Xpert MTB/RIF False Detection of Rifampin-Resistant Tuberculosis from Prior Infection

To the Editor:

A 71-year-old man with two prior episodes of pulmonary tuberculosis (TB) in China (2008–2009) and San Francisco, California (2009–2010) presented in July 2013 with progressive shortness of breath. The patient immigrated to San Francisco in November 2008, at which time he was noted to have right upper lobe scarring with cavity formation (Figures 1A and 1B); three sputa were smear-negative and culture-negative for acid-fast bacilli (AFB). In July 2009, the man developed right upper lobe (RUL) consolidation in the area of the previously noted cavitary lesion, as well as pleural effusion (Figure 1C). Pan-susceptible *Mycobacterium tuberculosis* complex (MTBC) was isolated from both sputum and pleural biopsy. The patient completed standard short-course TB treatment in March 2010, when follow-up chest radiograph demonstrated interval decrease of the 3 × 2 cm RUL opacity; three sputa were again smear-negative and culture-negative. Persistent RUL opacity was noted at 12-month post-treatment follow-up in 2011, although sputum cultures remained negative. The patient returned intermittently to China and declined 18-month follow-up.

In July 2013, the patient presented to our emergency department with 2 days of progressive shortness of breath without fevers, night sweats, cough, or weight loss. Computed tomography showed bilateral pleural effusions and persistent RUL opacification (Figures 1D and 1E). On hospital day 2, a sputum specimen sent for concentrated smear demonstrated few AFB. Xpert MTB/RIF (Cepheid, Sunnyvale, CA) testing of this specimen showed MTBC at very low quantity (mean cycle threshold, 30.6), with rifampin resistance detected within the region of probe A (*see* Figure E1 in the online supplement). On hospital day 3, rifampin, isoniazid, pyrazinamide, ethambutol, moxifloxacin, capreomycin, and linezolid were initiated pending confirmatory testing. Complete blood count was within normal limits, hemoglobin A1c level (15.7 mg/dL) indicated poorly controlled diabetes mellitus, HIV testing was negative, and blood cultures demonstrated no growth.

The concentrated sputum specimen tested by Xpert was sent to the Microbial Diseases Laboratory of the California Department of Public Health for pyrosequencing (1). The molecular target of *IS6110* for MTBC identification and six loci, including *rpoB* for drug resistance detection, yielded no sequences, implying insufficient DNA or no MTBC present. Repeat sputum smears were AFB-negative. Diagnostic thoracentesis demonstrated transudative pleural fluid and no AFB on smear examination. On hospital day 8, the specimen tested by Xpert became culture-positive, with morphology consistent with non-TB mycobacteria (NTM). Repeat pyrosequencing and Xpert testing from the broth culture (BacT/ALERT 3D system; bioMerieux Inc., Durham, NC) were
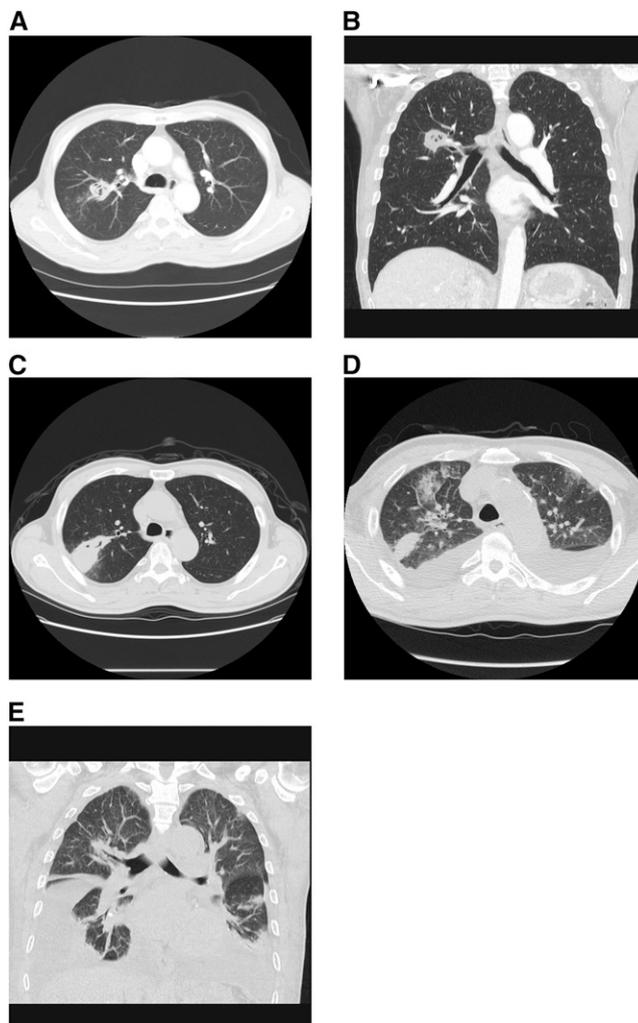
Figure 1. Computed tomography of the lungs. Computed tomographic scans of the lungs without administration of contrast at time of immigration to the United States (*A* and *B*), second diagnosis of pulmonary tuberculosis in 2011 (*C*), and re-presentation in 2013 (current case report; *D* and *E*).

negative. The culture tested negative for MTBC twice, using AccuProbe (Hologic, Inc., San Diego, CA); was plated to Remel Middlebrook 7H11 Agar, where it demonstrated growth after 3 days; and was identified as a member of the *Mycobacterium fortuitum-chelonae* group. Three additional sputum cultures showed no growth of any organism. These data, along with low clinical suspicion for TB, prompted discontinuation of all anti-TB therapy on hospital day 14, and the patient was discharged. At 12-month follow-up, the patient remained asymptomatic without radiographic progression or clinical evidence of active TB, and three additional sputum specimens were smear-negative and culture-negative.

Our patient's short interval of symptoms and evidence of volume overload strongly suggested a non-TB etiology of respiratory distress. In addition, reinfection with multidrug-resistant TB (MDR-TB) or acquisition of MDR-TB during treatment of pan-susceptible disease in California is rare (∼0.05%)

(2). Nevertheless, population risk factors (geographic origin [3], recent travel, diabetes [4], and tobacco smoke [4, 5]), prior TB, and abnormal radiographic findings prompted his care providers to "rule out" TB and to initiate treatment in response to the positive Xpert result.

Ultimately, although active disease from acquired rifampin-resistance or reinfection by a rifampin-resistant strain were considered, the growth of NTM, the failure to detect MTBC by other molecular methods, and the atypical clinical presentation raised questions about the reliability of the Xpert results. Potential explanations included test error, mixed infection of MTB and NTM, or nonviable MTB from prior active disease. Xpert test errors in rifampin-resistance determination have been documented, in particular in the setting of low pretest probability (6, 7). The Xpert graphical read-out showing four of five probes with threshold-crossing cycle values and complete signal "drop-off" for probe A (indicating an unambiguous mutation present within the probe A detectable range [codons 507–512]) made test error less likely. With regard to the possibility of mixed infection, neither *M. fortuitum* nor *M. chelonae* has homology with MTBC within the core region of *rpoB*, and thus they do not result in false-positive Xpert results (6). Inability to determine *M. tuberculosis* viability is a well-known and important limitation of current-generation molecular tests (8). In a recent study, 27% of patients remained Xpert-positive after 26 weeks of therapy for pan-susceptible disease (9), and Xpert false-positivity has been noted more than 5 years after completion of prior TB treatment (10). Although Xpert is strongly recommended as an initial diagnostic test in the setting of suspected drug resistance (11), it remains unclear how previous TB episodes impact such testing.

Given the uncertainty regarding the relative sensitivity of Xpert versus pyrosequencing in a specimen containing a low quantity of AFB, we decided to sequence the *rpoB* core region from the initial 2009 isolate. Remarkably, the 511CCG mutation (within the rpoB region of probe A) was detected in the 2009 isolate. Strains harboring the 511CCG mutation are often phenotypically susceptible to rifampin (12). In the absence of clinical indication of recurrent TB, these findings strongly suggested shedding of genetic material from nonviable organisms related to the prior TB episode. Hypothetically, such longstanding shedding could relate to a reduced ability to clear MTBC organisms because of architectural distortion of the airways and/or diabetes-associated immunocompromise. Notably, inability to grow MTBC from culture in this case does not unequivocally prove shedding of nonviable MTBC, as overgrowth of NTM is known to hinder recovery of MTBC in culture. Nevertheless, at 12-month follow-up, the patient remains asymptomatic and without radiographic progression of disease, making active TB unlikely.

In conclusion, as use of automated nucleic acid amplification tests increase within TB control programs and hospitals in the United States, such tests will likely play an increasingly important role in the exclusion of TB among inpatients (13). Our case emphasizes the importance of clinical judgment and sequence-based molecular assays in evaluating seemingly contradictory molecular and phenotypic drug susceptibility test results (14). Limited resource settings, where confirmatory testing is typically unavailable, must balance the risk of potentially unnecessary treatment with patient and community morbidity and mortality, with the understanding that cases such as ours remain rare and case-reportable events (11). ∎

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Effect of Parapharyngeal Fat on Dynamic Obstruction of the Upper Airway in Patients with Obstructive Sleep Apnea

To the Editor:

The pathophysiology of obstructive sleep apnea syndrome (OSAS) seems to result from a combination of predisposing anatomical and neuromuscular factors, but the exact mechanisms have not been fully elucidated. Obesity can contribute to OSAS in various ways, including mechanical compression of the upper airway by extrinsic fat accumulation in the neck or intrinsic fat infiltration into the upper airway (1). A distinctive fat tissue called the parapharyngeal fat pad exists in the upper lateral pharyngeal airway. Several studies have reported that collapsibility of the upper airway is related to localized fat tissue in the lateral pharyngeal wall (2–4). However, previous studies were based on static examination, which only reflects a single moment in time. Upper airway obstruction in patients with OSAS is a dynamic process, and therefore, a study on upper airway obstruction is effective when based on tests that show the entire course of the obstruction, such as drug-induced sleep endoscopy (DISE). We hypothesized that the volume of the parapharyngeal fat pad would have an effect on dynamic obstruction of the upper airway in patients with OSAS.

This study was conducted on 33 consecutive adult patients with OSAS diagnosed through overnight polysomnography from March to October 2013. All patients received a full otolaryngologic evaluation and facial computed tomographic (CT) scan. Tonsillar grade and modified Mallampati score were evaluated according to Friedman's classification. On the basis of the lateral scout view of CT, several cephalometric indexes were obtained, including PNS-P (distance from posterior nasal spine to tip of the soft palate) MP-H (distance from hyoid to mandibular plane), and SNB (angle between SN and NB [S, midpoint of fossa hypophysialis; N, anterior point at frontonasal suture; B, deepest anterior point in concavity of anterior mandible]) (5). Lateral pharyngeal wall thickness (distance between airway and medial margin of the parapharyngeal fat pad) were measured at the level of smallest retropalatal area in axial CT scan (6, 7). OSA severity was evaluated with the apnea-hypopnea index and classified into mild, moderate, and severe according to the 2007 clinical guidelines of the American Academy of Sleep Medicine. The study was approved by an institutional review board of Samsung Medical Center.

DISE was performed as previously described at different times with CT scans (8). Briefly, after placement of a 3.4-mm fiber rhinolaryngoscope in the wider side of the nasal cavity with topical anesthesia, sleep was induced using propofol (target-controlled infusion system), with bispectral index (BIS) monitoring by an anesthesiologist in the operating room. Video recording was performed simultaneously to reduce observer error, and an evaluation of the upper airway followed (BIS level < 70 for 30 s). First, the pattern (anteroposterior, lateral, and concentric) and degree (I, 0–25%; II, 25–30%; III, 50–75%; IV, 75–100%) of retropalatal obstruction were evaluated, followed by an evaluation of retroglossal obstruction.

The volumes of the parapharyngeal fat pad, the retropalatal airway and the retroglossal airway, and the smallest area in the retropalatal airway were measured using facial CT and a validated imaging software AMIRA 5.4 (Mercury Computer Systems/3D Viz group, San Diego, CA). Fat tissue was defined by voxels ranging from -200 to -50 Hounsfield units, whereas the Hounsfield unit value of the airway ranged from $-1{,}024$ to -201 (9, 10). Superior and inferior boundaries for measuring airway volume were defined as the hard palate (upmost axial CT slice, which does not include inferior turbinate) and epiglottis (lowermost slice, which does not include epiglottis), respectively. At the uvular base, the airway was divided into retropalatal and retroglossal compartments (Figure 1B).

The Kruskal-Wallis test with Tukey test using ranks was used to evaluate the association between the volume of parapharyngeal fat pad and obstruction type and grade on DISE. All data were reported as the mean $\pm$ SD, and statistical significance was determined as $P < 0.05$.

The mean age, body mass index (BMI), and apnea–hypopnea index were 42.2 ± 11.6 years, 25.7 ± 3.2 kg/m^2, and 30.4 ± 17.6 events/hours, respectively. The mean tonsillar grade was 1.7 ± 0.7; modified Mallampati score, 2.7 ± 0.9; MP-H, 17.27 ± 4.55 cm; PNS-P, 40.94 ± 3.55 cm; SNB, 82.55 ± 4.98 degree; and lateral pharyngeal wall thickness, 2.96 ± 0.68 cm. Regarding the severity of OSAS, the number of patients diagnosed with mild, moderate, and severe OSAS was 9, 8, and 16, respectively. The mean volume of the retropalatal and retroglossal airways was 5.62 ± 2.00 cm^3 and 4.78 ± 2.52 cm^3, respectively. The mean smallest area of retropalatal airway was 0.78 ± 0.34 cm^2.

Interestingly, in most cases, the parapharyngeal fat pad was not found in any level under the uvular base, and there was no case showing the fat pad beneath the level of the uvular tip (Figure 1C). The mean volume of parapharyngeal fat pad was 4.81 ± 1.95 cm^3.