Leucocyte Scintigraphy

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1. Introduction

An infection starts with colonization of tissue or organs by pathogenic exogenous noxae. In many cases, microorganisms are considered to be the cause of an infection. Tissues or organs react with an associated phenomenon, called acute inflammation. The presence of non-self-antigens or tissue degradation products activates mechanisms, such as the release of histamine and serotonin, increase of vascular permeability, hyper-expression of adhesion molecules on endothelial cells and secretion of chemotactic factors. All of these phenomena induce leukocyte rolling along the endothelium and migration of these leukocytes through the capillary wall.

Scintigraphy using radiolabelled white blood cells (WBCs) was developed in the 1970s. It was used for years in a variety of different clinical situations and is still considered the gold standard nuclear imaging technique to diagnose infections.

Leucocytes are isolated from the patient's blood and labelled with ^{111}In-oxine or ^{99m}Tc-exametazine (^{99m}Tc-HMPAO). Autologous leucocytes are preferred. Autologous leucocytes are characterized by high specificity, because they accumulate only as a consequence of active migration into infectious tissues. After intravenous reinjection, radiolabelled WBCs show rapid clearance from the lungs and blood pool, with progressive migration into the spleen, liver, bone marrow and into sites of infection where a neutrophilic infiltrate predominates. Radiolabelled WBCs are a specific indicator for leucocytic infiltration. ^{99m}Tc-labelled leucocytes have replaced ^{111}In-labelled leucocytes for most indications, because of the more optimal physical characteristics, availability, costs and lower radiation burden. However, an advantage of ^{111}In-leucocytes is that there is no major kidney, bladder or bowel excretion. In contrast, ^{99m}Tc-exametazine is released from the leucocytes, which starts a few minutes after administration. Up to 7% per hour of the released Tc is excreted by the kidneys. The remainder is excreted by the liver and gut, thereby disturbing the imaging of the abdomen at 3 h post administration. Therefore, the use of ^{111}In-leucocytes is preferred for evaluation of the kidneys, bladder, gall bladder and intestines. For all other indications, ^{99m}Tc-leucocytes are preferable.

Either isolated granulocytes or mixed leucocytes can be used. Labelling of mixed leucocytes is preferred, despite the fact that this may cause higher uptake in the blood pool on the 4 h images due to the presence of labelled erythrocytes. Labelling of mixed leucocytes causes irradiation damage to the lymphocytes as a result of self-irradiation by low-energy Auger electrons. However, this damage is considered negligible.

2. Methodology

This guideline is based on available scientific literature on the topic, the previous guideline (Aanbevelingen Nucleaire Geneeskunde 2007), international guidelines from EANM and/or SNMMI if available and applicable to the Dutch situation.

3. Indications

a. Osteomyelitis of the appendicular skeleton
b. Infected joint prosthesis and other orthopaedic hardware
c. Diabetic foot infection
d. Inflammatory bowel diseases
e. Soft tissue infections (postoperative infections, cardiovascular infections)
f. Vascular graft infections
g. Endocarditis
h. Fever of unknown origin
i. Pulmonary infections
j. Central nervous system infections

4. Relation to other diagnostic procedures

Depending on the above mentioned indications, radiological techniques, such as ultrasound, CT and MRI play an important role, complemented by radionuclide imaging procedures. Furthermore, for some indications ^{18}F-FDG-PET may be the nuclear imaging modality of choice (e.g. patients with fever of unknown origin with low probability of infection, vascular graft infections, endocarditis). In patients with suspicion of osteomyelitis and in patients with suspected joint prosthesis infection (> 2 years after placement of a hip prosthesis and > 4 years after placement of a knee prosthesis) three phase bone scintigraphy with spect-ct may be the first choice.

5. Medical information necessary for planning

a. Probability of an infection (low or high) and expected location
b. Symptoms and complaints
c. Infection parameters: leucocyte count, ESR, CRP
d. Results of other relevant, recently performed diagnostic imaging
e. Presence of joint prosthesis or other orthopaedic hardware and date of insertion
f. Presence of a vascular graft, date of insertion and type of graft
g. Use of antibiotics

6. Radiopharmaceutical

Tracer:	^{99m}Tc-exametazine or ^{111}In-oxine labelled leucocytes
Nuclide:	Technetium-99m or Indium-111
Activity:	370-740 MBq ^{99m}Tc-exametazine, 10-18,5 MBq ^{111}In-oxine
Administration:	intravenous re-injection. Since the investigation involves blood/blood products, it is essential that correct patient identification procedures are adhered to in order to avoid sample identification errors.

7. Radiation safety

During the labelling procedure, blood and blood elements from a patient, who could potentially be infected with pathogens, need to be handled with care. To prevent contamination of the operator who is performing the labelling, waterproof gloves should be worn throughout the procedure. Since the labelled leucocytes have to be re-injected into the patient, strict aseptic conditions are required for the labelling procedure. For this purpose, only sterile reagents and disposable plastic-ware should be used. Sterile gloves,

cap and mask should be worn. Usually, the labelling of leucocytes is performed in a laminar flow cabinet or cell isolator, installed according to local regulations. Simultaneous labelling of leucocytes from multiple patients is discouraged in order to prevent cross-contamination. Labelling of leucocytes from different patients should be carried out at physically separated locations unless closed devices are used. At all times correct identification of the patient's blood products should be guaranteed. All syringes, tubes and other material which comes in contact with the patient's blood components should be clearly labelled with the patient's name, bar-code and/or colour code.

8. Patient preparation/essentials for procedure

a. The patient should fast prior to the investigation in order to facilitate the isolation of leucocytes (better separation of WBC band and plasma). The patient may eat and drink again once the blood sample has been taken.
b. The potential interference of antibiotics with cell labelling has to be considered. However, patients receiving antibiotic treatment should not be excluded since reports regarding their effect on WBC scintigraphy give varying results. The decision whether to perform or cancel the study depends entirely on the clinical setting and must be discussed case-by-case with the referring clinician.
c. Using a large-bore needle (1,2 mm or 19 gauge), a 50-60 ml sample of venous blood is obtained from the patient without using a tourniquet. After labelling, the leucocyte suspension is returned to the patient intravenously using a similar needle.
d. During the labelling of WBC with ^{99m}Tc-HMPAO care should be taken not to damage the leucocytes, as this will result in leakage of the radioactivity from the cells, followed by adhesion of labelled leucocytes to the vascular endothelium (especially in the microvasculature of the lungs) and loss of motility. To avoid degradation of the radiopharmaceutical and radiation damage to labelled cells, ^{99m}Tc-HMPAO-labelled WBC should be re-injected as soon as possible, and certainly no later than 1 h after labelling.
e. For labelling procedures and quality controls: see the Radiopharmacy part of these recommendations or the EANM guidelines for the labelling of leucocytes (reference list).

9. Acquisition and processing

Camera

^{99m}Tc:

Large-field-of-view gamma camera with a low-energy high-resolution collimator.

^{111}In:

Large-field-of-view camera with a medium energy collimator. Both ^{111}In photopeaks should be acquired, 173 and 245 keV (±10%).

Imaging time

^{99m}Tc:

Due to the different bio-distribution of labelled WBC in blood, bone marrow, infection and sterile inflammation, 3 sets of images are recommended:

- Early images (within 30 min and 1 h post-injection)
- Delayed images (between 2 and 4 h post-injection)

- Late images (between 20 and 24 h post-injection)

The early images provide useful information on the lung transit, liver/spleen ratio, bone marrow distribution and vascular pattern. These early images are recommended, but not obligatory.
The delayed and late images must be acquired. Sites of infection should be visible on the delayed images with further accumulation of labelled leucocytes on the late images due to increased uptake in infected areas and reduction of background activity. Over time there is also reduction or stable activity in bone marrow and non-specific activity in the bowel.
For patients with abdominal infections or IBD, early and delayed images should be performed, late images are not necessary. The investigation should be completed within 4 h when assessing abdominal pathology. There is normal bowel activity seen in 20-30% of children at 1 h and 2-6% of adults at 3-4 h after injection.

^{111}In:
If ^{111}In is used, imaging may also be performed at 48 h. For abdominal infections or IBD, early imaging is useful to differentiate between mucosal uptake that will decrease with time due to intraluminal transit, sub-mucosal uptake that remains stable, and an abscess which will show increasing uptake between 3-4 h and 20-24 h.

Views
Depending on the indication, anterior and posterior views or whole body images are obtained. Supplementary images (oblique or lateral views) may be obtained as required. The late images can usually be limited to the views that best illustrate the leucocyte uptake at earlier time points. SPECT-CT imaging at delayed and/or late time points may be useful for most indications and are essential for selected indications (e.g. endocarditis, diabetic foot, vascular prosthesis, differentiation between osteomyelitis and soft tissue infection etc.). If SPECT/CT is used, a 128x128 matrix is recommended.

Acquisition protocols
Several acquisition protocols have been suggested. Fixed counts per image or fixed time per image at all time-points are the most commonly used protocols. These are also the most difficult to interpret because of operator bias. For fixed counts or time acquisitions, images of the regions of interest are acquired for at least 500.000 counts or 5-10 min per large field of view including a region of normal bone marrow as a reference (e.g. iliac bone, sternum or skull). This method is recommended for expert readers.
In order to reduce operator dependence upon final image interpretation it is recommended to acquire acquiring images that are time-corrected for isotope decay (see table 1). If delayed and late images are acquired with the same number of counts, late images should be corrected for the radioisotope half-life.

Table 1

A

Hours*	Acquisition time (in sec) for Tc decay corrected images				$exp^{(lt)}$	lt
0	**100**	**150**	**200**	**300**		
1	112	168	224	337	0,8909	0,1155
2	126	189	252	378	0,7937	0,231
3	141	212	283	424	0,7072	0,3465
4	159	238	317	476	0,6300	0,462
6	200	300	400	600	0,5001	0,693
8	252	378	504	756	0,3969	0,924
14	504	756	1008	1511	0,1985	1,617
20	1007	1511	2015	3022	0,0993	,2,31
22	1269	1904	2538	3808	0,0788	2,541

B

Hours*	Acquisition time (in sec) for In decay corrected images				$exp^{(lt)}$	lt
0	**100**	**200**	**300**	**400**		
1	101	202	303	404	0,9897	0,0103
2	102	204	306	408	0,9795	0,0206
3	103	206	309	413	0,9694	0,0310
4	104	208	313	417	0,9595	0,04137
6	106	213	319	426	0,9398	0,06205
8	109	217	326	435	0,9206	0,08274
14	118	236	354	472	0,8475	0,16549
20	123	246	369	492	0,8131	0,20686
22	125	250	374	499	0,7965	0,22755

Time 0 is the time of the first scan, not the time of injection.

Image display

Given a correct acquisition protocol, images must be correctly displayed on the screen to allow their interpretation. Most display units are pre-set to display multiple images in % of maximum counts/pixel. This display does not allow the reader to evaluate modifications of activity with time in the regions of interest. Furthermore, this kind of display needs the reader to adjust the intensity scale of each image to make bone marrow activity comparable and therefore introduces an operator bias.

Therefore, all images acquired with a time-decay corrected protocol should be displayed with the same intensity scale in total counts. Any adjustment of the intensity count scale must be applied to all images together, thereby avoiding operator bias.

10. Interpretation

Visual analysis:

Diagnosis of infection is made by comparing delayed (2-4 h) and late (20-24 h) images. Images are then classified as:

- Negative if there is no uptake or if there is a significant decrease in uptake from delayed to late images.
- Positive when uptake is seen in both delayed and late images, increasing in time or increasing in size
- Equivocal when the uptake in delayed and late images is similar or slightly decreasing in intensity

Semi-quantitative analysis

Following visual assessment semi-quantitative evaluation can also be performed, and has added value in equivocal cases as an adjunct for the differentiation between infection and non-specific uptake. Regions of interest (ROIs) are drawn over the area with the highest uptake and copied to presumed normal reference tissue (contralateral region or bone marrow region e.g. anterior-superior iliac crest). The mean counts per pixel in these ROIs are recorded and used to calculate the Lesion-to-Reference (L/R) ratio in both the delayed ($L/R_{delayed}$) and late (L/R_{late}) images. Calculations are then classified as:

- Negative when the L/R_{late} decreases compared to $L/R_{delayed}$
- Positive when the L/R_{late} increases compared to $L/R_{delayed}$
- Equivocal when the L/R_{late} is similar to or decreases slightly compared to $L/R_{delayed}$

In equivocal cases (both on visual and semiquantitative analysis)

In summary:

Criteria for a positive scan:

Increase in intensity and/or size over time.

Visual analysis:

Evaluate images without too many operator modifications (use decay-corrected images and display the images with the same intensity scale in total counts). Look at iliac bone (or sternum or skull) uptake at different time points as a reference region for bone marrow whenever possible.

Semi-quantitative analysis:

Use only in visually equivocal cases. Use the contralateral region or the anterior superior iliac crest as the reference region.

11. Report

Describe regions of abnormal and normal (bone marrow, liver, spleen, kidneys, bladder) leucocyte uptake both on the delayed and late images (and on the early images, when performed). Describe the uptake pattern over time.
When performing semi-quantitative analysis, describe the reference tissue taken and the L/R ratio in both the delayed and late images. When a SPECT/CT is acquired, describe the exact location of the leucocyte uptake.

12. Literature

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