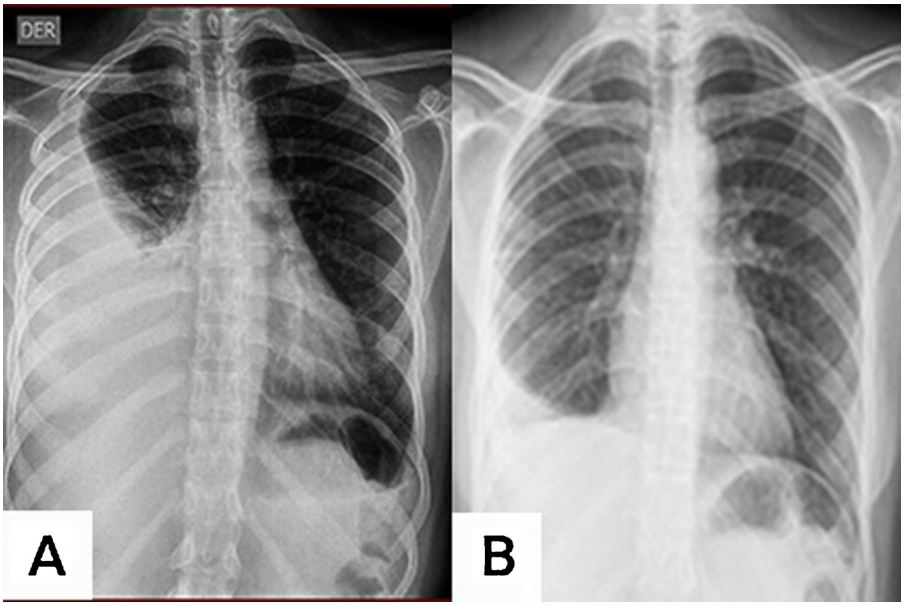

Figure 1. A. Obliteration of the costophrenic angle and Damoisseau sign with large pleural effusion. B. Reduction of pleural effusion without consolidation or cavitations.

Aliquots of pleural fluid were sent to an external laboratory and to the national TB reference laboratory for cultivation on Middlebrook 7H9 broth using BACTEC Mycobacteria Growth Indicator Tube-MGIT (Becton Dickinson, San Diego, USA). Additionally, in both laboratories the samples were inoculated in Lowenstein-Jensen medium.

A second sample was processed 48 hours later, yielding the same results. The clinician prescribed directly observed treatment (DOT).

Two months later, all cultures remained negative and clinical and radiological improvements were observed. Chest radiograph revealed right-sided pleural effusion (Figure 1B). Complete clinical remission and radiographic improvement was achieved in the fourth month of treatment.

3. Discussion

Molecular assays have been reported to be suitable for diagnosis of pleural tuberculosis. Hillemann *et al.*, in Germany, used Xpert® MTB/RIF in 113 pleural liquid samples and found a specificity of 98.1% compared to the culture.[5] In India, Alvarez-Uria *et al.*, used Xpert® MTB/RIF in 75 samples of pleural liquid and obtained twice the positive results as with microscopy of fluorescence,[6] and recently Peñata *et al.*[7] tested 48 pleural liquid samples from patients in a high-level Colombian hospital using Xpert® MTB/RIF, reporting 100% sensitivity and specificity compared with the culture.

On the contrary, WHO performed a meta-analysis of 17 studies using Xpert® MTB/RIF and cultures as the standard reference for detection of MTB from pleural fluid. Average sensitivity was low at 43.7% and specificity was around 98.1%.[8]

The detection of bacterial DNA in non-viable cells is common in samples from patients undergoing pharmacological treatment.[5,9] That can explain the cases in which NAAT was positive and culture was negative. In our case, the patient declared she had not been treated with antituberculosis drugs.

Other possible explanations for discordant results are technical errors, the reduction of bacterial load caused by the decontamination procedure, and the presence of latent TB.[9] In this case, the molecular tests were repeated by different technicians, with the same results; the sample came from a closed cavity, usually considered sterile, so it was not subjected to decontamination and the patient had signs and symptoms of TB, which eliminate the possibility of latent tuberculosis.

There are no studies that compare Abbott RealTime MTB and Xpert MTB/RIF using extrapulmonary samples. Wang *et al.* used both tests in 255 sputum specimens, and they did not find statistical differences in sensitivity and specificity between them. Discordant results between NAAT and culture were resolved using 16S–23S ITS rDNA sequencing.[10]

Sequencing is not available for routine clinical practice, which is a limitation of this work. Another limitation was the use of pleural fluid instead of pleural biopsy, which is the preferred sample for the confirmation of pleural tuberculosis.[8] However, NAAT are not intended for testing extrapulmonary samples, except for Xpert MTB/RIF in the case of cerebrospinal fluid.

Xpert® MTB/RIF and Abbott RealTime MTB provide greater sensitivity than smear microscopy and more rapid results than cultures. Both assays have been described in recent years, but to date, only Xpert® has been endorsed by WHO. The detection limit of the Xpert® MTB/RIF test is 131 CFU/mL and of the Abbott RealTime MTB is 17 CFU/mL.[3,9]

In this case it was not possible to evaluate and discard the presence of fastidious microorganisms. However, it is important to mention that various authors have determined the analytical specificity of both techniques: Tang[9] tested 80 microorganisms with Abbott RealTime MTB, including fastidious bacteria such as *Haemophilus influenzae*, *Legionella pneumophila*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Bordetella parapertussis*; viruses; nontuberculous Mycobacteria (NTM); common bacteria and yeasts; the result was "MTB Not Detected" in all cases. Chen[4] also tested 42 NTM using Abbott and all samples were determined as MTB negative. On the other hand, Lawn[3] used Xpert ® MTB/RIF to test 20 isolates of NTM and all samples were negative for MTB, showing high specificity, and Blakemore et al.[11] tested 89 organisms, including nutritionally demanding bacterias such as *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydia pneumoniae*, *Corynebacterium diptheriae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Haemophilus parahaemolyticus*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*; common bacterias; fungi and

viruses; and the Xpert assay did not mistakenly classify any of these organisms as *M. tuberculosis*.

In the present case, the detection limit difference between the NAAT could explain the discordant results, whereas the negative result in cultures could be due to the paucibacillary nature of pleural fluid and to the effect of conservation and transportation conditions on the viability of *Mycobacterium*.

Neither Abbott nor Xpert assays are recommended for pleural fluid samples according to their manufacturers. However, some authors have used them to test this kind of sample and the principal modification in the testing of liquid samples was the concentration by centrifugation before transferring them to the cartridge.[7,12]

In our institution, the techniques are not validated for pleural fluid samples, however, the concentration step by centrifugation was done as proposed by the authors mentioned.

Although other authors had already reported discrepancies between NAAT and the culture, to date this is the first report of contradictory results in extrapulmonary samples using both assays. Consequently, aside from the clinical findings, the diagnosis of pleural tuberculosis must consider the limitations of the methods for each kind of sample.

The findings reported here should be considered as a starting point for projects with a larger number of non-respiratory samples for comparing both techniques, and estimate the real advantage between the two assays.

Acknowledgments

We would like to thank to Ilda Jaimes (Laboratorio Clínica Reina Catalina, Barranquilla- Colombia) for technical help.

Source(s) of support in the form of grants, equipment, drugs, or all of these: The tests reported were carried out by the following institutions: Laboratorio Clinica Reina Catalina, (Barranquilla, Colombia), Laboratorios Nancy Flórez García, SAS, (Valledupar, Colombia) and the Instituto Nacional de Salud -INS (Bogotá, Colombia). No additional financial aid was received.

Conflict of interest: None of the authors report conflict of interest.

Ethics statement: Written informed consent was obtained from the patient for the publication of this case report. A copy of the informed consent has been included.

Previous publication/presentations mentioned: There are no studies that compare Abbott RealTime MTB and Xpert MTB/RIF using extrapulmonary samples. Therefore, this is the first report. Previously, this year, Wang used both tests but with sputum specimens.

References

1. Policy Statement: Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System [Internet]. Geneva: World Health Organization. 2011 - [cited 2016 Sep 18]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK304235/pdf/Bookshelf_NBK304235.pdf.
2. Global tuberculosis report 2015 [Internet]. Geneva: World Health Organization. 2015 - [cited 2016 Sep 18]. Available from: http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf.
3. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol* 2011;**6**:1067–82.
4. Chen JH, She KK, Kwong TC, Wong OY, Siu GK, Leung CC, et al. Performance of the new automated Abbott RealTime MTB assay for rapid detection of *Mycobacterium tuberculosis* complex in respiratory specimens. *Eur J Clin Microbiol Infect Dis* 2015;**34**:1827–32.
5. Hillemann D, Ru S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF System. *J Clin Microbiol* 2011;**49**:1202–5.
6. Alvarez-Uria G, Azcona JM, Midde M, Naik PK, Reddy S, Reddy R. Rapid Diagnosis of Pulmonary and Extrapulmonary Tuberculosis in HIV-Infected Patients. Comparison of LED Fluorescent Microscopy and the GeneXpert MTB/RIF Assay in a District Hospital in India. *Tuberc Res Treat* 2012;**2012**:1–4.
7. Peñata A, Salazar R, Castaño T, Bustamante J, Ospina S. Diagnóstico molecular de tuberculosis extrapulmonar y sensibilidad a rifampicina con un método automatizado en tiempo real. *Biomédica* 2016;**36**:78–89.
8. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF Assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children: Policy update [Internet]. Geneva: World Health Organization. 2013 - [cited 2016 Sep 18]. Available from: http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf.
9. Tang N, Frank A, Pahalawatta V, Lampinen J, Coblenz-Korte A, Dunn C, et al. Analytical and clinical performance of Abbott RealTime MTB, an assay for detection of *Mycobacterium tuberculosis* in pulmonary specimens. *Tuberculosis* 2015;**95**:613–9.
10. Wang SF, Ou XC, Li Q, Zheng HW, Wang YF, Zhao YL. The Abbott RealTime MTB assay and the Cepheid GeneXpert assay show comparable performance for the detection of *Mycobacterium tuberculosis* in sputum specimens. *Int J Infect Dis* 2016;**45**:78–80.
11. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, et al. Evaluation of the analytical Performance of the Xpert MTB/RIF Assay. *J Clin Microbiol* 2010;**48**:2495–501.
12. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a New Pillar in Diagnosis of Extrapulmonary Tuberculosis? *J Clin Microbiol* 2011;**49**:2540–5.