LEUKOCYTE SCINTIGRAPHY, SPECT/CT AND PET/CT IN THE DIAGNOSIS OF PROSTHETIC JOINT INFECTION

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Leukocyte scintigraphy, SPECT/CT and PET/CT in the diagnosis of prosthetic joint infection

THESIS FOR DOCTORAL DEGREE (PhD)

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To Asa, the love of my life
Arthroplasty, or joint replacement surgery, is a surgical procedure where the original joint surface is replaced by artificial material, most often metal and polyethene. These new surfaces are anchored to the bone either by direct bone ingrowth, i.e. uncemented implants, or by cement, i.e. cemented implants. The main indication is to relieve pain and increase function secondary to conditions causing joint deformity, e.g. osteoarthritis, rheumatoid arthritis or fractures close to a joint. The overall long-time results are excellent. However, if the arthroplasty becomes infected, the consequences can be very troublesome. A prosthesis is a good place for bacteria to adhere to where the white blood cells (leukocytes) have difficulty reaching them. If the bacteria survive long enough, they can produce a biofilm protecting them from white blood cells and other parts of the innate immune system resulting in a chronic periprosthetic joint infection (PJI). In addition, the bacterial metabolism and growth cause bone loss surrounding the prosthesis and eventually loosening. The biofilm might also disperse bacteria into the bloodstream that can cause infections at other locations and even septic shock and death.

Detection and visualisation of chronic PJI:s can be challenging. Blood tests analysing inflammatory markers are an unspecific and direct sampling of joint fluid or joint-adjacent tissues for bacterial culture, and other tests are not always easy. Other non-invasive alternatives have therefore been developed, such as nuclear medicine imaging techniques. Leukocyte scintigraphy is an imaging technique in which blood is withdrawn from the patient, the white blood cells (leukocytes) are separated and labelled with a radioactive substance and then reinjected into the patient. The radioactivity or “uptake” can then be detected by a gamma camera device that produces two-dimensional pictures in black and white. Infections will attract many leukocytes leading to darker black spots in the picture. Some gamma cameras also can spin around the patient to gather a three-dimensional picture. This technique is called single-photon emission computed tomography or SPECT as the radioactive substances emit particles called photons, which the camera detects. The SPECT examination can be combined with computed tomography (CT) X-ray scan that depicts the body in three-dimensional image data to more accurately see where the uptake is located in the body.

Another imaging method is positron emission tomography (PET) that, like SPECT, presents three-dimensional image data and is often combined with a CT scan. This method is more technically complicated and expensive but has better resolution than SPECT.
Study I in this thesis demonstrate that leukocyte scintigraphy is challenging to use for the detection of chronic PJI in patients with ongoing antibiotic treatment. Study II compared different ways to perform leukocyte scintigraphy to detect chronic PJI but did not find any method to be superior. Study III showed increased uptake of radionucleotides visible up to two years after uneventful total hip arthroplasty, obstructing the use of PET/CT for detection of infections the first years following surgery. Study IV compared the diagnostic performance of leukocyte SPECT/CT and leukocyte PET/CT in patients with suspected chronic PJI. Both methods performed poorly, with no method being superior in sensitivity or specificity.
POPULÄRVETENSKAPLIG SAMMANFATTNING


En annan avbildningsmetod är positronemissionstomografi (PET) som, liksom SPECT, presenterar tredimensionella bilddata och ofta kombineras med en CT. Denna metod är mer tekniskt komplicerad och dyr men har bättre upplösning än SPECT.

Studie I i denna avhandling visar att leukocytscintigrafi har svårt att upptäcka kronisk PJTI hos patienter med pågående antibiotikabehandling.
Studie II jämförde olika sätt att utföra leukocytscintigrafi för att upptäcka kronisk PJI men fann ingen metod som var överlägsen.

Studie III visade ökat upptag av radionukleotider kring protesleden som var synliga upp till två år efter en okomplicerad total höftartroplastik, vilket försvårar användningen av leukocyt PET/CT för detektion av infektioner de första åren efter operationen.

Studie IV jämförde diagnostiska prestanda för leukocyt SPECT/CT och leukocyt PET/CT hos patienter med misstänkt kronisk PJI. Båda metoderna fungerade dåligt, ingen metod som var överlägsen den andra i sensitivitet eller specificitet.
ABSTRACT

Background: A known complication of arthroplasty is prosthetic joint infection (PJI). It can be challenging to diagnose and treat PJI due to the formation of a biofilm protecting the bacteria in chronic infections from the innate immune system and antibiotics. The reference standard of PJI imaging is dual time point 99mTc-HMPAO-WBC scintigraphy, where a gamma camera detects the activity of radiolabelled leukocytes which migrate towards infection locations.

Purpose: This thesis aims to evaluate the performance of different types of 99mTc-HMPAO-WBC imaging techniques and 18F-FDG-WBC PET/CT to detect infectious activity in patients with suspected chronic PJI.

Study I, a retrospective cohort study, evaluated the sensitivity and specificity of a combination of dual time point 99mTc-HMPAO-WBC scintigraphy, 99mTc-HMPAO-WBC SPECT/CT and 99mTc-Nanocolloid (bone marrow) scintigraphy in a clinical setting for treatment evaluation and detection of chronic PJI. It showed a worse performance of the method than is reported in the literature for PJI in general but is consistent with studies on chronic PJI.

Study II, a subset analysis of the cohort in study I, compared visual and semi-quantitative evaluation of dual time point 99mTc-HMPAO-WBC scintigraphy, visual evaluation of single time point 99mTc-HMPAO-WBC scintigraphy combined with 99mTc-Nanocolloid (bone marrow) scintigraphy and visual evaluation of single time point 99mTc-HMPAO-WBC SPECT/CT combined with 99mTc-Nanocolloid (bone marrow) SPECT/CT for treatment evaluation in patients with on-going antibiotic therapy. No method was shown superior.

Study III, a prospective study, evaluated hip prostheses after uneventful primary arthroplasty without any signs of infection with 18F-FDG-WBC PET/CT and found a remaining uptake surrounding the prosthesis stem for at least 24 months. It seemed to decline over time.

Study IV, a prospective study, compared single time point 99mTc-HMPAO-WBC SPECT/CT and 18F-FDG-WBC PET/CT, both combined with 99mTc-Nanocolloid SPECT/CT, in the diagnosis of PJI. Both methods had a poor performance, with no method being superior.

Conclusions: In chronic PJI and treatment evaluation, dual time point 99mTc-HMPAO-WBC scintigraphy is the preferred method out of the ones tested in these studies as no other method showed itself superior to all other methods result in an increased cost and radiation.
dose to the patient. Its performance is worse in chronic PJI than reported for PJI in general. 18F-FDG-WBC PET/CT does not seem to be a superior alternative.
LIST OF SCIENTIFIC PAPERS

This thesis is based on these four papers, referred to in the text by their roman numerals.

I. Is 99mTc-HMPAO-leukocyte imaging an accurate method in evaluating therapy results in prosthetic joint infection and diagnosing suspected chronic prosthetic joint infection?

II. Dual Tracer Approach Versus Dual Time Point Approach in Leukocyte Scintigraphy in Treatment Evaluation of Persistent Chronic Prosthetic Joint Infection
Nuclear Medicine Communications, 2021; 42 (7): 719-724 DOI: 10.1097/MNM.0000000000001403

III. Post-Surgical Uptake of 18F-FDG-Labelled Leukocytes After Uneventful Hip Arthroplasty
In manuscript

IV. 99mTc-HMPAO-WBC SPECT/CT versus 18F-FDG-WBC PET/CT in Chronic Prosthetic Joint Infection – a Pilot Study
Accepted for publication in Nuclear Medicine Communications
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<th>Description</th>
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<tbody>
<tr>
<td>BM</td>
<td>Bone Marrow</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DAIR</td>
<td>Debridation, Antibiotics and Implant Retention</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EANM</td>
<td>European Association of Nuclear Medicine</td>
</tr>
<tr>
<td>EBJIS</td>
<td>European Bone and Joint Infection Society</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte Sedimentation Rate</td>
</tr>
<tr>
<td>FDG</td>
<td>Fluorodeoxyglucose</td>
</tr>
<tr>
<td>HMPAO</td>
<td>Exametazime (Hexamethylpropyleneamine oxime)</td>
</tr>
<tr>
<td>HPF</td>
<td>High Powered Field</td>
</tr>
<tr>
<td>ICG</td>
<td>International Consensus Group</td>
</tr>
<tr>
<td>IDSA</td>
<td>Infectious Diseases Society of America</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>MDP</td>
<td>Methylene Diphosphonate</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MSIS</td>
<td>Musculoskeletal Infection Society</td>
</tr>
<tr>
<td>PMN%</td>
<td>Polymorphonuclear Neutrophil Percentage</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PJI</td>
<td>Prosthetic Joint Infection</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell, Erythrocyte</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell, Leukocyte</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

1.1 PROSTHETIC JOINT INFECTION

1.1.1 Definition and criteria for prosthetic joint infection

Prosthetic joint infection or PJI is caused by microorganisms invading a prosthetic joint and adhering to the surface of the prosthesis. PJI can often be difficult to diagnose, which is reflected in the different definitions suggested over the years. The five-year risk of developing a PJI after primary arthroplasty was estimated at 1.09 % for hip prostheses and 1.38 % for knee prostheses (1). The number of procedures performed worldwide is above two million with an increasing demand projected over the coming years (2, 3).

The European Bone and Joint Infection Society (EBJIS) presented the latest generally accepted definition in 2019 (4). This definition ranks infection as confirmed, likely or unlikely based on clinical, microbiological, histological, synovial fluid and nuclear medical criteria, as shown in Figure 1.

In Sweden, the criteria used since 2018 for confirmed infection are the presence of a fistula communicating with a prosthetic joint or presence of pus surrounding a prosthesis or acute inflammation consistent with infection in histopathological examination of periprosthetic tissue or an elevated number of leukocytes in synovial fluid or with a neutrophil dominance. Infection is considered likely when the same microorganism (as far as can be established by standard laboratory methods) grows in at least two perioperative tissue cultures or cultures from a combination of preoperative joint fluid aspirate and perioperative tissue. If only one culture is positive, an infection should be considered possible if the microorganism is highly virulent (e.g. Staphylococcus aureus or Escherichia coli). A single positive culture finding a low virulence microorganism (for example, coagulase-negative staphylococci and Cutibacterium acnes) is more likely to be contaminated but could be relevant, and other criteria for infection must be considered. (5)

PJI is divided into an early postoperative infection (less than four weeks after joint surgery), acute haematogenous infection (less than three weeks of symptoms after a previously asymptomatic arthroplasty – an infection spread to the joint through septicaemia at any time) and chronic infection (infection with more than three weeks of symptoms after a previous arthroplasty) (6). In addition, an older division used in many studies is early infection (diagnosed within three months of joint surgery), delayed infection (diagnosed
three to twenty-four months after joint surgery) and late infection (diagnosed more than twenty-four months after joint surgery) (7).

<table>
<thead>
<tr>
<th>Infection Unlikely (all findings negative)</th>
<th>Infection Likely (two positive findings)</th>
<th>Infection Confirmed (any positive finding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical and blood workup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical features</td>
<td>Clear alternative reason for implant dysfunction (e.g., fracture, implant breakage, malposition, tumour)</td>
<td>1) Radiological signs of loosening within the first five years after implantation 2) Previous wound healing problems 3) History of recent fever or bacteremia 4) Purulence around the prosthesis</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>&gt; 10 mg/l (1 mg/dl)²</td>
<td>Sinus tract with evidence of communication to the joint or visualization of the prosthesis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Synovial fluid cytological analysis²</th>
<th>Leukocyte count (cells/µl)</th>
<th>≤ 1,500</th>
<th>&gt; 1,500</th>
<th>&gt;3,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN (%)</td>
<td>≥ 65%</td>
<td>&gt; 65%</td>
<td>&gt; 80%</td>
<td></td>
</tr>
</tbody>
</table>

| Alpha-defensin³                  |                          | Positive immunoassay or lateral-flow assay⁴ |

| Microbiology⁴                   |                          |                                          |
| Aspiration fluid                | Positive culture         |                                          |
| Intraoperative (fluid and tissue) | All cultures negative   | Single positive culture⁵                |
| Sonication⁶ (CFU/ml)            | > 1 CFU/ml of any organism⁶ | ≥ two positive samples with the same microorganism |
| Histology⁷,²                    | No growth                | > 50 CFU/ml of any organism⁶            |

<table>
<thead>
<tr>
<th>High-power field (400x magnification)</th>
<th>Negative</th>
<th>Presence of ≥ five neutrophils in a single HPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of visible microorganisms</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Others                               |            |                                          |
| Nuclear imaging                      | Negative three-phase isozone bone scan⁷ | Positive WBC scintigraphy⁸ |

Summary Key

a. Infection is only likely if there is a positive clinical feature or raised serum C-reactive protein (CRP), together with another positive test (synovial fluid, microbiology, histology or nuclear imaging).

b. Except in adverse local tissue reaction (ALTR) and crystal arthropathy cases.

c. Should be interpreted with caution when other possible causes of inflammation are present: gout or other crystal arthropathy, infection, active inflammatory joint disease (e.g., rheumatoid arthritis), periprosthetic fracture, or the early postoperative period.

d. These values are valid for hips and knee periprosthetic joint infection (PJI). Parameters are only valid when clear fluid is obtained and no lavage has been performed. Volume for the analysis should be ≥ 250 µL, ideally 1 mL, collected in an EDTA containing tube and analyzed in <1h, preferably using automated techniques. For viscous samples, pre-treatment with hyaluronidase improves the accuracy of optical or automated techniques. In case of bloody samples, the adjusted synovial WBC = synovial WBC observed − (WBC <10⁴ RBC blood x RBC synovial ratio) should be used.

e. Not valid in cases of ALTR, haematomas, or acute inflammatory arthritis or gout.

f. If antibiotic treatment has been given (not simple prophylaxis), the results of microbiological analysis may be compromised. In these cases, molecular techniques may have a place. Results of culture may be obtained from preoperative synovial aspiration, preoperative synovial biopsies or (preferred) from intraoperative tissue samples.

g. Interpretation of single positive culture (or < 50 UFC/ml in sonication fluid) must be cautious and taken together with other evidence. If a preoperative aspiration identified the same microorganism, they should be considered as two positive confirmatory samples. Uncommon contaminants or virulent organisms (e.g., Staphylococcus aureus or Gram negative rods) are more likely to represent infection than common contaminants (such as coagulase-negative staphylococci, micrococcus, or Cutibacterium acnes).

h. If centrifugation is applied, then the suggested cut-off is 200 CFU/ml to confirm infection. If other variations to the protocol are used, the published cut-offs for each protocol must be applied.

i. Histological analysis may be from preoperative biopsy, intraoperative tissue samples with either paraffin, or frozen section preparation.

j. WBC scintigraphy is regarded as positive if the uptake is increased at the 20-hour scan, compared to the earlier scans (especially when combined with complementary bone marrow scan).

Figure 1. The EBJS criteria for PJI (4) Unmodified from The EBJS definition of periprosthetic joint infection - a practical guide for clinicians © 2021 by Martin McNally, Ricardo Sousa, Marjan Wouthuyzen-Bakker, Antonia F. Chen, Alex Soriano, H. Charles Vogely, Martin Claus, Carlos Higuera, Rihard Trebiš is licensed under Attribution-NonCommercial-NoDerivatives 4.0 International. To view a copy of this license, visit
There are similarities among the different types of infection. Both acute and haematogenous infections are more accessible to cure than chronic infections as the infecting microorganisms have not had time to establish a biofilm fully; see chapter 1.1.3 below. Both the acute and chronic infection can be established in the immediate perioperatively period through the wound before its closure. Therefore, prolonged wound secretion is a significant risk factor for PJI and is, in turn, affected by several factors, including post-operative haematoma (8-10). On the other hand, the acute haematogenous infection is spread, as the name states, haematogenously from another site of infection or through general septicaemia.

1.1.2 Microbiology

The microbiological spectrum of PJI is broad and includes both Gram-positive and Gram-negative bacteria and anaerobic bacteria, mycobacteria, and fungi. However, staphylococcus aureus and coagulase-negative staphylococci are the most common infecting microorganisms, present in 55% of all PJI (11). Both are common in the bacterial flora of the skin and can migrate into the wound after surgery.

1.1.3 Chronic infection and biofilm formation

Many bacteria and other infectious microorganisms possess the ability to create an extracellular matrix of polysaccharides, macromolecules and DNA, a so-called biofilm. This mainly occurs when the bacteria adhere to a surface such as a tooth's enamel, the plastic of a catheter, or a prosthetic implant's foreign material. The biofilm has many effects and functions: it connects bacteria, enabling them, among other things, to share nutrition and exchange DNA, increasing antibiotic resistance and virulence factors in the population. The bacteria in the biofilm can also lower their metabolism, express more surface pumps, and increase their mutation rate to resist antibiotics further. On top of this, the biofilm reduces the mobility of antibiotics and the cells and other components of the immune system, causing less exposure to the bacteria inside. (12)

The most well-known biofilms in the body are dental plaques that, over time, cause degradation of enamel called caries (13). In the same way, a PJI can form a biofilm on the surface of a hip or knee prosthesis, making the infection chronic. As the biofilm matures and expands, it will destroy the bone surrounding the prosthesis, causing loosening that leads to joint pain and loss of mobility. How long it takes for the biofilm to mature depends on both factors associated with the infecting microorganism and factors involving the host.
A general estimation is that a biofilm has been produced and matured between two to six weeks after the start of infection. (14, 15)

1.1.4 Clinical signs

The clinical signs of infection in a prosthetic joint can be discreet or completely absent, as evidenced by the fact that the diagnosis of PJI can be made several years after the moment the microorganisms enter the joint. In a fulminant infection, the common signs are redness and swelling of the joint. In early PJI, there may also be wound dehiscence, sometimes with suppuration. Pain is another common symptom but may be hard to distinguish from postoperative pain. (5)

1.1.5 Treatment

The definite treatment of chronic PJI is total revision of the prosthetic joint where the old prosthesis (and the biofilm on its surface) is removed. A new prosthesis can then be implanted, either in the same surgical session or in a later session, with a prosthesis-free period in between with or without an anti-biotic infused spacer. A less extensive treatment option is debridement of inflamed tissue, irrigation of the joint and exchange of removable parts combined with antibiotic therapy in a treatment strategy called DAIR. The strategy's rationale is that the debridement removes tissue that the biofilm might adhere to, the antibiotics kill the bacteria, and the irrigation causes mechanical distortion of the biofilm, lessening its protective effect. The debridement and irrigation can be repeated several times. DAIR is most successful in acute infections, either postoperative or haematogenous, and if started within four weeks after surgery or within three weeks after the debut of infectious symptoms. It also requires the prosthesis to be well fixed without signs of loosening. The antibiotic therapy is continued for at least six weeks, but a recent study suggests that twelve weeks of treatment have a higher chance of success (16). If a patient is unsuitable for any surgical intervention, life-long suppression treatment with antibiotics is an option if the infecting bacteria are sensitive to an oral antibiotic without severe side effects. (17-21)

1.2 DIAGNOSTIC OPTIONS

1.2.1 Serum biomarkers

The most common biomarkers for inflammation that can indicate a systemic response to infection is the white-blood-cell count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). These markers are very unspecific for PJI but can be used together with
other clinical signs. Interleukin-6 (IL-6) is another inflammatory marker detectable in serum that has been used in the diagnosis of PJI. A meta-analysis from 2010 (22), with varying cut-offs used in the included studies, showed pooled sensitivity levels of 0.45, 0.75, 0.88 and 0.97 for the white-blood-cell count, ESR, CRP and IL-6, respectively. The pooled levels of specificity of the biomarkers were 0.87, 0.70, 0.74 and 0.91, respectively. A later meta-analysis (23) focusing solely on chronic infection showed pooled sensitivity levels for the same biomarkers of 0.42, 0.82, 0.85 and 0.88, respectively. The pooled levels of specificity were 0.90, 0.79, 0.81 and 0.87. This meta-analysis also included serum procalcitonin (PCT) with a pooled sensitivity of 0.58 and a pooled specificity of 0.89.

1.2.2 Synovial fluid analysis

Several analyses can be made on synovial fluid from the infected joint regarding the number and types of cells and the levels of different enzymes and other biomarkers. However, prosthetic joints differ in the composition of their synovial fluid compared to normal joints, so the cut-off levels for determining PJI are not the same as in determining septic arthritis in a non-arthroplastic joint.

The general leukocyte count in synovial fluid is one of the more common analyses performed after a diagnostic joint puncture. A meta-analysis showed a sensitivity of 0.73 and a specificity of 0.96 for hip prostheses and a sensitivity of 0.9, and specificity of 0.91 for knee prostheses with cut-off values ranging from 2500 to 50000 leukocytes/mL (24). Another meta-analysis that did not discriminate between prosthesis locations reported an estimated sensitivity of 0.9 and specificity of 0.93 (23).

The composition of the leukocyte population is also an essential factor, with the percentage of polymorphonuclear neutrophils as the measured factor. A meta-analysis showed a sensitivity of 0.85 and a specificity of 0.83 for hip prostheses and a sensitivity of 0.9, and specificity of 0.88 for knee prostheses with cut-off values ranging from 60-89% (24). Another meta-analysis that did not discriminate between prosthesis locations reported an estimated sensitivity of 0.91 and specificity of 0.88 (23).

Neutrophil granulocytes release a peptide called alpha-defensin when activated as a part of the immune response to infection. This can be measured in the synovial fluid using either an enzyme-linked immunosorbent assay (ELISA) or a standalone analysis kit called a lateral flow test. The first method has an estimated sensitivity of between 0.92 and 0.97 and a specificity of between 0.97 and 0.99 in two meta-analyses (23, 25) and the second an estimated sensitivity of between 0.82 and 0.85 and an estimated specificity of 0.95 and 0.96 in three meta-analyses (23, 25, 26).
1.2.3 Microbiological culture

Many factors can have an impact on the accuracy of a microbiological culture. For example, the composition of the sample and how it is acquired, concurrent or recent treatment with antibiotics, the culture medium and the time in incubation can all affect the result (27). The most common method is to aspirate synovial fluid by a joint puncture for culture, which has been shown to have a sensitivity of 0.46 to 0.86 and a specificity of 0.88 to 1 (23, 27-31). During surgery and arthroscopy or using a core biopsy needle, it is possible to extract periprosthetic tissue for culture with a reported sensitivity of 0.41 to 0.7 and specificity of 0.96 to 1 (23, 29-32). If the prosthesis is explanted, then all matter attached to its surface can be removed by ultrasound in a process called sonicating. The sonicate can then be deposited into a blood culture medium for culture with a reported sensitivity between 0.63 and 1 and specificity of between 0.81 and 1 (31, 33-36). All microbial culture sampling, whether joint fluid aspiration or tissue biopsies, is routinely performed after an antibiotic-free interval of at least two weeks (37).

An alternative to culture is detecting bacterial DNA through polymerase chain reaction (PCR) that in two meta-analyses showed an estimated sensitivity of between 0.67 and 0.76 and a specificity of between 0.86 and 0.95 (23, 38).

1.2.4 Morphological imaging

All radiologic imaging can provide morphological information regarding the prosthesis, the joint and the surrounding bone and soft tissue. Signs like increased lucency of the bone surrounding the prosthesis, swelling of the joints and soft tissue oedema can raise suspicion of a PJI, but the diagnosis can seldom be made from structural imaging alone, especially in the early postoperative period. Imaging can be used to ascertain whether a suppurate opening in the skin is connected to a sinus tract that communicates with the joint, which is a definite sign of PJI. (15, 39)

1.2.5 Conventional X-ray imaging and Computed tomography

Conventional X-ray imaging is a cheap method with a low radiation dose that visualizes the bone surrounding the prosthesis. However, bone resorption must be significant to be visible and is not a specific sign of infection as it can also indicate aseptic loosening (39). The sensitivity of conventional X-ray imaging is reported to be between 0.14 and 0.89, and the specificity to be between 0.50 and 0.93 (40, 41). If computed tomography (CT) is used, a better view of soft tissue, in general, can be achieved, but the metal prosthesis will result in artefacts reducing the visibility in the periprosthetic region (42). This can, to some extent,
be lessened by image processing but remains a considerable drawback of the technique (43). The sensitivity and specificity of the method are reported to be 0.83 and 0.96, respectively (44), and a recent study illustrated several imaging findings with high correlation with PJI, although it did not report sensitivity and specificity (42).

1.2.6 Magnetic resonance imaging
Magnetic resonance imaging (MRI) is more sensitive to fluid, such as joint swelling or soft tissue oedema than CT. Like CT, it suffers from metal artefacts, but these manifest differently. In CT, the artefacts of light and dark streaks spread from the prosthesis like bright light (43) However, in MRI, the artefacts lack signal, resulting in a black void surrounding the prosthesis (45). Both increase the difficulty in evaluating the tissue closest to the prosthesis where the infection is situated. The sensitivity of different MRI findings for PJI is reported to be between 0.08 and 0.95 and the specificity between 0.46 and 0.98 (46-49).

1.2.7 Ultrasound
Ultrasound (US) cannot penetrate bone or the metal of the prosthesis but provides good information regarding soft tissue oedema and joint swelling. However, the method mainly guides needle biopsies or joint fluid aspiration to relevant spots, especially in hip prostheses where the joint can be hard to reach without image guidance. (50)

1.3 GENERAL NUCLEAR MEDICINE THEORY

1.3.1 Radiopharmaceuticals and tracers
Nuclear medicine is the field of medicine where radiopharmaceuticals are used for functional imaging or radionuclide therapy. A radiopharmaceutical consists of two parts. The first part is the radioactive isotope that emits radiation that can be detected to produce an image or that affects the pathological tissue being treated. The second part is the molecule that brings the isotope to the target: a particular type of tissue or a metabolic process. This can, for example, be a molecule used in the metabolism of the target organ-like iodine for the thyroid gland, an antibody that adheres to particular cells or a drug whose distribution needs to be tracked. In some cases, like 131-Iodine, the radiopharmaceutical consists of only one part that is both radioactive and functionally active at the same time (51).
This thesis will not cover interventional or therapeutic nuclear medicine as the focus is on diagnostic imaging. Nuclear medicine imaging differs from radiography, which also
produces images through radiation in many ways. X-ray images are generated by an 
external radiation source, while the radiation source in nuclear medicine is internal. The 
radiopharmaceutical is often injected into the patient, but it can, for example, also be 
inhaled or ingested. Radiography produces morphological images of the anatomy, while 
nuclear medicine produces functional images of the distribution of the molecule in 
question. (52)

1.3.2 Scintigraphy

The oldest type of nuclear medicine imaging technology is planar scintigraphy with a 
gamma camera. The radiopharmaceutical emits gamma radiation that consists of photons. 
The photon enters the crystal part of the detector that reacts by emitting light. The process 
is called scintillation and gives the technology its name. The emitted light photon is 
multiplied in a photo multiplier tube, and a computer counts the number of photons 
exiting the tube. The activity detected this way is often measured in “counts”. Each count 
is then represented as a dot in a two-dimensional image where an increasing number of 
counts in the exact location led to an increasingly more intense dot. Activity in a specific 
location is referred to as uptake in that location. Images are usually acquired in at least two 
projections, anterior and posterior, to adjust for the attenuation of the radiation within the 
body. Other projections, such as oblique images, can be acquired if necessary. (53)

1.3.3 Single-photon emission computed tomography

Single-photon emission computed tomography or SPECT is another image acquisition 
technique using the same gamma camera. Although planar scintigraphy acquires two-
dimensional images from single projections, SPECT computes three-dimensional images 
from multiple projections where the detector rotates around the patient. This enables better 
localization of the uptake and can increase the chances of detecting minor uptakes that 
might be lost in the background activity of a planar image. The spatial resolution of SPECT 
images with a standard low-energy high-resolution collimator is 7.4 mm for imaging with 
99mTechnetium (99mTc), the most common SPECT radionuclide (54).

1.3.4 Positron emission tomography

Positron emission tomography or PET produces three-dimensional images like SPECT but 
by an entirely different mode of action. The SPECT radiopharmaceutical emits a single 
photon that is directly acquired in the detector. The PET radiopharmaceutical, commonly 
called a tracer, emits a positively charged positron that interacts with a negatively charged
electron in the patient. The interaction produces two high-energy photons called annihilation photons. The annihilation photons travel in opposite directions and are detected by a ring of detectors around the patient. When a photon is detected by a detector on one side of the patient within nanoseconds of another photon detected on the opposite side of the patient, they are considered a photon pair indicating that the annihilation event took part on a straight line between the two detectors. The three-dimensional images are then computed from these lines, like how the SPECT image is computed from counts. The positron may travel up to a millimetre in the patient's tissue before interacting with an electron, making the uptake's location slightly more imprecise. PET can achieve a spatial resolution of 4 mm for clinical studies (55). One of the significant limits is the size of the detector ring as the localization error increases with the distance the annihilation photons have to travel. (53)

1.3.5 Hybrid imaging

Both SPECT and PET can be combined with another imaging modality, most commonly computed tomography or CT. This enables more accurate anatomical localization of the uptake by displaying it as an overlay of the morphological CT images. The CT images are computed from the attenuation of radiation from an external source in the patient's tissue. This can be used to correct the detected activity in a SPECT or PET scan by the attenuation map generated from the CT to increase the accuracy of the nuclear medicine images. An uncorrected PET scan, for example, would show significant activity in the skin as the photons generated there travel through less tissue than the ones generated in the centre of the body and therefore has a lower risk of being diverted. With the attenuation of all tissue considered, the skin uptake displayed is lowered accordingly. (56, 57)
2 LITERATURE REVIEW OF NUCLEAR MEDICINE
IMAGING OF PROSTHETIC JOINT INFECTIONS

2.1 BONE SCINTIGRAPHY

Bone scintigraphy is performed with 99mTc-methylene diphosphonate (MDP), a radiopharmaceutical that indicates osteoblast activity, e.g., bone growth. The examination is primarily performed to find sites of bone metastases, but it can also show sites of infection. The biggest drawback of bone scintigraphy for PJI is that postoperative bone remodelling will result in a positive examination for years after the primary arthroplasty (58, 59).

Therefore, the method is primarily used as a rule-out test with a negative scan making PJI unlikely (4).

The injected activity of 99mTc-MDP bone scintigraphy is between 550 and 777 MBq resulting in an effective dose of 2.7 to 3.8 mSv (59-64). An overview of the reported sensitivity and specificity of 99mTc-phosphonate bone scintigraphy is presented in Figure 2 (58-63, 65-71). A meta-analysis including some of the studies referenced above estimated the sensitivity to be 0.83 (95% CI 0.72-0.90) and the specificity to be 0.73 (95% CI 0.65-0.80) (72).
2.2 LEUKOCYTE SCINTIGRAPHY

Leukocyte scintigraphy is today considered the reference standard method of functional PJI imaging, although there has been some controversy regarding this status in later years (73-75). Guidelines for the labelling of the leukocytes were published in 2010 (76, 77) and a guideline for image acquisition and evaluation were published in 2019 (78). A positive scan is, together with clinical features of infection or elevated serum CRP, considered a sign of likely infection (4).

A sample of the patient’s blood is extracted, and the leukocytes are separated by centrifugation. The most critical leukocytes in the setting of PJI are the granulocytes, but lymphocytes are also present in the wound in early PJI. Therefore, all leukocytes are labelled, or another round of centrifugation can separate only the granulocytes for the labelling. The granulocytes or the mixed leukocytes are then incubated in a solution containing the radioligand with known activity. After incubation, the leukocytes are extracted from the radioligand solution, and their activity is measured. The fraction of the original activity incorporated in the radiolabelled leukocytes is referred to as labelling efficacy. According to the European Association of Nuclear Medicine (EANM) guidelines, if labelling efficacy consistently is lower than 40%, the labelling procedure should be evaluated more closely to avoid systematic errors (76, 77).

There are three possible time-points for leukocyte scintigraphy, of which the two latter are obligatory. The optional early time-point is around 30 minutes after the radiolabelled leukocytes have been injected into the patient. This represents the blood pool, and bone marrow distribution before the inflammation surrounding the infection attracts the leukocytes to the joint (79). The delayed time-point is 2-4 hours after injection and allows the leukocytes to migrate to the infected joint by chemotaxis. The late time-point is 18-30 hours after injection and provides additional time for the leukocytes to migrate to the infected joint and periprosthetic tissue.

The diagnosis is made by observing increased uptake in the joint between the delayed and the late images or, if semi-quantitative analysis is used, by noting an increased target to background ratio between the two time points. The target, in this case, is the joint with suspected PJI, and the background can be the contralateral joint or bone marrow, commonly in the femur or the iliac crest.

There are three options when deciding the length of acquisition time at each time point: fixed number of counts, fixed time, and decay time-corrected acquisition. A fixed number of counts means stopping the acquisition after the same predetermined number of counts have been acquired at each time point. Fixed time means stopping the acquisition after the
same predetermined time has passed at each time point. Finally, decay time-corrected acquisition means increasing the acquisition time at later time points to take the isotope’s radioactive decay into account and provide equal contrast in the images. Therefore, decay time-corrected acquisition is considered the superior method in later studies (79, 80).

The radiolabelling of leukocytes in vitro is time-consuming and thereby expensive and requires contact with blood products. It also carries the risk of a patient being injected with another patient’s leukocytes (81). There were also some concerns that if a mixed leukocyte population was labelled that the lymphocytes could suffer DNA damage from the radiation (82). However, the risk of this damage leading to the development of lymphoma is regarded as virtually non-existent as the damaged cells would be removed through apoptosis or phagocytosis (83).

### 2.2.1 111Indium leukocyte scintigraphy

Several radioligands have been used for the labelling of leukocytes (84, 85). Among the most suited for imaging of PJI is 111-Indium (111In), often connected to oxine (also called oxyquinoline) that has been used since the 1970s (84, 86, 87). 111In-oxine can enter through the cell membrane through passive diffusion, whereafter the 111In disassociates and attaches itself to transferrin or other cytoplasmic components (76). The isotope emits radiation at energy levels of around 173 keV and 240 keV and is relatively stable with a half-life of 67.9 hours (64). Indium requires a medium energy collimator. The regular injected activity is 14.8 to 36 MBq, which results in an effective dose of 7.2 to 17.5 mSv (81, 86-91). The majority of the radiation is deposited in the spleen. There is negligible excretion through the liver or kidneys of the radioligand itself, so the clearance from the body is mainly through the radioactive 111-Indium decaying to stable 111-Cadmium.

An overview of the reported sensitivity and specificity of 111Indium-oxine-WBC-scintigraphy for PJI from some studies is presented in Figure 3 (86, 87, 89, 90, 92).
2.2.2 99mTechnetium leukocyte scintigraphy

99m-Tc--WBC

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Hip 99m-Tc--WBC

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<th>TN</th>
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Knee 99m-Tc--WBC

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<th>FN</th>
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Figure 4. Sensitivity and specificity reported in a selection of studies on 99mTc-HMPAO-WBC scintigraphy

99m-Technetium (99mTc) can be used for labelling leukocytes if connected to exametazime (hexamethylpropylene amine oxime, HMPAO) and for examination of ventilation and perfusion in the lung, excretion of bile and many other studies. As 111In-oxine, 99mTc-HMPAO diffuses passively through the cell membrane and dissociates, trapping the radioisotope intracellularly (85). It emits radiation at around 140 keV and has a half-life of 6 hours (64). 99mTc can be used with a low energy high-resolution collimator that increases spatial resolution compared to higher energy radionuclides like 111In. The binding between the radioligand and the leukocytes is weaker than that between 111In and oxine, resulting in some of the injected activity being secreted through the kidneys and liver. The regular injected activity is 185 to 740 MBq, which results in an effective dose of 2 to 8.1 mSv (64, 81, 91, 93-95).

An overview of the reported sensitivity and specificity of 99mTc-HMPAO-WBC-scintigraphy is presented in Figure 4 (58, 65, 70, 71, 79, 80, 92, 96-98). Note that Lauri et al. (71) only included doubtful cases evaluated by semi-quantitative analysis and that Blanc et al. (70) and Glithero et al. (92) only included suspected chronic infection.
2.2.3 Addition of SPECT and SPECT/CT to leukocyte scintigraphy

Using SPECT technique instead of planar imaging will add the ability to localize the uptake more precisely and help differentiate between leukocytes uptake in a PJI and soft tissue infection. This is important as the treatment regime might differ between the two diagnoses. The addition of concomitant CT to the SPECT examination results in even more improved localization. The usage of SPECT acquisition does not increase radiation dose but increases acquisition time. The addition of a concomitant CT scan, of course, increases the radiation dose by that of the CT. The amount of added radiation differs depending on whether the CT is a full-dose CT used for diagnosis or a low-dose CT used only for attenuation correction and rough localization.

An overview of the reported sensitivity and specificity of $^{99m}$Tc-HMPAO-WBC-SPECT and $^{99m}$Tc-HMPAO-WBC-SPECT/CT is presented in Figure 5 (94, 97). Adding a concomitant CT scan to SPECT leads to higher specificity (97).

2.2.4 Addition of bone marrow scintigraphy to leukocyte scintigraphy

The trauma of arthroplastic surgery results in a reaction of the bone marrow surrounding the prosthesis that can simulate the infection uptake. It is possible to use bone marrow (BM) scintigraphy to define the bone marrow and then compare it with the leukocyte images. A matching uptake on both images suggests that there is only reactive bone marrow and no infection. However, if there is an uptake on the leukocyte images not present on the bone marrow images, the examination is positive for infection. BM scintigraphy can be performed by $^{99m}$Tc-sulfur colloids or $^{99m}$Tc-nanocolloids (99). The injected activity for the bone marrow scintigraphy is 370 to 740 MBq resulting in an effective dose of $3.4 - 6.7$ mSv (64, 88, 99, 100). The dose of the leukocyte scintigraphy is added to this.
An overview of the reported sensitivity and specificity of 111In-oxine-WBC scintigraphy combined with BM scintigraphy is presented in Figure 6 (88, 89, 101-103).

2.2.5 Anti-granulocyte antibody and antibody fragment scintigraphy

As an alternative to labelling leukocytes, anti-granulocyte antibodies and antibody fragments marked with 99mTc were produced. These were administered directly to the patient and then distributed themselves through the bloodstream and interstitial space of the patient, adhering to granulocytes when they interacted with them. Some claim that the smaller antibodies would more easily permeate the inflamed tissue surrounding a chronically infected joint due to their smaller size, but no conclusive proof of this leading to increased diagnostic performance has been shown (104-107).

A meta-analysis (108) including both studies using antibodies and studies using antibody fragments showed a pooled sensitivity of 0.83 and a pooled specificity of 0.79.

The injected activity of the antibodies ranges between 600 and 877 MBq resulting in a radiation dose of 5.2 to 7.6 (63, 100, 109, 110). In addition, the injected activity of the antibody fragments ranges between 444 and 1130 MBq resulting in a radiation dose of 5 mSv to 11 mSv (107, 111, 112).

2.3 18F-FDG-WBC-PET/CT

To use the higher spatial resolution of the PET/CT, leukocytes can also be labelled with 18-Fluoride-fluorodeoxygenase (18F-FDG), which is the most common PET tracer (113). 18F-FDG cannot pass the cell membrane passively but are transported by glucose transporter proteins. Intracellularly the 18F-FDG is phosphorylated, which traps it in the cell.

However, 18F-FDG can leave the cell before it is phosphorylated, making the labelling less stable than 111In-oxine or 99mTc-HMPAO (85). Only two previously published studies
have used this technique to diagnose PJI (103, 114). The injected activity was 285 – 925 MBq resulting in an absorbed dose of 4.4 – 14.2 mGy (103, 114, 115). An overview of the reported sensitivity and specificity of 18F-FDG-WBC-PET/CT is presented in Figure 8 (103, 114).

2.4 18F-FDG-PET/CT

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<td>0.87 [0.74, 0.94]</td>
<td>0.91 [0.86, 0.95]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chatzis 2002</td>
<td>113</td>
<td>1</td>
<td>1</td>
<td>28</td>
<td>0.99 [0.95, 1.00]</td>
<td>0.97 [0.82, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysikos 2008</td>
<td>28</td>
<td>5</td>
<td>87</td>
<td>1</td>
<td>0.85 [0.68, 0.95]</td>
<td>0.95 [0.85, 0.97]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kumar 2016</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>24</td>
<td>0.94 [0.70, 1.00]</td>
<td>0.92 [0.75, 0.99]</td>
<td></td>
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</tr>
<tr>
<td>Leve 2004</td>
<td>13</td>
<td>17</td>
<td>11</td>
<td>15</td>
<td>0.54 [0.33, 0.74]</td>
<td>0.47 [0.29, 0.65]</td>
<td></td>
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</tr>
<tr>
<td>Murne 2005</td>
<td>42</td>
<td>2</td>
<td>4</td>
<td>22</td>
<td>0.91 [0.79, 0.98]</td>
<td>0.92 [0.73, 0.99]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pill 2006</td>
<td>20</td>
<td>5</td>
<td>1</td>
<td>66</td>
<td>0.95 [0.76, 1.00]</td>
<td>0.95 [0.84, 0.98]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reinartz 2005</td>
<td>31</td>
<td>3</td>
<td>2</td>
<td>56</td>
<td>0.94 [0.80, 0.99]</td>
<td>0.95 [0.86, 0.99]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rein 2006</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>1.00 [0.29, 1.00]</td>
<td>1.00 [0.77, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stumpfe 2004</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>21</td>
<td>0.33 [0.07, 0.70]</td>
<td>0.81 [0.61, 0.93]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Acker 2001</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>11</td>
<td>1.00 [0.54, 1.00]</td>
<td>0.73 [0.45, 0.92]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 9. Sensitivity and specificity reported in a selection of studies on 18F-FDG PET/CT

A regular 18F-FDG-PET/CT is commonly used to show areas of high metabolism indicating cancer cells or inflammation. This can also diagnose infection and is widely used in spinal and vascular graft infections. In addition, it can be used in the diagnosis of PJI but is less specific as, for example, the postoperative healing after surgery might result in an increased uptake surrounding the prosthesis. (116)

The injected activity of 18F-FDG is between 150 – 400 MBq resulting in an effective dose of 2.9 – 7.6 mSv (64, 94, 95, 99, 101, 117-119). If a concomitant CT scan is used, it adds to the dose. An overview of the reported sensitivity and specificity of 18F-FDG-WBC-PET/CT is presented in Figure 9 (61, 62, 66, 88, 94, 99, 101, 118, 119). A meta-analysis that includes some of the studies reported here shows a pooled sensitivity of 0.85 (95% confidence interval: 0.71-0.93) and a pooled specificity of 0.84 (95% CI: 0.68-0.93) (120).

2.5 REFLECTIONS ON THE LITERATURE

In the setting of PJI diagnosis, there are many occasions where a clinical examination can be limited in establishing the presence of PJI. To exemplify this, I present three scenarios:

The first occurs early in the immediate post-surgical period when it can be hard to distinguish an acute PJI from post-operative pain. The second occurs late when diffuse joint pain slowly increases and can be explained by a low-virulent chronic PJI or aseptic loosening. Finally, the third scenario can occur at any time in patients with a chronic inflammatory joint disease where both the joint disease and a PJI can cause joint pain and elevated inflammatory serum markers. If the suspicion of PJI is high, then a rule-in test
with high specificity should be chosen to prove it. If the suspicion is low, then a rule-out test with high sensitivity should be chosen instead.

The diagnostic tests performed on synovial fluid have very good to excellent specificity, even if the sensitivity varies (23). This makes them suitable as rule-in tests where, if the symptoms in general support chronic PJI, one can proceed to revision arthroplasty after a positive test.

Leukocyte scintigraphy is also used as a rule-in test in the latest guidelines (4) to determine if PJI is likely. However, if the examination is positive, infection still must be verified and a microbiological diagnosis is needed to choose the proper antibiotic treatment meaning that a joint puncture or synovial biopsy will be performed. Thus, if used, the function of leukocyte scintigraphy would be selecting the patients with the highest need for joint puncture and sparing other patients the risk of infection through an unnecessary puncture.

An example of a rule-out test is bone scintigraphy (99mTc-MDP scintigraphy), which is relatively cheap, widely available, and has excellent sensitivity (4). The risk of infection after a negative scan is very low, and other causes must be sought to explain the patient’s symptoms.

In most previously published studies, no data is presented regarding whether the PJI in question is acute or chronic (60, 65, 67, 79, 80, 89, 91, 93, 97, 98, 114, 121). The results for both acute and chronic infections are pooled together without distinction. Noteworthy, the studies with a pure chronic PJI cohort (70, 92) are also the studies where the reported results are worse than generally expected, especially regarding sensitivity. The proportion of patients in the cohort that suffer from inflammatory joint disease is seldom reported.

It can be argued that a study population is likely to be mainly chronic based on the time passed from the primary operation (66) but this does not consider that PJI can originate from any trauma to the joint that pierces the skin. This includes, among other things, revision surgery, arthroscopy or joint puncture for aspiration or injection. It also does not take into account the possibility of acute haematogenously spread infection.

The majority of the diagnostic methods for PJI require at least the puncture of a vein for a blood test or the injection of a radiopharmaceutical, and some require the puncture of the affected joint itself, in some cases even arthroscopy or surgery. Apart from the risk of infection through the puncture site or surgical wound, one must also consider the radiation dose to the patient of computed tomography or nuclear medicine examinations. The effect of radiation is negligible if the patient is elderly and extremely unlikely to develop a malignancy as a result thereof. However, as the numbers of arthroplasties performed in
middle-aged patients are rising (2), the radiation dose to the patient cannot be disregarded entirely.

The only two diagnostic methods that are without any risk to the patient are ultrasound and MRI, where the latter cannot be used if the patient has a non-MRI-safe pacemaker or similar implanted device. Conventional X-rays also have a radiation dose low enough to be disregarded entirely. Out of these three methods, only MRI has shown the potential to be accurate enough to be reliable in routine clinical use. However, it would require further research evaluating MRI in chronic PJI and comparing it directly with another imaging method, primarily the reference standard of 99mTc-HMPAO-WBC scintigraphy. Leukocyte scintigraphy with 99mTc-labeled leukocyte is a well-studied imaging method and today the reference standard for functional imaging (78). However, as referenced above, there is some variance in the study results. This accentuates the need for standardized labelling, examination and evaluation using decay time-corrected acquisitions, images displayed in absolute counts and high labelling efficacy. In addition, the method is time-and labour-consuming due to the labelling process and repeat examinations. Due to the routine use of 18F-FDG-PET/CT examinations in oncological imaging, the method is becoming increasingly available. It shows some promise in PJI diagnosis but has difficulty discerning between infectious uptake and non-septic loosening of the prosthesis. In addition, as the evaluation is based on criteria that vary between studies, it would also be valuable to standardize which criteria are to be used (116).
3 RESEARCH AIMS

The overall purpose of this thesis was to evaluate leukocyte scintigraphy and SPECT/CT in the setting of chronic prosthetic joint infection of the hip or knee and evaluate whether leukocyte PET/CT could be an alternative method to use.

The specific objectives of each study were:

Study I To evaluate a combination of dual-timepoint 99mTc-HMPAO leukocyte scintigraphy, 99mTc-nanocolloid bone marrow scintigraphy and 99mTc-HMPAO leukocyte SPECT/CT in the setting of chronic prosthetic joint infection.

Study II To compare single time-point 99mTc-HMPAO leukocyte scintigraphy combined with 99mTc-nanocolloid bone marrow scintigraphy, 99mTc-HMPAO leukocyte SPECT/CT combined with 99mTc-nanocolloid bone marrow SPECT/CT to the reference standard method of dual time-point 99mTc-HMPAO leukocyte scintigraphy.

Study III To visualize the uptake of 18F-FDG-WBC PET/CT in patients after prosthetic joint surgery without any suspicion of infection.

Study IV To compare 18F-FDG leukocyte PET/CT and 99mTc-HMPAO leukocyte SPECT/CT in the setting of chronic hip or knee prosthetic joint infection.
4 MATERIALS AND METHODS

4.1 ETHICAL CONSIDERATIONS

All studies were approved by the Regional Ethical Review Board in Stockholm (government agency, now a part of the Swedish Ethical Review Authority) with registration number 2012/2093-1/3 and revisions 2015/525-32 and 2017/1554-32 and were approved by the radiation protection committee of Karolinska University Hospital Huddinge. The prospective studies III and IV were also registered in the European Union Drug Regulating Authorities Clinical Trials Database (EUDRA-CT) with registration number 2013-001607-36 and approved by the Swedish Medical Products Agency. Participants and Clinical Evaluation

The inclusion criteria for studies I and II were a verified infection before the examination and a possibility to verify infection or cure after the examination. To verify infection a positive culture from aspirated joint fluid, two or more positive perioperative cultures (with the same organism) or a fistula to the joint were needed. To verify cure negative perioperative cultures or at least two years of symptom-free follow up after the cessation of antibiotic therapy were needed. All patients examined with 99mTc-HMPAO leukocyte scintigraphy between June 2010 and December 2013 were eligible. Study II excluded all patients without ongoing antibiotic treatment, with labelling efficacy below 40% and injected activity below 200 MBq.

The inclusion criteria for study III were complication-free implanted prosthesis in the hip or knee, age ≥ 65 years, ability to give informed consent and availability to be imaged at the study time points. The exclusion criteria were any suspicion of prosthetic joint infection, age < 65 years, haematological disease, leukocyte count below 2 x 10⁹/L, diabetes mellitus, any previously reported anaphylactic reaction or ongoing treatment with steroids, chemotherapy, or antibiotics.

The inclusion criteria for study IV were suspicion of delayed or late PJI, clinical signs of infection, elevated CRP or ESR and age > 45 years. The exclusion criteria were less than three months since primary operation or revision surgery, ongoing treatment with chemotherapy or steroids, intravenous antibiotic treatment longer than five days, haematological disease, diabetes mellitus with plasma glucose above 13 mmol/L, pregnancy, previous anaphylactic reaction.
4.2 LEUKOCYTE LABELLING

4.2.1 99mTc-HMPAO
The radiolabelled leukocytes were prepared according to the EANM Guidelines (24), but per the recommendation in the guidelines, the optional step of isolating the granulocytes was not used.

4.2.2 18F-FDG
Two 60 ml syringes per patient were filled with 0.7 ml (350 IU) sodium chloride diluted Heparin (Heparin LEO, 5000 IU/ml diluted by Natriumklorid Braun 9 mg/ml to 500 IU/ml). 35 ml of whole blood was collected from the patient and deposited into each syringe. In each syringe, 5 ml Leukokit HES 6% was added as a plasma expander after the blood. Leukocyte-containing plasma (after sedimentation) was aspirated from the syringes. If any syringe contained less than 17 ml of plasma, the plasma from both syringes was deposited in a single sterile 50 ml tube. If both syringes contained sufficient plasma, then it was deposited in two tubes.

The tube/s were then centrifuged for 5 minutes at 150 g. After centrifugation, the plasma was aspirated and discarded, leaving a leukocyte pellet at the bottom of the tube/s. After dilution with 1 ml saline with phosphate buffer (Leukokit PBS) and, if previously in two tubes, collection into a single sterile tube, the leukocytes were mixed with ≤1 ml fluid containing 1000 MBq FDG. Labelling proceeded for 30 minutes at 37 degrees Celsius until it was halted by adding 2 ml chilled PBS.

After centrifugation at 150 g for 5 minutes, the activity in the tube was measured. The free FDG was then removed together with the plasma, and the activity in this fluid was measured. After resuspension in 2-4 ml, phosphate-buffered saline, the activity in the radiolabelled leukocytes was measured. To estimate the labelling efficacy, the activity of the radiolabelled leukocytes was compared to the activity of the leukocytes and the discarded plasma together. If less than 15 %, then the leukocytes were discarded, and the process restarted.

4.3 LEUKOCYTE SCINTIGRAPHY AND SPECT/CT
350 MBq activity of 99mTc-HMPAO radiolabelled leukocytes were planned to be injected in each patient, after which images were to be acquired at one (study IV) or two (study I and II) time-points. At the first time-point, delayed, two hours post-injection, both planar
(study I and II) and SPECT/CT (study I, II and IV) images were acquired. At the second time-point, late, at 24 hours post-injection, only planar images were acquired. Planar images were acquired in both anterior and posterior projections with a 256 x 256 matrix. Delayed images were acquired for 2 minutes and late images for 25 minutes to adjust for activity loss due to radioactive decay between the time points. SPECT was performed with a 128x128 matrix size using 40 seconds per projection in 64 projections with step and shoot technique. At the same time, a CT scan was performed with 130kV, ten mAs and a pitch factor of 1.5. Unfortunately, the CT scan could not be used for attenuation correction because of the metal artefacts from the prostheses. Both planar images and SPECT/CT used a dual-head SPECT/CT system (Siemens Symbia T16, Erlangen, Germany) with LEHR collimators (low energy high-resolution).

4.4 LEUKOCYTE PET/CT

18F-FDG-WBC-PET/CT was performed in studies III and IV 2 hours after injection of radiolabelled leukocytes on a Biograph 64 True Point V PET/CT-system with four rings (Siemens Medical Solutions, Erlangen, Germany). A low dose concurrent CT was used for anatomic navigation. PET images were reconstructed with an ordered subsets expectation optimization (OSEM) algorithm using two iterations and 24 subsets, a 5.0 mm full width half maximum (FWHM) Gaussian filter and matrix size 200×200. Due to metal artefacts in the CT images caused by the prostheses, no attenuation correction nor scatter correction was applied.

4.5 BONE MARROW SCINTIGRAPHY AND SPECT/CT

The patients examined with bone marrow scintigraphy were injected with 500 MBq 99mTc-nanocolloid (BM), and imaging was performed one h after injection on a SPECT/CT system (Siemens Symbia T16, Erlangen, Germany) with low energy high-resolution collimators. Both planar and SPECT/CT images were acquired with similar acquisition parameters as the delayed leucocyte imaging described above. The time between leukocyte and bone marrow scintigraphy were at least 72 h to ensure that no significant trace of the isotope from the previous study remained.

4.6 IMAGE RECONSTRUCTION

SPECT and PET reconstruction, fusion and interpretation were performed on a Hermes workstation (Hermes Medical solution AB, Stockholm, Sweden) using an OSEM (ordered subset expectation maximization) algorithm with four iterations and eight subsets. For
SPECT images, resolution recovery and a Gaussian postfilter with 0.9 cm FWHM (Full width at half maximum). The SPECT examination was combined with a CT scan with 5 mm slices used for the fusion. A B08 kernel was used to reduce metal artefacts from the prostheses.

4.7 IMAGE EVALUATION

All examinations were evaluated separately by two board-certified specialists in both Nuclear Medicine and Radiology. In studies I and II they are Professor Axelsson and Dr Savitcheva and in studies III and IV they are Professor Axelsson and Dr Gabrielson. Any case where the evaluations differed were reviewed in a joint reading where a consensus was reached. All examinations in studies I, II and IV were blinded. In study III, the evaluators were not blinded.

4.7.1 Evaluation of 99mTc-HMPAO-WBC scintigraphy and SPECT/CT

At the start of the period when the examinations included in studies I and II were performed, the imaging protocol for 99mTc-HMPAO-leukocyte imaging at Karolinska University Hospital Huddinge consisted of delayed and late 99mTc-HMPAO-leukocyte planar images as shown in Figure 10 and delayed 99mTc-HMPAO-leukocyte SPECT/CT. Shortly after that, the imaging protocol was expanded to include 99mTc-HMPAO-nanocolloid planar images as shown in Figure 11 and 99mTc-HMPAO-nanocolloid SPECT/CT as shown in Figure 12 for bone marrow visualization. The images were displayed using the grayscale of percentages of maximum counts per pixel, not absolute counts for all examinations.

Study II divided dual time point 99mTc-HMPAO-leukocyte scintigraphy (which was evaluated both visually and semi-quantitatively), single time point 99mTc-HMPAO-leukocyte scintigraphy combined with 99mTc-HMPAO-nanocolloid scintigraphy and 99mTc-HMPAO-leukocyte SPECT/CT combined with 99mTc-HMPAO-nanocolloid SPECT/CT into three settings.
The first setting compared the relative uptake between delayed and late planar leukocyte images, both visually and semi-quantitatively, where an increase in the late images indicated infection, as shown in Figure 10. The target region of interest (ROI) for the semi-quantitative evaluation was any suspected site of infection, and the background ROI was the corresponding area in the contralateral joint. Any increase indicated infection.

Figure 10 Example of setting one with the examination positive for persistent infection in the right knee. There is an uptake in the right knee (arrows) that increases in intensity between the delayed (a) and late (b) images. Unmodified from Teiler J, Åkerlund B, Brismar H, et al. Dual-tracer approach vs dual time-point approach in leukocyte scintigraphy in treatment evaluation of persistent chronic prosthetic joint infection. Nucl Med Commun. 2021;42(7):719-724. © 2021 Wolters Kluwer Health, Inc. The content is licensed under Attribution-NonCommercial-NoDerivatives 4.0 International. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/ Published with permission from The British Editorial Society of Bone & Joint Surgery. Available at: https://doi:10.1097/MNM.0000000000001403

Figure 11 Example of setting two with the examination positive for persistent infection in the left hip. The increased uptake (arrow) on the leukocyte image (a) has no matching increase of uptake in the same area (arrow) on the bone marrow image (b). Unmodified from Teiler J, Åkerlund B, Brismar H, et al. Dual-tracer approach vs dual time-point approach in leukocyte scintigraphy in treatment evaluation of persistent chronic prosthetic joint infection. Nucl Med Commun. 2021;42(7):719-724. © 2021 Wolters Kluwer Health, Inc. The content is licensed under Attribution-NonCommercial-NoDerivatives 4.0 International. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/ Published with permission from The British Editorial Society of Bone & Joint Surgery. Available at: https://doi:10.1097/MNM.0000000000001403
The second setting visually compared the uptake on delayed leukocyte planar images and bone marrow planar images where any mismatch indicated infection, as shown in Figure 11.

Figure 12 Example of setting three with the examination positive for persistent infection. The increased uptake (arrows) on the 99mTc-HMPAO-WBC SPECT (a) and SPECT/CT (b) images have no matching uptake in the same area (arrows) on the bone marrow SPECT (c) and SPECT/CT (d) images. Unmodified (except for shortened description) from Teiler J, Åkerlund B, Brismar H, et al. Dual-tracer approach vs. dual time-point approach in leukocyte scintigraphy in treatment evaluation of persistent chronic prostatic joint infection. Nucl Med Commun. 2021;42(7):719-724. © 2021 Wolters Kluwer Health, Inc. The content is licensed under Attribution-NonCommercial-NoDerivatives 4.0 International. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/ Published with permission from The British Editorial Society of Bone & Joint Surgery. Available at: https://doi:10.1097/MNM.0000000000001403

The third setting visually compared the uptake on delayed leukocyte SPECT/CT images and bone marrow SPECT/CT images. Again, any mismatch indicated infection, as shown in Figure 12. This was the sole 99mTc-HMPAO-leukocyte setting used in study IV. In study IV, a formalized visual evaluation was performed to determine whether uptake was present at different locations. For the hips, the locations were the areas surrounding different parts of the prosthesis – cup, neck, proximal/middle/distal stem – and the more peripheral soft tissue. For the knees, the locations were the synovium, the bone-prosthesis interface, and the soft tissue.

4.7.2 Evaluation of 18F-FDG-WBC PET/CT

All leukocyte PET/CT images, even those in study III with no suspicion of infection, were evaluated visually by comparing them to bone marrow SPECT/CT corresponding to setting three above. Any mismatch indicated infection.

In study III, a visual estimation was made whether the uptake surrounding the prosthesis changed in intensity over time in the patients with repeated examinations. Finally, a formalized visual evaluation was performed in study IV to determine whether uptake was present at the exact locations defined for 99mTc-HMPAO-leukocyte SPECT/CT above. Semi-quantitative evaluation was used in studies III and IV, but the target and background volumes of interest (VOI) differed. Both studies used a target VOI containing the entire...
prosthesis (VOI<sub>total</sub>). Study IV, which included both hip and knee prostheses, used no other target VOI. In study III, target VOI:s were also placed in each Gruen zone (122, 123) apart from zone 11 as it coincides with zone 4 in a three-dimensional volume. The mean of these VOI:s was calculated and designated VOI<sub>prosthesis</sub> to indicate the uptake surrounding the bone-covered stem of the prosthesis. In addition to this, four target VOI:s were placed anterior, posterior, medial and lateral to the prosthesis neck. The mean of these VOI:s was designated VOI<sub>joint</sub> to indicate the uptake surrounding the neck of the prosthesis accessible by arthroscopy.

Both studies used a mirrored VOI<sub>total</sub> at the contralateral joint (VOI<sub>contra</sub>), a VOI in the ipsilateral iliac crest (VOI<sub>crest</sub>) and a VOI in the ipsilateral femur (VOI<sub>femur</sub>) as background VOI:s. Study III also included background VOI:s placed in the contralateral iliac crest (VOI<sub>cocrest</sub>) and femur (VOI<sub>cofemur</sub>). The knee prostheses in study IV only used VOI<sub>contra</sub>, VOI<sub>femur</sub> and VOI<sub>cofemur</sub> because the iliac crest was not included in the examined volume.

4.8 STATISTICAL ANALYSIS

The following statistical approaches were applied: For comparing sensitivity and specificity between settings (study II) or imaging modalities (study IV), McNemar’s test was used. Binary logistic regression was used in study I to evaluate the influence of labelling percentage on true positive results among infected joints and evaluate the influence of injected activity on sensitivity. Study I also used Fisher’s Exact Test to evaluate if the examinations' sensitivity was affected by leukocyte labelling above or below 40%, adequate antibiotic treatment at the time of scan or not, and whether the prosthesis was located in the hip or knee. Pearson’s correlation was used to evaluate uptake related to time passed since surgery. All tests used a 95% confidence interval and were considered significant when p ≤ 0.05. All statistical analysis was performed with SPSS Statistics for Macintosh, versions 23.0 - 25.0, except for the 95% confidence intervals of sensitivity and specificity for the individual settings in study II that were calculated using Review Manager 5.3.
5 RESULTS

5.1 STUDY I AND II

For the retrospective studies, 139 patients with a leukocyte examination for suspected prosthetic joint infection were reviewed, and 62 patients with 63 prosthetic joints were included in study I, out of which 31 patients with 31 joints were included in study II.

In study I, the patients were divided into a treatment evaluation group (49 patients with 49 joints), where patients had on-going antibiotic therapy or had stopped antibiotic therapy less than 14 days before imaging, and a suspected chronic infection group (13 patients with 14 joints), where patients had either no on-going antibiotic treatment or had started treatment less than 30 days before imaging. Study II only included patients under antibiotic therapy for treatment evaluation (31 patients). The patient characteristics of studies I and II are reported in Table 1.

<table>
<thead>
<tr>
<th>Table 1: Patient characteristics in studies I and II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
</tr>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Men / Women</td>
</tr>
<tr>
<td>Hip n (%)</td>
</tr>
<tr>
<td>Revision surgery</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Malignancy</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Antibiotic therapy</td>
</tr>
<tr>
<td>Days since arthroplasty</td>
</tr>
<tr>
<td>Days of follow-up</td>
</tr>
<tr>
<td>Labelling efficacy</td>
</tr>
<tr>
<td>Below 40%</td>
</tr>
<tr>
<td>Injected activity (MBq)</td>
</tr>
<tr>
<td>Below 200 MBq</td>
</tr>
</tbody>
</table>
Figure 13. Examination results of studies I and II

The results of the examinations in studies I and II are reported in Figure 13, and the likelihood ratios are reported in Table 2.

<table>
<thead>
<tr>
<th>Study</th>
<th>Examination group</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I entire cohort</td>
<td>All 3.79 Hips 2.18 Knees</td>
<td>4.50</td>
<td>All 0.46 Hips 0.83 Knees</td>
</tr>
<tr>
<td>Study I suspected chronic PJI</td>
<td>All 11.0 Hips cnc Knees 5.00</td>
<td>0.00 Hips cnc Knees 0.00</td>
<td></td>
</tr>
<tr>
<td>Study I treatment control</td>
<td>All 2.94 Hips 1.36 Knees</td>
<td>4.50</td>
<td>All 0.54 Hips 0.91 Knees</td>
</tr>
<tr>
<td>Study II setting one semi-quantitative</td>
<td>All 1.11 Hips 1.21 Knees</td>
<td>1.14</td>
<td>All 0.96 Hips 0.90 Knees</td>
</tr>
<tr>
<td>Study II setting one visual</td>
<td>All 2.46 Hips 0.91 Knees</td>
<td>3.41</td>
<td>All 0.59 Hips 1.03 Knees</td>
</tr>
<tr>
<td>Study II setting two</td>
<td>All 6.33 Hips cnc Knees 5.73</td>
<td>0.00 Hips 0.00 Knees</td>
<td>0.42</td>
</tr>
<tr>
<td>Study II setting three</td>
<td>All 2.71 Hips cnc Knees 1.91</td>
<td>0.00 Hips 0.80 Knees</td>
<td>0.55</td>
</tr>
</tbody>
</table>

cnc = cannot be computed

The levels of CRP and ESR, labelling efficacy, and the presence of infection were evaluated in study I for their effect on the scintigraphic result in a binary logistic regression analysis. Only the presence of infection had any significant effect on the result (p = 0.004). Factors were also evaluated using Fischer’s exact test that compared the proportions of patients with and without the factor whose examinations were positive for infection and
whose examinations result was true or false. The comparison between proportions of positive and negative examinations let us evaluate if any factor increased the risk of a positive examination, for example, by coinciding with or causing inflammation in the examined joint. If the same analysis is performed separately in the infected and cured patients, then it compares the proportion of true positives to false negatives (sensitivity) and true negatives with false positives (specificity), respectively. Finally, the comparison between true and false examinations let us evaluate if any factor generally lowers the accuracy of the examination. The comparisons are reported in Table 3.

<table>
<thead>
<tr>
<th>Evaluated factor</th>
<th>Positive yes/no</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated CRP</td>
<td>0.13</td>
<td>0.14</td>
<td>1</td>
<td>0.48</td>
</tr>
<tr>
<td>Elevated ESR</td>
<td>0.03* (+)</td>
<td>0.15</td>
<td>0.57</td>
<td>1</td>
</tr>
<tr>
<td>Injected activity &gt;200 MBq</td>
<td>0.24</td>
<td>0.02* (+)</td>
<td>0.52</td>
<td>0.02* (+)</td>
</tr>
<tr>
<td>Leukocyte labelling &gt;40%</td>
<td>0.35</td>
<td>0.37</td>
<td>0.56</td>
<td>0.73</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>0.17</td>
<td>1</td>
<td>0.07</td>
<td>0.45</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.43</td>
<td>1</td>
<td>0.53</td>
<td>0.67</td>
</tr>
<tr>
<td>Hip prosthesis</td>
<td>0.01* (-)</td>
<td>0.00* (-)</td>
<td>0.37</td>
<td>0.24</td>
</tr>
<tr>
<td>More than one prosthesis</td>
<td>0.79</td>
<td>0.38</td>
<td>0.67</td>
<td>1</td>
</tr>
<tr>
<td>On-going effective antibiotics</td>
<td>0.75</td>
<td>0.26</td>
<td>0.65</td>
<td>0.09</td>
</tr>
</tbody>
</table>


c = the factor is associated with a decreased proportion.

As the results in study I were extracted from electronic files used in clinical practice, no consensus reading was used. However, in study II, consensus reading was used for visual evaluation four times in setting one, one time in setting two and five times in setting three.

When comparing the different settings in study II there was no difference in sensitivity and specificity or accuracy; see table 4.

<table>
<thead>
<tr>
<th>Setting</th>
<th>One Visual</th>
<th>One Semiquantitative</th>
<th>Two</th>
<th>Three</th>
</tr>
</thead>
<tbody>
<tr>
<td>One visual</td>
<td>X</td>
<td>Sens p = 0.38</td>
<td>Sens p = 0.13</td>
<td>Sens p = 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spec p = 1</td>
<td>Spec p = 0.38</td>
<td>Spec p = 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acc p = 0.34</td>
<td>Acc p = 1</td>
<td>Acc p = 1</td>
</tr>
<tr>
<td>One Semiquantitative</td>
<td>Sens p = 0.38</td>
<td>Spec p = 1</td>
<td>Spec p = 0.38</td>
<td>Spec p = 0.63</td>
</tr>
<tr>
<td></td>
<td>Spec p = 1</td>
<td>Acc p = 0.34</td>
<td>Acc p = 0.51</td>
<td>Acc p = 0.69</td>
</tr>
<tr>
<td>Two</td>
<td>X</td>
<td>Sens p = 0.13</td>
<td>X</td>
<td>Sens p = 0.50</td>
</tr>
<tr>
<td></td>
<td>Sens p = 0.13</td>
<td>Spec p = 1</td>
<td>Spec p = 0.50</td>
<td>Spec p = 0.38</td>
</tr>
<tr>
<td></td>
<td>Spec p = 0.38</td>
<td>Acc p = 0.51</td>
<td>Acc p = 1</td>
<td>Acc p = 1</td>
</tr>
<tr>
<td>Three</td>
<td>Sens p = 1</td>
<td>Sens p = 0.63</td>
<td>Sens p = 0.50</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Spec p = 1</td>
<td>Spec p = 0.69</td>
<td>Spec p = 0.38</td>
<td>Spec p = 1</td>
</tr>
<tr>
<td></td>
<td>Acc p = 1</td>
<td>Acc p = 0.34</td>
<td>Acc p = 1</td>
<td></td>
</tr>
</tbody>
</table>

5.2 STUDY III

Nine patients were recruited. One withdrew their consent resulting in eight patients included. Seven patients had unilateral hip prostheses, and one had bilateral hip prostheses,
with both included in the study. Two patients were subjected to sequential imaging: one at three and six months after arthroplasty and the other at three, six and twelve months after arthroplasty. The other six patients were imaged at twenty-four months after arthroplasty, with the contralateral prosthesis imaged at thirty-four months after arthroplasty. 18F-FDG-WBC-PET/CT, 99mTc-HMPAO-WBC-SPECT-CT and 99mTc-Nanocolloid-SPECT/CT were performed on all patients. No patient had symptoms of PJI, but one patient had a respiratory tract infection and temporarily elevated serum inflammation markers which normalized. No adverse events were reported. No changes in vital parameters greater than or equal to ten per cent were recorded.

Uptake was detected at the bone-prosthesis interface surrounding the prosthesis stem in all patients. However, on visual evaluation, it decreased over time in both patients with sequential imaging.

Comparing the different combinations of target and background VOIs showed a negative correlation between time since arthroplasty and the ratio between VOI\textsubscript{prosthesis} and VOI\textsubscript{crest} ($p = 0.03$, $r = -0.64$). There was no significant change over time in the ratios of VOI\textsubscript{total} and VOI\textsubscript{crest} ($p = 0.08$, $r_{\text{adj}} = -0.53$) and VOI\textsubscript{joint} and VOI\textsubscript{crest} ($p = 0.17$, $r_{\text{adj}} = -0.42$). The VOI\textsubscript{total}/VOI\textsubscript{crest} ratio increased between examination in the two patients with sequential imaging, while the VOI\textsubscript{prosthesis}/VOI\textsubscript{crest} ratio decreased or was stationary.

5.3 STUDY IV

Out of 34 reviewed patients, 19 were included in the study. The reasons for exclusion were that seven patients refused to give or withdrew their consent, two patients’ serum markers of infection normalized before imaging, there were no longer any suspicions of infection in two patients at the time of imaging, one patient was not able to give informed consent, one patient had a previous anaphylactic reaction, one patient’s leukocyte count was below the threshold, and finally one patient died before completing six months of anti-biotic free follow-up.

Positive microbial culture indicating infection before treatment started was available for all patients.
The sensitivity and specificity of visual evaluation of the 99mTc-HMPAO-WBC-SPECT-CT and the 18F-FDG-WBC-PET/CT examinations of Study IV are reported in Figure 14, and the positive and negative likelihood ratios are reported in Table 5. Examples of both types of images are shown in Figure 15. Consensus evaluation was used in three of the 99mTc-HMPAO-WBC-SPECT-CT examinations and four of the 18F-FDG-WBC-PET/CT examinations. Comparing the results of the two methods in infected (p=1) and cured (p=1) patients using McNemar’s test showed no significant difference. The only false-positive result was false positive for both examinations; otherwise, all positive SPECT examinations were negative on PET and vice versa. Neither method showed any effect of low labelling efficacy on sensitivity or specificity.

![Figure 14](image)

### Table 5: Likelihood ratios for the examinations in study IV

<table>
<thead>
<tr>
<th>Examination group</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECT/CT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1.85</td>
<td>0.83</td>
</tr>
<tr>
<td>Hips n/a</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Knees 0.50</td>
<td></td>
<td>1.25</td>
</tr>
<tr>
<td>PET/CT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1.92</td>
<td>0.77</td>
</tr>
<tr>
<td>Hips n/a</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Knees 1.00</td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>

Figure 15 A 99mTc-HMPAO-WBC SPECT image (a) compared with an 18F-FDG-WBC PET image (b) showing the difference in resolution. Both examinations were combined with a concurrent CT and compared with a 99mTc-Nanocolloid SPECT/CT in the same manner as shown in Figure 12.
The clinical characteristics and characteristics of the examinations are reported in Table 6. The different locations of uptake did not vary significantly between the two methods, and there was no location with a significant correlation with infection in any of the two methods.

<table>
<thead>
<tr>
<th>No</th>
<th>Joint</th>
<th>Days since latest surgery</th>
<th>Days with antibiotic treatment</th>
<th>Infecting bacteria before imaging</th>
<th>SPECT labelling efficacy</th>
<th>PET labelling efficacy</th>
<th>SPECT result</th>
<th>PET result</th>
<th>Final diagnosis of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Knee</td>
<td>214</td>
<td>216</td>
<td>A. defectiva</td>
<td>40 %</td>
<td>32 %</td>
<td>True negative</td>
<td>True negative</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Knee</td>
<td>1284</td>
<td>224</td>
<td>S. lugdunensis</td>
<td>53 %</td>
<td>79 %</td>
<td>False negative</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Hip</td>
<td>270</td>
<td>297</td>
<td>S. epidermidis</td>
<td>19 %</td>
<td>65 %</td>
<td>False negative</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Hip</td>
<td>571</td>
<td>No ongoing treatment</td>
<td>E. faecalis</td>
<td>59 %</td>
<td>75 %</td>
<td>True positive</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Hip</td>
<td>145</td>
<td>162</td>
<td>Polymicrobial</td>
<td>Data missing</td>
<td>69 %</td>
<td>True negative</td>
<td>True negative</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Hip</td>
<td>1692</td>
<td>63</td>
<td>Polymicrobial</td>
<td>Data missing</td>
<td>66 %</td>
<td>True positive</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Knee</td>
<td>145</td>
<td>137</td>
<td>S. epidermidis</td>
<td>60 %</td>
<td>65 %</td>
<td>False negative</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Hip</td>
<td>190</td>
<td>196</td>
<td>S. aureus</td>
<td>77 %</td>
<td>27 %</td>
<td>False negative</td>
<td>True positive</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>Hip</td>
<td>2129</td>
<td>210</td>
<td>P. micra</td>
<td>37 %</td>
<td>69 %</td>
<td>True negative</td>
<td>True negative</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Knee</td>
<td>337</td>
<td>No ongoing treatment</td>
<td>R. planticola</td>
<td>66 %</td>
<td>69 %</td>
<td>True positive</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>Hip</td>
<td>151</td>
<td>153</td>
<td>Polymicrobial</td>
<td>S. aureus</td>
<td>54 %</td>
<td>74 %</td>
<td>True negative</td>
<td>True negative</td>
</tr>
<tr>
<td>12</td>
<td>Hip</td>
<td>307</td>
<td>No ongoing treatment</td>
<td>Polymicrobial</td>
<td>Data missing</td>
<td>65 %</td>
<td>False negative</td>
<td>True positive</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>Knee</td>
<td>159</td>
<td>150</td>
<td>S. aureus</td>
<td>Poly microbial</td>
<td>70 %</td>
<td>90 %</td>
<td>False positive</td>
<td>False positive</td>
</tr>
<tr>
<td>14</td>
<td>Hip</td>
<td>252</td>
<td>180</td>
<td>Poly microbial</td>
<td>68 %</td>
<td>62 %</td>
<td>False negative</td>
<td>True positive</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>Knee</td>
<td>114</td>
<td>No ongoing treatment</td>
<td>Poly microbial</td>
<td>66 %</td>
<td>92 %</td>
<td>False negative</td>
<td>True positive</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>Knee</td>
<td>133</td>
<td>135</td>
<td>Alpha streptococcus</td>
<td>Data missing</td>
<td>66 %</td>
<td>True negative</td>
<td>True negative</td>
<td>No</td>
</tr>
<tr>
<td>17</td>
<td>Knee</td>
<td>512</td>
<td>52</td>
<td>S. lugdunensis</td>
<td>49 %</td>
<td>86 %</td>
<td>False negative</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>Hip</td>
<td>3399</td>
<td>&gt;365</td>
<td>Poly microbial</td>
<td>69 %</td>
<td>93 %</td>
<td>True positive</td>
<td>False negative</td>
<td>Yes</td>
</tr>
<tr>
<td>19</td>
<td>Knee</td>
<td>263</td>
<td>&gt;365</td>
<td>Poly microbial</td>
<td>Data missing</td>
<td>55 %</td>
<td>90 %</td>
<td>False negative</td>
<td>True positive</td>
</tr>
</tbody>
</table>
The results of the AUROC analysis of the semi-quantitative evaluation of the 18F-FDG-WBC-PET/CT examinations are shown in Table 7.

Measurement of vital parameters before and after the injection of leukocytes labelled with 18F-FDG showed an increase of 30% or more in three patients and 10-29% in three patients. However, this was not associated with any symptoms or distress in any of the patients.
6 DISCUSSION

6.1 STUDY I AND II

Studies I and II focus on suspected chronic PJI and mainly the evaluation of ongoing antibiotic therapy effect. The first significant implication of these studies is that the diagnostic performance of 99mTc-HMPAO-WBC scintigraphy and SPECT/CT for therapy evaluation is poor compared to its reported diagnostic performance in PJI in general. The second significant implication is that 99mTc-HMPAO-WBC scintigraphy should primarily be performed as the standard dual-time examination, even in the chronic and therapy evaluating setting.

At Karolinska University Hospital Huddinge, leukocyte scintigraphy has been recommended for patients where it has been hard to reach a definite diagnosis of chronic PJI by clinical examination alone. This might, for example, be due to inflammatory joint disease causing elevated inflammatory blood markers and local symptoms or due to some remaining symptoms after full-length antibiotic treatment. In Study I, we reported our results using leukocyte scintigraphy and SPECT/CT under these premises. In Study II, we performed a blinded head-to-head evaluation of the different parts of our leukocyte imaging examinations used in Study I in a refined subset of patients under antibiotic treatment.

The purpose of Study I was to evaluate 99mTc-HMPAO-WBC imaging methods in a pure suspected chronic PJI cohort. For Study II the aim was to evaluate if one of the available 99mTc-HMPAO-WBC imaging methods was more accurate in a patient cohort where all patients are under ongoing treatment, and the method is used for treatment evaluation and not the primary diagnosis of PJI.

Only patients with an infection proven by microbial culture before imaging were included. All patients had ongoing antibiotic treatment for PJI or had already completed a full-length treatment, so any infection found at the examination was interpreted as chronic. The clinical evaluation of infection used the reference standard was strict, with an infection required to be confirmed by microbiological culture. To confirm the absence of infection in a cured patient, we required at least 24 months of symptom-free follow-up after the cessation of antibiotics or negative tissue cultures at revision surgery. This removed all equivocal patients from the analysis.

The strict criteria mentioned above is the greatest strength of studies I and II. Their weakness is the small patient cohort of study I and even smaller one in study II. Another significant weakness is that the efficacy of leukocyte labelling was generally low compared to reported levels in other studies (67, 79), even in study II, with all patients with labelling...
efficacy below the suggested threshold of 40% excluded. However, several studies (70, 80, 97) did not report the labelling making it hard to evaluate its effect on their results.

Among the most significant limitations of almost all other studies is using a mixed patient cohort with an unknown number of acute, chronic and acute haematogenous infections. Another limitation is an unspecified or arbitrary clinical evaluation with a short follow-up period used as the reference standard. This might contribute to the fact that in Study I and II, the diagnostic performance of the methods evaluated were worse than generally reported but consistent with the few other studies on chronic PJI (70, 92) or equivocal examinations (71).

Another aspect that might explain the difference in diagnostic performance in other studies on leukocyte scintigraphy is the previous lack of standardization of the method. This is now rectified through the guideline published by the EANM (78) and will hopefully improve the diagnostic performance in future studies and clinical practice. However, study I and II differ from these guidelines in several aspects.

Image acquisition was performed using standardized fixed times at each time point. The acquisition time at the later time point was longer to take the decay of the radiopharmaceutical into account. A correct approach would have been to fine-tune the acquisition to an exact number of seconds depending on the exact interval between examinations in an exact decay time-corrected protocol. In addition, the delayed images in studies I and II were performed around two hours after injection, whereas the guideline recommends three to four hours after injection for better performance.

All patients in study II and most patients in study I had ongoing antibiotic treatment at the time of examination. Guidelines (37, 124) recommend a two-week antibiotic-free interval before microbiological testing but not before leukocyte scintigraphy, but several studies fail to show any antibiotic effect on diagnostic performance (70, 80, 97). The antibiotic-free group in study I had higher accuracy than the treatment control group under antibiotic treatment. This might be caused by the absence of infected hip prostheses in the antibiotic-free group as the sensitivity for hips is generally lower than for knees (80, 92, 97) but an effect of antibiotics cannot be excluded.

Concomitant diseases might theoretically affect the diagnostic performance of leukocyte imaging by attracting leukocytes towards or away from the suspected infected joint. Inflammatory joint diseases like rheumatoid arthritis have been shown to attract leukocytes to native joints (125-127) but there are no studies performed in prosthetic joints, and there was no effect shown in studies I and II. Malignant tumours might attract leukocytes and
thus reduce the number available to aggregate in the infected joint (128, 129) but this is unlikely to be of a magnitude to have any clinically relevant effect.

### 6.2 STUDY III AND IV

Studies III and IV examined 18F-FDG-WBC PET/CT as a possible alternative to the reference standard of 99mTc-HMPAO-WBC scintigraphy. The main implication of study III is that a gradually diminishing post-surgical tracer uptake surrounding the prosthesis remains for at least two years and could influence the interpretation of the examinations if no additional bone marrow imaging is performed. The main implications of study IV are that there was no difference in sensitivity or specificity between 18F-FDG-WBC PET/CT and 99mTc-HMPAO-WBC SPECT/CT when used with complementary 99mTc-Nanocolloid SPECT/CT in the diagnosis of PJI but that there was a discrepancy in results indicating that different leukocyte populations might be labelled. The strength of study III is its novelty, as it is, to our knowledge, the first study on the topic. The greatest strengths of study IV are comparing the methods on the same patients and the strict criteria for verified infection as in studies I and II. The most significant weaknesses of studies III and IV are the small patient cohorts. They should therefore be viewed as pilot studies to be possibly expanded upon in the future.

As the interpretation of regular 18F-FDG PET/CT in diagnosis depends on the uptake pattern (130) all examinations in study IV were evaluated visually in a formalized manner to see if any pattern of uptake correlated with infection. Unfortunately, no such correlation was observed. The uptake surrounding the prosthesis was shown to remain at 24 months but could be expected to remain longer. Therefore, a complementary bone marrow scan is needed for visual evaluation as a late (18-30 h post-injection) scan is not feasible. However, a recent study (131) on 99mTc-HMPAO-WBC found that images eight hours post-injection had better results than late images. Eight hours is roughly four times the half-life of 18F-FDG, which is similar to the ratio between the half-life of 99mTc and the 24-hour average of late imaging. Thus, it could be possible to perform dual time-point 18F-FDG-WBC PET/CT with imaging at two and eight hours post-injection. However, a factor that could make this difficult is that 18F-FDG is a less stable labelling agent than 99mTc-HMPAO, making the biological half-life of 18F-FDG-WBC less than a fourth of that of 99mTc-HMPAO-WBC. An alternative to visual evaluation is semi-quantitative evaluation. There is no late imaging, so the target/background ratio is evaluated independently and not compared with another time point. This showed more promising results than visual comparison with bone marrow
images, but further research would be needed to establish an optimal cut-off. In addition, it is necessary to use bone marrow in the iliac crest or femur as the background (rather than the contralateral joint as commonly used in other studies (80)) because the presence or absence of a prosthesis in the contralateral joint would affect the ratio too much. All patients in study IV with a true positive examination with one method had a false negative examination with the other method. An explanation could be that the 99mTc-HMPAO would label all available leukocytes equally, whereas 18F-FDG would label leukocyte types according to their number of active glucose transporters with activated polymorphonuclear cells expressing the highest (132). The four cases where both methods produced false negative results were infections with low virulence bacteria (S. lugdunensis and S. epidermidis) that can be hard to detect by any leukocytes.

### 6.3 GENERAL DISCUSSION

The most important questions concerning a diagnostic test for a disease are if, when and how it should be applied. Is it a screening test to discern which seemingly healthy persons need further investigation and possible treatment for a yet asymptomatic disease? Is it a general indication of the level of inflammation in the patient? Is it a method to discern the cause of the patient’s localized symptoms? Is it the final divider deciding which patients need certain kinds of treatments?

The role of leukocyte scintigraphy investigated in studies I, II and IV is primarily that of therapy evaluation, deciding which patients with chronic PJI under antibiotic therapy require revision surgery for cure. Therefore, the consequence of a positive examination is that the patient could be planned for surgery and the effect of a negative examination is that the antibiotic therapy is discontinued. Therefore, to decide whether the examination needs to have high sensitivity or high specificity, one must first evaluate the benefit of a true result and the potential risk of a false result.

A true positive result will result in revision surgery with prosthesis removal at the earliest time with the infection suppressed by antibiotic treatment. However, the surgery should preferably take place after an antibiotic-free interval of two weeks to increase the sensitivity of the microbiological culture from the perioperative biopsies.

A false negative result means that the patient’s antibiotic treatment will be discontinued without the infection being cured. Thus, it is likely that the infection will progress with local manifestations such as pain, abscesses and prosthetic loosening. In the worst scenario, the infection will spread systemically and cause septic shock and even death.
A true negative result means that the patient’s PJI is cured, and the antibiotic treatment will be discontinued, sparing the patient further unnecessary treatment. On a population level, this could also reduce the development of antibiotic-resistant bacteria.

A false positive result means that the patient will be a candidate for unnecessary major surgery with the risk for complications like bleeding, lung embolism and death. In addition, there is also a risk of a prosthetic joint infection which is increased in revision arthroplasty compared to primary arthroplasty.

Out of these outcomes, the true positive result has the most significant impact on patients with a chronic PJI who need prosthesis removal for cure as they are spared unnecessary antibiotic treatment. More importantly, however, the false positive result leads to the most significant risk to the patient. Thus, the specificity of the examination is the most important factor to gauge its value.

In the clinical practice at Karolinska University Hospital Huddinge, the risk of severe complications of a relapsed infection after discontinuation of antibiotic therapy after the minimum treatment time has passed is estimated to be very low. Because of this, the recommended method used here to evaluate therapy effect is to end the anti-biotic treatment, re-evaluate the patient for reoccurring symptoms and increased inflammatory biomarkers and plan for surgery as symptoms appear. In this praxis, the only place for leukocyte scintigraphy is in the patients where a renewed infection cannot be risked, for example, when amputation is the only surgical option, or the patient previously has had systemic spread infection.

Another aspect that supports this approach is the antibiotic-free interval before surgery to ensure good sensitivity of the microbial cultures from perioperative tissue biopsies. This means that even the most vulnerable patients will be without antibiotic treatment for at least that period.

Regarding the antibiotic-free interval, there is some controversy regarding whether a similar interval should be used before leukocyte imaging, as used in some studies (71). A way to test this in a study would be to image patients with planned revision surgery for both suspected septic and aseptic loosening at two times: first during the last days of antibiotic treatment and then in the days leading up to surgery. Unfortunately, no such study has yet to be published.

There is a high value of standardisation and quality control in a procedure as complicated as leukocyte scintigraphy with so many different tasks to be performed. This applies to all personnel and departments involved in the process. Therefore it is laudable that EANM has published guidelines for the labelling of leukocytes (77), the acquisition of images and the
evaluation of the examinations (78). However, if there is significant variance in how the examination is performed, its performance will vary depending on who works on the day in question.
7 CONCLUSIONS

In summary, study I show that a combination of dual time point 99mTc-HMPAO-WBC scintigraphy, 99mTc-HMPAO-WBC SPECT/CT and 99mTc-Nanocolloid (bone marrow) scintigraphy in a clinical setting have average sensitivity and specificity for detecting chronic PJI in knee prostheses but very low sensitivity and good specificity for detecting chronic PJI in hip prostheses. Furthermore, the performance was better in a small subset of patients without or just recently started antibiotic treatment.

Study II compared visual and semi-quantitative evaluation of dual time point 99mTc-HMPAO-WBC scintigraphy, visual evaluation of single time point 99mTc-HMPAO-WBC scintigraphy combined with 99mTc-Nanocolloid (bone marrow) scintigraphy and visual evaluation of single time point 99mTc-HMPAO-WBC SPECT/CT combined with 99mTc-Nanocolloid (bone marrow) SPECT/CT in a subset of patients from study I, all under antibiotic treatment for PJI. As no method was shown to be superior dual time point 99mTc-HMPAO-WBC scintigraphy should still be the first-hand choice for functional PJI imaging due to it being a quicker and cheaper examination with less radiation dose to the patient.

Study III demonstrated that on 18F-FDG-WBC PET/CT examinations, there is a residual uptake surrounding the prosthesis stem after uneventful primary arthroplasty of the hip. The uptake seems to decrease over time but is detectable at least 24 months post-surgery.

Study IV compared single time point 99mTc-HMPAO-WBC SPECT/CT and 18F-FDG-WBC PET/CT, both combined with 99mTc-Nanocolloid SPECT/CT, in the diagnosis of PJI. There was no observed difference in the sensitivity and specificity of visual evaluation. The area under the receiver operating characteristic analysis showed some promise for semiquantitative evaluation of 18F-FDG-WBC PET/CT, but further research and comparison to the reference standard of dual time point 99mTc-HMPAO-WBC scintigraphy is needed before any method could be recommended in clinical practice.
8 POINTS OF PERSPECTIVES

8.1 THE DIAGNOSTIC DILEMMA
One of the problems of researching new diagnostic methods is that they must be tested against the presently available methods that seldom are perfect. The risk for an error of estimate thus is greater than the α and β errors of the study and must consider the inaccuracies of the control method. Therefore, a combination of available methods can be used in concert to improve the study's accuracy.

It is common to use clinical examination as a control for imaging in the cases where cultures and other tests were inconclusive or unavailable (96, 103). Clinical judgment is a good tool in daily hospital work, but there is reason to require more definite evidence of especially chronic PJI in a scientific context, as the risk for observer bias, unfortunately, is substantial. A study shows that a third of PJI diagnoses are made without the patient meeting the MSIS criteria (133).

Another important aspect that relates to the standardisation of the imaging procedures is the reproducibility of results, best evaluated through intra- and inter-observer agreement. This is a factor that is often missing in nuclear medicine studies, as shown by a recent meta-analysis (134).

8.2 BIOFILM IN PROSTHETIC JOINT INFECTION
What sets the chronic PJI apart from the two types of acute infection is the presence of the biofilm and its significant impact on detection and treatment. In addition, the difficulty of making the diagnosis and the severity of the cure if revision arthroplasty is needed should make chronic PJI the focus of all research concerning diagnostic tests for PJI, including imaging.

The ideal would be to detect the biofilm or the infecting microorganisms themselves. To take this into account and reduce the number of false-negative examinations perceived as true negative, one of the most critical parts of study design is to ensure that the follow-up time is sufficient. For example, a chronic infection treated with only antibiotics and debridement may be suppressed but not cured and can reappear years after treatment is ended. However, no further follow-up is necessary if a definite diagnosis of chronic PJI can be made through a microbiological culture (59, 99) or the recurring of a sinus tract.

It is unfeasible to prolong follow-up indefinitely, but a good start is that any follow-up should be defined as the time passed after all treatment for the infection is ended, not after the examination itself. An ordinary follow-up time has been twelve months (60, 63, 91, 93,
after examination, although some studies have had six months (61, 79, 80, 89, 95) or an even shorter minimum follow-up time (102, 109, 114).

The less invasive the diagnostic technique is, the better. Nuclear medicine techniques are not invasive, and the risks associated with ionizing radiation are lower in an elderly population, making these techniques suitable for imaging for PJI.

8.3 FUTURE NUCLEAR MEDICINE METHODS

Leukocyte imaging for infection diagnosis can be used to visualize the present state at the time of the examination, i.e., the location of the leukocytes or a process over time, i.e., leukocytes migrating to a site of infection. The first requires additional imaging of the places where leukocytes can aggregate without a sign of infection, i.e., the bone marrow. The second requires the leukocytes to be labelled with an isotope with a half-life that enables repeated examinations after a sufficient time has passed. Thus, in developing a novel leukocyte labelling isotope, these aspects must be considered, and a choice must be made between a dual time-point or dual tracer approach.

However, if a tracer can be developed that does not visualize the immune system's response to infection but the infection itself, then a single examination would be sufficient, and the time-consuming and not risk-free work of labelling the leukocytes could be omitted. Such a tracer could ideally target the bacteria or biofilm itself. One example of this is the method of Schoenmakers et al. (137) which consists of injecting the antibiotic agent vancomycin coupled with a fluorophore into a prosthetic knee joint, enabling the direct visualisation of the biofilm in real-time during arthroscopy in a cadaver model. Other studies have labelled the antibiotic ciprofloxacin and anti-bacterial peptides for scintigraphy (60, 121, 138).

In future research focused on new radiopharmaceuticals for leukocyte imaging, it is vital to either continue using dual time point imaging with an interval of 18-30 hours (78) between time points or evaluate if a shorter interval between time points is feasible (131). If the longer interval is to be used, then the half-life of the radiopharmaceutical must be sufficient, regardless of whether SPECT or PET imaging is performed.
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10 REFERENCES


