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A N I N T E R N A T I O N A L J O U R N A L

Infection Imaging with Radiopharmaceuticals in the 21st Century

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ABSTRACT

Infection continues to be a major cause of morbidity and mortality worldwide. Nuclear medicine has an important role in aiding the diagnosis of particularly deep-seated infections such as abscesses, osteomyelitis, septic arthritis, endocarditis, and infections of prosthetic devices. Established techniques such as radiolabelled leucocytes are sensitive and specific for inflammation but do not distinguish between infective and non-infective inflammation. The challenge for Nuclear medicine in infection imaging in the 21st century is to build on the recent trend towards the development of more infection specific radiopharmaceuticals, such as radiolabelled anti-infectives (e.g. ^{99m}Tc-ciprofloxacin). In addition to aiding early diagnosis of infection, through serial imaging these agents might prove very useful in monitoring the response to and determining the optimum duration of anti-infective therapy. This article reviews the current approach to infection imaging with radiopharmaceuticals and the future direction it might take.

Key words: Radiopharmaceuticals, imaging, infection, inflammation, radiolabelled anti-infectives, ^{99m}Tc- ciprofloxacin

INTRODUCTION

At the dawn of the 21st century, infection still remains a major cause of mortality and morbidity globally, although the developing countries bear the greatest burden. Tuberculosis and multi-drug resistant bacteria are increasing at an alarming rate and provide diagnostic, therapeutic, and infection control challenges. In addition, deep-seated infections e.g. intra-abdominal abscesses, endocarditis and osteomyelitis, due to these and other infective agents can be difficult to detect, resulting in delayed diagnosis, treatment, and sometimes even death. Clinicians use a variety of clues, e.g. clinical, laboratory, and radiological tests, to aid diagnosis and influence decision-making. Although commonly employed and useful, the demonstration of a lesion by conventional radiological techniques such as X-ray, ultrasound, computerized tomography (CT), magnetic resonance imaging (MRI), depends on the presence of structural abnormalities. These may take some time to become visible, may not always be present, and their resolution lag behind cure of the infection (e.g. monitoring of treatment of emphysema in the lung by serial chest X-rays or CT scans). In addition they are neither inflammation nor infection specific.

The introduction of radiopharmaceuticals in Nuclear Medicine has enhanced infection imaging,

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because it depends on the demonstration of pathophysiological and pathobiological changes, which occur earlier in the infection process and also resolve quicker after cure of the infection compared with gross changes in structure. They are often employed in the context of fever of unknown origin (FUO), where 30% (higher in older age groups) of the causes are non-infective, or suspected deep-seated infection to aid diagnosis and establish the need for anti-microbial therapy. However, the currently available agents target or label components of the inflammatory response, e.g. immune globulin, neutrophils, and cytokines, and are thus inflammation specific but unable to distinguish between infective and non-infective inflammation. Hence the expanding range of radiopharmaceuticals over the last few years, such as radiolabelled anti-infectives, which aim to be infection specific has been of particular interest to Clinical Microbiologists and Infectious Disease physicians as well as specialists in Nuclear Medicine [Becker and Meller, 2001; Wareham et al., 2000; Corstens et al., 1999]. In this review, the utility and limitations of the various radiopharmaceuticals (old, new, and experimental) for imaging infection are discussed, as well as the direction for future research in this important area.

Imaging agents

Irrespective of the cause, acute inflammation manifests as swelling, redness, and pain, leading to protective loss of function. It results from vasodilatation and increased capillary permeability, extravasation of proteins and cells, complement activation and release of soluble pro-inflammatory cytokines. Neutrophils adhere to the vascular endothelium by binding to adhesion molecules (e.g. E-selectin, ICAM-1, V-CAM, the expression of which are up-regulated locally by inflammation), exit the circulation by diapedesis and migrate to the site of inflammation by chemotaxis, down the chemoattractant gradient.

Chronic inflammation is characterised by a reduction in vasodilatation, capillary permeability and neutrophil infiltration, but an increase in the number of lymphocytes and macrophages at the focus of inflammation.

Established radionuclide imaging agents target components of these inflammatory responses and those that depend on vascular permeability (e.g. radiolabelled leucocytes) have greater sensitivity for detecting acute than chronic inflammation. However, none of these are infection specific. The properties of an ideal infection imaging agent are shown in Table 1.

Table 1 - Properties of an ideal infection imaging agent

Properties
• No side effects
• Specific to infection
• Applicable to immunocompromised patients
• Low non specific uptake
• Low marrow, gut, renal uptake
• Safe and easy to prepare and administer
• Not too expensive

The various radiopharmaceuticals, which have been developed for imaging infection and inflammation are discussed below and summarized at the end in Table 2.

Gallium-67 citrate (^{67}Ga)

Gallium-67 citrate (^{67}Ga) was one of the first radiopharmaceuticals developed for imaging infection [Hoffer, 1980]. It is transported in the blood either in ionic form or bound to transferrin, but at sites of inflammation it leaks out of the capillaries into the tissues after binding to the transferrin receptor CD71. In the tissue it binds with high affinity to lactoferrin, which is present in abundance in abscess fluid and neutrophils. Additionally ^{67}Ga may be taken up by siderophores produced by microorganisms as a mechanism for scavenging iron from the host in low-iron environment found in infected tissues. However, ^{67}Ga has a number of major drawbacks, which include: 1) usually it has to be ordered from an external supplier, which takes time; 2) imaging is usually over 48 h; 3) high radiation exposure; and 4) unfavourable physical characteristics for gamma camera imaging. Hence it is not widely used, having been superseded by ^{99m}Tc-labelled pharmaceuticals, which have more favourable properties as infection imaging agents. It is sometimes useful for the investigation of malignancy and autoimmune diseases, and is occasionally used for the investigation of FUO, chronic (but not acute) infections, including spinal osteomyelitis, and pulmonary infections,

particularly in immunocomromised patients. However, it may be unreliable post surgery or if fracture is present.

Radiolabelled Leucocytes (White cells)

Radiolabelled polymorphonuclearleucocyte (PMN) imaging is regarded as the "gold standard" in nuclear medicine technique for imaging infection and inflammation in many countries. PMN migrate to and concentrate at the site of infection through diapedesis and chemotaxis. These cells can be labelled with Indium 111 (^{111}In), using oxine, tropolone or mercaptopyridine N-Oxide (MERC) as a chelating agent, or ^{99m}Tc using hexamethylpropyleneamineoxime (HMPAO) as the chelating agent. The techniq ue is highly sensitive, exceeding 95% in some studies [Peters, 1994] and specific for acute inflammation (less useful in chronic inflammation because the influx of neutrophils into the lesion is greatly reduced) but does not distinguish between infective and non-infective inflammatory conditions, e.g. inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, which are key indications for leucocyte imaging (figure 1), from infective enteritis [Datz, 1994]. Uptake of white cells by bone marr ow and fractures makes bone images difficult to interpret, e.g. in osteomyelitis and septic arthritis. Neutropaenic patients do not have sufficient number of neutrophils to migrate to sites of infection and form abscesses and hence the technique is unreliable in these patients. In addition to being technically demanding and labour -intensive (about 3 hours to prepare), a major drawback is that white cell imaging involves *ex-vivo* labelling with the attendant hazards of blood borne infections from needle stick injuries, such as HIV, hepatitis B and C, as well as deterioration of the white cells themselves.

111In Leucocytes

^{111}In labelled leucocytes have been used for many years for the localisation of infection, after the labelling process was first describe d by Thakur et al. in 1977. It is lipophilic with a long half -life, which allows imaging beyond 24 h post injection. The agent is rapidly cleared from the blood pool and normal lungs and is taken up by the spleen and liver, but not the kidneys, bladder, ga ll bladder, and the gut. This makes it a good agent for imaging the abdomen, thorax, and regions near the blood pool, such as vascular prostheses. The technique is highly sensitive in acute infections involving these areas but in chronic infections and infections of the central skeleton the sensitivity is lower.

99mTc labelled Leucocytes

^{99m}Tc is one of the most commonly used radioisotopes in Nuclear Medicine and has a number of important advantages over ^{111}In-leucocytes. The radiation dose is low, quali ty of the images is superior, and the agent is readily available since it is obtained from generators on site rather than having to be obtained from an outside source. ^{99m}Technetium-leucocytes localise to the sites of infection more rapidly than ^{111}In-leucocytes and its sensitivity is higher [Mountford et al., 1990]. Thus it has replaced the latter for imaging inflammation and infection in most situations. However it is less stable than ^{111}In-leucocytes, with the radiolabel starting to leach out of the leucocytes soon after injection, accompanied by renal and then biliary excretion [Becker, 1995]. This does not occur with ^{111}In, which is, therefore, sometimes preferred for imaging renal, genitourinary, and gastrointestinal infections/inflammation.

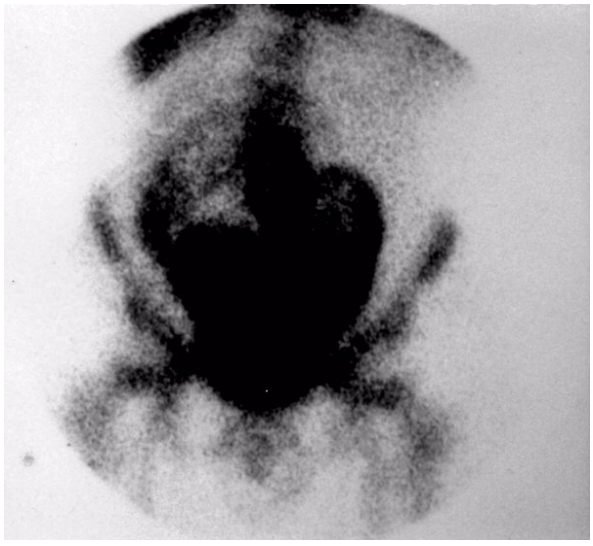

Figure 1 - Sterile pouchitis. ^{99m}Tc-lecucocyte imaging showing inflamed loops of bowel.

Human Polyclonal Immunoglobulin G (HIG)

HIG may be labelled with ^{111}In or ^{99m}Tc [Buscombe, 1995]. They accumulate at sites of inflammation mainly via capillary leakage, with smaller contribution from binding of the Fc portion of IgG to Fc receptors on granulocytes and, depending on the source of the pooled human immune globulin, binding of IgG to bacteria. Because HIG localises at sites of bacterial infection, sterile inflammation, and inflammatory tumours, it is a good "catch all" agent in FUO, especially in neutropaenic and immunocompromised patients. It has a half-life of 67 h, allowing imaging 24 h post injection with ^{111}In-HIG. It has been reported to be more sensitive than leucocyte imaging [De Kleijn et al., 1997] and does not require *ex vivo* labelling. Due to high blood pool activity, a limitation is that it is unable to image vascular lesions.

Radiolabelled Monoclonal antibodies (MCAs)

Radiolabelled murine MCAs against surface antigens of granulocytes were initially developed as a method of labelling leucocytes *in vivo* [Locher et al., 1986]. But non-specific extravasation through leaky capillaries at sites of inflammation is now recognised as the main mechanism of localisation of these agents at infective/inflammatory foci [Morrel et al., 1990]. ^{99m}Tc labelled anti-NCA-95 antibody (anti-CD66/CD67) is the most commonly used antibody (Granuloscint) in this category. Because of their high molecular weight they penetrate poorly and are slowly cleared from the blood and hence usually require 24 hr images to accurately locate inflammatory foci. This and the problem of the induction of human anti-mouse antibodies (HAMAs) have been overcome to a large extent by using antibody fragments, which are smaller, more rapidly cleared, and are less immunogenic. Examples include ^{99m}Tc labelled antigranulocyte Fab'-fragment (Leukoscan) [Becker et al., 1994]. ^{99m}Tc labelled anti-CD15 IgM monoclonal antibody (LeuTec) however is a large molecule, which has been used to image patients with appendicitis [Mozley et al., 1999]. MCAs against endothelial adhesion molecules, e.g. E-selectin have also been evaluated [Jamar et al., 1995] but like the above, are not specific for infection.

The development of MCAs directed against microbial antigens is a promising, and in theory perhaps the most specific, approach for imaging infection. Promising results were obtained with radiolabelled monoclonals for the detection of syphilitic lesions [Lee et al., 1990], *Pnemocystis carinae* pneumonia [Goldenberg et al., 1994] and *Pseudomonas aeruginosa* infection. Clinical efficacy may be enhanced by using a pool of monoclonals or monoclonals against genus-specific epitopes. However, currently data is limited to support their use in human infections.

Bacterial Chemotactic peptides

These small molecules, the prototype of which is N-formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe), produced by bacteria bind to granulocyte surface receptors with high affinity and stimulate chemotaxis. However, their development as radiopharmaceuticals have been curtailed because they cause significant leucopenia at physiological concentrations.

Cytokines

Agents such as radiolabelled interleukin-1 (IL-1), IL-1 receptor antagonist (IL1-ra), IL-8, platelet factor 4 (PF4), and tuftsin antagonist bind to surface receptors on polymorphs with high affinity and may be promising for imaging acute infections [Van der Laken et al., 1998]. Other cellular messengers may be superior for targeting surface receptors of the predominant cell types in chronic inflammation and infection, e.g. substance P targets mononuclear cells, and IL-2 and ^{111}In-octreotide target activated T-cells [Procaccini et al., 1999]. However, as with the bacterial chemotactic peptide analogues, these agents demonstrate agonist activity, causing toxicity (e.g. leucopaenia) even at low (physiological) doses. The development of the receptor antagonists as imaging agents was a way around this problem but they are taken up to a much lower extent by infective and inflammatory foci than the agonists [Van der Laken et al., 1996]. In addition, cytokines and the receptor antagonists are inflammation but not infection specific.

Nanocolloids

Nanocolloids are colloids of human serum albumin (HSA) less than 50nm in size [Streule et al., 1988], which localise at sites of inflammatory

foci through increased capillary permeability. ^{99m}Tc-labelled nanocolloids have been most commonly used for marrow and lymphatic imaging and for patients with musculoskeletal infection, with reported sensitivity and specificity of 87% and 93% respectively. The greatest disadvantage is their inability to image infections outside the musculoskeletal system and, as with most of the currently available radiopharmaceuticals, distinguishing infection from inflammation.

Radiolabelled Liposomes

Liposomes are bilayer vesicles formed spontaneously when amphiphilic phospholipids are exposed to aqueous solutions. They have been used as drug delivery agents, but can also trap radiopharmaceuticals [Datz, 1993]. Radiolabelled liposomes are phagocytosed by polymorphs and macrophages, which accumulate at infective foci. Their rapid clearance from the circulation by cells of the mononuclear phagocytic system (MPS) and accumulation in organs such the liver, spleen and bone marrow limit their usefulness as radiopharmaceuticals, particularly for imaging the upper abdomen. Long-circulating liposomes (LCLs) or sterically stabilised liposomes have been developed, e.g. polyethylene glycol or PEG-liposomes, which allow more prolonged circulation time in the blood and increased uptake at sites of inflammation [Torchilin, 1996]. However, the prolonged blood pool activity is a disadvantage for imaging infections in well perfused tissues. Biotinylated liposomes overcome this problem by clearing rapidly from the circulation, following the intravenous administration of avidin [Ogihara-Umeda et al., 1993]. Factors such as size and lipid composition affect the ability of liposomes to localise at inflammatory foci. In a study of 35 patients with predominantly musculoskeletal pathology, ^{99m}Tc-PEG-liposome scintigraphy was shown to have a sensitivity of 94% and specificity of 89% for infection [Dams et al., 2000]. Radiolabelled liposomes are difficult to prepare and do not distinguish between infective and non-infective inflammation. However, labelling liposomal anti-infective agents could be a potentially promising adaptation of this approach and might make it more specific for infection imaging.

Streptavidin-Biotin

Streptavidin is a small protein, which diffuses rapidly through leaky capillaries at sites of inflammation. Biotin has both high affinity for streptavidin and rapid whole body clearance. When it is radiolabelled with ^{111}In, the Streptavidin ^{111}In-Biotin complex can be detected at the site of inflammation. Hence the technique is called pretargetting. A preliminary study on 4 patients with chronic osteomyelitis gave promising results [Rusckowski et al. 1992], but larger studies are lacking.

^{18}F-Deoxyglucose (FDG)

Fluorine-18 fluorodeoxyglucose (FDG) is a positron emitter and is preferentially taken up by cells, which predominantly metabolise glucose as a source of energy, e.g. cancer cells and inflammatory cells such as neutrophils and macrophages. When stimulated, inflammatory cells express high concentrations of glucose transporters (GLUT-1 to GLUT-7), facilitating entry of FDG into these cells [Shepherd and Kahn, 1999]. Although clinical experience with this agent is limited, clearly FDG is not an infection specific agent [Alavi and Zhuang, 2001]. However, a prospective study showed it to be superior to ^{67}Ga scintigraphy in imaging FUO, with sensitivity and specificity of 81% and 86% respectively [Meller et al., 2000]. FDG-PET was reported to be highly accurate in the evaluation of musculoskeletal infections in 60 patients, with a sensitivity of 100% and specificity of 88% [De Winter et al., 2001]. In chronic osteomyelitis [Guhlmann et al., 1998] and infected lower limb prosthesis implants [Zhuang et al., 2001], sensitivities of 100% and 90.5% and specificities of 92% and 81% respectively have been reported.

Radiolabelled Antimicrobial peptides

A variety of endogenous antimicrobial peptides are found in abundance in mammals, birds, amphibians, insects and plants, providing protection against microbial attacks and contribute to the innate resistance to infection [Lehrer and Ganz, 1992, Ganz and Lehrer, 1995]. Defensins are small peptides containing 29-35 amino acid residues and are synthesized by several

mammalian cell types but particularly neutrophil precursors in the bone marrow. They make up 5-7% of the total protein in neutrophils, being present in high concentrations in the azurophil granules and phagocytic vacuoles. Hence they are also called human neutrophil peptides, of which there are four types, HNPs 1-4. They are released into the phagosome, where they contribute to the oxygen-independent cytotoxic killing of the phagocytosed microorganism. Their broad spectrum antimicrobial activity includes Gram-positive and Gram-negative bacteria, mycobacteria, spirochaetes, many fungi and enveloped viruses. They act by inserting into the target cell membrane, forming voltage-sensitive or ion channels, causing the microbial cell membrane to become permeable and the death of the microbe. However the killing mechanism is not specific for microorganisms [Lichtenstein, 1991], and normal cells may be destroyed if the process is not carefully controlled and confined to the phagocytic vacuole within the neutrophils. Additionally functions independent of cytotoxicity have been reported in many instances, raising the possibility that HNPs subserve multiple roles in inflammation and thus are not specific to infection [Levy, 1996]. This is compatible with our current understanding of the immune system, i.e. the innate immune system is critical in the rapid host response to challenge by foreign antigens, infective and non-infective, whereas humoral (antibody produced by B-lymphocytes/plasma cells) and cell mediated (T-lymhocytes and macrophages, augmented by cytokines) immunity take longer to appear but are specific to the antigen and may provide long term protection against infection through the generation of memory cells.

The plasma concentration of HNPs rise to high level with infection [Ihi et al., 1997]. Pro-HNPs circulate in the plasma as free forms. By contrast, HNPs 1-3 bind to α_2-macroglobulin, albumin, and immunoglobulins, which may function to scavenge these biologically very potent agents, restricting their activity to the phagolysomes in neutrophils. There is still a lot to be learnt about these important molecules, e.g. physiological role and pathophysiological implications, metabolism (site and rate of degradation), permeability into different tissues and pharmakokinetics including plasma half-life (particularly of extracellular defensins) [Lehrer, 1997].

However, promising results were reported in animal models (mouse and rabbit) of infection using ^{99m}Tc-labelled antimicrobial peptides [Welling et al., 1999]. In this study, ^{99m}Tc-labelled HNP-1 allowed rapid visualisation of *Staphylococcus aureus* and *Klebsiella pneumoniae* induced infections (peritonitis and thigh abscess) but not sterile inflammation in mice, which was considerably faster than that for ^{99m}Tc-labelled IgG (as early as 5 min after injection compared with 4 h for IgG)). The exogenously administered HNP-1 was rapidly cleared from the circulation, mainly through renal excretion into the bladder, with half-lives of 170 and 55 min for *S. aureus* and *K.pneumoniae* induced infections respectively. Similar results were obtained with the antimicrobial peptide ubiquicidin (UBI) labelled with ^{99m}Tc (UBI 18-35 and UBI 29-41) but results were less favourable for human lactoferrin (hLF) and related peptides [Welling et al., 2000]. Infection due to *Candida albicans* could also be imaged [Welling et al., 2001]. Welling and colleagues preferred ^{99m}Tc-labelled UBI peptides over defensins for imaging infection because they can be prepared synthetically under good manufacturing conditions in large amounts and so far appear to lack immunological side-effects, e.g. on leucocyte function. However, as yet there are no studies of these agents in human patients with infection.

Radiolabelled Antimicrobial agents

The property of selective toxicity (i.e. destroying the microbe but causing little harm to the patient) is the basis of the use of antimicrobial compounds, e.g. antibiotics, to treat infections and has been life saving in this respect. The same property provides the potential for these agents to be exploited as radiopharmaceuticlas for infection imaging. The principle is simple: the specificity for infection is provided by the antimicrobial agent binding to the microbe, which in turn can be visualised by a gamma camera because the antimicrobial agent is labelled with a gamma emitter such as ^{99m}Tc.

^{99m}Tc-Ciprofloxacin (Infecton)

The first antibiotic to be developed as a radiopharmaceutical was ^{99m}Tc-ciprofloxacin (Infecton), which possesses many of the properties of the ideal infection-specific agent (Table 1). Ciprofloxacin is a broad spectrum quinolone antibiotic, which binds to bacterial DNA gyrase

and inhibits DNA synthesis. It is retained at sites of infection and associates freely with metal ions, allowing it to be radiolbelled with technetium. Ciprofloxacin also binds to the equivalent mammalian enzyme, topoisomerase II, but with 100 to1000 times lesser affinity and the binding is readily reversible. Similarly, although they may penetrate neutrophils, macrophages and other cells and tissues, they are not retained at these sites for prolonged periods. Thus after the initial distribution phase, as the serum concentration falls, the antibiotic readily leaches out of the cells and tissues into tissue fluid and then the blood, and excreted freely, predominantly in the urine. By contrast ciprofloxacin is retained at sites of infection, giving high target to back ground ratio and permitting infection specific imaging to occur when sequential images are taken at 1,4, and if required 24 h post injection.

In vitro, Infecton is taken up by a wide variety of Gram-positive, Gram negative and anaerobic bacteria (including ciprofloxacin resistant bacteria as long as the resistance is not mediated by cel l membrane impermeability, which prevents entry of the antibiotic into the bacterial cell) but not by dead bacteria or white cells [Hall et al., 1996]. In a rabbit thigh model, the agent was taken up by infected abscess induced by injection of *S.aureus* but not by sterile abscess produced by injection of turpentine. In man, the normal Infecton image shows high uptake by the kidneys, with excretion to the urinary bladder, moderate uptake by the liver and spleen and no uptake by bone or bone marrow (fig. 2).

Early images show predominantly blood pool activity. The gall bladder may be seen occasionally and bowel activity is commonly seen at 4 h in patients from the Asian subcontinent but rarely in Europeans or South Americans. An abnormal image, as well as show ing normal uptake, shows diffuse uptake at sites of bacterial infection (fig. 2).

In a comparative study in 51 patients, Infecton demonstrated greater specificity (96%) for imaging infection than white cell imaging (84%) [Vinjamuri et al., 1996]. The high specificity for bacterial infection was confirmed in a subsequent study involving 90 patients [Hall et al., 1997]. It also showed that some infections due to ciprofloxacin resistant bacteria could be imaged by Infecton and that prior antibiotic treatment d id not significantly affect the imaging result. The sensitivity and specificity of Infecton imaging has been validated further in a large multi -centre study involving 879 patients, with a wide variety of infective and non-infective conditions (including non-infective inflammatory disorders), in eight countries, under the auspices of the International Atomic Energy Agency (IAEA) [Britton et al., 2002]. Internationally recognised criteria (e.g. CDC, Duke's for infective endocarditis, and WHO for tuberculosis) were used to define infections, as were agreed criteria for the interpretation of Infecton images.

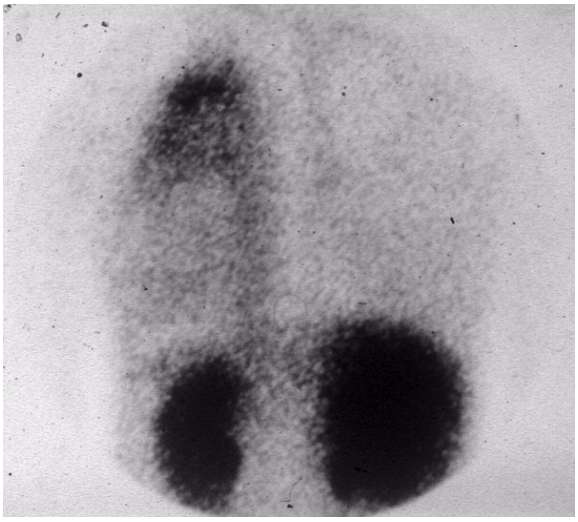

Figure 2 - Left lung abscess demonstrated by ^{99m}Tc-ciprofloxacin (Infecton) imaging. The lack of bone marrow uptake seen here in the vertebrae is an advantage with this technique

No adverse reactions occurred and antibiotic resistant organisms did not emerge as a result of the administration of Infecton into patients. This was expected because only a tracer dose of ciprofloxacin is present in Infecton - 2 mg, which is $1/200^{th}$ of a single therapeutic intravenous dose of ciprofloxacin (400 mg). Overall, the sensitivity (85.4%) and specificity (81.7%) for imaging sites of infection were good, but varied according to the type of infections imaged. The highest sensitivities (in excess of 90%) were seen in osteomyelitis, septic arthritis, infection of orthopaedic prostheses (which is often difficult to diagnose by standard techniques and differentiate from aseptic loosening), and culture proven soft tissue and abdominal infections and tuberculosis. The greatest specificities (above 90%) were seen in orthopaedic prosthesis infections, surgical wound infections, and infective endocarditis. Where serial

images were done, conversion from positive to negative Infecton image correlated well with the resolution of the infection with antibiotic therapy (figure 3) with or without other treatment modalities, such as surgery.

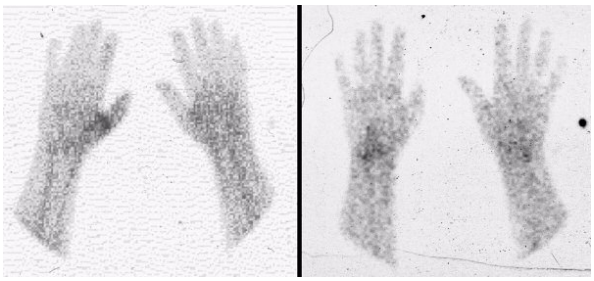

Figure 3 - Osteomyelitis of the left thumb.

^{99m}Tc-ciprofloxacin (Infecton) images showing resolution of infection with successful treatment Left: before treatment, Right: after treatment.

Serial imaging with Infecton, therefore, might be useful to monitor the response to antibiotic therapy and help to decide when to stop it or continuing it if no conversion has occurred.

In addition, Infecton has several advantages over established, e.g. radiolabelled leucocytes, and other methods for imaging infection, which include: 1) Specificity for infection, 2) Lack of bone marrow uptake, which is a significant advantage in imaging bone and joint and orthopaedic prostheses infections, 3) Ease and cost of preparation of the agent, 4) *Ex-vivo* labelling, which avoids contact with blood and hence the risk of acquiring blood borne infections such as HIV and Hepatitis B and C, 5) Independence of the host inflammatory response and neutrophil count and hence it can be used to image infections in immunocompromised patients, including those who are neutropaenic, where culture is often negative and white cell imaging unreliable, and 6) Availability in a kit format with long shelf-life, making it user friendly and more widely available.

^{99m}Tc- Ethambutol

Ethambutol is a narrow spectrum antibiotic, which is active against mycobacteria (inhibits cell wall mycolic acid synthesis) and is used as a first line drug for the treatment of tuberculosis (TB). Hence radiolabelled ethambutol is an attractive candidate for specifically imaging mycobacterial infections, including early TB. Good results were obtained by Verma et al. (2002) in a thigh model of *Mycobacterium tuberculosis* infection in mice and rabbits. ^{99m}Tc- Ethambutol accumulated at the site of infection as early as 2 h post injection, which increased at 4h and persisted till 24 h. Further studies with this agent are eagerly awaited.

^{99m}Tc-Fluconazole

Lupetti et al. (2002) reported that the antifungal agent fluconazole radiolabelled with technetium could distinguish *C.albicans* infections from bacterial infections and sterile inflammation in mice. This is encouraging and one hopes that, given the difficulty of early diagnosis with existing methods and the significance of *C.albicans* as a major pathogen in immunocopromised patients, ^{99m}Tc-fluconazole might be successful in imaging invasive *C.albicans* and other yeast infections in humans.

CONCLUSION

A plethora of radiopharmaceuticals is now available for imaging infection/inflammation and the list is expanding every year. The problem for the nuclear medicine physician is to select the best agent for the condition being investigated. This may depend on whether this is acute or chronic, the body location, tissue type of the lesion (e.g. skeletal or soft tissue), patient's immune status, as well as side effect, pharmacokinetic, and imaging properties of the radiopharmaceutical. Thus the following options have been suggested by Corstens and van der Meer [1999]: 1) Bone infection- ^{99m}Tc-methylenediphosphonate (MDP), ^{99m}Tc-HIG, and ^{99m}Tc-antigranulocyte MCA (in peripheral skeletal infection); 2) Soft-tissue infection- ^{99m}Tc-leucocytes (in acute lesion but not chronic because of the low influx of leucocytes), ^{67}Ga for pulmonary conditions (but not abdominal because of the excretion of the agent in the gut), ^{99m}Tc-antigranulocyte MCA (in appendicitis, vascular graft infection, and native and prosthetic valve endocarditis); 3) Neutropaenic patient- ^{99m}Tc-HIG, ^{67}Ga (in viral, fungal, mycobacterial, and protozoal infections); 4) FUO- ^{111}In-leucocytes and ^{67}Ga; 5) Septicaemia (to locate a focus of infection, if present)- radiolabelled leucocytes and ^{18}FDG. It is unlikely that most nuclear medicine departments would have access to all these agents.

Furthermore, while sensitive and specific for inflammation, none of these radio-pharmaceuticals are specific for infection.

In infection imaging the key problem is the distinction between infection and inflammation. There are many causes of inflammation, infection being only one, though a very important one. In addition, the degree and type of the inflammatory response varies according to the type of infection and the infectious agent as well host factors such as nutrition, neutropaenia and immunosuppression whether due to underlying diseases such as cancer and HIV infection or treatment with drugs, e.g. cytotoxic agents and steroids. Radiopharmaceuticals that target the host inflammatory response are not, therefore, ideal for imaging infection, most importantly because they are not specific to it.

Table 2 - Radiopharmaceuticals for infection imaging

RADIOPHARMACEUTICAL	METHOD OF LOCALISATION	DISADVANTAGES
Gallium-67 citrate	Lactoferrin binding	Non specific for infection, poor image quality, superseded by better agents
Radiolabelled leucocytes	Diapedesis and chemotaxis	Inflammation but not infection specific
Polyclonal immunoglobulin G	Unclear but mainly capillary leakage	Non specific for infection, localise tumours also
Monoclonal antibodies	a) Binding to leucocyte surface antigens	a) Non specific for infection
	b) Binding to microbial antigens	b) Limited animal studies
Chemotactic peptides	Bind to receptors on polymorphs	Cause a fall in peripheral white count
Cytokines	Bind to receptors on inflammatory cells	Agonist activity (side-effects) Inflammation specific
Cytokine receptor antagonists	As above	Lower uptake at inflammatory foci
Nanocolloids	Increased vascular permeability	Non-specific for infection
Liposomes	Phagocytosis by polymorphs and macrophages	Difficult to prepare, accumulate in liver and spleen, non-specific for infection
Streptavidin-Biotin	Increased vascular permeability Pretargetting	Non-specific for infection
F-18-Deoxyglucose	Uptake by metabolically active cell	Non-specific for infection
Antimicrobial peptides	Binding to microbial and other cell membranes	May have multiple roles in inflammation
Antimicrobial agents:		
-Ciprofloxacin (Infecton)	-Binding to bacterial DNA gyrase	Emergence of resistant organisms a potential problem but the risk is probably minimal with tracer doses of the anti-infective agent
-Ethambutol	-Inhibition of mycolic acid synthesis (mycobacterial cell wall)	
-Fluconazole	-Inhibition of ergosterol synthesis (yeast cell membrane)	

The sensitivities and specificities reported in the literature for these agents for localising infective foci are in reality those of inflammation, which often (but not always) accompany infection, but not of infection itself. To be infection specific, the agent must target the microbe, which is causing the infection. The analogy here is the management of a patient with suspected infection, where leucocytosis, raised ESR and CRP, and if available sometimes changes in cytokine levels or their receptor expression, may be useful as non specific (inflammatory) markers of infection and are thus often measured, but by themselves are not diagnostic of infection - this depends on culture of the micro-organism or detection of its components, e.g. antigens, DNA, or failing these specific antibody against the organism. The challenge for nuclear medicine for infection imaging in the 21st century is, therefore, to move away from over dependence on labelling components of the host inflammatory response, which is not always reliable, and instead focus on and develop radiopharmaceuticals, which bind to the microbe directly and are specific to it.

These include antimicrobial agents, such as antibiotics, which interfere with important metabolic processes or molecules within the micro-organism such as DNA synthesis (e.g. ciprofloxacin), cell wall/membrane components (e.g. ethambutol and fluconazole) and protein synthesis, and monoclonal antibodies against microbial specific epitopes (e.g. radiolabelled monoclonals against the outer membrane proteins of *P.aeruginosa*).

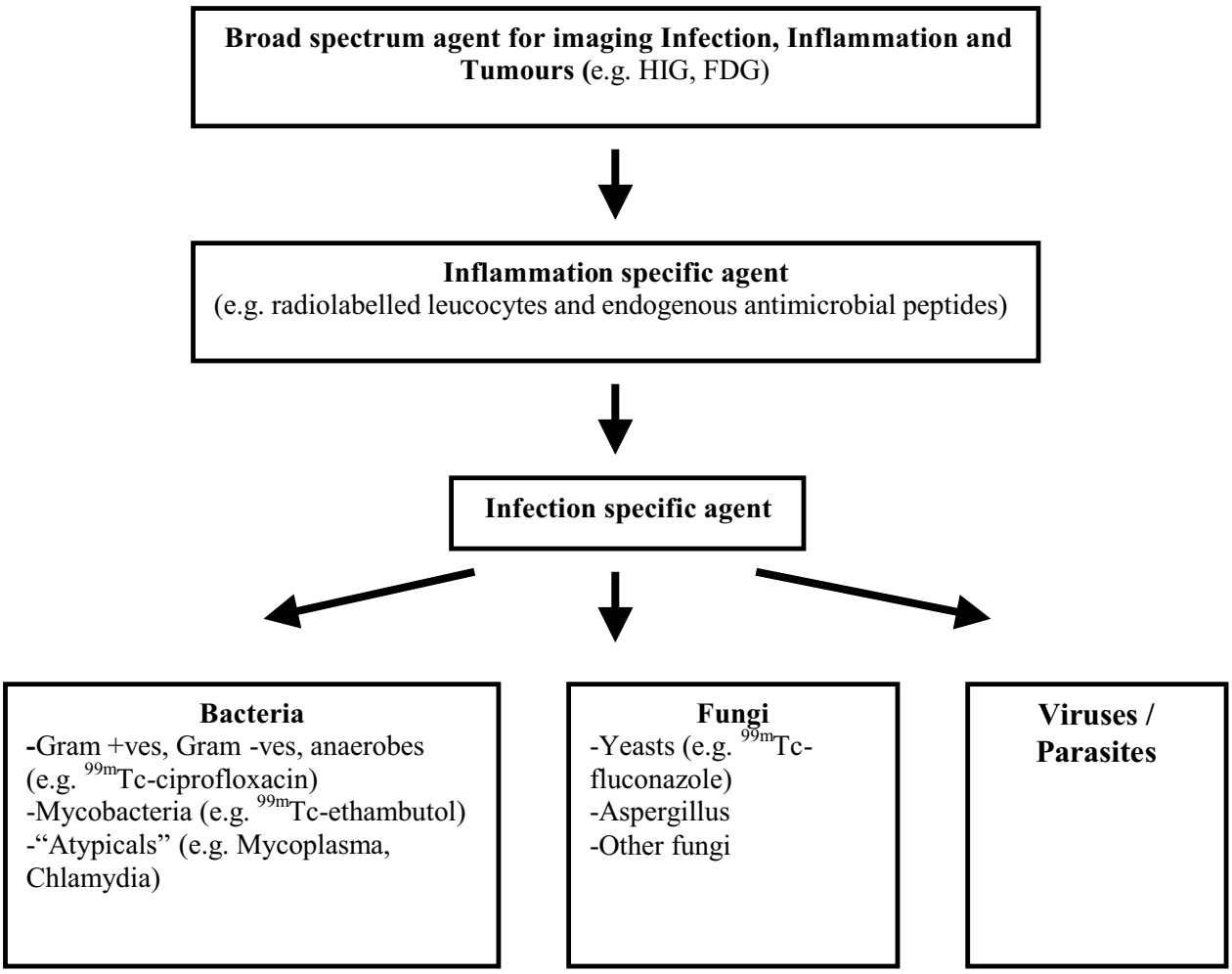

Figure 5 - Schematic diagram of the approach to infection imaging.

The exploitation of these targets for imaging bacterial (it may be possible to design agents that are specific against particular species of bacteria, e.g. ^{99m}Tc- ethambutol for mycobacteria), fungal, viral, and parasitic infections will depend on closer collaboration between nuclear medicine specialists and their colleagues in microbiology and infectious diseases to select the best targets and conduct well designed clinical trials, incorporating standardised criteria for infection diagnosis and image interpretation, to validate efficacy of these agents for imaging sites of infection, which will stand up to close scientific scrutiny.

This approach is more likely to yield radiopharmaceuticals that are infection specific and generate confidence in clinicians about nuclear medicine's ability to offer tests, which can reliably locate sites of infection and distinguish these from inflammatory and other lesions and thus aid patient management. Broad-spectrum agents with high sensitivity, e.g. ^{99m}Tc-HIG, will still be helpful in the context of FUO to pick up both infective and non-infective (e.g. tumours and inflammatory disorders such as connective tissue and inflammatory bowel diseases) causes. But a positive result should then prompt imaging with more specific agents, such as Infecton, to confirm or exclude infection (fig. 5).

Conversely, depending on the clinical urgency, infection specific agents could be used first to permit early diagnosis and treatment of serious infections, followed by imaging with an inflammation specific agent if the test is negative. Such techniques will have greatest impact if, through serial imaging, they can be used to monitor the patient's response to anti-infective therapy. An agent able to predict the requirement for and the duration of antibiotic treatment will not only decrease costs and side effects but also contribute to the fight against the growing and sinister problem of anti-microbial resistance. The recent trend towards the development of infection specific radiopharmaceiticals is long over due and to be welcome, but there is still much to be done if the full potential of these exciting agents is to be realised.

RESUMO

A infecção continua a ser uma das grandes causas de morbidade e mortalidade mundiais. A Medicina Nuclear tem um importante papel em contribuir elucidar o diagnóstico de infecções tais como abscessos, osteomielites, artrite séptica, endocardite e infecção nos aparatos de prótese. As técnicas já estabelecidas tais como leucócitos marcados são sensíveis e específicas para inflamação, mas não distinguem entre inflamação infecciosa e não-infecciosa. O desafio para a Medicina Nuclear para obtenção de imagem de infecções no século 21 é trabalhar na recente tendência para o desenvolvimento de radiofármacos mais específicos para o estudo de infecções, tais como agentes anti-infecciosos marcados (por exemplo, ^{99m}Tc-ciprofloxacina). Além disto, para ajudar no diagnóstico precoce das infecções, através de imagens seriadas, estes agentes podem se provar muito úteis no monitoramento das respostas e para determinar a duração ótima de uma terapia anti-infecciosa. Este artigo revê a atual tendência da utilização do uso da imagem de processos infecciosos com radiofármacos e o seu futuro direcionamento.

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