Advanced Imaging for Tuberculosis: Insights into Disease Pathogenesis

Unite to End TB by Making the Connections
Maryland Department of Health and Mental Hygiene
March 9, 2017

Sanjay K. Jain, MD
Director, Center for Infection and Inflammation Imaging Research
Associate Professor of Pediatrics & International Health
Department of Pediatrics & Center for TB Research
Johns Hopkins University
Disclosures

- **Grant funding:** NIH
  Gilead Biosciences

- **Patent:** International patent PCT/US13/59897, ‘Bacteria-specific labeled substrates as imaging biomarkers to diagnose, locate and monitor infections’ filed

Sanjay K Jain
Objectives

• Describe a clinical vignette to demonstrate the power of imaging in monitoring drug-resistant TB

• Discuss limitations of current tools for diagnosis and monitoring of infections, and how imaging could overcome some of these limitations

• Discuss “perceived” and real risks of clinically available imaging modalities

• Describe two molecular imaging studies at Hopkins currently enrolling TB patients, and approved by the Maryland Health Department

• Describe some bacteria-specific imaging agents currently in development
A two year-old child from the United States developed pneumonia after a three-month visit to India:

• On arrival in India, she was immunized with BCG, stayed with her grandparents and attended a local day-care facility. During the last week of her visit, she developed fevers that continued after her return to the USA.
• Based on clinical presentation and CT imaging findings, she was started on empiric first-line TB treatment after obtaining sequential gastric-aspirates.
• Symptoms resolved with first-line TB treatment but extensively drug-resistant (XDR) *Mycobacterium tuberculosis* grew in culture.
• The child was started on an individualized drug regimen for XDR-TB consisting of intravenous streptomycin, linezolid, PAS, cycloserine and clofazimine.
• But treatment was complicated by uncertainties about drug selection, and lack of child-friendly formulations. *So, we needed to know (quickly), whether the individualized drug regimen was working or not!!!*
• However, assessing response to treatment was challenging, as clinical response was noted with suboptimal regimens, and microbiology could also not be used in this child.

CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child

- Clinical response was noted with a suboptimal regimen.

- Gastric aspirate smears were negative at the time of initiation of XDR-TB treatment, and cultures remained negative subsequently.

- Therefore, in the absence of clinical or microbiological markers, low-radiation exposure pulmonary CT imaging was used to monitor treatment response, and guide the individualized drug regimen for XDR-TB.
CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child

Salazar-Austin et al. Lancet Infect Dis. 2015
Initial diagnoses of pulmonary TB
Initiation of XDR-TB treatment
6 weeks after initiating XDR-TB treatment
6 months after initiating XDR-TB treatment

CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child

Salazar-Austin et al. Lancet Infect Dis. 2015
Initial diagnoses of pulmonary TB

Initiation of XDR-TB treatment

6 weeks after initiating XDR-TB treatment

6 months after initiating XDR-TB treatment

1. Growth of acid-fast bacilli in liquid broth;
2. Identification of \textit{M. tuberculosis} complex by 16S rRNA sequencing;
3. Persistent left lower lobe infiltrate on chest radiography;
4. Sanger sequencing and initial TREK panel confirming XDR strain;
5. Agar proportion results (Maryland State);
6. CT demonstrating significant reduction in lesion volume;
7. Agar proportion results (National Jewish);
8. Consistent weight gain.

Salazar-Austin et al. \textit{Lancet Infect Dis.} 2015
CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child

• This report highlights the:
  • risks of acquiring drug-resistant (DR) TB overseas
  • *challenges of diagnosing DR-TB, especially in young children*
  • lack of dosing and child-friendly drug formulations
  • controversy regarding the infectious risk of young child and
  • *lack of tools to rapidly monitor DR-TB treatments*

• In this child, low-radiation exposure pulmonary CT demonstrated marked improvement as early as 6 weeks after initiation of treatment for XDR-TB, and corroborated by consistent weight gain, which however lagged improvement on CT imaging by 10 weeks.

• *Treatments for drug resistant TB are often empiric, and the risks of treatment failure substantially outweigh the use of relatively advanced, but promising technologies that can provide rapid and reliable means to monitor treatments.*
Food for thought . . .

• Most experts agree that Infections will not be eradicated for decades, or maybe never . . .

• Are we planning for the future, and utilizing the advances in technology that may be applicable to Infectious Diseases?

• Does diagnosis and monitoring of infections in special situations (hard to get locations, difficult to grow bugs, e.g. *M. tuberculosis* or other fastidious bugs) merit the development and / or use of technologies, that may be different from those being developed currently?
The future is not what it used to be . . .

Fundamental diagnostic: 1882
Need to isolate the bug

Fundamental diagnostic: 2017
Need to isolate the bug
Problem(s) – Counting bugs

- Bugs: “need to bring them out, or go in and get them”
- Often impractical or dangerous
- Whole organ / body view of disease not available

Solution(s) – Take a picture instead

- Inject radiotracer
- Uptake by target cells or bugs
- Take pictures
- X-ray
- CT
- PET

- Rapid, and *easily scalable to humans*

- Major investment by Oncologist to develop this technology for cancers
- Now used extensively for monitoring cancer patients during clinical trials and also for patient care
- Can these technologies be used in Infectious Diseases?
How can imaging help with TB trials and patients?

- Tomographic imaging can evaluate disease processes deep within the body, noninvasively and relatively rapidly.
- Longitudinal assessments can be conducted in the same individual, which is a fundamental advantage over the traditional invasive tools.
- Provides holistic, 3D views of the whole organ or body representative of the overall disease, and also less prone to sampling errors.

Purpose
- Diagnosis
- Monitoring and prognostication; end-points for treatment trials enable adaptive designs
- Understanding pathogenesis host-directed therapies multi-compartment PK
Risk of Imaging: Let’s be real . . .

- Even with treatment, the risk of mortality is high for XDR and MDR forms of TB, and similar to 5-year morality that due to cancers.

- Even drug-susceptible TB with adequate treatment has substantially higher risk of mortality (~1000 times) than radiation induced cancers with optimized imaging.

- Radiation due to:
  - Optimized PET (Pediatric torso), similar to annual background radiation
  - Chest CT – Pediatric protocol, similar to a mammogram or four round trip trans-Atlantic flights

Only 52% and 28% of MDR- and XDR-TB treated successfully. WHO 2016
https://www.ncbi.nlm.nih.gov/books/NBK218704/
Following Cavity formation and Treatment Failure using High-Resolution CT Imaging

Development of a cavity during treatment failure, due to inadequate TB treatment

TB treatment

<table>
<thead>
<tr>
<th>Week 9</th>
<th>Week 14</th>
<th>Week 20</th>
<th>Week 26</th>
</tr>
</thead>
</table>

He = Heart

TB granulomas (tumors) in mouse lungs

Noninvasive Pulmonary $[^{18}\text{F}]-2$-Fluoro-Deoxy-$d$-Glucose Positron Emission Tomography Correlates with Bactericidal Activity of Tuberculosis Drug Treatment

Stephanie L. Davis,¹,² Eric L. Nuermerberger,¹,³ Peter K. Um,¹,² Camille Vidal,⁵,⁷ Bruno Jedynak,⁶,⁷ Martin G. Pomper,⁴ William R. Bishai,¹,³ and Sanjay K. Jain¹,²

Center for Tuberculosis Research,¹ Department of Pediatrics,² Department of Medicine,³ and Department of Radiology,⁴ Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, and Department of Biomedical Engineering,⁵ Department of Applied Mathematics and Statistics,⁶ and Center for Imaging Science,⁷ Johns Hopkins University, Baltimore, Maryland 21218

Received 12 June 2009/Returned for modification 15 July 2009/Accepted 26 August 2009

18F-FDG PET/CT correlates with treatment outcome in patients with MDR-TB

• Prospective imaged 35 adults (median age 37 years) with MDR-TB, on second-line TB treatment, using 18F-FDG PET and CT at 2 and 6 months after starting treatment.

• Imaging assessed by radiologists or automated analyses.

• 18F-FDG PET at 2 months and automated CT at 6 months were more sensitive than sputum smear or solid culture conversion at 2 months, these differences were not statistically significant, possibly because of the small sample size in our study.

• Automated methods were more reliable than radiologists.

Chen et al. Sci Transl Med. 2014
Imaging TB-associated inflammation with iodo-DPA-713

- Iodo-DPA-713 is a ligand for translocator protein (TSPO)
- Up-regulated in inflamed microglia and macrophages
- TB lesions full of activated macrophages

TSPO expression in macrophages within TB lesions

Foss et al. J Infect Dis 2013 (Cover article)
Imaging TB-inflammation to monitor treatments: \( ^{125}\text{I}-\text{DPA-713-SPECT} \) versus \( ^{18}\text{F-FDG-PET} \)

Pulmonary \( ^{125}\text{I}-\text{DPA-713 SPECT}, \) but not \( ^{18}\text{F-FDG PET} \), correctly identified the bactericidal activities of the TB treatments as early as 4 weeks after starting treatment \((P < 0.03)\)

Iodo-DPA-713 bound activated (CD68 \(^+\)) antigen presenting cells and imaging correlated with tissue TNF-\(\alpha\) (Spearman’s \(\rho = 0.94; P < 0.01\))

Significant correlation was found between an increase in \( ^{125}\text{I}-\text{DPA-713 SPECT} \) activity (but not with \( ^{18}\text{F-FDG PET} \)) with bacterial burden at relapse (Spearman’s \(\rho = 0.79; P < 0.01\))

Foss et al. J Infect Dis. 2013 (Cover Article)
Imaging TB-associated inflammation with $^{124}$I-DPA-713-PET

- $^{124}$I-DPA-713 synthesized under a research contract
- Mouse studies demonstrate ($^{124}$I-DPA-713 PET in blue-green-red; and skeletal outline in grey) excellent signal-to-noise ratio: 4.0-fold higher in TB lesions versus uninfected controls ($P = 0.03$)
- Toxicology studies completed and first-in-human studies have started under an FDA Exploratory Investigational New Drug application (#121615)
- **Half life of I-124 is 4.2 days and so this can be shipped worldwide.**

Proposed $^{124}$I-DPA-713 PET human studies to specifically localize sites of TB infections

- Age: 18-65 years old
- Culture or molecular (GeneXpert, etc.) confirmation of TB
- On TB treatment for ≤ 4 weeks by the time of study
- Can understand English

If so, fill the screening script and refer to the study staff (JHU). The study, funded by the U.S. National Institutes of Health, requires three visits:

- Screening visit: history, written consent, screening labs
- Imaging visit day 1 (~half a day); injection of tracer
- Imaging (day 2 or 3) may last up to 3 hours
- During the imaging visit, we will use a new investigational PET/CT imaging technique
- Paid a total of $200 for participation in this study
Radiosynthesis and Bioimaging of Rifampin

- First-line drug essential for shortening therapy against *M. tuberculosis*
- Dosing based on serum / plasma concentrations (confirmed by post mortem resection)
- Drug concentration within necrotic pulmonary lesions (post-mortem) lower than blood concentrations*
- Labeled rifampin with $^{11}$C using methods described by Liu *et al*

\[ \text{[}^{11}\text{C}]\text{RIF} \]

Liu *et al.* J Med Chem. 2010
**Radiosynthesis and Bioimaging of Rifampin**

- Dynamic $^{11}$C-Rifampin PET/CT of a *M. tuberculosis* infected mouse
- Granulomatous tissue depicted by yellow circle
- Purple represents concentration of rifampin (highlighted by orange arrow)

DeMarco and Ordonez *et al.* *Antimicrob Agents Chemother.* 2015
Radiosynthesis and Bioimaging of Rifampin
Lower concentrations in infected lung tissues

Data are represented as medians and interquartile ranges. 5 mice were used for each group.

DeMarco and Ordonez et al. Antimicrob Agents Chemother. 2015
**Radiosynthesis and Bioimaging of Rifampin**

*Lower concentrations in infected lung tissues*

---

*Human studies:* Dynamic $^{11}$C-rifampin PET imaging studies in TB patients have begun to evaluate the penetration of rifampin in target, infected tissues – necrotic, cavitary and CNS lesions. cGMP syntheses complete and RDRC / IRB approvals obtained.

---

Proposed $^{11}$C-rifampin PET human studies in patients with Rifampin-susceptible TB

- Age: ≥18 years old
- Culture or molecular (GeneXpert, etc.) confirmation of TB susceptible to rifampin
- On TB treatment for ≤ 6 weeks by the time of study
- Can understand English

If so, fill the screening script and refer to the study staff (JHU). The study, funded by the U.S. National Institutes of Health, requires two visits:

- Screening visit: history, written consent, screening labs
- Imaging visit day 1 (~half a day)
- During the imaging visit, we will use a new investigational PET/CT imaging technique
- Paid a total of $150 for participation in this study
Current Imaging Agents:
Lack of Infection-specific tracers

Pros
- Readily available
- Excellent sensitivity

Cons
- Non-specific for bacteria or infection
- Non-specific; host-dependent

CT / MRI / US

18F-FDG PET/ WBC scan
Developing Bacteria-specific Imaging Tracers

**AIM:** To develop a pipeline of class specific bacterial imaging probes that would provide a platform to identify, localize and monitor a wide range of pathogenic bacteria

**HYPOTHESIS:** Small molecules metabolized by prokaryotic-specific pathways could be utilized as bacteria-specific imaging tracers to:

a) discriminate infection from non-infectious processes
b) categorize the causative bacterial species and
c) provide information on antibiotic efficacy

Ordonez and Weinstein *et al.* *J Nucl Med.* 2017
## Candidate Bacteria-specific Imaging Tracers

<table>
<thead>
<tr>
<th>Name</th>
<th>S. aureus (Gram-positive)</th>
<th>E. coli (Gram-negative)</th>
<th>P. aeruginosa</th>
<th>Mycobacteria*</th>
<th>Macrophages (J774)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arabinose [1-14C]</td>
<td>0.41 ± 0.03</td>
<td>41.61 ± 9.91</td>
<td>0.21 ± 0.02</td>
<td>0.28 ± 0.01 (Mtb)</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Cellobiose [3H]</td>
<td>1.81 ± 0.10</td>
<td>0.80 ± 0.05</td>
<td>--</td>
<td>0.13 ± 0.02 (Ms)</td>
<td>--</td>
</tr>
<tr>
<td>D-Lyxose [1-14C]</td>
<td>0.03 ± 0.01</td>
<td>1.86 ± 0.14</td>
<td>0.12 ± 0.04</td>
<td>0.35 ± 0.08 (Mtb)</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>D-Mannitol [1-14C]</td>
<td>68.40 ± 7.39</td>
<td>81.80 ± 1.96</td>
<td>0.69 ± 0.05</td>
<td>0.29 ± 0.13 (Mtb)</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Methyl-α-D-glucopyranoside [methyl-14C]</td>
<td>11.01 ± 0.71</td>
<td>26.78 ± 0.59</td>
<td>--</td>
<td>0.11 ± 0.01 (Ms)</td>
<td>--</td>
</tr>
<tr>
<td>para-Aminobenzoic acid [3,5-3H]</td>
<td>16.82 ± 1.03</td>
<td>18.99 ± 5.80</td>
<td>4.02 ± 1.11</td>
<td>32.93 ± 4.73 (Mtb)</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>L-Rhamnose [3H]</td>
<td>4.96 ± 0.13</td>
<td>4.73 ± 0.07</td>
<td>0.24 ± 0.04</td>
<td>3.82 ± 0.84 (Mtb)</td>
<td>0.60 ± 0.01</td>
</tr>
<tr>
<td>Shikimic acid [3-3H]</td>
<td>7.54 ± 0.01</td>
<td>1.52 ± 0.07</td>
<td>1.31 ± 0.02</td>
<td>0.17 ± 0.01 (Ms)</td>
<td>--</td>
</tr>
<tr>
<td>D-Sorbitol [14C] (18F-FDS)†</td>
<td>0.47 ± 0.09</td>
<td>72.20 ± 9.09</td>
<td>0.52 ± 0.46</td>
<td>--</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>D-Xylose [1-14C]</td>
<td>0.31 ± 0.01</td>
<td>73.94 ± 2.06</td>
<td>0.53 ± 0.08</td>
<td>0.18 ± 0.02 (Mtb)</td>
<td>0.19 ± 0.01</td>
</tr>
</tbody>
</table>

*FDS or Fluorodeoxysorbitol is a fluoro analog of Sorbitol – FDA approved sugar-free sweetener

$^{18}$F-FDS can be synthesized from $^{18}$F-FDG (in 30 min) and is specifically accumulated by *Enterobactericaceae*.

Weinstein and Ordonez *et al.* *Sci Transl Med* 2014 (Cover article)
$^{18}$F-FDS PET can differentiate infection sites from sterile inflammation
18F-FDS Uptake by 15 Random Clinical Multidrug-Resistant Enterobacteriaceae strains

- Notorious source of life-threatening nosocomial infections due to multidrug resistant organisms including:
  - Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae
  - Carbapenem-resistant Enterobacteriaceae (CRE)
  - Enterobacteriaceae resistant to colistin (“last line of defense”)
- All 15 random ESBL-producing clinical strains (clinical MDR E. coli), demonstrated substantial 18F-FDS uptake

Weinstein and Ordonez et al. Sci Transl Med 2014 (Cover article)
An increase in bacterial load corresponds with disease progression to sepsis and death in seriously ill patients.

Weinstein and Ordonez et al. Sci Transl Med 2014 (Cover article)
Acknowledgments

**JHU**
Nicole Salazar-Austin
Alvaro Ordonez
Alice Jenh Hsu
Jane Benson
Mahadevappa Mahesh
Aaron Milstone
Nicole Parrish
Eric Nuermberger
Richard Chaisson
Nursing and Pharmacy

**Others**
Jeffrey Starke
Max Salfinger

**Public Health**
Nancy Baruch
Elizabeth Menachery
Jafar Razeq
Dorothy Freeman

Maryland Department of Health and Mental Hygiene
Howard County Health Department
Centers for Disease Control and Prevention
Food and Drug Administration

Jain Lab
Alvaro Ordonez
Ed Weinstein
Supriya Pokkali
Liz Tucker
Alvin Kalinda
Vikram Saini
Mariah Klunk
Peter DeMarco
Lauren Bambarger
Yongseok Chang
Allison Murawski

Stony Brook
Peter Tonge
Hui Wang
Zhang Zhuo

Maryland TB Counties
Kelly Russo
Elizabeth Menachery
Lucia Donatelli
Kimberly Townsend

**JHU Radiology**
Catherine Foss
Ronnie Mease
Martin Pomper
Bob Dannals
Steve Rowe
Akimosa Jeffrey-Kwanisai
Ghedem Solomon
Mahadevappa Mahesh

JHU
Carlton Lee
Maunank Shah
Kelly Dooley

**Funding**

*NIH Director's Transformative Research Award R01-EB020539*

*NIH Director’s New Innovator Award DP2-OD006492*

*NHLBI R01-HL116316, R01-HL131829 and NIAID DAIDS supplement*