The Journal of International Medical Research
2009; 37: 54 – 67 [first published online as 37(1) 12]

^{99m}Tc-Fab′ Fragments (Sulesomab) for Imaging Septically Loosened Total Knee Arthroplasty

S Gratz[1,2], TM Behr[1], P Reize[3], A Pfestroff[1], WU Kampen[4] and H Höffken[1]

[1]Department of Nuclear Medicine, Philipps University, Marburg, Germany; [2]Department of Nuclear Medicine, Centre Bad Cannstatt, Stuttgart, Germany; [3]Department of Trauma, Reconstructive and Orthopaedic Surgery, Centre Bad Cannstatt, Stuttgart, Germany; [4]Department of Nuclear Medicine Spitaler Hof, Centre Spitaler Hof, Hamburg, Germany

The diagnostic accuracy of infection scintigraphy with ^{99m}Tc-labelled monoclonal antibody Fab′ fragments (sulesomab) was studied in patients with suspected total knee arthroplasty (TKA) infection. Images from 26 patients were evaluated by two independent readers and compared with a quantitative interpretation of time–activity courses. Microbiological examinations and joint aspiration results were used as reference standards. Histologically, aseptic TKA loosening occurred in two patients and severe, moderate or mild septic loosening in four, nine and 11 patients, respectively. Diagnostic accuracy for severe infection was 100% for both readers, whereas for moderate infection accuracy decreased by 12% and 12% for readers one and two, respectively. For mild infection a further decrease of approximately 61% and 52% occurred for readers one and two, respectively. Quantitative evaluation gave significantly better results over visual interpretation with a diagnostic accuracy of 100% for severe infection and decreased by only 10% and 15% in patients with moderate and mild infection, respectively. Quantitative evaluation of ^{99m}Tc-Fab′ fragments is highly sensitive and specific for diagnostic imaging of infection in patients with septically-loosened TKA.

KEY WORDS: Total knee arthroplasty (TKA); Diagnostic imaging; ^{99m}Tc-Fab′ fragments; Sulesomab; Infections

Introduction

Some complications of joint replacement surgery, such as fracture or dislocation of arthroplasty, are easily diagnosed, however differentiating infection from aseptic loosening is difficult because these entities may appear remarkably similar on clinical and histopathological examination. Clinical signs and symptoms, laboratory tests, radiography and joint aspiration have tended to have poor sensitivity or specificity, or both. In addition, cross-sectional imaging modalities are hampered by artefacts produced by the prosthetic devices.[1 – 5] Radionuclide imaging is unaffected by the presence of metallic hardware and is, therefore, useful for evaluating a painful prosthesis. To date, the gold standard for the

nuclear medicine imaging of infection has been the use of radiolabelled autologous leucocytes,[6,7] however this procedure requires *ex vivo* handling of blood, time-consuming techniques and special training, and puts laboratory personnel and patients at risk of infection.[8] There are several ways to overcome such problems. Intact murine antibodies are easy to handle and show rapid results, with high sensitivity and specificity.[9 – 15] The major disadvantage of using intact murine antibodies is the development of human antimouse antibodies (HAMA)[16] and associated allergic reactions. The HAMA response can be addressed in several ways. The first option is the administration of human or humanized antibodies.[17,18] A disadvantage of these antibodies is their low degree of specificity, since a correct differentiation between specific uptake in an infectious focus and non-specific accumulation in an inflamed lesion is often impossible. The second option is the use of antibody fragments instead of intact immunoglobulin G.[19] Several multicentre studies have used ^{99m}Tc-labelled monoclonal antibody fragments (^{99m}Tc-Fab′ fragments, sulesomab) to image infection in pre-selected groups of patients with bone and soft-tissue infections; such studies have demonstrated a high degree of sensitivity and specificity in these patient populations.[20,21] The small size of the Fab′ fragments (50 kD) means that fast imaging is possible, as early as 1 – 4 h post-injection, due to rapid uptake into the lesion and a lower degree of background activity, and results in excellent imaging quality.[19]

There is an increasing demand for novel imaging methods to establish diagnosis, especially in patient populations with suspected low-grade infections. Such methods should, ideally, be without any patient side-effects, since repeated imaging is frequently necessary. In different studies, ^{99m}Tc-Fab′ fragment imaging has already proven to be an easy-to-handle antibody with high sensitivity and specificity for targeting infectious lesions, and without negative side-effects.[20 – 23]

The present study sought to determine whether the ^{99m}Tc-labelled Fab′ fragment, sulesomab, is a reliable imaging tool for resolving the problem of suspected septic total knee arthroplasty (TKA). Specifically, we investigated whether a quantitative evaluation of sulesomab was superior to a standard reading. Furthermore, we investigated whether there was a correlation between the imaging results and the histological and laboratory findings.

Patients and methods

PATIENTS

Between February 2006 and December 2007, consecutive patients who had undergone surgery for ≥ 1 year previously for either cemented or uncemented TKA, and who were suspected of having septic loosening, were identified at the outpatient clinic of the Department of Trauma, Reconstructive and Orthopaedic Surgery of the Centre Bad Cannstatt, Stuttgart, Germany. Patients who had undergone surgery within 1 year were excluded from the study owing to the potentially confounding effects of post-operative reactive changes on scintigraphic imaging. All eligible patients presented with a gradual deterioration in function and increasing pain at the site of the prosthesis, lasting > 6 weeks. No patient was receiving antibiotic treatment at the time of imaging. The patients had been referred to the radiology department for conventional radiography of the knee joint and fluoroscopically guided joint aspiration, according to our standard diagnostic protocol for cases of suspected infection.

The study was approved by the local ethics committee of the Centre Bad Cannstatt. All patients were fully informed about the study purpose and any potential risks, and gave their informed consent.

STANDARDS OF REFERENCE

In most patients (n = 24/26), final diagnosis was determined by microbiological evaluation of surgical specimens and on the basis of intra-operative findings. The criterion for infection was based on the detection of micro-organisms in cultures. If no micro-organisms were found, the detection of local abscess formation and the presence of neutrophilic granulocytes were also considered, to indicate that infection was definitely present. In 2/26 patients, diagnosis of infection was based on the results of joint aspiration together with clinical findings after a minimum follow-up of 6 months (range 6 – 14 months). No infection was assumed to be present in patients who had negative microbiological results after joint aspiration; normal erythrocyte sedimentation rates (ESR), C-reactive protein (CRP) levels and white blood cell (WBC) counts; or improvements in clinical symptoms > 6 months.

Histological specimens

Histological specimens were classified as: (i) severe (pus, necrotic-granular plasma cells, lymphocytic infiltrate, or plasma cells and lymphocytes only); (ii) moderate (plasma cells, lymphoplasmacellular infiltrates, beginning of granulation and scar tissue); and (iii) mild (very little lymphoplasma-cellular infiltrate; mostly sclerosis and scar tissue present).[24]

Laboratory evaluation

A minimum of 2 months' follow-up was required during which WBC, ESR and CRP levels were monitored.

^{99m}Tc-LABELLED MONOCLONAL ANTIBODY Fab′ FRAGMENT SCINTIGRAPHY

Characterization of the antibody, labelling procedure and application

Sulesomab (LeukoScan®, Immunomedics® GmbH, Darmstadt, Germany) was used, which is a ^{99m}Tc-labelled Fab′ fragment of IMMU-MN3, an immunoglobulin G1 murine monoclonal antibody produced from a hybridoma, developed by fusion of murine myeloma (SP 2/0) cells with spleen lymphocytes obtained from a mouse immunized with carcinoembryonic antigen. The antibody reacts strongly with non-specific cross-reacting antigen 90 (NCA-90, $K_a = 0.5 \times 10^8 \pm 0.2 \times 10^8$ l/mol) present on human granulocytes.[25,26] The Fab′ fragment was provided in a ready-to-label lyophilized kit from Immunomedics® Inc. (Morris Plains, NJ, USA). Labelling was achieved by adding 1000 – 1500 MBq of ^{99m}Tc-pertechnetate in isotonic saline solution directly into a vial containing 0.3 mg of the monoclonal antibody Fab′ fragment and shaking sporadically for 5 min. By carrying out radiolabelling in this manner, previous studies have demonstrated < 1% free Tc in the labelled product.[26] For imaging of patients, about 0.25 mg of sulesomab labelled with 1110 ± 185 MBq of ^{99m}Tc-sodium pertechnetate was diluted with saline and injected intravenously. Patients for whole-body and single-spot views received an activity of 555 ± 111 MBq.

Anterior and posterior whole-body and single-spot view scans of the knees (anterior, posterior and lateral views) were obtained 4 and 24 h post-injection, using a double-headed gamma camera (Prism 2000; Picker International, Cleveland, OH, USA) with a parallel hole, low energy, high resolution collimator, using the 140-keV ^{99m}Tc peak, a 256 × 256 matrix and a pre-selected time of 10 min/image for single-spot views.

Visual image analysis

Visual image analysis of sulesomab scans was performed by two independent, board-certified, experienced nuclear physicians, each with > 5 years' experience with sulesomab scans, who were blinded to the results of other studies. These readers were different from those involved in the evaluation of other assessments. The intensity of sulesomab uptake in each zone was graded on a four-point scale as follows: 1, sulesomab uptake was similar to that in the bone marrow; 2, sulesomab uptake was increased minimally over that in bone marrow; 3, sulesomab uptake was distinctly higher than uptake in bone marrow; 4, sulesomab uptake was two or more times greater than uptake in bone marrow.

Quantitative scintigraphic evaluation

Activity around the knee prosthesis was compared with activity of the pelvic bone marrow using regions of interest. Bone marrow regions were drawn as large as possible, with the shape and size of regions around the knee being dependent on the shape and size of activity in this area. Regions were copied from early- to late-phase views. A septic arthroplasty was present if the ratios in one of the regions around the TKA increased > 10% from the early to the late phase; the 10% threshold allowed differentiation between septic and aseptic abnormalities of joint prostheses.[27]

A scan was regarded as true-positive when an infectious or inflammatory cause for the abnormal uptake was confirmed by biopsy, culture and laboratory findings. It was scored as false-positive when an infectious or inflammatory basis for the uptake was unconfirmed. A false-negative scan was defined as a scan without abnormal uptake around the TKA, although infectious or inflammatory aetiology was subsequently established. A true-negative scan was defined as a normal scan obtained in a patient, with no infectious or inflammatory basis found during subsequent extensive investigations.

STATISTICAL EVALUATION

Sensitivity, specificity and accuracy values for ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) in the diagnosis of TKA infection were calculated separately for each reader, and quantitative measurements were made according to standard algorithms. Data were expressed as mean ± SD. The inter-observer agreement for each imaging modality was determined with κ-statistics: κ-values < 0.40 indicated poor agreement; 0.40 – 0.75 indicated fair to good agreement; and > 0.75 indicated excellent agreement.[28]

Diagnostic confidence was classified according to the following system: 0, high likelihood that no infection was present (i.e. both readers diagnosed no infection); 1, presence of infection was uncertain (i.e. readers' diagnoses were conflicting); 2, high likelihood of infection (i.e. both readers diagnosed infection). Diagnostic confidence was calculated separately for sensitivity and specificity values and was compared with the sign test. Non-parametric Mann–Whitney *U*-tests were used to analyse variables across the patients studied. *In vitro* laboratory tests were analysed for significant differences using the paired Student's *t*-test. *P*-values < 0.05 were considered to be statistically significant.

Results

DEMOGRAPHIC DATA

A total of 26 patients, 18 with cemented and eight with uncemented TKA, were evaluated. Twenty of the patients had undergone primary implantation and six had undergone revision arthroplasty of the knee. Patient demographic data for the 24 patients with infection are shown in Table 1. There

TABLE 1:
Demographic data, laboratory evaluation and infection details for the 24 patients with severe, moderate or mild septic total knee arthroplasty (TKA) loosening

Septic TKA loosening[a]	Patient	Age (years)	Gender	Fever (weeks)	CRP (mg/l)	ESR (mm/h)	WBC (x10^9/l)	Isolated micro-organism	Sulesomab
Severe	1	71	M	3	104	44	16.4	Coagulase-negative *Staphylococcus*	+/+/+
	2	85	M	2	87	51	18.9	*Staphylococcus epidermidis*	+/+/+
	3	66	F	3	116	46	15.0	Coagulase-negative *Staphylococcus*	+/+/+
	4	49	F	2	121	55	19.5	*Pseudomonas aeruginosa*	+/+/+
	Mean ± SD	67.75 ± 17.25		3.0 ± 1.0	107 ± 13.1	49.0 ± 9.04	17.4 ± 2.5		
Moderate	1	71	M	—	43	25	14.1	*Peptostreptococcus magnus*	+/+/–
	2	85	M	—	44	22	9.6	*Staphylococcus epidermidis*	+/+/–
	3	66	F	4	48	23	8.5	*Staphylococcus aureus*	+/+/–
	4	49	F	—	56	22	12.8	*Staphylococcus epidermidis*	+/+/–
	5	74	F	2	34	25	15.6	*Staphylococcus epidermidis*	+/+/–
	6	69	F	3	39	18	14.2	*Staphylococcus aureus*	+/–/–
	7	72	F	—	51	31	7.2	—	+/+/–
	8	81	M	—	39	36	15.1	*Staphylococcus epidermidis*	+/–/–
	9	67	F	3	46	34	12.5	*Pseudomonas aeruginosa*	+/+/–
	Mean ± SD	70.44 ± 14.56		3.0 ± 1.0	44.4 ± 11.1	26.2 ± 10.04	12.1 ± 3.7		
Mild	1	68	M	—	33	22	11.3	*Staphylococcus epidermidis*	–/+/+
	2	56	F	3	36	23	11.2	*Streptococcus viridans*	–/+/–
	3	54	F	—	25	17	13.2	*Staphylococcus epidermidis*	–/+/–
	4	62	F	—	44	8	12.2	—	+/+/–
	5	78	M	—	45	22	13.2	*Escherichia coli*	+/+/+
	6	62	F	2	10	10	7.3	—	+/+/+
	7	76	M	—	8	16	8.4	—	+/–/–
	8	75	F	—	15	16	9.4	—	+/+/–
	9	59	F	—	22	6	8.3	—	+/+/+
	10	83	M	4	36	10	8.8	—	+/+/–
	11	84	F	—	22	15	9.8	—	+/+/–
	Mean ± SD	68.81 ± 15.19		3.0 ± 1.00	26.9 ± 15.1	15.00 ± 9.22	10.2 ± 2.6		

+ or – signs indicate intensity of blood flow visually and increase or decrease, respectively, in uptake over time for ^{99m}Tc-monoclonal Fab' fragments (sulesomab). Normal values: C-reactive protein (CRP) ≤ 8.0 mg/l, erythrocyte sedimentation rate (ESR) < 15 mm/h, white blood count (WBC) 4.0 – 11.0 × 10^9/l.
[a]Histological specimens: severe – pus, necrotic-granular plasma cells, lymphocytic infiltrate, or plasma cells and lymphocytes only; moderate – plasma cells, lymphoplasmacellular infiltrates, beginning of granulation and scar tissue; mild – very little lymphoplasmacellular infiltrate, and mostly sclerosis and scar tissue.

were 17 females (age range 49 – 84 years, mean 71 years) and nine males (age range 68 – 85 years, mean 76 years). According to the results of the Mann–Whitney *U* test there were no significant differences between the mean ages of males and females. The mean time between the last surgical intervention at the site of the involved TKA and study inclusion was 71 months (range 12 – 260 months).

INFECTION DATA

Of the 26 patients who underwent surgery, 24 patients were found to have infection on the basis of the results of microbiological tests. The results of joint aspiration and laboratory findings excluded infection in two patients.

Histologically, in the 24 patients with infection, symptoms were classified as severe in four patients (pus, necrotic-granular plasma cells, lymphocytic infiltrate [three patients], plasma cells and lymphocytes only [one patient]); moderate in nine patients; and mild in 11 patients. Those with severe infection had elevated infection parameters: three patients had fistulae and one patient had a soft-tissue abscess adjacent to the prosthesis. Patients with moderate and mild infections had decreasing infection parameters. The micro-organisms found in the three classes of patients are shown in Table 1. In patients where no micro-organisms were found, the diagnosis of infection was based on the presence of abundant neutrophilic granulocytes, plasma cells, lymphoplasmacellular infiltrates and the formation of scar tissue, in combination with local abscess formation. Two of the 26 patients who underwent surgery had no infection on the basis of findings at joint aspiration. One of these patients had a foreign-body reaction to polyethylene debris with granuloma formation and the other had an aseptic loosening of the entire prosthesis in combination with a tibial fatigue fracture that had not been prospectively diagnosed at conventional radiography.

^{99m}Tc-LABELLED MONOCLONAL ANTIBODY Fab′ FRAGMENT SCINTIGRAPHY

Visual image analysis and inter-observer agreement

With the use of diffusely increased grade 3 or 4 sulesomab uptake as a criterion for infection, results of sulesomab were true-positive in all four patients with severe infection (images from one patient shown in Fig. 1).

Of the nine patients with moderate infection, results were true-positive in four patients for both readers (images from one patient shown in Fig. 2). Results of sulesomab were false-positive in two patients (reader one) and three patients (reader two) of the nine patients in whom a foreign body reaction with granuloma and a hyperplastic capsule were seen at surgery. For three of these patients, histopathological analysis revealed inflammatory cells – including histiocytes, giant cells and fibrovascular tissue – associated with foreign-body reactions. Results were false-negative in three of the patients for reader one and in two of the patients for reader two. In two patients with false-negative findings, focally increased grade 2 peri-prosthetic uptake was observed and, in one patient, uptake in the tibial region was difficult to interpret owing to overlying venous varicosis.

Of the 11 patients with mild infection (Fig. 3), true-positive results were found for three patients for reader one and four patients for reader two. False-negative results were found in six patients by both readers; in all these patients, grade 2 sulesomab uptake was similar to the surrounding normal bone marrow uptake. Unequivocal results with

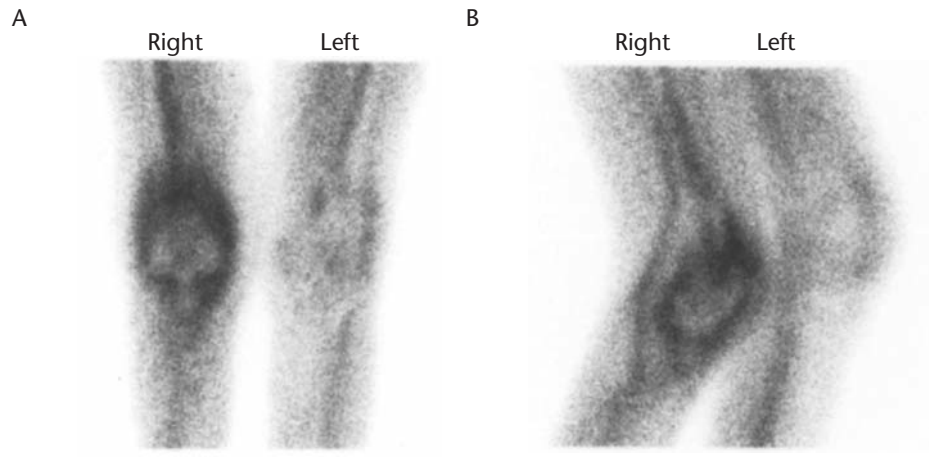

FIGURE 1: Patient with severe coagulase-negative *Staphylococcus* total knee arthroplasty infection. (A) Anterior and (B) lateral single spot views of ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) images showing pathologically increased uptake around the prosthesis at 4 h post-injection

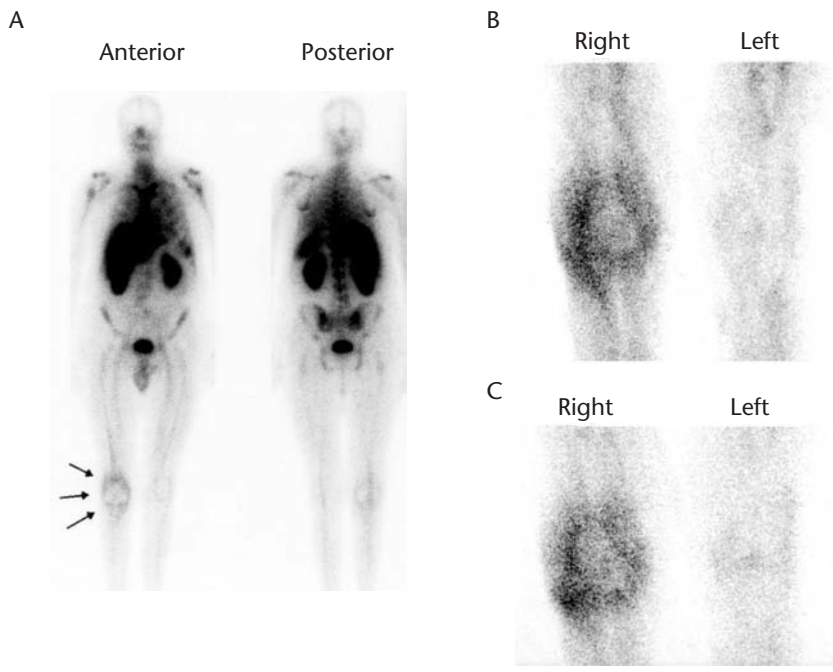

FIGURE 2: Patient with moderate *Staphylococcus epidermidis* total knee arthroplasty (TKA) infection. (A) Infection in a whole body scan at 4 h post-injection at the right TKA; single view spots with ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) showing increased uptake around the TKA at (B) 4 h post-injection and (C) 24 h post-injection

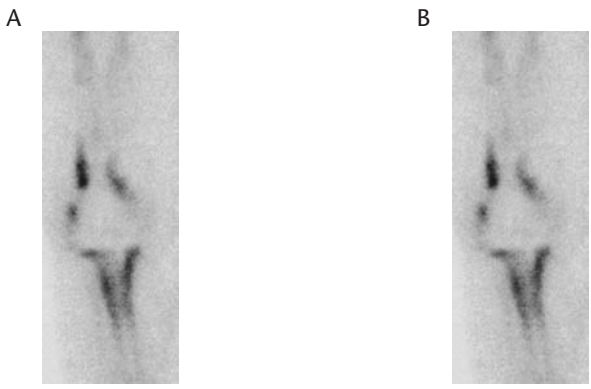

FIGURE 3: Patient with mild *Streptococcus viridans* total knee arthroplasty (TKA) infection. Single spot views of ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) showing decent uptake around the TKA at (A) 4 h post-injection and (B) 24 h post-injection with slightly increasing target to background ratio

grade 2 uptake or less were found in two patients for reader one and one patient for reader two, which for final analysis were added to the group of false-negative results. In most of these patients, histopathological analysis revealed lymphoplasmacellular infiltrate and sclerosis.

Sulesomab sensitivity, specificity and accuracy for readers one and two are shown in Table 2. The ^{99m}Tc-labelled monoclonal antibody Fab′ fragment scintigraphy (sulesomab) had excellent inter-observer agreement in patients with severe infection, with a κ-value > 0.75. Inter-observer agreement decreased with a decrease in grade of infection (moderate infection, κ-value 0.49; mild infection, κ-value 0.25). Calculating the diagnostic confidence for sensitivity and specificity values separately revealed that sulesomab had excellent results in patients with severe infections (P = 0.009), whereas values of confidence decreased in patients with moderate and mild infections (P = 0.035).

TABLE 2:
Inter-observer agreement between two readers for visual sulesomab interpretation (qualitative evaluation). Sensitivity, specificity and accuracy values for ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) in the diagnosis of the different grades of total knee arthroplasty infection calculated separately for readers one and two

	Reader one			Reader two		
	Severe infection[a]	Moderate infection[a]	Mild infection[a]	Severe infection[a]	Moderate infection[a]	Mild infection[a]
Sensitivity	100%	57%	27%	100%	66%	36%
Specificity	100%	66%	34%	100%	57%	39%
Accuracy	100%	88%	27%	100%	88%	36%

[a]Histological specimens: severe – pus, necrotic-granular plasma cells, lymphocytic infiltrate, or plasma cells and lymphocytes only; moderate – plasma cells, lymphoplasmacellular infiltrates, beginning of granulation and scar tissue; mild – very little lymphoplasmacellular infiltrate, and mostly sclerosis and scar tissue.

Quantitative image analysis and statistical evaluation

Quantitative evaluations of sulesomab in patients with severe infection gave true-positive results in all four patients, with ratios in one of the regions around the TKA that increased between 45% and 145% from the early to the late phase. For the nine patients with moderate infection, true-positive results were found in seven patients, with increased uptakes between 25% and 55%, false-negative results in one patient with uptake that decreased by 8% and a false-positive result in one patient. Histopathological analysis of the patient with the false-negative result revealed inflammatory cells with predominantly histiocytes, giant cells, fibrovascular tissue and few granulocytes only. For the 11 patients with mild infection (such as the patient in Fig. 4) true-positive results were found in eight patients, with increase of

4h post-injection

Bone marrow region	SIZE pixels	AVG counts/pixel	SUM counts
1	877	13.4	45 932
2	2103	54.5	22 432
3	841	13.2	10 900
4	432	19.4	3955
BG	499	0.2	125
Region	[1.5]	[1.1]	RATIO
SUM	29%	71%	0.41
AVG	22%	78%	0.28
Region	[1.4]	[1.1]	RATIO
SUM	31%	69%	0.45
AVG	24%	76%	0.32
Region	[1.6]	[1.1]	RATIO
SUM	11%	89%	0.12
AVG	22%	78%	0.28

24h post-injection

Bone marrow region	SIZE pixels	AVG counts/pixel	SUM counts
1	877	12.9	19 112
2	2103	3.4	7955
3	841	5.3	3824
4	432	3.5	955
BG	499	0.2	83
Region	[2.5]	[2.1]	RATIO
SUM	41%	66%	0.62
AVG	28%	72%	0.38
Region	[2.4]	[2.1]	RATIO
SUM	30%	74%	0.40
AVG	31%	72%	0.43
Region	[2.4]	[2.2]	RATIO
SUM	13%	86%	0.15
AVG	24%	71%	0.33

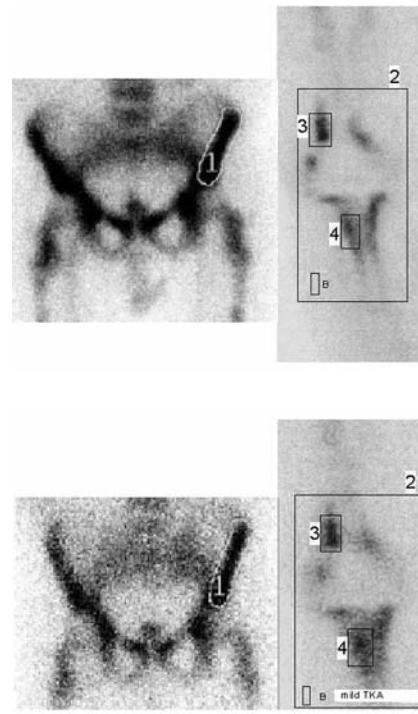

FIGURE 4: The same patient with mild *Streptococcus viridans* total knee arthroplasty (TKA) infection as shown in Fig. 3, showing ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) quantitative evaluation of the time–activity course in sections of whole-body scans. Number 1 indicates the bone marrow region; numbers 2 – 4 indicate regions around the TKA; and B/BG denotes the background region. For each region, pixel size, counts/pixel and the sum of counts are given. For each region around the TKA, the ratio to the bone marrow region was calculated (SUM, without and AVG, with plain normalization). The numbers in square brackets denote the regions for which the ratio was calculated. Percentages indicate the single region counts as a percentage of the sum of the counts in the two compared regions. For final count evaluation the counts/pixel ratios were applied. Increase in the plain corrected ratio ranged from 31% to 52%, indicating the diagnosis of infection

uptake between 13% and 27% and false-negative results in three patients. Histopathological analysis in these three patients revealed lymphoplasmacellular infiltrate, mostly sclerosis, scar tissue and no granulocytes. There were no false-positive results following quantitative evaluation in patients with mild infection. The quantitative analysis of sulesomab for sensitivity, specificity and accuracy in patients with severe, moderate and mild infections is depicted in Table 3. No correlation could be found for increase of sulesomab uptake within 24 h and the type of micro-organism responsible for infection.

Calculation of diagnostic confidence showed that quantitative evaluation of sulesomab increased significantly in sensitivity and specificity with the decrease in grade of infection compared with visual interpretation. A gain of specificity was especially seen for the quantitative evaluation of sulesomab in patients with moderate and mild infection (P = 0.008 and P = 0.0011, respectively).

STANDARDS OF REFERENCE

Histological findings

There was no correlation between the type of micro-organism found and sulesomab, but there was a good correlation between cellular infiltrates (such as number of granulocytes) and sulesomab (P = 0.008). The greatest correlation was found when sulesomab was evaluated quantitatively, especially in patients with moderate and mild TKA infections (P = 0.0011) (Fig. 5).

Laboratory findings

There was a strong correlation between WBC (16.4 – 19.5 × 10^9/l), ESR (44 – 55 mm/h) and CRP (87 – 121 mg/l) levels and the results of quantitatively evaluated sulesomab (increase of uptake 45 – 145% within 24 h; P = 0.001) in patients with severe TKA infections. WBC levels tended to be normal in patients with moderate/mild TKA infections while ESR and CRP levels were moderately increased in these patients (Table 1). In patients with moderate/mild TKA infections the best correlation was found between CRP levels (8 – 56 mg/l), ESR levels (6 – 36 mm/h) and sulesomab (increase of uptake 25 – 55% within 24 h; P = 0.009), whereas no significant correlation could be found with WBC levels. CRP levels showed significant differences (P = 0.0001) between the three classes of infection and greatest overall correlation with sulesomab (P = 0.009).

Discussion

An increasing number of surgeons express

TABLE 3:
Significances for quantitative evaluation of sulesomab. Sensitivity, specificity and accuracy values for ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) in the diagnosis of different grades of total knee arthroplasty infection calculated quantitatively using the regions of interest method

	Severe infection[a]	Moderate infection[a]	Mild infection[a]
Sensitivity	100%	88%	72%
Specificity	100%	> 90%	66%
Accuracy	100%	> 90%	85%

[a]Histological specimens: severe – pus, necrotic-granular plasma cells, lymphocytic infiltrate, or plasma cells and lymphocytes only; moderate – plasma cells, lymphoplasmacellular infiltrates, beginning of granulation and scar tissue; mild – very little lymphoplasmacellular infiltrate, and mostly sclerosis and scar tissue.

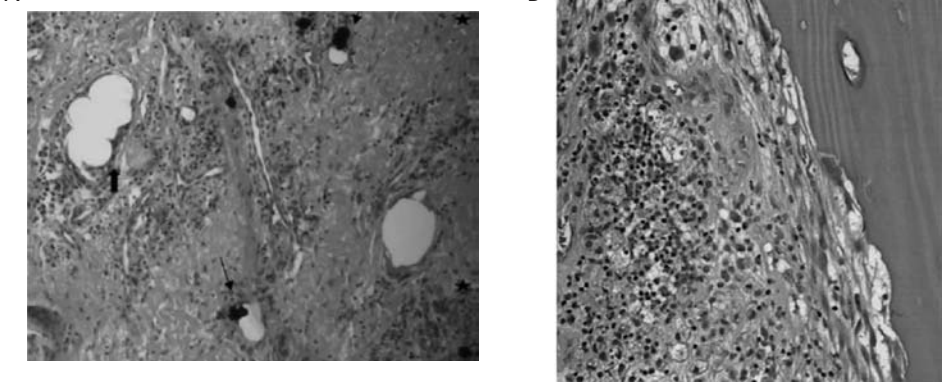

FIGURE 5: Histological specimens from two different patients with severe total knee arthroplasty (TKA) infection. (A) Strong cellular infiltrates with severe coagulase-negative *Staphylococcus* infection (patient 1) showing polyethylene wear particles (small arrow) surrounded by leucocytes and parenchymal cell debris (star) (septic loosening) as well as macrophages and multinuclear giant cells (large arrow). Polarization microscopy, haematoxylin and eosin (H & E) staining, iron reaction, original magnification ×100. (B) Tibial bone cross-section showing severe *Staphylococcus epidermidis* TKA infection within the bone (patient 2). H & E staining.

interest in the early and specific differential diagnosis of septic or aseptic TKA loosening and this will become even more important because the total number of TKA procedures performed in the USA is expected to increase more than five-fold, from approximately 428 000 in 2005 to nearly 2.16 million by 2030.[6]

The present study achieved excellent results with ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) in patients with severe TKA infections, whereas inter-observer agreement and diagnostic accuracy decreased in patients with moderate and mild TKA infections. This is consistent with similar studies using ^{99m}Tc-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO)-labelled granulocytes or ^{111}In-oxine-labelled granulocytes for imaging infection of suspected prosthesis loosening.[29] Love *et al.*[30] showed that labelled leucocytes accumulate not only in infected areas but also in the bone marrow. Historically, haematopoietically active marrow in adults has been assumed to be limited to the axial skeleton and proximal appendicular skeleton, with any labelled leucocyte activity outside this distribution ascribed to infection. In fact, the distribution of haematopoietically active marrow is extremely variable and can be affected by various entities, including tumours, fractures, haemolytic anaemias and even orthopaedic hardware.[7,30] Clearly, the explanation for the inconsistent results reported for labelled leucocyte imaging is thus neither chronicity nor non-specific inflammation, but rather a visual inability to distinguish uptake of labelled leucocytes in infection from uptake in aberrantly located, but otherwise normal, bone marrow.

This problem can be overcome in two ways: by the addition of complementary bone marrow imaging, which is usually performed with ^{99m}Tc-sulphur colloid,[7,31] or by quantitative evaluation of granulocyte migration within 24 h of imaging. The first method requires a further radionuclide

investigation which, taking into consideration the cost effectiveness and considerable time involved, is less preferable. The second method allows a quantitative estimate to be made of the migration of ^{99m}Tc-monoclonal Fab′ fragments (sulesomab)-labelled circulating granulocytes into the infected focus and normal bone marrow. While there is also non-specific extravasation of the free monoclonal Fab′ fragments into infected areas, specific accumulation of activity in the infected focus is largely caused by chemotaxis of monoclonal Fab′ fragments (sulesomab)-labelled circulating granulocytes. This is supported by the results of Steinsträsser *et al*,[32] who found that, for complete antibodies, 25% of the injected activity was cell bound, with a nearly constant level of cell bound activity over time. This also seems to be true for fragmented antibodies.[22,23]

To our knowledge, the present study is the first to evaluate the diagnostic efficacy of radionuclide imaging in patients with histologically established different grades of TKA infection. We demonstrated that quantitative interpretation using early- and late-phase views of ^{99m}Tc-monoclonal Fab′ fragments (sulesomab)-labelled circulating granulocytes gave significantly better results over visual observer interpretation, especially in patients with moderate and mild infections. In the latter patients, a visual differentiation between uptake in infectious foci and normal surrounding bone marrow was often not possible. Instead, with quantitative evaluation of ^{99m}Tc-monoclonal Fab′ fragment (sulesomab)-labelled circulating granulocytes, normal uptake in bone marrow physiologically decreased within 24 h, whereas in infectious foci there was a significant increase in uptake. Thus, in this particular group of patients with moderate TKA infection, a specificity > 90% and an overall diagnostic accuracy > 90% was found. In patients with mild TKA infection diagnostic accuracy still was 85%. This is very important since the majority of patients seen in daily clinical settings are patients with 'low grade' infections.

Furthermore, in the present study an excellent correlation was found between WBC, ESR and CRP, and the results of ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) in patients with severe and severe-to-moderate infections. In particular, CRP correlated best with the various grades of infection; it showed significant differences between the three classes of infection and a valuable correlation with the imaging results of ^{99m}Tc-monoclonal Fab′ fragments (sulesomab).

Nevertheless, it is important to compare the results of the present study with those obtained using other radionuclide imaging modalities for the evaluation of knee prostheses. Ciprofloxacin (sensitivity 86%, specificity 78%)[33] and ^{111}In-labelled WBC (sensitivity 83%, specificity 94%)[34] were unable to exclude infection of knee prostheses with certainty. In addition, fluorine-18 fluorodeoxyglucose positron emission tomography (sensitivity 91%, specificity 72%)[35] could not exclude infection in all cases. Compared with these studies, quantitatively evaluated ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) (moderate infection sensitivity 88%, moderate infection specificity > 90%) targeted at circulating granulocytes seem to be superior.

Limitations of the present study included a high infection prevalence – this was related to the prospective nature of the study – therefore there was a high confidence for sensitivities. The low κ-values in our study may not exclusively reflect the uncertainty of the various readers in diagnosing an infection in TKA with the current imaging modalities. Moreover, κ-values are strongly

influenced by disease prevalence.

Further studies are needed to evaluate whether ^{99m}Tc-monoclonal Fab′ fragment (sulesomab) scintigraphy is able to identify painful TKAs with occult infections not identified by aspiration, and whether both imaging methods can identify infection in patients with normal knee aspiration and screening laboratory tests (WBC, ESR, CRP). In conclusion, however, the present study showed ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) to be useful in the assessment of septic TKA. In combination with CRP imaging, suspected TKA infection with ^{99m}Tc-monoclonal Fab′ fragments (sulesomab) seems to be a promising diagnostic tool.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

• Received for publication 6 May 2008 • Accepted subject to revision 7 May 2008
• Revised accepted 14 July 2008

References

1 Daniels AU, Tooms RE, Harkess JW: Arthroplasty: introduction and overview. In: *Campbell's Operative Orthopaedics,* 9th edn (Canale ST, ed). St Louis: Mosby, 1998; pp 211 – 227.
2 National Institutes of Health: *Deep Infections of Total Joint Replacement*. Publication No. PA-00-014. Bethesda: National Institutes of Health, December 1999.
3 Guyton JL: Arthroplasty of ankle and knee. In: *Campbell's Operative Orthopaedics*, 9th edn (Canale ST, ed). St Louis: Mosby, 1998; pp 232 – 285.
4 Harkess JW: Arthroplasty of hip. In: *Campbell's Operative Orthopaedics*, 9th edn (Canale ST, ed). St Louis: Mosby, 1998; pp 296 – 456.
5 Steinberg DR, Steinberg ME: The early history of arthroplasty in the United States. *Clin Orthop Relat Res* 2000; **374:** 55 – 89.
6 Love C, Tomas MB, Marwin SE, *et al*: Role of nuclear medicine in diagnosis of the infected joint replacement. *Radiographics* 2001; **21:** 1229 – 1238.
7 Palestro CJ, Torres MA: Radionuclide imaging in orthopedic infections. *Semin Nucl Med* 1997; **27:** 334 – 345.
8 Rojas-Burke J: Health officials reacting to infections mishaps. *J Nucl Med* 1992; **33:** 13N – 14N, 27N.
9 Becker W, Borst U, Fischbach W, *et al*: Kinetic data of *in-vivo* labeled granulocytes in humans with a murine Tc-99m-labelled monoclonal antibody. *Eur J Nucl Med* 1989; **15:** 361 – 366.
10 Becker WS, Saptogino A, Wolf FG: The single late 99Tcm granulocyte antibody scan in inflammatory diseases. *Nucl Med Commun* 1992; **13:** 186 – 192.
11 Hotze AL, Briele B, Overbeck B, *et al*: Technetium-99m-labeled anti-granulocyte antibodies in suspected bone infections. *J Nucl Med* 1992; **33:** 526 – 531.
12 Lind P, Langsteger W, Költringer P, *et al*: Immunoscintigraphy of inflammatory processes with a technetium-99m-labeled monoclonal antigranulocyte antibody (MAb BW 250/183). *J Nucl Med* 1990; **31:** 417 – 423.
13 Locher JT, Seybold K, Andres RY, *et al*: Imaging of inflammatory and infectious lesions after injection of radioiodinated monoclonal anti-granulocyte antibodies. *Nucl Med Commun* 1986; **7:** 659 – 670.
14 Sciuk J, Brandau W, Vollet B, *et al*: Comparison of technetium 99m polyclonal human immunoglobulin and technetium 99m monoclonal antibodies for imaging chronic osteomyelitis. First clinical results. *Eur J Nucl Med* 1991; **18:** 401 – 417.
15 Sciuk J, Puskás C, Greitemann B, *et al*: White blood cell scintigraphy with monoclonal antibodies in the study of the infected endoprosthesis. *Eur J Nucl Med* 1992; **19:** 497 – 502.
16 Goldenberg DM, Goldenberg H, Sharkey RM, *et al*: Clinical studies of cancer radioimmunodetection with carcinoembryonic antigen monoclonal antibody fragments labeled with ^{123}I or ^{99m}Tc. *Cancer Res* 1990; **50(3 suppl):** 909s – 921s.
17 Oyen WJ, Claessens RA, van Horn JR, *et al*: Scintigraphic detection of bone and joint infections with indium-111-labeled nonspecific polyclonal human immunoglobulin G. *J Nucl Med* 1990; **31:** 403 – 412.
18 Rubin RH, Fischman AJ, Callahan RJ, *et al*: ^{111}In-labeled nonspecific immunoglobulin scanning in the detection of focal infection. *N Engl J Med* 1989; **321:** 935 – 940.
19 Goldenberg DM, Larson SM: Radioimmunodetection in cancer identification. *J Nucl Med* 1992; **33:** 803 – 814.
20 Delcourt A, Huglo D, Prangere T, *et al*: Comparison between LeukoScan (sulesomab) and gallium-67 for the diagnosis of

osteomyelitis in the diabetic foot. *Diabetes Metab* 2005; **31:** 125 – 133.
21 Kerry JE, Marshall C, Griffiths PA, *et al*: Comparison between Tc-HMPAO labelled white cells and Tc LeukoScan in the investigation of inflammatory bowel disease. *Nucl Med Commun* 2005; **26:** 245 – 251.
22 Gratz S, Schipper ML, Dorner J, *et al*: LeukoScan for imaging infection in different clinical settings: a retrospective evaluation and extended review of the literature. *Clin Nucl Med* 2003; **28:** 267 – 276.
23 Gratz S, Raddatz D, Hagenah G, *et al*: ^{99m}TC-labelled antigranulocyte monoclonal antibody FAB′ fragments versus echocardiography in the diagnosis of subacute infective endocarditis. *Int J Cardiol* 2000; **75:** 75 – 84.
24 Mirra JM, Marder MD, Amstutz HC: The pathology of failed total joint arthroplasty. *Clin Orthop Relat Res* 1982; **170:** 175 – 183.
25 Hansen HJ, Goldenberg DM, Newman ES, *et al*: Characterization of second-generation monoclonal antibodies against carcino-embryonic antigen. *Cancer* 1993; **71:** 3478 – 3485.
26 Hansen HJ, Jones AL, Sharkey RM, *et al*: Preclinical evaluation of an "instant" ^{99m}Tc-labeling kit for antibody imaging. *Cancer Res* 1990; **50(3 suppl):** 794s – 798s.
27 Pelosi E, Baiocco C, Pennone M, *et al*: ^{99m}Tc-HMPAO-leukocyte scintigraphy in patients with symptomatic total hip or knee arthroplasty: improved diagnostic accuracy by means of semiquantitative evaluation. *J Nucl Med* 2004; **45:** 438 – 444.
28 Fleiss JL: The measurement of interrater agreement. In: *Statistical Methods for Rates and Proportions,* 2nd edn (Fleiss JL, ed). Toronto: John Wiley, 1981; pp 212 – 236.
29 Simonsen L, Buhl A, Oersnes T, *et al*: White blood cell scintigraphy for differentiation of infection and aseptic loosening: a retrospective study of 76 painful hip prostheses. *Acta Orthop* 2007; **78:** 640 – 647.
30 Love C, Tomas MB, Marwin SE, *et al*: Role of nuclear medicine in diagnosis of the infected joint replacement. *Radiographics* 2001; **21:** 1229 – 1238.
31 Palestro CJ, Swyer AJ, Kim CK, *et al*: Infected knee prostheses: diagnosis with In-111 leukocyte, Tc-99m sulfur colloid, and Tc-99m MDP imaging. *Radiology* 1991; **179:** 645 – 648.
32 Steinsträsser A, Berberich R, Kuhlmann L, *et al*: Binding of the monoclonal antibody BW 250/183 to human granulocytes. *Nuklearmedizin* 1992; **31:** 57 – 63 [in German, English abstract].
33 Larikka MJ, Ahonen AK, Niemelä O, *et al*: ^{99m}Tc-ciprofloxacin (Infecton) imaging in the diagnosis of knee prosthesis infections. *Nucl Med Commun* 2002, **23:** 167 – 170.
34 Scher DM, Pak K, Lonner JH, *et al*: The predictive value of indium-111 leukocyte scans in the diagnosis of infected total hip, knee, or resection arthroplasties. *J Arthroplasty* 2000; **15:** 295 – 300.
35 Zhuang H, Duarte PS, Pourdehnad M, *et al*: The promising role of 18F-FDG PET in detecting infected lower limb prosthesis implants. *J Nucl Med* 2001; **42:** 44 – 48.

Author's address for correspondence
Dr S Gratz
Department of Nuclear Medicine, Centre Bad Cannstatt, Seelbergstraße 11, 70372 Stuttgart, Germany.
E-mail: Nuklearmedizin-Gratz@gmx.net