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Clinically Proven Radiopharmaceuticals for Infection Imaging: Mechanisms and Applications

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Gallium-67 (^{67}Ga)-citrate was initially introduced as a tumor imaging agent in the early 1970s, but it was soon recognized that it was useful in the identification of both acute and chronic inflammation. Because of its physical characteristics (multiple γ photons energy; long half life) and binding to the plasma protein transferrin (resulting in relatively high background and therefore reduced lesion-to-background contrast), it was less than ideal as an imaging agent. Several years later, it became possible to radiolabel leukocytes, which had the advantage of greater specificity for acute infections characterized by a granulocytic response, but had the disadvantage of requiring the removal of blood and isolation of the leukocyte component. Several radiolabeled antibody preparations and a radiolabeled antibacterial agent have been introduced and evaluated, but none of these have been used widely. Most recently, it has been recognized that the use of ^{18}F-fluorodeoxyglucose can be used to identify infection/inflammation, probably based on the focal increase in anaerobic glucose metabolism associated with the cellular response. In this work, we review the features of each of these agents and discuss the issues involved in their use as radiopharmaceuticals for the identification of inflammation and/or infection.
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During the last 4 decades, nuclear medicine has played an important role in the detection and localization of inflammation and infection. Many radiopharmaceuticals (Table 1) have been evaluated extensively in both preclinical and clinical studies as potential diagnostic agents to identify the sites of infection. In general, nuclear medicine techniques, however, do not necessarily provide a specific diagnosis and may require puncture, biopsy, or culture of tissue or fluids to confirm the presence of infectious foci identified by the radiopharmaceuticals. Although ^{67}Ga-citrate has proven to be extremely useful in the identification of the sites of infection in vivo, autologous leukocytes, labeled in vitro with ^{111}In or ^{99m}Tc, are still considered the "gold standard" for infection imaging because labeled leukocytes are the only agent that is specific to image acute infection. Because labeling leukocytes in vitro is both a time-consuming and potentially risky procedure, the search for an ideal radiopharmaceutical for infection imaging that does not require ex vivo cell separation continues to provide a major challenge for nuclear medicine and molecular imaging.

At present, there are several functional (planar scintigraphy, single-photon emission computed tomography and positron emission tomography [PET]) and morphological (computed tomography, magnetic resonance imaging) imaging modalities that are being used to identify infectious and inflammatory foci of soft tissue and/or bone. The choice of modality depends on many factors. It is important that the physician understands and appreciates the pathophysiological changes involved in the inflammatory process as well as the coexistence of other diseases such as cancer to select the most efficacious procedure. Although computed tomography and magnetic resonance imaging have high anatomic resolution and can identify the exact location of the pathologic process, only radionuclide techniques provide specificity and functional characterization of the infectious focus. The design and development of new radiopharmaceuticals for infection imaging is based on the definition and description of the ideal properties of a radiopharmaceutical for infection imaging, which are summarized in Table 2. With the current resurgence of interest in molecular imaging strategies, the design of target-specific molecular imaging probes based on better understanding of the biochemistry of the inflammatory response may ultimately lead to the development of an ideal agent for infection imaging.

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Table 1 Radiopharmaceuticals for Imaging Inflammation/Infection

Approved Radiopharmaceuticals	Investigational Radiopharmaceuticals
^{67}Ga-citrate	^{111}In-DTPA-human IgG (HIG)
^{111}In-Oxine to label leukocytes in vitro	^{99m}Tc-HYNIC-IgG (HIG)
^{99m}Tc-HMPAO (Ceretec) to label leukocytes in vitro	^{99m}Tc-anti-NCA-95 IgG (BW 250/183)
^{99m}Tc-anti-NCA-90 Fab' (LeukoScan) anti-granulocyte antibody	^{111}In-F(ab)$_2$-anti-E-selectin antibody
^{99m}Tc-anti-SSEA-1 IgM (LeuTech) to label leukocytes in vivo	^{99m}Tc-Interleukin-8 (IL-8)
^{99m}Tc-ciprofloxacin (Infecton)	^{99m}Tc-labeled chemotactic peptides
	^{99m}Tc-labeled nanocolloids
	^{18}F-Fluorodeoxyglucose (FDG)
	^{18}F-FDG-Leukocytes (labeled in vitro)

In this article, we plan to first discuss the pathophysiology of inflammation and infection, followed by a critical review of the mechanisms of several radiopharmaceuticals that are either used routinely in the clinic or have been investigated as potential diagnostic agents in patients with infection. This review will provide the necessary background for other articles in this issue; future diagnostic agents and the role of PET as a diagnostic tool in infection.

The routine applications and limitations of clinically proven radiopharmaceuticals will be described briefly. Several specific applications of nuclear medicine techniques in the diagnosis of osteomyelitis and arthritis, diabetic foot, and prosthetic joint infection are discussed more extensively elsewhere in this issue.

Pathophysiology of Inflammation and Infection

Inflammation is a complex tissue reaction to injury that may be caused by physical, chemical, or immunological agents or even by radiation. If the injury is caused by or involves living microbes, the injury leads to infection. Whatever the cause may be, inflammation is a nonspecific response to an injury and is a protective response to the cause as well as the consequences of such an injury.[1,2] In general, the inflammatory response is characterized by local hyperemia (rubor, calor), edema or swelling (tumor) and, in some locations, pain (dolor).

Inflammation may be classified broadly as acute or chronic depending on the duration of inflammatory reaction (the interval since the initial injury) and also on other pathological and clinical features. Acute inflammation is the early or an immediate response to injury that lasts for a short duration (8-10 days), whereas the condition characterized as chronic inflammation is of longer duration, lasting for several weeks to even years. The systemic changes associated with inflammation are leukocytosis (caused by stimulation by complement component C3a and colony stimulating factors), fever, and an increase in plasma antiinflammatory proteins such as C-reactive protein, fibrinogen, and haptoglobin.[2] Abscess formation is an occasional consequence, resulting in isolation of the inflammatory focus, which may result in hindering radiotracer access and hence reducing detection.

Acute Inflammation

As an immediate consequence of injury, acute inflammation is associated with many regional and systemic changes, such as vasodilation, increased vascular permeability, and formation of exudate. These events are followed by local cellular events, such as chemotaxis, leukocyte margination, and emigration (diapedesis). Many of these changes are regulated by chemical mediators produced endogenously by cells at the infectious foci or present in circulation.[2,3]

Vasodilation of local arterioles and capillary bed is one the most important local vascular changes associated with acute inflammation. Increased vascular permeability results because of (1) widening of intercellular gaps, (2) leukocyte mediated endothelial injury, (3) endothelial necrosis and detachment, and (4) formation of new capillaries (angiogenesis) that are leaky. As a consequence, circulation is slowed in the local vessels resulting in stasis.

Another hallmark of acute inflammation is formation of exudate, an inflammatory extravascular fluid with a high protein (globulins and fibrin) content and cell debris. The formation of exudate is a consequence of vasodilation and increased capillary permeability. Exduate varies in composition and may become thick and clotted; when rich in leukocytes, it is called purulent and when it contains red blood cells, it is described as hemorrhagic.

After stasis develops, leukocytes accumulate at the site of infection as result of leukocyte margination and emigration. Chemotaxis is the process of leukocyte migration to the site of injury. Leukocytes have specific receptors for a number of chemotactic factors produced by bacteria or present in plasma that result in attraction of the leukocytes to the site of infection. Several inflammatory cells, particularly polymorphonuclear leukocytes (PMNs) accumulate and aggregate at the site of inflammation/infection. As part of the response to

Table 2 Ideal Characteristics of a Radiopharmaceutical for Inflammation/Infection Imaging

High sensitivity for inflammation/infection
Should differentiate between infection and inflammation
Should differentiate between acute and chronic infection
No toxicity and/or immunogenic response
High specificity
Rapid clearance from circulation
No uptake in gastrointestinal tract
Ease to prepare, low cost, and wide availability

infectious material, PMNs and macrophages ingest foreign particles, bacterial and tissue debris in a process known as phagocytosis. In the PMN, this is accompanied by the release of peroxides (the superoxide burst) that destroys bacteria.

Chronic Inflammation

After acute inflammation, healing of the injured tissue may lead to resolution (restoration of normal structure and function) or repair (formation of a scar consisting of collagen). Alternatively, instead of healing, the acute inflammation may progress to a chronic inflammatory stage characterized by reduction in the number of PMNs and an increased infiltration of macrophages, lymphocytes, plasma cells, and fibroblasts.

Abscess Formation

Abscess can be present with both acute and chronic inflammation. Abscess is generally caused by bacterial infection and is defined as a collection of pus in tissues, organs, or confined spaces. During this period of development, it is characterized by hyperemia, leukocytosis (increased production of leukocytes), and edema, with or with out cellular necrosis (also known as phlegmon). During the period of abscess formation, there is a vigorous PMN response but, after stabilization of the abscess, depending on the degree of isolation of the abscess contents, there is a diminution in the pathophysiologic response and hence functional imaging procedures may be less sensitive.

Major Sites of Inflammation

Although the basic pathophysiology of infection/inflammation has a characteristic pattern, there are subtle differences in the microenvironment at different sites of soft tissue and bone infections.

Abdominal Infection/Inflammation

Abdominal inflammation may involve an organ diffusely, as in hepatitis, colitis, or peritonitis, or focally, as in intraperitoneal abscess, retroperitoneal abscess, or visceral abscess (hepatic, pancreatic and splenic). Several factors such as hyperemia and fibrin formation may predispose infection/inflammation in the abdominal cavity to abscess formation.[2] Inflammatory bowel disease (such as ulcerative colitis and Crohn's disease) is an idiopathic disease, and several factors, such as environmental, infectious, genetic, and autoimmune reactions, may be involved in the etiology of the inflammatory process that can be either acute or chronic.[2] The pathophysiology may include inflammation of the mucosal lining of intestinal tract, causing ulceration, edema, bleeding, and fluid and electrolyte loss.

Pulmonary Infection/Inflammation

The common chest inflammatory conditions include bacterial and viral pneumonia, fungal infections, infection involving organisms that are usually nonpathogenic such as *Pneumocystis carinii* pneumonia (PCP), granulomatous disease (either infectious, such as tuberculosis, histoplasmosis or coccidiomycoses, or presumably noninfectious, such as sarcoidosis), and diffuse interstitial inflammation. Bacterial and even viral pneumonias are associated with an acute inflammatory process and a vigorous PMN response. Frequently, the initial injury may be attributable to one organism but the injured tissue becomes vulnerable to secondary infection involving other organisms thus complicating the specific cellular content of the inflammatory response. In this setting, labeled leukocytes are quite sensitive and provide images with the greatest contrast between infected/inflamed tissue and background.

Pulmonary sarcoidosis starts as a diffuse interstitial alveolitis, followed by characteristic granulomas, which are present in the alveolar septa as well as in the walls of bronchi, and pulmonary arteries and veins. During periods of active inflammation, the response is characterized by macrophages and other elements typical of chronic inflammation. PCP is common in immunocompromised conditions, particularly the acquired immunodeficiency syndrome (AIDS) but is also found in congenital immunodeficiency and in immunocompromised patients who are receiving chemotherapy and corticosteroids.[4] The inflammatory reaction to this type of infection in the lungs consists of monocytes, histocytes, lymphocytes, and plasma cells rather than PMNs, unless there is a superimposed bacterial infection. Accordingly, labeled leukocyte imaging based on labeling PMNs is less useful than ^{67}Ga scintigraphy or ^{18}F-fluorodeoxyglucose (^{18}F-FDG) imaging, which readily identify the hypercellularity of the inflammatory response even though it may be devoid of PMNs.

Renal Inflammation/Infection

There are 2 main kinds of acute renal infection; pyelitis (confined to renal pelvis), and pyelonephritis (involving both pelvis and parenchyma). Acute pyelonephritis is a bacterial infection and most often occurs because of ascending urinary tract infection. The kidney is usually enlarged and edematous with small foci of abscess formation in the cortex. Microscopically, there is intense inflammation and abscess formation with infiltration of PMNs throughout the interstitial tissue.[2] Chronic pyelonephritis occurs mostly in patients with major anatomic anomalies including urinary tract obstruction, calculi, renal dysplasia or, most commonly, vesicoureteral reflux in young children.[2] The interstitial tissue is predominantly infiltrated by lymphocytes and plasma cells.

Skeletal Inflammation

Osteomyelitis is an infection involving the cortical bone as well as the myeloid (bone marrow). The infection may be limited to the periosteum (periosteitis) without involvement of cortex and marrow but when the cortex is involved, it is called osteitis and osteomyelitis. Osteomyelitis may be classified based on several factors such as route of infection (hematogenous or nonhematogenous), underlying etiology (diabetic foot), age of onset (infantile).[2] These classifications are relevant as they also represent differences in the pathophysiologic responses involved and hence influence the choice of radiopharmaceutical selected to identify the inflammatory

process. Although many different organisms have been encountered in osteomyelitis, *Staphylococcus aureus* is the most common Gram-positive bacterium involved.[5] One of the consequences of osteomyelitis is reactive new bone formation resulting in increased blood flow. Chronic osteomyelitis is characterized by less marked infiltration of inflammatory cells than seen in the acute state and may exhibit variable amount of necrotic tissue. Osteomyelitis in the diabetic foot is a unique clinical and pathologic problem. It is a common complication of diabetes and generally occurs as a result of the spread of infection from adjacent foot ulcers.[2] Patients undergoing hip or knee arthroplasties may experience discomfort due to loosening with or without infection. The extent of reactive bone formation, however, depends on the nature of prosthetic material; the cementless porous coated prosthesis induces more reactive bone formation than the cemented prosthesis. Finally, infectious or septic arthritis refers to the invasion of synovial space by microorganisms; acute infection is normally caused by bacteria and is a medical emergency, whereas the chronic arthritis may be caused by fungal and mycobacterial pathogens.

Mechanism(s) of Radiopharmaceuticals Localization

The increased uptake and localization of various diagnostic radiopharmaceuticals at the sites of infection and inflammation can be explained by both specific and nonspecific mechanisms (Table 3). A number of nonspecific radiopharmaceuticals such as ^{67}Ga-citrate and ^{111}In and ^{99m}Tc-labeled proteins and peptides show increased extravasation and uptake at the sites of inflammation due to enhanced vascular permeability. ^{111}In and ^{99m}Tc-labeled leukocyte preparations (labeled in vitro or in vivo) are the only target specific radiopharmaceutical localizing at the sites of acute infection due to cellular migration. Most of the radiopharmaceuticals, specific or nonspecific, cannot distinguish between infection and sterile inflammation. Several molecular imaging probes with target specificity for the infectious foci are under development and clinical evaluation. In preclinical studies, the localization of radiolabeled tracers at the sites of experimental infection is erroneously interpreted as specific accumulation even though leakage of tracer molecules due to increased vascular permeability is the major mechanism of tracer accumulation.[6] For many of these radiopharmaceuticals, the mechanisms of localization, advantages and disadvantages are discussed herein.

Nonspecific Radiopharmaceuticals

^{67}Ga-Citrate

Gallium is a trivalent metal. It is relatively insoluble at normal pH and forms insoluble gallium hydroxides and the gallate ion. In the presence of citrate ions, however, gallium exists as a soluble gallium citrate complex. Because ^{67}Ga is a cyclotron produced radionuclide, it is essentially carrier-free and is available in very high specific activity (10-16 Ci/μmol). As a result, the routine clinical dose of ^{67}Ga-citrate (370 MBq) represents $<$50 ng.

After intravenous administration, ^{67}Ga quickly binds to the iron-binding protein, transferrin in blood.[7] The thermodynamic stability of ^{67}Ga-transferrin complex is very high at normal pH in blood but in tissues where pH is relatively acidic such as in tumor and abscess, ^{67}Ga dissociates from transferrin.[8] The first step in ^{67}Ga localization at the sites of

Table 3 Radiopharmaceuticals for Inflammation/Infection Imaging: Mechanisms of Localization

Mechanism	Radiopharmaceutical	Comments
Increased capillary permeability of radiolabeled proteins and small molecules	^{67}Ga-citrate	^{67}Ga binds to transferrin in blood
	^{111}In-human IgG (HIG)	Nonspecific IgG immunoglobulin
	^{99m}Tc-HYNIC-IgG (HIG)	Nonspecific IgG immunoglobulin
	^{99m}Tc-anti-NCA-90 Fab′ (LeukoScan)	Antigranulocyte antibody with no in vivo binding to leukocytes in blood
	^{99m}Tc-anti-NCA-95 IgG (BW 250/183)	Antigranulocyte antibody with no in vivo binding to leukocytes in blood
	^{99m}Tc-Albumin nanocolloids	Nonspecific uptake
Vasodilation and hyperemia	^{99m}Tc-MDP	Nonspecific flow tracer
Target specific Cellular migration	^{111}In-oxine-labeled leukocytes	Autologous leukocytes labeled in vitro
	^{99m}Tc-HMPAO-labeled leukocytes	Autologous leukocytes labeled in vitro
	^{18}F-FDG-labeled leukocytes	Autologous leukocytes labeled in vitro
	^{99m}Tc-anti-SSEA-1 (LeuTech)	Antigranulocyte IgM antibody labels leukocytes in the blood after injection
Metabolic trapping	^{18}F-FDG	Increased glucose utilization by activated leukocytes and microbes
Receptor binding	^{123}I and ^{99m}Tc-labeled interleukin-2	Specific binding to activated lymphocytes
	^{123}I and ^{99m}Tc-labeled interleukin-8	Specific binding to receptors (CXCR1, CXCR2) on granulocytes and promotes chemotaxis
Specific binding to microbes	^{99m}Tc-ciprofloxacin (Infecton)	Specific binding to *DNA gyrase* in viable bacteria

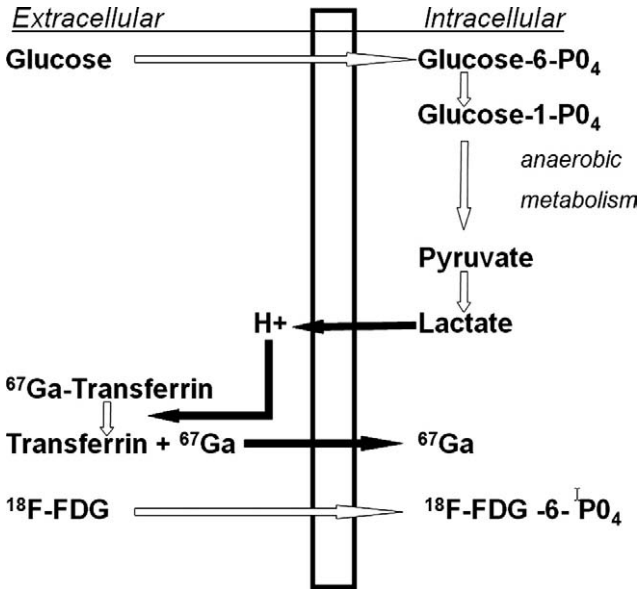

Figure 1 Diagrammatic representation of anaerobic glucose metabolism demonstrating the production of hydrogen ion which favors dissociation of ^{67}Ga from the ^{67}Ga-transferrin complex. Hence, increased anaerobic metabolism favors both ^{18}F-FDG and ^{67}Ga accumulation.

infection/inflammation is the leakage of ^{67}Ga-transferrin complex from blood into the extracellular fluid space of inflamed tissue through gaps in the endothelial junctions of the capillaries.[9] At inflamed/infected sites, cell debris and relative anaerobic conditions result in a localized decline in pH, which favors dissociation of the ^{67}Ga moiety from the ^{67}Ga-transferrinn complex. ^{67}Ga then binds to other iron-binding molecules such as lactoferrin or siderophores which bind ^{67}Ga more avidly than transferrin at the acidic pH present at inflamed tissue sites (Fig. 1).[10]

^{67}Ga-citrate has demonstrated high sensitivity for both acute and chronic infection as well as noninfectious inflammation.[9,11] The specificity, however, is relatively poor because of the physiological bowel excretion, accumulation in malignant tissue and in the areas of bone healing. In addition, optimal imaging often requires delayed imaging up to 3 days after injection to allow for clearance from normal tissue. In patients with vertebral osteomyelitis, ^{67}Ga imaging appears to be more sensitive than labeled leukocytes, probably related to the chronic nature of the infection. In immunocompromised patients, ^{67}Ga imaging is the procedure of choice to detect opportunistic infections because of the nature of the cellular response to the infections (PCP, fungal) usually seen in this population (Fig. 2). In patients with fever of unknown or uncertain origin (FUO), ^{67}Ga scintigraphy had been the gold standard to detect both acute and chronic infection and inflammations. Since the diagnostic potential of ^{18}F-FDG-PET is comparable to that of ^{67}Ga scintigraphy, and since the results are available in hours rather than days, ^{18}F-FDG-PET may now be the preferred procedure[11] (see below).

^{111}In and ^{99m}Tc-Labeled Nonspecific Human IgG

In early 1990s, it was proposed that human polyclonal immunoglobulin (HIG) labeled with ^{111}In or ^{99m}Tc is retained at the sites of infection and inflammation because of a specific interaction (binding) of HIG with Fc-γ receptors expressed on leukocytes.[12] Subsequently, it was shown that radiolabeled HIG localization at the infectious foci is mainly attributable to nonspecific extravasation or leakage (similar to ^{67}Ga-transferrin complex) of the labeled protein due to increased vascular permeability. Unlike ^{67}Ga, however, the ^{99m}Tc does not bind to the other proteins at the site of inflammation.

HIG was initially labeled with ^{111}In with the use of DTPA-conjugated HIG (^{111}In-HIG). Subsequently, HIG labeled with ^{99m}Tc (^{99m}Tc-HIG) with hydrazinonicotinamide as the chelator to complex the metal became available. In various subacute infections, ^{111}In-HIG scintigraphy showed a slightly, but significantly better, overall accuracy compared with ^{111}In-WBC imaging. Radiolabeled HIG appeared to be clinically useful for infection imaging studies in patients with musculoskeletal infections, rheumatoid arthritis, and in pulmonary infection, particularly in immunocompromised patients.[11-14] Radiolabeled HIG may have some clinical utility, but commercial kits are no longer available for routine clinical studies.

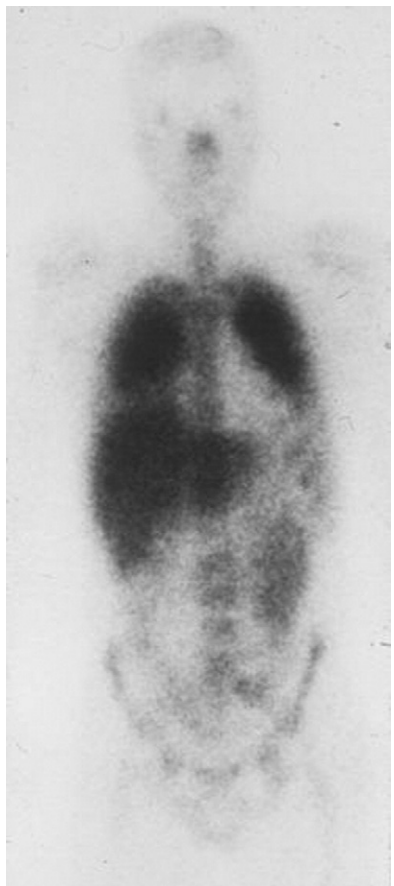

Figure 2 ^{67}Ga scintigraphy (planar) demonstrating bilateral intense ^{67}Ga activity in the lungs (equal or greater than hepatic activity). Although not totally specific, this is a typical image from an immunosuppressed patient with *Pneumocystis carinii* pneumonia.

^{18}F-FDG

^{18}F-FDG-PET can be used to image the increased glucose metabolism of inflammatory cells (PMNs, lymphocytes, macrophages) at sites of infection and inflammation. The uptake in the activated leukocytes is a consequence of the use of glucose as the primary energy source (Fig. 1). After transport into the cells by facilitated diffusion, ^{18}F-FDG is phosphorylated and ^{18}F-FDG-6-phosphate is trapped within the cells. Because the expression of glucose-6-phosphatase is relatively high in mononuclear cells[15] compared with that in malignant cells, ^{18}F-FDG-6-phosphate may undergo dephosphorylation. As a result ^{18}F-FDG molecules may diffuse back into extracellular fluid. In preclinical studies, ^{18}F-FDG showed significant uptake in animal models of experimental infection[16,17] but ^{18}F-FDG accumulation was greater in chronic inflammation than in acute infection.[16] ^{18}F-FDG uptake at the site of infection provides a better signal than the uptake of ^{67}Ga-citrate, radiolabeled thymidine, methionine, and human serum albumin.[18] ^{18}F-FDG-PET has demonstrated significant diagnostic potential and advantage compared with standard nuclear medicine tracers (^{67}Ga-citrate and radiolabeled leukocytes) in patients with soft-tissue and bone infections (Fig. 3).[11,19] In addition, because of the superior signal provided by ^{18}F as well as the utility in both acute and chronic inflammation, ^{18}F-FDG may become the agent of choice for the initial screening of patients suspected of having occult inflammation. Despite this enthusiasm by the nuclear medicine community, currently many 3rd party insurers including Medicare will not reimburse for this procedure.

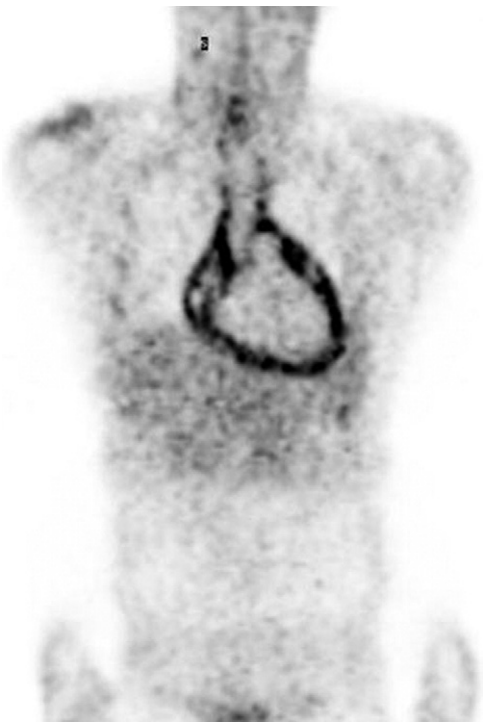

Figure 3 ^{18}F-FDG-PET coronal section demonstrating pneumococcal pericarditis in a 38-year-old man.

Specific Radiopharmaceuticals

^{111}In and ^{99m}Tc-Labeled Leukocytes

Among the circulating leukocytes, the normal differential demonstrates that PMNs account for about 59%, lymphocytes are about 34%, and monocytes are about 2%. In clinical studies, a mixed leukocyte population is isolated by differential centrifugation of anticoagulated blood and labeled in vitro with ^{111}In-oxine or ^{99m}Tc-HMPAO. Because lymphocytes are radiosensitive, they are usually damaged (mutilated) during the labeling procedure. In 1970s, imaging infection and inflammation using ex vivo labeled autologous ^{111}In-leukocytes was first introduced by McAfee and Thakur.[20] After intravenous administration, the labeled leukocytes are first sequestered in the lung, but clear rapidly from normal lungs and accumulate in spleen, liver and bone marrow. With radiolabeled leukocytes, the principle mechanism of uptake at the sites of infection is cellular migration and target specific localization.

The most important clinical indications for radiolabeled leukocytes include FUO, inflammatory bowel disease, osteomyelitis, and follow-up of patients with vascular or orthopedic prostheses (Figs. 4 and 5).[9,21] Leukocyte imaging provides high sensitivity for both acute infection (90%) and chronic infection (86%). Compared with ^{99m}Tc-leukocytes, ^{111}In-leukocytes are more stable in vivo and are better for infection imaging. ^{99m}Tc-leukocytes may provide early diagnosis (2-4 hours) but physiological ^{99m}Tc activity in the abdominal area may be seen and results in false positive images.[22]

^{99m}Tc-Labeled Antigranulocyte Monoclonal Antibodies

Because radiolabeled leukocytes are specific to detect infection and inflammation, radiolabeled antigranulocyte antibodies have been developed to label leukocytes in vivo.[23-26] The murine anti-NCA-95 IgG antibody (BW 250/183) recognizes the nonspecific cross-reacting antigen 95 (NCA-95) expressed on human granulocytes and myelocytes. On the basis of in vitro studies, it was shown that 95% of the activity was bound to granulocytes rather than lymphocytes and monocytes. It has been used successfully to image various infections and inflammatory processes including subacute infectious endocarditis, lung abscesses and septic loosening of hip and knee prostheses.[23,24] The major drawbacks of murine IgG are relatively slower blood clearance of IgG antibody and the production of HAMA after intravenous administration.

On the basis of another antigranulocyte antibody known as anti-NCA-90 antibody, ^{99m}Tc-anti-NCA-90 Fab′ fragments (LeukoScan; Immunomedics GmbH, Darmstadt, Germany) were developed for in vivo leukocyte labeling and infection imaging.[25] The tracer had faster clearance from circulation but showed significant nonspecific bowel activity. There was no clear evidence that Leukoscan had any binding to leukocytes in vivo. Leukoscan did, however, show rapid localization of soft tissue and bone infections.[26-28]

^{99m}Tc-labeled antistage specific embryonic antigen-1 (anti-SSEA-1) monoclonal IgM class antibodies, known as LeuTech (Mallinckrodt, Hazelwood, MO) binds specifically to the CD-15 antigen epitope on the cell membrane of activated

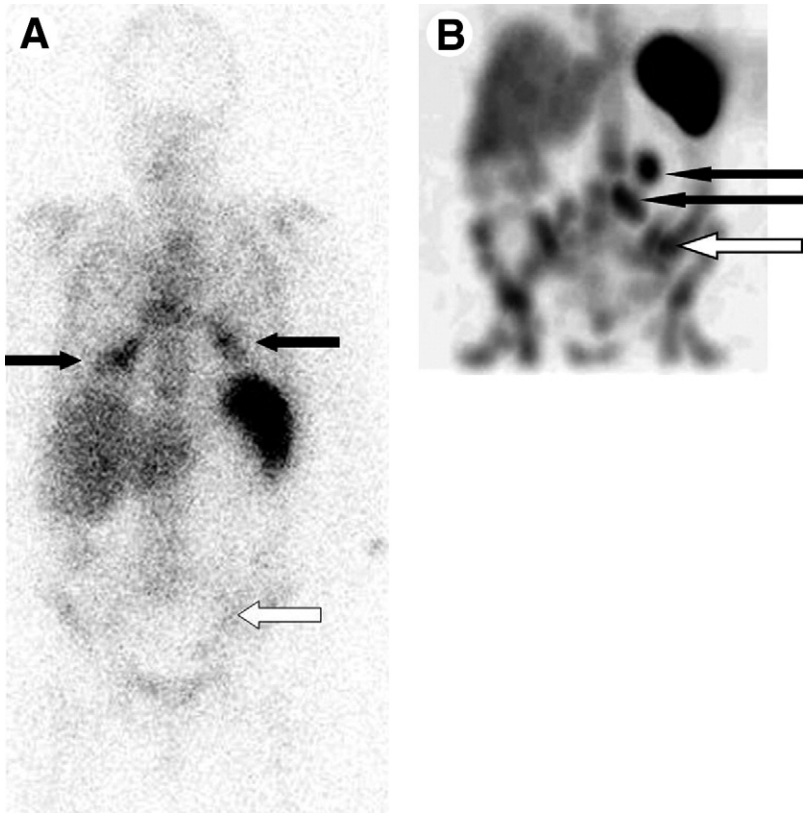

Figure 4 ^{111}In white blood cell (WBC) scintigraphy in soft-tissue infection. (A) Patient with bilateral bronchopneumonia. Minimal left lower quadrant activity represents swallowed sputum. (B) Maximum intensity projection in a patient with infected aortic graft. Interpretation based on single-photon emission computed tomography/computed tomography (not shown) localization of accumulated labeled leukocytes. Left lower quadrant tubular activity identifies concomitant inflammatory bowel disease.

neutrophils with very high affinity ($K_d = 10^{-11}$ mol/L). Because CD-15 antigen expression on activated neutrophils is increased, significant in vivo labeling of neutrophils (>50%) has been observed with LeuTech.[29] Because LeuTech is available as a commercial kit formulation, labeling with ^{99m}Tc pertechnetate can be performed rapidly when needed. Because of the specific binding of circulating neutrophils, LeuTech is rapidly cleared from circulation, making it an ideal radiopharmaceutical for infection imaging studies.[30] LeuTech was shown to be a highly sensitive imaging procedure for detection and diagnosis of equivocal appendicitis.[31] In addition, this agent was successfully used to identify various foci of infection and inflammation associated with osteomyelitis, diabetic foot ulcers, and post surgical infection.[30] Because of specific neutrophil binding and sequestration by the liver, transient neutropenia is observed. This phenomenon may be

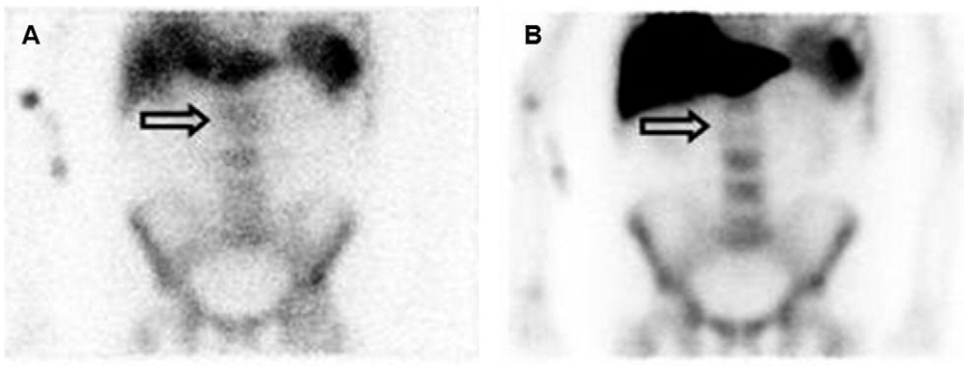

Figure 5 (A) ^{111}In-WBC scintigraphy in vertebral body infection. In this instance, ^{111}In-WBC activity in involved vertebral body is virtually uniform with other vertebral activity. (B) Accompanying ^{99m}Tc-sulfa-colloid imaging to identify bone marrow demonstrates absence of functioning bone marrow elements.

only a minor disadvantage compared with the disadvantage of ex vivo labeling requiring blood removal and separation of leukocytes from blood followed by ex vivo radiolabeling procedures. Unfortunately, LeuTech is not available commercially at this time, since the death of two patients was reported following administration of the radiopharmaceutical even though this serious adverse event may be unrelated to LeuTech.

Among the antigranulocyte antibodies discussed in this article, LeuTech is the only radiolabeled antibody that provides in vivo labeling of neutrophils, and has target specific localization at the sites of inflammation and infection. In general, radiolabeled antibodies and fragments localize at the sites of infectious foci to a large extent by nonspecific extravasation due to increased capillary permeability.[6]

^{99m}Tc-Labeled Ciprofloxacin (Infecton)

The broad-spectrum antibiotic ciprofloxacin is a fluoroquinolone analog with specific binding to bacterial *DNA gyrase* that is present in all viable bacteria. Because of its specific binding to bacteria, it was hypothesized that ^{99m}Tc-labeled ciprofloxacin (known as Infecton [Draximage, Quebec, Canada]), a tracer designed to image microorganisms, may identify only the sites of infection and thus distinguish between sterile inflammation and infection.[32] Localization, however, appears to be based primarily on extravasation and stasis at the sites of increased vascular permeability. It is rapidly cleared from circulation by the kidneys and shows no uptake in bone marrow, and minimal localization in liver and abdominal area. In clinical studies, Infecton showed acceptable sensitivity and specificity in identifying a wide variety of infectious foci,[33-35] but it does not appear to distinguish between infection and sterile inflammation.[36]

^{99m}Tc-Labeled Cytokines

Cytokines are a category of signaling proteins and glycoproteins, like hormones and neurotransmitters, and are used extensively in cellular communication. Cytokines are produced by a wide variety of hematopoietic and nonhematopoietic cell types and can have effects on both nearby cells or throughout the organism. Cytokines interact with leukocytes and other inflammatory cells via specific cell surface receptors and bind with high affinity in nanomolar range. Cytokines are of human origin, non immunogenic, have low molecular weight (<25 kDa) and clear rapidly from circulation. Therefore, radiolabeled cytokines may be ideal molecular imaging probes for imaging infection.

Interleukin-2 (IL-2) is a glycoprotein (15.5 kDa), synthesized and secreted by stimulated T-lymphocytes. Since activated lymphocytes are known to express high affinity IL-2 receptors, ^{123}I and ^{99m}Tc-labeled IL-2 were developed for imaging chronic infection.[37,38] In patients with active Crohn's disease, type-1 diabetes, Hashimoto's thyroiditis and Grave's disease, radiolabeled IL-2 targeted mononuclear cell infiltrations occur at the sites of chronic inflammation.[37,38]

IL-8, a chemotactic cytokine is a small protein (8.5 kDa) and binds with high affinity to specific receptors (CXCR1, CXCR2) on granulocytes. ^{123}I and ^{99m}Tc-labeled IL-8 were developed as target specific molecular probes to image infection. In animal models with experimental infection, radiolabeled IL-8 showed significant localization.[39] Recent clinical studies in patients with suspected infection, ^{99m}Tc-IL-8 imaging studies demonstrated diagnostic potential with no side effects.[40]

Overview and Conclusions

Among the radiopharmaceuticals that have been evaluated to identify sites of infection and/or inflammation, there are three principle choices available in the United States: ^{67}Ga- gallium citrate, ^{111}In- and/or ^{99m}Tc-labeled leukocytes, and ^{18}F-FDG. A fourth choice, ^{99m}Tc-anti-SSEA-1 IgM (LeuTech), is approved and marketed in many European countries. ^{67}Ga-citrate and ^{18}F-FDG have been described as useful to identify both acute and chronic inflammatory conditions. At the present time, however, although ^{18}F-FDG is available as an imaging agent for neurologic, oncologic, and cardiac indications, it is not yet approved as an agent for the identification of inflammatory/infectious foci. Neither radiotracer, however, is specific for inflammation; both radiotracers identify focal areas of increased anaerobic glucose metabolism, which occurs in most tumors and even healing scars. Labeled leukocytes provide the greatest specificity to identify infection but have the limitation that they are most sensitive to detect acute inflammation, that is, the inflammatory response dominated by polymorphonuclear leukocytes (granulocytes). With chronicity, the granulocyte response decreases and the inflammatory process is dominated by macrophages and other mononuclear cells.

Chronic inflammation as well as acute inflammation is nevertheless readily detectable with either ^{67}Ga-citrate or ^{18}F-FDG because the accumulation of both occurs with localized pH reduction. It is appropriate to briefly consider the choice or preference between these two tracers as well as the differences observed between ^{111}In and ^{99m}Tc-labeled leukocytes.

In general a shorter radionuclide half-life is favorable because it allows for the administration of a larger dose. Different radionuclides, however, have different rates of blood clearance. Because ^{67}Ga binds to transferrin, it clears slowly from the circulation whereas ^{18}F-FDG clears rapidly. Accordingly, one would expect better quality images and detection of inflammation with ^{18}F-FDG provided the inflamed/infected site is well perfused such as at soft-tissue sites (Fig. 5), whereas it is less satisfactory (and not recommended) for the detection of osteomyelitis. ^{18}F-FDG is probably the agent-of-choice for FUO and suspected abdominal or other soft tissue sites of infection (or inflammation). ^{111}In- and/or ^{99m}Tc-labeled leukocytes are more specific than those agents for acute soft-tissue infection (Figs. 4 and 5) but the short half-life of ^{99m}Tc is somewhat of a mismatch given the time involved in leukocyte migration to most inflammatory sites. In this regard, it is not surprising that it has had some use for inflammatory bowel disease but is not used at all for osseous infections. As good as ^{18}F-FDG is for soft-tissue infections, it not sensitive for the detection of less well perfused osseous foci as it clears from the circulation rapidly.

In acute inflammation, although both ^{67}Ga-citrate and ^{111}In-labeled leukocytes can be used, ^{111}In-leukocytes usually provide better images because of the greater contrast between the infected/inflamed focus and the background. Infections such as PCP which are characterized by a nongranulocytic response (unless there is a superimposed secondary bacterial infection) are identified by ^{67}Ga-gallium citrate with greater intensity than would be observed with labeled leukocytes (Fig. 2).

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