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Future Diagnostic Agents

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Timely and specific diagnosis of infectious diseases can be clinically challenging but essential for the patient's outcome. Laboratory tests, such as a blood culture or urine specimen, can detect the responsible micro-organism but cannot discriminate between sterile inflammatory disease and truly infectious disease. Imaging tests, like scintigraphic techniques, can pinpoint the infection in the body. There are a number of clinical scintigraphic tests from which to choose, and no single test is optimal for the various presentations of clinical infectious disease. The currently available radiopharmaceuticals often are not capable of distinguishing between sterile inflammation, and bacterial or fungal infections. Neutrophil-mediated processes, characteristic for both inflammatory and infectious processes, can be targeted in situ by radiolabeled leukocytes, antibodies or fragments, or even by cytokines and ^{18}F-fluorodeoxyglucose. Unfortunately those techniques are not infection-specific markers, and ongoing research is in progress to tackle this problem. The most promising option in this respect is directly targeting bacteria or fungi with radiolabeled antibiotics or antimicrobial peptides. These theoretically highly infection-specific radiopharmaceuticals could be used for monitoring the success of antimicrobial therapy of infectious disease. Although results from preclinical experiments and pilot studies in patients are promising, radiolabeled anti-infective agents are not currently in routine clinical use and studies are continuing to prove their effectiveness for diagnostic imaging of infections in the future.
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Accurate and prompt diagnosis of infectious disease is crucial for initiating appropriate therapy and patient management. Although superficial lesions can be readily accessed and rapidly diagnosed, deep-seated sites of obscure or persistent infection pose a serious challenge to the clinician and result in the use of procedures that may reveal inconclusive findings. This difficulty is commonly encountered in the management of disorders such as pulmonary, intra-abdominal, and cerebral processes, but also in fever of unknown origin, spinal infections, and complicated osteomyelitis. The latter conditions are more observed frequently in the elderly and oncological patients, both of which are immunocompromised populations.[1-5] In view of the increasing use of immunosuppressive agents in patients who receive transplants, more intensive anticancer therapies, and patients with acquired immunodeficiency syndrome (AIDS), far more knowledge and greater awareness of the presence of opportunistic infections must be acquired.

Detecting and monitoring infectious processes can be achieved with the use of various techniques, including conventional radiography (radiograph), ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI). The cross-sectional imaging techniques, such as CT and MRI have proven useful in detecting organ infections and musculoskeletal infections.[6,7] Because of their excellent spatial resolution, these methods have allowed clinicians to assess more accurately the depth of a superficial infection, thereby aiding in diagnosis and surgical planning.[8] However, these techniques rely solely on *morphological* changes and, therefore, most abnormalities can only be detected at advanced stages of disease and diagnosis of early infections and differentiation between active and structural but indolent alterations following surgery or other interventions can be difficult. Furthermore, in situations in which normal anatomy is distorted by postsurgical changes, scarring, or the presence of implants and/or vascular grafts, the diagnostic role of these techniques is limited. Morphological imaging methods cannot differentiate between sterile inflammation and infection

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and do not allow for monitoring the success of antimicrobial therapy.[9]

In these settings, functional imaging procedures can in many cases provide useful and complementary information. In contrast to morphological imaging studies like computed tomography (CT) and magnetic resonance imaging (MRI), nuclear medicine procedures can determine the location and the degree of disease activity in infectious processes based on physiologic and/or metabolic changes that are associated with these diseases. Today, there remains a need for a diagnostic radiopharmaceutical agent that can distinguish between infection and sterile inflammation.[10] To quote from a 20-year-old seminal article on infection imaging, Rubin and coworkers[11] state:

> *. . . the need for techniques for anatomically delineating the primary and metastatic sites of infection; the need for noninvasive imaging techniques that could be performed repetitively to assess the response to therapy; the need for a noninvasive technique for in vivo, specific diagnosis that would obviate the need for invasive biopsy procedures; and the need for targeted therapy of such infections as those caused by fungi that would permit more aggressive therapy of the process with lesser amounts of systemic toxicity.*

This statement is still valid in patients with infection. The need for such an infection-specific imaging tracer is even emerging as the result of several reasons. First of all, the spectrum of involved micro-organisms is changing as the result of the intensive treatments in cancer patients but also because of the increased use of devices, shunts, and implants.[5,12] Second, increasing aging of the global population is a major risk factor for developing iatrogenic and opportunistic infections. Third, HIV and tuberculosis infections are rapidly increasing, especially in developing countries. Last, but not least, inappropriate use and overuse of antibiotic treatment in developed countries induces the major problem of bacterial resistance to antibiotics. In these cases, imaging studies assume a major role in diagnosing/delineating the infectious focus and in planning appropriate/targeted antibiotic therapy.

Recent developments in the field have substantially improved the ability of nuclear techniques to detect infectious disease. These new methods include single-photon emission computed tomography (SPECT) tracers such as radiolabeled chemotactic peptides,[13] radiolabeled liposomes,[14] avidin-mediated imaging,[15,16] radiolabeled antibiotics (eg, ciprofloxacin),[17,18] and monoclonal antibodies. Some of these agents also can be labeled with positron emitters such as fluorine-18 (^{18}F) and gallium-68 (^{68}Ga) and can be applied for positron emission tomography (PET) imaging.[19]

It is crucial to understand the pros and the cons of each of these future diagnostic agents. It depends on several factors that are based on the knowledge of the pathophysiological aspects of different forms of infection and also the cascade of cellular and immunological reactions in inflammation and infection (Fig. 1).[20] This overview focuses mainly on the development and testing of new infection-targeting radiopharmaceuticals dedicated to specific imaging of infectious diseases and their possible role in the future.

Imaging of Infectious Disease by New SPECT and PET Tracers

Infection imaging may appear simple but is actually quite complicated because of a multitude of factors influencing and interfering with the imaging process by the choice and design

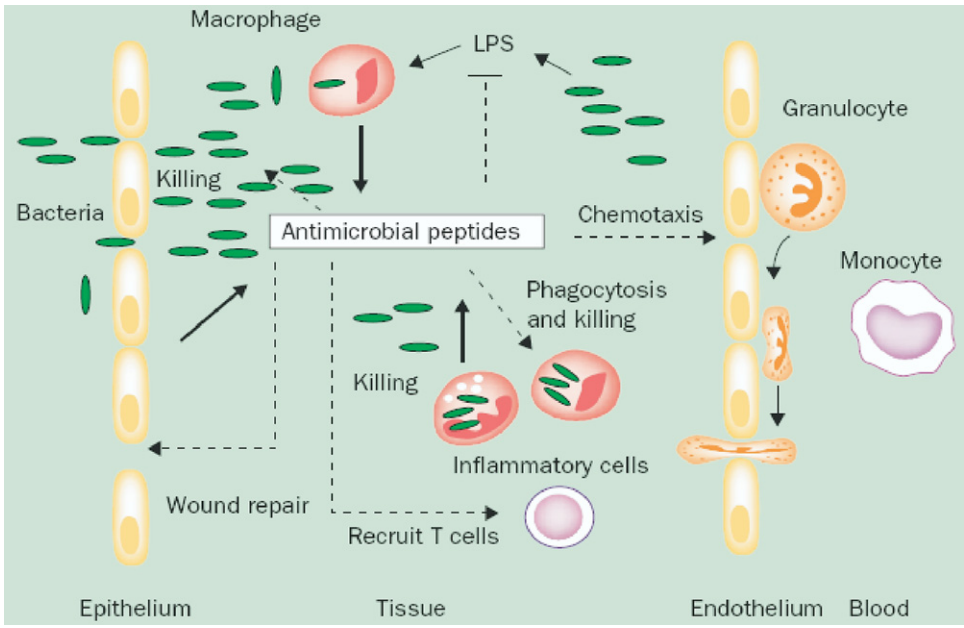

Figure 1 Schematic representation of the role of antimicrobial peptides on the complex network of innate immunity in the pathogenesis of bacterial infection. LPS, lipopolysaccharides. (Reprinted with permission from Lupetti et al.[20])

Table 1 Requirements for An "Ideal" Radiopharmaceutical for Imaging Infection: Magic Bullet Concept

1.	Rapid localization and good retention at site of infection
2.	Rapid clearance from noninfected tissues (high target to background ratios)
3.	Uptake in infection not in sterile inflammation, identifying bacterial, viral and fungal infections
4.	The uptake of the radiotracer should be proportional to the degree of infection, therapy response can be monitored
5.	No pharmacological effect/immunological response, safe use, repeated injection is feasible
6.	Labeling should be simple and uncomplicated and well characterized
7.	Preferably ^{99m}Tc-labeling, positron-label is still experimental.
8.	Does not depend on host leukocyte functions
9.	Less expensive than other combined modalities
10.	Radiation dosimetry should be acceptable

of radiopharmaceuticals.[21] Superimposing factors from infection and inflammation further complicate their use in clinical infectious diseases, leading frequently to a combination of different techniques. Following the pioneering work by McAfee and Thakur, who used labeled leukocytes for infection imaging, new radiopharmaceuticals have emerged on a regular basis.[21] Nevertheless, although not specific for infection, the visualization of radiolabeled leukocytes (with labeling both by in vivo and in vitro methods) have taken a central role in infection imaging during the last decades in clinical practice.[9,22-24]

The ideal radiopharmaceutical (Table 1) should enable early diagnostic imaging, a low absorbed radiation dose, and make a distinction between inflammation and infection, which is of paramount importance in the field of various infections, including musculoskeletal, soft-tissue, and parenchymal infections. Furthermore, it should be nontoxic, inexpensive, readily available, and rapidly cleared from the blood and the body. Finally, it should not accumulate in major target organs, and its preparation should be simple and rapid.[25-27] Many of our currently available radiopharmaceuticals share some of these criteria, but all of them lack specificity.[28] Most importantly, until now, no single radiopharmaceutical has emerged in the clinical routine that can clearly distinguish infection from sterile inflammation.[9,21] Furthermore, in the modern era of multiresistant organisms and immunosuppressed patients, any new "perfect" diagnostic agent needs to be effective in an environment with leukocytes reduced in numbers and functioning.[28] There are 3 main areas of development, namely the introduction of specific micro-organism–targeted agents such as radiolabeled antibiotics, antifungals, and antimicrobial peptides; secondly the introduction of monoclonal antibodies against specific components of micro-organisms; and last but not least, the introduction of PET technology with ^{18}F and ^{68}Ga.

Targeting the Leukocyte

After activation by leukocytes residing at the site of infection, blood leukocytes expressing several receptors become activated and migration from the bloodstream into the interstitial space can take place (diapedesis). Small diffusible molecules, such as proteins and peptides, which can readily cross the activated endothelium, include radiolabeled chemokines, cytokines, and somatostatin analogs, targeting respectively circulating and migrated neutrophils, lymphocytes, and mononuclear cells.[29] These receptor-based imaging tests are not solely specific for infectious processes but also are positive in inflammatory diseases. Nevertheless, they can be used in the future as a useful inflammation-seeking radiotracer, similar to ex vivo radiolabeled leukocytes, which are the gold standard for imaging inflammation and infection.[22] Unfortunately, some of these, such as cytokines and chemotactic peptides, even at low doses, are highly immunogenic and cytotoxic, which can be a major limitation when these types of agents are used in the future.[21,30] Furthermore, their radiochemistry is still unclear and, according to Food and Drug Administration regulations, this prevents it's clinical use. More promising nowadays is the acute phase cytokine interleukin-8 (IL-8).

IL-8 is a member of a family of proinflammatory cytokines, and its best-characterized activities include the chemoattraction and activation of neutrophils.[31] Holmes and coworkers[31] reported the IL-8 receptor, which is a member of the G protein-coupled super family, is partially identical to receptors involving other neutrophil chemoattractants, such as fMet-Leu-Phe and C5a. Moreover, they found that mammalian cells transfected with the IL-8 receptor cDNA clone bind IL-8 with high affinity. The binding mechanism of this small protein (8.5 kDa) to neutrophil receptors CXC1 and CXC2 has been quite extensively studied in vitro.[32,33] Two major drawbacks of the model are the use of an enormous amount of micro-organisms (10^{11} CFU) and the choice of *Escherichia coli* bacteria for soft-tissue infections, which are both clinically irrelevant.

The Nijmegen group has recently developed a pulmonary infection rabbit model, exploring the feasibility of targeting 3 different pulmonary infections experimentally induced by *Aspergillus, Streptococcus*, and *Escherichia coli* species with ^{99m}Tc-labeled IL-8.[1] They demonstrated excellent visualization of localization and extent of pulmonary infection in all the 3 models, which was to be expected because of the large number of bacteria and infiltrating leukocytes present at the site of infection. Moreover, further analysis confirmed a relationship between the amount of circulating neutrophils and uptake of ^{99m}Tc-labeled IL-8 in the affected tissues, reflecting greater uptake in immunocompetent versus neutropenic rabbits, also compatible with the neutrophil-driven power of IL-8 agents. Therefore, the lower levels of IL-8 receptor-expressing cells in immunosuppressed animals makes this tracer unsuitable for infection imaging in immunocompromised patients, who are the major group of patients eligible for infection imaging. Finally, the authors emphasized the

Table 2 Physical, Radiochemical Properties, Presumed Uptake Mechanisms, Advantages, and Disadvantages of Various

Agent	Interleukin-8	LTB-4 Antagonist	AB Fluoroquinolones
Proposed mechanism of uptake/activity	Receptor mediated (CXC1 and 2); Chemoattraction and activation of neutrophils; thus migration of in vivo labeled WBCs	Receptor mediated, expressed on activated/infiltrated neutrophils, thus migration of in vivo labeled WBCs	Blocking bacterial DNA replication by inserting itself between DNA and the enzyme DNA gyrase, causing double-stranding breaks in the bacterial chromosome
Biodistribution routes of excretion	Kidney, bone marrow, liver, spleen	Kidney, bone marrow (variable), liver, spleen	Kidney, spleen
Preparation/ labeling procedure	In vivo labeling/HYNIC	In vivo labeling/HYNIC	Kit
Dose (adult)	400 MBq	—	370 MBq
Physical properties	Generator $T_{½}$ = 6 hours Photopeak: 140 keV	Generator $T_{½}$ = 6 hours Photopeak: 140 keV	Generator $T_{½}$ = 6 hours Photopeak: 140 keV
Effective dose equivalent (mSv/dose)	2.7	—	3
Timing/imaging characteristics	4 to 24 hours	—	4 to 24 hours SPECT
Instrumentation	Gamma camera	Gamma camera	Gamma camera
Advantages	High specific activity, no risks of infection or cross contamination	High T/NT ratio; no risks of infection or cross contamination	Low bone marrow uptake, low accumulation in liver and intestines; PET derivates
Limitations/ disadvantages	Lack of in vivo studies testing the affinity; Neutrophil-tracer, present in acute infections, less useful in neutropenic and/or non-neutrophil-mediated and/or low-grade infections, possible side-effects, no differentiation from fungi infections; until now human results comparable to in vitro WBCs	Specific receptor mechanism is not known; Neutrophil-tracer, present in acute infections, less useful in neutropenic and/or non-neutrophil-mediated and/or low-grade infections, possible side-effects, no differentiation from fungi infections; molecule not available	No exact radiochemical structure; a-specific binding (wash-in/out) thus responsible for accumulation in bacterial infected tissues as well in sterile inflammatory lesions; no differentiation from fungi/tuberculous infections

AB (antibiotic) fluoroquinolones (ciprofloxacin-norfloxacin-sparfloxacin-enrofloxacin-levofloxacin-pefloxacin-lomefloxacin-ofloxacin).

potential to cover a broad range of future clinical applications in detection of human pulmonary infections.[1]

Subsequently, the same group tested the safety of a 10 μg ^{99m}Tc-labeled IL-8 in humans and assessed the value of ^{99m}Tc-labeled IL-8 scintigraphy in patients with suspected musculoskeletal infections, including prosthetic joint and osteomyelitis.[34] Scintigraphy with ^{99m}Tc-labeled IL-8 correctly identified the infection after 4 hours in 10 of the 12 patients being tested for an infection, 2 patients being scored as false-negative (1 with vertebral osteomyelitis and 1 with a low-grade knee prosthesis infection). There were no false-positive cases. Together for the 20 patients in this study, the sensitivity, specificity, and accuracy of ^{99m}Tc-labeled IL-8 scintigraphy were calculated as 83%, 100%, and 90%, respectively. In addition, in 3 patients with coexisting malignant disease, ^{99m}Tc-labeled IL-8 uptake was observed in none of them, reflecting the specificity for bacterial infections over malignancy of this agent. Early biodistribution analysis showed physiologic uptake of ^{99m}Tc-labeled IL-8 in the kidneys, bone marrow, liver, and spleen with rapid clearance from the blood pool and nontarget tissues. Later images showed migration of radioactivity that accumulated earlier in the bone marrow to the infected tissues indicating migration of radiolabeled leukocytes to the site of infection. Most importantly, there were no significant side effects after the injection of ^{99m}Tc-labeled IL-8 and no significant adverse effect was noted in the peripheral leukocyte counts. In summary, technetium-IL-8 is an interesting candidate to replace radiolabeled leukocytes by in vivo labeling of neutrophils (Table 2). Clinical studies with larger numbers of patients with various infec-

Promising Infection-Specific Future Diagnostic Agents Tested Both In Vitro, in Animals and Results in Human Trials

Antimicrobial Peptides	^{18}F-FDG/FDG-WBC	Bacteriophages
Perforation of membranes (bacteria, viruses. fungi, parasites), membrane instability, and bacteriolysis intracellular: DNA and mitochondrial inhibition immunostimulatory activity	Up-regulation of GLUT in activated granulocytes, lymphocytes and macrophages, migration of WBCs (diapedesis/chemotaxis)	Viruses, attachment to specific surface receptors, transferring their genetic material into the host cell for reproduction
Variable to low accumulation in the liver, no bowel deposits, kidney	Brain, heart, liver, bone marrow (mildly), GU tract, GI tract	Liver, lung, gut
Kit	WBCs in vitro labeling by HMPAO	Kit/MAG-3
74 to 222 MBq (children)	370 MBq	—
Generator	Cyclotron	Generator
$T_{½}$ = 6 hours	$T_{½}$ = 110 minutes	$T_{½}$ = 6 hours
Photopeak: 140 keV	Photopeak: 511 keV	Photopeak: 140 keV
<2	8 to 10	—
1 to 2 hours	Fast (2 to 4 hours), CT if available	—
Gamma camera	PET camera or coincidence gamma camera	gamma camera
Specific and fast targeting living micro-organisms; low affinity for host cells (not tumors); development of resistant bacteria unlikely; suitable for therapy monitoring; UBI well tolerated, kit formulation; can be prepared synthetically in large amounts and lack immunological side-effects; PET derivates	Superior imaging characteristics; high target-to-background ratio, low bone marrow/bone uptake for FDG; all-in-one technique (with CT); high interobserver agreement; quantifying FDG uptake	Specific targeting bacterial cells, and not mammalian cells, cocktail of phages for each bacterial strain; some of them high T/NT ratio
Cannot discriminate between bacterial or not-bacterial infection; bacteriolysis; eliminating the target antigen; not able to detect intracellular infectious sources; anti-viral peptides still under research	Poor specificity (activated leucocytes/neoplastic cells); poor availability; high cost; WBCs not useful in leukopenia (WBC < 2000/mm^3); labor-intensive; risks of infection; cross contamination Tc-HMPAO: unstable complex	Binding both living and heat-killed bacteria, partly non-specific diffusion across the endothelial lining; safety and efficacy have yet to be validated

tions are warranted in the future but the lack of radiochemical characterization and the possible dangers of side effects may, according to Food and Drug Administration regulations, prevent its use in humans.[34]

The leukotriene (LT) B4 receptors, expressed on polymorphonuclear granulocytes, are involved in leukocyte function during the inflammatory response, chemotaxis, and chemokinesis, and are therefore not specific for the detection of infections.[35,36] Several studies have been performed with ^{111}In-labeled LTB4 antagonist to visualize infection and inflammation by targeting specific receptors expressed on the membrane of activated and infiltrated neutrophils.[37] The BLT subtype 1 receptor is of the high-affinity type and is found primarily on neutrophils.[35] The receptor–interaction process was highlighted by adding excess cold molecules in experiments.[38,39] Moreover, the same Nijmegen group showed that in infected animals, there is an increasing accumulation in the abscess and a decreasing accumulation in the bone marrow, in contrast to an increasing accumulation in the bone marrow of healthy control animals. This observation indicates specific migration of labeled LTB4 labeled bacteria and/or white blood cells into the inflamed region.[37] ^{111}In-labeled DPC11870 (a bivalent DTPA-conjugated LTB4 oligopeptide) rapidly revealed infectious and inflammatory lesions in several rabbit models of acute inflammation.[40] Biodistribution analyses demonstrated physiologic uptake in kidney, bone marrow, and spleen and also substantial uptake in the infected region.

Recently, 2 new 6-hydrazinonicotinic-conjugated ^{99m}Tc-labeled compounds structurally related to DPC11870 were

developed with similar success to the ^{111}In-labeled derivate, avoiding the unfavorable radiation characteristics of ^{111}In.[41] Comparison of the ^{99m}Tc-labeled bivalent and labeled monovalent LTB4 antagonists revealed that images after injection of the monovalent ^{99m}Tc-labeled MB88 allowed, compared with the bivalent ^{99m}Tc-labeled-MB81, superior abscess visualization because of rapid clearance from nontarget tissues and preserved accumulation in the abscess. Additionally, the muscle abscess was delineated earlier with ^{99m}Tc-labeled MB88 than with the bivalent analog ^{99m}Tc-labeled MB81. Also compared with the ^{111}In-labeled DPC11870 molecule, despite similar pharmacology in abscess and kidney uptake, better images were obtained because of lower radioactivity concentrations in the bone marrow of animals injected with the monovalent ^{99m}Tc-labeled MB88 LTB4 antagonist than for the bivalent analog ^{99m}Tc-labeled-MB81. Therefore, among the 3 LTB4 antagonists, the monovalent ^{99m}Tc-labeled MB88 is the most potent and promising agent for visualizing and evaluating infection and inflammation in patients, allowing further a fair comparison with the other currently studied agents by this group (Table 2).[41]

Targeting the Micro-Organism

A totally different strategy is to target micro-organisms directly in vivo without the need for intervening leukocytes, an approach that has been adopted in the development of new radiopharmaceuticals such as radiolabeled ciprofloxacin and antimicrobial peptides.[21] Since the 1980s, several groups have reported on the potential role of using radiolabeled antibiotics as SPECT or PET tracers,[42] ^{18}C- and ^{3}H-labeled antibiotics,[43] ^{18}F-fluconazole,[44] ^{99m}Tc-erythromycin, and ^{99m}Tc-streptomycin.[45]

Still controversial, one of the most tested radiolabeled antibiotics is ^{99m}Tc-labeled ciprofloxacin (Infecton; Draximage Inc, Quebec, Canada), introduced by Britton and colleagues.[46] Ciprofloxacin is a fluoroquinolone antibiotic in which the antimicrobial activity is mediated by insertion between DNA and the bacterial DNA gyrase and/or topoisomerase IV enzymes, which are present in all dividing bacteria, even in those resistant to ciprofloxacin for the pathogens tested.[17,47] ^{99m}Tc-labeled ciprofloxacin (Infecton) has been reported to successfully evaluate various infectious diseases in humans.[48,49] It has a favorable biodistribution because it is mainly excreted by the kidneys and shows has low liver metabolism. Consequently, low nonspecific bowel uptake is seldom a problem for abdominal imaging.[18] Moreover, the lack of bone marrow uptake is particularly useful for the detection of osteomyelitis.[50] Although the exact chemical structure of ^{99m}Tc-labeled ciprofloxacin has never been determined, the radiolabeled compound is believed to retain most of the pharmacokinetic and pharmacodynamic properties of unlabeled ciprofloxacin and accumulates in vivo within bacterial cells in a nonspecific fashion.[49]

It has been suggested that Infecton only binds to living bacteria and, thus, it has been proposed as an agent that is able to distinguish sterile inflammation from bacterial infection. However, since its introduction in 1993, this has never been confirmed. Initial clinical studies showed high accuracy in various infectious conditions, including orthopedic infections, arthritis, pelvic inflammatory disease, and a variety of bacterial and nonbacterial infections. The largest trial for Infecton in about 900 patients was conducted in the framework of an International Atomic Energy Agency Coordinated Research Project. In this study, the overall calculated sensitivity for this tracer was 88%, whereas the specificity was determined at 82%.[48] Other clinical work showed a lower specificity of this radiopharmaceutical for infections than data obtained with earlier studies.[50-52] Unfortunately, there are still a lot of controversial data for imaging in vitro, both in animals and in human studies on the infection-specific targeting capacity of Infecton. Possible explanations for these contradicting results in both the preclinical and clinical settings include the presence of ciprofloxacin-resistant bacteria, insufficient numbers of viable intralesional bacteria and nonspecific binding to dead intralesional bacteria, and the similar use of antibiotic therapy before imaging.[53] Additionally, there is clear evidence that the part of the ciprofloxacin molecule that interacts with bacterial DNA is also responsible for binding to mammalian DNA abundantly present in infiltrating leukocytes (Table 2).[54]

Siaens and coworkers of the Ghent group reported on the binding of ^{99m}Tc-labeled ciprofloxacin and ^{99m}Tc-labeled enrofloxacin in vitro in a rat model of intramuscular infection versus inflammation.[55] Similar to the results of Alexander and coworkers, they confirmed that neither of the 2 tracers showed specific binding to living or heat-killed bacteria. Moreover there was no difference in fluoroquinolone uptake between infectious and inflammatory lesions. Siaens and coworkers argued that this was possibly the result of vital changes in the conformation of the fluoroquinolone molecules or modification of active sites as the result of complexation with technetium. Differences in labeling conditions cannot be excluded.[55] The exact binding potential mechanism of the ciprofloxacin complex was recently revealed by Langer and coworkers and Zijlstra and coworkers. They labeled ciprofloxacin with a fluorine positron tracer, thereby preserving the pharmacokinetic and pharmacodynamic properties.[56,57] Furthermore, they used ^{18}F-ciprofloxacin PET both in vitro and in a clinical study with a small group of patients presenting with soft-tissue bacterial infections.[58] Their results demonstrate low uptake of ^{18}F-ciprofloxacin by bacteria and poor retention in granulocytes as well.

Moreover, as others have suggested, the specific binding to bacterial DNA/DNA gyrase was masked by a high level of nonspecific binding.[57,58] It was suggested that increased local blood supply, together with increased vascular permeability, expansion of the extracellular space, and probably an increase in leukocyte accumulation, may enhance transudation of the radiolabeled (both SPECT and PET) ciprofloxacin at the inflammatory-postsurgical sites, as is commonly reported for the other currently available SPECT tracers, like ^{67}Ga-citrate and radiolabeled leukocytes (Table 2).[9,55,58,59] Given that the structure of the investigational agent Infecton is still unknown, controversial results could be attributed mainly to the formation of various radiolabeled species and stability of

Table 3 Imaging Experimental Infections With (Directly) Labeled Nonfluoroquinolone Antibiotics

^{99m}Tc-Labeled Antibiotics	Mechanism	Evaluation
Macrolides Erythromycin Streptomycin	Binding to the 50S subunit of the bacterial 70S rRNA complex, inhibiting protein synthesis and replication	Accumulation in inflammatory and infected tissues. High uptake in bile and liver with intestinal deposits.
Cephalosporins Ceftizoxime Cefoperazone	Disrupt the synthesis of peptidoglycan layer and thus the structural integrity of the bacterial cell walls	Accumulation in inflammatory and infected tissues. High uptake in bile and liver with intestinal deposits.
Kanamycin	Frameshift mutation and prevents the translation of RNA	Accumulation in *S. aureus* infected tissues. No deposits in intestines.
Isoniazid	Inhibits the synthesis of mycolic acid in the mycobacterial cell wall	Specific tracer for the detection of *M. tuberculosis* infected lesions especially in the lungs

the complex. Draximage recently published data on a Phase II clinical study performed in the United States. Despite these improvements in the standardization of preparation of ^{99m}Tc-labeled ciprofloxacin, this radiopharmaceutical showed poor specificity and accuracy in patients with osteomyelitis at 2-hour (early) as well as at 24-hour (late) images.[9] In their experience, the tracer disappeared from sites of infection as well as from inflammation with equal rapidity. This results raises again the issue of whether the uptake is specific or only a blood pool effect.[9]

Because Infecton showed the detection of bacterial infections with poor specificity and accuracy, Palestro and coworkers considered that it is unlikely that this radiolabeled antibiotic will ever be a valid method for imaging infection. Moreover, a recent press release from Draximage clearly stated that formulation development of Infecton targeting orthopedic indications has not been successful and Draximage will allocate the resources devoted to this product to other projects (http://www.draxishealth.com/pdf/Draxis_Q3_PR_US_GAAP.pdf). Differences in the outcome in the specificity of detecting micro-organisms may be the result of (1) inability of the compound to discriminate infection from sterile inflammation; (2) different ways of reading the scans; (3) differences in the performance of the labeling procedure, which lead to the formation of different complexes; or (4) insufficient quality control.[54] Taken together, these findings indicate that both ^{99m}Tc-labeled ciprofloxacin (Infecton) and ^{18}F-ciprofloxacin are not suitable as bacteria-specific infection imaging agents.[9,55,58,59] Finally, before introducing Infecton or any other new compound into clinical routine, some dedicated preclinical experiments should be performed to show its usefulness. For instance in vitro studies of binding mechanism and affinity of ^{99m}Tc-labeled compounds to pathogens and host cells answered many of these questions related to the controversial results obtained. Of course, each compound needs to be tested in experimental infection models in animals in comparison with animals having sterile inflammatory lesions to evaluate its usefulness.

Current studies focus on developing other well-defined ^{99m}Tc-labeled complexes that carry fluoroquinolone antibiotics (Tables 3 and 4).[60] Preliminary studies with sparfloxacin, norfloxacin, pefloxacin, lomefloxacin, and ofloxacin derivates have shown significant in vitro uptake in bacteria as well as accumulation in sterile inflammatory sites. Critical evaluation (eg, interaction with host cells and adequate control experiments) of these investigational compounds is warranted to ultimately prove their value for specific imaging of infections.[3]

The concept of handling radiolabeled antimicrobials has been further extended to include antifungal agents. This may be of particular interest in the severely immunocompromised patient, especially in and around the time of bone marrow transplantation.[61] Fungal infections are life-threatening in immunocompromised patients, and early recognition and

Table 4 Imaging Experimentally Infections With Other (Direct) Labeled Antiinfectives

Anti-infective	Mechanism	Evaluation
^{125}I-FIAU	Nucleoside analogue used in the treatment of viral infections acting via inhibition of thymidine kinase involved in nucleic acid replication	(Late) imaging of bacterial-infected tissues; elaborate synthesis and purification steps; risk of liver failure
^{99m}Tc-labeled fluconazole	Inhibits cytochrome P450 enzyme 14α-demethylase and fungal cytoplasmatic membrane synthesis	Specific marker for fungal infections
^{123}I-labeled ornidaole	Entrapment by hypoxic cells	Cannot discriminate between infections and sterile inflammatory lesions

appropriate treatment are extremely important.[61] Furthermore, the distinction between bacterial versus fungal infection is crucial for tailored treatment and has led to the development of ^{131}I-labeled MoAb and F(ab′)$_2$ fragments directed against cell wall glycoproteins of *Candida albicans* in guinea pigs and more recently the development of ^{99m}Tc-labeled fluconazole.[62] Fluconazole, the fungal antibiotic, is specifically taken up by fungi and retained inside by binding to cytochrome P450. After labeling with ^{99m}Tc, it is not taken up in tissues infected with bacteria or by mammalian cells and does not accumulate in inflammatory processes.[62] Furthermore, the Leiden group found also a good correlation between the accumulation of ^{99m}Tc-fluconazole in *Candida albicans*-infected thigh muscles in mice and the number of viable yeast present at the site of infection, indicating that ^{99m}Tc-fluconazole may be suitable for monitoring the efficacy of antifungal therapy in *Candida albicans* infections. Fluconazole has also been labeled with ^{18}F, and although the initial results are promising, further research is warranted to consider this compound suitable for imaging fungal infections in patients (Table 2).[44]

Recently, the Ghent group has developed a new antifungal imaging agent, chitinase labeled with iodine-123, for specifically targeting fungal and not bacterial or human cell walls. It was tested for specificity in a mouse model to localize fungal infections with both *Candida* and *Aspergillus* species. Furthermore, the chitinase uptake appears to correlate well with the number of viable fungal cells.[61,63] However, the radioligand was dehalogenated, resulting in high uptake in the thyroid and stomach. Therefore, the same group reported on the possible use of chitin-binding proteins in vitro and in vivo, like CBP21. Because the binding of ^{99m}Tc-HYNIC-CBP21 to fungi, and not to *Escherichia coli*, could be altered by previous incubation in the presence of a 50-fold molar excess of cold CBP21, it was concluded again that the binding was caused by a specific interaction of the compound with fungi, presumably by binding of the tracer to chitin in the cell wall.

In muscle thigh infections induced in leukocytopenic mice, the target/nontarget (T/NT) ratios for *Aspergillus fumigatus* were significantly greater than those for *Candida albicans*, *Escherichia coli*, and also compared to sterile inflammation, reaching a maximum between 5 and 7 hours after injection. Biodistribution analysis demonstrated typical characteristics of 21-kDa molecular weight labeled proteins with predominant renal and rapid blood clearance. Unfortunately, prominent stomach uptake was observed, indicating instability of the tracer-binding in vivo. Because CBP21 has no enzymatic function and binds chitin with high affinity, this protein seemed an interesting experimental compound for the development of a novel fungi-specific tracer. Its possible advantages included binding widespread fungi or yeasts, its immunity to resistance development, and its ability to be labeled with technetium as favorite radionuclide.[64] Finally, because a diagnosis of fungal infection, particularly in patients undergoing chemotherapy, is a major challenge in the clinical setting, there is good reason to believe that fungi-related diagnostic agents will be developed in the future.[61]

Another application of the antimicrobial principle is the introduction of radiolabeled antituberculosis agents, such as ^{99m}Tc-isoniazid and ^{99m}Tc-ethambutol. Those products bind to the cell walls of living mycobacteria. Singh, Verma, and coworkers reported the promising imaging results of *Mycobacterium tuberculosis* cold abscesses in rabbits, suggesting that both agents may be very useful in the future for the detection and follow-up of tuberculous lesions in humans.[65,66]

A new class of radiopharmaceuticals that specifically target bacteria, fungi, and viruses are the antimicrobial peptides.[20] They are found in abundance in mammals, birds, amphibians, and insects, and they protect these animals against infection.[67] They exhibit different chemical features. Most of them are cationic and interact with negatively charged bacterial phospholipids or with other compounds such as lipoteichoic acid present on the bacterial cell wall. They are produced mainly by macrophages and polymorphs and act by the production of pores into the bacterial membrane that cause their death. The specificity of these interactions for micro-organisms is caused by the differences in charge and composition of bacterial and human cell membranes.[68] Various natural and synthetic peptides have been investigated for the imaging of bacterial infections, such as synthetic peptides derived from domains on human antimicrobial peptide ubiquicidin (UBI), human lactoferrin (hLF), and natural peptide human defensin.

The hLF peptides and defensin, even at low doses, also demonstrated chemoattractive activity for monocytes and lymphocytes, although this was not observed for UBI and UBI-derived peptides. The Leiden group reported that radiolabeled human neutrophil defensin accumulates rapidly (within 5-15 minutes) at sites of infection in mice infected intramuscularly with Gram-positive and Gram-negative bacteria.[69] Moreover, binding of this peptide to bacteria is the major factor contributing to its accumulation in foci of bacterial infection in mice.[70] Biodistribution studies in these animals have revealed that the radiolabeled peptide is rapidly cleared from the circulation and excreted in the urine, with limited accumulation in kidney and liver. A major drawback of the application of radiolabeled antimicrobial peptides for imaging infections could be that, by successfully attacking the infectious agent, they eliminate the target for the labeled peptide.[68]

To circumvent this, one could develop synthetic peptides representing the microbial binding domain of antimicrobial peptides without the domain encompassing microbicidal activities. UBI and synthetic fragments were chosen because this antimicrobial peptide is produced and stored inside the cell independent from microbial triggering. It is believed to be the first defense against cell-penetrating micro-organisms and therefore acts as a broad-spectrum antimicrobial peptide. Furthermore, Welling and coworkers synthesized small peptides based on 10 to 18 amino acid residues from various domains of a recently discovered human antimicrobial peptide called UBI. Some of these oligopeptides, labeled with ^{99m}Tc, accumulated in mice bearing sites of bacterial and fungal infection but not at sites where heat-killed or ultravi-

olet-inactivated bacteria, endotoxins, serum, or other inflammatory agents were injected. ^{99m}Tc-labeled synthetic fragments UBI 18-35 and UBI 29-41 showed favorable in vitro and in vivo behavior over radiolabeled human natural antimicrobial peptides defensins and hLF.

Furthermore hLF and synthetic peptides hLF 1-11 and hLF 21-31, representing 2 n-terminal domains on hLF and for defensin, show a greater binding affinity to bacteria than to activated leukocytes in vitro and in vivo.[71,72] Most tested UBI peptides do not accumulate in sterile inflammatory foci. They can be prepared synthetically in large amounts and do not provoke immunological side-effects. After intravenous injection in mice, ^{99m}Tc-labeled UBI peptides accumulate specifically in tissues infected by bacteria or by *Candida albicans* and can be potentially used for the detection of both bacterial and fungal infections.[72] In general, antimicrobial peptides can be synthesized or produced by genetically engineered bacteria and animals. It is believed that the cationic domain of the peptide and the anionic charge of the outer membrane of the micro-organism are responsible for the binding potential between the 2 systems and that subsequent pore formation leads to insertion in the membrane. Intracellular targeting of mitochondria and DNA cannot be excluded (Tables 2 and 5).[73]

Among the human-derived antimicrobial peptides tested, the UBI 29-41 showed the greatest promise. ^{99m}Tc-labeled UBI 29-41 is a small synthetic peptide (amino acid sequence TGRAKRRMQYNRR; 1693 D), derived from human UBI and binds preferentially to bacteria in vitro and not to activated leukocytes.[74] This small peptide demonstrated the ability to distinguish bacterial infections from inflammation with greater specificity than other UBI peptides. The UBI 29-41 has also shown specificity for *Candida albicans* and *Aspergillus fumigatus* infections.[72] Other studies revealed that this agent is able to distinguish *Staphylococcus aureus* infections better than *Escherichia coli* infections, although the mechanism of this selectivity is not understood.[75] Probably, a difference in the outgrowth (virulence) of the pathogen is the most plausible explanation of these findings. Recently, in a rabbit model of prosthetic joint infection inoculated with *Staphylococcus aureus*, ^{99m}Tc-UBI 29-41 scintigraphy permitted the early detection of acute infection and excluded infection in chronic sterile prosthetic joint inflammation.[76] Rapid kidney clearance within 24 hours and absence of adverse effects are positive properties of this agent, allowing it to be entered into phase I clinical trials.[20] In a small series of 18 patients with suspected bacterial bone or soft-tissue infections of the limbs (including prosthesis), Akhtar and coworkers discovered first in rabbits, and then in humans, the absence of adverse effects or significant changes in vital signs.[67] Biodistribution analysis demonstrated renal clearance and liver uptake, rapidly decreasing after 2 hours, as showed in animals by Welling and coworkers[71,72] and Akhtar and coworkers[75] and also in humans as observed by Melendez-Alafort and coworkers[77] In infected cases, both visual and quantitative analysis (T/NTs of mean 2.75) were significantly positive at 30 minutes after intravenous injection. A sensitivity and a specificity of 100% and 80% were calculated.

Table 5 Characteristics of Imaging Infections With ^{99m}Tc-Labeled Antimicrobial Peptides (AMPs)

AMPs	Defensins	Lactoferrins	Ubiquicidins	Histatins
Formulation	HNP 1 to 3, natural peptides	hLF, rhLF, and synthetic fragments from N-terminus	Natural UBI 1 to 59 and synthetic linear fragments from α-helix domains	Synthetic linear peptides, derivates and dimers
Amino-acids	30 aa	692 aa	10 to 18 aa	12 to 24 aa
Imaging characteristics	Fast (<1 hour)	Fast (<1 hour)	Fast (<1 hour)	No improvements
Specificity for infections	Yes	No	Yes	Yes
Binding to host cells	No	Yes	No	No
Bactericidal and immunomodulatory effects	Yes, chemotactic	Yes	No	Yes
Biodistribution	Liver, kidneys	Liver, gallbladder, intestines	Low accumulation in the liver, no bowel deposits, kidneys and urinary bladder	Low accumulation in the liver, no bowel deposits, kidneys and urinary bladder

The fact that the amount of accumulated radiolabeled UBI 29-41 depends on the number of viable bacteria present at the site of infection could make this tracer suitable for monitoring the success of antimicrobial therapy even of drug-resistant pathogens in animals with compromised immune systems.[78] Furthermore, in a very recent study of Akhtar and coworkers,[79] ^{99m}Tc-labeled UBI 29-41 was tested as potential therapy monitoring agent in an infectious rabbit model. After induction of thigh muscle infection with increasing bacterial concentrations of viable and drug-sensitive *Staphylococcus aureus*, 9 rabbits were imaged with ^{99m}Tc-labeled UBI 29-41 before and after ciprofloxacin treatment at different time points. Similar to the findings of Nibbering and coworkers,[68] Akhtar and coworkers found specific accumulation of radiolabeled UBI 29-41 in the infected region with the highest uptake at 1 hour after injection, in contrast to a poor accumulation in the animals of the control group, ie, mice with sterile inflammatory lesions. Furthermore, they found a significant correlation between the tracer accumulation and the number of viable bacteria present at the site of infection indicating a direct relationship between the number of viable bacteria and ^{99m}Tc-labeled UBI 29-41 uptake. Moreover, it was demonstrated clearly that ciprofloxacin treatment of infections with drug sensitive *Staphylococcus aureus* significantly reduced the mean T/NT ratios in these animals, showing that effective antibacterial therapy could lower the sensitivity of an infection-specific agent like ^{99m}Tc-labeled UBI 29-41. They concluded that serial imaging can predict the optimum duration of antibacterial therapy, and that ^{99m}Tc-labeled UBI 29-41 could serve as a potential very useful, noninvasive radiopharmaceutical for monitoring efficacy of any anti-infectious therapy, possibly reducing resistance emergence and lowering the global costs.[79] Despite the lack of distinction between bacterial and fungal infections, some of these peptides may fulfill the criteria of an ideal infection tracer in the future.[67,75,80] Further studies are warranted to select pathogen-specific antimicrobial peptides.

UBI 29-24 derivate also was synthesized recently with ^{18}F.[57] Zijlstra and coworkers reported pharmacokinetic analyses and in vitro experimentation with ^{18}F-UBI 29-41 and found a substantial binding to *Staphylococcus aureus*. More recently, the same group compared the distribution of respectively ^{99m}Tc-labeled UBI 28-41, ^{18}F-labeled UBI 29-41, and ^{18}F-labeled UBI 28-41, and ^{3}H-deoxyglucose in a rat abscess model, inoculated with *Staphylococcus aureus* suspension, to that of anti-*Staphylococcus aureus* immunofluorescent imaging.[81] Different antibodies against the bacteria and macrophages were used to delineate the infected region with an immunofluorescence technique. The additional radiolabeled deoxyglucose permitted dual tracer autoradiography of the infected tissue. They used various time intervals (1-6 days) for scanning the region of interest. They found that radiolabeled UBI derivates in vivo exhibited variably increased uptake in the area of bacterial abscesses, but did not show congruity with areas that were highly positive for bacteria on immunofluorescent imaging. Moreover, the uptake pattern partly matched deoxyglucose uptake and macrophage infiltration. This was in contrast to in vitro results, where clear and significant binding was demonstrated for the different radiolabeled UBI derivates, but they found no specific binding in vivo although the number of experiments was too low to draw a consistent conclusion from these results. Most likely, the rat model is not comparable to mice as the healthy rat eliminates bacterial infections quickly. Thus at the intervals of testing most of the viable bacteria could be phagocytized and were not detectable with ^{99m}Tc-labeled UBI 29-41. Unfortunately, the viability of the bacteria at the site of infection was not assessed which might clarify this hypothesis. However some other groups have successfully tested the ^{99m}Tc-labeled UBI 29-41 peptide in humans.[67,77] UBI derivates should be further analyzed in the future, hopefully revealing the exact binding mechanism and their possible applications in clinical infectious disease.[81]

Another application of a potential infection-specific imaging agent is the introduction of radiolabeled bacteriophages, which are highly specific for bacterial species. In contrast to radiolabeled antibiotics and antimicrobial peptides, bacteriophages (phages) are viruses that show no specificity for mammalian cells and infect bacteria exclusively.[10] Furthermore, most phages infect several bacterial species, but in a more narrow range than that exhibited by antibiotics, eg, showing specificity toward a single bacterial strain. The binding mechanism consists of the attachment of the phages to specific surface receptors or domains located on the surface of the bacterium, subsequently transferring their genetic material into the host cell dedicated for phage replication/reproduction. Before the antibiotic era, phages were used clinically in the 1920s, and they are presumably nontoxic, although their safety and efficacy have yet to be validated in modern toxicology studies.

Rusckowski and coworkers described the first investigations of a ^{99m}Tc-labeled bacteriophage M13 in vitro and in vivo in an infection/inflammation mouse model, infected with 2 *Escherichia coli* strains that are susceptible to this strain of phages.[10] They have shown that the labeling technique with ^{99m}Tc using a chelator MAG_3 is feasible, and that the radiolabeled phage binds immediately and essentially equally to both living and heat-killed bacteria. Also the complex is highly stable. Biodistribution analysis in normal mice showed the highest accumulation in the liver followed by a rapid decrease from this organ and subsequent rapid accumulation of radioactivity in the lungs that also decreased rapidly shortly thereafter. When tested in the mouse infection/inflammation model, significantly greater accumulations in the target (bacterially infected) thigh muscle than in the nontarget (control and/or inflamed) contralateral thigh muscle were noted. The differences in biodistribution patterns between normal and inflamed thigh muscle in mice, with lung and gut uptake, need further investigation.

Recently, the same group investigated the promising role of 4 ^{99m}Tc-labeled bacteriophages against different microorganisms as potential infection-specific imaging tracers.[82] In each case, specific binding to bacteria was demonstrated in vitro. Subsequently, in a bacteria-infected mouse model, the authors emphasized difficulties in explaining the results, mainly attributable to the variable role of each of the infection

models in the pharmacokinetics of the radiolabeled phage. In general, they showed that for each of the 4 animal models, the infected thigh had a substantial increase in weight of approximately 1.5- to 2.5-fold compared with the weight of the normal thigh muscle, explaining partly the nonspecific diffusion across the endothelial lining resulting in nonspecific accumulation of the radiolabeled phages. Nevertheless the most encouraging results were obtained with a *Pseudomonas aeruginosa* bacteriophage, demonstrating that 1.93% of the injected dose accumulated in the infected thigh muscle with a T/NT ratio dose of 14.2. They concluded that the increased accumulation in infection therefore argues in favor of a mechanism, that at least in part, involves specific binding to bacteria, but this hypothesis needs to be further tested. As suggested before, extensive toxicology experiments are warranted before human studies can be considered.[10,82]

Targeting Hypermetabolism

As the result of work substantially performed and reviewed a few years ago by the Gent University Group,[19,83] PET with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) has been introduced as a promising imaging modality for the evaluation of various clinical infectious conditions. The most challenging patient populations are those in the postoperative or post-traumatic musculoskeletal setting. Therefore, ^{18}F-FDG can easily compete with ^{67}Ga-SPECT and, on the other hand, with MRI or CT, especially in the presence of orthopedic devices or spinal implants.[84,85] Since the 1990s, it is known that, in AIDS patients, ^{18}F-FDG accurately localizes foci of infection as well as tumor and is particularly useful for differentiating central nervous system lymphoma from toxoplasmosis.[86,87] Increased ^{18}F-FDG uptake in inflammation and infection is thought to be caused by an increased number and expression of transmembrane glucose transporters by activated inflammatory cells.[88] The affinity of glucose transporters for deoxyglucose is apparently up-regulated in inflammatory processes by various cytokines and growth factors.[89]

Other groups demonstrated by autoradiography that the greatest FDG uptake, in experimental bacterial abscesses in rats, was observed in areas of inflammatory cell infiltrates. Moreover the FDG uptake was predominantly associated with neutrophils in the acute phase and with macrophages in the chronic phase of soft-tissue infection.[90] PET has several potentially important advantages over conventional camera imaging that use SPECT agents.[9,19,91] First, PET intrinsically is a high-resolution tomographic technique that enables the precise localization of suspected sites of infection, further improving in the future by the use of coregistered PET-CT technique. Second, semiquantitative analysis or handling a dual-imaging acquisition method could be useful for differentiating infectious from noninfectious conditions and for monitoring a patient's response to therapy. These are some of the reasons it would be useful to have an infection-specific PET tracer available for use in clinical practice.[9,19]

Unfortunately, ^{18}F-FDG is of limited value for differentiation between infectious bacterial and nonbacterial inflammatory processes, such as autoimmune conditions, including vasculitis, fever of unknown origin, fungal, tuberculosis infections, and cancer. Nevertheless, in the right clinical perspective, ^{18}F-FDG can be a very good alternative to conventional radiopharmaceuticals and morphological imaging methods to image various clinical infectious diseases, as reported in numerous reports from different centers. In particular, PET and combined PET/CT will have a role in imaging difficult-to-manage patient populations and/or in the evaluation of infection in a difficult and complex region, such as complicated orthopedic conditions, diabetic foot infections, fever of unknown origin, the immunosuppressed patient, and probably also in vascular graft infection.[2,4,85,92-94]

The role of the CT devices in PET or SPECT/CT machines is not negligible.[9,93] Indeed, the superior anatomic detail provided by the CT portion also provides valuable information to aid in differentiation of inflammation from infection, such as the anatomic distribution and overall appearance of uptake (eg, in the paravertebral soft tissues and/or paraspinal fluid collection rather than the bone in case of spinal infection, collapse of a vertebra in case of osteoporotic fracture), association with osteolysis (in case of any malignancy, chronic osteomyelitis or implant loosening), and/or solely soft-tissue reaction/cellulitis (in the case of diabetic foot infection or chronic osteomyelitis), soft-tissue air (suggesting abscess in the case of vascular graft infection) and possible drainage tracts (in case of prosthetic joint infection or chronic osteomyelitis).

Mahfouz and coworkers[2] reported 165 sites of verified infection in 143 patients with multiple myeloma predominantly undergoing treatment with myelo-ablative chemotherapy in preparation for stem cell transplantation. They found pneumonia in 60% of the cases and in the other 40% musculoskeletal infections, septic thrombophlebitis, colitis and periodontal abscesses with various verified etiologies of infection including bacteria, mycobacteria, viruses, and fungi. Moreover ^{18}F-FDG-PET and PET/CT scanning was specifically able to detect and localize infection associated with severe pancytopenia and also identified occult infection, which is highly relevant for patients who will undergo stem cell transplantation, avoiding possible life-threatening infections afterward. They concluded that ^{18}F-FDG-PET and PET/CT have a definite role in the detection of a wide range of relevant infections under clinically adverse conditions and demonstrated the usefulness of both techniques in making a significant contribution to the clinical management of immunocompromised patients.[2]

Unfortunately, less-promising news was reported in a recent issue of the *Journal of Nuclear Medicine* that, according to the Centers for Medicare and Medicaid Services, "evidence is inadequate to conclude that ^{18}F-FDG-PET for chronic osteomyelitis, infection of the hip arthroplasty, and FUO improves health incomes."[95] The main reason for this conclusion is the absence of "any systematic reviews" in the literature evaluating the use of ^{18}F-FDG-PET for the requested indications, a paucity of data supporting the ability of PET imaging to improve treatment or enhance long-term outcomes, and the absence of evidence-based guidelines for the use of PET in the requested indications. Articles submitted for support of ex-

panded approval were criticized for small sample sizes and methodological and statistical flaws. A lack of interest was noted in this issue by practicing orthopedic surgeons or their professional societies and physicians, generally infectious disease specialists, who would routinely be consulted in cases of fever of unknown origin. Finally, some research centers promote dual-time point imaging for differentiating FDG avid inflammatory/infectious lesions from malignancy, but this topic is still under investigation, both in animal models as well as in human studies.[96-99]

Attempts have been made to combine the neutrophil-mediated power of human leukocytes with the tomographic resolution of PET technology, but until now, human studies on ^{18}F-FDG-labeled white blood cells (^{18}F-FDG-WBCs) are scarce in the literature.[91,100,101] Early in the 1990s, Osman and coworkers demonstrated the feasibility of in vitro labeling of human leukocytes with ^{18}F-FDG.[102] The influence of glucose concentration was very important for a successful labeling. In 2000, Forstrom and coworkers also demonstrated a good leukocyte labeling efficiency with predominantly labeled granulocytes.[103] Two years later, the same group reported the results of biodistribution and dosimetry studies on normal volunteers showing that the leukocyte activity primarily was distributed in the reticuloendothelial system as expected.[104] Recently, Pellegrino and coworkers compared the relative uptakes of ^{18}F-FDG with ^{18}F-FDG-WBCs in a rat muscle inflammation-infection model.[105] They demonstrated a better specific binding mechanism of ^{18}F-FDG-WBCs compared with ^{18}F-FDG. They suggested that the enhanced imaging properties of ^{18}F-FDG-labeled leukocytes over ^{18}F-FDG could have clinical implications.

Dumarey and coworkers recently reported their initial experience with ^{18}F-FDG-WBC PET/CT in a small series of 21 patients with various infections.[100] Using a visual score of at least 2 as positive for infection, sensitivity, specificity, and accuracy were each 86% on a patient-per-patient basis and 91%, 85%, and 90% on a lesion-per-lesion basis. The absence of gastrointestinal and renal uptake and the faint brain and myocardial uptake makes ^{18}F-FDG-WBCs likely to do well in the assessment of intra-abdominal, renal, intracerebral, and cardiac infectious diseases.[100] As described previously, ex vivo radiolabeling of WBCs either labeled with ^{18}F-FDG or with ^{99m}Tc does not contribute to the issue of specific imaging of infections. Hybrid ^{18}F-FDG-WBC PET/CT delineated the infection with high precision. Moreover, the 100% negative predictive value virtually excluded infection when the study was negative.

Palestro and coworkers studied 43 patients who were suspected of having infection by using both coincidence PET imaging with ^{18}F-FDG-WBCs and conventional ^{111}In-labeled leukocyte scintigraphy.[101] Overall accuracies of ^{18}F-FDG-WBC and ^{111}In-labeled leukocyte scintigraphy were similar, approximately 80%, but were disappointingly low in the subgroup of patients with suspected osteomyelitis. For the subgroup of patients with suspected infection of a prosthetic joint, accuracies were highly promising, and values >90% were calculated for both radiolabeling techniques. The authors found an important difference between the 2 modalities concerning the labeling efficiency, which was significantly lower for ^{18}F-FDG-WBCs versus ^{111}In-labeled WBCs. In addition, 6 patients were excluded from the study because their ^{18}F-FDG-WBC labeling efficiencies were unacceptably low. These effects need further investigation in the future, but probably high serum glucose levels or the nonfasting state at the time of labeling can, as for imaging infections with ^{18}F-FDG itself, result in a different uptake mechanism of the radiopharmaceutical.[101] Also, the same group hypothesizes on other possible drawbacks involving the usefulness of ^{18}F-FDG-WBCs in the future. Mainly, the short half-life of ^{18}F, in comparison with ^{111}In, and the application in chronic musculoskeletal infections, limiting late imaging with PET to not much later than up to 5 to 6 hrs after injection, can be a drawback for FDG-labeled WBC imaging in the future.[9]

Besides the nonspecificity of the ^{18}F-FDG label, the problem is further that an early healing process in the body involves an inflammatory phase that represents a highly activated state of cell metabolism and glucose consumption, subsequently mimicking an infection on FDG-PET imaging.[106] Therefore, the Turku group introduced ^{68}Ga, which is a positron-emitting radionuclide with a short half-life of 68 minutes. In a recent rabbit osteomyelitis model, bone infection could be distinguished from aseptic bone healing by means of PET using ^{68}Ga-chloride as soon as 2 weeks after onset of the osteomyelitis.[107] Moreover, they found that ^{68}Ga could better discriminate than ^{18}F-FDG between bacterial infections and normal bone healing. Very recently, the same group explored the application of ^{68}Ga-DOTA-labeled VAP-P1, a new PET radiopharmaceutical, for the assessment of the physiological inflammation process in bone healing and osteomyelitis in a standardized animal model.[106]

The hallmark question was the accurate differentiation between bacterial superimposed infection and inflammatory reactions associated with the early phases of bone healing. Therefore, they introduced an endothelial marker VAP-1, which is an inflammation-inducible dual function endothelial glycoprotein with adhesion properties and amine oxidase activity. It plays a critical role in lymphocytic trafficking and, in humans, the expression of VAP-1 is up-regulated on endothelial cells at sites of inflammation as is reported in numerous diseases. The authors observed a significant difference in the immunohistological evaluation of VAP-1 expression between the osteomyelitis and control animals at 24 hours after surgery but, unfortunately, this difference was not reflected on PET. Furthermore, although they found that this new peptide complex is capable of accurately demonstrating the phase of inflammation in healing bones and the progress of bacterial infection in osteomyelitis sites, they argued that the tracer does not specifically accumulate to infectious sites. Finally, maybe the most important question answered is that this novel imaging agent allowed for the differentiation of bone infection as the result of *Staphylococcus aureus* and normal bone healing at 7 days after surgery. High-resolution dedicated animal PET should be used in the future to accurately delineate periosteal inflammation from that of surrounding soft tissues. From the clinical point of view, this

new PET tracer could be valuable for the detection of infection in its early stages.[106]

Recently, the influence of antibiotic treatment, using ceftriaxone on ^{18}F-FDG uptake, was investigated by histological analysis and high-resolution small animal PET in experimental soft-tissue infections.[108] Unfortunately, ^{18}F-FDG uptake in treated and untreated animals on consecutive days did not reveal any significant difference, indicating that ^{18}F-FDG is not suitable either for the specific detection of infections nor for therapy monitoring.

More recently, different radiotracers based on uracil nucleosides, labeled with different SPECT and PET tracers, have been synthesized.[109,110] These radiopharmaceuticals allow the noninvasive detection of bacteria or viruses by targeting thymidine kinase (tk), whose substrate is distinct from that of the major human tk. Because those uracil nucleoside derivates are incorporated into bacteria rather than into inflammatory cells, it should be specific for the infectious process, as demonstrated in a pilot study of musculoskeletal bacterial infection in patients.[111] Recently, in the United States, ^{124}I-FIAU has become commercially available and therefore should be relatively simple to test as an imaging agent for various infections. Another application is the noninvasive detection of *Herpes simplex* or *Varicella zoster* viruses type 1 thymidine kinase (HSV1/VZV-tk) reporter gene expression.[109,112] These new radiopharmaceuticals have been intended to be used to image successful gene transfer; nevertheless, potentially they could be used to image HSV infections in immunocompromised patients.[113] Targeting microorganismal tk with radiolabeled nucleoside analogs such as FIAU provides an unique approach to the noninvasive diagnosis and therapeutic monitoring of infection.[111]

Conclusions and Future Perspectives

Nuclear medicine techniques have a lot to offer in the visualization of infectious foci. Quite often, these techniques do not immediately lead to a definitive diagnosis, ie, histological or a microbial diagnosis.[9] However, they point to parts in the body in which a particular metabolic process is ongoing, leading to increased uptake of a dedicated radiopharmaceutical.[23] With the help of other complementary techniques, such as CT/MRI, and more invasive tests, such as aspiration, biopsy, and culture, a definitive diagnosis can be made.[9,23] There are several reasons to explain why imaging of infection has become increasingly important in the past decades. The population is aging, with subsequent increasing use of orthopedic or cardiovascular devices, implants, or grafts. The number of immunocompromised patients is still growing in the era of HIV and tuberculosis, and neutropenic fever is frequently encountered as the result of cytotoxic treatments, such as chemotherapy and transplantation. Furthermore, multidrug-resistant micro-organisms are a global burden because of an inefficient use of some of the broad-spectrum antibiotics.

To improve the clinical management of patients with complicated osteomyelitis and also to confirm the presence of pneumonia and to define the precise etiology, the availability of a SPECT or PET imaging diagnostic agent that is able to differentiate between infection and sterile inflammation would be desirable.[114] Among the myriad of imaging tests currently available, none are truly specific for infection, and the search continues for new and better agents.[9,115] The currently available radiopharmaceuticals do not have a perfect profile for infection detection due to disadvantageous kinetics (slow clearance, delayed imaging capability), possible side effects (HAMAs, radiation burden, immunomodulatory effects) and, moreover, some of them are time-consuming in preparation and may encompass a risk due to blood handling.[116] The requirements for infection-specific tracers are of utmost importance especially the interaction with the pathogens and not with the host cells, and also a well defined radiochemistry, predominantly lacking in current experimental agents.

During the past decade, the focus in the development of new radiopharmaceuticals has gradually shifted from large proteins with a nonspecific uptake mechanism via large receptor-specific proteins to receptor-specific small proteins and peptides, such as radiolabeled antibiotics and antimicrobial peptides.[117] According to the 10 criteria for an ideal radiopharmaceutical for infection imaging and based on the properties of antimicrobial peptides, those small peptides could be considered as the most promising infection-specific imaging agent, already used in a few human trials.[67,77] Furthermore, some synthetic UBI derivates, especially, are probably the most promising vehicles to monitor the efficacy of antibiotic treatment in patients because they give further insight into the minimum numbers of bacteria that can be detected (approximately 10^4 to 10^5) on monitoring the effect of antimicrobial therapy-using radiolabeled antimicrobial UBI peptides are now available.[78,79]

Unfortunately, some of the published data on diverse antibiotics are contradictory, ie, the in vitro and animal studies cannot be easily translated to human studies. In conclusion, the challenges for the development of future infection imaging agents are multiple. First, optimization and characterization of ^{99m}Tc and ^{18}F radiochemistry is urgently needed. Second, in vitro tests and preclinical imaging without any evidence of nonspecific binding (wash-in/out) is of utmost importance. Therefore, control experiments are greatly needed for this proof of principle. Further, more work is warranted on the role of ^{18}F-FDG, ^{18}F-FDG-WBCs or ^{68}Ga molecules and other possible PET infection-specific compounds. Finally, last but not least, the fusion of hybrid imaging, ie, SPECT or PET/CT, will play an important role in delineating infections. By providing information on pathophysiological and pathobiochemical processes, nuclear medicine has an important and increasing role to play in overcoming these difficulties encountered in noninvasive infection imaging.[118]

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