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Case Reports

The limitations of polymerase chain reaction in the setting of possible recurrent tuberculosis: 2 instructional cases

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Abstract

The interpretation of a positive result for *Mycobacterium tuberculosis* by nucleic acid amplification such as polymerase chain reaction (PCR) can be challenging. We present 2 cases that illustrate the limitations of tuberculosis PCR on respiratory secretions in previously treated patients, even years after the previous disease episode.

Keywords: Tuberculosis; Nucleic Acid Testing; Polymerase Chain Reaction; False positive

1. Illustrative cases

1.1. Case 1

The patient was a 62-year-old indigenous woman with a history of chronic rheumatoid arthritis treated with weekly methotrexate, who smoked 20 to 30 cigarettes per day.

She had previously been treated for pulmonary tuberculosis (TB) on 2 occasions. The first episode was in the 1960s, at which time treatment included 5 months of injections followed by oral medication, and resulted in a good clinical response. (Treatment details are incomplete for this episode.) The second episode was 2 years previously. She was treated for fully sensitive pulmonary TB with rifampicin (R), isoniazid (H), pyrazinamide (Z), and ethambutol (E) for 2 months, followed by HR for a further 7 months. Therapy was successfully completed 14 months before the current episode.

On this occasion, she presented with a 4-day history of fever, cough, dyspnea, and left-sided chest pain. A chest radiograph showed left mid and upper zone opacification.

Initial routine cultures, including mycobacterial and fungal culture of blood and sputum, were negative. Acid-fast bacilli (AFB) smears of 2 sputum specimens and urinary pneumococcal and *Legionella* antigen test results were also negative. She remained febrile with increasing opacification on chest radiograph and underwent a bronchoscopy. Bronchial washings were AFB smear and culture negative but positive by TB polymerase chain reaction (PCR). Three subsequent sputum specimens were also PCR positive but AFB smear and culture negative (Table 1). All other microbiologic studies, including PCR for respiratory viruses and *Pneumocystis jiroveci* pneumonia, were negative.

Chest computed tomography (CT) showed 3 areas of peripheral nodular consolidation, one of which showed cavitation and several showed calcified mediastinal nodes and right upper lobe bronchiectasis. A CT-guided biopsy of the pleurally based lesion revealed nonspecific mild interstitial inflammation and fibrosis, with no granulomas or necrosis.

The patient had good clinical improvement with 5 days of conventional antibiotics. She was reviewed 5 weeks after discharge and was well, and her chest radiograph showed almost complete resolution of the previously noted acute changes.

1.2. Case 2

The second patient was an 89-year-old lady living independently and in good health. Her medical history included diabetes, peripheral vascular disease, myocardial

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Table 1
Summary of investigations for TB during admission in case 1

Day	Sample	Smear Result	PCR result[a]	Culture result
2	Sputum	No AFB		Negative
3	Sputum	No AFB		Negative
3	Blood cultures: 2 sets			Negative
4	Sputum	No AFB		Negative
5	Bronchoscopy	No AFB	Positive	Negative
12	Sputum	No AFB	Positive	Negative
13	Sputum	No AFB	Positive	Negative
14	Sputum	No AFB	Positive	Negative
17	Lung tissue	No AFB	Insufficient sample	Negative

[a] PCR using TaqMan with IS6110 target (Globan and Fyfe, 2010).

infarction, stroke, and pulmonary hypertension. She previously had breast cancer and a retinal vein thrombosis.

Twenty-eight months before the current presentation, she had been diagnosed with fully drug-susceptible, smear-positive pulmonary TB. She was treated with 2 months of HRZ followed by 4 months of HR and made a full clinical recovery.

Nine months later, she presented with a productive cough. Sputum was AFB smear positive and TB PCR positive, but AFB cultures were negative. A CT scan showed a right lower lobe lung mass with surrounding nodules. Despite the negative sputum cultures, she was retreated for TB with 6 months of HRZE (the pyrazinamide was ceased after 2 months). The right lung lesion was unchanged on a repeat CT scan after completion of therapy. A positron emission tomography scan did not show any increased uptake in the corresponding area of the lung.

Seven months later, she presented with an acute upper respiratory illness on the background of ongoing chronic cough. Three sputum specimens were smear positive for AFB, 2 of which were also TB PCR positive (Table 2). Chest CT scan was unchanged from 13 and 7 months previously. A repeat sputum sample was smear and PCR negative for TB.

Table 2
Summary of investigations for TB in case 2

Date	Sample	Smear result	PCR result	TB culture result
7/9/2006	Sputum	No AFB	Positive	Negative
8/9/2006	Sputum	Positive		Positive
6/12/2007	Sputum	No AFB	Positive	Negative
6/12/2007	Sputum	Positive		Negative
6/12/2007	Sputum	No AFB		Negative
15/12/2007	Sputum		Positive	
17/12/2007	Sputum	Positive		Negative
18/12/2007	Sputum	Positive		Negative
29/12/2008	Sputum	Positive	Negative	Negative
30/12/2008	Sputum	Positive	Positive	Negative
31/12/2008	Sputum	Positive	Positive	Negative
13/1/2009	Sputum	No AFB	Negative	Negative
3/2/2009	Sputum	No AFB		Negative

Although she had already recovered from her respiratory illness, she was commenced on 4-drug antituberculous therapy with HRZ and moxifloxacin. At 2 months of review, all 3 sputum cultures were negative, and so all antituberculous therapy was discontinued. A further 5 months later, she remains well and continues to live independently at home.

2. Discussion

TB diagnosis has traditionally been made on the basis of clinical presentation, radiograph findings, microscopy, and culture. Nucleic acid amplification tests (NAAT) are now used widely as an additional diagnostic test. Recently, the US Centers for Disease Control and Prevention (2009) has recommended that PCR testing is performed on at least 1 respiratory specimen from each patient for whom TB is being considered.

TB PCR has greater sensitivity and specificity than microscopy but is not as sensitive as culture for the detection of pulmonary TB (Catanzaro et al., 2000; Wobeser et al., 1996). In a meta-analysis of various studies, sensitivity and specificity of TB PCR in smear-negative pulmonary disease are 66% and 98%, respectively, and in smear-positive pulmonary disease, sensitivity is 96% and specificity only 85% (Greco et al., 2006).

Organism detection by PCR requires the presence of preserved genetic material, but a positive result does not necessarily indicate that the organism is viable. In patients with untreated disease, a positive PCR can generally be assumed to indicate that viable *Mycobacterium tuberculosis* is present. After treatment is started, PCR may remain positive in the absence of viable bacteria, as judged by conversion of cultures to negative (Yuen et al., 1993). The length of time that the PCR remains positive is not clearly defined, and the role of PCR in patients being investigated for possible TB recurrence is poorly understood.

These 2 patients had both been treated for TB on 2 previous occasions. They then presented with respiratory illnesses, compatible in case 1 with acute community-acquired bacterial pneumonia and in case 2 with an upper respiratory tract infection. In both cases, the subsequent clinical courses were in keeping with these diagnoses, although no laboratory confirmation was achieved. The dilemma that arose on both occasions was how to interpret a repeatedly positive sputum TB PCR results when there was a clear discordance between this finding and the patient's clinical presentation. This dilemma was heightened in case 2, who was also sputum AFB smear positive. In both cases, the final decision was to accept that these PCR results are due to nonviable bacilli. The TB PCR used (Globan and Fyfe, 2010) is specific for the *M. tuberculosis* complex of organisms; however, because *M. tuberculosis* had been cultured during prior episodes and because no alternative species was ever cultured, it would be reasonable to assume this was the organism detected by PCR.