OPEN
Original article

Nuclear Medicine Communications

Dual-tracer approach vs. dual time-point approach in leukocyte scintigraphy in treatment evaluation of persistent chronic prosthetic joint infection

Johan Teiler[a,b], Börje Åkerlund[c,d], Harald Brismar[a,e], Irina Savitcheva[f], Marcus Ahl[c,d], Annie Bjäreback[f], Håkan Hedlund[a,g], Maria Holstensson[a,f] and Rimma Axelsson[a,f]

Background **Both dual time-point ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO)-leukocyte scintigraphy and dual-tracer ^{99m}Tc-HMPAO-leukocyte scintigraphy (with the addition of ^{99m}Tc-nanocolloid bone marrow scintigraphy) have been used to diagnose prosthetic joint infection (PJI). A treatment evaluation of persistent PJI using these imaging protocols has yet to be presented.**

Objective **The purpose of this study was to compare the accuracy of dual time-point ^{99m}Tc-HMPAO-leukocyte scintigraphy to the dual-tracer alternative of single time-point ^{99m}Tc-HMPAO-leukocyte scintigraphy or single-photon emission computed tomography/computed tomography (SPECT/CT) combined with a ^{99m}Tc-nanocolloid bone marrow scintigraphy or SPECT/CT, for treatment evaluation of PJI.**

Material and methods **Thirty-one PJI patients under antibiotic treatment were included in this retrospective study. Examinations were organized into three settings. Setting one used dual time-point approach with delayed (2 h) and late (24 h) planar ^{99m}Tc-HMPAO-leukocyte scintigraphy, including both visual and semiquantitative analysis. Setting two used delayed (2 h) planar ^{99m}Tc-HMPAO-leukocyte scintigraphy combined with ^{99m}Tc-nanocolloid bone marrow scintigraphy and for setting three SPECT/CT replaced planar imaging.**

Results **Accuracy was 0.68 for visual evaluation and 0.55 for semiquantitative evaluation of setting one; 0.71 for setting two; and 0.68 for setting three. Sensitivity was 0.54 for visual evaluation and 0.31 for semiquantitative evaluation of setting one; 0.38 for setting two; and 0.46 for setting three. Specificity was 0.78 for visual evaluation and 0.72 for semiquantitative evaluation of setting one; 0.94 for setting two; and 0.83 for setting three.**

Conclusion **No significant difference in accuracy, sensitivity, or specificity between the approaches for treatment evaluation of suspected persistent PJI in the hip or knee was observed.** *Nucl Med Commun* **42: 719–724**

Nuclear Medicine Communications 2021, **42**:719–724

Keywords: biofilm, chronic infection, leukocyte scintigraphy, nuclear medicine, prosthetic joint infection, single-photon emission computed tomography, ^{99m}Tc-HMPAO-white blood cell, treatment evaluation

[a]Department of Clinical Science, Technique and Intervention (CLINTEC), Karolinska Institutet, [b]Department of Radiology, Karolinska University Hospital Huddinge, [c]Department of Medicine, Karolinska Institutet, [d]Unit of Infectious Diseases Karolinska University Hospital Huddinge, [e]Department of Orthopaedic Surgery, Karolinska University Hospital Huddinge, [f]Function Medical Radiation Physics and Nuclear Medicine, Karolinska University Hospital Huddinge and [g]Department of Orthopaedic Surgery, Visby General Hospital, Stockholm, Sweden

Correspondence to Johan Teiler, MD, Department of Clinical Science, Technique and Intervention (CLINTEC), Karolinska Institutet, SE-171 77 Stockholm, Sweden
Tel: +46 858584699; e-mail: johan.teiler@ki.se

Received 11 January 2021 **Accepted** 9 February 2021

Introduction

Early detection and optimal treatment of a prosthetic joint infection (PJI) is crucial for the outcome. Debridement, antibiotics, irrigation, and prosthetic retention (DAIR) treatment may prevent infecting microorganisms from establishing a persistent chronic biofilm infection. If a chronic infection develops, total revision surgery is required for cure. However, surgery is not suitable for all patients and a lifelong suppressive antibiotic therapy with varying efficacy could be an alternative [1].

Evaluation of the treatment effect following DAIR in suspected persistent PJI is of utmost importance, especially in patients with ongoing antibiotic treatment where an early cessation of antibiotics would cause a relapse of infection with risk for the development of sepsis and chronic illness. The main reason for an early treatment failure is the establishment of a so-called biofilm protecting the bacteria from the innate immune system and antibiotics and thereby making the infection persistent [2,3].

To evaluate whether a treated infection is cured or has become persistent, a diagnostic tool such as nuclear

0143-3636 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/MNM.0000000000001403

imaging would be of great value. Its utility would be even greater in patients with inflammatory diseases such as rheumatoid arthritis, where clinical signs, for example, joint pain and elevated inflammatory markers often are present and could be misinterpreted as persisting PJI.

During the last three decades, different scintigraphic approaches were proposed for the diagnosis of PJI. Dual time-point acquisition protocol with leukocyte scintigraphy gained increased popularity and was recently proposed as the primary nuclear imaging method for PJI diagnosis [4,5]. This method uses reinjected radiolabeled autologous leukocytes and planar images that are obtained at two or more time points where an increased relative uptake in the joint over time is considered a sign of infection [6,7].

Another frequently applied approach is dual-tracer imaging where leukocyte scintigraphy is combined with bone marrow imaging to subtract unspecific post-surgically induced activity in activated or redistributed bone marrow [8]. This method is commonly used to complement inconclusive dual time-point leukocyte scintigraphy examinations. There is no reported direct comparison between dual time-point ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO)-leukocyte scintigraphy and dual-tracer imaging when ^{99m}Tc-HMPAO-leukocyte scintigraphy is combined with ^{99m}Tc-HMPAO-nanocolloid bone marrow scintigraphy for treatment evaluation of PJI [6].

Images can also be acquired in a three-dimensional mode using single-photon emission computed tomography (SPECT), commonly combined with a concomitant (often low-dose) computed tomography (CT) scan for additional anatomical detail. Only a few studies have been published in patients with orthopedic prostheses with this technique, generally showing increased sensitivity and/or additional information of anatomical details and location of uptake when SPECT was used compared to planar imaging [9–11].

An established biofilm affects leukocyte aggregation [12] which could influence the way the infection is visualized in scintigraphic images, emphasizing the need to evaluate all versions of leukocyte scintigraphy in patients with suspected persistent chronic PJI.

This study aims to compare the accuracy, sensitivity, and specificity of dual time-point ^{99m}Tc-HMPAO-leukocyte scintigraphy with dual-tracer imaging combining single time-point ^{99m}Tc-HMPAO-leukocyte scintigraphy and ^{99m}Tc-nanocolloid bone marrow scintigraphy using planar imaging or SPECT/CT for treatment evaluation of suspected persistent chronic PJI.

Methods

Study design

All patients referred for leukocyte scintigraphy with suspected hip or knee prosthetic infection between June 2010 and December 2013 at a single institution were screened for inclusion in this retrospective study. Inclusion criteria were verified PJI prior to scintigraphic imaging, completed scintigraphic imaging using both dual time-point and dual-tracer scintigraphy and SPECT/CT, on-going antibiotic treatment at the time of imaging, and 24 months of follow-up after the cessation of antibiotics. Patients with labeling efficacy below 40%, injected activity below 150 MBq were excluded.

The PJI verification followed international criteria [13]: the presence of a fistula from the joint and/or positive microbial culture(s) from tissue biopsies or aspirated joint fluid. The endpoint of this study was defined as either cured infection or relapse with new microbiological verification of infection after imaging. If the patient had only negative microbial cultures and no recurring symptoms of PJI 24 months after the cessation of antibiotic therapy, the infection was considered cured.

Ethical considerations

Ethical approval was given by the Regional Ethical Review Board ahead of patient screening and inclusion. All patients gave their informed consent to access their electronic medical records before in-depth screening and inclusion or exclusion.

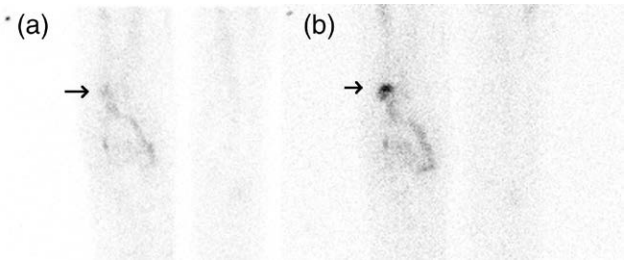
Imaging methods

^{99m}Tc-labeled white blood cells (^{99m}Tc-HMPAO-WBCs) were prepared following the guidelines of the European Association of Nuclear Medicine (EANM) without isolating granulocytes from lymphocytes [14].

Imaging was performed with low-energy high-resolution collimators on a dual-head SPECT/CT system (Siemens Symbia T16, Erlangen, Germany). Planar images were acquired for 2 min at 2 h post-injection using a 256 × 256 matrix and followed by SPECT/CT. The late planar images at 24 h post-injection were acquired for 25 min to account for decay and physiological clearance of the radiopharmaceutical.

^{99}Tc-HMPAO-WBC SPECT/CT was performed using 40 s per projection, 64 projections, step and shoot mode with

Fig. 1



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.

an acquisition matrix of 128×128 combined with an ultra-low dose CT (130 kV, 10 mAs, pitch factor 1.5). SPECT reconstruction, fusion, and interpretation were performed on a Hermes workstation (Hermes Medical solution AB, Stockholm, Sweden) using an ordered subset expectation maximization algorithm with four iterations, eight subsets, resolution recovery, and a Gaussian postfilter with 0.9 cm full width at half maximum. Attenuation correction was not performed as the CT contained metal artifacts. Fusion was made with 5 mm thick CT slices using a B08 kernel to reduce metal artifacts from the prosthesis.

The bone marrow examination was performed at least 72 h before or after the leukocyte scan as a 2-min static scan 1 h after injection of 500 MBq ^{99m}Tc-nanocolloid. ^{99m}Tc-nanocolloid SPECT/CT was performed with the same parameters as for ^{99}Tc-HMPAO-WBC.

Evaluation of images

The standard imaging protocol consisted of delayed and late leukocyte planar images, delayed leukocyte SPECT/CT images as well as bone marrow planar and SPECT/CT images. The images were divided into three settings with each patient being represented in all settings.

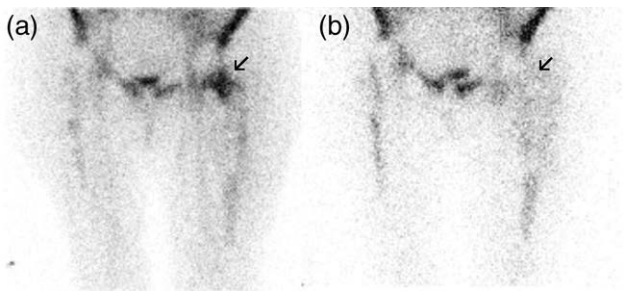
Setting one included planar ^{99m}Tc-HMPAO-WBC images at 2 and 24 h evaluating dual time-point leukocyte scintigraphy as shown in Fig. 1. For this setting, both visual and semiquantitative analyses were performed. The visual analysis was considered positive for infection if the uptake in the joint increased between the two time-points. For the semiquantitative analysis, the mean uptake in a region of interest (ROI) placed over the suspected infectious focus (lesion) was related to the mean uptake in a similar ROI on the contralateral side (reference) to calculate a lesion-to-reference ratio (L/R) for both the delayed and the late scan. An increase of L/R of 10% or more between the delayed and the late images was considered positive for infection [4].

Setting two included planar ^{99m}Tc-nanocolloid images and delayed planar ^{99m}Tc-HMPAO-WBC images at 2 h evaluating the dual-tracer approach as shown in Fig. 2. The examination was considered positive for infection if an increased ^{99m}Tc-HMPAO-WBC uptake was observed in the joint that was not matched by a similar uptake on the bone marrow images.

Setting three included ^{99m}Tc-nanocolloid SPECT/CT images and delayed ^{99m}Tc-HMPAO-WBC SPECT/CT images, for example, dual-tracer approach by tomographic imaging as shown in Fig. 3. The criteria for infection were identical to setting two except that SPECT/CT images were used instead of planar images.

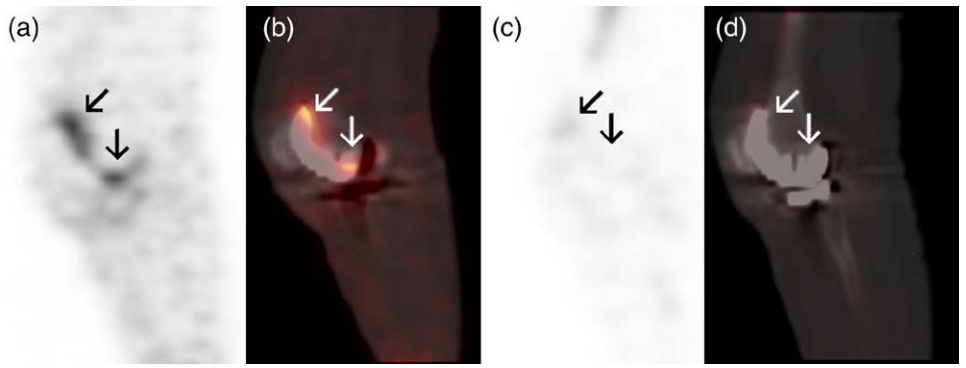
Two experienced nuclear medicine physicians with national board certifications in Nuclear Medicine and Radiology performed the analysis of the blinded investigations separately. In the case of diverging opinions, a consensus evaluation was made through discussion. For the semiquantitative evaluation of setting one, both observers measured the L/R and an average of the two ratios was used.

Fig. 2



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).

Fig. 3



Example of setting three with the examination positive for persistent infection. The increased uptake (arrows) on the ^{99m}Tc-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. CT, computed tomography; HMPAO, hexamethylpropylene amine oxime; SPECT, single-photon emission computed tomography; ^{99m}Tc-HMPAO-WBC, ^{99m}Tc-HMPAO-white blood cell.

Statistical analysis

For comparing sensitivity and specificity between the settings, McNemar's test was used with a 95% confidence interval and was considered significant when $P \leq 0.05$. Power calculations were performed using G*Power [15]. All other statistical analyses were performed with SPSS Statistics for Macintosh, version 26.0.

Results

A total of 139 patients with suspected PJI in the hip or knee were evaluated for inclusion in the study. One hundred eight patients were excluded, 52 due to lack of verification of infection (according to the definition) previous to imaging, 12 due to loss of follow-up because of death or moving to other region, 11 due to incomplete examination, 14 due to low labeling efficacy or low injected activity, 8 due to no on-going antibiotic treatment at the time of imaging and finally 11 due to difficult verification after the examination (for example, due to on-going suppression treatment or infection in different location). Thirty-one patients with 31 joints were included for further evaluation.

Table 1 Patient characteristics

Age	69 (67.6±10.2)
Male, *n* (%)	24 (77%)
Knee, *n* (%)	17 (55%)
Hip, *n* (%)	14 (45%)
Days with antibiotic therapy	123 (196±275)
Days since arthroplasty	615 (1815±2302)
Days since last surgery	208 (732±1328)
Cemented prosthesis	19 (61%)
Revision surgery	11 (35%)
Rheumatoid arthritis	5 (16%)
Diabetes mellitus	6 (19%)
Malignant disease	1 (3%)
Labelling efficacy	51% (42.1%±7.5%)
Injected activity (MBq)	305 (308±45)

Values reported as number and percentage or median (mean±SD).

The patients' characteristics are presented in Table 1.

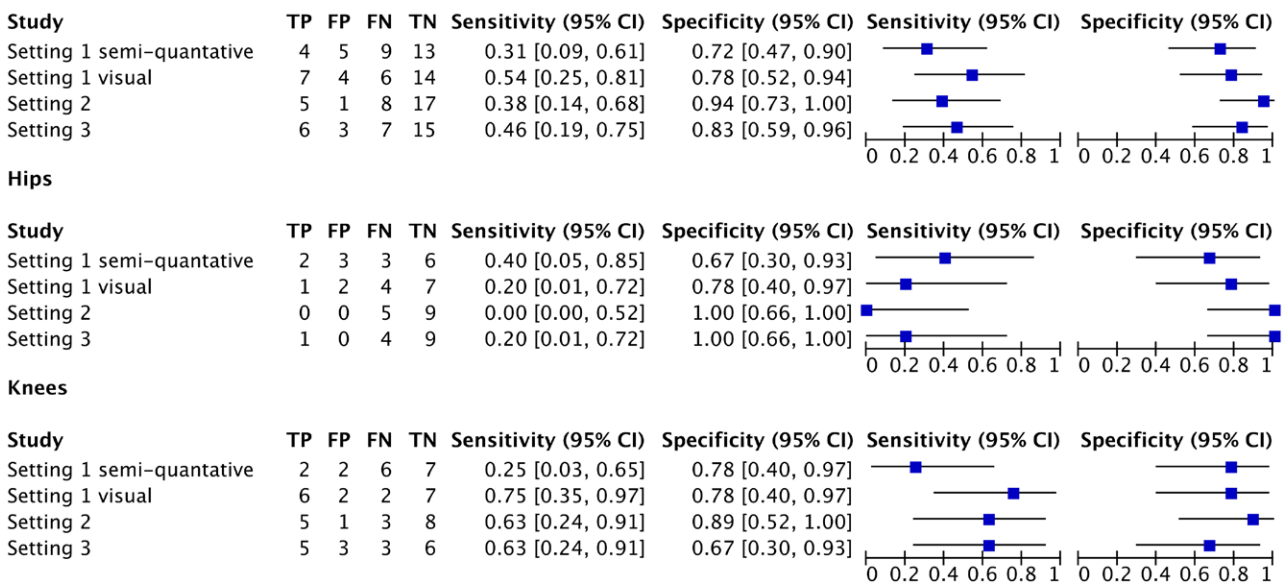
At last follow-up, at least two years after antibiotic cessation, 58 % had a cured infection and 42% a failure, that is, persistent chronic PJI.

Consensus evaluation was used for four joints in the visual evaluation of setting one, one joint in setting two, and five joints in setting three.

For the leukocyte scintigraphy, the injected activity was between 222 and 454 MBq. Labeling efficacy varied between 40 and 71%.

Accuracy was 0.68 for visual evaluation and 0.55 for semiquantitative evaluation of setting one; 0.71 for visual evaluation of setting two and 0.68 for visual evaluation of setting three. The results of all examinations and the sensitivity and specificity of the three settings are reported as a forest plot in Fig. 4. None of the differences in accuracy, sensitivity, or specificity were statistically significant.

Fig. 4



The results of all examinations with sensitivity and specificity were reported for all joints combined as well as hips and knees separately. FP, false positive; FN, false negative; TP, true positive; TN, true negative.

Discussion

The results of this study show average accuracy, mixed specificity, and generally poor sensitivity of all investigated approaches for evaluating chronic persistent PJI in patients on antibiotic treatment for previously diagnosed PJI of knee or hip. The dual-tracer approach performed using planar or tomographic imaging was not superior to the standard dual time-point leukocyte scintigraphy.

All methodological approaches evaluated in this study on chronic PJI have previously been investigated in patients either with early/acute or non-specified PJI with very promising results [16] that encouraged us to introduce the technique in our hospital. We found the method most valuable in patients with a suspected persistent chronic PJI and we, therefore, decided to perform this retrospective study on this group. Since the study period, the technique and protocol of leukocyte scintigraphy have improved [4,6,7].

Planar dual time-point leukocyte scintigraphy was included in our study as setting one, using a protocol similar to the fixed time protocol reported by Glaudemans *et al.* [7]. In previous studies, this method has shown sensitivity between 0.50 and 1.00 as well as specificity between 0.30 and 1.00 [6,7,9,17–21]. In our study, we used a protocol with a fixed longer acquisition time for the late images. The highest diagnostic accuracy using the method was reported in the study by Erba *et al.* [6], who reported a sensitivity of 0.93, a specificity of 1.00, and an accuracy of 0.98 in 84 patients with hip or knee prostheses. In their study, visual analysis of decay time-corrected scans had significantly better accuracy than visual analysis of fixed time scans and also than semiquantative evaluation regardless of which acquisition timing that was used. Based on that the accuracy of setting one may have been higher if we had used decay time-corrected scans as is the modern standard [4]. As their cohort is not presented in terms of acute or persistent PJI it is difficult to make a more accurate comparison.

Setting two, the planar dual-tracer approach, used bone marrow images combined with delayed leukocyte scintigraphy images as described by Palestro *et al.* [22]. The published data by Fuster *et al.* [18] and Jung *et al.* [21] have shown sensitivity of 0.92 and 1.00, specificity of 0.98 and 0.83 as well as accuracy of 0.97 and 0.91, respectively. Fuster *et al.* [18] showed very good results and had similar criteria as ours for established infection but excluded all patients with previously treated infection and on-going antibiotic treatment and therefore their patient cohort is not comparable with ours. Most of their patients presented with late infection, which would suggest that they had either a persistent chronic or an acute hematogenous PJI, the latter of which is associated with bacteremia and strong local inflammatory response that might explain a higher diagnostic yield.

The experience of hybrid imaging with SPECT/CT in patients with orthopedic prostheses is still limited to a few studies. Kim *et al.* [9] and Filippi and Schillaci [10] used this 3D technique with ^{99m}Tc-HMPAO-WBC-SPECT/CT and showed sensitivity of 0.93 and 1.00, respectively, and specificity of 0.93 and 1.00, respectively, as well as accuracy of 0.93 and 1.00, respectively. Tomographic acquisition was only used with the dual time-point approach. Kim *et al.* [9] report very good results in both hip and knee in 164 patients where 26% had received antibiotic treatment up to 2 weeks before the study. SPECT/CT was used as an addition to dual time-point leukocyte scintigraphy. However, all patients in their study presented with early/acute infection which, due to the higher inflammatory response would probably have been easier to detect than patients with chronic infection and ongoing antibiotic treatment.

The labeling efficacy of ^{99m}Tc-HMPAO-WBC scintigraphy is expected to be between 40 and 80%. The mean labeling efficacy in the present study was in the lower part of this interval (see Table 1.) A higher labeling efficacy had been desirable from a methodological point of view [14] and might partially explain our generally low specificity. However, as all patients were examined with all three settings the labeling efficacy and any confounders would have had an equal effect on all analyses.

The result of our study differs from the majority of the previously published literature. There are several possible explanations.

First, our applied strict criteria for verification of cured infection after imaging are stronger than the above-mentioned studies as we required either negative cultures from tissue biopsies or at least 24 months follow-up after cessation of antibiotics for defining an infection as cured. We also avoided using clinical evaluation as the sole criteria of PJI diagnosis and relied on a stricter and internationally accepted PJI confirmation such as positive bacterial cultures and/or fistula from the joint [13].

Second, all patients in this study were suspected to have a persistent chronic PJI, that is, having implants with colonized bacteria shielded by a biofilm influencing the innate immune system [9] and reducing leukocyte chemotaxis and thus modifying the expected increase in leukocyte uptake surrounding the prosthesis. Blanc *et al.* [19] recently presented a sensitivity of 0.72, a specificity of 0.60, and an accuracy of 0.67 for dual-time leukocyte scintigraphy on 150 patients with suspected chronic PJI. The similarity of our results suggests that the presence of a biofilm might lower the accuracy of the method compared to when it is used on acute infections.

Third, all patients in our study were under treatment with antibiotics. Antibiotic therapy has in other studies commonly been discontinued 14 days before a leukocyte scan [23]. This was not the clinical practice in our hospital for patients with suspected persistent chronic PJI and might explain the lower sensitivity of the scan. Antibiotics suppress bacteria, thereby reducing the

stimulus for leukocyte aggregation [24]. In the study by Blanc *et al*. [19], 37 patients were still on antibiotics at the time of the scan without any reported reduction of sensitivity or specificity. These results give support to examining patients without interrupting antibiotic treatment as we did in our study.

This study supports the recommendations of EANM in that planar dual time-point leukocyte scintigraphy should be the first alternative for nuclear medicine imaging of PJI. We find that replacing the standard dual time-point approach with dual-tracer approach cannot be motivated due to the increase in radiation dose to the patient with no significant increase in diagnostic yield. However, ^{99m}Tc-Nanocolloid bone marrow scintigraphy and SPECT/CT might be used as additive imaging in doubtful cases, as suggested by EANM [4].

In our opinion, nuclear imaging is primarily demanded in cases of suspected chronic PJI with difficulties in decision making between cessation of antibiotics and retainment of a prosthesis, prosthesis revision, or chronic suppressive antibiotic treatment. In light of our result, further development of nuclear medicine methods enabling the detection of persistent infection with the presence of biofilm and antibiotic treatment is warranted.

In conclusion, dual-tracer leukocyte scintigraphy using either planar imaging or SPECT/CT was not more sensitive, specific, or accurate than dual time-point leukocyte scintigraphy for treatment evaluation of suspected persistent chronic PJI.

Acknowledgements

The study was funded by a grant from the Stockholm County Council (ALF project 20140182). The funding body did not influence any part of the design of the study, collection, analysis or interpretation of data, or writing of the article.

Data presented previously at the 11th symposium of the Japanese Scandinavian Radiological Society and an abstract was published in the symposium programme.

Conflicts of interest

There are no conflicts of interest.

References

1 Zimmerli W. Clinical presentation and treatment of orthopaedic implant-associated infection. *J Intern Med* 2014; **276**:111–119.
2 Römling U, Kjelleberg S, Normark S, Nyman L, Uhlin BE, Åkerlund B. Microbial biofilm formation: a need to act. *J Intern Med* 2014; **276**:98–110.
3 Shoji MM, Chen AF. Biofilms in periprosthetic joint infections: a review of diagnostic modalities, current treatments, and future directions. *J Knee Surg* 2020; **33**:119–131.
4 Signore A, Jamar F, Israel O, Buscombe J, Martin-Comin J, Lazzeri E. Clinical indications, image acquisition and data interpretation for white blood cells and anti-granulocyte monoclonal antibody scintigraphy: an EANM procedural guideline. *Eur J Nucl Med Mol Imaging* 2018; **45**:1816–1831.
5 Signore A, Sconfienza LM, Borens O, Glaudemans AWJM, Cassar-Pullicino V, Trampuz A, *et al*. Consensus document for the diagnosis of prosthetic joint infections: a joint paper by the EANM, EBJIS, and ESR (with ESCMID endorsement). *Eur J Nucl Med Mol Imaging* 2019; **46**:971–988.
6 Erba PA, Glaudemans AW, Veltman NC, Sollini M, Pacilio M, Galli F, *et al*. Image acquisition and interpretation criteria for 99mTc-HMPAO-labelled white blood cell scintigraphy: results of a multicentre study. *Eur J Nucl Med Mol Imaging* 2014; **41**:615–623.
7 Glaudemans AW, de Vries EF, Vermeulen LE, Slart RH, Dierckx RA, Signore A. A large retrospective single-centre study to define the best image acquisition protocols and interpretation criteria for white blood cell scintigraphy with ^{99}mTc-HMPAO-labelled leucocytes in musculoskeletal infections. *Eur J Nucl Med Mol Imaging* 2013; **40**:1760–1769.
8 Palestro CJ, Kim CK, Swyer AJ, Capozzi JD, Solomon RW, Goldsmith SJ. Total-hip arthroplasty: periprosthetic indium-111-labeled leukocyte activity and complementary technetium-99m-sulfur colloid imaging in suspected infection. *J Nucl Med* 1990; **31**:1950–1955.
9 Kim HO, Na SJ, Oh SJ, Jung BS, Lee SH, Chang JS, *et al*. Usefulness of adding SPECT/CT to 99mTc-hexamethylpropylene amine oxime (HMPAO)-labeled leukocyte imaging for diagnosing prosthetic joint infections. *J Comput Assist Tomogr* 2014; **38**:313–319.
10 Filippi L, Schillaci O. Usefulness of hybrid SPECT/CT in 99mTc-HMPAO–labeled leukocyte scintigraphy for bone and joint infections. *J Nucl Med* 2006; **47**:1908–1913.
11 Weon YC, Yang SO, Choi YY, Shin JW, Ryu JS, Shin MJ, *et al*. Use of Tc-99m HMPAO leukocyte scans to evaluate bone infection: incremental value of additional SPECT images. *Clin Nucl Med* 2000; **25**:519–526.
12 Hirschfeld J. Dynamic interactions of neutrophils and biofilms. *J Oral Microbiol* 2014; **6**:26102.
13 Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, *et al*. New definition for periprosthetic joint infection: from the workgroup of the musculoskeletal infection society. *Clin Orthop Relat Res* 2011; **469**:2992–2994.
14 de Vries EF, Roca M, Jamar F, Israel O, Signore A. Guidelines for the labelling of leucocytes with (99m)Tc-HMPAO. Inflammation/infection task group of the European Association of Nuclear Medicine. *Eur J Nucl Med Mol Imaging* 2010; **37**:842–848.
15 Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; **39**:175–191.
16 Palestro CJ, Love C. Radionuclide imaging of musculoskeletal infection: conventional agents. *Semin Musculoskelet Radiol* 2007; **11**:335–352.
17 Glithero PR, Grigoris P, Harding LK, Hesslewood SR, McMinn DJ. White cell scans and infected joint replacements. Failure to detect chronic infection. *J Bone Joint Surg Br* 1993; **75**:371–374.
18 Fuster D, Duch J, Soriano A, Garcia S, Setoain X, Bori G, *et al*. Potential use of bone marrow scintigraphy in suspected prosthetic hip infection evaluated with 99mTc-HMPAO-leukocytes. *Revista Española de Medicina Nuclear* 2008; **27**:430–435.
19 Blanc P, Bonnet E, Giordano G, Monteil J, Salabert AS, Payoux P. The use of labelled leucocyte scintigraphy to evaluate chronic periprosthetic joint infections: a retrospective multicentre study on 168 patients. *Eur J Clin Microbiol Infect Dis* 2019; **38**:1625–1631.
20 Pelosi E, Baiocco C, Pennone M, Migliaretti G, Varetto T, Maiello A, *et al*. 99mTc-HMPAO-leukocyte scintigraphy in patients with symptomatic total hip or knee arthroplasty: improved diagnostic accuracy by means of semiquantitative evaluation. *J Nucl Med* 2004; **45**:438–444.
21 Jung KP, Park JS, Lee AY, Choi SJ, Lee SM, Bae SK. The clinical usefulness of (99m)Tc HMPAO Leukocyte/(99m)Tc phytate bone marrow scintigraphy for diagnosis of prosthetic knee infection: a preliminary study. *Nucl Med Mol Imaging* 2012; **46**:247–253.
22 Palestro CJ, Love C, Tronco GG, Tomas MB, Rini JN. Combined labeled leukocyte and technetium 99m sulfur colloid bone marrow imaging for diagnosing musculoskeletal infection. *Radiographics* 2006; **26**:859–870.
23 Glaudemans AW, Galli F, Pacilio M, Signore A. Leukocyte and bacteria imaging in prosthetic joint infection. *Eur Cell Mater* 2013; **25**:61–77.
24 Majeski JA, McClellan MA, Alexander JW. Evaluation of leukocyte chemotactic response in the presence of antibiotics. *Surg Forum* 1975; **26**:83–85.