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Diagnosis of infective endocarditis with nuclear medicine techniques: use of SPECT/CT with [^{99m}Tc]Tc-HMPAO-labelled leukocytes

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Abstract

According to European Society of Cardiology (ESC) guidelines, imaging techniques form an important part of infective endocarditis (IE) diagnostics. Several reports have shown promising results for radiolabeled white blood cell single photon emission computed tomography/computed tomography (SPECT/CT) scintigraphy, especially in patients with a high level of clinical suspicion of IE on a prosthetic valve but without a confirmation in microbiological and echocardiographic studies. SPECT/CT allows the assessment of localisation and extension of infection even early after the prosthesis implantation. In addition, the whole body imaging performed with radiolabeled leukocytes enables the detection of peripheral embolic and metastatic infectious events. SPECT/CT studies report high specificity in the diagnosis of prosthetic valve endocarditis (PVE) and also native valve endocarditis (NVE) and cardiac device-related infective endocarditis (CDRIE). However, to establish the diagnostic accuracy of SPECT/CT in PVE, NVE and CDRIE, further studies on large groups of patients are required, including groups with individual pathogenic bacteria. Also, further research on clinical usefulness of extracardiac findings should be carried on, as well as on the effect of antibiotics and other drugs on the results, and on a role in monitoring response to antimicrobial treatment. It should be pointed out that inflammation diagnostics with the use of autologous leucocytes radiolabeled in vitro is a highly specialized method. It requires highly qualified personnel for blood handling and radiopharmaceutical preparation, multiple and long lasting scintigraphic acquisitions, laborious and specialized analysis and interpretation of the results.

Key words: infective endocarditis, scintigraphy, radiolabeled leukocytes, [^{99m}Tc]Tc-HMPAO, SPECT/CT

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Introduction

Infective endocarditis (IE) is a life-threatening condition with a multitude of possible clinical presentations and a complex diagnostic process. Despite measures undertaken to prevent development of IE, its incidence has not decreased significantly over the recent years [1], and the mortality rate remains high. Early diagnosis and institution of effective therapy are critical for patient survival [2]. The diagnostic workup for IE is based on showing an association between symptoms of infection and the evidence for endocardial involvement. Currently, the established diagnostic approach to patients with a clinical suspicion of IE is to apply the modified Duke criteria which allow patient categorization to one of the three groups: definite IE, possible IE, and rejected IE. The diagnosis is based on the microbiological criteria (positive blood cultures or cultures from a vegetation/removed valve, serological testing) and clinical criteria, along with identification of lesions typical for IE in the imaging studies [3].

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In the diagnostic process, transthoracic echocardiography (TTE) and transoesophageal echocardiography (TOE) are the gold standard imaging techniques. However, standard diagnostic tools, i.e., microbiological testing and echocardiography, may not yield a clear diagnosis in many cases [4–7]. For this reason, additional imaging techniques including computed tomography (CT), positron emission tomography (PET), and single photon emission computed tomography (SPECT) have been incorporated in the most recent guidelines on the diagnosis and management of IE. In addition, three new diagnostic criteria have been added compared to the previous guidelines [3]:

1. Identification of perivalvular lesions in cardiac CT is considered a major criterion.
2. In suspected prosthetic valve endocarditis (PVE), abnormal periprosthetic inflammation activity detected by PET/CT using fluorodeoxyglucose labelled with fluorine-18 ([^{18}F]FDG) (only if the prosthetic valve was implanted more than 3 months ago) or by SPECT/CT using radiolabelled leukocytes should be considered a major criterion.
3. Confirmation of recent embolic events or an inflammatory aneurysm in the imaging studies only (i.e., clinically silent lesions) should be considered a minor criterion.

While microbiological testing and echocardiography combined with CT or less available and thus less frequently employed magnetic resonance imaging (MRI) are sufficient for the diagnosis in most cases of native valve endocarditis (NVE), echocardiographic and CT/MRI imaging may be inconclusive in patients with PVE, those with implanted cardiac devices (pacemakers, cardioverter-defibrillators, cardiac resychronization therapy devices), and at a very early disease stage. In addition, echocardiographic evaluation may be rendered difficult by acoustic shadowing in patients with severe vascular calcifications. Also, in the early postoperative period, symptoms of infection require differentiation from other causes of fever (mediastinal infection, sternal or postoperative wound infection).

Basic imaging modalities for the diagnosis of IE, i.e., TTE and TOE, as well as CT and MRI are based on the detection of specific morphological lesions, particularly vegetations, abscesses or pseudoaneurysms, or the presence of new leak. The need to acquire information about the activity of the inflammatory process, particularly in subacute IE, and to look for extracardiac inflammation foci suggests a role of molecular diagnostic modalities, SPECT/CT and PET/CT, which provide functional data on the presence of tissue inflammation.

The goal of the present review is to summarize the current recommendations and published study results regarding the use of SPECT/CT with radiolabelled leukocytes for the diagnosis of IE.

Principles of SPECT/CT with [^{99m}Tc]Tc-HMPAO-labelled leukocytes

Nuclear medicine techniques are used to evaluate organ function following administration of small amounts of specific radiopharmaceuticals. Gamma radiation emitted by the radioisotope component of the radiopharmaceutical is recorded by a gamma camera (classical scintigraphy) or a PET scanner, allowing visualization of spatiotemporal changes in radiotracer uptake.

Mechanism of leukocyte accumulation in infective endocarditis

The pathogenetic process underlying IE is bacterial colonization of the endocardium, mostly within the valves, but also involving the subvalvular apparatus, and at the sites of a ventricular or atrial septal defect (due to turbulent blood flow resulting in endothelial damage) [8]. Formation of a bacterial vegetation, which is the most characteristic lesion of IE, involves accumulation of plasma proteins, platelets, and fibrin at the site of endothelial damage, mediated by cytokines and tissue factors released by endothelial cells. Particularly predisposed are those endothelial structures that previously sustained damage due to rheumatic or degenerative changes, but also with direct activity of the circulating pathogens. The initial lesion is thus a sterile thrombus but concomitant bacteriaemia poses a risk for adhesion of circulating pathogens and development of a bacterial vegetation [9, 10]. Vascular changes develop at the site of inflammation, including vasodilatation and increased endothelial permeability, which enable migration of activated leukocytes from the microcirculation (diapedesis). Extravasated leukocytes accumulate at the site of inflammation by chemotaxis, migrating within the parenchyma towards the increased intensity of signals originating at the inflammation site (mostly bacterial products and cytokines). Further vegetation growth by accumulation of inflammatory cells and platelets leads to an increased risk of peripheral embolism, abscess formation, and valvular damage [11, 12].

The radiopharmaceutical employed in PET/CT is [^{18}F]FDG. Its intracellular uptake is related to the rate of intracellular glucose metabolism. This modality is used to identify inflammation foci due to increased glucose metabolism in activated inflammatory cells. In vivo, [^{18}F]FDG is extensively taken up by activated granulocytes, monocytes, macrophages and lymphocytes at the site of inflammation [13].

An increased number of neutrophils accumulating at the site of infection is the basis for the diagnostic use of scintigraphy with labelled leukocytes in IE.

Leukocyte tagging

Classical scintigraphy with leukocytes radiolabelled in vivo or in vitro has been used as a diagnostic modality in osteomyelitis, infections involving joints, joint prostheses and vascular grafts, chronic inflammation in the diabetic foot syndrome, and to investigate the cause of protracted fever of unknown origin [14]. While in vivo labelling with radioactive technetium-99m (^{99m}Tc) (using murine monoclonal antibodies or their fragments) has not been adequately documented in the investigation of IE [15], in vitro leukocyte labelling has been used for the diagnosis of IE since mid-1970s. For this purpose, indium-111 (^{111}In) radioisotope was initially used but over the years, it was nearly completely replaced with ^{99m}Tc due to its more favourable physical properties, lower cost, wider availability, and lower radiation dose for the patient.

Autologous leukocytes are tagged in vitro with [^{99m}Tc]Tc-hexamethylpropyleneamineoxime (HMPAO, exametazime), and then the cells are administered intravenously to the patient. Properly performed tagging does not impair the chemotaxic properties of the tagged leukocytes. [^{99m}Tc]-HMPAO is a lipophilic compound which passively diffuses through the leukocyte membranes and becomes intracellularly trapped by transformation into a hydrophilic compound and/or by binding to non-diffusible proteins and cellular organelles.

The study encompasses several steps. It does not require specific patient preparation. The first step is to collect about 50 mL of patient's blood and isolate leukocytes (by erythrocyte sedimentation and then separation of leukocytes from platelets by centrifugation). The second step is tagging of HMPAO with technetium-99m to obtain the radiopharmaceutical, [^{99m}Tc]Tc-HMPAO. The third step is in vitro leukocyte tagging with [^{99m}Tc]Tc-HMPAO. The fourth step is leukocyte reinjection by intravenous administration of the tagged leukocytes to the patient. Finally, images are acquired within the set temporal and structural settings and then subjected to specialist interpretation.

Image acquisition

Digital acquisition of scintigraphic images is also a multi-step process. Due to varying biodistribution of administered leukocytes in blood, bone marrow, sites of infection, and sterile inflammation foci, radiotracer uptake images are recorded at three intervals from the injection of tagged leukocytes: early images at 30 minutes, delayed images at 3–6 hours, and late images at 20–24 hours.

The 30-minute image (planar chest image in the anteroposterior and posteroanterior views, encompassing the lungs, liver, and spleen) serves as a leukocyte tagging quality control: lung uptake should be low and homogeneous (without local signal increases corresponding to aggregates of administered tagged leukocytes), and spleen uptake should be at least 2 times higher compared to liver uptake. The cycle of delayed scintigraphic image acquisitions in patients with suspected IE includes planar chest image acquisition, SPECT/CT chest image acquisition, and whole body (WB) imaging. WB imaging is performed to detect possible septic emboli (often accompanying IE and caused by fragments of bacterial vegetations forming on the heart valves) [3] and/or other inflammatory foci. If WB imaging suggests foci outside the chest, additional SPECT/CT imaging of the suspicious areas is usually performed. Late acquisitions (at 20–24 hours) are most commonly a replication of acquisitions performed at 3–6 hours [15]. During all acquisitions, the patient is placed supine and lays still on the examination table for 5 to 30 minutes, with hands along the torso or behind the head depending on the type of examination. By combining SPECT and CT technologies, SPECT/CT allows precise anatomical determination of radiotracer uptake sites. In hybrid SPECT/CT machines, X radiation from multidetector row CT (also used for the purpose of correcting gamma radiation attenuation in the patient body) is employed to localize SPECT imaging findings.

Evaluation of scintigraphic findings

Evaluation of scintigraphic findings requires an extensive experience from the nuclear medicine specialist. Difficulties with interpretation result from radiotracer accumulation that is not specific for inflammation, mostly within the gastrointestinal tract, urinary system, lungs, liver, and spleen.

The diagnosis of infection is based on the comparison of delayed and late images. Inflammation in a given location is evidenced by a characteristic pattern of increasing radiotracer activity in serially acquired images — areas of infection should be visible on delayed images, with further leukocyte accumulation on late images. Regarding the diagnosis of IE, the scintigraphic findings may be considered positive if increasing radiotracer uptake is found in the cardiac area. The result is negative when no abnormal uptake is seen in the cardiac area, and unequivocal when stable or decreasing radiotracer uptake is seen [15].

The sensitivity of SPECT/CT with labelled leukocytes depends on neutrophil accumulation at the site of an infection and is highest in the acute inflammation phase [16]. In individuals with leukopenia and neutropenia, the result may be non-diagnostic despite an appropriate testing procedure.

Diagnostic workup for IE according to the 2015 European Society of Cardiology (ESC) guidelines

According to the current diagnostic approach based on the modified Duke criteria (Table 1), the diagnosis is based on microbiological testing and echocardiography, the sensitivity of which for NVE and PVE has been estimated

Table 1. Definitions of the terms used in the European Society of Cardiology 2015 modified criteria for the diagnosis of infective endocarditis (based on [3])

I. Major criteria
1. **Blood cultures positive for IE**
• Typical microorganisms consistent with IE from 2 separate blood cultures: *Viridans streptococci*, *Streptococcus gallolyticus* (*Streptococcus bovis*), HACEK group, *Staphylococcus aureus*; or community-acquired enterococci, in the absence of a primary focus
• Microorganisms consistent with IE from persistently positive blood cultures: ≥ 2 positive blood cultures of blood samples drawn > 12 h apart; *or* All of 3 or a majority of ≥ 4 separate cultures of blood (with first and last samples drawn ≥ 1 h apart)
• Single positive blood culture for *Coxiella burnetii* or phase I IgG antibody titre > 1:800
2. **Imaging positive for IE**
• Echocardiogram positive for IE: Vegetation; abscess, pseudoaneurysm, intracardiac valvular perforation or aneurysm; new partial dehiscence of prosthetic valve
• Abnormal activity around the site of prosthetic valve implantation detected by [^{18}F]FDG PET/CT (only if the prosthesis was implanted for > 3 months) or SPECT/CT with radiolabelled leukocytes
• Definite paravalvular lesions by cardiac CT
II. Minor criteria
1. Predisposition such as predisposing heart condition, or injection drug use
2. Fever defined as temperature > 38°C
3. Vascular phenomena (including those detected by imaging only): major arterial emboli, septic pulmonary infarcts, infectious (mycotic) aneurysm, intracranial haemorrhage, conjunctival haemorrhages, and Janeway's lesions
4. Immunological phenomena: glomerulonephritis, Osler's nodes, Roth's spots, and rheumatoid factor
5. Microbiological evidence: positive blood culture but does not meet a major criterion as noted above or serological evidence of active infection with organism consistent with IE

CT — computed tomography; FDG — fluorodeoxyglucose; HACEK — *Haemophilus parainfluenzae*, *H. aphrophilus*, *H. paraphrophilus*, *H. influenzae*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella kingae*, and *K. denitrificans*; IE — infective endocarditis; Ig — immunoglobulin; PET — positron emission tomography; SPECT — single photon emission computed tomography

at 70% and 50%, respectively, for TTE, and 96% and 92%, respectively, for TOE [17]. The specificity of both TTE and TOE has been estimated at about 90%. Identification of such lesions as vegetations, abscess, pseudoaneurysm or new perivalvular risk constitutes a major criterion for the diagnosis of IE.

Normal echocardiographic findings do not exclude IE. Degenerative valvular changes, primary native valve disease, and the presence of a valve prosthesis or an implantable device hinder visualization of valvular lesions. Another challenge for the echocardiographer may be the need to differentiate with thrombi, Lambl excrescences, tumors, e.g., fibroelastoma, and non-infectious lesions (e.g., Libman-Sacks endocarditis). In these situations, additional diagnostic modalities should be employed to arrive at the proper diagnosis.

According to the current guidelines, a nuclear medicine modality (PET/CT or SPECT/CT) should be considered in patients with suspected PVE who have been categorized as possible or rejected IE based on the modified Duke criteria but in whom the clinical presentation strongly suggests endocardial involvement (Figure 1) [3]. This is justified by the added value of these investigations compared to the conventional echocardiographic imaging, resulting in an increased sensitivity of the modified Duke criteria.

Incorporation of nuclear medicine modalities to the diagnostic algorithms in IE has been generally based on three clinical studies that evaluated the utility of SPECT/CT and PET/CT and showed a significant role of these modalities, particularly in the diagnosis of PVE [18–20]. The number of patients in these study groups was low — 131 patients with suspected NVE or PVE (the proportions were not disclosed), and 39 and 72 patients with suspected PVE, respectively.

In the 2012 study by Erba et al. [18], SPECT/CT with [^{99m}Tc]Tc-HMPAO-labelled leukocytes was performed in 131 patients with suspected IE, of whom 51 ultimately received the diagnosis of IE based on the microbiological and clinical criteria. The study included patients with biological and mechanical valve prostheses, and those with suspected NVE. SPECT/CT findings were true positive in 46 and false negative in 5 of 51 patients with IE (sensitivity 90%), while no false positive findings were noted (specificity 100%). Positive SPECT/CT findings with increased radiotracer uptake only within the heart were noted in 23 of 51 patients, while both intracardiac and remote foci were

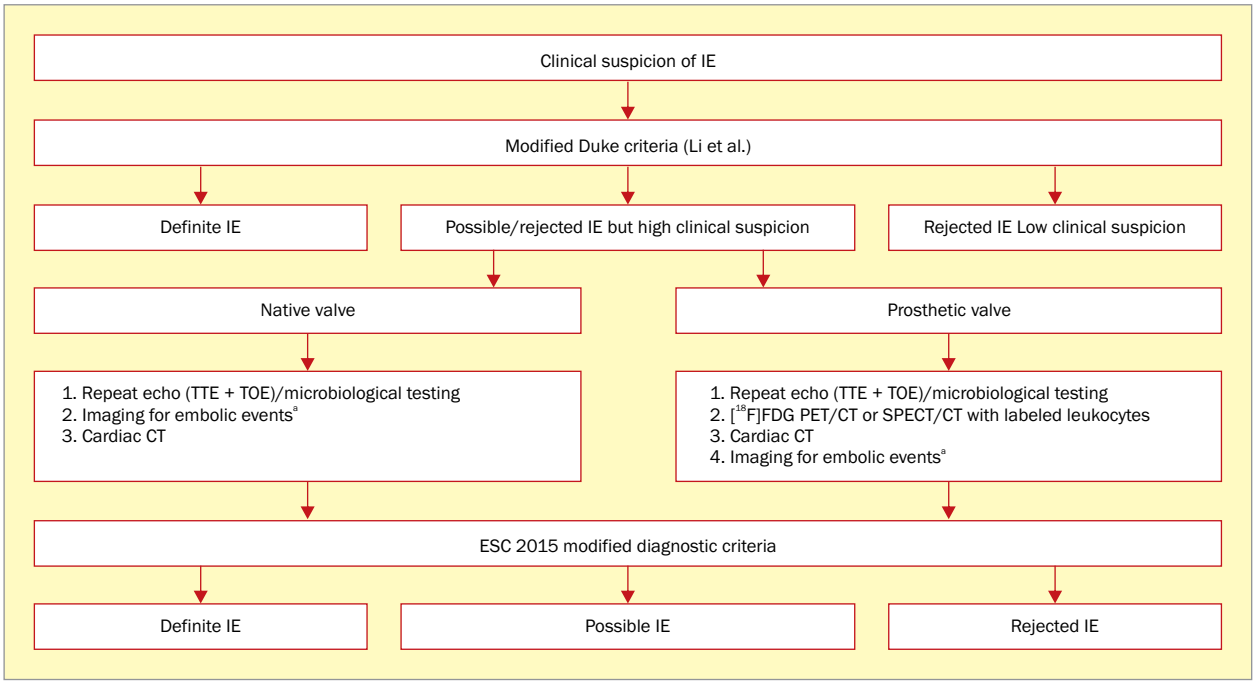

Figure 1. The European Society of Cardiology (ESC) 2015 algorithm for the diagnosis of infective endocarditis (IE) (based on [3]); [a]May include cerebral magnetic resonance imaging, whole body CT, and/or PET/CT; CT — computed tomography; FDG — fluorodeoxyglucose; PET — positron emission tomography; SPECT — single photon emission computed tomography; TOE — transoesophageal echocardiography; TTE — transthoracic echocardiography

found in the remaining 23 patients with positive SPECT/CT findings. This modality was particularly useful in patients with negative or equivocal echocardiographic findings, as SPECT/CT allowed the diagnosis of IE in 11 of 88 such patients. In addition, in 3 patients with false positive echocardiographic findings, SPECT/CT showed no infectious foci, which highlights the possibility of employing scintigraphy to clarify the nature of valvular lesions identified by echocardiography (infected versus sterile vegetations).

In the 2014 study, Rouzet et al. [19] compared two nuclear medicine modalities in the investigation of IE: [^{18}F]FDG PET/CT and [^{99m}Tc]Tc-HMPAO SPECT/CT [19]. The study group (39 patients) was limited to patients with suspected PVE in whom the diagnosis was made based on the clinical grounds and echocardiography was equivocal. Ultimately, based on clinical and pathological criteria, 14 patients were categorized as definite IE, 4 as possible IE, and 21 as rejected IE. The sensitivity and specificity for SPECT/CT was estimated at 64% and 100%, respectively, and for PET/CT at 93% and 71%, respectively. Discordant PET/CT and SPECT/CT findings were noted in 12 patients. In the definite IE category, 5 patients had a true positive PET/CT result and a false negative SPECT/CT result (in 3 of them, a non-pyogenic *Coxiella* or *Candida* infection was found). In the rejected IE category, 6 patients had a true negative SPECT/CT result and a false positive PET/CT result (all these patients were examined within first 2 months after a surgical procedure). The study findings confirmed high specificity of scintigraphy with labelled leukocytes as reported in previous studies. In particular, a role of SPECT/CT in patients with suspected IE in the early postoperative period was highlighted, particularly during the first 2 months when [^{18}F]FDG PET/CT may give false positive results.

In both these studies [18, 19], no false positive [^{99m}Tc]Tc-HMPAO SPECT/CT results were obtained, indicating a very high specificity of this technique in the investigation of IE.

Another study that was included in the ESC guidelines was the 2013 paper by Saby et al. [20], focusing on the use of PET/CT in the investigation of PVE. An added value of this technique was shown, as it increased the sensitivity of the modified Duke criteria for the diagnosis of IE from 70% to 97% by reducing the number of patients categorized as possible IE. The role of this modality in the diagnosis of peripheral septic embolism was also highlighted.

Peripheral embolism

Peripheral embolism is a major complication of IE which is associated with worse outcomes. It occurs in 10–50% of IE cases, and the risk increases with larger vegetations (> 10 mm) and their mobility. The most common location of embolism is the central nervous system — occurring in

20–40% of all IE cases — followed by the lungs, peripheral arteries, spleen, and kidneys. Very rarely, ocular and coronary artery embolism occurs [21, 22]. The highest risk of peripheral embolism is observed within the first 2 weeks of antibiotic therapy. A factor increasing the risk of recurrent embolism during treatment is the occurrence of an embolic event before the initiation of antimicrobial therapy [23]. Due to complex symptomatology of IE, embolism may be sometimes the initial presentation of the disease, and if embolism is found in a patient with fever, cardiac lesions should always be sought. On the other hand, nearly half of embolic events, particularly in the spleen, may remain asymptomatic and peripheral embolism is identified only after further diagnostic workup of fever with positive blood cultures. An active search for primary and secondary infection foci is critical before planning the surgery. If not all infection foci are identified, microbial dissemination may reoccur after the therapy is finished.

According to the current guidelines on the diagnosis and management of IE [3], imaging for embolism may include brain MRI, whole body CT and/or PET/CT. However, the protocol of imaging using leukocytes labelled in vitro does include WB imaging (and chest SPECT/CT). Thus, the published studies on cardiac SPECT/CT often include information about the foci of increased, non-physiological accumulation of labelled leukocytes in other body areas.

In the 2012 study by Erba et al. [18], in which SPECT/CT was true positive in 46 of 51 patients with the diagnosis of IE, both intracardiac and remote inflammation foci were identified in 23 patients (including 20 remote foci corresponding to septic emboli related to IE, and 3 remote foci that were considered false positives). In a later study that compared the clinical utility of [^{99m}Tc]Tc-HMPAO SPECT/CT and [^{18}F]FDG PET/CT for the identification of extracardiac infection foci, SPECT/CT scintigraphy was found to be inferior [24]. In the group of 55 patients with confirmed IE (involving a native valve, valve prosthesis, or an implanted cardiac device), SPECT/CT identified 37 inflammation foci in 24 patients, and PET/CT identified 91 foci in 32 patients (these were likely all foci, including those within the heart). However, these results for SPECT/CT were obtained with the methodology that is currently unacceptable, using general purpose collimators (currently replaced with high resolution collimators, used since 2018) and performing image acquisition at 1 and 3 hours (while the current protocol calls for image acquisition at 3–6 hours and importantly also at 20–24 hours) [15, 25].

In a study by Holcman et al. [26], published in 2019, that targeted mostly patients with implantable cardiac devices (80%), with the diagnosis of IE ultimately made in 14 of 40 patients, extracardiac foci were identified in 19 patients (47.5%), including 7 of 16 patients with intracardiac foci and in 12 of 24 patients without intracardiac foci. Extracardiac foci were most commonly identified in the gastrointestinal tract (47%), bones (16%), respiratory system (11%), and urinary system (5%), but the clinical significance of these findings was not determined.

Evaluation of the clinical significance of extracardiac foci detected during investigations for IE using leukocytes labelled in vitro requires further systematic studies (in previous studies, extracardiac foci were secondary endpoints only) and collecting more precise data on WB imaging.

Recent publications regarding the use of leukocytes labelled in vitro in the investigation of IE (including IE related to implanted cardiac devices)

Since incorporation of nuclear medicine techniques into the investigation for IE in the most recent 2015 ESC guidelines, further studies have been performed to evaluate the clinical utility of SPECT/CT and PET/CT and the use of these modalities in clinical practice. The study groups increasingly include patients with cardiovascular implantable electronic devices (CIED) in whom an additional challenge is the need to differentiate between a local inflammatory process limited to the area of an implanted device and cardiac device-related infective endocarditis (CDRIE). Infections related to an implantable device are a growing problem, particularly among the elderly patients [27], and are associated with potentially dangerous complications. Local infectious process is limited to the device pocket and extravascular lead segments, while CDRIE is defined as an infection involving intravascular and intracardiac lead segments. Proper diagnosis affects further management and therapy, and thus it is important to define the extent of the infectious process [3]. Echocardiography remains the basic diagnostic tool in suspected CDRIE but due to limited visualization related to possible acoustic shadowing, similarly to valve prostheses, use of other imaging modalities is warranted. In 2013, Erba et al. [28] performed SPECT/CT in 63 patients with suspected infection related to CIED and reported the sensitivity of 94% and specificity of 100%.

A metaanalysis of studies regarding the use of SPECT/CT with labelled leukocytes in the investigation of IE included 4 studies, including the 2012 study by Erba et al. [18] and the 2014 study by Rouzet et al. [19] discussed above (overall, 246 patients with suspected IE) [29]. The sensitivity was estimated at 86%, and the specificity at 97%. This metaanalysis included patients with suspected NVE, PVE, and CDRIE. In the 2018 recommendations on nuclear and hybrid imaging, Erba et al. [25] indicated that the specificity of SPECT/CT in suspected CDRIE was similarly high as in suspected NVE and PVE. The overall sensitivity based on, among others, the metaanalysis of Juneau et al. [29], has been estimated at 80–86%, and the specificity at 97–100%.

A study by Holcman et al. [26], published in 2019, that compared the diagnostic performance of echocardiography

and [^{99m}Tc]Tc-HMPAO SPECT/CT in the investigation of IE, included mostly patients with implantable cardiac devices (80%) and patients with a suspected infection involving a native or prosthetic valve (22.5%). The diagnosis of IE was ultimately made (based on microbiological and clinical criteria) in 14 of 40 patients (35%), infection of the device pocket was diagnosed in 2 patients, and IE was excluded in 24 patients. In this study, the sensitivity and specificity of SPECT/CT for the diagnosis of IE was 93% and 88%, respectively (higher sensitivity but lower specificity compared to previous studies), while the sensitivity and specificity of TTE was 93% and 42%, respectively. Adding SPECT/CT to the diagnostic algorithm reduced the number of false positive diagnoses compared to echocardiography only (3 patients vs. 15 patients). In another study by the same authors, the study group was limited to patients with a suspicion of IE related to the lead of an implanted cardiac device, in whom a lead-related mass was identified by TTE or TOE [30]. The study included 40 patients. A true positive SPECT/CT result was obtained in 14 patients, and a false negative result in 5 patients (sensitivity of 74% and specificity of 81%, lower than in the studies by Erba et al.). All false negative results were obtained in patients who received antibiotic therapy. When these patients were excluded, the sensitivity increased to 100%. The authors highlighted the role of SPECT/CT in the differentiation between bacterial vegetations and thrombi when a lead-related mass is identified by echocardiography. A notable finding was also an association between antibiotic therapy and false negative SPECT/CT results.

Effect of medications and other mechanisms on the diagnostic utility of testing with leukocytes labelled with [^{99m}Tc]Tc-HMPAO

In the currently available literature, there is no clear evidence of the effect of antibiotic therapy on leukocyte migration to the infection site, although many authors indicated a role of antibiotic therapy in reducing the sensitivity of scintigraphy [15, 18, 24, 30, 31]. In the study by Erba et al. [18], antibiotic use in high doses during the testing was indicated as one likely cause of false negative SPECT/CT results. On the other hand, a significant number of patients had a positive scintigraphy result despite negative blood culture results. Definite assessment of the effect of antibiotic therapy on the sensitivity of scintigraphy requires further studies. According to the current guidelines, concomitant antibiotic therapy is not a contraindication for testing.

There are also reports in the literature on the effect of other medications on the efficiency of leukocyte tagging, and thus on the sensitivity of scintigraphy with labelled leukocytes. Possible interactions with cephalosporins, azathioprine, prednisolone, cyclophosphamide, nifedipine, sulfasalazine, naproxen, ranitidine, iron salts, and heparin have been suggested. Although a direct causal effect has not been established, known adverse effects of the above medications and their effect on the function of blood components may affect the efficiency of leukocyte tagging [32, 33].

Other possible causes of false negative SPECT/CT results include the aetiology of infection and the ability of some pathogens to form a biofilm (e.g., *Candida spp*, *Enterococcus spp*, *Staphylococcus epidermidis*). Biofilm is a complex multi-layer bacterial cellular structure which reduces their penetration by inflammatory cells and complement components, this helping these pathogens avoid host defence mechanisms.

In addition, a chronic inflammatory condition resulting from the ability of microorganisms to persist on the endothelial surface without inducing an acute inflammatory response of the immune system reduces neutrophil accumulation in a given location [34].

In case of *Candida* infections, a key factor is the type of cells accumulating at the site of inflammation. It has been indicated that monocytes and lymphocytes predominate over neutrophils in the inflammatory infiltrate, which may result in a reduced sensitivity of SPECT/CT [35].

Defence mechanisms specific for particular bacterial species are also known, such as extracellular proteases produced by *Enterococcus faecalis* which may modulate the host defence response, leading to a reduced circulating neutrophil recruitment at the site of infection [36].

Diagnostic utility of testing with leukocytes labelled with [^{99m}Tc]Tc-HMPAO in IE — summary

Based on the 2018 metaanalysis, the overall diagnostic performance of SPECT/CT in the investigation of IE in patients with suspected NVE, PVE and CDRIE was estimated as follows [29]:

- sensitivity: 86% (95% confidence interval [CI] 77–92%);
- specificity: 97% (95% CI 92–99%).

Regarding infections related to CIED, the diagnostic performance of SPECT/CT was estimated as follows [28]:

- sensitivity: 94% (95% CI: 84–98%);
- specificity: 100% (95% CI: 93–100%).

The sensitivity and specificity values reported in patients with suspected NVE, PVE or CDRIE in individual studies discussed in the present review were in the ranges of 64–94% and 81–100%, respectively.

Reduced sensitivity of the technique, provided that leukocyte labelling was properly performed, may result, among others, from:

- reduced overall leukocyte count in the patient, also due to long-term antibiotic therapy before the testing

(lymphocyte accumulation relatively prevailing over granulocyte accumulation with increasing treatment duration);
— the aetiology of the ongoing infection (lower sensitivity, e.g., in *Candida spp., Enterococcus spp.*, and *Staphylococcus epidermidis* infections).

The technique has also other limitations, the most important of which are:
— time-consuming preparation of leukocytes labelled in vitro;
— need for a direct contact with patient blood preparations;
— patient exposure to ionizing radiation.

Of note, use of radioisotope imaging techniques with leukocytes labelled in vitro in the investigation of inflammatory processes is a highly complex diagnostic approach. It requires a high level of personnel expertise regarding leukocyte isolation, radioisotope tagging and readministration; necessitates compliance with the timing constraints at each step of the procedure. In addition image acquisition must be performed over a long period (in addition to baseline quality control image acquisition at 30 minutes, images are recorded at 3–6 hours and then at 20–24 hours after administration of labelled leukocytes). Visual data processing and analysis of multiple images for specific scintigraphic criteria indicating the presence of an inflammatory process are a complex and time-consuming procedure. Additional competencies are needed for SPECT and CT image fusion, required for determination of the anatomical location of scintigraphic changes characteristic for inflammation.

Areas requiring further studies:
— evaluation of the sensitivity and specificity of SPECT/CT in the investigation of NVE, PVE, and CDRIE in large patient populations (feasible in multicentre studies with established central image analysis systems);
— more definite assessment of the effect of antibiotic therapy on the testing results;
— evaluation of the sensitivity and specificity of the technique in regard to specific pathogens;
— evaluation of the prognostic value of the testing [37];
— evaluation of the clinical significance of detected extracardiac foci;
— determination of the potential role of SPECT/CT in the monitoring of anti-inflammatory treatment efficacy.

Conflict of interest

The authors declare no conflict of interest.

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