



What's Lurking Out There? WSLH Case Studies

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Case Study: Patient History and Clinical Presentation

- 61 year old male
- History of mild COPD
- Chronic hepatitis C and underlying cirrhosis
- Abnormal CT scan of chest: “bilateral tree-in-bud opacities”
- Clinician is thinking:
 - Indolent infection with non-tuberculous mycobacteria or fungus
 - Sequelae of dust/soil inhalation due to occupation (pouring and setting concrete)



Case Study: Patient History and Clinical Presentation

- Although the patient is asymptomatic, (due to abnormal CT scan), bronchoscopy ordered and BAL collected
- Few AFB seen (smear microscopy)
- TB symptoms and risk factors:
 - Patient denies fever, chills, anorexia, weight loss or night sweats
 - Patient denies TB exposures (girlfriend had latent TB)
 - Skin test/IGRA results unknown



WSLH PCR Testing

- For detection of *M. tuberculosis* complex (TB) and *M. avium* complex (MAC) directly from patient specimens
- Automatically performed on smear positive respiratory and non-respiratory specimens
- Testing takes less than two hours
- Testing is fee-exempt for patients suspected to have active TB



Case Study

- TB PCR result: Positive (low level)
- MAC PCR result: Negative
- TB PCR result confirmed by repeat testing
- “Despite the 2 positive test results, I have great difficulty believing the patient actually has tuberculosis, given the profound paucity of his symptoms and a CT scan which certainly is not strongly suggestive of this possibility”



Possibilities??

- False-positive result
 - Equipment contamination
 - Specimen mix-up
 - Cross contamination during specimen processing
 - False-positive TB PCR result
- Unexpected: “real” TB



How rare are false-positive laboratory results?

- Burman and Reeves. Clin. Infect. Dis. 2000. 31: 1390.
 - Reviewed articles and abstracts from Medline, ATS, IUATLD from 1966 to 1999, selected 14 studies evaluating >100 patients
 - False positive culture results are not rare
 - Median false positive rate= 3.1% (range 2.2-10.5%)
 - Clerical errors found to be as common as lab contamination
 - 67% of patients with false positive cultures were treated for TB



How to proceed?

- Patient started on therapy and placed in respiratory isolation
- More (sputum) specimens collected
- Diagnostic laboratory reviews procedures
- Await culture growth (culture confirmation of MTBC)
 - Refer to national center for TB genotyping



TB Genotyping

- Performed at the Michigan Public Health Laboratory and CDC
- Molecular method for distinguishing different strains of *M. tuberculosis* complex
- Used for epidemiology purposes:
 - Discover unsuspected transmission
 - Identify TB outbreaks
 - **Detect laboratory cross-contamination event**
- Genotyping performed for all culture-positive TB patients in Wisconsin



TB Genotyping

- “Spoligotyping”: detects presence or absence of 43 spacer oligo sequences in the direct repeat region of TB DNA
- MIRU-VNTR: calculates that number of repeats at 24 tandem repeat loci of the TB DNA

Spoligotype	MIRU1	MIRU2
777777477760771	2x3226133321	242534233525



TB Genotype Possibilities

- Possible sources of MTBC in laboratory contamination:
 - Contamination with QC strain of *M. tuberculosis* complex (H37Rv)
 - Contamination with another patient specimen that contained MTBC organisms (handled in the laboratory during the same time period)



TB Genotype Results

Isolate	Spoligotype	MIRU1	MIRU2
QC Strain H37Rv	777777477760771	2x3226133321	242534233525
Patient Isolate	777760077760771	124326153224	323124123226



TB Genotype Results

Isolate	Spoligotype	MIRU1	MIRU2
Patient Isolate	777760077760771	124326153224	323124123226

Year	Number of Wisconsin TB Patients with exact genotype match
1997-1999	4
2000-2002	6
2003-2005	1
2006-2008	2
2009-2011	6
2012-2014	2 (most recent in 2014)



Case Study: Follow-up Specimens

Collection Date	Smear Result	Culture Result
3/2/16	1+ smear positive	<i>M. tuberculosis</i> complex (MTBC)
3/3/16	2+ smear positive	MTBC
3/4/16	1+ smear positive	MTBC
3/22/16	2+ smear positive	MTBC
3/23/16	1+ smear positive	MTBC
3/24/16	2+ smear positive	MTBC
4/9/16	2+ smear positive	pending
4/10/16	2+ smear positive	pending
4/11/16	1+ smear positive	pending



Result Summary

- Genotype results indicated that the isolation of MTBC from this patient was likely not due to a false positive result or cross contamination event:
 - Not a laboratory QC strain
 - Not a recently-isolated strain or a common strain (last seen in 2014)
- MTBC was isolated from culture of subsequent respiratory specimens, confirming the laboratory diagnosis of tuberculosis.



Summary

- Clinicians in areas with low incidence of tuberculosis don't always include tuberculosis in their differential diagnosis
- The patient in this case study is now being treated for active tuberculosis disease. He will be in respiratory isolation (at home) until:
 - Two weeks of adequate therapy have been administered and there is evidence of clinical improvement
 - Three consecutive smear negative respiratory specimens are obtained
 - Arrangements for post-isolation care have been made



Summary

- WSLH has access to molecular methods for rapid detection of MTBC and genotyping for epidemiology purposes
- Laboratory contamination events occur and are not as rare as you might think
 - Serious implications for patient management
 - Correlate laboratory results with clinical picture
- If any doubts about laboratory results:
 - Troubleshoot
 - Collect more specimens
 - Open communication with health care provider and health departments is necessary
 - Call WSLH for assistance with genotyping



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Comments or Questions??