

## SPECIAL TOPIC

# The Promise of Molecular Imaging in the Study and Treatment of Infectious Diseases

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### Abstract

Infectious diseases are a major threat to humanity, and it is imperative that we develop imaging tools that aid in their study, facilitate diagnosis, and guide treatment. The alarming rise of highly virulent and multi-drug-resistant pathogens, their rapid spread leading to frequent global pandemics, fears of bioterrorism, and continued life-threatening nosocomial infections in hospitals remain as major challenges to health care in the USA and worldwide. Early diagnosis and rapid monitoring are essential for appropriate management and control of infections. Tomographic molecular imaging enables rapid, noninvasive visualization, localization, and monitoring of molecular processes deep within the body and offers several advantages over traditional tools used for the study of infectious diseases. Noninvasive, longitudinal assessments could streamline animal studies, allow unique insights into disease pathogenesis, and expedite clinical translation of new therapeutics. Since molecular imaging is already in common use in the clinic, it could also become a valuable tool for clinical studies, for patient care, for public health, and for enabling precision medicine for infectious diseases.

**Key words:** Bacteria, PET, Influenza, TB, Optical imaging, Microbiome

## Introduction

Humans in the modern era are increasingly prone to virulent infections. Selection and dissemination of “super bugs” by overuse (misuse) of antimicrobials, global travel and the rapid spread of infections, and the increasing use of medical procedures—implants, catheters, immunosuppressive/cancer therapies—are leading to life-threatening infections that are a growing threat to humanity [1–4]. In 2011, about 722,000 Americans developed hospital-acquired infections, resulting in 75,000 deaths in the USA. Bacteria, notably from the Enterobacteriaceae family (26.8 %), *Clostridium difficile* (12.1 %), *Staphylococcus aureus* (10.7 %), and *Pseudomonas aeruginosa* (7.1 %), accounted for the majority of these infections [5, 6]. Similarly, viral infections such as influenza cause millions of new cases and several thousand deaths annually in the USA [7]. The

burden of hospital-acquired and other infections is substantially higher globally. For example, *Mycobacterium tuberculosis*, the causative agent for tuberculosis (TB), was responsible for 10.4 and 1.8 million new cases and deaths in 2015 alone [8]. The alarming rise of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains, as well as HIV co-infection, continues to fuel this epidemic [8–10]. Global travel and rapid spread of infections—swine flu, SARS-CoV, Ebola, and Zika virus—have led to several recent global pandemics [11, 12]. Finally, several infectious pathogens, e.g., *Yersinia pestis* (causes plague) from the Enterobacteriaceae family, are also recognized as biothreat agents [13].

## Imaging of Infection Interest Group

Developing a better understanding of the pathogenic mechanism(s) of disease and new tools that could visualize

these processes *in vivo* is a key to controlling infections. Therefore, the World Molecular Imaging Society has made a commitment for advancing imaging tools for infectious diseases and inflammation by creating the Imaging of Infection Interest Group (IOI-IG). This interest group is tasked with the mission to globally advance the development of new imaging technologies for infectious diseases and the translation of effective imaging tools to provide unique insights into disease mechanisms, enable drug and vaccine development, guide treatments, and benefit patients (Fig. 1). As part of these efforts, the IOI-IG organized several sessions at the 2016 World Molecular Imaging Congress (WMIC). This included a 1-day workshop focused on imaging of infection and inflammation, which brought together individuals from multiple scientific disciplines and highlighted the latest developments in the field. In addition, the workshop also had a panel discussion on the challenges in funding research, with panelists from the US National Institutes of Health. A similar workshop is planned at the 2017 WMIC (Philadelphia, PA) and will focus on leveraging current resources, promoting collaborations, clinical translation, and finding strategies to overcome the challenges in funding research in this emerging field.


## The Clinical Problem

Rapid and accurate diagnosis and localization of infections are essential for effective early interventions and appropriate management. However, traditional diagnostic tools, namely microscopy, microbiology, and molecular techniques, are dependent upon sampling suspected sites of infection, often blindly, and then performing assays that can be time

consuming. While major advances are being made in the application of molecular techniques such as nucleic acid amplification (NAA), deep sequencing, and matrix-assisted laser desorption/ionization, they are all dependent on the availability of a relevant clinical sample. Easily obtained clinical samples such as blood or urine may lack relevance and not contribute to a specific diagnosis, especially with deep-seated infections, and invasive biopsies are often required. Biopsies can be costly, risky (anesthesia, dangers of surgery), and prone to incorrect sampling (limited to the tip of the biopsy needle) as well as lead to the introduction of artifacts or contamination. Molecular imaging techniques detect crucial biological and biochemical changes at the sites of infection, often at the earliest phase of the disease, thereby allowing the clinician to promptly identify not only the infective or inflammatory focus but also the class of the causative pathogen and establish the best therapeutic approach for patients.

## The Solution

Tomographic imaging can evaluate disease processes deep within the body, noninvasively and rapidly. Moreover, the ability of imaging to conduct noninvasive, longitudinal assessments in the same individual is a fundamental advantage over current traditional tools. Other major advantages of imaging are its ability to provide a holistic, three-dimensional assessment of the whole organ or body, less likely to be limited by sampling errors and co-relating well with the overall disease process (Table 1). Selected recent examples highlighting the unique role of imaging in infectious diseases are briefly described in this overview.



**IOI Interest Group**  
Imaging of Infections

**Mission**  
To globally advance the development of new imaging technologies for infectious diseases and the translation of effective imaging tools to provide unique insights into disease mechanism(s), enable drug and vaccine development, guide treatments and benefit patients

- Identify and address shortcomings in imaging technologies for studying, diagnosing and monitoring infections
- Provide a stronger, higher visibility network of scientists involved with research of infectious diseases imaging at all career levels
- Attract students from multiple disciplines (chemistry, biology, clinical medicine, etc.) to pursue a career in imaging of infections
- Liaison with other scientific organizations and funding agencies to disseminate information and establish the needs for this field
- Develop academic and industry collaborations globally

**WMIS**  
World Molecular Imaging Society

Discover. Visualize. Learn. Cure.

**Fig. 1.** Imaging of Infection Interest Group. The Imaging of Infection (IOI) Interest Group was formed as part of the World Molecular Imaging Society to advance the field of infectious disease imaging.

**Table 1.** Role of imaging in infectious diseases

Advantages of tomographic imaging over traditional techniques used for infections		
Evaluate disease processes deep within the body, noninvasively, and relatively rapidly		
Longitudinal assessments in the same individual—fundamental advantage over traditional tools		
Provide holistic, three-dimensional assessment of the whole organ or body representative of the overall disease (versus tip of a biopsy needle) and therefore less prone to sampling error		
Imaging in infectious diseases		
Role	Setting	Overall goal(s)
Pathogenesis	Preclinical	Unique insights into disease pathogenesis, <i>e.g.</i> , assessing hideouts of infections, defining the diversity of the microbial populations (microbiome)
	Clinical trials	Studying multi-compartment antimicrobial pharmacokinetics Expedite bench-to-bedside translation of new therapeutics, <i>e.g.</i> , surrogate end points to assess antimicrobial or vaccine efficacy or predict stable cure
	Patient settings	Unique insights into disease pathogenesis—noninvasive visualization of processes deep inside the body Phase 0 studies to determine compartment-specific antimicrobial penetration/binding (sites of infection, necrotic/fibrotic lesions, privileged sites—CNS) to inform appropriate dosing of novel drugs; determine accumulation at non-target sites to assess potential toxicities; current US Food and Drug Administration (FDA) guidelines require tissue drug distribution studies at the infected sites
Diagnosis	Clinical trials and patient settings	Enabling precision medicine by providing unique insights into disease pathogenesis, antimicrobial pharmacokinetics, <i>etc.</i> Rapidly and specifically distinguish an infectious process from other diseases (malignancy, sterile inflammatory processes, <i>etc.</i> ) Determine the site ( <i>e.g.</i> , extension/metastasis to other organs or privileged sites) and extent of disease Provide information on the class of the infectious pathogen, which could help in targeted empiric antimicrobial treatments
	Monitoring and prognostication	Noninvasive longitudinal assessments, especially in studies utilizing larger, more expensive animal species; serial assessments in the same animal could significantly reduce sample size, inter-animal variability (outbreed animals), and therefore cost of the studies
Monitoring and prognostication	Preclinical	Early end points for treatment trials to assess activity of treatments and to predict stable cure
	Clinical trials	Assessing host-directed treatments for infections Enable adaptive designs
	Patient settings	Rapidly detect treatment failures due to drug-resistant organisms or other reasons Rapidly monitor treatment responses in patients with drug-resistant organisms and individualize treatments
	Public health	Early end points for duration of treatment and predict stable cure enabling precision medicine Rapid determination of the infectious risk of a patient to the population based on response to treatment and extent and location of disease Rapid diagnosis and monitoring of biothreat agents

### Pathogenesis

Optical imaging methods have been used extensively to study disease pathogenesis, largely in small laboratory animals. For example, real-time analysis of *Mycobacterium marinum* infection in the transparent zebrafish has provided new insights into the temporal kinetics of host-pathogen interactions [14]. Similarly, immune responses to central nervous system (CNS) viral infections were visualized in real time using intravital two-photon laser scanning microscopy [15]. While highly sensitive, optical imaging is limited by the depth of the signal and is therefore well suited for the study of laboratory animal models but has limited clinical utility. The limited depth of penetration of signal is not a problem using nuclear medicine tools. For example, 2-deoxy-2-[<sup>18</sup>F]fluoro-*D*-glucose ([<sup>18</sup>F]FDG) positron emission tomography (PET) and computed tomography (CT) were used to monitor the spatial evolution of individual pulmonary lesions in a cohort of infected mice that develops TB lesions akin to humans [16–18] and study the temporal evolution of reactivation

pulmonary TB (relapse) [19]. It was noted that the fate of individual pulmonary lesions in the same animal varied substantially. More interestingly, several lesions also arose *de novo* within regions with no prior lesions suggesting that dormant bacteria may also reside outside TB lesions. Similar variability of TB lesion within the same host was also reported in non-human primates [20]. Molecular imaging can therefore provide unique insights into disease pathogenesis that are not possible with traditional tools. Other applications include assessing hideouts of infections, defining the diversity of the microbial populations (microbiome), and providing end points to assess antimicrobial or vaccine efficacy or predict stable cure.

### Drug Development

Current antimicrobial dosing regimens are based on achievable serum concentrations. However, a growing number of studies support the importance of monitoring drug concentration in

infected tissues, rather than serum alone. Inadequate drug concentrations in target tissues can lead to treatment failure and selection of drug-resistant organisms. Additionally, altered metabolism in diseased states may lead to organ toxicities. Vancomycin, a widely used antimicrobial to treat drug-resistant, Gram-positive bacteria, highlights these limitations. Studies performed in response to treatment failures noted in patients with standard doses of vancomycin revealed that it penetrates poorly into infected tissues, with levels only one sixth of plasma [21]. Conversely, high levels of vancomycin can cause nephrotoxicity. Based on these data, new recommendations have been developed for vancomycin [22], which, unfortunately, had been inappropriately under-dosed for decades.

Noninvasive bioimaging can be used to assess the distribution of drugs into multiple compartments of interest, simultaneously. For example, given the importance of rifampin for successful cure, potential for shortening the duration of TB treatments, and the limited availability of *in situ* data [23], dynamic PET imaging was performed over 60 min after injection of  $^{11}\text{C}$ -rifampin in live, *M. tuberculosis*-infected mice [24].  $^{11}\text{C}$ -rifampin rapidly distributed and quickly localized to the liver. Areas under the concentration-time curve for the first 60 min ( $\text{AUC}_{0-60}$ ) in infected and uninfected mice were uniformly low in the brain (10 to 20 % of blood values). However, lower concentrations were noted in necrotic lung tissues of infected mice than in healthy lungs, demonstrating the need for higher dosing than those determined by serum levels alone.

### Specific Diagnosis

An ideal imaging agent for diagnosing infections needs to be both sensitive and specific. While current, noninvasive techniques, such as CT, magnetic resonance imaging (MRI), and ultrasound, can rapidly detect anatomic pathology, they are non-specific and cannot reliably differentiate infectious lesions from non-infectious processes such as cancer or autoimmune diseases. Similarly, highly sensitive, current nuclear imaging tools such as [ $^{111}\text{In}$ ]oxine-tagged white blood cell, single-photon emission computed tomography (SPECT), and [ $^{18}\text{F}$ ]FDG PET, which are increasingly being used for infections, are non-specific [25]. Finally, all these imaging tools are dependent upon host responses, which could be altered in immunosuppressed states (*e.g.*, cancer, AIDS).

Radiolabeled antibiotics or antimicrobial peptides have been previously tested as pathogen-specific imaging tracers [26, 27]. However, radiolabeled antibiotics have demonstrated variable specificity and an inability to reliably differentiate infection from sterile inflammation [28]. Radiolabeled peptides lack mechanistic binding or uptake, but recent PET tracers may show promise [29]. Tracers targeting pathogen-specific metabolic processes have also been evaluated in preclinical studies [30, 31]. [ $^{124}\text{I}$ ]FIAU, a nucleoside analog substrate for thymidine kinase, was developed as a  $\gamma$ -herpesvirus and bacteria-specific tracer [32] but was later found to lack specificity, presumably due to host mitochondrial metabolism

[33]. Ning *et al.* and Gowrishankar *et al.* have demonstrated the feasibility of F-18-labeled maltohexaose and [ $^{18}\text{F}$ ]fluoromaltose to specifically image bacteria *in vivo* [34, 35]. These tracers target the maltodextrin transporter present in a wide range of Gram-negative and Gram-positive bacteria. Enterobacteriaceae are an important class of Gram-negative bacteria that cause serious infections in humans and include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., and *Yersinia* spp. Weinstein and Ordonez *et al.* demonstrated that 2-deoxy-2- [ $^{18}\text{F}$ ]fluorosorbitol ([ $^{18}\text{F}$ ]FDS) PET could specifically detect Enterobacteriaceae *in vivo* in various different sites including the brain, and the PET signal was not affected by immunosuppressive cancer treatments [36]. F-18 analogs of trehalose have also been developed as potential imaging agents for mycobacteria [37], while *Aspergillus fumigatus* has been imaged *in vivo* using Ga-68-radiolabeled siderophores [38]. Investigators have also utilized radiolabeled antibodies to image *A. fumigatus* and *Yersinia* in murine models [39, 40]. Similarly, Santangelo *et al.* developed a technique to image simian immunodeficiency virus (SIV) replication using a Cu-64-labeled SIV Gp120-specific antibody and were able to detect virus-specific PET signals in treated and untreated monkeys [41]. Other approaches have utilized Tc-99m-labeled oligomers to specifically target the ribosomal RNA in the pathogen [42, 43]. Finally, using endogenous CEST contrast, Liu *et al.* were able to image bacteria-specific MRI signals from *C. novyi*-NT-infected tumors in mice [44].

### Monitoring

Noninvasive imaging, including non-specific agents, can be used to monitor disease, and serial imaging has been used to assess antimicrobial treatments in animals and humans [9, 45–47]. For example, phase III TB trials entail treating hundreds of patients  $\geq 6$  months and monitoring for  $\geq 1$  year thereafter for relapse [48]. As *M. tuberculosis* can infect any part of the lung (or extra-pulmonary sites), traditional tools such as sputum culture or NAA (*e.g.*, Xpert® MTB/RIF) do not always correlate with the overall disease burden. Recently, Chen *et al.* reported that [ $^{18}\text{F}$ ]FDG PET and CT imaging were superior to traditional (sputum) microbiology for monitoring response to treatments in adults with MDR-TB [49]. In this study, quantitative changes in lesion volumes on CT imaging were predictive of treatment responses. Moreover, quantitative changes in [ $^{18}\text{F}$ ]FDG PET not only correlated with treatment responses but were also predictive of long-term treatment outcomes. In another more recent study, given the absence of clinical or microbiological markers of disease, low-radiation exposure pulmonary CT imaging was successfully used to monitor treatment response in a 2-year-old child with XDR-TB and guide an individualized drug regimen [9]. Imaging agents with higher specificity for TB-associated inflammation have been shown to be promising in animal models [45]. Pathogen-specific imaging could be an even better predictor



of treatment responses [36]. For example, both  $^{18}\text{F}$ -labeled maltohexaose and [ $^{18}\text{F}$ ]FDS PET could rapidly identify therapeutic failures associated with drug-resistant infections [34, 36].

### Public Health

Given that several infectious agents are easily transmissible (e.g., via aerosol), imaging could also help with rapid determination of the infectious risk of a patient to the population. This could be especially relevant for highly drug-resistant organisms or biothreat agents, where rapid diagnosis and therapeutic monitoring would be critical to prevent spread to others in the community.

### Biocontainment

A large majority of infectious pathogens can be handled using standard precautions. However, animals infected with pathogens designated as biosafety level 3/4 (BSL-3/4) require appropriate containment. In addition, work with biothreat pathogens requires regulatory approvals, as well as highly trained personnel [50]. To achieve containment, several groups have installed imaging equipment inside the BSL-3/4 barrier (e.g., NIAID, University of Pittsburg). While advantageous, maintaining expensive equipment

within these barriers can be complicated. Therefore, other groups have utilized animal isolation approaches achieved by isolating infected-animals inside sealed, biocontainment devices that can be transported to the imaging equipment housed in standard environments [46, 51, 52] or polycarbonate plastic tubes extending from the biocontainment space to the imaging equipment [53]. A tail vein catheter system was also used for on-table drug delivery to infected animals while sealed inside the biocontainment device [24, 54]. Animal containment allows for easier maintenance of the equipment, which can also be shared with other investigators that do not work within the BSL-3/4 barrier.

### Clinical Translation

Unlike traditional microbiological or molecular techniques, imaging can provide key spatial information to dictate treatment, enabling detection and therapeutic monitoring of infections in patients with deep-seated infections for whom traditional clinical samples (e.g., blood, urine) would be insensitive and high risk/impractical (e.g., biopsy for brain infection) or where rapid assessment of therapeutic effect is needed. In fact, in our clinical practice at Johns Hopkins Hospital, oncology patients with fever and neutropenia or patients with fever of unknown origin (FUO) routinely undergo whole body imaging to look for foci of

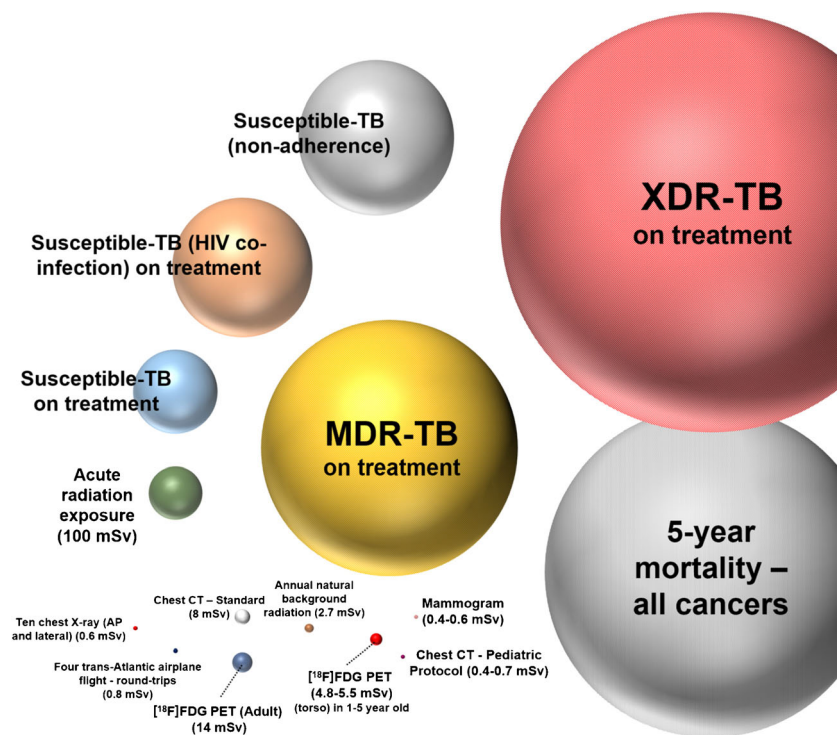


Fig. 2. Relative risk of infections with treatment- and radiation-induced cancer. The risk of mortality due to infections (TB as an example) compared to the risk of radiation-induced cancer due to commonly used imaging techniques. The risk of mortality for MDR- and XDR-TB patients on treatment is similar to that due to cancers. Even drug-susceptible TB patients on treatment have significantly higher risk of mortality than radiation-induced cancers with optimized imaging. The risk of radiation-induced cancer and mortality has been theoretically estimated based on acute radiation exposure (100 mSv), and actual risks are likely to be much lower for smaller amounts of radiation.

infection. However, current imaging modalities are not specific, and biopsies are often non-diagnostic, leading to uncertainty and overuse (or misuse) of broad-spectrum antimicrobials.

CT and other nuclear medicine techniques are often perceived to deliver high levels of radiation. However, recent technological developments have significantly lowered radiation exposure and also allowed rapid scans that avoids the need for sedation in children. For example, the effective dose for each chest CT in the child treated for XDR-TB was 0.4–0.7 mSv [9]. This is equivalent to 3 months of natural background radiation, a single screening mammography, or four trans-Atlantic airplane round trips [55–57]. Moreover, no sedation was required for this child as scan times were short (3 s). We calculated the risks related to infections, with TB as an example, and compared them to the various imaging techniques used currently (Fig. 2). The risk of mortality for patients with MDR- and XDR-TB on treatment is similar to that due to cancers [8, 52, 58]. Moreover, even patients with drug-susceptible TB on treatment have several orders of magnitude higher risk of mortality than the theoretical risk due to radiation-induced cancers [59, 60]. Development of nuclear imaging tracers with short half-life isotopes and rapid elimination from the body could further limit radiation exposure. While no studies should be performed without an excellent rationale and clinical indication, we need to be pragmatic about the (minimal) risks of imaging, especially when dealing with infections due to MDR organisms.

Given sub-pharmacological dosing, there are several approaches for translating PET tracers to humans [61], allowing more rapid translation of new imaging tools to the clinic. Some PET tracers discussed above are currently being tested in humans. Recently, a study in healthy volunteers demonstrated that  $^{18}\text{F}$ -FDS was safe, well tolerated, and rapidly cleared, following a single, intravenous dose [62], suggesting significant potential for use in humans.

## Imaging in the Developing World

During the past decade, developing countries, especially the BRICS nations (Brazil, Russia, India, China, South Africa), have witnessed significant increases in the installation and use of advanced imaging. For example, New Delhi (India) alone has >100 clinical MRI scanners (several with 3 T strength) [63, 64]. Moreover, CT, MRI, and PET/CT costs are substantially lower, ~\$50–\$100 for CT and MRI and \$300 for PET per scan at private (for-profit centers) in developing nations such as India than in the USA [53]. Mobile PET scanners and  $^{68}\text{Ga}$  PET agents that can be generated without the need of a cyclotron hold great promise for use in remote areas [29]. China has already overtaken the USA to become the largest economy in purchasing power parity, with India expected to become the second largest economy in purchasing power parity by 2050 [65]. Infections are rampant in the developing world, and given the considerable number of people living in big cities, advanced imaging has the potential to become a powerful routine clinical tool for the early diagnosis and monitoring of infectious diseases in developing countries.

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### Compliance with Ethical Standards

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### Conflict of Interest

The author declares that he has no conflict of interest.

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