RECENT EVOLUTIONS IN THE DIAGNOSIS OF MYCOBACTERIAL INFECTIONS

Emmanuel André
Epidemiology of TB

- 2.5 billion are infected
- 10% Will evolve to active TB disease
- 10.4 million people developed TB disease in 2016
- 1.7 million people died from TB in 2016

Global plan du End TB

- Prevention
- Poverty reduction
- Treatment
- Drug resistance
TB and poverty

J-P. Janssens, H. L. Rieder
European Respiratory Journal 2008 32: 1415-1416; DOI: 10.1183/09031936.00078708
TB infection and TB disease

a Latent infection

- Mycobacterium tuberculosis
- Alveolus
- Dendritic cell
- Migration to the lymph nodes for T cell priming

b Active disease

- Granuloma
- Lymph node
- Infected lymph node

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Traditional approach for the diagnosis of TB infection

**Infection eliminated**
- With innate immune response
- With acquired immune response

**Latent TB infection**
- Mycobacterium tuberculosis
- Granuloma

**Subclinical TB disease**

**Active TB disease**

<table>
<thead>
<tr>
<th>Test</th>
<th>TST</th>
<th>IGRA</th>
<th>Culture</th>
<th>Sputum smear</th>
<th>Infectious</th>
<th>Symptoms</th>
<th>Preferred treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>No</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>No</td>
<td>None</td>
<td>Preventive therapy</td>
</tr>
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<td>Positive</td>
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<td>No</td>
<td>Mild or none</td>
<td>Multidrug therapy</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Usually negative</td>
<td>Sporadically</td>
<td>Mild to severe</td>
<td>Multidrug therapy</td>
</tr>
<tr>
<td>Usually positive</td>
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<td>Positive or negative</td>
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</table>
Drug resistance in TB

Percentage of new TB cases with MDR/RR-TB

- 0–2.9
- 3–5.9
- 6–11
- 12–17
- ≥18
- No data
- Not applicable
Non-TBc Mycobacteria
Epidemiology of NTM

Nontuberculous mycobacteria in Denmark, incidence and clinical importance during the last quartercentury. Nature Scientific reports 2017
Clinical significance of positive NTM cultures

<table>
<thead>
<tr>
<th>NTM species</th>
<th>Total</th>
<th>Definite NTM disease</th>
<th>Possible NTM disease</th>
<th>NTM colonization</th>
<th>Age Median [IQR]</th>
<th>Localisation Pulmonary %</th>
<th>Localisation Non pulmonary %</th>
<th>Sex male%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. abscessus/chelonae</em></td>
<td>195</td>
<td>93</td>
<td>37</td>
<td>65</td>
<td>46.6 [45.2]</td>
<td>82.6</td>
<td>15.9</td>
<td>54.4</td>
</tr>
<tr>
<td><em>M. avium complex</em></td>
<td>1,757</td>
<td>1,093</td>
<td>403</td>
<td>261</td>
<td>55.0 [59.1]</td>
<td>56.9</td>
<td>35.9</td>
<td>52.6</td>
</tr>
<tr>
<td>Adults aged 15+</td>
<td>1,309</td>
<td>652</td>
<td>400</td>
<td>257</td>
<td>64.2 [26.0]</td>
<td>75.1</td>
<td>15.5</td>
<td>56.3</td>
</tr>
<tr>
<td>Children aged 0-14</td>
<td>448</td>
<td>441</td>
<td>3</td>
<td>4</td>
<td>2.5 [2.0]</td>
<td>3.8</td>
<td>95.3</td>
<td>41.7</td>
</tr>
<tr>
<td><em>M. gordonae</em></td>
<td>527</td>
<td>0</td>
<td>0</td>
<td>527</td>
<td>65.9 [21.9]</td>
<td>94.1</td>
<td>5.5±</td>
<td>58.4</td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>55</td>
<td>34</td>
<td>15</td>
<td>6</td>
<td>50.0 [36.4]</td>
<td>83.6</td>
<td>12.7</td>
<td>58.2</td>
</tr>
<tr>
<td><em>M. malmoense</em></td>
<td>139</td>
<td>97</td>
<td>23</td>
<td>19</td>
<td>59.5 [42.0]</td>
<td>71.2</td>
<td>28.8</td>
<td>55.4</td>
</tr>
<tr>
<td><em>M. marinum</em></td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>44.4 [27.4]</td>
<td>1.0</td>
<td>98.0</td>
<td>72.0</td>
</tr>
<tr>
<td><em>M. fortuitum/peregrinum</em></td>
<td>123</td>
<td>15</td>
<td>24</td>
<td>84</td>
<td>59.4 [31.0]</td>
<td>91.9</td>
<td>8.1</td>
<td>59.3</td>
</tr>
<tr>
<td><em>M. xenopi</em></td>
<td>140</td>
<td>61</td>
<td>42</td>
<td>37</td>
<td>63.6 [21.6]</td>
<td>95.0</td>
<td>2.9</td>
<td>60.0</td>
</tr>
<tr>
<td>Others $^\dagger$</td>
<td>426</td>
<td>125</td>
<td>110</td>
<td>191</td>
<td>62.1 [25.5]</td>
<td>81.0</td>
<td>17.5</td>
<td>55.8</td>
</tr>
<tr>
<td>Total</td>
<td>3,462</td>
<td>1,618</td>
<td>654</td>
<td>1,190</td>
<td>58.5 [37.2]</td>
<td>69.1**</td>
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Nontuberculous mycobacteria in Denmark, incidence and clinical importance during the last quartercentury. Nature Scientific reports 2017
Immunodiagnosis of latent TB infection

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Immunodiagnosis of latent TB infection

Classification of the Tuberculin Skin Test (PPD) Reaction

- **> 5 mm**
  - HIV positive
  - Recent contact with an active TB patient
  - Nodular or fibrotic changes on chest X-ray
  - Organ transplant

- **> 10 mm**
  - Recent arrivals (< 5 yrs) from high-prevalence countries
  - IV drug users
  - Resident/employee of high-risk congregate settings
  - Mycobacteriology lab personnel
  - Chronic conditions
  - Children < 4 yrs old
  - Infants, children, & adolescents exposed to high risk categories

- **> 15 mm**
  - Persons with no known risk factors for TB

TST vs IGRA
Immunodiagnosis of latent TB infection

Fig. 6 Concordance between the QFT-Plus tubes TB1 and TB2
**Bacteriological diagnosis of active TB infection**

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N.B. **Mycobacterium tuberculosis**

*Innate immune response*
Recent innovations for the **diagnosis of active TB infection**

- Sensitivity
- Rapidity

- **Microscopy**
- **Culture**

**PCR**

- Real-time PCR, automatization (*)
Xpert MTB/RIF

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Semi-nested real-time amplification and detection in integrated reaction tube
8. Printable test result

Time to result, 1 hour 45 minutes
Strategies for increasing the sensitivity of direct MTBc PCR

Single copy target

ie: Xpert MTB/RIF: ropB

Multiple copy target

ie: Xpert MTB/RIF Ultra: IS6110

Decreased Ct for the same amount of bacteria in the sample
Recent innovations for the detection of drug resistance in TBc

- Direct PCR
- Direct NGS
- Culture-based DST; culture-based WGS

Number of drugs tested

Rapidity
Detection of Rifampicin resistance by the Xpert MTB/RIF Ultra test
Detection of Rifampicin resistance by the Xpert MTB/RIF Ultra test

Soumitesh Chakravorty et al. mBio 2017;
doi:10.1128/mBio.00812-17
rpoB gene and Rifampicin resistance
Refugee (Somalia) with Pott disease

Smear microscopy : -
Direct TBc PCR : -
Culture : +
TBc on positive culture : TB+ / Rif sensitive
Phenotypic DST: RIF S, INH R, PZA S, Ethol S
OUTBREAK OF UNDETECTED MDR-TB IN SOUTH-AFRICA AND SWAZILAND

*rpoB* Ile491phe mutation is undetected by commercial molecular tests.

Patients receive inappropriate first-line therapy and no extensive drug susceptibility testing.

Ongoing transmission and acquisition of further drug resistance mutations.
Recent innovations for the diagnosis of NTM infections

- Microscopy, Direct sequencing
- Culture, Culture-based MALDI-TOF
- (direct PCR)
- Culture-based PCR

NTM species which can be identified
Severe systemic infection (HIV infected patient)

Smear microscopy : +
Direct TBc PCR : -
Culture : -
Leg ulcer (regular travels to rural Africa)

Smear microscopy : +
Direct TBc PCR : -
Culture : -
Conclusion

• Tuberculosis is a major international public health challenge
• Mycobacteriology is rapidly evolving
• Moving towards molecular-based diagnostic

• Anticipated challenges
  – Limitations of rapid molecular tests
  – NGS implementation in routine settings
  – NTM diagnosis (and drug susceptibility testing)