# DIAGNOSIS OF ORTHOPAEDIC PROSTHESIS INFECTIONS WITH RADIONUCLIDE TECHNIQUES; CLINICAL APPLICATION OF VARIOUS IMAGING METHODS

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## *Abstract*

A variety of radiopharmaceuticals and imaging techniques are currently available for scintigraphic imaging of infections. However, comparisons on the clinical value of such techniques have been limited, especially in prosthesis infections.

This series included 138 cases with suspected prosthesis infections – 94 in hip and 44 in knee prostheses of patients whose final diagnoses were based on clinical, operative and microbiological findings, and who underwent three-phase bone, $^{99m}$Tc -leukocyte and $^{99m}$Tc-ciprofloxacin imaging in the Department of Clinical Chemistry, Oulu University Hospital and in the Laboratory, Länsi-Pohja Central Hospital, during the years from 1993 to 2001.

The normal arterial and soft-tissue phase images of three-phase bone imaging practically excluded infection in hip prostheses, whereas these techniques frequently yielded false positive findings in patients with knee prostheses, resulting in specificity of 23% or less. In combined $^{99m}$Tc-leukocyte/bone imaging, diagnostic accuracy was 80–86% at two- to four-hour images and 87–98% at 24-hour images. The $^{99m}$Tc-ciprofloxacin images showed unspecific accumulation of tracer in the one-hour and four-hour images, which disappeared in the 24-hour images in most hip and knee prostheses. $^{99m}$Tc-ciprofloxacin imaging yielded almost as good diagnostic accuracy as combined $^{99m}$Tc-leukocyte/bone imaging.

In conclusion, in suspected hip prosthesis infections, normal findings in three-phase bone imaging exclude infection, whereas abnormal results in the arterial and soft-tissue phases should be confirmed with $^{99m}$Tc-leukocyte imaging using 24-hour images. Contrary-wise, in suspected knee prosthesis infections, $^{99m}$Tc-leukocyte imaging with 24-hour images is the first-line examination, and abnormal results in 24-hour images should be confirmed by using $^{99m}$Tc-bone-metabolic imaging. $^{99m}$Tc-ciprofloxacin yielded almost equally good results as $^{99m}$Tc-leukocyte/bone-metabolic imaging, but unfortunately, the tracer is not commercially available, although it has been patented.

*Keywords:* $^{99m}$Tc-ciprofloxacin, hip prosthesis, infection, knee prosthesis, labelled leukocytes, technetium-99m, three-phase bone imaging

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Kemi, November 2003 Martti Larikka

# Abbreviations

| | |
|---|---|
| DNA | deoxyribonucleic acid |
| DXA | dual energy x-ray absorptiometry |
| FDG | fluorodeoxyglucose |
| HAMA | human anti-mouse antibody |
| HDP | hydroxydiphosphonate |
| HIG | human non-specific immunoglobulin |
| HMPAO | hexamethylenepropyleneamine oxime |
| IgG | immunoglobulin G |
| IgM | immunoglobulin M |
| IL | interleukin |
| MDP | methylene diphosphonate |
| NCA | non-specific cross-reacting antigen |
| NPV | negative predictive value |
| PCR | polymerase chain reaction |
| PEG | polyethylene glycol |
| PET | positron emission tomography |
| PPV | positive predictive value |
| SPET | single photon emission tomography |

# List of original publications

I Larikka M, Ahonen A, Junila J, Niemelä O, Hämäläinen M & Syrjälä H (2001) Extended combined $^{99m}$Tc-white blood cell and bone imaging improves the diagnostic accuracy in the detection of hip replacement infections. European Journal of Nuclear Medicine 28: 288–293.

II Larikka M, Ahonen A, Junila J, Niemelä O, Hämäläinen M & Syrjälä H (2001) Improved method for detecting knee replacement infections based on extended combined $^{99m}$Tc-white blood cell/bone imaging. Nuclear Medicine Communications 22: 1145–1150.

III Larikka M, Ahonen A, Niemelä O, Puronto O, Junila J, Hämäläinen M, Britton K & Syrjälä H (2002) $^{99m}$Tc-ciprofloxacin (Infecton) imaging in the diagnosis of knee prosthesis infections. Nuclear Medicine Communications 23: 167–170.

IV Larikka M, Ahonen A, Niemelä O, Junila J, Hämäläinen M, Britton K & Syrjälä H (2002) Comparison of $^{99m}$Tc ciprofloxacin, $^{99m}$Tc white blood cell and three-phase bone imaging in the diagnosis of hip prosthesis infections: improved diagnostic accuracy with extended imaging time. Nuclear Medicine Communications 23: 655–661.

This thesis also includes some previously unpublished data. The permission of the copyright owners Springer Verlag (I) and Lippincott Williams & Wilkins (II, III and IV) to reproduce the original material is gratefully acknowledged.

# Contents

# 1 Introduction

In developed countries, the population is getting older than ever before, and with the increasing longevity of citizens, the incidence of degenerative joint diseases, and hence the need of joint prosthesis surgery, is constantly growing. A small fraction of these patients eventually develop an infection of their prosthesis, and as the absolute number of operations continues to grow, so does the number of infected prostheses (Birrell *et al.* 1999, Moran & Horton 2000, Paavolainen *et al.* 1991, Puolakka *et al.* 2001, Tennant *et al.* 1995). Infection following joint replacement is a clinically serious problem (Goldman *et al.* 1996, Widmer 2001).

In the diagnosis of prosthesis infections, clinical history, physical examination, blood tests, radiography, joint aspiration and microbial cultures suffer from insensitivity and non-specificity (Itasaka *et al.* 2001, Tunney *et al.* 1998, Widmer 2001). The presence of metallic prostheses causes artefacts in computed tomography and magnetic resonance imaging (Tormanen *et al.* 1996, White *et al.* 2000). Nuclear medicine plays an important role in the diagnosis. The techniques used for this purpose include bone imaging, $^{67}$Ga-citrate imaging and radiolabelled leukocyte imaging (Kraemer *et al.* 1993, Merkel *et al.* 1986, Reing *et al.* 1979, Rosenthall *et al.* 1979, Tumeh *et al.* 1986).

Leukocytes can be labelled with $^{111}$In (Thakur *et al.* 1977a) and with $^{99m}$Tc (Holmes *et al.* 1985, Peters *et al.* 1986). Accumulation of leukocytes in normal bone marrow and at sites of operative trauma causes uncertainty in the interpretation of images. Bone imaging and bone marrow imaging have been combined with leukocyte imaging to overcome these uncertainties (Johnson *et al.* 1988, Moragas *et al.* 1991, Oswald *et al.* 1989, Oswald *et al.* 1990, Palestro *et al.* 1990, Palestro *et al.* 1991, Schauwecker *et al.* 1984, Scher *et al.* 2000). Bone imaging alone has proven unspecific in prosthesis infections (Rosenthall *et al.* 1987, Rosenthall 1997, Rubello *et al.* 1995, Rubello *et al.* 1996, Weiss *et al.* 1979). Recently, new methods, including $^{99m}$Tc-labelled ciprofloxacin (Infecton®, Draximage Inc., Kirkland, Quebec, Canada) (Britton *et al.* 1997, Britton *et al.* 2002, Hall *et al.* 1998, Sonmezoglu *et al.* 2001, Vinjamuri *et al.* 1996) and $^{18}$F-FDG-PET-imaging (Chacko *et al.* 2002, Manthey *et al.* 2002, Van Acker *et al.* 2001, Zhuang *et al.* 2002) have been introduced as methods to image infection.

# 2 Review of the literature

## 2.1 History of prosthesis surgery

Although major surgical procedures were occasionally performed in the early 1800s, it was not until the introduction of general anaesthesia and antiseptic techniques during the latter half of the nineteenth century that bone and joint surgery could be developed. During the 19th century, the conditions affecting joints were generally different from those encountered today. Surgeons treated patients with acute and chronic infections, both septic and tuberculous, poliomyelitis and untreated developmental dysplasia of the hip. These conditions often led to joint instability and ankylosis. Fracture treatment was relatively primitive, and non-unions, mal-unions and osteonecrosis of the hip were commonly encountered. Thus, arthroplasty was used to treat patients with numerous conditions, which differed considerably from the contemporary situation, in which the majority of patients suffer from degenerative joint diseases and other forms of arthritis. (Steinberg & Steinberg 2000)

Resection arthroplasty of the hip was first reported in Europe in the early 1800s and became well established by the middle of the 19th century. This procedure was performed primarily for the treatment of patients with chronic bacterial and tuberculous arthritis. Between 1921 and 1945, G. R. Girdlestone, Professor of Orthopaedic Surgery at Oxford, refined the indications and techniques for resection arthroplasty. This procedure eventually became known as the Girdlestone arthroplasty. The results were thereby improved substantially, and before the development of total hip replacement, Girdlestone pseudoarthrosis was often used as a primary treatment for patients with degenerative arthritis of the hip. (Steinberg & Steinberg 2000)

The era of modern arthroplasty began when Sir John Charnley developed the predecessors of today's hip replacement during the late 1950s and early 1960s (Steinberg & Steinberg 2000). Hip replacements soon revolutionised the treatment of hip arthritis in elderly patients (Moran & Horton 2000). Modern hip prostheses are modular, which means that the surgeon can modify the different components to suit each patient's needs. They are made of metal and plastic, and the components can be attached to bone in several ways. In cemented fixation, the prosthesis is secured by polymethylmethacrylate.

The fixation of a cementless prosthesis takes place by means of bony ingrowth into a porous coating on the surface of the device. Acetabular components can be press-fit and secured with screws (Kingston & Walsh 2001, Love *et al.* 2001).

The success with hip replacements led to interest in other joints, particularly the knee. A great variety of knee replacements were produced, and hinged knees caused particular problems. Regardless of how well the hinged components were designed, the basic problem seemed to be that rigidly fixed components with motion in only one plane failed because of the stresses applied to the device to its attachment to bone. Thus, in the 1970s and early 1980s, knee replacement was widely considered to result in a poor outcome. Condylar components were also used. There was controversy, which still continues, concerning the advisability of replacing only one compartment of the knee. Some of the designs were successful, however: these aimed to resurface the joint and to reproduce the knee anatomy with a low-friction joint. The remaining knee ligaments provided stability, allowing some rotational movement. Total knee replacements have undergone a period of convergent evolution, and most implants now adhere to the same basic design principles (Moran & Horton 2000). Nowadays, mobile-bearing prostheses have improved mobility and lessened plastic breakdown compared to the older fixed-bearing models (Love *et al.* 2001, Scuderi *et al.* 2001, Steinberg & Steinberg 2000).

## 2.2 Indications and clinical statistics of hip and knee prosthesis surgery, the increasing need

Data on hip and knee prostheses have been gathered on a nationwide basis into the Finnish Arthroplasty Register since 1980. Between 1980 and 1988, 25966 operations were reported, of which 56% had been made for primary osteoarthrosis, 22% for rheumatoid arthritis and 6.3% for secondary arthrosis. In 1988, the total number of arthroplasties was 4628: about two thirds were hip and a third knee replacements. The annual incidence of primary total hip arthroplasties in 1988 was 58 per 100000 inhabitants. More than 40% of the patients were under 65 years old (Paavolainen *et al.* 1991). The annual frequency of re-arthroplasty increased from 9.8% in 1980 to 13.6% in 1988, indicating an increasing need in the future (Paavolainen *et al.* 1991). 62841 primary and 12224 revision total hip arthroplasties were recorded between 1980 and 1999. The frequency of both primary and revision total hip arthroplasties has increased, and in 1999 the incidence of primary total hip arthroplasties was 93/100000 (Puolakka *et al.* 2001).

Nearly 500000 hip and knee arthroplasties are performed annually in the United States and nearly 100000 in the United Kingdom. With the increasing longevity of citizens, it is estimated that the total number in the United States may exceed 700000 by the year 2030. Half of all prostheses show radiographic evidence of loosening by 10 years after installation, and 30% require revision. (Love *et al.* 2001, Moran & Horton 2000, Widmer 2001)

The long-term survival of modern knee replacements appears to be better than that of hip replacements, indicating 85% survival at 13 years (Moran & Horton 2000). Knee

arthrosis is slightly more common than hip arthrosis in the general population aged over 55 years, and 2% would benefit from knee replacement; one fifth of these patients have extreme disability but many are not referred for surgery (Tennant *et al.* 1995). Among women aged over 74 years, the incidence is 4.3%, and demographic changes are likely to increase the demand for knee replacement by 40% over the next 30 years (Birrell *et al.* 1999). There is already a large unmet need for knee replacements, and the waiting time for surgery is often unacceptably long. Waiting list management is becoming a major political issue in many countries, and it is possible that standardised priority assessment criteria will be introduced (Hadorn & Holmes 1997).

## 2.3 Complications of prosthesis surgery

Primary thromboembolic complications have been reported in 1.4%, luxations in 1.4%, infections in 0.9% and evacuated hematomas in 0.6% of operations in the Finnish Arthroplasty Register. Data from the register indicate that the results of replacements are improving. The most common reasons for revision are aseptic loosening (65%), dislocation (9%) and infection (7%). (Paavolainen *et al.* 1991, Puolakka *et al.* 2001)

### *2.3.1 Pathophysiology involved in the loosening of prostheses*

There is evidence to suggest that a significant number of cases of aseptic loosening result from an inflammatory reaction. Histological examinations of failed prostheses show a pseudo-membranous structure that develops at the cement-bone interface, the cellular composition of which varies: histiocytes are seen most frequently (95% of specimens), followed by giant cells (80%) and lymphocytes and plasma cells (25%). Neutrophils are present in less than 10% of cases. It is believed that particulate debris from component fragmentation activates phagocytes around the prosthesis. This debris is resistant to enzymatic destruction, leading to repeated attempts at phagocytosis, which stimulate the secretion of cytokines and proteolytic enzymes that damage bone and cartilage (Peersman *et al.* 2001, Spangehl *et al.* 1999, Ure *et al.* 1998). Tunney *et al.* (1999) found infiltration of neutrophils, lymphocytes or macrophages into tissue associated with prostheses in 73% of 120 patients undergoing total hip revision surgery.

### *2.3.2 Incidence of prosthesis infection and contamination with bacteria*

High rates of infection complicated the early experience of arthroplasty, and infection is still a source of considerable morbidity. In the 1960s, Charnley considered a rate of infection of 7% as unacceptable (Charnley & Eftekhar 1969). More recently, authors have reported infection-related failures in 1% to 2% of primary total hip prostheses and in 2%

of knee prostheses. The rate is higher after revision procedures, being about 3% for hip prostheses and 5% for knee prostheses (Spangehl *et al.* 1998). In a recent review of 6489 total knee replacements carried out between 1993 and 1999, as low an infection incidence as 0.39% was reached in primary operations, while the incidence in revision operations was 0.97% (Peersman *et al.* 2001).

In a study using immunological and DNA detection methods and culture for the detection of bacteria in the hip prostheses retrieved from 120 patients undergoing total hip revision surgery, bacterial DNA was detected in 72% of samples by PCR amplification techniques. All the culture-positive samples were also positive for bacterial DNA. Bacteria were also detected by immunolabelling and fluorescence microscopy after the prostheses had been subjected to ultrasonic treatment in 62% of the cases. The authors concluded that unrecognised infection is a potential major cause for prosthetic hip failure (Tunney *et al.* 1999). The question remains open at present.

### *2.3.3 Risk factors of joint prosthesis infections*

Although the risk factors for joint prosthesis infections have not been established, there is evidence that at least diabetes and psoriasis increase the risk of joint replacement infections. Long operating time is a considerable risk factor, and it has been shown that an operating time longer than 2.5 hours increases the risk of infection significantly. (England *et al.* 1990, Peersman *et al.* 2001, Stern *et al.* 1989)

### *2.3.4 Countermeasures against prosthesis infections related to operations*

In addition to standard aseptic techniques, there are other documented countermeasures available to reduce the incidence of infections. These include the use of ultra-clean air and laminar flow in the operating theatre, the use whole-body exhaust-ventilated suits by the surgeons and antibiotic prophylaxis for the patient.

The effect of ultra-clean air was shown to reduce the incidence of infections to about half, and when whole-body exhaust-ventilated suits were added, the incidence was further reduced to about one quarter of the original incidence in a series of over 8000 operations (Lidwell *et al.* 1982). In a recent series of over 6000 operations with an especially low incidence of infections, the operations were made in a theatre with vertical laminar flow and with the surgical team using body exhaust suits (Peersman *et al.* 2001).

The effect of antibiotic prophylaxis was proved in a double-blind, placebo-controlled trial of 2137 patients undergoing hip replacement in nine centres, in which five days of prophylactic treatment reduced the number of infections significantly from 3.3% to 0.9% (Hill *et al.* 1981). The duration of antibiotic prophylaxis has varied, and in a recent study with excellent results, there was no change in the infection rate when the duration of antibiotic prophylaxis was decreased from 48 to 24 hours after surgery (Peersman *et al.* 2001).

### *2.3.5 Epidemiology of prosthesis contamination and infection*

*Staphylococcus epidermidis* (31% of cases) and *Staphylococcus aureus* (20%) are the most common bacteria, whereas *Streptococcus viridans* (11%), *Escherichia coli* (11%), *Enterococcus faecalis* (8%) and group B *Streptococci* (5%) are less frequently encountered (Davis *et al.* 1999, Evans *et al.* 1998, Gaine *et al.* 2000, Peersman *et al.* 2001, Segawa *et al.* 1999, Spangehl *et al.* 1998). Microorganisms also include anaerobes, gram-negative bacilli, such as *Pseudomonas* species or, especially in hematogenous infections, *Streptococci*. *Propionibacterium* species were isolated in 60% of orthopaedic device infections by using anaerobic bacteriologic practices (Tunney *et al.* 1999). *Propionibacterium* species are also the second most frequent contaminant observed in joint aspiration (Widmer 2001).

### *2.3.6 Pathogenesis of prosthesis infection, biofilm formation*

Understanding of the pathogenesis of biofilm formation facilitates optimal diagnosis and treatment. It also explains why signs and symptoms are relieved by short-term treatment with antimicrobial agents but reoccur soon after withdrawal of treatment. All prostheses undergo physiological changes after implantation. The earliest and probably clinically the most important step is the competition between tissue cell integration and bacterial adhesion to the same surface. Upon contact, body fluids immediately coat all surfaces with a layer of material, primarily serum proteins and platelets. Albumin, the major serum component, is rapidly deposited on foreign material and prevents non-specific neutrophil activation and deposition of matrix proteins on the surfaces. Adherence of *Staphylococcus aureus* to bioprosthetic materials is mediated by adhesins, such as fibronectin, fibrinogen, fibrin, collagen, laminin, vitronectin, thrombospondin, bone sialoprotein, elastin and matrix-binding proteins, which promote attachment onto polymeric or metallic surfaces by specific receptors. Adherence of microorganisms progresses to aggregation on the surface of the prosthesis, forming a biofilm. As the colonies mature, sessile bacteria detach and disperse as planktonic bacteria. Costerton *et al.* (1999) defined bacterial biofilms as ”structured communities of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface”. These types of surfaces are frequently present in prostheses. Biofilms grow slowly and resist cellular and humoral immune responses. Moreover, several mechanisms render bacteria protected by biofilm less susceptible to antimicrobial agents than their planctonic counterparts. Clinically established mechanisms include adherence, slime production, and a slow rate of bacterial growth. Bacteria become sessile in the biofilm, their features change considerably, and they become resistant through several mechanisms. Two clinically important mechanisms are the failure of antimicrobial agents to penetrate the biofilm and the stationary phase of growth. In addition, some bacteria form small-colony variants, characterised by a reduced growth rate, diminished exoprotein production, decreased susceptibility to aminoglycosides and, possibly, intracellular persistence. Standard antibiotic therapy typically alleviates symptoms caused by planktonic bacteria released from the biofilm but fails to kill the bacteria in the biofilm. Therefore, successful

treatment of prosthesis infections with retention of the implant must incorporate treatment against both planktonic and sessile bacteria. Another possibility is to kill planktonic bacteria by antimicrobial agents and to remove the implant to get rid of sessile bacteria. (Widmer 2001)

A variety of microorganisms develop slime, an amorphous extracellular glycocaliceal substance based on polysaccharide, which promotes intracellular adhesion, captures nutrients and protects microorganisms from the effects of antimicrobial agents. Slime can also block penetration of antibiotics into the bacterial cell and decrease chemotaxis of neutrophil granulocytes. (Widmer 2001)

Bacteria in biofilm do not grow exponentially. They exist in a slow-growing or stationary phase. Studies of orthopaedic device-related infections in an animal model have confirmed the slow growth of *Staphylococcus aureus* and *Escherichia coli*. (Costerton *et al.* 1999)

## 2.4 Classification of prosthesis infections

Coventry (1975), and later Fitzgerald *et al.* (1977), described perhaps the most common system for the classification of infections in total hip arthroplasty. This classification is based on the mode or timing of infection.

Type I infections originate at the time of operation and become symptomatic in the immediate postoperative period. They are called early postoperative infections. The patient is usually seen during the first month after the operation, and the diagnosis can be made on the basis of a medical history and a physical examination. Systemic signs of infection may be present, and there is usually continuous pain. The wound may be erythematous, swollen, fluctuant and tender and there may be purulent wound drainage on examination. These infections are caused by infected hematomas and wound infections spreading to the periprosthetic space.

Type II infections are also believed to originate at the time of operation, but the onset of symptoms in these cases is delayed. These infections are also called late chronic infections. The patient is usually seen between six months and two years after the operation. This type of infection is associated with deteriorating function and increasing pain. Pain is often present from the time of the procedure, and it may be activity-related or occur during rest. Early loosening of the components is often the only clue to infection, and systemic symptoms are not part of the presentation. The findings on examination are non-specific and similar to those associated with aseptic loosening.

Type III infections, also called hematogenous infections, are the least common and are caused by hematogenous spread to the hip, which usually occurs two years after arthroplasty or later. Dental manipulation, catheter-associated urinary tract infections and remote infections may trigger hematogenous seeding. *Streptococci* are more frequently isolated in this type of infections than in others. There is generally a febrile episode accompanied by sudden deterioration of the hip. These infections are likely to occur in patients who are immunosuppressed for inflammatory arthropathy or transplantation and in patients with recurrent episodes of bacteremia (Spangehl *et al.* 1998).

## 2.5 Microbiological diagnosis of prosthesis infections

Microbiological samples can be obtained by aspiration of the prosthetic joint or during operation. The sensitivity of aspirated samples for culture has been poor, although it can be somewhat improved by sonication. Multiple organisms are often isolated in the samples, which may indicate polymicrobial infection, but also raises the possibility that one microorganism may be responsible for the infection and the other may be a contaminant. New diagnostic tools, such as PCR, tend to render the interpretation of the results even more difficult. However, multiple specimens for culture should be obtained from any suspected infection site, and the clinician should place the samples into transport media for anaerobic microorganisms. Multiple specimens will facilitate interpretation of the culture results. A concordant positive result for a particular microorganism from three specimens indicates infection. Additional information from the microbiology laboratory, such as massive growth in cultures and the resistance pattern of the pathogen, may help to suggest true infection. However, some cases remain unclear even after a review of all data available. The high likelihood of contamination precludes the routine use of microbiological culture for prosthesis-related infections without clinical signs or symptoms of infection, unless multiple specimens are taken. Nevertheless, some patients scheduled for routine revision do not present with signs or symptoms of infection, and the diagnosis of prosthesis-related infection is made exclusively based on intraoperative culture and histopathology. This applies specifically to patients with a suspected diagnosis of aseptic loosening of the prosthesis, who require careful workup. (Widmer 2001)

## 2.6 Non-invasive diagnosis of prosthesis infections

Many complications of joint prosthesis surgery can be diagnosed easily. However, differentiating infection from aseptic loosening is difficult, because these conditions are very similar clinically and histopathologically. Clinical signs, symptoms, laboratory tests and radiography are insensitive, non-specific or both. In the postoperative period, signs and symptoms that are associated with infection may be masked by normal postoperative changes. Anatomic imaging modalities provide high-quality details and are widely available. However, x-ray cannot exclusively differentiate infection from loosening, and cross-sectional imaging modalities, e.g. computer tomography and magnetic resonance imaging, are complicated by artefacts produced by metallic prostheses. Radionuclide imaging is not significantly affected by metallic objects and is therefore useful in the diagnosis of complicated prostheses. Another advantage is that, since scintigraphic images are based on functional tissue changes, infectious and inflammatory foci can be visualised in their early phases, when anatomical changes are not yet apparent. Over the past 30 years, many approaches have been developed to visualise infection and inflammation using radionuclides. Understanding the action of radionuclide tracers requires, however, understanding of the pathophysiology of inflammation and infection. (Itasaka *et al.* 2001, Palestro 1995, Rennen *et al.* 2001, Rosas *et al.* 1998)

Inflammation can be described as a reaction of the body to any kind of injury. Injuries range from trauma to ischaemia, neoplasm or invasion by microorganisms. There can be infection without overt inflammation, as in the case of a severely neutropaenic patient. In such cases, it is clear that, for the purposes of imaging, one would need an agent that directly interacts with the microorganims. Conversely, there is often inflammation without infection when tissue injury is not due to microorganisms but is caused by other stimuli, such as trauma, ischaemia, neoplasm or foreign particles. (Rennen *et al.* 2001)

The response to infection or inflammation is characterised by locally increased blood supply, increased vascular permeability, enhanced transudation of plasma proteins and enhanced influx of leukocytes. Defence mechanisms consisting of cells and plasma proteins are activated in response to tissue damage. Many mediators, both vasoactive and chemotactic, are involved in the process. These mediators are generated at the focus of inflammation or infection and amplify the local response by recruiting cells and plasma components from blood. Vasodilatation and increased endothelial permeability facilitate extravasation of proteins and cells. Expression of adhesion molecules on endothelial cells and leukocytes is also stimulated, which causes leukocytes to actively migrate from the circulation into the inflamed tissue. First, leukocytes adhere to the vascular endothelium due to locally enhanced expression of adhesion molecules (rolling, arrest and adhesion). Then they pass through the endothelium and the basal membrane (diapedesis) and migrate into the inflammatory focus (chemotaxis). In acute inflammation or infection, the infiltrating cells are predominantly polymorphonuclear cells. In chronic inflammation or infection, the cellular response is different from that in acute situation, and the infiltrating cells are predominantly mononuclear cells: lymphocytes, monocytes and macrophages. (Etzioni *et al.* 1999, Gahmberg *et al.* 1992, Gahmberg 1997, Gahmberg *et al.* 1997, Gahmberg *et al.* 1998, Rennen *et al.* 2001)

## 2.7 Nuclear medicine procedures for imaging infection and inflammation

Both specific and non-specific radiopharmaceuticals are used to image infection. Increased blood supply, increased vascular permeability and enhanced transudation are processes that result in accumulation of tracers, and it must be emphasised that all radiopharmaceuticals accumulate, to some extent, in this non-specific way at the site of infection or inflammation. (Rennen *et al.* 2001)

The main uptake mechanisms of radiopharmaceuticals used to image infection and inflammation are presented in table 1.

*Table 1. Main uptake mechanisms of radiopharmaceuticals used to image infection and inflammation.*

| Radiopharmaceuticals | Main uptake mechanism |
|---|---|
| Non-specific radiopharmaceuticals | |
| Bone-imaging tracers | Increased vascular permeability and increased bone metabolism |
| $^{67}$Ga-citrate | Transferrin and lactoferrin receptor binding |
| $^{99m}$Tc-nanocolloids | Increased vascular permeability and uptake in activated endothelial cells |
| $^{99m}$Tc- and $^{111}$In-labelled human immunoglobulin (HIG) | Increased vascular permeability and antigen binding |
| Radiolabelled liposomes | Extravasation due to increased vascular permeability |
| Avidin-biotin | Increased vascular permeability and antigen binding |
| $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) | Increased glucose uptake by activated leukocytes |
| Specific radiopharmaceuticals | |
| $^{99m}$Tc- and $^{111}$In-labelled leukocytes | Migration of activated leukocytes |
| $^{99m}$Tc-labelled granulocyte antibodies | Increased vascular permeability and specific binding or uptake as antibody labelled granulocytes |
| Antimicrobial and chemotactic peptides | Binding to receptors on known cell populations |
| $^{99m}$Tc-ciprofloxacin | Binding to DNA-gyrase enzyme in living bacteria |

### *2.7.1 Non-specific infection and inflammation imaging*

#### *2.7.1.1 Bone imaging*

Bone imaging with its ability to image osteoblastic activity is one of the greatest triumphs of nuclear medicine. Today, it is a widely available, accurate and cost-effective examination that has improved the treatment of many patients.

The body load transfer to a normal femur is uniform, and as a result, the femoral bone mineral density and radio-phosphate uptake are also uniform. The implantation of a prosthesis radically changes the load transfer distribution in the femur in such a way that there are areas with little or no transfer, where the bone is stress-shielded, and other areas where transfer takes place. Bone demineralisation or atrophy takes place in the area of shielding, whereas the region of load transfer is associated with a normal mineral content or hypertrophy. Generally, stresses are transferred through the stem to the distal femur rather than to the periprosthetic bone, and this often results in proximal cortical atrophy. Uncemented stems cause more proximal stress shielding than cemented stems. (Rosenthall 1997)

The changes in mineral density in joint prostheses can be evaluated with quantitative and semi-quantitative radiographic assessments and with dual energy x-ray

absorptiometry (DXA) (Rosenthall 1997). In hip prostheses, the stress-shielding phenomenon has been documented to take place within 6 to 24 months after implantation (Gruen *et al.* 1979). In knee prostheses, loss of bone mineral density has been documented in relation to both the femoral (Petersen *et al.* 1995b) and the tibial (Petersen *et al.* 1995a) components for up to eight years after implantation (Levitz *et al.* 1995). Stress shielding in the tibia is related especially to long-stemmed knee prostheses (Lonner *et al.* 2001).

It should be noted that the degree of radiophosphate deposition reflects bone turnover and may not correlate with bone mineral density. Radiophosphate deposition is a function of osteoblastic activity, and the osteoblastic and osteoclastic activities are interrelated. When there is an increase in bone formation or resorption, an increase in resorption or formation, respectively, will try to maintain the mineral density, but radiophosphate deposition will be enhanced in both cases, because osteoblastic activity is increased. If the compensatory response is not balanced, there will be mineral gain or mineral loss (Rosenthall 1997). Using SPET and DXA in a prospective one-year follow-up study, Kröger *et al.* (1997) found that increased periprosthetic radiophosphate uptake seemed to be associated with increased bone mineral loss in 15 patients who had undergone uncemented total hip arthroplasty without complications.

*Static bone imaging*. In 1979, investigators found increased $^{99m}$Tc-MDP uptake related to the tip of the femoral component or the acetabular component to be a good indicator of infection or loosening with 100% sensitivity and 72% specificity. Increased uptake in the lesser trochanter was considered a normal variant, while increased uptake in the greater trochanter was indicative of heterotopic ossification or could be a consequence of osteotomy. Static bone imaging was recommended as an initial study, because negative results could be treated conservatively. Arthrography and aspiration for culture to diagnose possible infection were recommended for positive results. (Weiss *et al.* 1979)

In studies of asymptomatic porous coated prostheses, the bone-metabolic phase was positive in many cases until 24 months after surgery, and in most patients the intensity of uptake decreased or remained unchanged over time (Oswald *et al.* 1989, Oswald *et al.* 1990). In a study of 97 patients with painful un-cemented hip prostheses implanted three or more years previously, at least one area of significantly increased uptake was observed in all the patients with loosened or infected prostheses as well as in 66% of the controls. The most frequent pattern of loosening was simultaneous positivity of the lesser trochanter and the tip (Rubello *et al.* 1995). Increased uptake was found diffusely around whole the prosthesis in seven of the nine infected prostheses. A follow-up of any focal uptake was suggested, since it was thought that this type of increased activity could signify early loosening (Lieberman *et al.* 1993). In a group of 20 patients who had revision surgery and prior bone scans, none had focal concentration limited to the femoral stem tip (Rosenthall & Aldis 1985). Isolated findings of focal stem tip accretion do not seem to be common in loosening and have been a source of false positive interpretations (Weiss *et al.* 1979).

The natural history of periprosthetic uptake has been qualitatively documented for cemented femoral components in a review of the results of 267 bone scans in 97 asymptomatic patients. This study included a subset of 193 scans in 59 patients monitored prospectively for up to 3 years. The following scoring system was used: normal was Grade 0, and uptake equal to that of the iliac crest or the sacroiliac joint was Grade 2. At

the femoral tip, 10% of the cases showed Grade 2 or higher uptake that persisted for 1 to 3 years. In the greater trochanter, Grade 2 or higher uptake was seen in 20% of the prostheses at 2 years, but the uptake decreased slightly by 3 years. Uptake around the femoral shaft and in the lesser trochanter was less than Grade 2 from 9 months onwards. There was Grade 2 or higher acetabular activity in 12% of the implants at 2 years, which decreased somewhat thereafter. It was concluded that the singular finding of a focal concentration at the stem tip in the absence of other abnormalities after one year is likely to be a normal variant rather than a sign of loosening, and it is also possible that a combination of stem tip and trochanteric uptake is normal. The probability of loosening is greatly increased by the presence of enhanced uptake around the femoral component. (Utz *et al.* 1986)

Based on thorough quantitative analyses of the periprosthetic bone-metabolic changes in different stem models of hip prostheses, it was concluded that prostheses of different designs provoke disparate remodelling patterns that can be studied by bone densitometry and by quantitative radiophosphate imaging (Rosenthall 1997). Static bone imaging is limited by the fact that the operation itself causes increased bone metabolism, which takes a long time to resolve and is dependent on the type and location of the prosthesis. In cemented hip prostheses the increased uptake may resolve in a year, but in uncemented hip prostheses the uptake often remains increased for up to two years after the operation even in uncomplicated cases. Uptake around the acetabulum is more intensive in implants under 12 months old than in older implants (Rosenthall 1997). In cemented hip prostheses, the abnormally elevated uptake generally disappears within 6–12 months of surgery, and if persistent increased uptake is found, loosening or infection should be suspected (Schauwecker *et al.* 1984).

To improve the accuracy of bone imaging in hip prosthesis patients, investigators have recommended that a baseline scan be taken between 9 and 12 months after surgery, at a time when the patient is asymptomatic, so that relative changes can be found when the patient is being investigated for symptoms. (Oswald *et al.* 1989, Oswald *et al.* 1990, Utz *et al.* 1986)

In knee prostheses, persistent uptake is common, particularly adjacent to the tibial component. The uptake reflects mechanical stresses specific to knee prostheses. Patterns of uptake have been evaluated for indicators of infection or other complications of knee prostheses. In 1982, Rozing *et al.* reported that a radiolucent line was often accompanied by an increased uptake over the same condyle of a knee prosthesis, but there was no relationship with pain or loosening. However, in another study published in 1987, the degree of isotope uptake did not correlate with the development of radiolucent lines around the components, and the uptake did not differ between the painful and non-painful knees, either (Duus *et al.* 1987).

Rosenthall *et al.* (1987) published a study on radiophosphate uptake in asymptomatic knee arthroplasty. The series consisted of 37 total knee replacements in 30 patients, who underwent scanning one month to 12 years after surgery, with half of the implants imaged at one year or less. The knees demonstrated diffuse sub-prosthetic uptake of radiophosphate in both the femurs and the tibias. The femoral components showed significantly less uptake in prostheses that were over one year old compared to more recently implanted prostheses, while the difference in the tibial components was very small. In knees studied 13 months to 12 years after surgery, 89% of the tibial components

and 63% of the femoral components showed clearly increased uptake. An analysis of the intensity of periprosthetic uptake showed a significantly greater concentration at the tibial component relative to the femoral component in prostheses over one year old, but not in newer implants. This finding was due to a reduction in the femoral concentration over time, and it also correlated with the increased incidence of loosening in the tibial component compared to the femoral component. The findings suggested that the diagnosis of postoperative complications could not be based only on an assessment of the degree of overall uptake in a single study. (Rosenthall *et al.* 1987)

Hoffman *et al.* (1990) studied 28 cemented and 31 uncemented total knee prostheses. The increased uptake was found to gradually decrease towards the baseline levels over 10–12 months. The average uptake was roughly similar in the cemented and uncemented groups, but highly variable in individual patients and in each of the follow-up periods. The authors concluded that a single postoperative bone scan could not differentiate component loosening from early bone remodelling. However, it was reported in the same year, based on a series of 98 total knee prostheses, that symptomatic knee prostheses showed significantly greater uptake in the patella, the femur and the medial and lateral tibial plateau regions than asymptomatic prostheses (Kantor *et al.* 1990).

Rubello *et al.* (1996) reported findings on 30 uncemented Hoffman total knee prostheses imaged 2 to 4 years after surgery. In a comparison of the groups of patients examined at 2, 3 and 4 years after surgery, an increasing number of cases showed normal uptake, while the number of cases with mildly or moderately increased uptake progressively diminished. Two years after surgery, the lowest uptake values were observed in the femoral component; similar values were recorded for the lateral tibial component after 3 years and for the medial tibial component after 4 years, suggesting different time intervals for the stabilisation of the prosthesis components. The highest uptake values were always found in the medial tibial component. The authors concluded that different knee prosthesis designs may have different bone scan normality parameters, and that the criteria of a complication could differ, too, and should be determined for each type of prosthesis. With regard to the studied design, the criteria for a failed implant could be a high or very high periprosthetic uptake in a single study or a progressive increase in uptake in respect to a baseline assessment, which should be obtained in asymptomatic conditions at 2 years or more after surgery.

Smith *et al.* (2001), in their series of 80 scans obtained from 75 total knee replacement patients, found a 95% negative predictive value for excluding complications, and none of the patients with infected knee prostheses had normal bone scans. Quantification was felt to be of little value, and comparison with the other knee was often difficult because of bilateral prostheses or contralateral degenerative changes. The ability of bone imaging to distinguish loosening from infection was considered poor.

*Dynamic bone imaging*. A two-year follow-up study of 22 asymptomatic patients with uncemented hip prostheses revealed slightly increased blood flow at the prosthetic tip in only one of the 136 scans, and none of them showed increased flow in the acetabulum. Increased blood pool activity in the acetabulum was initially seen in 22% and in the muscles of the thigh by 44%, but negative results were obtained within three months of surgery (Oswald *et al.* 1989, Oswald *et al.* 1990). In a group of complicated uncemented prostheses, focal blood pool activity was seen in both aseptic loosening and infection, but there was no evidence of increased blood flow (Rosenthall *et al.* 1991). The arterial phase

was positive only in two patients with hip prosthesis infection and negative in all uninfected cases in another study (Rubello *et al.* 1995). The blood pool phase was negative in the patients with asymptomatic prostheses, but positive in 71% of the complicated uncemented hip prostheses. It was concluded that dynamic bone imaging seems to be a good screening test despite its low accuracy, and the addition of a more specific test to bone imaging was considered necessary for the final diagnosis.

In 30 uncemented asymptomatic Hoffman knee prostheses, a negative arterial phase and a mildly positive blood pool phase were reported in 41.6%, 25% and 16.6% of cases at 2, 3 and 4 years, respectively (Rubello *et al.* 1996). In a series of 75 patients with painful total knee replacements, complication was most likely if both blood pool and static images were abnormal, but the ability of these images to distinguish infection from loosening was poor (Smith *et al.* 2001). However, none of the nine patients with infected prostheses had a normal scan. Unfortunately, the arterial phase was not imaged in this study. The negative predictive value of a normal scan was 95%.

*Three-phase bone imaging*. Three-phase bone imaging combines dynamic and static bone imaging in a three-phase study including arterial, soft-tissue and bone-metabolic phases. This procedure has been used in several studies of patients with hip and knee replacements.

It seems reasonable to conclude that, in hip prostheses, a positive dynamic and/or blood pool phase as well as a progressive increase in the uptake intensity in the bone-metabolic phase suggest the presence of a complication, while slight uptake in the bone-metabolic phase seems to be a non-specific finding. Marked focal uptake involving the lesser trochanter and the tip or appearing in the shaft seems to be related to loosening. Diffuse periprosthetic uptake, especially when associated with positive dynamic and blood pool phases, is suggestive of infection. The results are quite similar in cemented and uncemented hip prostheses, the main difference being that, in cemented prostheses, the bone-metabolic phase should become normal within a year from operation, whereas in uncemented prostheses the uptake often remains elevated for two years or longer. (Lieberman *et al.* 1993, Rosenthall 1997, Rubello *et al.* 1995, Weiss *et al.* 1979)

The situation with knee prostheses is more complicated, as increased uptake takes a long time to resolve and is often persistent adjacent to the tibial part even in the absence of complications. Thus, the diagnosis of postoperative complications cannot be based solely on an assessment of the degree of uptake in a single study. Also, cemented and cementless prostheses fail to show the same kind of logical differences in uptake seen in hip prostheses. In view of the dynamic phases, the evidence in the literature seems to be inconclusive, though the blood pool phase often seems to remain positive for a long time after surgery even in uncomplicated cases. (Rosenthall *et al.* 1987, Rubello *et al.* 1996, Smith *et al.* 2001)

### *2.7.1.2 Other non-specific imaging methods*

$^{67}$Ga is a cyclotron-produced radionuclide, and $^{67}$Ga-citrate was first used in 1969 for infection imaging. There are many factors that govern the uptake of gallium in infection. In addition to increased blood flow and vascular permeability, gallium is also transported

by leukocytes to some degree. Siderophores, produced by bacteria, have a high affinity for gallium. It is believed that the siderophore-gallium complex is transported into the bacterium, where it remains until phagocytosis occurs. In addition to siderophores, gallium also binds to transferrin, which is present in inflammatory foci. It is believed that gallium, delivered to the site of infection primarily as a gallium-transferrin complex, dissociates from the transferrin and forms a gallium-lactoferrin complex. Gallium imaging is typically performed 48 to 72 hours after injection. (Chianelli *et al.* 1997)

Bauer *et al.* (1973) and Reing *et al.* (1979) reported the results of trials of bone scans and gallium scans in patients suspected of having infection or loosening of prostheses. Abnormal scans could not reliably distinguish between infection and loosening. Abnormal gallium scans can be associated with granulation tissue related to chronic loosening (Alazraki 1990). Rosenthall *et al.* (1979) reported that incongruent gallium-technetium uptake was a sign of infection in prosthetic joints. Evidence confirming this conclusion was later also obtained from other studies (Merkel *et al.* 1985, Schauwecker *et al.* 1984, Tumeh *et al.* 1986). For example, Schauwecker *et al.* (1984) studied 56 patients suspected of having osteomyelitis with gallium-technetium bone scans and reported that a normal combined scan was excellent for ruling out osteomyelitis. Gallium-technetium scans were reliable indicators of osteomyelitis when the relative uptake of gallium exceeded the uptake of technetium, or when the distribution was incongruent with the distribution of technetium. However, only 28% of patients with osteomyelitis showed these patterns. Tumeh *et al.* (1986) reported a study in which they defined various patterns of gallium-bone scan uptake in patients with suspected osteomyelitis and noted that only the patterns of gallium uptake exceeding bone scan uptake and/or incongruence of gallium and bone scan uptake correlated well with osteomyelitis. Schauvecker *et al.* (1984) and Tumeh *et al.* (1986) concluded similarly that, although gallium could be relied upon to exclude osteomyelitis when normal, the combination of gallium and bone scan was a good indicator of infection in only about one-fourth to one-third of patients with infection. Gallium uptake relates generally to inflammation and, with its accuracy of about 70%–80%, this technique does not seem to be accurate enough for distinguishing an aseptically loosened prosthesis from an infected one. (Kraemer *et al.* 1993, Palestro & Torres 1997, Williams *et al.* 1981)

$^{99m}$Tc-nanocolloids can be used for the diagnosis of infection and inflammation. Nanocolloids are actively taken up in the reticuloendothelial system, after which they are lysosomally degraded and 54.5% are renally excreted after 24 hours (Becker 1995). Approximately 86% of nanocolloid particles are 30 nm in diameter or smaller, and the remainder are between 30 and 80 nm (De Schrijver *et al.* 1987). The passage of these particles into pericapillary spaces and their accumulation are due to the increased permeability of the capillary basal layer. Blood clearance is rapid, and imaging may be completed within 2 to 4 hours. Only a few studies have evaluated $^{99m}$Tc-nanocolloids clinically in patients with osteomyelitis. The results indicate values with sensitivity ranging from 87% to 95% and specificity between 77% and 100%. One study reported a calculated sensitivity of 75% with $^{111}$In-labelled leukocytes and 94% with $^{99m}$Tc-nanocolloids and specificities of 90% and 84%, respectively (Flivik *et al.* 1993). The authors got improved specificity when patients with slightly increased activity were regarded as being negative. The advantage of nanocolloids is the rapid localisation of an

infectious process within 30–60 minutes, after which the study can be terminated (De Schrijver *et al.* 1987, Ooi *et al.* 1993, Streule *et al.* 1988, Vorne *et al.* 1989a).

Rubin *et al.* (1988) discovered that human non-specific polyclonal immunoglobulin G (IgG) in their rat model of infection provided results similar to those obtained with a specific monoclonal antibody. However, $^{111}$In-IgG accumulates both in infection and in sterile inflammatory processes. The reported sensitivity and specificity for bone, joint and joint prosthesis infections have been 93–97% and 85–88%, respectively (Oyen *et al.* 1991, Oyen *et al.* 1992). The main problem with $^{111}$In-HIG is that it is not commercially available (Becker 1995). Nijhof *et al.* (1997) studied infections with $^{111}$In-HIG in 85 hip and 17 knee prostheses and found its sensitivity to be 100% and its specificities for hip and knee 80% and 50%, respectively. Human non-specific immunoglobulin G can also be labelled with technetium-99m ($^{99m}$Tc-HIG). Images with $^{99m}$Tc-HIG can be obtained at 24 hours, but not at 48 hours. $^{99m}$Tc-HIG and a monoclonal $^{99m}$Tc-labelled antibody have been compared in patients with suspected osteomyelitis. In centrally located osteomyelitis, these two tracers performed differently: injection of the labelled polyclonal IgG resulted in hot lesions, whereas injection of the monoclonal antibody resulted in cold lesions (Buscombe 1995). In a study of $^{99m}$Tc-HIG in 27 patients with suspected hip and knee prosthesis infections, sensitivity was 100% and specificity 41%. The method was considered useful only as a screening test for prosthesis infections. (Demirkol *et al.* 1997)

Liposomes are spheres consisting of one or more lipid bi-layers surrounding an aqueous space. They were tested as tools to image infection about 20 years ago, but the preparations were cleared from circulation rapidly by the mononuclear/phagocyte system. If the surface of the liposomes is coated with a hydrophilic polymer, such as polyethylene glycol (PEG), they circumvent recognition by this system. Such PEG liposomes can be labelled with $^{111}$In and $^{99m}$Tc. The first clinical evaluation showed good results for detecting focal infection, but another study revealed side effects in three out of nine patients. (Awasthi *et al.* 1998, Boerman *et al.* 1995, Dams *et al.* 2000, Erdogan *et al.* 2000, Laverman *et al.* 1999)

Avidin binds to biotin with high affinity. Biotin is a low molecular weight compound that can be radiolabelled. This approach is based on the fact that avidin will non-specifically localise at the sites of infection due to their increased vascular permeability. Avidin is injected as a pre-targeting agent, followed by an injection of radiolabelled biotin. Good diagnostic accuracy has been demonstrated in preliminary clinical investigations of vascular infection and chronic osteomyelitis with $^{111}$In-labelled biotin. (Hnatowich *et al.* 1987, Lazzeri *et al.* 1999, Rusckowski *et al.* 1996, Samuel *et al.* 1996)

$^{18}$F-FDG is a deoxyglucose labelled with $^{18}$F, which is taken up by cells like glucose but is not metabolised (Love *et al.* 2001). $^{18}$F-FDG imaging correctly diagnosed the presence or absence of active infection in a study of 11 patients, many of them with suspected osteomyelitis (Sugawara *et al.* 1998). $^{18}$F-FDG imaging with a coincidence camera was found to be superior to $^{111}$In-leukocyte imaging in the diagnosis of chronic osteomyelitis in the central skeleton (Meller *et al.* 2002). $^{18}$F-FDG imaging was found to be accurate for infected hip prostheses, but false positive results were obtained for knee prostheses. Furthermore, following hip arthroplasty, persistent, non-specific $^{18}$F-FDG uptake was found around the head and neck portions of the prostheses for many years, even in patients without complications (Zhuang *et al.* 2001). Van Acker *et al.* (2001) found that, in total knee prostheses, $^{99m}$Tc-leukocyte imaging combined with bone

imaging had high specificity, whereas $^{18}$F-FDG-PET imaging seemed to offer no additional benefit. One group recently suggested that they could, to some extent, differentiate between isolated synovitis, loosening and infection by addressing the location and extent of increased glucose metabolism around suspicious hip and knee prostheses (Manthey *et al.* 2002). Chacko *et al.* (2002) declared that they could differentiate aseptic loosening from infection based on the location of increased glucose metabolism in hip prostheses. In infection, metabolism was increased along the bone-prosthesis interface, whereas aseptic loosening was accompanied by intense uptake around the head or neck of the prosthesis. On the other hand, Zhuang *et al.* (2002) found increased $^{18}$F-FDG-uptake around the head or neck of the prosthesis in 26 out of 30 asymptomatic hip arthroplasties, including all the nine prostheses, which were evaluated prospectively during one year after surgery. In conclusion, although $^{18}$F-FDG imaging seems to be sensitive in studies with joint prosthesis infections, it does not allow reliable differentiation between aseptically loosened and infected prostheses. (Love *et al.* 2001)

## *2.7.2 Specific infection and inflammation imaging*

### *2.7.2.1 Leukocytes, in vitro labelling*

Leukocytes accumulate in infection by chemotaxis and can be used to transport radiotracers to the infected area. Leukocyte labelling requires withdrawal of blood from the patient, purification of leukocytes and labelling and re-injection of the cells. Use of autologous leukocytes labelled with $^{111}$In or $^{99m}$Tc leads to positive imaging because leukocytes retain their capacity to migrate to the inflamed area. $^{111}$In, with its physical half-life of 67 h and 173 keV and 247 keV photopeaks, allows delayed imaging with gamma cameras. $^{99m}$Tc has an ideal 140 keV photopeak, but its physical half-life is only 6 hours. The agents used for leukocyte labelling, $^{111}$In-oxine, $^{111}$In-tropolonate and $^{99m}$Tc-HMPAO, have one property in common, namely lipophilicity, which enables cell labelling, and they label all cell types. For this reason, leukocytes must be separated before labelling. Thakur *et al.* first described the labelling technique with $^{111}$In and used the labelled leukocytes in patient studies (Thakur *et al.* 1977b, Thakur *et al.* 1977a). Uptake of labelled cells is dependent on functioning chemotaxis, the number and type of cells labelled and the principal cell component of the inflammatory response.

Neutrophils have a life cycle of two weeks. Originating from stem cells in bone marrow, neutrophils are released into peripheral blood after 6–12 days. There they are distributed into two pools; about half of the cells are in a circulating pool, while the other half are in a marginating pool, being temporarily sequestered in capillaries or adhering to the vascular endothelium. Cells move between the two pools after physical exercise, adrenaline administration and exposure to bacterial endotoxin. In all conditions, cells move from the marginating to the circulating pool. In inflammatory and infectious diseases, the response of leukocytes to a chemotactic stimulus occurs as early as 30 to 40 minutes after stimulation. Labelling of inflammatory cells that migrate to sites of

infection may be the most important achievement in the diagnosis of infection in the field of nuclear medicine. The numbers of leukocytes that accumulate at sites of inflammation are very high: as many as 10% of circulating leukocytes accumulate at such sites each day (Becker 1995).

The hallmark of positive leukocyte images is the migration of polymorphonuclear leukocytes. While acute infection is indeed characterised by that migration, mononuclear cells, lymphocytes and fibroblasts migrate in chronic inflammation. Local reproduction of lymphocytes may also take place in chronic inflammation, and indirect evidence supporting this was found by Jaakkola *et al.* (1997). The group successfully labelled purified lymphocytes with $^{99m}$Tc-HMPAO and imaged patients with inflammatory bowel disease, but the results did not encourage the use of labelled lymphocytes for such purposes. In a standard procedure, a mixed population of cells is injected, and the success of imaging thus depends somewhat on the white count differential (Alazraki 1990). Schauwecker *et al.* (1988) found mixed leukocytes slightly superior in sensitivity to purified granulocytes in chronic infections in a series of 211 patients. Also, when Datz *et al.* (1986) studied the effect of chronicity on the sensitivity of $^{111}$In-labelled mixed leukocyte imaging, sensitivity was 90% for the 69 patients who had had infection for 0–14 days and 86% for the 86 who had had infection for 15 days or longer.

When labelled leukocytes are used for the diagnosis of orthopaedic prosthesis infections, there are some difficulties. Firstly, the operation to insert the prosthesis causes bone trauma, and $^{111}$In-labelled white blood cell uptake has been demonstrated in non-infected fractures, although usually less in intensity and extent than $^{99m}$Tc bone-metabolic uptake (Al Sheikh *et al.* 1985, Kim *et al.* 1987, McAfee & Samin 1985, Van Nostrand *et al.* 1988). Secondly, the activity normally seen in bone marrow may be misleading in the evaluation infection (Palestro *et al.* 1990, Palestro *et al.* 1991). The prosthesis also causes bone marrow displacement, and even in the absence of infection, there may be photopenia in the bone marrow image. The presence of heterotropic bone that contains bone marrow adjacent to the prosthetic joint may also confuse interpretation. Thirdly, abnormal uptake without infection is sometimes seen in rheumatoid arthritis, which may be the disease that necessitated the implantation of a prosthesis (Alazraki 1990). The interpretation of labelled leukocyte images is therefore limited, and a reference method is usually necessary. The methods used for this purpose are bone-metabolic imaging and bone marrow imaging (Cunningham 1991, Johnson *et al.* 1988, King *et al.* 1990, Moragas *et al.* 1991, Oswald *et al.* 1989, Oswald *et al.* 1990, Palestro *et al.* 1990, Palestro *et al.* 1991, Schauwecker *et al.* 1984, Scher *et al.* 2000). Luckily, leukocytes have been found to show increasing sensitivity in visualising infection in bones that are peripherally located compared to centrally located ones (Schauwecker *et al.* 1984).

Another concern about labelled leukocytes is the handling of blood needed to separate, label and re-inject cells. The process is prone to errors, and not even strict adherence to quality control and good medical practices, in order to maintain the lowest possible misadministration levels, will totally eliminate the possibility of misadministration. Statistics in nuclear medicine show very low incidences of misadministration, about 1 in 10000. However, the prevalence of HIV- and hepatitis-positive blood poses a risk, which, unfortunately, will rarely manifest as misadministration. (Alazraki 1990)

*$^{11}$1In-labelled leukocytes.* There are two sources of radiation to leukocytes after $^{111}$In-labelling. The first is external radiation absorbed by leukocytes during the labelling

process when the cells are incubated. The second, and more important, source is internal radiation from the $^{111}$In incorporated in the cells. Most of the internally derived dose comes from low-energy Auger electrons with a range smaller than the cell diameter, which cause the radiation burden to be high, up to 14.8 Gy. Nonetheless, there is significant evidence to suggest that even in mixed-cell populations of leukocytes labelled with 500 μCi of $^{111}$In, the oncogenic risk to the patient is extremely low (Becker 1995, Thakur *et al.* 1977a, Thakur & McAfee 1984). Due to the characteristics of $^{111}$In, i.e. its medium energy, long physical half-life and high radiation burden, combined with its production in a cyclotron, causing limited availability, and the sub-optimal resolution achieved with gamma camera imaging, $^{111}$In is not favoured in practice. When it is possible to label leukocytes with $^{99m}$Tc-HMPAO, there are only a few indications for $^{111}$In labelling (Becker 1995).

Mulamba *et al.* (1983) reported a series of 30 hip prostheses, 13 of which were infected, subjected to $^{111}$In-labelled leukocyte and $^{99m}$Tc sulphur colloid imaging. With this method, they diagnosed all but one the infections, and there were no false positive findings. McKillop *et al.* (1984) found better sensitivity in prosthesis infections with $^{67}$Ga-citrate imaging than with $^{111}$In-labelled leukocyte imaging in 15 patients with a painful joint prosthesis. Merkel *et al.* (1985) published a study on a comparison of $^{111}$In-labelled leukocytes with $^{99m}$Tc-bone/$^{67}$Ga scanning. In patients with a prosthetic component, the accuracy of $^{111}$In-labelled leukocytes was 94% and that of $^{99m}$Tc-bone/$^{67}$Ga scanning 75%. $^{111}$In-labelled leukocyte imaging was superior on all parameters (sensitivity 86% vs. 57%, specificity 100% vs. 89%, positive predictive value 100% vs. 80% and negative predictive value 90% vs. 73%). The study also included receiver operating characteristic curves for three observers, and all of them had better results with $^{111}$In-labelled leukocyte imaging than with $^{99m}$Tc-bone/$^{67}$Ga scanning.

$^{111}$In-labelled leukocytes have been used in the diagnosis of prosthesis infections by many other investigators, too. Sensitivity of 88% and specificity of 73% were obtained in a series of 225 patients with possible orthopaedic implant infection using $^{111}$In-labelled leukocytes alone (Magnuson *et al.* 1988). Another study indicated 100% sensitivity and 50% specificity for $^{111}$In-labelled leukocytes alone in a series of 21 suspected hip and 2 suspected knee prosthesis infections. The low specificity was accounted for by the fact that $^{111}$In-labelled leukocyte scanning may also be positive in the absence of culture-proven infection in patients with painful loose hip prostheses. When correlated with bone imaging, sensitivity was 88%, specificity improved to 95%, and the authors recommended this technique for the detection of infected hip prostheses (Johnson *et al.* 1988).

Oswald *et al.* (1989) prospectively studied the uptake of $^{111}$In-labelled leukocytes in the prosthetic tip of 25 uncomplicated porous coated hip prostheses and observed two patterns of uptake, focal and diffuse linear, which occurred at approximately equal frequencies in 80% of prostheses during the first two years after implantation. They recommended further investigation of this observation with sulphur colloid marrow imaging. Concerning the acetabulum, in an article on the same series, they found increased leukocyte uptake in 92% of prostheses. The intensity of uptake was less than or equal to the intensity of $^{99m}$Tc-MDP uptake in 98% of prostheses and always less than or equal to that in the iliac crest, and it always remained stable or decreased during the two years of study. Based on these findings, they recommended comparison of leukocyte

images with bone images (Oswald *et al.* 1990). However, a recent publication of 166 $^{111}$In-labelled leukocyte and static bone imaging studies done during 1984–1995 indicated only 64% sensitivity and 78% specificity (Teller *et al.* 2000). Scher *et al.* (2000) published a study using $^{111}$In-leukocyte/$^{99m}$Tc-HDP imaging. For 91 hip prostheses, they found 60% sensitivity with 93% specificity and for 40 knee prostheses 88% sensitivity with 78% specificity. They also did dynamic imaging with $^{99m}$Tc-HDP, but did not report the results.

King *et al.* (1990), and especially Palestro *et al.* (1990, 1991), achieved good results with combined $^{111}$In-labelled leukocyte and bone marrow imaging, with 86–100% sensitivity and 97–100% specificity in hip and knee prosthesis infections. Joseph *et al.* (2001) also noted the ability of added sulphur colloid scanning to eliminate the false positive results observed earlier in many cases with $^{111}$In-labelled leukocyte imaging alone. In their hands, however, this combined method produced many false negative results and only 46% sensitivity in a series of 58 hip and knee prosthesis patients. They criticised the previous studies for underestimating the number of true infections, because of lacking operative treatment and histological data in many cases and short follow-up periods of only 6 months.

Datz and Thorne (1986) studied the effects of antibiotic therapy on the sensitivity of $^{111}$In-leukocyte imaging in 312 scans. There were 128 soft-tissue and 15 bone infections in the series. The sensitivity of leukocyte scanning was 88.7% in patients on antibiotic therapy and 92.1% in those not receiving antibiotics, and the difference between the two groups was not significant ($p>0.05$).

With $^{111}$In-labelled leukocyte imaging, uptake by soft tissues has sometimes not been separable from abnormal bone uptake because of image resolution problems. (Alazraki 1990)

*$^{99m}$Tc-labelled leukocytes*. There was a clear need for a $^{99m}$Tc labelling procedure for leukocytes, because of the possible improvements in cost efficiency, convenience, image resolution and dosimetry compared to $^{111}$In-labelled leukocyte imaging. The first promising results were obtained in 1986, when Peters *et al.* proposed a new lipophilic brain imaging agent, $^{99m}$Tc-hexamethylpropyleneamine oxime or $^{99m}$Tc-HMPAO, synthesized by Holmes *et al.* in 1985, as a new agent also applicable to leukocyte labelling. Since their introduction, $^{99m}$Tc-HMPAO-labelled leukocytes have been widely used for infection imaging. The only reliable $^{99m}$Tc-labelling method is still the $^{99m}$Tc-HMPAO labelling of leukocytes. $^{111}$In is highly stable in all labelled cell types, while $^{99m}$Tc-HMPAO is not. Hourly elution of 6–9% out of labelled leukocytes has been measured *in vitro*, while the corresponding figures for $^{111}$In-label have been 1–2%. Because of this instability, secondary hydrophilic complexes of $^{99m}$Tc are excreted via the kidneys, and excretion in bile may also occur. No differences in cell viability have been seen between the two labels (98% versus 96.5%). A disadvantage of $^{99m}$Tc-HMPAO imaging is the appearance of kidney, bladder, biliary and bowel activities, which reduce the diagnostic accuracy of scannings in the abdominal and retroperitoneal regions. However, most inflammatory abdominal diseases can be diagnostically visualised by imaging 30 minutes after the injection of $^{99m}$Tc-labelled leukocytes. This problem of unspecific activity is not seen outside the abdominal and retroperitoneal locations, and late scanning is thus feasible in prosthetic joint infections. One difficulty in patients with bone infection is the slow leukocyte accumulation in some of them, which makes late

scans requiring also long acquisition times, necessary. The image quality of $^{99m}$Tc-leukocyte scans is better than the quality of $^{111}$In-leukocyte scans. (Becker *et al.* 1988, Becker 1995, Hovi *et al.* 1993, Wolf *et al.* 2001)

Paakkinen *et al.* (1987) were one of the first groups to publish a study with $^{99m}$Tc-HMPAO-labelled leukocytes in 1987 using a modified labelling method of Peters *et al.* and imaging patients with soft-tissue infections. Later, they also achieved good results in suspected bone and joint infections, except in two out of three patients with chronic disease, and concluded that the utility of this method in chronic osteomyelitis warrants further evaluation. The results were superior to those of $^{67}$Ga-citrate imaging (Vorne *et al.* 1989b). In comparison with $^{99m}$Tc-nanocolloids, $^{99m}$Tc-HMPAO-labelled leukocytes were more sensitive in bone and joint infections (90% vs. 95%), giving 100% specificity and true positive findings in four cases of prosthetic joint infections (Vorne *et al.* 1989a).

Moragas *et al.* (1991) published an article on combined $^{99m}$Tc-HMPAO-labelled leukocyte and bone-metabolic imaging in the diagnosis of bone and joint infections. No false negative results were found in this series of 25 suspected hip and 5 suspected knee prosthesis infections. Recently implanted prostheses were considered to reduce specificity, as aseptic inflammation associated with the operative injury might accumulate leukocytes. The bone-metabolic images were considered useful in detecting such accumulation. There were two false positive findings in this study, one was caused by inflammatory trochanteric bursitis and the other involved a change in the distribution of bone marrow. The authors recommended the use of $^{99m}$Tc-sulphur colloid scanning whenever there is doubt in distinguishing normal from abnormal bone marrow activity in a suspected focus. The study did not address late 24-hour imaging. Lantto *et al.* (1992) discovered that $^{99m}$Tc-leukocyte imaging detected osteomyelitic lesions better than $^{99m}$Tc-bone imaging in the growing bone of children.

Devillers *et al.* (1994) published a retrospective study of $^{99m}$Tc-labelled leukocyte imaging in 116 patients for the diagnosis of bone and joint infections involving 74 patients with orthopaedic implants, 52 of whom had hip prostheses. They achieved 97% sensitivity and 89% specificity in the orthopaedic implant group without using late imaging. De Lima Ramos *et al.* (1996) published a comparison of $^{99m}$Tc-labelled leukocytes with $^{111}$In-labelled non-specific polyclonal human immunoglobulin G in bone and joint infections, also using 24 h images with $^{99m}$Tc-leukocytes and bone imaging for comparison. Overall, $^{99m}$Tc-labelled leukocyte imaging was superior to $^{111}$In-IgG, but in the subgroup of 12 prostheses, the authors only got 50% accuracy. Palermo *et al.* (1998) used late $^{99m}$Tc-labelled leukocyte imaging in a patient series for detecting musculoskeletal inflammation and compared the method with $^{99m}$Tc-MDP and $^{99m}$Tc-HIG imaging. Unfortunately, late images were only available of two prostheses, but the inflammation index used by them decreased in the negative case and increased in the positive case over time. Rosas *et al.* (1998) combined $^{99m}$Tc-labelled leukocyte imaging with bone scanning and colloidal sulphide bone marrow scanning in a series of 20 hip and 17 knee prostheses and achieved 100% sensitivity and 87% specificity with the combined procedure, which did not include 24 h images. In the same year, a consensus protocol for labelling white blood cells with $^{99m}$Tc-HMPAO was published (Roca *et al.* 1998).

The $^{99m}$Tc-labelling of leukocytes made high-quality SPET imaging available with labelled leukocytes. Weon *et al.* (2000) reported increased sensitivity (69% to 94%) and specificity (67% to 78%) with the use of SPET imaging technique in a series of 25

prostheses, concluding that additional SPET images may be helpful in localising the site of infection more accurately. Wolf *et al.* (2003) published a retrospective series of 54 hip and 11 knee prostheses imaged with $^{99m}$Tc-HMPAO-labelled leukocytes at 4.5–5 hours post-injection. They found the preferred localisations of the infectious focus to be the intertrochanteric region and the middle part of the shaft in the hip prostheses and the proximal shaft of the tibia in the knee prostheses.

*Heterologous labelled leukocytes*. Gratz *et al.* (2002) used heterologous $^{99m}$Tc-HMPAO-labelled leukocytes to image infection in rabbits. They found that purified granulocytes from infected donor rabbits were superior to purified granulocytes from non-infected donor rabbits. In addition, autologous granulocytes gave similar results compared to heterologous granulocytes from infected donor rabbits, suggesting that intrinsic cell activation is needed for specific cell migration. The authors concluded that further identification of factors that might be found in the donor blood to improve migratory capacity of the cells is needed. Heterologous leukocytes would, theoretically, be useful for infection imaging in neutropaenic patients.

### *2.7.2.2 Leukocytes, in vivo labelling*

A distinction can be drawn between tracers that bind leukocytes by receptor binding (relatively small molecules of molecular weight <20000) and tracers that bind by antibody-antigen interaction (relatively large molecules). The molecular weight of the antibodies ranges from 50000 (antibody fragments) through 150000 (IgG) to 900000 (IgM). Ever since it became clear that labelled leukocytes could visualise infectious foci, investigators have been developing a method that could label leukocytes *in vivo*. At least three antigranulocyte antibodies have been tested: anti-NCA-95 IgG (BW 250/183), anti-NCA-90 Fab' (Immu-MN3, LeukoScan®: anti-CD66) and anti-SSEA-1 IgM (LeuTech®: anti-CD15). All of these antibodies, labelled with $^{99m}$Tc or $^{123}$I, allowed infection imaging. (Rennen *et al.* 2001)

It soon turned out that the *in vivo* behaviour of anti-granulocyte antibody preparations did not mimic the behaviour of radiolabelled leukocytes. In general, blood clearance of IgG preparations was slower, resulting in higher background activity. On the other hand, preparations based on antibody fragments had higher renal excretion. An IgM antibody had higher liver uptake than labelled white blood cells. Becker *et al.* (1989) found that less than 10% of the radiolabelled BW250/183 antibody present in blood was actually associated with granulocytes. Observations indicated that antigranulocyte antibody imaging was not a feasible method for labelling white blood cells *in vivo*. It is now generally accepted that radiolabelled antigranulocyte antibodies image infectious foci mainly by non-specific extravasation, and that binding of the antibody to infiltrated leukocytes in the inflamed tissue may contribute to the retention of the radiolabel in the focus. (Rennen *et al.* 2001)

In bone infections, the sensitivity of antigranulocyte antibody scintigraphy in 106 patients was 69% for the hip, 79% for the thigh, 85% for the knee and 100 % for the lower leg and ankle. Sensitivity decreased from the periphery to the central parts. This may result from physiological uptake of the antibody in bone marrow, where normal

uptake cannot be distinguished from uptake related to infection (Becker 1995). Antigranulocyte antibody-based radiopharmaceuticals visualised infectious foci in patients with sensitivity between 80% and 90% (Rennen *et al.* 2001).

A disadvantage of murine monoclonal antibodies is that they induce human anti-mouse antibodies (HAMAs), which may result in altered distribution after subsequent injections. The production of such antibodies is dose-dependent, their frequency ranging from more than 30% in patients receiving repeated injections down to 4.5% in patients with a fixed dose of 125 μg of the antibody (Becker *et al.* 1994b). In this respect, the use of antibody fragments instead of whole antibodies seems to be advantageous, since fragments appear to be less immunogenic. In addition, antibody fragments have faster blood clearance and may thus enable earlier diagnosis. (Rennen *et al.* 2001)

Initial studies with $^{99m}$Tc-labelled monoclonal antigranulocyte-antibody fragments were successful (Becker *et al.* 1994a), and two subsequent studies from Becker *et al.* (1996) and Hakki *et al.* (1997) demonstrated high sensitivity and specificity. In a recent study, however, only 85% sensitivity and 77% specificity were demonstrated for orthopaedic imaging in clinical practice (Ryan 2002).

### *2.7.2.3 Antimicrobial and chemotactic peptides*

The first report on antimicrobial peptides dates back to 1930, but the discovery of antibiotics drew researchers' attention away from these antimicrobial proteins. These peptides can be categorised into three main structural classes: linear peptides adopting α-helical structure, peptides with disulphide bridges and peptides that are rich in one amino acid. Microbial membranes expose the negatively charged phospholipids on their surface, and insertion of these peptides into the bacterial cytoplasmic membrane results in increased permeability and leakage of cellular constituents. There is also evidence that some antimicrobial peptides affect yeast membranes through an effect on the mitochondrion. $^{99m}$Tc-labelled antimicrobial peptides have been used in different laboratory animals. A peptide derived from human ubiquicidin appears a promising tracer, because it can discriminate between infections and sterile inflammatory processes. Further research, such as identification of the mechanisms underlying the labelling method, the toxicology of $^{99m}$Tc-peptide complex, and adverse immunological reactions, are needed before considering the use of $^{99m}$Tc-ubiquicidin in humans. (Lupetti *et al.* 2003)

Radiolabelled cytokines are low molecular weight (<20000) protein pharmaceuticals. Cytokines act by interacting with cell surface receptors, and their binding affinities are usually high. Low levels of cytokine receptors are expressed on non-excited cells, and their expression can be up-regulated during activation (Rennen *et al.* 2001). Radiolabelled cytokines may have diagnostic potential in many diseases, as cytokine-cytokine receptor complexes operate in infections, autoimmunity, tumours and other pathologies. Receptors are often up-regulated by activation of the receptor-bearing cells, and receptor expression can be used for the diagnosis of the disease under investigation (Signore *et al.* 2000).

Interleukin 1 (IL-1) binds to the receptors expressed on leukocytes with high affinity. Unfortunately, toxicity limits the clinical application of IL-1. The IL-1 receptor antagonist

is an alternative. $^{123}$I labelled IL-1 receptor antagonist was tested in rheumatoid arthritis, and inflamed joints were visualised. Lymphocytic infiltrates have been successfully imaged with radiolabelled interleukin-2. Interleukin-8, which belongs to chemotactic cytokines, binds to receptors on neutrophils with high affinity. $^{123}$I and $^{99m}$Tc-labelled interleukin-8 has been tested in rabbits. (Babich *et al.* 1993, Fischman *et al.* 1991, Fischman *et al.* 1993, Lupetti *et al.* 2002, Palestro *et al.* 2001, van der Laken *et al.* 2000, Welling *et al.* 1999, Welling *et al.* 2001)

### *2.7.2.4 Radiolabelled antibiotics, $^{99m}$Tc-ciprofloxacin*

Tetracycline was labelled with $^{131}$I and $^{99m}$Tc in the 1970s and used as a bone tumour-localising agent before being replaced by newer tracers. Although not used in infection imaging, it is radiolabelled antibiotic and might be worth studying even in this indication. (Breslow *et al.* 1974, Chauncey *et al.* 1976b, Chauncey *et al.* 1976a, Hagan *et al.* 1977, Holman *et al.* 1974, Riihimaki *et al.* 1976, Robinson, Jr. & Battaglia 1975)

A new way to specifically detect infection is to use radiolabelled antibiotics, which accumulate in microorganisms. Interesting results have been obtained by using $^{99m}$Tc-labelled ciprofloxacin. It has been found that the site of infection can be successfully detected, even before the migration of radiolabelled leukocytes is evident. Radiolabelled leukocyte scanning can point the site of inflammation, but it may fail to distinguish between bacterial infection and non-bacterial inflammation. $^{99m}$Tc–ciprofloxacin is assumed to be more specific for bacterial infection, because it binds to the DNA-gyrase enzyme in living bacteria and should therefore visualise the bacteria directly instead of inflammation. This agent, which was developed in the 1990s, combines the advantages of the $^{99m}$Tc-label and bacteria-localising capability of ciprofloxacin. Initial *in vitro* and animal studies showed that $^{99m}$Tc–ciprofloxacin localises in abscesses caused by living bacteria, but does not localise in areas of sterile inflammation or abscesses with dead bacteria. (Britton *et al.* 2002)

The first published clinical study included 56 patients with suspected bacterial infections and achieved 84% sensitivity and 96% specificity in the whole series, which was better than white blood cell imaging with 81% sensitivity and 77% specificity. The reason for the false negative cases in $^{99m}$Tc-ciprofloxacin imaging was thought to be that several patients had received antibiotics before the imaging. The patients were imaged at 1 hour, 4 hours and, occasionally, 24 hours after injection with $^{99m}$Tc–ciprofloxacin and $^{99m}$Tc–labelled white blood cells, and at 4 hours and 24 hours after injection with $^{111}$In-labelled white blood cells. The study included 33 patients with suspected skeletal infections. The authors thought that $^{99m}$Tc-ciprofloxacin had several advantages over radiolabelled white blood cells. First, $^{99m}$Tc-ciprofloxacin does not require handling of blood during preparation, thus reducing the risks of hepatitis and HIV infection. Second, $^{99m}$Tc-ciprofloxacin is technically easier and less labour-intensive to prepare than radiolabelling white cells. Third, this method is independent of the patient's white-cell status, which is advantageous in leukopaenic patients. Finally, $^{99m}$Tc-ciprofloxacin is not taken up by bone marrow and is thus better able to identify infection in the spine and the proximal parts of limbs. (Vinjamuri *et al.* 1996)

The second clinical trial included 99 patients, 61 of them with a suspicion of a skeletal infection. In this subgroup, $^{99m}$Tc–ciprofloxacin achieved 89% sensitivity, 91% specificity and 90% accuracy. In the total series, its accuracy was calculated to be 83–87%. Antibiotic resistance of the bacterial strain was associated with positive $^{99m}$Tc–ciprofloxacin imaging in six of the seven patients in whom this was documented. 24-hour images were obtained in 15 of the initial studies, but they did not alter the interpretation at 4 hours and were thus not considered necessary for diagnosis. (Britton *et al.* 1997)

The third paper presented data on the efficacy of $^{99m}$Tc-ciprofloxacin imaging in the first 90 patients with suspected infective disorders to have undergone such investigation. The images were acquired at approximately one and four hours and, occasionally, at 24 hours after injection. The study included four patients with infected surgical prostheses and positive $^{99m}$Tc-ciprofloxacin images. However, total sensitivity was only 70.3%, while specificity was 93.1%. There were no significant differences with respect to antibiotic treatment between the $^{99m}$Tc-ciprofloxacin-positive and negative groups. The study included three false positive patients, who presented with rheumatoid and psoriatic arthritis of the hands and with non-union of a wrist fracture. The authors' experience was that most of the false positive images seen at four hours in chronic non-infective inflammatory disorders became negative when imaged at 24 hours. (Hall *et al.* 1998)

Neutrophils and activated macrophages can also take up ciprofloxacin (Easmon *et al.* 1986). However, there is only very little evidence that this is an important factor in imaging. In animal experiments $^{99m}$Tc-ciprofloxacin is taken up only by bacterial, but not by sterile abscesses; non-infective inflammatory conditions, such as ulcerative colitis and Crohn's disease, are negative by $^{99m}$Tc-ciprofloxacin imaging but positive by white blood cell imaging; and *in vitro* results indicate that, compared with bacteria, human white blood cells exhibit either no uptake or markedly less uptake of $^{99m}$Tc-ciprofloxacin. The main factor of $^{99m}$Tc-ciprofloxacin imaging is considered to be the uptake of the tracer by bacteria, which is distinct from its antibacterial activity, as bacteria resistant to ciprofloxacin can still take up $^{99m}$Tc-ciprofloxacin if their resistance is mediated only through DNA gyrase alteration. Positive images were obtained in five cases of resistant bacteria causing infection (Hall *et al.* 1998).

Amaral *et al.* (1999) described a cold-hot mismatch between $^{99m}$Tc-labelled leukocytes and $^{99m}$Tc-ciprofloxacin in three cases of axial skeleton infections, concluding that $^{99m}$Tc-ciprofloxacin should be a better tracer than radiolabelled leukocytes for detecting osteomyelitis in the axial skeleton. They mentioned that, in some patients, 24-hour post-injection imaging was needed, although the findings were already visible in the 4-hour images published in the paper.

DeWinter *et al.* (2001) published a study on the bio-distribution and dosimetry of $^{99m}$Tc-ciprofloxacin. Approximately 60% of the injected activity was recovered in urine by 24 hours post-injection. The urinary bladder wall, the thyroid, the upper large intestine, the lower large intestine and the uterus received the highest absorbed doses. The estimated mean effective dose was 8.30E-03 mSv/MBq, which is lower than that for the other tracers used in infection imaging, such as radiolabelled leukocytes (59.0E-02 mSv/MBq for $^{111}$In and 17.0E-03 mSv/MBq for $^{99m}$Tc-HMPAO) and $^{67}$Ga (12.0E-02 mSv/MBq) (Datz *et al.* 1997, Seabold *et al.* 1997b, Seabold *et al.* 1997a). It was concluded that the distribution of $^{99m}$Tc-ciprofloxacin demonstrated low brain, lung, bone marrow and liver uptake, allowing imaging of the whole body and specifically of the spine and the

limbs, and that the amount of $^{99m}$Tc-ciprofloxacin required for planar and tomographic imaging results in an acceptable effective dose to the patient (De Winter *et al.* 2001).

In 2001, two Turkish groups published good results with $^{99m}$Tc–ciprofloxacin imaging in orthopaedic infections, comparing the method with either $^{99m}$Tc–HMPAO leukocyte imaging (Sonmezoglu *et al.* 2001) or sequential bone/gallium imaging (Yapar *et al.* 2001). The studies consisted of 51 patients and 22 patients, respectively.

The first study included a subgroup of 18 patients with orthopaedic implantation material, giving a total of 23 sites of suspected infection (11 hip prostheses, 11 knee prostheses and 1 femoral fixation nail). In this group, $^{99m}$Tc-ciprofloxacin imaging was positive in 11 of the 12 infected sites, and there was one false positive finding in a case with soft-tissue infection, while $^{99m}$Tc-leukocyte imaging gave positive results at only 7 sites. With these results, the sensitivity, specificity, positive and negative predictive value and accuracy of $^{99m}$Tc–ciprofloxacin imaging in prosthesis infections were between 91% and 92%. Images were obtained at 1 hour and at 4 hours and only in suspicious cases, which were not specifically documented, at 24 hours. In the total series, 16 of 24 knee joints imaged showed $^{99m}$Tc–ciprofloxacin accumulation in synovial cavities, which sometimes diminished after 24 hours. Three of the joints were affected by septic arthritis, one had rheumatoid arthritis, six had prostheses with unproven infection and six had possible degenerative changes. False positive findings were recorded in patients with fibrous dysplasia of the ulna and avascular hip necrosis. Only three months' follow-up was used in some cases to decide whether infection was actually present. One of the main conclusions was that the lack of bone marrow uptake makes $^{99m}$Tc–ciprofloxacin a powerful and promising tracer for vertebral infections. (Sonmezoglu *et al.* 2001)

In the second study, only 1-hour and 4-hour imaging was done. The material included 15 patients with hip prostheses and four patients with knee prostheses. The best results were achieved when slightly increased activity was considered negative for infection, and sensitivity was 85%, specificity 92% and accuracy 88%. If slightly increased activity was considered positive for infection, sensitivity improved to 92%, but specificity decreased to 23% and accuracy to 58%. The corresponding figures for bone/gallium imaging were 78%, 100% and 90%. The authors concluded that slight accumulation in the periprosthetic region is seen without infection, but significant uptake suggests infection. (Yapar *et al.* 2001)

Dumarey *et al.* studied $^{99m}$Tc–ciprofloxacin imaging in a series consisting of 30 patients with suspected osteomyelitis or septic arthritis and 41 controls without any inflammatory or infectious disease. All were imaged at 4 hours and five also at 8 hours, and imaging at 24 hours was done on 51 subjects, including 24 with suspected infections and 27 controls. The uptake of $^{99m}$Tc–ciprofloxacin was scored from 0 to 2. When only patients with a score of 2 were considered positive, sensitivity was 84.2%, specificity 54.5% and accuracy 73.3%. When patients with a score of 1 were also considered positive, specificity decreased to 27.3%. The authors found no relationship between tracer kinetics, i.e. the evolution from 4 hours to 8 or 24 hours, and the diagnosis of infection. This study included three children, in whom intense uptake was present in the growth cartilage. False positive findings were seen in non-infected knee prostheses, pseudo-arthrosis with bone graft necrosis, rheumatism and postoperative fibrosis of the lumbar spine. In the control group, uptake was observed in 17% of hip joints, 49% of lumbar spines, 58% of sacro-iliac joints, 58% of knee joints, 75% of wrist joints and 82.1% of

shoulder joints. The authors concluded that $^{99m}$Tc–ciprofloxacin is sensitive but not specific for bone and joint infection. (Dumarey *et al.* 2002)

Malamitsi *et al.* (2003) found sensitivity of 97.2%, specificity of 80%, a positive predictive value of 94.6% and a negative predictive value of 88.9% in their series of 45 patients with known or suspected bone infection, who underwent 50 scans with $^{99m}$Tc-ciprofloxacin. They concluded that $^{99m}$Tc-ciprofloxacin is a very sensitive and quite specific tracer of bone infection, but care must be taken in cases of excessive new bone formation and primary bone tumours, where false positive results may be obtained.

The largest study on $^{99m}$Tc-ciprofloxacin up to date is the worldwide multi-centre study on 879 patients with suspected bacterial infection, including 194 orthopaedic prostheses, sponsored by International Atomic Energy Agency. In this study, many false positive findings were obtained, especially during the first year and particularly in patients with active arthropathies in large joints, such as rheumatoid arthritis, when only one-hour and four-hour images were taken. The value of the 24-hour images became apparent only after this initial learning phase. The study group reported that the most successful results were seen in osteomyelitis (sensitivity 90.5% and specificity 72.8%, n=228) and orthopaedic prosthesis infections (sensitivity 96% and specificity 91.6%, n=194). The sensitivity and specificity of $^{99m}$Tc-ciprofloxacin in bacterial infection were determined with respect to the Centres of Disease Control, World Health Organisation and Duke's criteria (Garner *et al.* 1988). The sensitivity was, however, 100% in the 63 prosthesis infections that were confirmed by bacterial cultures. (Britton *et al.* 2002)

In all of the abovementioned studies using $^{99m}$Tc–ciprofloxacin, none of the patients were reported to have had any adverse reactions from the radiopharmaceutical.

## 2.8 Examples of studies utilising nuclear medicine procedures in the diagnosis of orthopaedic infections

In order to make this publication easier to read, exemplary data from some previous studies have been collected into Table 2. The selection of these examples is purely subjective and represents studies including the required documentation for this kind of tabulation and a series including hip and knee prosthesis infections, often among other orthopaedic infections. Because of varying aspects, including but not limited to the study methodology and documentation, no definite conclusions should be drawn based on this simplified table alone. However, particular attention should be paid to the variable follow-up periods of the patients in the reference methods column.

*Table 2. Examples of studies utilising nuclear medicine procedures in the diagnosis of orthopaedic infections.*

| Method | Publication | n prostheses | n patients | Sensi-tivity | Specifi-city | Reference method (follow-up time) |
|---|---|---|---|---|---|---|
| $^{67}$Ga/bone | Merkel *et al.* 1986 | hip 71, knee 14, other | 130 | 66 | 81 | Operative and clinical (2 years) |
| $^{99m}$Tc-nanocolloid | Flivik *et al.* 1993 | hip 14, knee 7 | 21 | 100 | 82 | Operative and clinical (not specified) |
| $^{111}$In-HIG | Nijhof *et al.* 1997 | hip 85, knee 17 | 100 | 100 | 80 in hip, 50 in knee | Operative and clinical (6 months) |
| $^{18}$F-FDG-PET | Zhuang *et al.* 2001 | hip 38, knee 36 | 62 | 90 | 81 | Operative and clinical (1 year) |
| $^{111}$In-leukocyte/bone | Johnson *et al.* 1988 | hip 21, knee 2 | 28 | 88 | 95 | Operative only |
| $^{111}$In-leukocyte/ marrow | Palestro *et al.* 1990 | hip 92 | 72 | 100 | 97 | Operative and clinical (6 months) |
| $^{111}$In-leukocyte/ marrow | Palestro *et al.* 1991 | knee 41 | 28 | 86 | 100 | Operative and clinical (6 months) |
| $^{99m}$Tc-leukocyte | Devillers *et al.* 1994 | hip 52, other implant 22 | 116 | 97 | 89 | Operative and clinical (1 year) |
| $^{99m}$Tc-leukocyte/ bone | Moragas *et al.* 1990 | hip 25, knee 5 | 50 | 100 | 91 | Operative and clinical (not specified) |
| $^{99m}$Tc-antigranulocyte antibody fragment | Ryan 2002 | hip 3, knee 23, other | 55 | 85 | 77 | Operative and clinical (6 months) |
| $^{99m}$Tc-ciprofloxacin | Sonmezoglu *et al.* 2001 | hip 11, knee 11, femur 1 | 51 | 92 | 91 | Operative and clinical (3 months) |

# 3 Aims of the study

The aim of this study was to develop and evaluate different nuclear imaging methods for diagnosing hip and knee prosthesis infections. The special aims were as follows:

1. To find out if three-phase bone imaging has any place in the diagnostics.
2. To further develop imaging methods and the interpretation of images for the diagnosis of prosthesis infections with $^{99m}$Tc-labelled leukocyte/bone imaging.
3. To develop suitable imaging and interpretation methods for a new tracer, $^{99m}$Tc-ciprofloxacin, in prosthesis infections.
4. To compare combined $^{99m}$Tc-labelled leukocyte/bone imaging with $^{99m}$Tc-ciprofloxacin imaging in the diagnostics.
5. To produce, if possible, a practical guide for the clinical use of imaging methods in the diagnostics of orthopaedic prosthesis infections.

# 4 Patients and methods

## 4.1 Patients

### *4.1.1 Patient series*

Patients were prospectively allocated in these studies. The inclusion criteria were: prosthesis with clinical symptoms and a clinical suspicion of infection. The symptom was usually pain at exercise or rest, and the diagnostic setting was the need to differentiate infection from aseptic loosening of the prosthesis. The main characteristics of the patients included in this work are presented in Tables 3a and b. The patients in the studies III and IV also underwent $^{99m}$Tc-labelled leukocyte/bone imaging, in addition to $^{99m}$Tc-ciprofloxacin imaging.

*Table 3a. Main patient characteristics in the studies 1–4.*

| Study | Main tracer | Prosthesis | n | Infections | Re-operations |
|---|---|---|---|---|---|
| 1 | $^{99m}$Tc-leukocytes | Hip | 64 | 6 | 30 |
| 2 | $^{99m}$Tc-leukocytes | Knee | 30 | 8 | 13 |
| 3 | $^{99m}$Tc-ciprofloxacin | Knee | 16 | 7 | 8 |
| 4 | $^{99m}$Tc-ciprofloxacin | Hip | 30 | 8 | 10 |

*Table 3b. Main patient characteristics in the studies 1–4*

| Study | Mean age (range) | Female / Male % | Arthrosis | Rheumatoid | Other |
|---|---|---|---|---|---|
| 1 | 65 (25–87) | 72 / 28 | 34 | 15 | 15 |
| 2 | 75 (47–82) | 87 / 13 | 17 | 13 | 0 |
| 3 | 70 (60–82) | 75 / 25 | 10 | 6 | 0 |
| 4 | 63 (25–86) | 63 / 37 | 13 | 10 | 7 |

### *4.1.2 Diagnostic criteria of infections*

The patient had prosthesis infection if bacterial cultures taken during the operation yielded bacteria, if there was a purulent finding at surgery or if repeated aspirated samples grew bacteria or were purulent. Purulence, as the sole criterion, was only accepted for the patients who had received prior antibiotic treatment or needed long-term antibiotic therapy for recovery. Correspondingly, operatively treated patients were considered uninfected if their operative cultures showed no growth and there was no purulence at surgery. Un-operated patients were considered uninfected if no clinical signs of infection were present during a follow-up period of at least one year. All patients were carefully assessed by the senior consultant for infectious diseases at Oulu University Hospital and by the orthopaedic surgeon, to evaluate the clinical outcome.

## 4.2 Methods

### *4.2.1 Bone-imaging radiopharmaceutical*

For three-phase bone imaging, a dose of 550 MBq of $^{99m}$Tc-HDP was used.

### *4.2.2 Leukocyte labelling*

Blood samples were collected for the preparation of labelled leukocytes, and the leukocyte fractions were labelled with $^{99m}$Tc-HMPAO (Ceretec®, Amersham) using a labelling dose of 370 MBq of technetium and standard techniques (Roca *et al.* 1998). The leukocytes were subsequently re-injected intravenously into each patient with a delay of no more than 2 h 30 min. The total white blood cell count of the patients at the time of the study was between 3.9 and 12.3 x $10^9$ / l and the labelling yield of white blood cells was between 80 and 96%.

### *4.2.3 Preparation of $^{99m}$Tc-ciprofloxacin*

$^{99m}$Tc-ciprofloxacin was prepared by reconstitution of a two-phase labelling kit, by mixing 2 mg of ciprofloxacin, 500 μg of stannous tartrate and 400 MBq of freshly eluted sodium pertechnetate (Britton *et al.* 1997). The mixture was allowed to react at room temperature for 15 minutes. Radiochemical purity was determined with a paper chromatography technique, using 1 mm paper (Whatman, Ann Arbor, MI), and it was between 80 and 100%. Injected activity was 370 MBq.

### *4.2.4 Imaging procedures*

In the protocol used in these studies, the three-phase bone imaging was done during the same day following the 24-hour $^{99m}$Tc-leukocyte imaging. In the studies including $^{99m}$Tc-ciprofloxacin imaging, at least one day was allowed between it and the other imaging protocols. All images were acquired with a 20% window centred at 141 keV as follows:

The routine $^{99m}$Tc-white-blood-cell imaging was done between two and four hours and the late imaging at about 24 hours after the injection of labelled leukocytes. The routine images were acquired with 10 minutes' imaging time using a high-resolution collimator. For the late images, a general-purpose collimator was used to keep the acquisition time at a reasonable 10 minutes in spite of the lower count rate at this time point.

In the three-phase bone imaging, the arterial-phase dynamic imaging was done in the anterior view using an acquisition time of 5 to 6 seconds per frame up to 2 minutes after the injection. A soft-tissue phase image was obtained between 4 and 8 minutes after the injection. A high-sensitivity or general-purpose collimator was used for dynamic imaging. $^{99m}$Tc-bone-metabolic images were acquired at about 3 hours after the injection, using an acquisition time of 10 minutes and a high-resolution collimator.

The $^{99m}$Tc-ciprofloxacin imaging was done at three different time points: at one hour, at four hours and at 24 hours after the injection of tracer. The one-hour and four-hour images were carried out using an imaging time of 10 minutes. In addition to the images of the prosthetic sites, whole body images in anterior and posterior views were also obtained at four hours. These images were acquired with a scanning speed of eight to ten centimetres per minute, to yield a minimum of $1.5 \times 10^6$ counts per image. A high-resolution collimator was used for the images acquired at one and four hours. For the 24-hour images, a general-purpose collimator was used.

Adac, Elscint and Siemens cameras were used for imaging. Altogether five different cameras were used, and one camera was double-headed, while the other cameras were single-headed.

### *4.2.5 Interpretation of images*

In the hip prostheses, blood flow, blood pool, bone metabolism and leukocyte activities were considered positive or negative compared to pre-defined background and reference areas, as presented in paper I. In the hip prostheses, the suspicion of infection was unilateral in all cases. In the case of knee prostheses, the non-suspected knee was used for comparison, except in the two cases with suspicion of bilateral infection, where interpretation was done only qualitatively.

A previously described method for a systematic evaluation of prosthesis infection with white-blood-cell and bone imaging was used (Johnson *et al.* 1988). Sites of interest were considered infected if the intensity of periprosthetic leukocyte uptake was higher than the activity in a corresponding bone-metabolic image, or if the leukocyte uptake was incongruent with the bone-metabolic uptake. Two experienced specialists in nuclear

medicine assessed the data blind to the clinical data, and consensus was used as the final interpretation.

The $^{99m}$Tc-ciprofloxacin images were evaluated by the specialists blinded to the clinical data and the other imaging results. The imaging was considered positive when there was distinct positive uptake of the tracer at the site of the symptomatic prosthesis. If the accumulation had a declining pattern of activity, it was considered unspecific and negative for infection (Britton *et al*. 1997). In study III, including knee prostheses, a third experienced nuclear medicine physician (OP), who had never seen the images before, determined the final interpretation according to the study criteria.

### *4.2.6 Statistics*

Sensitivity was calculated by dividing the number of cases with infection having positive imaging results by the total number of cases with infection. Specificity was calculated by dividing the number of cases without infection having negative imaging results by the total number of cases without infection. Positive predictive value (PPV) was calculated by dividing the number of cases with true positive imaging results with the total number of cases with positive imaging results. Negative predictive value (NPV) was calculated by dividing the number of cases with true negative imaging results with the total number of cases with negative imaging results. Accuracy was calculated by dividing the sum of cases with true positive and true negative imaging results with the total number of cases. (Gerhardt & Keller 1986)

# 5 Results

In addition to the results presented here, including some results not presented in the original publications, more information on the results is also available in these publications.

## 5.1 Verified infections

In the total series, 14 cases with hip prostheses and 15 cases with knee prostheses were verified as infected. Bacterial growth was found in 13 cases with hip prostheses and in 8 cases with knee prostheses. The rest of the cases had purulent findings, and one case had clinical infection with a favourable antibiotic response. The most common bacteria were *Staphylococcus epidermidis* in 10 cases and *Staphylococcus aureus* in 5 cases. The other bacteria were *Staphylococcus lugdunensis*, *Staphylococcus simulans*, *Enterococcus faecalis*, *Enterobacter aerogenes* and *Enterobacter cloacae*.

## 5.2 Hip prostheses

The first study included 64 symptomatic hip prosthesis patients imaged with three-phase bone and $^{99m}$Tc-leukocyte imaging. In six of them (9.4%), the clinical and microbiological investigations confirmed the presence of an infection. Three-phase bone imaging was positive in all of the six infected cases, being pathological in all phases. In the non-infected patients, the metabolic phase of the bone scan was also positive in all except one patient. There were ten cases with false positive results in the arterial and soft-tissue phases of the three-phase bone scan among the non-infected patients, giving a relatively poor specificity and a poor positive predictive value for bone imaging in correctly classifying the presence of infection. The ages of the prostheses that yielded false positive findings on dynamic imaging at the time of the study varied between 2 months and 22 years.

The routine leukocyte images combined with bone images were positive in nine of the hip prostheses patients, but in only three cases with true infections, i.e. six observations proved to be false positive findings, thus giving a positive predictive value of 33%. On the other hand, late leukocyte images combined with bone images were positive in five out of six infected cases, and there were no false positive findings, yielding the highest positive predictive value and a very high negative predictive value. The patients with verified infections had focally increased leukocyte uptake. In six cases, where false positive results were obtained on conventional leukocyte imaging, leukocyte uptake was found to be markedly decreased in the 24-hour images compared to the early 2-hour to 4-hour post-injection images. Contrariwise, the late leukocyte images of the infected patients revealed increasing leukocyte uptake in two cases and no essential change in uptake in four cases. In none of the patients with an infected prosthesis was there a trend for the uptake to decline with time. There was only one non-infectious patient with increased uptake still visible around the prosthesis on the late leukocyte images, and this uptake was diffuse in nature, which was atypical for infection.

In the series of 30 hip prosthesis patients imaged with all the three methods (study IV), the presence of infection was confirmed in eight cases (27%). The arterial phase of the three-phase bone imaging achieved 100% sensitivity, 86% specificity, 73% positive predictive value, 100% negative predictive value and 90% accuracy. The figures for the soft-tissue phase were 100% sensitivity, 77% specificity, 61% positive predictive value, 100% negative predictive value and 83% accuracy. All the infected patients were positive in both the arterial and the soft-tissue phases of dynamic bone imaging. However, there were three false positive results in the arterial and five false positive results in the soft-tissue phase. Nevertheless, with this method, infection could be ruled out in 17 (77%) of the non-infected patients.

The early 2-hour to 4-hour $^{99m}$Tc-leukocyte images correctly identified five of the eight infected patients when analysed together with the bone-metabolic images, whereas the late 24-hour $^{99m}$Tc-leukocyte images with the bone-metabolic images correctly identified all the infected patients, and there were no false positive findings at either imaging time.

$^{99m}$Tc-ciprofloxacin imaging correctly identified all the patients with infected hip prostheses, being positive at all imaging times (1 h, 4 h and 24 h). Thirteen (59%) of the 22 non-infected patients had false positive scans at the 1-hour imaging time. However, such uptake faded away with time; seven findings were false positive at 4 hours, and only one patient remained false positive at 24 hours.

The efficacies of the different methods in the diagnostics of hip prosthesis infections are shown summarised in Table 4.

*Table 4. Efficacies of the different methods for identifying hip prosthesis infections. Results derived from the original studies I (n=64) and IV (n=30).*

| Study | I | I | IV | I | IV | I |
|---|---|---|---|---|---|---|
| Method | Bone imaging | Bone imaging | Cipro-floxacin | Leukocyte/ Bone | Cipro-floxacin | Leukocyte/ Bone |
| Phase/ Time | Arterial phase | Soft tissue phase | 4 h imaging | 4 h imaging | 24 h imaging | 24 h imaging |
| Sensitivity | 100% | 100% | 100% | 50% | 100% | 83% |
| Specificity | 82% | 82% | 68% | 90% | 95% | 100% |
| PPV | 38% | 38% | 53% | 33% | 89% | 100% |
| NPV | 100% | 100% | 100% | 95% | 100% | 98% |
| Accuracy | 84% | 84% | 77% | 86% | 97% | 98% |

## 5.3 Knee prostheses

In the series of 30 symptomatic total knee replacements imaged with three-phase bone and $^{99m}$Tc-leukocyte imaging (study II), eight patients (27% of the prostheses) underwent clinical and/or microbiological investigations that confirmed the presence of infection. Three-phase bone imaging was positive in all of the infected cases, being abnormal in all of the three phases. Also, the majority of non-infected prostheses had false positive findings in the arterial (n=17), soft-tissue (n=20) and bone-metabolic (n=21) phases, giving poor specificity and poor positive predictive values for bone imaging in correctly classifying the presence of infection.

The routine leukocyte images combined with bone images were positive in 12 patients, including seven infected and five non-infected cases. In the false positive cases, increased leukocyte uptake disappeared in one patient in the late images and persisted in four patients. All the infected cases showed focally increased leukocyte uptake in the late leukocyte images, giving the highest positive and negative predictive values for the late images to predict the presence of infection. Compared to the routine leukocyte images, the patients with infections showed increasing leukocyte uptake intensity in two cases, and the intensity remained essentially similar in six cases. None of the patients with infected prostheses had a decreasing trend of intensity.

The series of 16 suspected knee prosthesis infections imaged with $^{99m}$Tc-ciprofloxacin (study III) included seven patients (44%) with clinically or microbiologically confirmed infections, and the $^{99m}$Tc-ciprofloxacin imaging correctly identified six of them at all available time points. Unfortunately, the 24-hour images could not be taken in two of these patients, although the earlier 1-hour and 4-hour images were positive. One infected patient had a constant negative finding, but she had received 3 weeks' treatment with a first-generation cephalosporin antibiotic prior to the imaging. There were two false positive findings, and both were seen at all imaging times; one of them was associated with a newly implanted prosthesis (operated due to arthrosis one month before the imaging), while the other patient had rheumatoid arthritis with an active synovial process.

There was positive uptake in five of the nine non-infected prostheses in the 1-hour and 4-hour images, which diminished in the 24-hour images. The $^{99m}$Tc-ciprofloxacin imaging achieved 86% sensitivity, 78% specificity, 75% positive predictive value, 88% negative predictive value and 81% accuracy for correctly identifying knee prosthesis infections.

All the 16 patients in study III also underwent extended $^{99m}$Tc-leukocyte and three-phase bone imaging, but the results were not published, except, in the case of two patients, in a figure giving examples of positive and negative imaging results. The arterial and bone-metabolic phases of three-phase bone imaging were positive in all except one patient, and the blood pool phase was positive in all patients. Routine $^{99m}$Tc-leukocyte/bone imaging achieved 86% sensitivity, 78% specificity, 75% positive predictive value, 88% negative predictive value and 81% accuracy, i.e. results similar to $^{99m}$Tc-ciprofloxacin imaging. The results improved in late $^{99m}$Tc-leukocyte/bone imaging, which achieved 100% sensitivity, 78% specificity, 78% positive predictive value, 100% negative predictive value and 88% accuracy in this series.

The efficacies of the different methods in the diagnostics of knee prosthesis infections are shown summarised in Table 5.

*Table 5. Efficacies of the different methods for identifying knee prosthesis infections. Results derived from the original studies II (n=30) and III (n=16).*

| Study | II | II | III | II | III | II |
|---|---|---|---|---|---|---|
| Method | Bone imaging | Bone imaging | Cipro-floxacin | Leukocyte/ Bone | Cipro-floxacin | Leukocyte/ Bone |
| Phase/Time | Arterial phase | Soft tissue phase | 4 h imaging | 4 h imaging | 24 h imaging | 24 h imaging |
| Sensitivity | 100% | 100% | 86% | 87% | 80% | 100% |
| Specificity | 23% | 9% | 22% | 77% | 78% | 82% |
| PPV | 32% | 29% | 46% | 58% | 67% | 67% |
| NPV | 100% | 100% | 67% | 94% | 87% | 100% |
| Accuracy | 43% | 33% | 50% | 80% | 79% | 87% |

## 5.4 Total series

The efficacies of the different methods in the diagnostics of all the cases in the original studies are shown summarised in Table 6.

*Table 6. Total efficacies of $^{99m}$Tc-ciprofloxacin imaging (studies III and IV, n=46) and $^{99m}$Tc-leukocyte/bone-metabolic imaging (studies I and II, n=94) in hip and knee prosthesis infections.*

| Method | Ciprofloxacin imaging | Leukocyte/Bone Imaging |
|---|---|---|
| Sensitivity | 93% | 93% |
| Specificity | 90% | 95% |
| PPV | 82% | 76% |
| NPV | 97% | 99% |
| Accuracy | 91% | 95% |

## 5.5 Figures

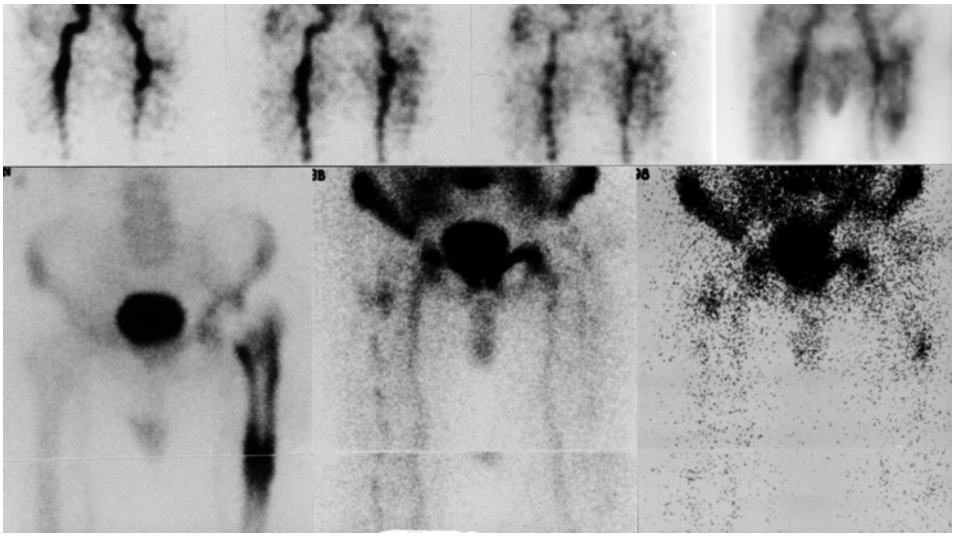

**Fig. 1. A 76-year-old male patient with an infected (9 months old) cemented prosthesis of the left hip. First row: arterial and soft-tissue phase images from a positive bone scan. The first three images demonstrate the arterial phase, while the fourth image demonstrates the soft-tissue phase. Second row: left, static image of the metabolic phase of the bone scan; centre, 4-hour leukocyte image; right 24-hour leukocyte image. The images demonstrate diffusely increased metabolic activity around the femoral shaft of the hip prosthesis and focal uptake of leukocytes at the prosthesis shaft, which becomes clearly visible over time. Bacterial cultures taken at revision operation 4 months after the imaging indicated the growth of *Staphylococcus aureus* and *Enterococcus faecalis*.**

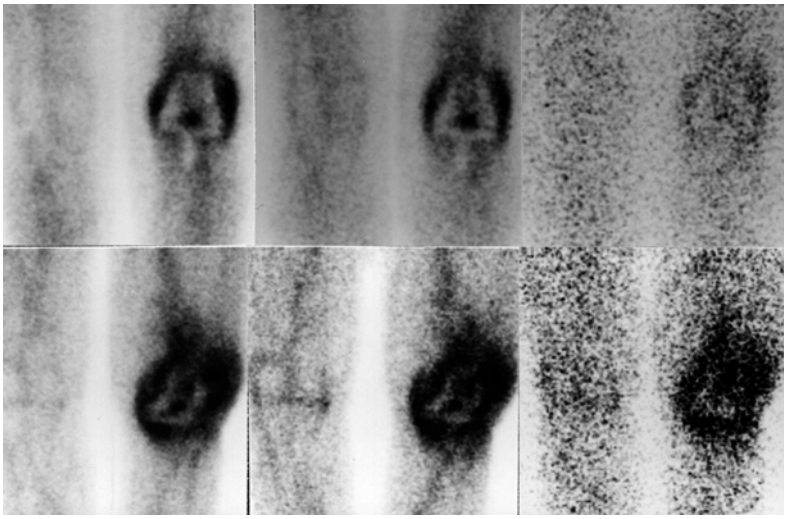

**Fig. 2. Upper row: a 71-year-old woman with post-traumatic arthrosis and a left knee prosthesis operation 1 year and 1 month before the imaging. She had had pain in her left knee after the operation, which continued until the imaging. $^{99m}$Tc-ciprofloxacin images (from left to right) at 1, 4 and 24 hours after injection demonstrate fading accumulation of the tracer. No infection could be verified, and the knee became symptomless during follow-up. Lower row: a 68-year-old man with arthrosis, a left knee revision prosthesis operation because of a worn prosthesis 10 months and patelloplasty operation 3.5 months before the imaging. He had suffered from pain and swelling of the left knee after the last operation. $^{99m}$Tc-ciprofloxacin images (from left to right) at 1, 4 and 24 hours after injection demonstrate increased uptake, which remains visible. The patient had verified *Staphylococcus epidermidis* prosthesis infection.**

# 6 Discussion

## 6.1 Patient series, incidence of infections and verification of final diagnoses

The patient series in these studies consisted of symptomatic patients with pain and a clinical suspicion of infection in their hip or knee prostheses. The number of suspected prosthesis infections in the studies varied between 16 and 64, being restricted by the resources available for this work during eight years. The suspicion of infection pertained to a single prosthesis in each patient, except in two patients with knee prostheses, who had a suspicion of bilateral prosthesis infections. The incidence of infections varied from 9.4% (6/64 patients) to 27% (8/30 prostheses, two studies) to 44% (7/16 patients), and the pre-test probability of infection was hence quite variable, which might have had an influence on the results. The difference in incidence was independent of the nuclear medicine unit and reflected patients referred by clinicians for the investigations in the different years, with higher incidences usually reported for $^{99m}$Tc-ciprofloxacin and knee prosthesis imaging. However, the interest in the new radiopharmaceutical might have affected the incidence.

Intra-operative confirmation of infection with bacterial cultures was used as the reference method in most infected cases. In the few prostheses considered infected based on purulent findings at surgery and/or repeated aspirations, the patients had had preceding antibiotic treatment and needed long antibiotic treatment for recovery. The non-operated cases were followed-up for at least one year after the imaging procedures, and all patients were carefully assessed for the clinical outcome by the senior consultant for infectious diseases and by the orthopaedic surgeon. The quality of the “golden standard” for confirming the final diagnoses can be considered adequate and even better than in many other studies in this field.

## 6.2 Three-phase bone imaging

The diagnostic value of bone scans in the detection of postoperative complications has been previously evaluated in many studies. In accordance with the earlier findings, the current studies showed a high sensitivity for three-phase bone scanning in the detection of infected joint replacements (Gelman *et al.* 1978, Weiss *et al.* 1979). Pathological findings were found in the arterial flow and soft-tissue phases in all the infected cases. Similarly, in harmony with the previous studies (Johnson *et al.* 1988, Palestro & Torres 1997), there was a high incidence of positive findings in the uninfected cases, indicating low specificity. In view of these and earlier findings, it appears that three-phase bone imaging alone has only limited value in diagnosing infections, but is useful in excluding infections in hip prostheses (Johnson *et al.* 1988, Palestro & Torres 1997, Williams *et al.* 1981). In study I, the majority of patients could be excluded as not having hip prosthesis infection based on 75% (48/64) negative findings in the arterial and soft-tissue phases of imaging. The relatively low frequency of false positive findings is valuable in light of the low incidence of infections, 9.4% (6/64), in this study.

In knee prostheses, dynamic images were almost always abnormal in both the arterial and the soft-tissue phases, although previous studies have shown normal results in many cases though usually in asymptomatic subjects (Rubello *et al.* 1995, Rubello *et al.* 1996). Anyway, this is in accordance with the previous findings indicating that three-phase bone scans are of limited value in guiding the clinical management of symptomatic patients with knee prostheses (Owen *et al.* 1995). There are many studies on radiophosphate uptake in knee prostheses, but most of them have failed to document the dynamic arterial and soft-tissue phases (Duus *et al.* 1987, Duus *et al.* 1990, Hofmann *et al.* 1990, Hunter *et al.* 1980, Kantor *et al.* 1990, Rozing *et al.* 1982, Ryd *et al.* 1993). In accordance with previous studies, the majority of the present patients had positive findings in the bone-metabolic imaging of knee prostheses.

## 6.3 $^{99m}$Tc-leukocyte imaging

The current data suggest that, in combined $^{99m}$Tc-leukocyte/bone-metabolic imaging, late $^{99m}$Tc-leukocyte imaging performed 24 hours post-injection improves both the sensitivity and the specificity of detection of hip and knee prosthesis infections compared to routine $^{99m}$Tc-leukocyte imaging performed at 2 to 4 hours post-injection. Although the number of verified infections in the hip prosthesis study (original publication I) was rather low (6/64, i.e. 9.4%), the specificity of the positive results was excellent (90% at the 2-hour to 4-hour and 100% at the 24-hour imaging), and the data indicate that late imaging with $^{99m}$Tc-leukocytes also improves sensitivity. The findings in study IV confirmed these findings by showing 100% accuracy in the late 24-hour $^{99m}$Tc-leukocyte/bone imaging.

In the knee prosthesis study (study II), infections were more frequent (8/30, i.e. 27%), but false positive findings, which often had logical explanations associated with trauma or inflammation, also occurred. This is in accordance with the earlier literature, in which fractures have been reported to cause false positive findings in $^{111}$In-labelled leukocyte

imaging (Van Nostrand *et al.* 1988). In addition, one patient showed signs of chronic infection in histological tissue samples taken at operation 10 days after the imaging, and no acute infection could be verified on clinical or microbiological grounds or on the basis of purulence. More sensitive methods, such as gene amplification techniques (Tunney *et al.* 1999), may be needed to confirm infection in such cases. When the time was extended to 24 hours, the number of unspecific findings diminished and sensitivity improved to 100% even in knee prostheses. The diagnostic accuracy of 24-hour $^{99m}$Tc-leukocyte/bone imaging was clearly superior to that of the conventional $^{99m}$Tc-leukocyte imaging protocol, and a clear improvement of the $^{99m}$Tc-leukocyte/bone imaging results by extending the time of leukocyte imaging was also obtained in the series of study III.

In the evaluation of the $^{99m}$Tc-leukocyte images, focal uptake sites and incongruence with bone-metabolic images proved to be notable signs of infection, although $^{111}$In-labelled leukocyte images and bone images have been reported to be incongruous in up to 15% of cases, even in the absence of infection in asymptomatic patients with porous-coated prostheses (Oswald *et al.* 1990). The diagnostic accuracy of late $^{99m}$Tc-leukocyte scans combined with bone-metabolic imaging (87%) in knee prostheses infections appears to be of the order previously reported by Palestro *et al.* (1991) with combined $^{111}$In-labelled leukocyte/bone imaging (75%) and combined $^{111}$In-labelled leukocyte/sulphur-colloid imaging (95%). The diagnostic accuracy of this procedure in hip prosthesis infections (98%) was equally good as that reported by Palestro *et al.* (1990) with combined $^{111}$In-labelled leukocyte/sulphur-colloid imaging (98%) and better than in most other studies with labelled leukocytes.

$^{99m}$Tc-leukocyte imaging is currently a widely used method for examining patients with suspected infections and available in most nuclear medicine facilities. $^{99m}$Tc is readily available, and compared with $^{111}$In, its radiation dose is lower and its gamma energies better suited for imaging (Corstens & van der Meer 1999, Flivik *et al.* 1993, Glithero *et al.* 1993, Rennen *et al.* 2001). $^{99m}$Tc-leukocyte imaging is usually carried out at 2 hours to 4 hours after the injection of cells, which is a suitable approach especially in soft-tissue infections (Corstens & van der Meer 1999). In suspected hip or knee prosthesis infections, 24-hour imaging seems highly recommendable.

## 6.4 $^{99m}$Tc-ciprofloxacin imaging

The present study focused on symptomatic patients with hip and knee prostheses. The incidence of infections was quite high, being 27% (8/30 patients) in the hip prostheses and 44% (7/16 patients) in the knee prostheses. $^{99m}$Tc-ciprofloxacin imaging gave positive results in 14 of the 15 infected hip and knee prostheses (93% sensitivity) at all the imaging time points. In one infected knee prosthesis patient, who had received 3 weeks of treatment with first-generation cephalosporin prior to the scanning, the images were false negative. As expected, there were many false positive findings in the early 1-hour and 4-hour images. In the case of knee prostheses, the number of false positive findings decreased from seven in the 1-hour and 4-hour images to two in the 24-hour

images. In the case of hip prostheses, the number decreased from 13 in the 1-hour to seven in the 4-hour to only one in the 24-hour images, giving a specificity of 95% in this indication. In the total hip and knee prostheses series, specificity was 90%.

Since the uptake of $^{99m}$Tc-ciprofloxacin depends on dividing bacteria, in situations where bactericidal antibiotic treatment of some weeks' duration has led to a significantly reduced number of living bacteria, the amount of $^{99m}$Tc-ciprofloxacin accumulating will also be reduced. Studies are currently under way to confirm whether $^{99m}$Tc-ciprofloxacin can be used through serial imaging to monitor the response to antimicrobial treatment. (Britton *et al.* 2002)

Several previous studies with $^{99m}$Tc-ciprofloxacin imaging have been reported (Britton *et al.* 1997, Hall *et al.* 1998, Vinjamuri *et al.* 1996) with promising results also in osteomyelitis, infected joint prostheses and axial skeleton infections (Britton *et al.* 2002, Sonmezoglu *et al.* 2001, Yapar *et al.* 2001). The largest study on $^{99m}$Tc-ciprofloxacin has been the worldwide multi-centre study on 879 patients, including 194 orthopaedic prostheses, sponsored by International Atomic Energy Agency. The study group reported that the most successful results were seen in osteomyelitis (sensitivity 90.5% and specificity 72.8%, n=228) and orthopaedic prosthesis infections (sensitivity 96% and specificity 91.6%, n=194). The additional value of 24-hour images was also apparent, as the false positive rate in bone and joint infections decreased from 5% to 0% in the UK arm of the study and from 4.1% to 2.5% in the Argentina arm of the study when 24-hour images were systematically acquired (Britton *et al.* 2002). The results of our studies confirm the need for 24-hour imaging, and the 93% sensitivity and 90% specificity are in line with this multi-centre study.

The imaging characteristics and dosimetry of $^{99m}$Tc-ciprofloxacin are favourable for clinical imaging (De Winter *et al.* 2001). Because only a tracer dose of ciprofloxacin (2 mg, which is only one 200$^{th}$ of a single intravenous therapeutic dose of ciprofloxacin) is used in the labelling process, the risk of side effects from $^{99m}$Tc-ciprofloxacin is very small, if any. This has been proved in all the studies referred to in this work, and there were no signs of side effects in this series, either. The risk of clinical resistance emerging is probably also very small when ciprofloxacin is used at very low concentrations for diagnostic purposes (Britton *et al.* 2002).

The present data indicate that $^{99m}$Tc-ciprofloxacin imaging is suitable for the diagnosis of hip and knee prosthesis infections when the imaging time is extended to 24 hours after the injection of the tracer. The accuracy of the results achieved in this way is comparable to the results described with labelled leukocyte imaging (Devillers *et al.* 1995, Flivik *et al.* 1993, Johnson *et al.* 1988, Magnuson *et al.* 1988, Palestro *et al.* 1991, Sonmezoglu *et al.* 2001, Vinjamuri *et al.* 1996). $^{99m}$Tc-ciprofloxacin imaging may thus be recommended for imaging hip and knee prosthesis infections.

## 6.5 Comparisons and recommendations

The data support the view that, in symptomatic patients with hip prostheses, three-phase bone imaging should be done first, and if the arterial and soft-tissue phases are negative, no leukocyte scan is needed. Positive results may be confirmed using $^{99m}$Tc-leukocyte

imaging with 24-hour images. Based on the present and previous observations, a practical approach to the diagnosis of hip prosthesis infections would include an initial screening of the patients with three-phase bone imaging, where a sensitivity of 100% and an accuracy of 80–90% can be achieved. Three-phase bone imaging is widely available and economical, and its radiation dose is low, especially when compared to $^{111}$In-labelled leukocyte imaging. With this approach, however, there would be a need for additional examinations in many patients. If the supplementary method were extended $^{99m}$Tc-leukocyte imaging, 98% accuracy would be achieved, whereas $^{99m}$Tc-ciprofloxacin imaging reached an accuracy of 97%. Use of $^{99m}$Tc-leukocytes may, however, be less convenient due to the time-consuming blood handling with the associated risks of infection. Lack of $^{99m}$Tc-ciprofloxacin uptake in normal bone marrow, which may sometimes be a confusing factor in the interpretation of leukocyte images, can also be considered an advantage related to this tracer. In addition, $^{99m}$Tc-ciprofloxacin imaging is independent of the number and function of white blood cells and thus advantageous in patients with neutropaenia.

In symptomatic knee prosthesis patients, the arterial and soft-tissue phases of the three-phase bone imaging were almost always abnormal, and this method hence does not seem suitable for screening purposes. It would seem adequate to begin with $^{99m}$Tc-leukocyte imaging with 24-hour images and to supplement this technique with comparative bone-metabolic and/or bone marrow imaging in positive cases. $^{99m}$Tc-ciprofloxacin may also prove to be useful, although our patient series was too small to allow definitive conclusions.

In the future, other specific infection/inflammation tracers may also become available for studying patients with a suspicion of infection in their orthopaedic prostheses.

# 7 Summary and conclusions

1. Three-phase bone imaging is useful in the diagnosis of hip prosthesis infections, as negative results in the dynamic arterial and soft-tissue phases exclude infection reliably in the majority of suspected cases. The bone-metabolic phase of imaging is also useful for comparison with leukocyte images in both hip and knee prostheses.
2. In addition to earlier images, 24-hour images are needed for reliable diagnosis of hip and knee prosthesis infections with $^{99m}$Tc-labelled leukocyte/bone imaging, and comparison of the 2- to 4-hour images with the 24-hour images is necessary.
3. In addition to earlier images, 24-hour images are needed for reliable diagnosis of hip and knee prosthesis infections with $^{99m}$Tc-ciprofloxacin imaging, and comparison of the earlier images with the 24-hour images is necessary. However, it does not seem necessary to obtain both 1-hour and 4-hour images, and in clinical practice, imaging between 3 and 4 hours post-injection would appear sufficient.
4. The results with $^{99m}$Tc-labelled leukocyte/bone imaging are slightly superior to $^{99m}$Tc-ciprofloxacin imaging in the diagnosis of hip and knee prosthesis infections. $^{99m}$Tc-labelling of leukocytes is time-consuming and includes a potential risk of disease transmission by misadministration, which could be avoided by the use of $^{99m}$Tc-ciprofloxacin. The $^{99m}$Tc-ciprofloxacin imaging procedure is less time-consuming than the combined $^{99m}$Tc-labelled leukocyte/bone imaging procedure.
5. A suitable method for everyday clinical practice in suspected hip prosthesis infections would include screening of the patients with three-phase bone imaging and confirmation of abnormal results in the arterial and soft-tissue phases with $^{99m}$Tc-leukocyte imaging, including 24-hour images. In suspected knee prosthesis infections, $^{99m}$Tc-labelled leukocyte imaging, including 24-hour images, could be done first, and no additional studies would be needed in negative cases. In positive cases, bone-metabolic imaging could be used for the confirmation of possible infections. Based on the literature, bone marrow imaging is also advantageous in comparison with leukocyte imaging. $^{99m}$Tc-ciprofloxacin imaging appears to be a promising method in suspected hip and knee prosthesis infections. Further research with larger patient populations is warranted to verify the validity of this technique.

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