Detection of Infection in Postoperative Orthopedic Patients with Technetium-99m-Labeled Monoclonal Antibodies Against Granulocytes

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A prospective study of 106 orthopedic patients was performed for the detection of infection in the early postoperative stage using ^{99m}Tc-labeled murine Mabs directed against epitopes on granulocytes. Accuracy was 81% in the hips (n = 26), 81% in the thigh (n = 21), 84% in the knee (n = 19), and 100% in the tibia (n = 27). The technique did not work well in the spine where false-negative results were observed in the three patients studied. One patient suffered transient swelling of the eyelids following injection. Optimal imaging results were obtained 2–6 hr postinjection.

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The late stages of postoperative infections can frequently be diagnosed on the basis of clinical findings. However, more sophisticated methods are required for the detection of early infections, when complete restitution is still possible (*1*). Scintigraphic methods for the diagnosis of infections of the musculoskeletal system include bone scintigraphy with diphosphonates (*2*), microcolloids (*3*), radiolabeled leukocytes (*4–10*), ^{67}Ga (*11*) and ^{111}In-chloride (*12,13*). These methods, however, lack specificity in the first postoperative weeks or require time-consuming methods for the isolation of leukocytes.

Antibodies directed against epitopes on granulocytes have the advantage of rapid preparation, do not require isolating leukocytes and accomplish white cell labeling in vivo. After more than two years using ^{99m}Tc-hexamethylpropyleneaminoxime (HMPAO) labeled leukocytes in orthopedic patients (*14–17*), we tested test a murine Mab directed against granulocytes. This report discusses our results in 106 patients.

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PATIENTS AND METHODS

Orthopedic patients in the early postoperative stage (mean: 17 days, range: 3–42 days after operation) were referred to our department, when the clinical findings concerning an infection in the area of the surgical intervention were equivocal. Seven patients were treated with antibiotics, but treatment had been started not longer than two days before. The study included 106 patients, 24 women and 82 men, treated surgically because of fracture (n = 79), arthrosis (n = 17), rupture of ligaments (n = 7), or slipped disk (n = 3). The mean age was 45 yr with a range of 16–86 yr.

Antibody Characteristics

Antibody BW250/183 (Behringwerke/Hoechst, Frankfurt FRG) is a mouse monoclonal antibody of IgG 1 isotype that is directed against epitopes (CEA, NCA-95) on granulocytes.

Contents

The kit is a two-vial kit. Content of component I: 1 mg lyophilized Mab, 2.9 mg PBS-buffer. Content of component II: 2.9 mg 1,1,3,3-propanetetraphosphone ~ acid, tetrasodiumsalt × 4 H_2O (PTP); 0.12 mg SnII-chloride × 2 H_2O.

Labeling Process

First, stannous(II)-chloride × H_2O was dissolved in 5 ml of sodium chloride. One milliliter of the solution then was added to the lyophilized and PBS-buffered Mab. After complete dissolution, 2 to 7 ml of fresh eluated ^{99m}Tc-$NaTcO_4$ was added with an activity of ca 1 GBq. Gentle inversion of the vial for a few times followed by incubation for 10 min at room temperature completed the labeling procedure.

After the patient was informed about the technique and its possible risks and consent was obtained, 300–400 MBq of the antibody solution (equivalent to 0.3 to 0.4 mg Mab) were slowly injected intravenously. The critical organ was the bone marrow with a calculated dose of 16.3 μGy per administered MBq (calculated per the MIRD concept, Behringwerke/Hoechst, Frankfurt FRG), which limits the amount of activity for injection.

Imaging Technique

Two to 4 hr after injection, images of the affected region were obtained with a large field of view gamma camera (Dyna Digit, Picker Int.). Images of the limbs were obtained from anterior and

lateral views, while the hip region was imaged both in anterior and posterior views.

Diagnostic Criteria

Scintigraphic diagnosis of an infection utilized the following criteria:

1. Uptake in bone marrow significantly higher than that in the bone marrow of the contralateral side.
2. Strong uptake in bone that contained no red bone marrow.
3. Well-circumscribed depositions in the hip area, even if enhancement over the normal soft tissue was low (experiences from a pre-study).

All cases of this prospective study were reviewed independently by two readers. Plain films were used to identify the anatomy after surgery. No additional information, such as clinical situation or wound culture results, were accessible. The scintigraphic findings were diagnosed as an acute infection only when both readers agreed on the diagnosis.

Scintigraphic diagnosis was confirmed or refuted either by intraoperative findings combined with the results from bacterial cultures or histologic findings, or by the clinical development, being either uncomplicated or leading to delayed surgical intervention.

RESULTS

Positive Signs of Infections

The scintigraphic patterns which occurred in hip lesions are shown in Figures 1 and 2. Patients without infection showed a symmetric uptake in the bone marrow of pelvis, unless bone marrow was destroyed by surgical manipulation (cold areas after hip prosthesis). The main problems were due to deposition in normal bone marrow, especially of the greater and lesser trochanters. Postoperative changes made it difficult to differentiate deposition in bone marrow from soft-tissue infection.

Another problem was caused by soft-tissue uptake in this area. Even faint uptake was indicative of severe bacterial infection (Fig. 1). Lesions were detected in 54 patients (Table 1). Combined soft-tissue infection and osteitis (Fig. 2) were seen in 31 patients, osteitis in 9, soft-tissue infection in 7, combined arthritis and osteitis in 4, and arthritis in 3. Uncomplicated hematomas of the knee joint after ligamental repair or replacement showed a faint deposition of granulocytes (Fig. 3). We found the same pattern in uncomplicated synovitis of the knee joint. The intense uptake in a hematoma with poor delineation against bone (Fig. 4A) was intraoperatively and histologically confirmed as a combination of infected hematoma and osteitis. The histologic section confirmed the strong migration of granulocytes into bone tissue (Fig. 4B). After operative revision of a fraction of the left forearm, we found no deposition of granulocytes in the tibia of a patient with psoriasis and pain in the shank. While the bone scan revealed a high uptake in the left tibia (Fig. 5A), there was no pathologic deposition of granulocytes in this area (Fig. 5B). Histologic findings with monocytes and lymphocytes were judged as acute granulomatous osteomyelitis on the base of psoriasis (Fig. 5C).

The sensitivity of detection varied in different regions. False-negatives tended to occur in the hips and spine, rather than at more peripheral sites. Specificity varied between 83% and 100% (Tables 1 and 2).

FIGURE 1. Patient with fever, pain, and soft-tissue swelling lateral on the right side of the pelvis (endoprosthesis in the right hip, 5 wk postoperatively). On the granulocyte scan, (3 hr p.i.), there is significant deposition lateral in the soft tissue of the right hip. Diagnosis: soft-tissue infection.

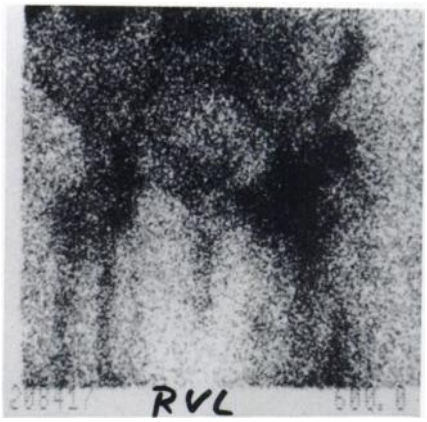

FIGURE 2. Marked uptake of granulocytes in the proximal left femur, the left hip-joint, and the surrounding soft tissue 2 wk after implantation of the second endoprosthesis (loosening of the first prosthesis). Bone marrow and the big vessels (pelvis, right groin, proximal right upper thigh) are well delineated 4 hr postinjection. Diagnosis: osteomyelitis of the left femur, empyema of the left hip, and soft-tissue infection of the proximal left upper thigh.

Cold Spots Demarcating Infections

Increased uptake of granulocytes may not occur in spondylodiscitis. In a patient with low back pain, two examinations were normal before a third study 5 wk after the onset of pain showed two photopenic lumbar vertebrae (Fig. 6). Hence, spondylodiscitis may not be evident until the normal marrow is destroyed by replacement with inflammatory tissues.

Laboratory Parameters

Increases in sedimentation rate and the number of leukocytes are uncertain findings in the early stage of an

TABLE 1
Site-Dependent Results of Infection Diagnostic in 106 Orthopedic Patients Six Weeks After Surgery (Two Conservatively Treated Fractures)

Region (surgical intervention)	True-positive	True-negative	False-positive	False-negative
Hip (11 endoprostheses, 13 bone plates, 2 marrow nailings)	9	12	1	4
Thigh (17 bone plates, 4 marrow nailings)	11	6	1	3
Knee (6 bones plates, 3 ligamentum repair, 4 ligamentum replacement, 6 prostheses)	11	5	1	2
Shank (18 plates, 9 fixateur externe)	15	12	0	0
Foot (3 wiring)	3	0	0	0
Upper arm (4 bone plates)	2	2	0	0
Forearm (1 bone plate, 1 conservatively treated shooting-fracture)	2	0	0	0
Hand (conservatively treated fracture)	1	0	0	0
Vertebral column (2 vertebral blocking, 1 hematogenic osteomyelitis after surgical revision of a symphysitis)	0	0	0	3

infection as shown by comparison with scintigraphic results and histologic findings (Table 3).

Side Effects

One patient developed eyelid swelling a few minutes postinjection. He was treated with corticosteroids for two days, after which the edema vanished without further complications.

DISCUSSION

In spite of high-standard surgical techniques, the most common and severe complication of trauma surgery is the development of infections (*1*). Blood clots, hematoma and necrotic tissue (marrow, muscle) in the surgical area create favorable conditions for bacterial growth. The normal healing process with pain, fever, and aseptic inflammation can cover very early symptoms of infection. Infections may be classified into stages of "threatening" infection, early manifestations and late infection (*18*). Therapy should be initiated in the first stage, leading to complete resolution, whereas progression to a serious infection may occur rapidly (*19,20*). Thus, the surgeon needs a final decision within hours.

FIGURE 3. Six days after repair of the cruciate ligaments there was faint visualization of the left joint space (3 hr p.i.). The small deposition of granulocytes compared with that in soft tissue and the contralateral side was correctly judged as noninfected hematoma.

We have found clinical findings as well as laboratory parameters, such as sedimentation rate and number of leukocytes, to be of limited value when infection is suspected (*14*). Also, plain x-ray films are known to lack specific signs in the early stage of infection, especially when anatomy is altered by previous surgical interventions (*21*).

Bone scanning with ^{99m}Tc-labeled phosphonates is very sensitive in detecting bone alterations. However, it cannot distinguish between trauma and infection or a combination of the two. Other nonspecific scintigraphic techniques like ^{67}Ga (*11*) and ^{111}In-chloride scans (*12,13*) use radionuclides with longer half-lives and less suitable energies for gamma cameras, resulting in a higher radiation dose to patients and poorer image quality.

The most physiologic way to detect infections is to follow the migration of leukocytes (*22*). Autologous leukocytes labeled with ^{111}In-oxine (*4–6*) have proven to be a very powerful tool for the detection of infections in bowel, soft tissue, and bone (*6,7,23*). However, scans as late as 24 hr postinjection are needed in order to obtain reliable results.

When ^{99m}Tc-HMPAO (*9,14–17*) is used to label white cells instead of ^{111}In-oxine, results are obtained after 6 hr,

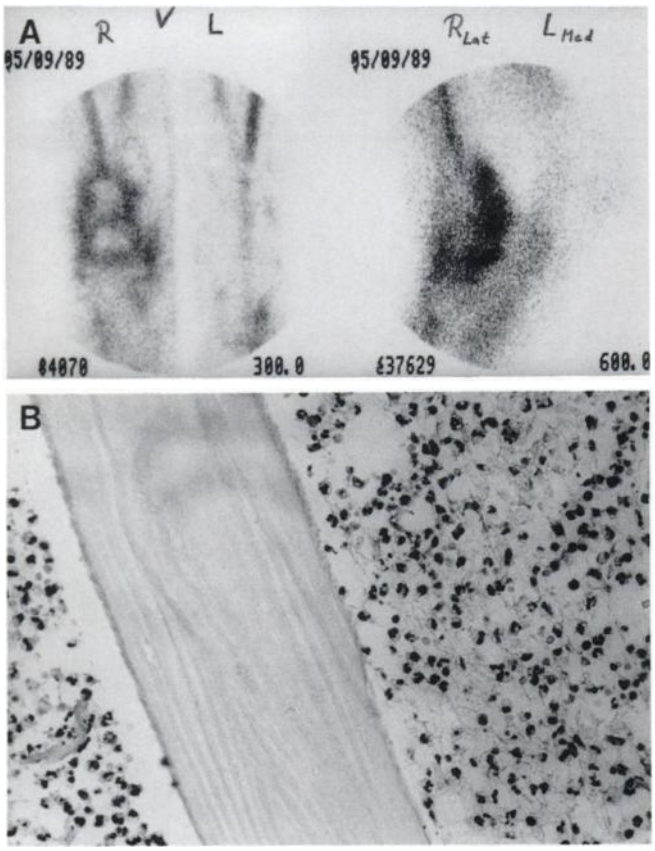

FIGURE 4. (A) Marked migration of granulocytes into the articular space of the right knee after replacement of the anterior cruciate ligament (2.5 hr p.i.), assessed as an infected hematoma. The diffuse delineation against the femoral bone is indicative for concomitant osteomyelitis. The symmetric delineation of the bone marrow down to the knee joints is unusual. (B) In the histologic section of the femoral bone, a massive migration of granulocytes with segmented nuclei is visualized beside cell-detritus. Histologic assessment was acute, purulent osteomyelitis. (Same case as in Fig. 5A).

allowing for a 2-hr period for leukocyte isolating and labeling.

The advantage of ^{99m}Tc-labeled murine Mabs directed against epitopes on granulocytes is the simplicity of the labeling process, requiring only 15 min to label the antibody, including 10 min of incubation time and in-vivo labeling of white cells. Images of high quality may be obtained as early as 2 hr postinjection.

When we started to examine patients in order to prove or rule out infections, we had to learn how to interpret the scintigraphic patterns. The greatest problem in image interpretation is the nonspecific deposition of leukocytes in hematoma, contusion and other sites of aseptic inflammation. However, in bacterial infections, the uptake of labeled granulocytes tends to be more intense and more localized. Detection of infection in the postoperative hip, with or without endoprostheses, has been most difficult. Normal marrow uptake can be nonuniform, especially in the greater trochanter. Correlation with x-ray films of the pelvis and hips is mandatory. In chronic infections with or without fistulas, the uptake of radioactivity may be weak leading to false negative results (*23–25*). Nonetheless,

TABLE 2
Dependence of Sensitivity and Specificity on the Location of Infection in the Lower Limb (96 Patients, < 6 Weeks After Surgery)

Region	Sensitivity	Specificity
Hip	69%	93%
Thigh	79%	86%
Knee	85%	83%
Shank/Ankle	100%	100%

FIGURE 5. (A) Two weeks after surgical intervention because of a fracture of the left forearm there was development of pain in the left shank. At 3 hr postinjection, there was strong enhancement of the left tibial bone in the bone scan (^{99m}Tc-MDP), suggesting osteomyelitis. (B) A granulocyte scan performed 1 wk later without preceding antibiotic therapy. No pathologic deposition of granulocytes is visualized (3 hr p.i.), ruling out an acute bacterial osteomyelitis. (C) Histologic section of the left tibia shows a massive infiltration of lymphocytes and monocytes. No granulocytes or cell detritus are visualized. The granulomatous structure of the inflammation specifies the diagnosis of an acute granulomatous osteomyelitis on the basis of psoriasis of 8 yr duration.

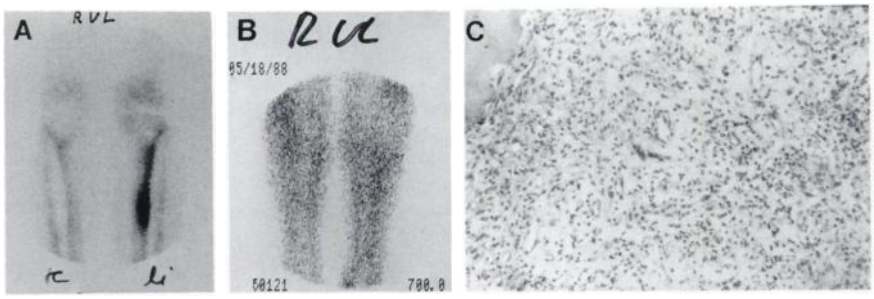

TABLE 3
Comparison of Number of Leukocytes, Sedimentation Rate, Fever and Confirmed Scintigraphic Results in Postoperative (< 6 Weeks) Orthopedic Patients

Scintigraphic diagnosis	No. of leukocytes > 10,000/mm³ (n = 99)	Sedimentation rate > 25/35 (n = 101)	Body temperature > 38°C (n = 75)
True-positive	50	51	26
True-negative	49	50	49

scintigraphy resulted in successful surgery in three of five patients with hip infections. On the other hand, pooling activity in varicose veins could be confused with positive uptake in infection. Labeled leukocytes do not appear useful in the detection of spondylodiscitis, and three-phase bone scans appear preferable in this condition (*26,27*).

CONCLUSION

Our results with Mabs directed against NCA-95 are similar to those obtained with ^{111}In-labeled leukocytes (*23*). However, this method has the advantage of in-vivo white cell labeling (*28*). Antibodies against granulocytes provide a useful tool for diagnosing postoperative infections that is more sensitive and specific than clinical findings or laboratory parameters. Involvement of bone, soft tissue and joints and their exact extent can be assessed in addition to the detection of infection. If necessary, surgical intervention can be performed within 2 hr.

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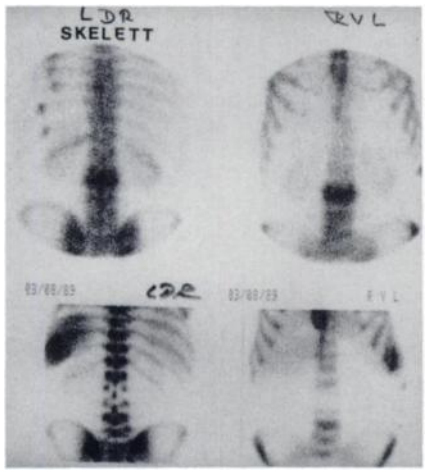

FIGURE 6. Patient with hematogenic spondylodiscitis after surgical revision of a symphysitis 6 wk earlier. Typical increased uptake in adjacent parts of two lumbar vertebrae in the bone scan (upper images, 3 hr p.i.). Second and third lumbar vertebrae produced localized photopenia in the granulocyte scan (lower images, 4 hr p.i.).

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Erratum

Due to a production error, Figures 1C and 2C in the article, Liver Transplant Rejection and Cholestasis: Comparison of Technetium-99m-DISIDA Hepatobiliary Imaging with Liver Biopsies, by Kuni et al, which appeared in the August issue of the *Journal*, were printed as black and white instead of color. Color reproductions are printed below.

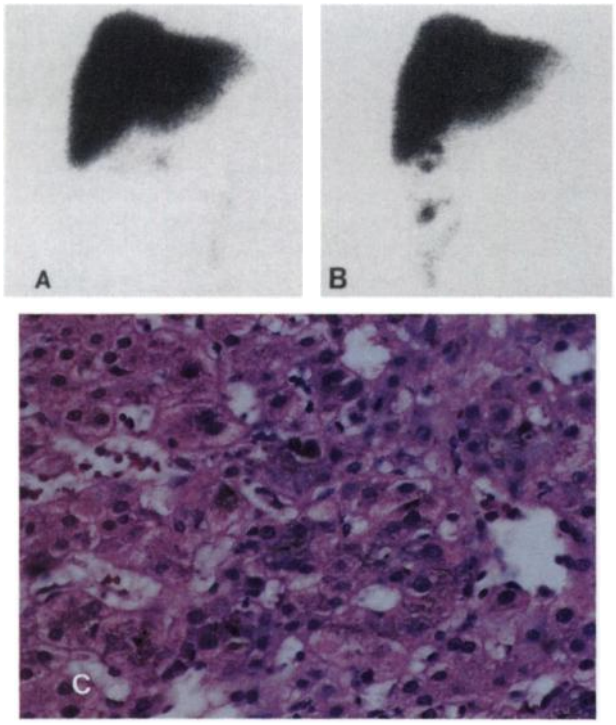

FIGURE 1. The cardiac blood pool is only barely visible at 1 min (A), indicating normal uptake. The 30-min image (B) shows intestinal radioactivity but no decrease in parenchymal intensity from 10 min, indicating severely abnormal excretion. Biopsy (C) shows normal hepatocytes (HD score = 0). Bile collections are seen in hepatocytes, canaliculi, and ductules (CS score = 2). These biopsy findings of normal hepatocytes and cholestasis correspond to the scintigraphic findings of normal uptake and impaired excretion.

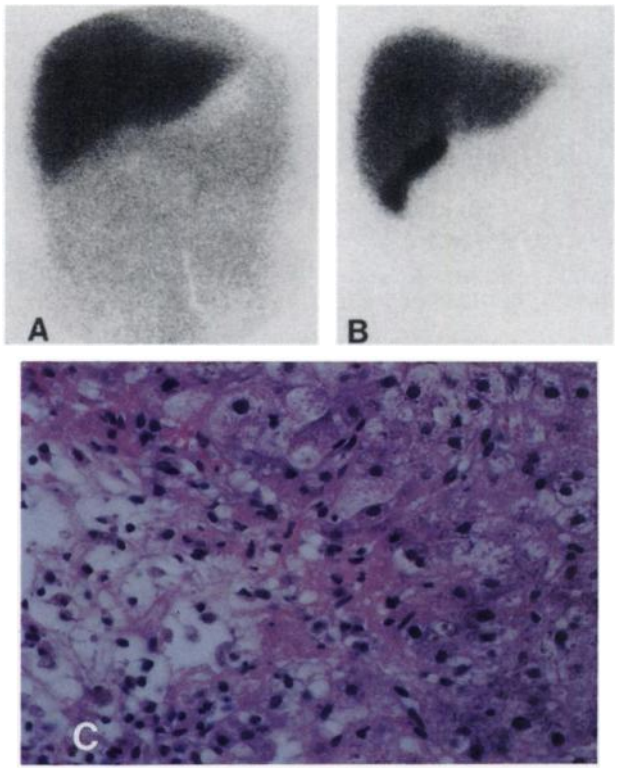

FIGURE 2. The cardiac blood pool is prominent at 10 min (A), indicating abnormal uptake. The 30-min image (B) shows intestinal radioactivity and a decrease in parenchymal radioactivity from 10 min; this decrease is less than expected in a normal liver. These findings suggest moderately impaired excretion. Biopsy (C) shows hepatocyte damage manifested as ballooning degeneration and spotty necrosis (HD score = 6). Only minimal bile stasis is present (CS score = 1). These biopsy findings correspond to the scintigraphic findings of abnormal uptake and ex cretion.