Radiopharmaceuticals for clinical infection imaging

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SUMMARY

- Introduction
- Ga-67 citrate
- Radiolabelled white blood cells (+ colloids)
- FDG
- Conclusions

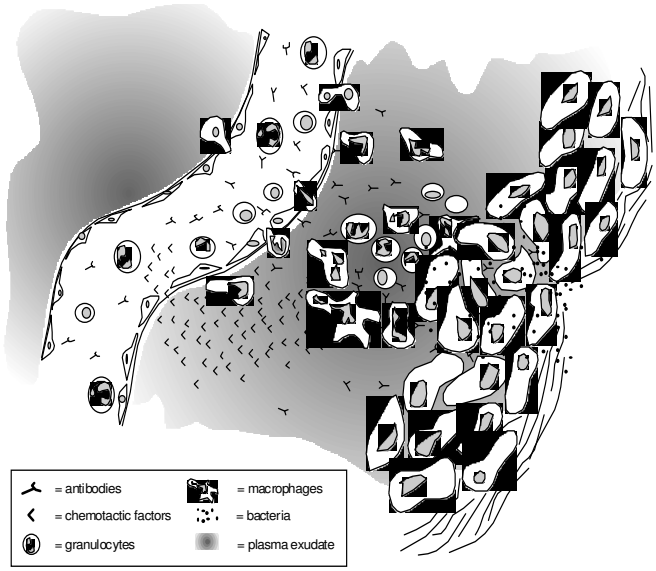
Introduction

- Demonstrates pathophysiological and pathobiological changes, which occur earlier in the infection process and also resolve quicker after cure of the infection compared with gross changes in structure.
- Currently available agents target or label components of the inflammatory response, e.g. immune globulin, neutrophils, and cytokines, are unable to distinguish between infective and non-infective inflammation.
- Thus the search for more infection specific imaging agents remains of interest to Clinical Microbiologists and Infectious Disease physicians as well as specialists in Nuclear Medicine

Table 1 - Properties of an ideal infection imaging agent

Properties
• No side effects
• Specific to infection
• Applicable to immunocompromised patients
• Low non specific uptake
• Low marrow, gut, renal uptake
• Safe and easy to prepare and administer
• Not too expensive

A model of acute inflammation/infection



Diseases characterized by acute inflammation

Trauma and degenerative diseases

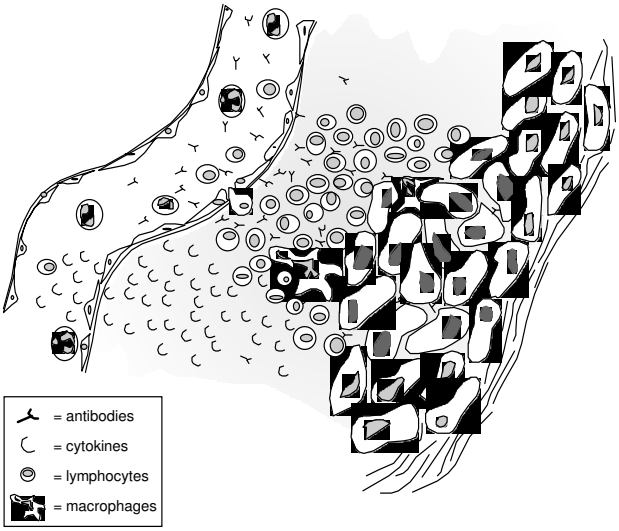
Inflammatory bowel disease
- Crohn's disease
- Ulcerative colitis

Bacterial infections

Acute graft rejection
- Kidney
- Lung
- Liver

Parasite infections, abscesses, spondilodiscitis, endocarditis, FUO, etc. are atypical inflammation.

A model of chronic inflammation



Diseases characterized by chronic inflammation

Organ specific autoimmune diseases
- Type 1 diabetes mellitus
- Multiple sclerosis
- Crohn's disease
- Coeliac disease
- Sjogren Syndrome
- Rheumatoid Arthritis
- Autoimmune thyroiditis
- Autoimmune infertility

Tumours

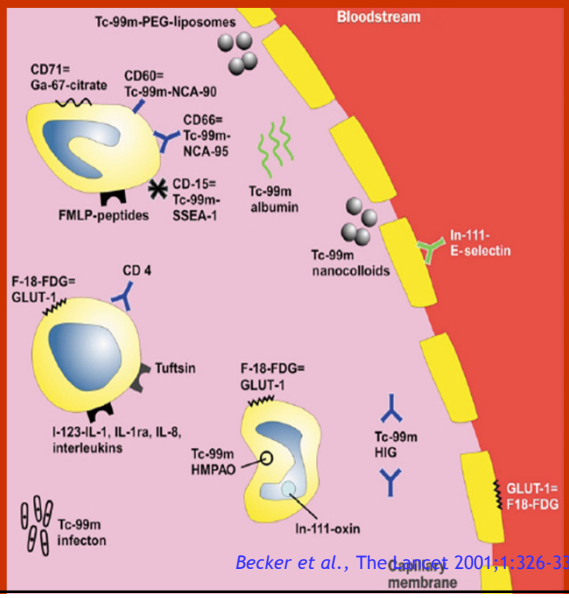
Atherosclerosis

Granulomatosis
- Tuberculosis
- Sarcoidosis

Infective diseases
- Fungal
- Viral

Graft rejection
- Kidney
- Lung
- Liver

Molecular imaging of infection and inflammation



Becker et al., The Lancet 2001;1:326-33.

Available radiopharmaceuticals for imaging Acute inflammation/infection

Acute inflammation

67Ga/68Ga-citrate
99mTc/111In/18FDG/64Cu-labelled WBC
99mTc-labelled MoAb (LeuTech®, Leukoscan®, Scintimun®)
99mTc-SnF_2-WBC
99mTc-HIG
99mTc-Nanocolloids
18F-FDG
123I-IL1ra (P)
99mTc-IL8 (P)
99mTc-P483H (P)
99mTc-EP1-HNE2 (P)
99mTc-a-E-Selectin (P)
99mTc-DMP444 (P)
99mTc-Chemotactic peptides (P)
99mTc-PEG-Liposomes

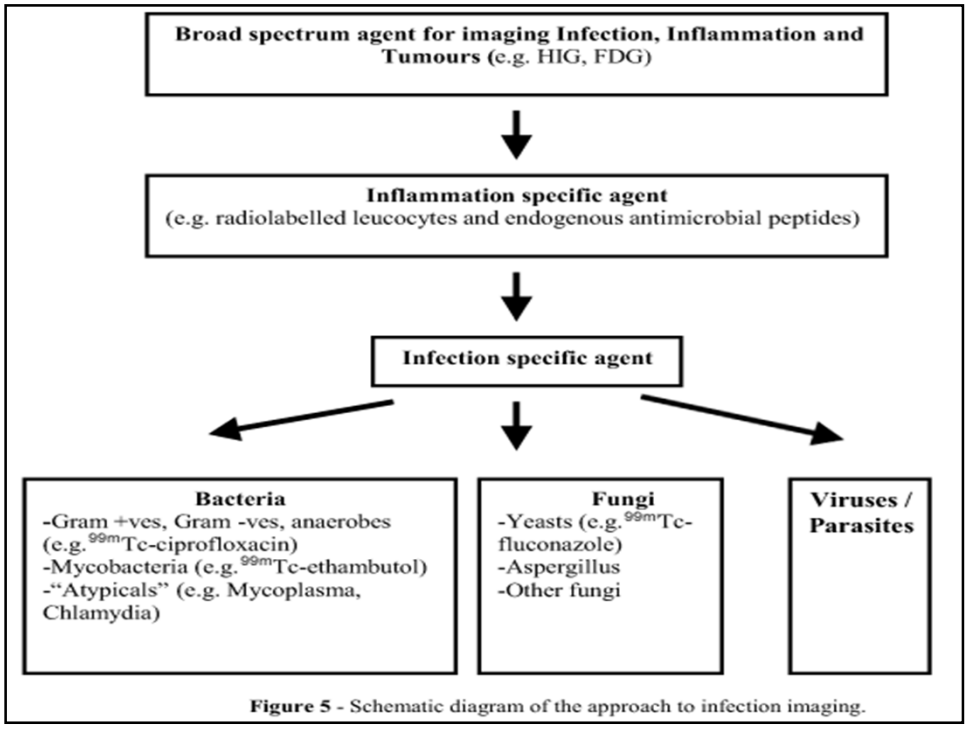


Figure 5 - Schematic diagram of the approach to infection imaging.

Infection imaging

↓

High sensitivity to detect small lesions

High specificity for differential diagnosis between sterile inflammation and infection

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1970-1975
67Ga-citrate for imaging infections

Lavender JP, Lowe J, Barker JR, Burn JI, Chaudhri MA
Gallium 67 citrate scanning in neoplastic and inflammatory lesions.
Br J Radiol 1971 May;44(521):361-6

- Increased vascularization
- Increased tissue metabolism
- Increased expression of transferrin receptors

Gallium-67 citrate (67Ga)

- Gallium-67 citrate (67Ga) was one of the first radiopharmaceuticals developed for imaging infection.
- It is transported in the blood either in ionic form or bound to transferrin, but at sites of inflammation it leaks out of the capillaries into the tissues after binding to the transferrin receptor CD71.
- In the tissue it binds with high affinity to lactoferrin, which is present in abundance in abscess fluid and neutrophils.
- Additionally 67Ga may be taken up by siderophores produced by microorganisms as a mechanism for scavenging iron from the host in low-iron environment found in infected tissues.

67Ga has a number of major drawbacks, which include:

1) Usually it has to be ordered from an external supplier, which takes time;

2) Imaging is usually over 48 hr

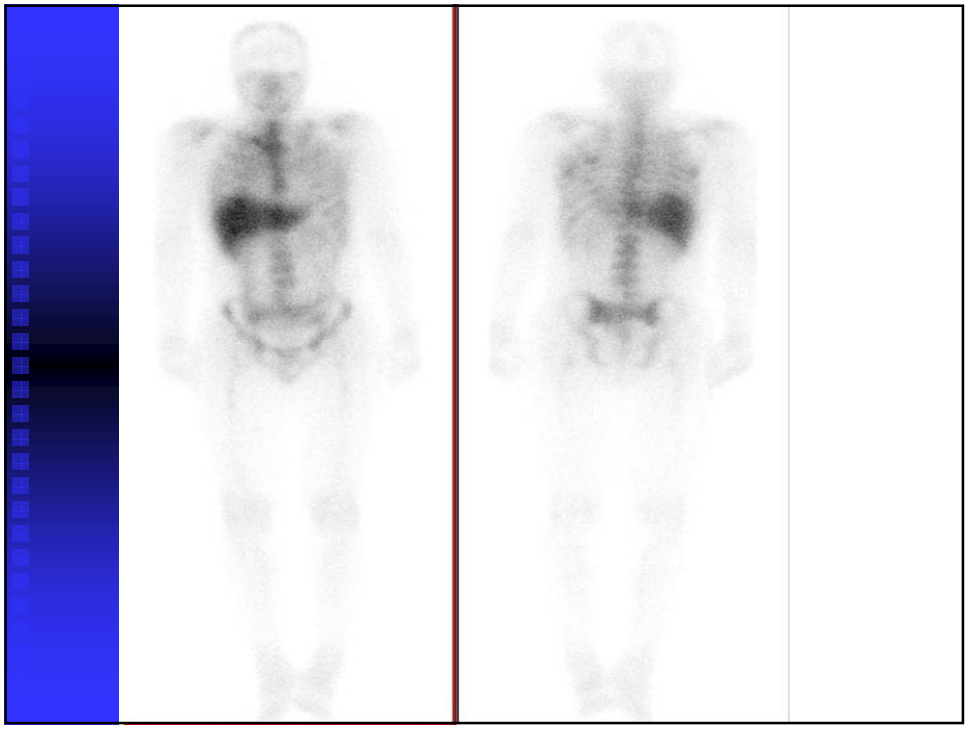
3) High radiation exposure; and

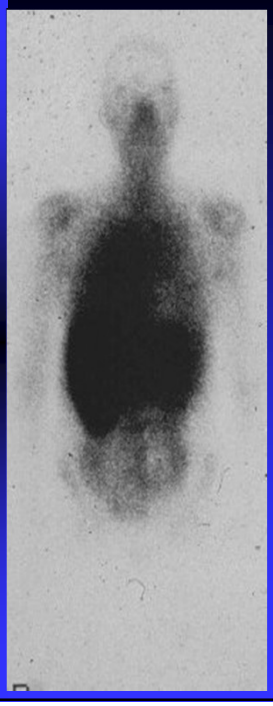
4) Unfavorable physical characteristics for gamma camera imaging.

Hence it is not widely used, having been superseded by 99mTc-labelled pharmaceuticals, which have more favorable properties as infection imaging agents. It is sometimes useful for the investigation of malignancy and autoimmune diseases, and is occasionally used for the investigation of FUO, chronic (but not acute) infections, including spinal osteomyelitis, and pulmonary infections, particularly in immunocompromised patients. However, it may be unreliable post surgery or if fracture is present.

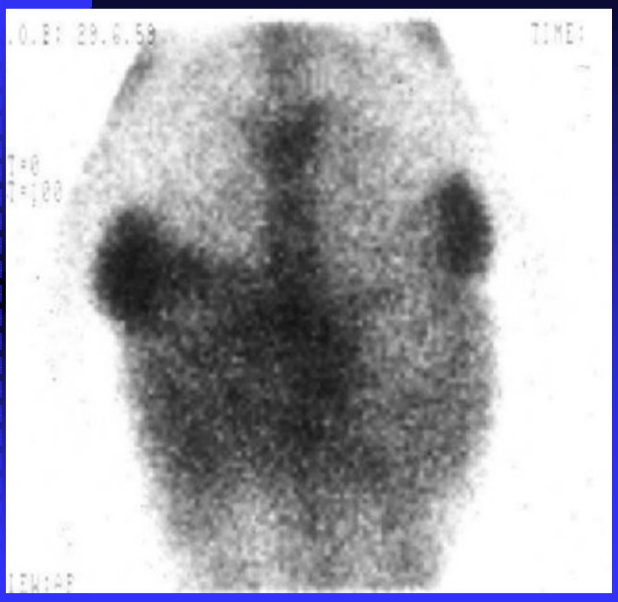
Comparison of gallium with other agents for imaging infections

Agent	Sensitivity	Specificity	Cost	Indications
Ga-67	+++	+	$	FUO, TB, AIDS, osteomyelitis, sarcoid Lymphoma, prosthetic joints
Tc-99m HIG	++	-	$$	Osteomyelitis of peripheral skeleton arthritis, FUO
In-111 HIG	+++	++	$$$	FUO, AIDS, osteomyelitis, intra abdominal sepsis, arthritis
WBC	++	+++	$$$	FUO, IBD, infected grafts and prosthesis osteomyelitis





Whole body Ga-67 image of an HIV positive man showing uptake in the chest due to pneumocystis carinii pneumonia. He also has uptake around his nose at the site of an infective sinusitis



Anterior chest image of Ga-67 uptake in the breast tissue of a man with gynaecomasti a

Teaching points

Learn 67Ga-citrate normal patterns

Good agent for FUO (high sensitivity)

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Radiolabelled Leucocytes

- "gold standard" in nuclear medicine for imaging infection and inflammation : PMN migrate to and concentrate at the site of infection through diapedesis and chemotaxis.
- In-vitro (^{111}In-oxine, ^{99m}Tc-HMPAO labelled, ^{99m}Tc-SnF_2, FDG)
- In-vivo (leucoscan)
- highly sensitive and specific for acute inflammation (less useful in chronic inflammation because the influx of neutrophils into the lesion is greatly reduced)
- does not distinguish between infective and inflammatory conditions

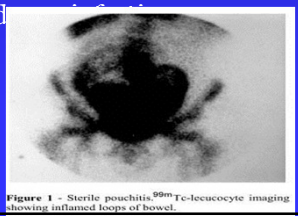


Figure 1 - Sterile pouchitis.^{99m}Tc-lecucocyte imaging showing inflamed loops of bowel.

Radiolabelled Leucocytes

- Rapid clearance from blood pool and normal lungs
- Up by the spleen, liver and bone marrow, but not kidneys, bladder, gall bladder, and gut. Thus making it a good imaging agent for the abdomen, thorax, and regions near the blood pool, such as vascular prostheses.
- The technique is highly sensitive in acute infections involving these areas but in chronic infections and infections of the central skeleton the sensitivity is lower.

1975-1980
111In-oxine for labelling WBC

Arseneau JC, Aamodt R, Johnston GS, Canellos GP
Evidence for granulocytic incorporation of 67gallium in chronic granulocytic leukemia.
J Lab Clin Med 1974 Mar;83(3):496-503

Thakur ML, Coleman RE, Mayhall CG, Welch MJ Jr.
Preparation and evaluation of 111In-labeled leukocytes as an abscess imaging agent in dogs.
Radiology 1976 Jun;119(3):731

1980-1985
99mTc-HMPAO for labelling leukocytes

English D, Andersen BR.
Organ distribution of canine leukocytes labeled with 99mTc-sulfur colloid.
J Nucl Med 1977 Mar;18(3):289-95

Peters AM, Danpure HJ, Osman S, Hawker RJ, Henderson BL, Hodgson HJ, Kelly JD, Neirinckx RD, Lavender JP.
Clinical experience with 99mTc-hexamethylpropylene-amineoxime for labelling leucocytes and imaging inflammation.
Lancet 1986 Oct 25;2(8513):946-9

Clinical indications for WBC

Disease	Cases	Sens.	Spec.	Acc.	PPV	NPV
Subcutaneous impl.	11	36.4	**100**	36.4	**100**	-
Neurological inf.*	159	**93.0**	**97.1**	**98.0**	-	-
Joint prosthesis	572	88.6	**96.5**	**93.1**	**94.2**	89.6
Endocarditis*	30	67.1	**95.0**	86.0	86.3	86.2
IBD	1286	**90.0**	**94.4**	86.1	**92.7**	87.5
Appendicitis	191	**93.7**	**90.6**	**92.7**	**90.0**	**95.2**
FUO*	637	73.2	**89.1**	80.6	74.2	76.0
Vascular prosthesis	434	**97.7**	**88.6**	**94.6**	**90.0**	**100**
Sec. Osteomyelitis*	376	88.2	**80.3**	79.3	66.2	87.4
Osteo-muscular inf.*	1803	84.8	78.9	81.6	62	92
Prim. Osteomyelitis*	617	85.4	75.5	74.0	64.1	73.0
Rheumatoid Arthritis	45	85.4	75.4	80.4	75.0	86.2
Diabetic Foot*	463	86.1	74.4	77.2	72.4	82.6
Sternal wound inf.*	369	83.9	67.3	75.3	**100**	94.7
Spondilodiscitis*	163	83.8	56.3	65.6	63.5	86.7

* indicates ^{111}In-WBC. All others are ^{99m}Tc-WBC. Metanalysis 1982-2002

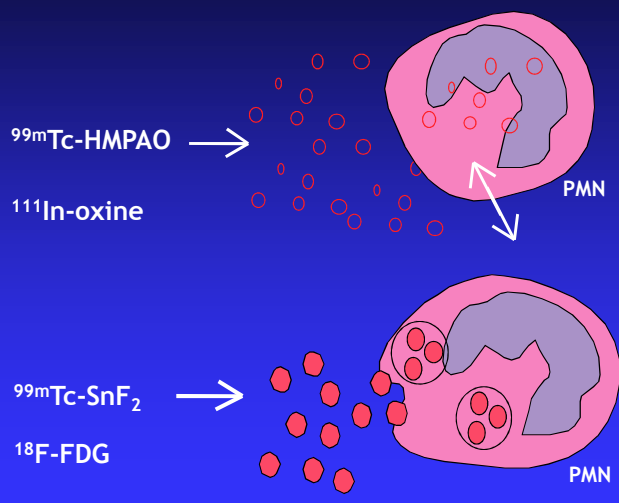
WBC as gold standard

in vitro labelling of WBC

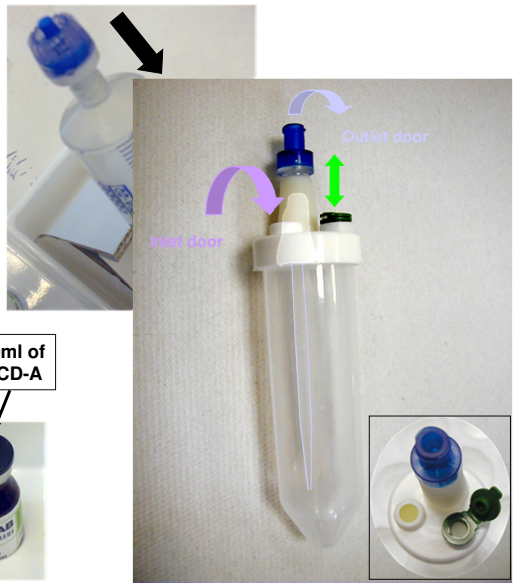
Methodological aspects

- How to label WBC?
- At what time post-inj. to acquire images?
- How long to acquire images for?
- What kind of images should we acquire?
- When should we use colloids after WBC?
- Is it any better to use SPECT/CT?
- Qualitative or quantitative analysis?

WBC labelling with ^{99m}Tc-HMPAO and ^{99m}Tc-SnF_2



Easy labelling of WBC with Leukokit®



WBC washing solution | 10% Haes Steryl | 10ml of ACD-A

GI PHARMA

Quality Controls for labelled WBC: Visual inspection

- In search for clumps, clots, fibrin and platelet aggregates (throughout the procedure and particular after resuspending the pellet of cells after centrifugation).
- At the end, before collecting the labelled cells in the syringe, inspection should be performed carefully by gently rotating the vial/syringe.
- Aggregates should be dissolved by gently shaking or pipetting the sample.
- If clumps cannot be dissolved, the preparation should not be injected.

QC to be performed:
- for method validation
- in case of method modification
- routinely

Quality Controls for labelled WBC: Labelling efficiency (LE)

After each production, the LE should be determined by measuring the amount of radioactivity in the supernatant and the pellet of the labelling solution after centrifugation. The labelling efficiency can be calculated using the formula:

$$LE(\%) = \frac{\text{radioactivity in pellet}}{\text{radioactivity in pellet} + \text{radioactivity in supernatant}} \times 100$$

If the LE is <50% for ^{111}In or <40% for ^{99m}Tc, further quality controls should be performed, such as microscopic inspection and Trypan blue exclusion test for cell viability.

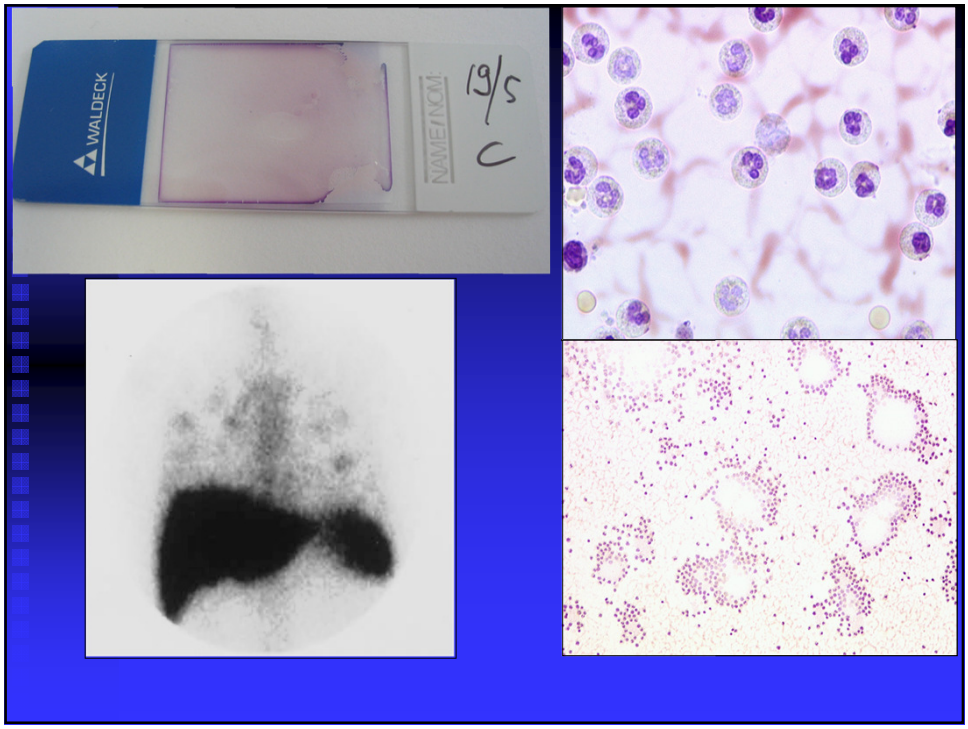
QC to be performed:
- for method validation
- in case of method modification
- routinely

^{111}In-oxine: Range 50-100%
^{99m}Tc-HMPAO: Range 40-85%

Quality Controls for labelled WBC: Microspopic inspection

- Simple test to perform but may require 15-20 min and thus delay cell-injection if performed routinely (thus to be performed only periodically).
-A drop of labelled cells is added to a slide and a smear is performed. After drying and fixation with a spray for cytology, the slide is stained with HE and observed with a light microscope at 20x, 40x to search for clumps. It also informs on the percentage of red blood cells and platelets.
-Limits of acceptability in the final cell suspension are:
RBC/WBC < 3 and PLT/WBC < 1.

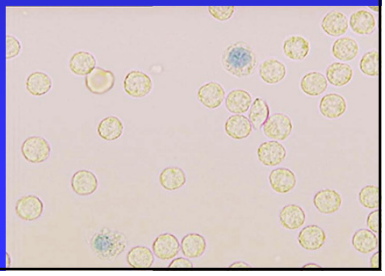
QC to be performed:
- for method validation
- in case of method modification
- periodically or in case of low LE



Quality Controls for labelled WBC: Trypan Blue Vitality test

- 25 µl of 0.4 % trypan blue solution (H_2O) + 25 µl of labelled WBC (gm).
- add a drop of the blue mixture in a haemocytometer and place the haemocytometer under a phase-contrast microscope (40x) ; control sample (unlabelled cells).
  - -Check for clumps and micro-aggregates of cells
  - -count the number of cells
  - -count the percentage of blue-stained cells (damaged by the labelling).
- If >4% of dead cells (blue stained cells), do not release + new tests for validation of the method should be undertaken.

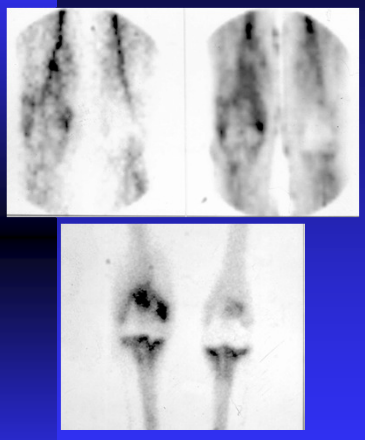
QC to be performed:
- for method validation
- in case of method modification
- periodically or in case of low LE



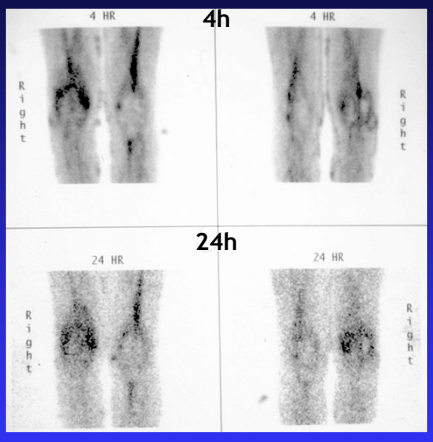
WBC as gold standard

in vivo labelling of WBC

In vivo WBC labelling with anti-granulocyte Fab Leukoscan®



3-phase bone scan



Leukoscan

Agreement rate between SCINTIMUN and ^{99m}Tc-WBCs

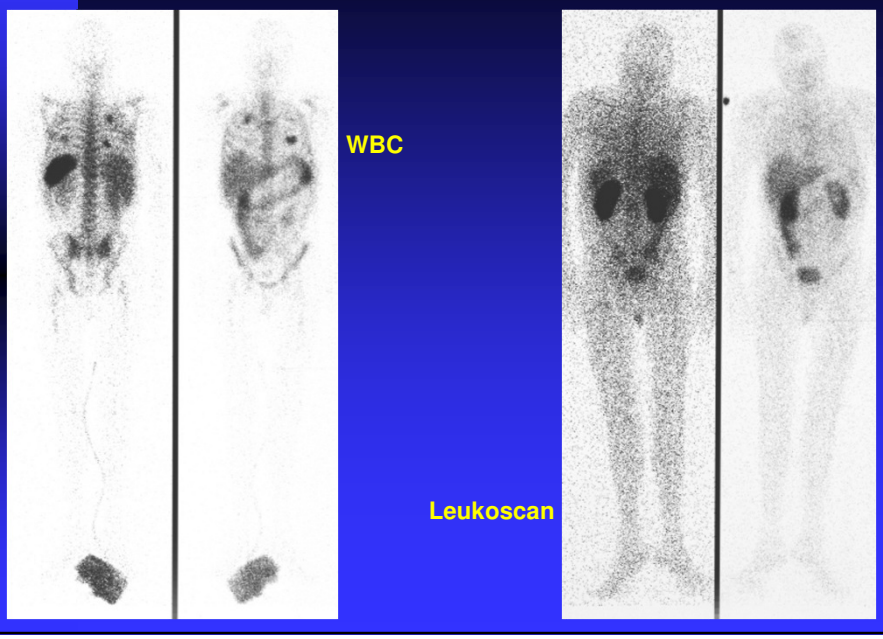
Across readers	SCINTIMUN	^{99m}Tc-WBCs
Sensitivity (N = 73)	0.75 [0.67-0.83]	0.59 [0.50-0.68]
Specificity (N = 39)	0.72 [0.59-0.84]	0.79 [0.70-0.89]

	SCINTIMUN		^{99m}Tc-WBCs	
Onset subgroup	Sensitivity	Specificity	Sensitivity	Specificity
>6 weeks (« chronic »)	0.73 [0.64-0.83]	0.73 [0.59-0.87]	0.54 [0.44-0.65]	0.77 [0.65-0.90]
<6 weeks (« acute »)	0.84 ND*	0.70 ND*	0.82 ND*	0.85 ND*

	SCINTIMUN		^{99m}Tc-WBCs	
Onset subgroup	Sensitivity	Specificity	Sensitivity	Specificity
Prosthesis	0.75 (n=39)	0.80 (n=22)	0.58 (n=39)	0.77 (n=18)
Osteomyelitis w/o prosthesis	0.57 (n=18)	0.78 (n=9)	0.42 (n=18)	0.74 (n=9)

Large European multi-center study (23 centers)
3 blind readers with no knowledge of clinical history of patients

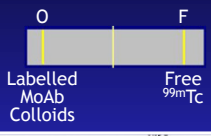
Comparison of WBC and Leukoscan scintigraphy In a patient with lung bacterial infection



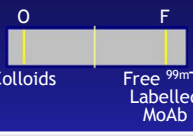
Quality Controls for labelled antibodies:

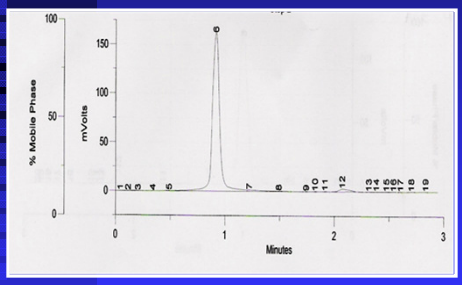
ITLC-SG

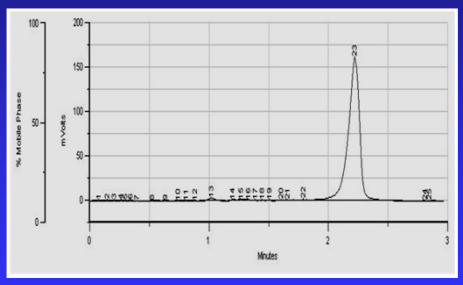
Saline
(normal strips)



EtOH:NH_4OH:H_2O(2:1:5)
(BSA coated strips)



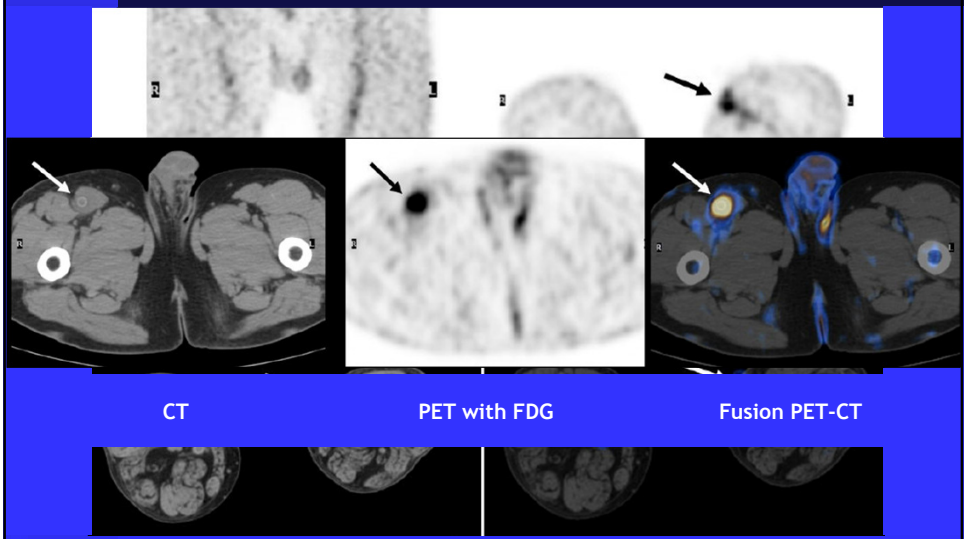




RESULTS: Labelling efficiency >98%
Free ^{99m}Tc <2%
Colloids <1%

In vivo WBC labelling with ^{18}F-FDG

Imaging infected vascular prosthesis with ^{18}FDG



CT PET with FDG Fusion PET-CT

Methodological aspects

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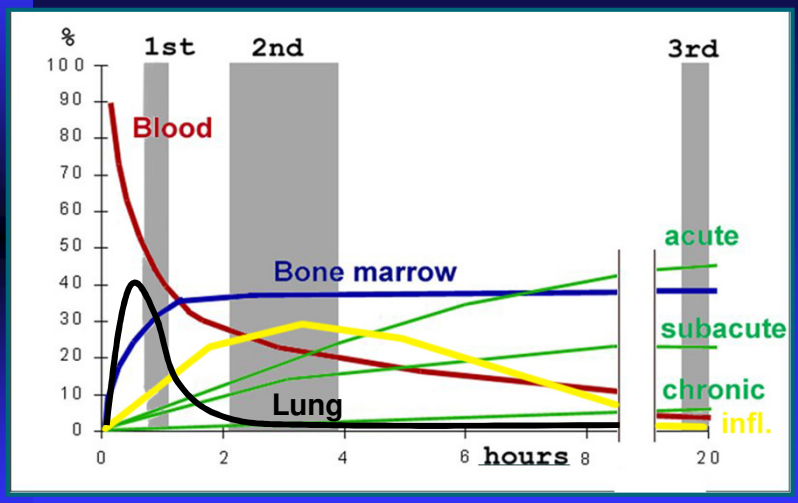
- Qualitative or quantitative analysis?

The accumulation of labelled WBC in infection sites is a dynamic process



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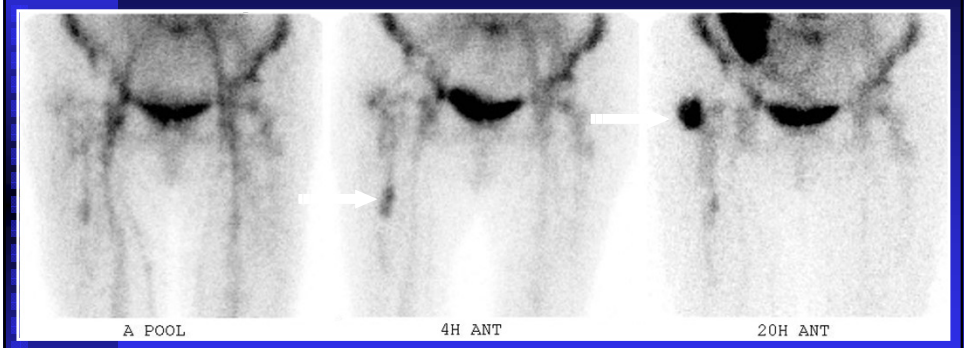

Criteria for positivity:

- Any uptake with increase of activity with time
- Any uptake with increase of size with time

Methodological aspects

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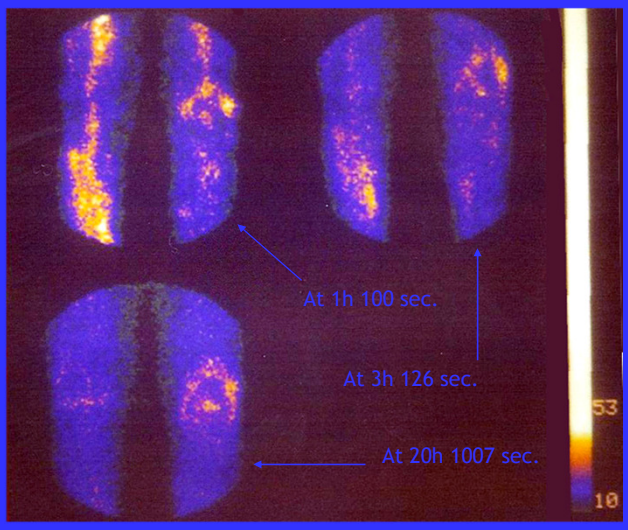
How long to acquire images?



Any uptake with increase of activity with time
Any uptake with increase of size with time

Methodological aspects

- How to label WBC?
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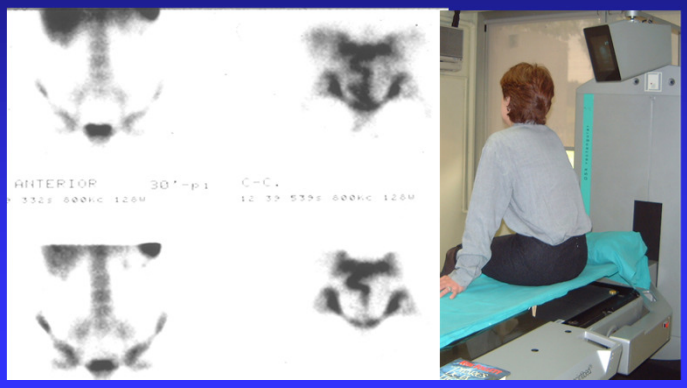
- Qualitative or quantitative analysis?

What kind of images should we acquire?

- planar antero-posterior views, lateral, obliques

- SPECT at 3h can be useful for peripheral OM

- additional sitting view in case of rectal IBD



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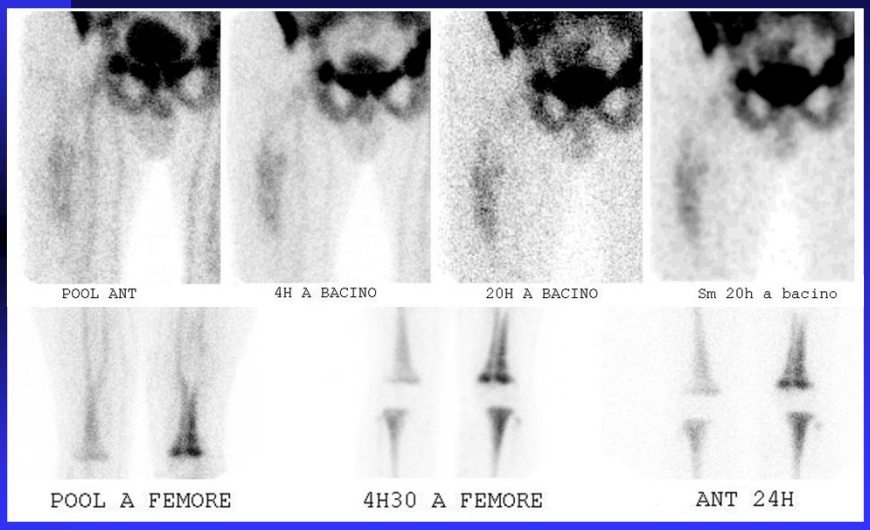
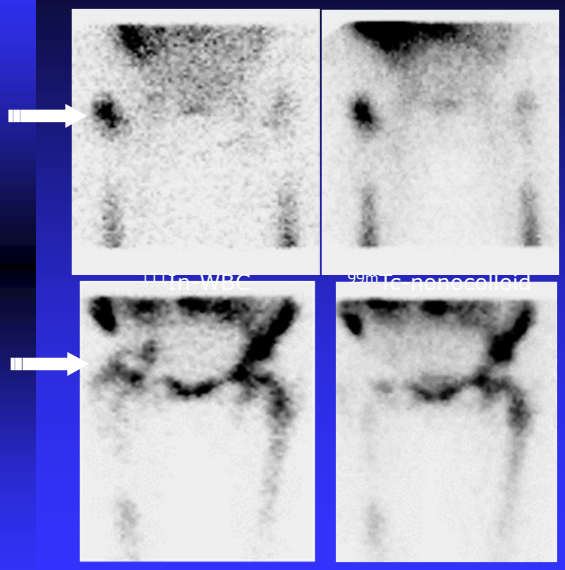
Bone Marrow displacement (frequent after joint prosthesis)



Bone Marrow imaging with ^{99m}Tc-nanocolloids



Non infected prosthesis

Infected prosthesis

Methodological aspects

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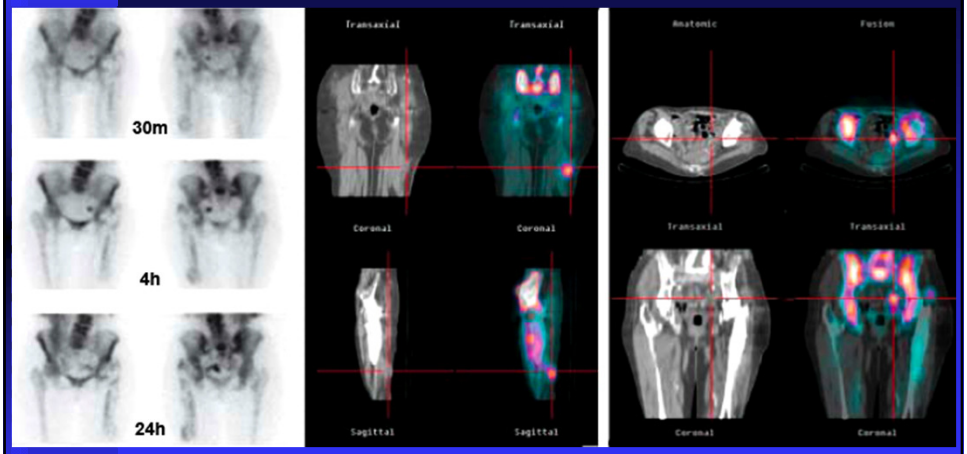
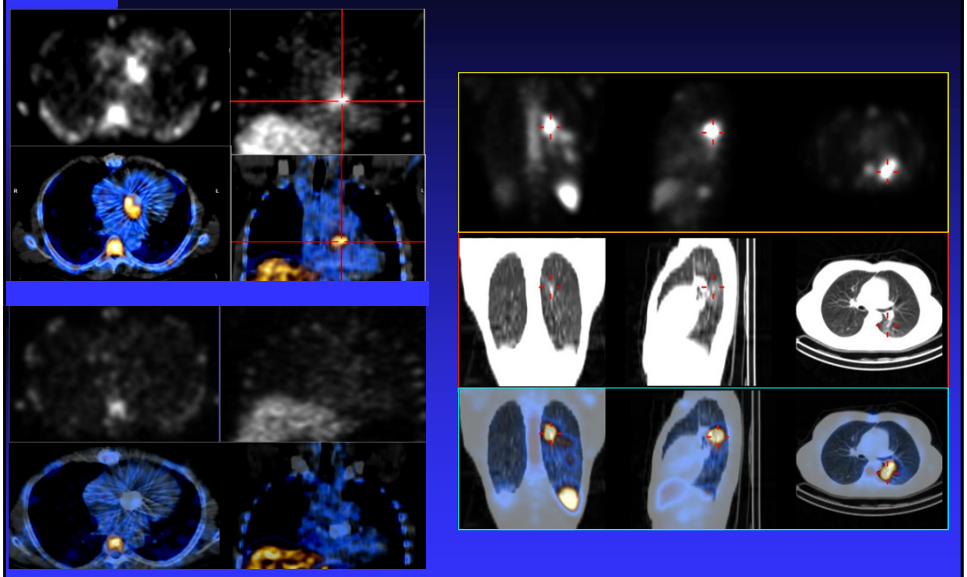
- What kind of images should we acquire?
- When should we use colloids after WBC?
- Is it any better to use SPECT/CT?
- Qualitative or quantitative analysis?

Is it any better to use SPECT/CT?



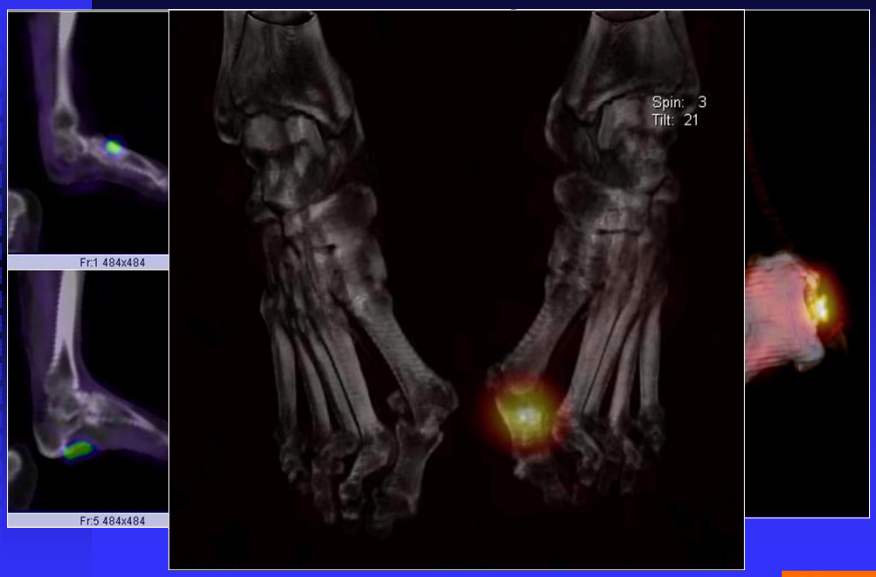
L.Filippi et al. J Nuc Med

SPECT-CT with ^{99m}Tc-WBC in endocarditis



Kindly provided by Dr. P. Erba

Is it any better to use SPECT/CT?

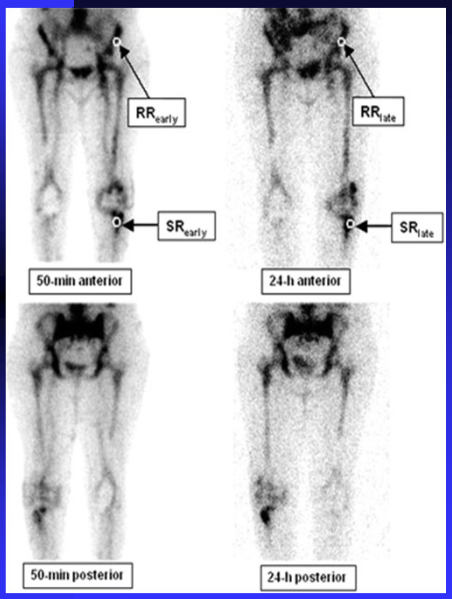


umcg

Methodological aspects

- How to label WBC?
- At what time post-inj. to acquire images?
- How long to acquire images for?
- What kind of images should we acquire?
- When should we use colloids after WBC?
- Is it any better to use SPECT/CT?
- Qualitative or quantitative analysis?

Qualitative or quantitative analysis?



^{99m}Tc-HMPAO-leukocyte scintigraphy in a patient with bilateral knee prostheses and suspected left knee prosthesis infection.

Quantitative analysis:
SR_{early} = 64.7; RR_{early} = 61.9;
SR_{late} = 28.1; RR_{late} = 13.7;

T/B_{early} = 1.05 T/B_{late} = 2.05

SUMMARY

- Introduction
- Ga-67 citrate
- Radiolabelled white blood cells (+ colloids)
- FDG
- Conclusions

FDG-PET in Infection

Inflammatory cells (activated lymphocytes, neutrophils, macrophages) exhibit (~ malignant cells) high intracellular levels of hexokinase & increased expression of surface glucose transporter proteins with high affinity to FDG

FDG-imaging – a good alternative for assessment of infection ("the blessing of the curse…")

SUMMARY

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Conclusions

- Infection continues to be a major cause of morbidity and mortality worldwide. Nuclear medicine has an important role in aiding the diagnosis of particularly deep-seated infections such as abscesses, osteomyelitis, septic arthritis, endocarditic, and infections of prosthetic devices. Established techniques such as radiolabel led leucocytes are sensitive and specific for inflammation but do not distinguish between infective and non-infective inflammation. The challenge for Nuclear medicine in infection imaging in the 21st century is to build on the recent trend towards the development of more infection specific radiopharmaceuticals. In addition to aiding early diagnosis of infection, through serial imaging these agents might prove very useful in monitoring the response to and determining the optimum duration of anti-infective therapy.