Rapid Impact of Effective Treatment on Mtb Transmission: 
Mechanisms and Practical Implications

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Questions Addressed:

1. When do pulmonary TB patients on effective therapy become non-infectious?
2. When can TB patients be taken out of isolation and sent home?
3. What is the mechanism by which effective treatment rapidly stops transmission?

G.A.

- 29 yo HCW (peds resident) presents to BWH ER after one episode of hemoptysis (while on call)
- No cough, no fever or night sweats, no weight loss, no decreased appetite, no malaise.
  - Just a funny feeling in the back of the throat, and expectorated a 1 tablespoon of red blood
  - On exam: no fever, normal BP, HR, RR and O2 sat
  - One additional similar episode of hemoptysis in ER
G.A. 12/6/2013

Additional history

- Other sources of bleeding:
  - No sinus problems
  - Recent dental work – no bleeding
- Negative TST (PPD) until 2 years ago when she converted to pos senior year in medical school
  - 2 other 4th year medical students converted
  - Travelled to Africa (6 mos) and Central America in medical school and volunteered in homeless shelter in Nashville
  - Normal CXR at that time – did not receive INH
- Born in Latin America, immigrated at age 4
- Uncomplicated pregnancy – delivered a healthy baby 7 mos ago – baby well
Non-hospital course

- **CT scan for PE** in the EW - neg
- Asked pulmonary to consider bronchoscopy for hemoptysis
  - Pulm: initially declined, but patient was anxious to know due to professional contact with sick children, etc
- **Bronch** performed the morning of presentation:
  - LUL clot, bilateral blood seen, no active source identified.

Non-hospital course continued

- Post bronch, patient started on **standard TB Rx**:
  - 2 mos IRPE, 4 mos IR, pending NAAT, culture and drug susceptibility tests
- **Patient discharged that same day against the wishes of DPH Boston.**
  - DPH was “concerned” about 7 mos old child.
  - PHN could not visit her home before Monday – insisted that we keep her in the hospital till visited.
  - Patient asked to stay out of work the following week (was on research block for 2 wks)
Rationale for discharge

1. TB suspects/cases need not be admitted to the hospital, so there is no required duration of hospitalization
   – Exceptions for medical and social reasons
2. Household contacts have already been exposed weeks or months before diagnosis
   – Baby was acting normally
3. Patient was not coughing – cough strongly associated with infectiousness
4. Patients started on therapy rapidly become non-infectious – how rapidly?

### Effects of Chemotherapy on Transmission

- Gunnels et al (ARRD 1974):
  - studied contacts of 155 patients sent home after 1 month of treatment in hospital
  - 69 Culture neg.
  - 86 Culture pos
    - 52 Smer and culture positive.

- No difference in infection rate among 284 contacts of culture positive cases versus 216 contacts of culture negative contacts.
Effects of Chemotherapy on Transmission

  - Sputum smear and culture positivity correlate with transmission before but not on therapy.
  - Evidence that smear and culture positive TB patients on effective therapy do not infect close contacts.

Effects of Chemotherapy on Transmission (Rouillon)

- "There is an ever-increasing amount of evidence in support of the idea that abolition of the patient’s infectiousness - a different matter from ‘cure,’ which takes months, and from negative results of bacteriological examinations, direct and culture, which may take weeks – is very probably obtained after less than 2 weeks of treatment."

- "These facts seem to indicate very rapid and powerful action by the drugs on infectivity…"

Outcome

- Bronch lavage AFB smear and NAAT negative for TB
  - Sputum – AFB negative (one specimen discarded – in improper container!)
  - Lavage culture ultimately positive – pan sensitive TB
- No further hemoptysis
- Completed therapy uneventfully
- Husband and Baby remained well and TST neg
Commentary

• Unusual presentation for TB
  – Hemoptysis usually a later feature of cavitary TB where necrosis erodes blood vessel
  • Exsanguination was the most feared cause of death in the pre-chemotherapy era
• RUL granuloma may have eroded into a small vessel, heralding TB before any other symptoms
• Patients on effective therapy are not infectious – almost immediately!
  – One what basis do I say that?

How Rapidly Does Treatment Stop Transmission?
Wells/Riley Experimental TB Ward 1960-62


• Riley RL. What nobody needs to know about airborne infection. (How It Really Happened) AJRCCM 2001; 163:7-8.

Wells/Riley Ward – Results (Exp 2)
• 2.6 GPs infected per month
  – strict criteria
• Relative infectivity of patients*:
  – Susceptible TB
    • 61 Untreated (29 GPs) 100%
    • 29 Treated (1 GP) 2
  – Drug-resistant TB
    • 6 Untreated (14 GPs) 28
    • 11 Treated (6 GPs) 5

*all smear positive patients, relative to the amount of time on the ward
Smear pos - started therapy the same time they entered the ward - not 2 weeks before.
Rapid impact of effective chemotherapy on transmission of drug-resistant tuberculosis

Airborne Infection Research Facility

Airborne Infection - Interventions

- Aerobiology
- Environmental stressors
- Temperature and humidity
- Oxygen and radiation
- Treatment
  - Drug resistance
- Host resistance
- Immunization
- Resp Protection
  - Masks
- Source control
  - Patients

Pathogenesis
- Treatment of latent infection

Isolation
- Dilution (ventilation)
- Filtration
- UVGI
- Locking

Controls
- Admin
- Isolation
- Source control
- Host resistance

Source strength
- Drug resistance
- Virulence
- Viability
- Viability

Drug resistance
- Oxygen and radiation
- Temperature and humidity
- Virulence

Host resistance
- Isolation
- Source control
- Dilution (ventilation)
- Filtration
- UVGI
- Locking
TB Spread in General medical Clinics, hospitals
- unsuspected case
- Unsuspected DR

Unsuspected, untreated TB
Active case finding, Lima Hospital
13 of 40 TB cases (33%) detected/yr – UNSUSPECTED!
(EMerg Inf Dis 2001; 7:123-7)

What is the mechanism for the rapid effect of treatment on transmission?
1. Not explained by smear and culture conversion
2. Likey related to the combine stress responses
   - to aerosolization and to treatment
3. Gene expression mediated?
4. Other phenotypic responses to environment?

*Mtb* studied in culture and in animal models – not at all in air – *aerobiology of Mtb virtually unknown.*
Mycobacterium tuberculosis phenotypes in patient aerosols

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Cough Aerosol Sampling
Kevin Fennelly, MD

Each patient will perform 2 cough sampling sessions over 2 successive days
Cough Aerosol Assays and Analyses

For each patient (n=50) and each sampling session (n=100):
- Andersen Sampler
- BioSampler with PBS/PANTA
- BioSampler with GTC
- Expectorated sputum

- Colony counts
- CFU/culture
- MPN counts
- RPF dependency assays
- MGIT Culture
- MGIT assay
- Transcriptional assays qRT-PCR
- Lipid body analysis (raw sputum)
- Transcriptional analysis qRT-PCR (samples in GTC medium)
- MGIT Culture
- Full genome sequences awaited for re-mapping
- Analysis on the Rockhopper 2.02 platform

From Patients in the AIR facility, Pretoria
- Seven pre-treatment aerosol samples from 3 patients in the cough aerosol sampling system (CASS).
- All Andersen samples positive for colony forming units with 10 min sampling (5-534 CFU)
- Liquid impactor samples taken for RNA analysis (lower air volume sampled than Andersen)
- 5 aerosols +ve for Mtb 16S rRNA (10^2.5 copies)

RNA from 4 aerosol/sputum pairs sent for RNAseq
- Illumina NextSeq analysis (Vertis), approx 10^7 reads per sample
- Good coverage in one aerosol/sputum pair
- Fair coverage in remaining 3 aerosols
- Reads mapped to Mtb H37Rv genome
- Full genome sequences awaited for re-mapping
- Analysis on the Rockhopper 2.02 platform
Differentially expressed (DE) transcripts (Aerosol vs. sputum)
- 111 DE: 52 UP, 59 DOWN
- 95 Protein encoding transcripts
- UP examples
  - cytochrome D ubiquinol oxidase subunit II CydB
  - ribonuclease VapC47
  - mycofactocin system protein MfB
  - ESX-1 secretion-associated protein EspA
- Remarkable consistency across 4 aerosol samples (2 patients sampled on 2 consecutive days)

Transcription factor associated regulatory patterns
- DosR significantly upregulated in both sputum and aerosol vs aerobic growth in vitro
- Mce2R genes significantly up in aerosol vs sputum
- Many additional regulatory patterns to be explored

RNA-seq reads from aerosol, VS. sputum and in vitro Mtb growth

Integrated Genome Viewer visualisation of RNA-Seq profiles of aerosol and sputum samples from a patient sampled by CASS, aligned to the Mtb H37Rv genome and compared to mid exponential growth. The profile demonstrates that genome-wide coverage has been obtained from a ten minute cough sample, that differential expression is readily detected and that both untranslated transcripts and potential novel RNAs were detected.
Summary of DE RNAs Aerosol vs. Sputum (q<0.01)

Mapping of 16S reads to compare microbiomes in aerosol and sputum

- Genus level assignments only
- Aerosol dominated by mycobacterium (>95%)
- Sputum – major Firmicute (streptococcal) signal and mycobacterial signal much lower than in aerosol

Interpretation

- Note that aerosol samples were RNA stabilised within seconds of expectoration
- Strong indications that aerosol is not simply a sample of sputum
- The Mtb present in aerosol show significantly different gene expression from those in contemporaneously sampled sputum
- The accompanying microbiomes in aerosol and sputum are also very distinct
Summary

- TB Patients need not be hospitalized at all – unless there are extenuating circumstances
- Effective treatment rapidly stops transmission
  - Known cases on Rx NOT the source of hospital transmission
  - Focus on active case finding (cough, rapid molecular diagnosis, and effective treatment)
- Environmental controls in general medical areas and waiting rooms important for unsuspected cases
- Aerosolization changes Mtb gene expression - stress response.
- Effective drugs also cause gene expression stress responses in vitro
- Other stress responses NOT associated with gene expression