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Labelled monoclonal antibodies used for the detection of inflammatory processes and bone marrow metastases

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Abstract

This review gives a short history of the developments of immunoscintigraphy using radiolabelled monoclonal antibodies and will look at some clinical indications studied in close cooperation with international groups. Examples are presented of infected orthopaedic prostheses and the diagnosis of osteomyelitis in diabetic foot in which the method is considered diagnostically helpful. Furthermore, the accuracy of the bone marrow immunoscintigraphy is discussed in evaluating results of a multi-centre study, clearly demonstrating its diagnostic superiority over bone scanning in metastatic cancer. It is concluded that the future diagnostic trend is going towards more specific agents (antibodies, peptides) and a speedier availability of the diagnostic results in cases of supposed infection.
Key words: immunoscintigraphy, infection, bone marrow metastases

General remarks

Acute inflammation is the immediate and early response to injury. A critical function of the response is to deliver leucocytes at the site of injury, where they can help clear invading infectious agents, as well as degrade necrotic tissues resulting from the damage. The adequate stimulation of leucocytes is a complex process induced and monitored by multiple local and general reagents and physical stimuli. A sequence of cellular events allows the migration of leucocytes towards the chemotactic stimulus and eventually their extravasation from the vascular bed. The identity of the emigrating leucocytes varies, depending on the nature of the inciting stimulus, and also changes as the inflammatory site ages. In most forms of acute inflammation, polynuclear neutrophils predominate for the first 24 hours and are followed by monocytes in the subsequent 24 to 48 hours. Therefore, different forms of radiolabelled leucocytes could be used as an ideal medium for imaging inflammation, infection or defects of leucocyte production and pooling in bone marrow and spleen.

By studying the key events of leukocyte activation and the biological activities resulting from leukocyte (such as activation, chemotaxis, modulation of adhesion molecules, elaboration of metabolites, etc.), it becomes clear that the imaging of such a complex system must lose specificity in many clinical cases and could certainly not be quick enough in others. One must make compromises and concentrate on some specific questions. Therefore, different methods have been developed which are proposed for and used in varying pathophysiological courses of development resumed above. Practically, in many clinical situations, one has to decide between the speed of obtaining adequate diagnostic information and the specificity of a diagnosis when choosing a diagnostic technique (Table 1).

Methodical considerations

In many cases an infection could be diagnosed easily by simple and cheap methods. Osteotropic and colloid tracers labelled

Table 1. Scintigraphic detection of infections

Non-specific tracers
1. Ga-67 citrate
2. Tc-99m phosphates
3. Tc-99m HSA-nanocolloid
4. Radiolabelled HSA
5. Tc-99m non-specific Human Immune-Globulin (HIG)
Specific tracers
6. Radiolabelled leucocytes
a) In vitro by In-111-oxine or Tc-99m-HMPAO
b) In vivo by monoclonal antibodies (I-123, Tc-99m), peptides, liposomes, etc.

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with Tc-99m are used for a methodical concept, which mainly refers to two basic pathophysiological phenomena of every inflammation, namely hyperaemia and increased permeability of vessels. Especially in cases looking for peripheral osteomyelitis, the so-called triple phase scintigraphy with Tc-99m-labelled phosphates ^{99m}Tc-MDP) is widely used. We strongly recommend starting with this method in all clinical indications to be discussed below in order to demonstrate hyperactive bone metabolism. Focal hyperaemia at the infection site is seen within minutes after the injection of the tracer, and, later on, the osteoblastic reaction of infected bone can be demonstrated on delayed scans. Certainly, this is an unspecific reaction of bone to all kinds of injury. But, apart from very few exceptions (such as peracute arthritis in children), a pathological bone scan is mandatory for further diagnostic evaluation of possible infection sites.

Since its introduction by McAfee [1] and Thakur [2], the in vitro labelling of white blood cells (WBC) with In-111 and, more recently, with ^{99m}Tc-HMPAO has become a standard technique and is used worldwide with numerous modifications. The method consists of an in-vitro labelling of primarily isolated leucocytes from large blood samples and re-infusion of the labelled cells to the patient. The method is, therefore, time-consuming and needs trained personnel. However, in our experience, a technical modification published by Laue [3] gave good results. The main risk of in-vitro WBC labelling must be always observed, namely that the viability and the function of cells could be hampered by various matters that eventually lead to different scintigraphic results.

The labelling of mature neutrophils with monoclonal antibodies could deliver an important answer, especially for proving local infection. We first started with this method in the mid 1980s using the monoclonal antibody Mab-47, and studied many clinical indications (Locher [4], Seybold [5]). At that time the main advantage of the method was its technical simplicity, the handling of the material for the labelling. No time-consuming *in vitro* laboratory work was needed. It was the first in vivo labelling of living non-tumour cells in routine diagnostics.

In early times we labelled the mab-47 with I-123, later with Tc-99m also. It was a murine mab of the IgG_1 class raised against CEA [6, 7]. During our cooperation with the Budapest group [8–10] we were able to optimise many methodological aspects, e.g. increasing the contrast at pathological sites as well as defining the best acquisition conditions for imaging. Using different kits, both labelling yield (65% ± 20%) and immunoreactivity (max. 80%) could be improved progressively [9]. Kinetic dates [11, 12] showed that the label is cleared from blood biexponentially (t/2 0.73 hours and 9.3 hours) About 14% of the material is bound to granulocytes, an amount that remains stable during the period of examination. Urinary excretion starts normally after 6 hours after tracer injection and 120 hours cumulative urinary excretion was about 60%. Because of logistic reasons the dispatching of mab-47 was discontinued some years ago, but almost the same results could be obtained with a competitive antibody BW-250/183 [13], commercially available as a kit ready for labelling with Tc-99m (Anti-Granulocyte 250/183, Schering AG, Germany). Some characteristics of these widely used antibodies are summarised in Table 2.

HAMA production was detected in 1.6% (^{123}I- mab-47) to 12% (BW 250/183) of cases and in 26% after repeated injections [14]. No adverse reaction or clinically relevant side effects have been seen. This is mainly because we were able to lower the amount of protein injected substantially below the 250 μg recommended initially. If HAMA were observed, they belonged to the short living IgM class, therefore disappearing from blood after a few weeks.

In several clinical studies we found that the diagnostic performance was different for various clinical indications listed in table 2, and, therefore, the interest of the clinicians changed over time, especially in the context of upcoming competitive methods.

Two important indications should be discussed in the following part:

— description of infectious sites in orthopaedic prosthesis infection and in the diabetic foot;

— imaging of metastatic bone marrow lesions.

Prosthesis infection

Differentiation of loosening from infection in surgically implanted joint prostheses is often a difficult clinical problem. The symptoms and signs of infection are frequently indolent and often are not associated with systemic signs and symptoms. No single diagnostic procedure has proved to be completely satisfactory. Even joint aspiration lacks sensitivity (12–66%). Radiography also has poor sensitivity, while bone scans have poor specificity for the diagnosis.

To diagnose a peri-prosthetic infection, therefore, labelled leucocyte imaging, combined with bone marrow imaging and bone scanning, is the procedure of choice. In this setting, the sensitivi-

Table 2. Comparison of technical characteristics

	I-123 Mab-47	Tc-99m Mab-47	Tc-99m BW 250/183
Injected dose (mCi)	3–5	15	15–30
Physical half-life (hrs)	11	6	6
Protein content (μg)	120	100–250	200–400
Radiochemistry	iodogen	terminal Aminogroup	double S-bridge
Subclass	IgG_1	IgG_1	IgG_1
Antigen	NCA-95	NCA-95	NCA-95
Binding sites	7.1×10^5		7.1×10^5
Association constant	3×10^8		2×10^9

ty, specificity and accuracy were 93%, 100%, and 97%, respectively, which are significantly better results than for every other scanning combination [15]. However, combining the three-phase bone scintigraphy with immunoscintigraphy, we found highly acceptable performances in cases of painful hip arthroplasties. The combined technique was equally helpful in patients with total knee replacement in whom infection was suspected. In post-operative orthopaedic adult patients, the accuracy of antibodies in localising infection in the lower limbs increases from proximal to distal parts. Accuracy was 81% in hip and thigh, 84% in the knee, and 100% in the tibia Anyhow, yielding values higher than 90% are also possible when observing kinetic antibody uptake over 24 hours, which means that the gradually increasing concentration of activity in infectious foci should be measured on delayed scans in comparison with surrounding tissues.

Some methodical limitations, however, should be noticed. When antigranulocyte antibodies are used for the search of infection sites, bone marrow labelling exceeds peripheral neutrophil granulocyte labelling (in vitro WBC-labelling) by an order of magnitude. This makes it difficult to localise florid granulocytic inflammatory foci in those skeletal regions that harbour active granulopoetic marrow. Hence, when interpreting mab scans, one must consider the distribution pattern of active bone marrow in order to avoid false positive imaging results in areas of reactive or displaced marrow (e.g. by performing an additional bone marrow scintigraphy using Tc-99m labelled sulphur colloids). Furthermore, in some cases of chronic osteomyelitis, the local conditions become unfavourable for migration of polynuclear neutrophils and, as we have seen, the relative number of monocytes not to be labelled increases. Therefore, a cold lesion could be e.g. a typical sign of spondylitis, and mab scans risk being classified as false negative in these cases.

Figure 1. Abscess connected to right hip replacement: **A.** Early and delayed bone scans demonstrate hyperaemic and osteoblastic reaction of the right trochanter bone, while the left hip prosthesis is inconspicuous; **B.** Immunoscintigraphy with ^{99m}Tc-mab-47 showed an increase of activity within the abscess between 6 hours pi. (Upper row) and 24 hours (below), which is the typical "filling-in" phenomenon.

Figure 2. Loosening of left hip prosthesis. **A.** Early and delayed bone scans demonstrate the same image pattern as in figure 1, that is hyperaemic and osteoblastic uptake around the femoral component of the prosthesis; **B.** However, no pathological leucocyte concentration is seen on immunoscintigraphy.

Figure 3. Infected knee prosthesis. **A.** Early bone scans of both knees in four projections (upper row) presenting intense hyperaemic distribution of activity around the right knee, and corresponding hyperactive bone reaction round the prosthesis on delayed images (lower row); **B.** Significant leucocyte concentration around the prosthesis at 6 hours (upper row) and 24 hours (lower row) after injection of ^{99m}Tc-labelled antibodies.

Diabetic foot

Some 14% of diabetics are hospitalised yearly for foot problems. Foot complications are the principal cause of the morbidity, disability and mortality of these individuals, and the most common cause of non-traumatic amputation of the lower extremity [16]. Precise and timely diagnosis is therefore paramount to the prompt institution of effective treatment. The most commonly encountered complication in the diabetic forefoot is the pedal trophic ulcer — or mal perforans. Its etiology is multifactorial. The majority of patients with pedal osteomyelitis arising via direct extension present without systematic illness, lacking obvious clinical signs and symptoms, and the diagnosis is often overlooked clinically (with the exception of pedal ulcer).

Very often in such cases the osteomyelitis is not seen on conventional radiographs, because its sensitivity is about 50% only in comparison to the 100% of bone scans. But there is inverse specificity for radiography: 83% versus 30%. In this situation, namely in

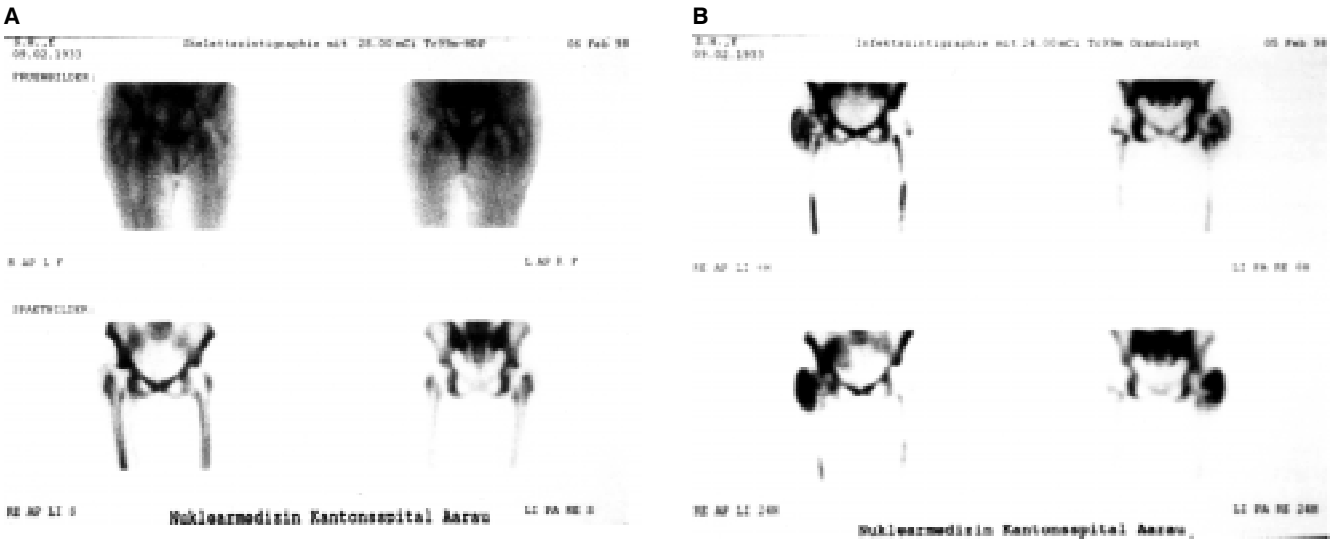

Figure 1. A. Infected prosthesis of the right hip. 2-phase 99mTc-MDP bone scan; **B.** Immunoscintigraphy: abscess formation in connection with the right hip prosthesis.

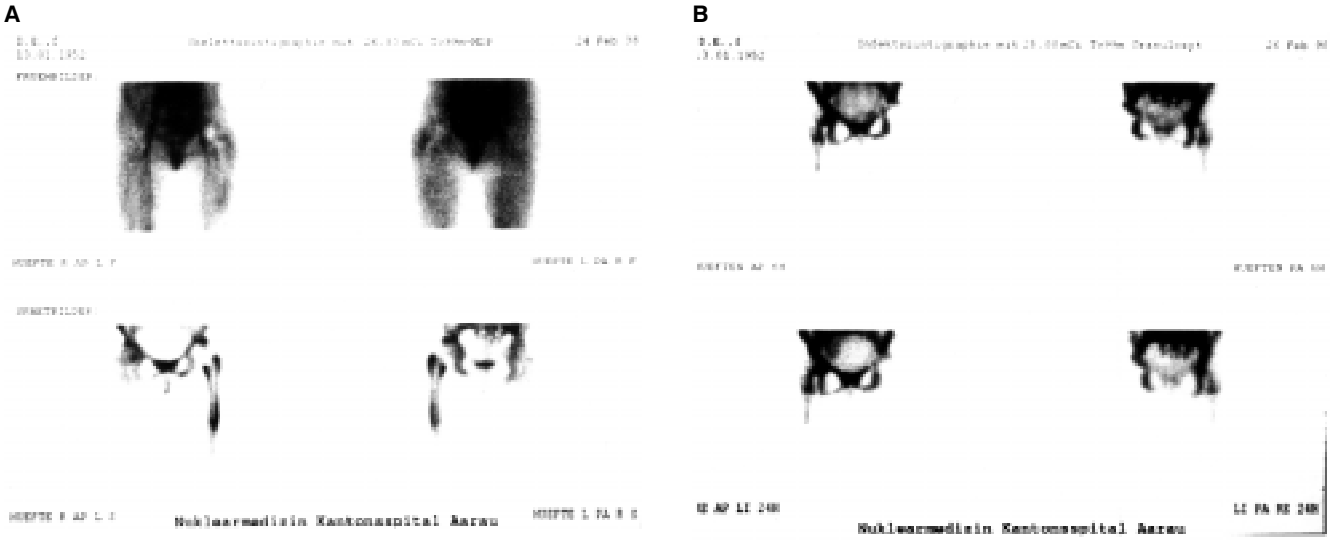

Figure 2. A. Loosening of left hip prosthesis without infection. Bone scan shows osteoblastic reaction; **B.** Immunoscintigraphy: No leucocyte accumulation around the prosthesis.

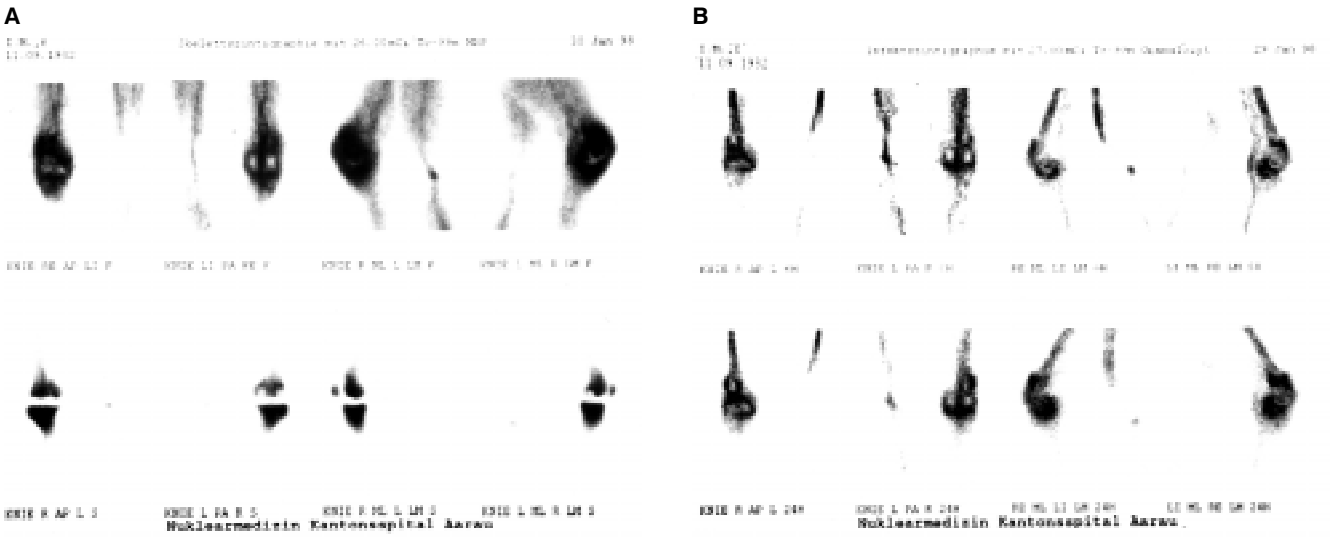

Figure 3. A. Infected prosthesis of right knee: Intense reaction on 2-phase bone scan; **B.** Immunoscintigraphy proves infection of knee prosthesis (4 views).

the case of osteomyelitis of forefoot bones, the labelled leucocyte scintigraphy is clearly superior to both of them, presenting a sensitivity of about 90% and a specificity of 97% [17].

Figure 4. Diabetic foot: under an only small skin lesion the whole calcaneus bone is osteomyelitic. Therefore, an amputation of the foot must be discussed.

Figure 5. A case with previous amputation of the right lower leg is shown. Multiple abscesses (arrows) are seen, most of them not supposed clinically.

To consider: In diabetic foot labelled leucocyte scanning is probably the most useful test for determining whether infection is present. However, conventional radiography is still the initial screening study performed on the diabetic foot, despite an accuracy of only 50%. The choice of any additional studies to be performed must be guided by the clinical situation. In the forefoot, early in the course of the disease process, when clinical suspicions are low and when medical therapy is contemplated, labelled leucocyte scanning is the procedure of choice.

Bone marrow scintigraphy

In the literature it has been reported many times that bone marrow invasion of tumours can precede the invasion of other tissues and, in addition, that bone marrow immunoscintigraphy detects more patients with metastatic bone disease and more lesions than conventional bone scans. A rapid and accurate localisation of tumour bone marrow invasions and/or infectious lesions may have substantial therapeutic impact in many clinical situations. During the last years our groups and others have shown that immunoscintigraphy with antigranulocyte monoclonal antibody (mab) is a very sensitive tool for both indications [18, 19].

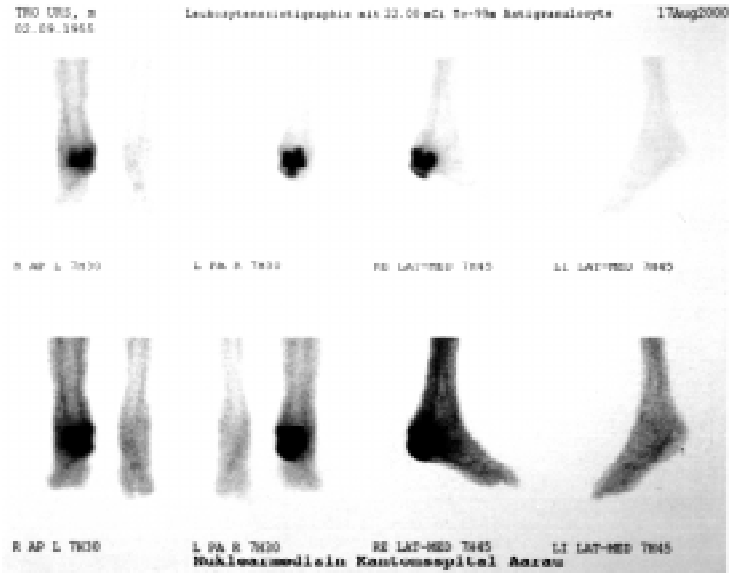

Figure 4. Diabetic foot: Osteomyelitis of the right calcaneus bone. Mab scans in 4 views approximately 7 hours post injection.

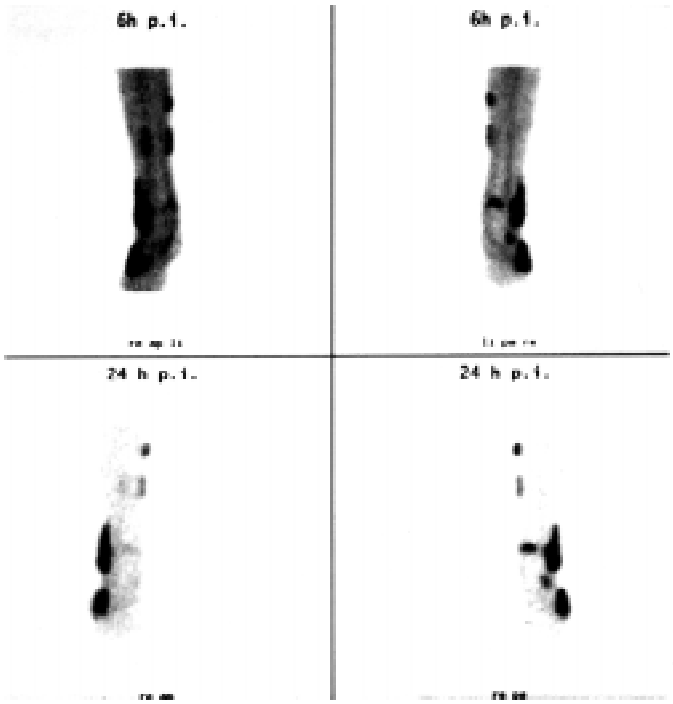

Figure 5. Diabetic foot. Lower left extremity presents multiple abscesses and local osteomylitis.

Considering these reports, we defined 24 easily distinguishable body regions (ROIs) for a semiquantitative comparison of the MDP-bone scans and the mab-47 BM scans, both performed within a short time delay (Table 3). The total number of evaluated topographic regions was 2304. We found concordant results in 94% (12% plus 82%) of the regions, equivalent to a concordant positive indication of metastases in 276 regions and a concordant negative result in 1889.

More interesting are the remaining 6% regions with discordant findings. Discrepant findings were either due to a focal osteoblastic spot on a MDP scan in a region with normal BM appearance, or due to a BM defect without a local osteoblastic bone reaction. All 139 regions with discrepant findings were further examined with CT, MRI or biopsy. The diagnostic outcome is summarised in Table 4. Out of 46 BM lesions not seen on MDP scans 34 (74%) turned out to be of tumour origin and only 12 (26%) were of benign origin. For comparison, in 93 regions with positive MDP but normal BM findings the quota was 32 (34%) metastases and 61 (66%) benign lesions (Table 5).

These results seem advantageous for the immunoscintigraphy (74% tumours found), but indicate at the same time that dis-

Table 3. Indications for immunoscintigraphy of infections

Bone	Acute osteomyelits
	Chronic osteomyelitis
	Prosthetic infection
	Septic arthritis
Soft tissue	Focal infection
	Brain abscess
	Diabetic foot
Abdomen	Abdominal abscesses
	Peritonitis
	Crohn's disease
	Colitis ulcerosa
Others	Vascular graft infection
	Fever of unknown origin
	Bacterial endocarditis

Table 4. Comparison of relative uptakes in ROIs

Institution	Regional Uptake (MDP versus mab-47 scans)				
	path/path	path/no*	no/path	no/no	Total
A	108	28	22	586	744
B	61	42	10	774	888
K	107	22	14	529	672
Total	276 (12%)	93	46	1889 (82%)	2304

Discordant results 139 (6%)

*) Path — pathological findings; no — normal findings

Table 5. Etiology of discordant results in tumour patients (n = 139)

Abnormality	MDP scan (n = 93)		mab-47 scan (n = 46)	
Final diagnosis	tumour	non-tumour	tumour	non-tumour
Number of ROIs	32	61	34	12
(%)	**34**	66	**74**	26

crepant findings always need further clarification, especially when therapeutic changes must be considered.

No diagnostic problems exist in these cases of breast and prostate cancer with multiple, concordant metastatic lesions (Fig. 6). In such situations the complementary contrast of lesions (positive on bone scan — negative on mab scans) must be observed.

Solitary lesions are always problematic. In Figure 7, in a case of breast cancer, a small focus in the lumbar spine is seen on a MDP scan, which could be overlooked easily, but is clearly demonstrated on SPECT images using mab-47. Biopsy revealed a metastasis. Furthermore, BM expansion in the large bones is often considered to be an indication of tumour invasion.

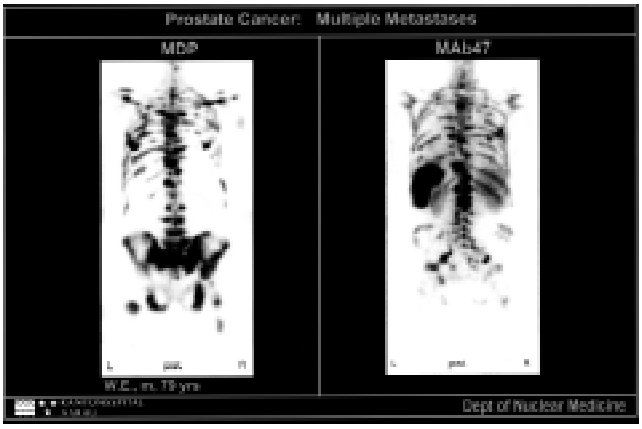

Figure 6. Prostate cancer: Multiple bone metastases demonstrated in positive contrast on bone scan (left) and negative contrast on immunoscintigraphy (right)

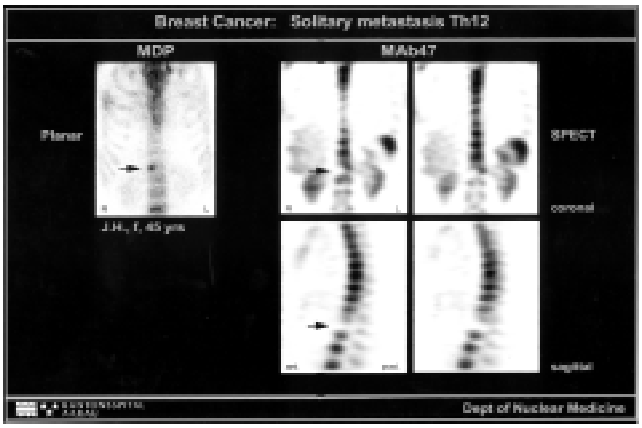

Figure 7. Solitary metastasis of breast cancer in lumbar spine; singular intense uptake on bone scan (left) — defect on immunoscintigraphy--SPET.

Future prospects

In the recent literature [20] you can find an increasing number of peptides under evaluation. As an example, I show a representative case of chronic osteomyelitis after open hand trauma (Fig. 8). The case was threefold studied: first by three-phase ^{99m}Tc-MDP bone scan (positive uptake), secondly by ^{99m}Tc-labelled antigranulocyte antibody BW 250/183 (positive). The third examination performed with the ^{99m}Tc-labelled peptide RP128 (tuftsin antagonist) [21] again showed intense uptake at the site of the lesion, but already after 30 minutes post injection. This is in great contrast to every other method used today, needing much more time for an exact diagnosis.

So, this last example demonstrates the actual direction of research: to obtain an accurate diagnosis of infection, *the trend is going towards higher specificity and increased speed.* That must also be our way to go.

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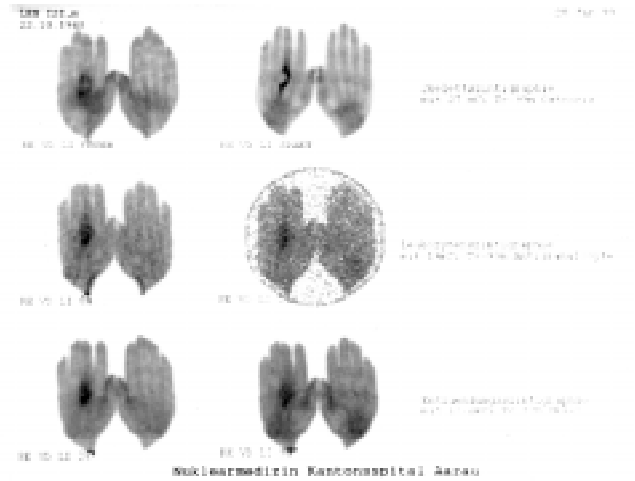

Figure 8. Osteomyelitis of metacarpal bone III. Peptide scanning bottom row.

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