Radioactive agents currently available for imaging infection by gamma scintigraphy do so by identifying inflammation in response to the infection, and are therefore non-specific in so far as they are unable to distinguish between infective and non-infective inflammation. The term 'infection imaging' is therefore generally synonymous with 'inflammation imaging'. This article will briefly review inflammation imaging, beginning with the functional properties of the agents and followed by a description of clinical circumstances in which radionuclide imaging of inflammation may be helpful.

**Radioactive agents**

The number of radioactive agents that may be useful for imaging infection is increasing, largely as a result of increased knowledge of the cellular biology of inflammation (Table 1). Autologous leukocytes, radiolabelled in vitro, are the most effective way of imaging acute inflammation. Macromolecules show increased accumulation in inflammatory lesions as a result of increased endothelial permeability and an expanded local interstitial fluid space. Other agents have targets located in the interstitial space; these include radiolabelled antibiotics, the glucose analogue deoxyglucose labelled with fluorine-18 (¹⁸F) (FDG) which is taken up by metabolically active inflammatory cells, and radiolabelled cytokines that target leukocyte receptors activated following migration.

**Gallium-67**

Gallium-67 (⁶⁷Ga), injected as gallium citrate, was the first agent to be used for imaging inflammation by gamma-camera scintigraphy. After injection it binds to transferrin and circulates as a metalloprotein. It therefore behaves like a macromolecule, although increased endothelial permeability is not the only factor involved in its localisation in inflammatory foci because it is known to bind to extravascular transferrin receptors expressed in inflammation. It is useful for imaging inflammation in the chest, especially granulomatous disease (Fig 1), but is not so reliable in the abdomen because of physiological excretion through the gastrointestinal tract. Imaging is generally performed 48–72 hours after injection. ⁶⁷Ga is taken up in a wide range of inflammatory, infective and neoplastic processes, so it is often useful in pyrexia of unknown origin (PUO) in which its non-specificity is turned to advantage²⁻⁴.

**Labelled leukocytes**

The principle of blood cell labelling is that lipophilic radiometal-chelate complexes, such as indium-111 (¹¹¹In)-oxine⁵, ¹¹¹In-tropolonate⁶ or technetium-99m (⁹⁹⁰Tc)-hexamethyl propyleneamineoxime (HMPAO)²⁻⁸, are able to penetrate the cell membrane. Once inside, the metal-chelate is irreversibly bound,
Table 1. Radionuclide agents for imaging infection classified according to location of target for radioactive agent.

Pre-endothelial:
- radiolabelled circulating leukocytes
  - in vitro labelling with lipophilic complexes
  - in vivo labelling with anti-granulocyte monoclonal antibodies
  - phagocytic labelling

Endothelial:
- increased permeability to macromolecules
  - proteins
  - colloids
  - streptavidin/biotin
  - liposomes
- activated adhesion molecules

Post-endothelial:
- ligands to leukocyte receptors expressed following migration
  - chemokines
  - interleukin-2
  - somatostatin analogues
- local metabolic activity of inflammatory cells
  - $^{18}$F-fluorodeoxyglucose
- microorganisms
  - labelled antibiotics
  - specific monoclonal antibodies

Fig 1. $^{67}$Ga-citrate whole-body scan in a patient with sarcoidosis. Diffusely increased uptake is seen in the lungs (anterior (Ant) and posterior (Post) projections).

$^{111}$In to intracellular proteins and $^{99m}$Tc-HMPAO as a result of transformation into a hydrophilic complex. Autologous leukocytes are separated from an intravenous blood sample of 50–100 ml and labelled in vitro. In some circumstances, it is advisable to purify the leukocytes further to obtain neutrophils for labelling. In any event, the active component of a labelled leukocyte preparation, the neutrophil, dictates the likelihood of a positive scan; thus, only those inflammatory processes with a predominantly neutrophilic infiltrate are likely to be positive. Lymphocytes are also labelled in a mixed leukocyte preparation, but are radiosensitive and fail to recirculate normally. The normal distribution includes prominent physiological uptake in the reticuloendothelial system (RES).

The need to isolate leukocytes in vitro exposes both patient and laboratory technician to an infection hazard, and represents a significant disadvantage of the technique. Because of this, off-the-shelf agents, such as monoclonal antibodies against leukocyte surface antigens, which can label circulating neutrophils in vivo, have been developed.

Molecules that label extravascular targets

Leukocytes express several receptors following migration into the interstitial space, so small diffusible molecules, such as labelled chemokines, which readily diffuse across the endothelium and target such receptors may be used to image inflammation. FDG is taken up by inflammatory cells as a result of increased metabolic requirements and is able to detect inflammation, including subacute and chronic infections (Fig 2). An approach for specific imaging of infection is the use of radiolabelled antibiotics which target microorganisms. Increased specificity has been claimed as an advantage, but there is a limited number of clinical scenarios for which the agent would be chosen.
Targeting the endothelium of inflamed tissue

Two approaches to targeting the endothelium for imaging inflammation have been described:

1. Techniques based on increased vascular permeability.
2. Direct targeting of activated endothelium.

Techniques based on increased vascular permeability

As a result of increased vascularity and endothelial permeability, and a locally expanded interstitial fluid space, radiolabelled macromolecules accumulate non-specifically in inflammation. $^{67}$Ga is taken up predominantly by this mechanism as a metal-transferrin complex. Polyclonal human immunoglobulin (HIG) has been introduced more recently for imaging inflammation$^{13}$ and, despite early claims for several active mechanisms of localisation, is now known to accumulate on the basis of increased permeability$^{14}$ (Fig 3).

Several groups have investigated radiolabelled liposomes (particles ca 100 nm), for imaging infections over many years. Early efforts were unsuccessful mainly because liposomes are rapidly removed from the circulation into the RES. Interest has recently been rejuvenated, however, by the development of so-called 'stealth' liposomes which have a much longer clearance time from blood. This has been achieved by surrounding the particle with an envelope of polyethylene glycol (pegylated liposomes). They can be labelled with either $^{99m}$Tc or $^{111}$In, utilising the same principles as cell labelling. Recent work has shown that stealth liposomes are effective for localising infection$^{15,16}$.

Direct targeting of activated endothelium

The second approach to imaging endothelium involves targeting activated endothelial adhesion molecules$^{17}$. Endothelium is metabolically active and orchestrates leukocyte migration through the activation of a cascade of adhesion molecules, which govern leukocyte margination, adherence, spreading and eventually transendothelial migration. Adhesion molecules useful for imaging inflammation are those which are:

- expressed only during inflammation
- present only on endothelium
- expressed on the luminal side of the endothelial cell
- not shed into the circulation
- internalised along with the targeting agent following binding.

E-selectin fulfils these specifications. It is synthesised $de$ $novo$ by the endothelial cell in response to several cytokines$^{18}$. Haskard et al have characterised E-selectin expression in several
models of experimental inflammation, and have used an anti-E-selectin monoclonal antibody for imaging acute and chronic inflammation17–19 (Fig 3).

**Clinical localisation of inflammation**

Inflammation imaging has become an important application of nuclear medicine over the last few years. Its clinical value must be viewed alongside the availability of other radiological techniques for imaging inflammation, especially ultrasonography and computed tomography (CT). Intra-abdominal abscess, for example, which several years ago was a common indication for leukocyte scanning, is now generally managed by interventional radiologists.

*Inflammatory bowel disease*

Inflammatory bowel disease (IBD) is the inflammatory disease for which 99mTc-HMPAO-labelled white cells are clearly preferred over any alternative agent. The clinical applications of scintigraphic imaging of IBD are:

- to define the extent of disease, either in a newly diagnosed patient or one with known disease
- to assess inflammatory activity of a segment of bowel in a patient with known disease, for example in a patient with a stricture, to address whether or not the stricture is inflammatory
- to quantify the overall intensity of inflammatory activity.

Labelled neutrophil localisation is usually clearly evident within the wall of inflamed bowel within one hour of injection (Fig 4). Neutrophils that migrate into inflamed bowel wall pass rapidly into bowel lumen and move distally within the lumen20 (Fig 5). The complete faecal excretion over several days of such neutrophils provides the basis of a sensitive, accurate and useful technique for quantifying disease activity in IBD. 99mTc has a physical half-life of only six hours and eluted 99mTc-HMPAO appears in faeces physiologically, so this technique must be based on 111In-labelled neutrophils which have a half-life of 2.8 days20. The 111In is counted in a complete four-day faecal collection, corrected for physical decay and expressed as a fraction of the injected dose. Alternatively, since 111In, as a heavy metal, has a long half-life in

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**Fig 4.** 99mTc-HMPAO scan in a patient with distal colitis. There is intense abnormal uptake in the distal half of the transverse colon and descending colon. Note physiological uptake in the liver, spleen and bone marrow: (a) and (b), anterior projection at one and four hours, respectively, and (c) left anterior oblique projection (LAO) at four hours.

**Fig 5.** 99mTc-HMPAO scan in a patient with terminal Crohn's ileitis. Note distal movement of activity between the image obtained one hour (left panel) and four hours (right panel) after injection.
the body, whole body retention of $^{111}\text{In}$ can be monitored over several days by whole-body counting. Obviously, pure neutrophils must be isolated for $^{111}\text{In}$ labelling.

**Bone infection**

Acute osteomyelitis is generally diagnosed with plain radiography and $^{99m}\text{Tc}$-methylene diphosphonate (MDP) bone scanning. Acute relapse of chronic osteomyelitis should be investigated with labelled leukocytes because MDP is likely to be abnormal even in the absence of infection. A bone scan in these circumstances, in addition to the leukocyte scan, may nevertheless be helpful for distinguishing between soft tissue infection and infection extending to involve bone (Fig 6). This is a frequent problem in soft tissue infection or ulceration in the peripheral skeleton, such as the diabetic foot, in which the critical clinical problem is often whether or not infection involves underlying bone.

Another common clinical scenario is the infected prosthetic joint. Neutrophils are taken up physiologically by bone marrow, and it is often difficult to distinguish neutrophil uptake at an

**Fig 6. Soft tissue infection in an amputation stump without involvement of underlying bone** (left = plain x-ray). All three images (above) were simultaneously obtained from the right lateral projection by dual photon acquisition on $^{99m}\text{Tc}$ and $^{111}\text{In}$ photopeaks (left = $^{99m}\text{Tc}$-MDP bone image; middle = $^{111}\text{In}$-leukocyte image; right = composite image).
infective focus from marrow uptake. A separate bone marrow scan is therefore recommended (Fig. 7). $^{67}$Ga is useful for chronic bone infection, including infection in a prosthetic joint. Its kinetics in bone are more complicated than those of labelled leukocytes because, in addition to showing physiological uptake in marrow, it is also bone-seeking. A bone scan is usually therefore required for comparison with the $^{67}$Ga scan (Fig. 8). Because of the possibility of a false-positive leukocyte or $^{67}$Ga scan in an inflamed but sterile prosthetic joint, agents that specifically target infecting organisms, such as labelled antibiotics, may be useful. Labelled antibiotics. FDG, HIG and anti-E-selectin monoclonal antibody have an advantage over leukocytes in that they show minimal physiological bone marrow uptake.

**Soft tissue sepsis**

Abdominal abscesses progressively and avidly accumulate labelled leukocytes and are usually obvious on scintigraphy (Fig. 9). An abdominal abscess not infrequently communicates with bowel lumen. Characteristically, it appears as a prominent focal abnormality on early imaging, followed by a decline in prominence over the next 24 hours in association with the appearance of activity in the gut lumen. Communication is typically seen in abscesses close to the pancreas, and in association with Crohn's disease or diverticular disease. They are easily missed on CT and ultrasonography because of their decompression. Sepsis close to or in the spleen or liver may be difficult to see because of surrounding physiological uptake of labelled cells by these organs. It may be helpful to perform a liver/spleen colloid scan for subtraction.

**Subacute/chronic bacterial infection**

As inflammatory lesions become chronic, they are associated with a decreasing neutrophilic turnover and an increasing number of mononuclear inflammatory cells. The leukocyte scan therefore becomes less effective.

Selective labelling of lymphocyte or monocyte subpopulations is difficult because of the related problems of cell radiosensitivity and of harvesting sufficient cells for labelling. Accumulation of macromolecules such as $^{67}$Ga-transferrin and HIG, on the basis of increased endothelial permeability, may be preferable to labelled leukocytes in chronic inflammation.

**Intrathoracic sepsis**

Pyogenic infection in the chest has been a relatively minor indication for inflammation-seeking agents because other imaging techniques are effective in diseases such as pneumonia, bronchiectasis, lung abscess and empyema. For non-pyogenic infection, such as tuberculosis or the complications of HIV (see...
below). $^{67}$Ga has been generally more helpful. Migrating labelled leukocytes in bronchiectasis are all eventually mobilised and either expectorated or excreted in the faeces (Fig 10), a fact exploited by Currie et al using whole-body $^{11}$In counting for the assessment of disease activity.

**Table 2. Some important causes of pyrexia of unknown origin.**

**Pyogenic infection:**
- soft tissue sepsis
- pneumonia
- musculoskeletal infection

**Non-pyogenic infection:**
- mycobacterial
- viral
- chronic bacterial
- fungal/rickettsial

**Non-infective inflammation:**
- sarcoidosis
- inflammatory bowel disease
- vasculitis
- collagen diseases
- organ rejection

**Neoplasia:**
- haemoproliferative
- renal cell carcinoma
- melanoma

**Non-pyogenic infections**

Viral, mycobacterial, fungal and parasitic infections are likely to be negative on leukocyte scanning unless secondary pyogenic infection is present. $^{67}$Ga and HIG are often preferable, with tuberculosis and other mycobacteria being particularly well visualised with $^{67}$Ga. Some centres are attempting to develop highly specific agents for some infections by raising radiolabelled monoclonal antibodies to specific microorganisms, such as *Pneumocystis carinii* and mycobacteria. These techniques, in common with labelled antibiotics, are likely to be useful only in well-defined clinical settings.

**Pyrexia of unknown origin and occult infection: undiagnosed fever (Table 2)**

In general, patients presenting with undiagnosed fever can be divided into two groups:

1. Those with no significant previous medical history who satisfy the classic criteria for PUO.
2. Patients, often with a significant previous medical history, such as diabetes mellitus or recent surgery, in whom there is a strong clue of infection but, as in PUO, the location of the pathology is unknown.

The second presentation may be called ‘occult’ infection. Most diseases causing PUO are not associated with a neutrophilic infiltrate and are therefore likely to be negative with labelled leukocytes. Making a clinical distinction between PUO and occult infection is helpful because it guides the choice between a leukocyte scan or a less specific alternative, such as $^{67}$Ga. Patients with occult infection should be investigated with labelled leukocytes because pyogenic infection is more likely, whereas patients with PUO should be investigated with a less specific agent such as $^{67}$Ga or HIG.

The likelihood of a positive labelled leukocyte image in a patient with an undiagnosed fever correlates with a history of surgery within six months, but not with a leukocytosis or raised C-reactive protein or erythrocyte sedimentation rate. Occult infection is one clinical setting in which labelled antibiotics may have a role, especially if bacteremia is present from which binding potential may be demonstrated by preliminary in vitro experiments. FDG may have a useful role in PUO because it shows increased uptake both in inflammation and in tumours.

**Imaging in HIV infection**

As with several forms of intrathoracic inflammation, the intrathoracic complications of AIDS are in general more effectively imaged with $^{67}$Ga than with labelled leukocytes. It is important to interpret the $^{67}$Ga scan alongside a current chest radiograph. A normal $^{67}$Ga scan with a normal chest x-ray excludes infection with a high degree of certainty. A normal $^{67}$Ga scan in the presence of obvious respiratory deterioration carries a very poor prognosis. Focal pulmonary $^{67}$Ga uptake usually indicates bacterial pneumonia or *P. carinii* pneumonia (PCP). Diffuse pulmonary $^{67}$Ga uptake is usually due to PCP but, especially if faint, may indicate the presence of other chest infections such as mycobacterial and cytomegalovirus.
infections. Coexisting normal chest x-ray, heterogeneous distribution of 67Ga activity and intense uptake all strongly suggest PCP. Lymph node uptake is most often due to mycobacterial infections or lymphoma. Kaposis's sarcoma does not take up 67Ga, so a normal scan with an abnormal chest x-ray suggests this diagnosis. Outside the chest, leukocyte scintigraphy is generally more useful than 67Ga. Centres with special expertise in cell isolation from HIV-positive blood are performing autologous leukocyte scintigraphy in patients with AIDS. An alternative is to use labelled donor leukocytes.

Conclusions
Nuclear medicine is increasingly being asked to search for infection in patients who have already undergone extensive imaging and other investigations. Labelled leukocytes made a significant clinical impact after their development in the late 1970s and early 1980s, but the technique is now being requested for increasingly difficult patients for many of whom an alternative agent might be more rational. This is providing the impetus to develop novel imaging strategies, an area in which success will depend on close collaboration between imaging specialists and basic scientists, especially molecular biologists and radiochemists.

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