When good genes go bad

Dr Kessendri Reddy
NHLS Tygerberg Hospital
Division of Clinical Microbiology

Fakulteit Geneeskunde en Gesondheidswetenskappe
Faculty of Medicine and Health Sciences
Overview

• Cases

• Tests

• Discussion

Disclaimer: cases are part of a TB diagnostic study in children
Case 1

4y ♀
- HIV negative
- No known TB contact
- 3w history of L supraclavicular LAD
- 1w history of cough, night sweats

<table>
<thead>
<tr>
<th>FNA Sample</th>
<th>Smear</th>
<th>GXP</th>
<th>LPA – direct</th>
<th>LPA – culture</th>
<th>Phenotypic DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>-</td>
<td>MTB low +</td>
<td>Rif R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GXP</td>
<td></td>
<td>MTB +</td>
<td>Rif R</td>
<td>INH S</td>
<td></td>
</tr>
<tr>
<td>LPA – direct</td>
<td></td>
<td>MTB +</td>
<td>Rif R</td>
<td>INH S</td>
<td></td>
</tr>
<tr>
<td>LPA – culture</td>
<td></td>
<td>MTB +</td>
<td>Rif R</td>
<td>INH S</td>
<td></td>
</tr>
<tr>
<td>Phenotypic DST</td>
<td></td>
<td>Rif S</td>
<td>INH S</td>
<td>Oflox S</td>
<td>Amik S</td>
</tr>
</tbody>
</table>
Case 2

9m ♀

- HIV exposed, PCR negative
- TB contact +
- Short history of: FTT, cough
- CXR: features suggestive of LRTI

<table>
<thead>
<tr>
<th></th>
<th>Gastric aspirate D1</th>
<th>Sputum D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>+ 8 AFBs/100f</td>
<td>-</td>
</tr>
<tr>
<td>GXP</td>
<td>MTB low +</td>
<td>Rif S</td>
</tr>
<tr>
<td></td>
<td>MTB very low +</td>
<td>Rif R</td>
</tr>
<tr>
<td>LPA – culture</td>
<td>MTB +</td>
<td>Rif S</td>
</tr>
<tr>
<td></td>
<td>INH S</td>
<td>MTB +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rif S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INH S</td>
</tr>
<tr>
<td>Phenotypic DST</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>
Case 3

4y ♂

- HIV+, CD4 2%
- Clinical diagnosis of DS-TB in 2011, started RHZE
- Re-admitted 4mo later with pneumonia, sputum taken
- Re-admitted 6mo later
  - Adherence problems: VL unchanged
  - Clinical deterioration: Hb 5.0g/dl, new cavitations on CXR
- MDR-TB treatment started empirically

<table>
<thead>
<tr>
<th></th>
<th>Sputum at 4mo</th>
<th>Gastric aspirate at 10mo</th>
<th>Induced sputum at 10mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GXP</td>
<td>Not done</td>
<td>MTB low +</td>
<td>MTB med +</td>
</tr>
<tr>
<td>LPA – culture</td>
<td>MTB+ Rif S INH S</td>
<td>MTB + Rif S INH S</td>
<td>MTB + Rif S INH S</td>
</tr>
<tr>
<td>Phenotypic DST</td>
<td>Not done</td>
<td>Not done (lost viability)</td>
<td>Not done</td>
</tr>
</tbody>
</table>
NHLS Diagnostic Algorithm

NHLS laboratory testing algorithm

SA NTBCP: ALL TB SUSPECTS

Xpert MTB/RIF

MTB present

susceptible to RIF

2nd specimen: TB microscopy only

RIFAFOUR 6 months; follow up patient

Xpert: resistant to RIF

2nd specimen: TB culture & RIF resistance confirmation by LPA

LPA: resistant to RIF

Phenotypic drug susceptibility testing (DST) to 2nd line drugs (and INH)

MTB absent

2nd specimen: TB culture & DST (LPA)

LPA: INH and RIF result

If RIF resistant: phenotypic drug susceptibility testing (DST) to 2nd line drugs

Slide courtesy of Dr Natalie Beylis, NHLS GSH
Rifampicin

• Mechanism of action

• Resistance
Determining rifampicin resistance

Approx 92% of mutations conferring rifampicin resistance

Somasundaram, S et al, J of TB Research 2013
Before we proceed...

- Phenotypic testing
  - Solid: Löwenstein-Jensen medium, Middlebrook 7H10 agar
  - Liquid: Bactec MGIT 960

- Critical concentration

- Low-level and borderline rifampicin resistance
Real time PCR for M. tuberculosis (GeneXpert):
PCR result (raw) Mycobacterium tuberculosis complex detected
PCR result Mycobacterium tuberculosis complex detected
Quantitative result Low
Rifampicin Resistant
Comment

This patient has presumptive MDR-TB. Please refer URGENTLY to an appropriate treatment facility. Send a 2nd sample for microscopy, TB culture and further susceptibility testing for confirmation.

Cartridge Lot Number 18111
Cartridge Serial Number 235025043
Module Serial Number 600784
Instrument Serial Number 703771
Probe A Ct value 22.2
Probe B Ct value 23.4
Probe C Ct value 22.9
Probe D Ct value 23.7
Probe E Ct value
SPC Ct value
GeneXpert

- Rifampicin resistance on GXP

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>95.1</td>
<td>98.4</td>
</tr>
</tbody>
</table>

(WHO 2011)

- Some limitations
  - Detects 80% of the population
  - Silent mutations
  - Mutations outside RRDR
  - Can’t detect specific mutations
Line Probe Assay
• hybridization failures with wild-type probe(s) in the absence of hybridization with mutant probes should be investigated by direct sequencing before assigning resistance (Alonso, M et al, JCM 2011)
Line Probe Assay

- Rifampicin resistance on LPA

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled</td>
<td>98.1</td>
<td>98.7</td>
</tr>
</tbody>
</table>

(Ling, D Eur Resp J 2008)

- Some limitations:
  - Detects 60% of the population
  - Silent mutations
  - Leu533Pro strain – false rifampicin susceptible
  - Can’t specify all mutations
Liquid culture

- Contamination
- Rifampicin resistance cut-off 1μg/ml
- MGIT-DST at a lower critical concentration did not improve the results except for a few mutations (Rigouts, L et al, JCM 2013)
- Consistently missed isolates with mutations:
  - Leu511Pro, Leu533Pro, Asp516Tyr, Ile572Phe
Possible reasons for discordant results

• Pre-analytical
  • Sampling variability
  • Patient mix-up, specimen mix-up

• Analytical
  • Lab error, lab contamination
  • GXP: false rifampicin resistance, silent mutation, heteroresistant population, mixed population
  • LPA: observer error, silent mutation, heteroresistant population, mixed population
  • Phenotypic liquid DST: contamination, technical errors, low-level resistance

• Post-analytical
  • Results incorrectly linked/entered
Case 1

4y ♀
- HIV negative
- no known TB contact
- 3w history of L supraclavicular LAD
- 1w history of cough, night sweats

DNA sequencing:
Leu511Pro

<table>
<thead>
<tr>
<th>FNA Sample</th>
<th>Smear</th>
<th>GXP</th>
<th>LPA – direct</th>
<th>LPA – culture</th>
<th>Phenotypic DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>-</td>
<td>MTB low +</td>
<td>Rif R</td>
<td>Rif R</td>
<td>Rif S</td>
</tr>
<tr>
<td>GXP</td>
<td></td>
<td>MTB +</td>
<td>Rif R</td>
<td>INH S</td>
<td>INH S</td>
</tr>
<tr>
<td>LPA – direct</td>
<td></td>
<td>MTB +</td>
<td>Rif R</td>
<td>INH S</td>
<td>Oflox S</td>
</tr>
<tr>
<td>LPA – culture</td>
<td></td>
<td>MTB +</td>
<td>Rif R</td>
<td>INH S</td>
<td>Amik S</td>
</tr>
<tr>
<td>Phenotypic DST</td>
<td></td>
<td>Rif S</td>
<td>INH S</td>
<td>Oflox S</td>
<td>Amik S</td>
</tr>
</tbody>
</table>
Case 2

9m ♀
- HIV exposed, PCR negative
- TB contact +
- Short history of: FTT, cough
- CXR: features suggestive of LRTI

**DNA sequencing:**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sputum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR result (raw data)</td>
<td>Detected: Very low</td>
</tr>
<tr>
<td>PCR result</td>
<td>Mycobacterium tuberculosis complex detected</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

*Rifampicin resistant. Please submit another sample for TB microscopy, culture and confirmation of drug resistance. If a second sample has already been submitted, TB microscopy, culture and drug sensitivity testing results to follow.*

**Cartridge lot #** 16912  
**Module serial #** 638903  
**Instrument serial #** 703771  
**Probe A Ct value** 33.7 (0.0)  
**Probe B Ct value** 31.7 (0.0)  
**Probe C Ct value** 32.6 (0.0)  
**Probe D Ct value** 33.9 (0.0)  
**Probe E Ct value** 36.4 (0.0)  
**SFC Ct value** 25.1 (27.1)
Case 3

4y ♂
- HIV+, CD4 2%
- Clinical diagnosis of dsTB in 2011, started RHZE
- Re-admitted 4mo later with pneumonia
- Re-admitted 6mo later
  - Adherence problems: VL unchanged
  - Clinical deterioration: Hb 5,0 and new cavitations on CXR
  - MDR-TB treatment started empirically

<table>
<thead>
<tr>
<th></th>
<th>Sputum at 4mo</th>
<th>Gastric aspirate at 10mo</th>
<th>Induced sputum at 10mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GXP</td>
<td>Not done</td>
<td>MTB low +</td>
<td>MTB med +</td>
</tr>
<tr>
<td>LPA – culture</td>
<td>MTB+</td>
<td>Rif S</td>
<td>Rif S</td>
</tr>
<tr>
<td>Phenotypic DST</td>
<td>Not done</td>
<td>Not done (lost viability)</td>
<td>Not done</td>
</tr>
</tbody>
</table>

DNA sequencing:
- Wild-type (spt)  
- Leu533Pro (GA)
A way forward?

- Changing methods of detection

- WHO recommendations
  - GXP pos Rif R: Clinical risk stratification and local epidemiology. Repeat GXP if no risk factors for MDR-TB. Discrepancies to be resolved by rpoB sequencing

- New susceptibility breakpoints

- Addressing the clinical approach to discordant results
The bottom line(s)

- Molecular diagnostics have ushered in a new generation of problems
- Seeing isn’t always believing
- No result should be looked at in isolation
- Phone a friend
Acknowledgments

- Prof HS Schaaf
- Dr Marieke van der Zalm
- Dr Liz Walters
- The Desmond Tutu TB Center
- Prof Andrew Whitelaw
- Dr Natalie Beylis
- Dr Lisa Frigati
- Corne Bosch

Please Lord... Don't let the Japanese find out about cricket!
@ninohendricks