Chapter 1
Introduction

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1.1 Global Burden of Infectious Diseases

Infectious diseases have been a major scourge to humankind for centuries and are likely to continue to do so for centuries to come in both the USA and globally. For example, in 2011, about 722,000 Americans developed hospital-acquired infections (HAIs) with *Enterobacteriaceae* (26.8%), *Clostridium difficile* (12.1%), *Staphylococcus aureus* (10.7%), and *Pseudomonas aeruginosa* (7.1%) accounting for the majority of infections [1], resulting in 75,000 deaths [2]. HAIs cost the US healthcare system tens of billions of dollars in direct costs annually, and additional loses in lost wages extended hospital stays and premature deaths annually [3, 4]. While major efforts are being made to curb such infection, increasing rates of drug-resistant pathogens [5–9], increasing use of invasive techniques such as implants, and immunosuppressive and cancer therapies [10] remain a major challenge in controlling these infections. Multidrug-resistant (MDR) bacteria can also be notorious sources of life-threatening infections with high rates of death [5–9]. Similarly, viral infections such as influenza cause millions of new cases and several 1000 deaths annually in the USA [11]. The burden of hospital-acquired and other infections is substantially higher globally. In addition, global diseases such as tuberculosis (TB) continue to be major killers worldwide. In 2015, *Mycobacterium tuberculosis*, the causative agent of TB, was responsible for 10.4 new cases of TB and 1.8 million deaths [12]. It is also estimated that one third of the world's population is latently infected with *M. tuberculosis*, and many develop reactivation disease (relapse), years after the initial infection. It is expected that care and prevention of

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S.K. Jain (ed.), *Imaging Infections*, DOI 10.1007/978-3-319-54592-9_1

tuberculosis (TB) patients in low and middle-income countries in 2016 alone will cost $8.3 billion. With alarming rise of MDR, extensively (XDR) and even totally drug-resistant (TDR) TB [13, 14], and poor treatment and monitoring options, we may be losing the battle, and *M. tuberculosis*, a single infectious agent, remains as one of the top ten causes of death worldwide [12]. Global travel and rapid spread of infections—swine flu, SARS-CoV, Ebola, and Zika virus—is also a major concern and led to several recent global pandemics [15, 16]. Finally, several infectious pathogens, e.g., *Yersinia pestis* (causes plague) from the *Enterobacteriaceae* family, are also recognized as biothreat agents [17].

1.2 Molecular Imaging

Molecular imaging focuses on understanding fundamental molecular pathways in live organisms and organ systems in a noninvasive manner. It is based on utilizing imaging biomarkers called "tracers" or "probes" which target specific molecular pathways by chemical interactions with the biological systems. The spatial localization of the biomarkers is determined by accurately measuring the source of the radionuclide attached to the biomarker, e.g., for nuclear medicine techniques such as single-photon emission computed tomography (SPECT) and positron emission tomography (PET), or by other methods (e.g., magnetic resonance spectroscopy (MRS)), providing two- or three-dimensional image representative of the molecular changes occurring within the area of interest. This allows exciting possibilities for medical application, including early detection and monitoring of diseases as well as study of basic biology and pathogenesis. Several molecular imaging techniques, e.g., PET, are also quantitative. Molecular imaging techniques are often used in conjunction with anatomic imaging, e.g., computed tomography (CT) and magnetic resonance imaging (MRI), for anatomic reference. The basic characteristics of common molecular imaging modalities are outlined in Table 1.1.

MRI is becoming widely available globally and has several advantages: (1) well established and widely available clinically, (2) does not utilize radiation, (3) capable of providing not only structural but also functional and metabolic information (e.g., MRS), (4) high tissue contrast and resolution, and (5) ability to provide both endogenous and exogenous contrast. However, current MRS techniques lack sensitivity, which are several magnitudes lower than that of nuclear medicine techniques (PET). One other limitation of MRI (compared to CT) is the relatively slower acquisition times. New MRI techniques are an area of active research and hold great promise.

While CT and nuclear medicine techniques are often perceived to deliver high levels of radiation, recent technological developments have significantly lowered radiation exposure and also allowed rapid acquisitions, which can avoid the need for sedation in children [18]. Figure 1.1 shows the risks of common imaging techniques compared with infections, nuclear medicine screening tests, and natural sources of radiation [19]. It should be noted that the risk of mortality for patients with drug-resistant infections can be similar to that due to cancers and substantially

Table 1.1 Characteristics of molecular imaging modalities

Modality	Agents	Sensitivity	Comments
Optical	Fluorescence, bio-luminance	Pico- to femtomolar	Excellent sensitivity but limited depth penetration and therefore applicable to imaging of small animals or superficial sites only; mostly two-dimensional
Nuclear SPECT PET	^{99m}Tc, $^{123/5}$I, ^{111}In ^{11}C, ^{18}F, ^{124}I	Nano- to picomolar	Excellent sensitivity and depth penetration with three-dimensional imaging, but radiation exposure; production facilities (e.g., cyclotron) for short half-life isotopes need to be close; relatively costly
MR spectroscopy	Endogenous	Micro- to millimolar	Excellent depth penetration with three-dimensional imaging without radiation exposure; low sensitivity and equipment is costly
Ultrasound	Functionalized microbubbles	Micro- to millimolar	Lower-cost imaging without radiation exposure; low sensitivity and generally operator dependent

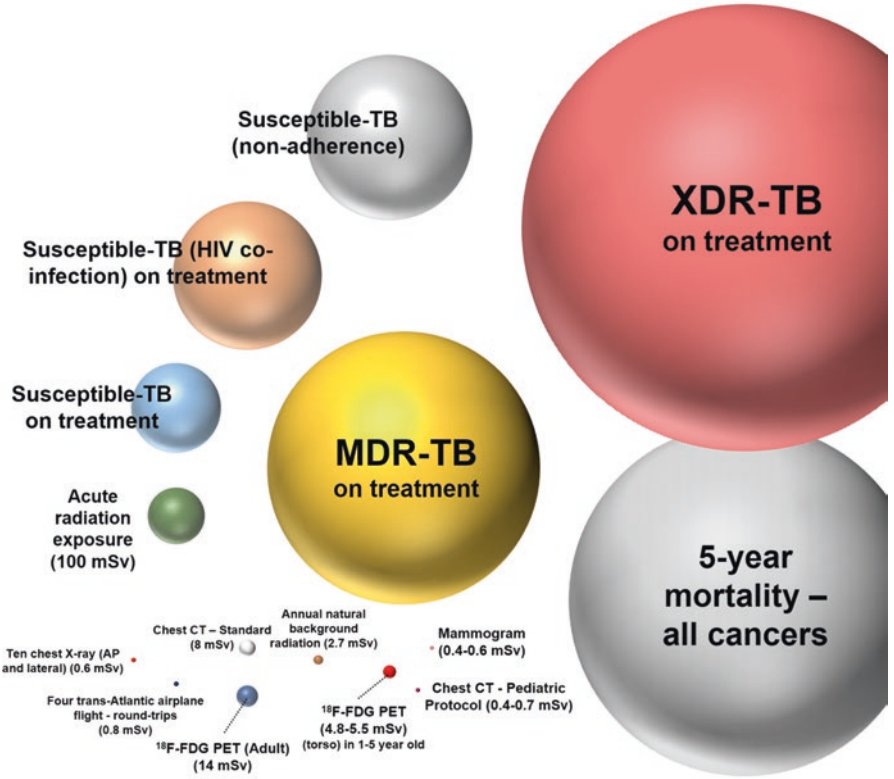

Figure 1.1 Risk of imaging. The risk of mortality due to infections (tuberculosis as an example) is compared to the risk of radiation-induced cancer due to commonly used imaging techniques. The risk of radiation-induced cancer and mortality have been theoretically estimated based on acute radiation exposure (100 mSv), and actual risks are likely to be much lower for smaller amounts of radiation. Adapted from Jain et al. [19]

higher than the theoretical risk due to radiation-induced cancers. Therefore, 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 drug-resistant organisms. Imaging tracers that are rapidly eliminated from the body could further limit radiation exposure.

1.3 The Problem

Traditional diagnostic tools, namely, microscopy, microbiology, and molecular techniques, are dependent upon sampling suspected sites of infection and then performing assays or tests. This approach is invasive, often terminal for animal experiments, and dangerous for deep-seated infections in humans, labor intensive, and time consuming (can take days), as well as subject to the uncertainties of incorrect sampling and contamination. Major advances have already been made in the use of molecular tools such as nucleic acid amplification (NAA), deep sequencing, matrix-assisted laser desorption/ionization, etc., for use in infectious disease research or patient care; however, they are all dependent on the availability of a relevant tissue sample(s). Given that infections are often localized, samples such as blood, urine, and bronchoalveolar lavage/washings cannot provide information on the crucial biological or biochemical changes at the sites of infection nor contribute to a specific diagnosis. This often warrants the use of invasive biopsies during patient care or necropsies in case of animal studies. Biopsies in patients 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.

1.4 The Solution

Tomographic imaging can evaluate disease processes deep within the body, noninvasively and rapidly. Noninvasive techniques, such as CT, MRI, and ultrasound, can provide a rapid means for diagnosing anatomical pathology and also provide a site of interest for invasive tissue sampling. However, CT and MRI are non-specific and cannot differentiate infections from noninfectious processes such as cancer or autoimmune diseases. Moreover, they reveal structural abnormalities that are often a late occurrence. Molecular imaging enables the visualization and monitoring of molecular processes and early events in living organisms noninvasively. It is therefore not surprising that nuclear molecular imaging tools such as SPECT and PET have powerfully augmented the investigation of various disease processes, both for research and patient care [20, 21]. Moreover, the ability to conduct noninvasive, longitudinal assessments in the same individual is a fundamental advantage over current methods utilized for infections. 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 therefore correlating well with the overall disease process. Some examples of the potential uses of molecular imaging for infectious diseases are outlined in Table 1.2.

Noninvasive imaging can be used to study disease pathogenesis in an unprecedented fashion as discussed here [22, 23]. For example, standard methodologies for studying TB cannot reliably monitor and identify the spatial location of lesions and

Table 1.2 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, e.g., assessing hideouts of infections, defining the diversity of the microbial populations (microbiome), etc. Studying multi-compartment antimicrobial pharmacokinetics Expedite bench-to-bedside translation of new therapeutics, e.g., surrogate end points to assess antimicrobial or vaccine efficacy or predict stable cure
	Clinical trials	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 and determine accumulation at nontarget sites to assess potential toxicities; current US Food and Drug Administration (FDA) guidelines require tissue drug distribution studies at the infected sites
	Patient settings	Enabling precision medicine by providing unique insights into disease pathogenesis, antimicrobial pharmacokinetics, etc.
Diagnosis	Clinical trials and patient settings	Rapidly and specifically distinguish an infectious process from other diseases (malignancy, sterile inflammatory processes, etc.) Determine the site (e.g., 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

(continued)

Table 1.2 (continued)

Role	Setting	Overall goal(s)
Monitoring and prognostication	Preclinical	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
	Clinical trials	Early end points for treatment trials to assess activity of treatments and to predict stable cure 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 Early end points for duration of treatment and predict stable cure enabling precision medicine
	Public health	Rapid determination of the infectious risk of a patient to the population based on response to treatment, extent, and location of disease Rapid diagnosis and monitoring of biothreat agents

Adapted from Jain et al. [19]

reactivation within the same animal because monitoring disease requires sacrificing animals. One study utilized ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/CT to study the temporal evolution of reactivation (relapse) pulmonary TB [23]. The same cohort of infected mice that develops TB lesions akin to humans [24–26] were serially imaged through pretreatment, TB treatment, and subsequent development of relapse. A novel diffeomorphic image registration method was successfully used to monitor the spatial evolution of individual pulmonary lesions. They found that while the majority of lesions during relapse developed in the same regions as those noted during pretreatment, several lesions also arose de novo within regions with no prior lesions. Contrary to the commonly held belief, this study suggested that dormant bacteria may also reside outside TB lesions and cause relapse in regions where no apparent lesions were present initially, which could prove valuable in the development of novel therapeutics. Another interesting application of pathogen-specific imaging is the detection of viral dynamics and localization in the context of human immunodeficiency virus (HIV). Santangelo et al. developed a technique to image total-body simian immunodeficiency virus (SIV) replication using antibody-targeted PET. They utilized a ^{64}Cu-labeled SIV Gp120-specific antibody, and we were able to detect virus-specific PET signals in treated and untreated monkeys [27]. This real time, virus-specific imaging method could have important applications for studying HIV pathogenesis, development of novel therapeutics, and even potential for clinical use.

1.5 Biocontainment

Several pathogens are designated as biosafety level 3/4 (BSL-3/4) and require appropriate handling and containment. Furthermore, work with biothreat pathogens requires regulatory approvals, as well as highly trained personnel [28]. There are two major ways of achieving containment: (A) installing imaging equipment inside the BSL-3/4 barrier (e.g., NIAID, University of Pittsburg). While this is logistically advantageous, maintaining equipment within these barriers can be expensive. (B) Therefore, some groups have utilized portable biocontainment devices that can seal infected animals within, which can then be transported to the imaging suite (housed in standard environments) (Figure 1.2) [29–31], or polycarbonate plastic tubes extending from the biocontainment space to the imaging equipment [32]. Weinstein and Liu et al. have also described a tail vein catheter system for on-table drug delivery to infected animals, while sealed inside the biocontainment device [33, 34].

Figure 1.2 Biocontainment devices. The imaging suite at the Center for Infection and Inflammation Imaging Research at the Johns Hopkins University School of Medicine is shown here. The imaging equipment is located in a standard environment inside the animal vivarium and adjacent to a biosafety level 3 (BSL-3) facility. Animals infected with *Mycobacterium tuberculosis* (requiring BSL-3 handling) are isolated inside airtight, self-contained biocontainment devices. Standard small animal anesthesia machines are used to deliver a mixture of isoflurane and oxygen during transport and imaging, and two 0.22 μm filters are used in series at both the inlet and the outlet of the biocontainment device to contain the infectious agents. The external surface of the biocontainment device is decontaminated and transported to the imaging suite for imaging. During prolonged anesthesia, an infrared thermometer and a heat lamp are used to measure and maintain ambient air temperature inside the biocontainment device

Containment of animals rather than the imaging equipment 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.

1.6 Human Translation of PET Tracers

Due to the use of sub-pharmacological doses used in most nuclear medicine studies, the risk to human subjects is limited due to the administration of the parent compound. Therefore, the requirements for preclinical safety testing are significantly simpler. The FDA currently accepts the use of extended (14-day) single-dose toxicity data in one mammalian species to support single-dose studies in humans [35], and this has streamlined this process for more rapid translation of new imaging agents to the clinic.

1.7 Imaging Infections: From Bench to Bedside

This book brings together multidisciplinary expertise to provide comprehensive information about molecular imaging of infectious diseases. We want to disseminate information about molecular imaging (to our infectious disease colleagues) and highlight the need for innovation and development of imaging technologies for infectious disease (to our molecular imaging colleagues). The overall goal is to spur interest and innovation to develop new imaging technologies for infectious disease and their translation to the clinic. Chapters 2–4 provide in-depth overviews, while Chapters 5–12 are more focused on discussing the concepts and techniques for infectious disease imaging.

1.7.1 Imaging in the Clinic

Unlike traditional tools used in infectious diseases, imaging can provide key spatial information about disease processes and enable whole-body detection and therapeutic monitoring of infections. This is especially relevant for patients with deep-seated infections or infections at unknown sites, for whom traditional clinical samples (e.g., blood, urine) would be insensitive, high risk, or impractical (e.g., bleeding risk, brain biopsy) or where rapid assessment of therapeutic effect is needed. In fact, oncology patients with fever and neutropenia or patients with fever of unknown origin (FUO) routinely undergo whole body imaging to look for foci of infection. Similarly patients with implants and orthopedic infections also utilize imaging extensively to guide management. Chapter 2 provides an authoritative overview of current imaging techniques used for infectious diseases, as well as the limitation of current tools, namely, that they are non-specific and dependent on host responses to infections.

1.7.2 Molecular Imaging Techniques

Optical imaging methods have been used extensively to study disease pathogenesis. Chapter 3 provides a detailed review of how in vivo optical imaging has permitted the noninvasive and longitudinal monitoring of host-pathogen interactions, including the dynamics of infection burden and dissemination, host immune responses, and the effects of treatments, all measured in the same live animals over time. These approaches have provided valuable information about microbial pathogenesis and treatment, while also significantly reducing animal numbers and cost. Although optical imaging was once considered to be applicable only to preclinical animal research, new methodologies such as photoacoustic imaging are advancing this technique into clinical practice.

While highly sensitive, optical imaging is limited by the depth of the signal, i.e., signals from deep inside the body cannot be visualized well, which however is not a limitation of nuclear medicine techniques. Chapter 4 provides a highly in-depth review of radiochemical methods to produce radiopharmaceuticals for SPECT and PET and also discusses the synthesis of several agents that have been used for infection imaging in the preclinical as well as clinical settings.

1.7.3 Imaging Host Responses

Chapter 5 discusses some of the emerging imaging techniques that measure host responses. These tracers can not only be used to study new biology but also to monitor disease. For example, imaging modalities targeting host inflammation, while non-specific, have been used to longitudinally monitor the natural history of some infections and/or responses to antimicrobial treatments in animal models as well as humans [18, 22, 23, 30, 36, 37]. Chen et al. reported that ^{18}F-2-fluoro-deoxy-D-glucose (^{18}FFDG) PET/CT imaging was superior to conventional (sputum) microbiology for monitoring response to treatments in adults with MDR-TB [38]. More recently, low-radiation exposure pulmonary CT imaging was used to monitor treatments for a highly drug-resistant form of TB (XDR-TB) in a 2-year-old child and guide an individualized drug regimen (Figure 1.3) [18]. Imaging agents with higher specificity for TB-associated inflammation have been shown to be even better at monitoring response to treatments in animal models [36].

1.7.4 Pathogen-Specific Tracers

An ideal imaging agent for diagnosing infections needs to be both sensitive and specific. However, current molecular imaging tools for infectious diseases are non-specific and dependent upon host responses that could be significantly altered in diseased states (e.g., cancer, AIDS). Therefore, there is a great need for the

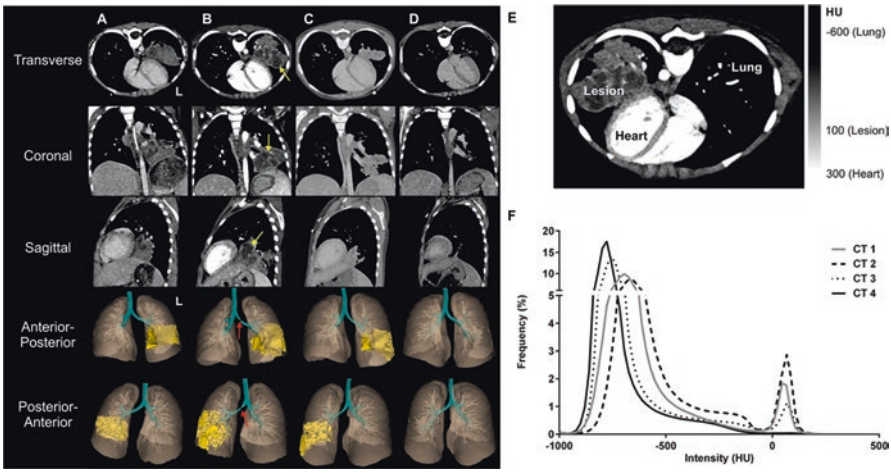

Figure 1.3 Serial CT to guide an individualized treatment in a 2-year-old with extensively drug-resistant (XDR) tuberculosis. (**a**–**d**) CT with intravenous contrast was performed using a protocol customized for children. The transverse, coronal, sagittal, and three-dimensional views of the lung parenchyma and the pulmonary infiltrates in the left lung are shown. Each panel corresponds to CT performed at initiation of first-line TB treatment (**a**, day 0), initiation of individualized XDR tuberculosis (TB) treatment (**b**, day 90), and 6 weeks (**c**, day 131) and 6 months (**d**, day 270) after initiation of XDR-TB treatment, respectively. Several necrotic (hypodense) central areas can be visualized in (**b**) (*yellow arrows*). Also, note the partial obstruction of the left main bronchus in (**b**) (*red arrows*). Marked improvement with resolution of necrotic areas is noted after 6 weeks (**c**) and near-complete resolution of the infiltrate after 6 months (**d**) of XDR-TB treatment, respectively. (**e**) Transverse CT image (post-contrast, day 90), showing the Hounsfield Unit (HU) densities in the chest cavity (lung, pulmonary lesion, and heart). (**f**) Histogram showing the HU densities of the segmented CT lung images. The pulmonary lesions correspond to −15 to 210 HU (smaller peaks on the right, which decrease with treatment). Lesion volumes were calculated at each time point by integrating the area under the corresponding curve. The lesion volumes were verified manually by drawing regions of interest. Each trace corresponds to CT performed at day 0 (CT1), day 90 (CT2), day 131 (CT3), and day 270 (CT4). *L* = left side. Adapted from Salazar-Austin et al. [18]

development of pathogen-specific imaging tracers. Chapters 6 and 7 focus on emerging targets and tracers for developing pathogen-specific imaging agents for bacterial and fungal infections, respectively. Chapter 7 also briefly discusses the use of imaging for select parasitic infections. There is also discussion on approaches for rationale selection of targets for pathogen-specific imaging tracers in Chapter 6. For example, there have been several recent studies showing the feasibility of pathogen-specific nuclear tracers in animal models [39–46]. MRI techniques have also been used to specifically image bacteria in mice [47]. It should be noted that clinically apparent (acute) infections often have high pathogen burden, e.g., 8.3 $\log_{10}$ CFU/ml of bacteria [48], and can be several centimeters in diameter with volumes of tens to hundreds of milliliters [49, 50]. Therefore, current pathogen-specific imaging agents in development could in principle provide early detection of infections in patients. In addition to diagnosis, pathogen-specific imaging can also be a great tool for predicting treatment responses [41, 42]. For example, ^{18}F-fluorodeoxysorbitol

(^{18}F-FDS) PET was able to rapidly (within 24 h) monitor the efficacy of antimicrobial treatment and identify therapeutic failures associated with drug-resistant, extended-spectrum β-lactamase (ESBL)-producing *E. coli* infections [42].

1.7.5 Neuroimaging

The field of neuroimaging is relatively advanced, and descriptions of cross-sectional imaging of the central nervous system (CNS) have been available for more than two decades. This has resulted in a large array of descriptive criteria capable, in most circumstances, of narrowing the differential diagnosis, detecting life-threatening complications, and establishing baseline for assessment of treatment response and is described briefly in Chapter 8. The availability of pathogen-specific imaging agents/ligands can have a great effect on the management of patients with CNS infection. Besides early diagnosis, avoidance of diagnostic brain biopsies can have significant effect on the mortality and morbidity of patients.

1.7.6 Antimicrobial Development

Vancomycin, a widely used antimicrobial to treat drug-resistant gram-positive bacteria, highlights some of the limitations of current tools for antimicrobial dosing. Methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia and methicillin-susceptible *S. aureus* (MSSA) pneumonia treated with vancomycin have been associated with mortality of 50% and 47%, respectively, versus only 5% for MSSA pneumonia treated with β-lactam antibiotics [51]. Vancomycin is a relatively large molecule and penetrates poorly into the alveolar lining fluid (ALF). As a result, levels attained in ALF are only one-sixth of the plasma concentration [52]. Conversely, high levels of vancomycin can cause nephrotoxicity. Based on these data, and recent studies demonstrating better efficacy with higher-dose vancomycin, new recommendations have been made for treating pulmonary infections [53]. Chapters 9 and 10 discuss the concepts behind developing PET imaging for multi-compartment pharmacokinetic and pharmacodynamics assessments of antimicrobials, with an eye toward clinical translation and as tools to optimize antimicrobial dosing strategies by achieving appropriate concentrations at the target, infected tissues.

1.7.7 Image Analyses

Current practice in the diagnosis and treatment of infections relies on radiologic image evaluation combined with clinical information. These tasks are challenging because radiologic manifestations of infections are associated with a large spectrum of nonspecific patterns. The qualitative judgments of radiologists can be improved by quantitative image analysis techniques. These concepts as well as automated and computer-aided image analyses tools for infectious diseases are discussed in Chapter 11.

1.7.8 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 [54, 55]. Moreover, the costs of advanced imaging are substantially lower in some of these countries [32]. Mobile scanners and ^{68}Ga-PET agents that can be generated without the need of a cyclotron hold great promise, especially in these settings [56]. China has already overtaken the USA in purchasing power parity, with India also expected to do so by 2050 [57]. Since infections are rampant in the developing world, and these countries have huge populations living in big cities, advanced imaging has great potential. Chapter 12 discusses some of the challenges and opportunities of imaging of infections in the developing world.

1.8 Summary

Infectious diseases are a major cause of morbidity and mortality worldwide and in the USA. Overall costs and morbidity are expected to continue to rise due to increasing rates of drug-resistant pathogens, use of invasive techniques such as implants, as well as immunosuppressive and cancer therapies. Tomographic molecular imaging techniques enable rapid visualization and monitoring of molecular processes noninvasively and promise unparalleled opportunities for field of infectious diseases. These technologies are an emerging field of research, overcome several fundamental limitations of current tools, and could have a broad impact on both basic research and patient care. Beyond diagnosis and monitoring disease, these technologies could also provide a uniform cross-species platform for animal studies, allow unique insights into understanding disease pathogenesis, and expedite bench-to-bedside translation of new therapeutics. Finally, since molecular imaging is readily available for humans, validated tracers could also become valuable tools for clinical applications and for enabling personalized medicine for infectious diseases.

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