Utility of Indium-111-Labeled Polyclonal Immunoglobulin G Scintigraphy in Fever of Unknown Origin

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We studied the role of ^{111}In-labeled immunoglobulin (^{111}In-IgG) scintigraphy in different subgroups of patients with fever of unknown origin (FUO). **Methods:** During a 2-yr period (January 1992 through January 1994), the internal medicine wards of eight university hospitals in The Netherlands participated in this study. A total of 167 patients with FUO were prospectively included to prevent unintended selection. Fifty-eight patients underwent ^{111}In-IgG scintigraphy. For 23 patients without potentially diagnostic clues (PDCs) or only misleading PDCs, the technique was used as a screening procedure. In 35 patients with PDCs pointing at local inflammation this technique was used when indicated. **Results:** After diagnostic work-up, infections were found in 17 patients (29%), neoplasms in 6 (10%), noninfectious inflammatory diseases in 14 (24%) miscellaneous disorders in 3 (5%) and no diagnosis in 18 (31%). Indium-111-IgG scintigraphy was helpful in the diagnostic process for patients with PDCs at local inflammation only. The diagnostic yield of this technique in this subgroup was 26%. Infection was found in only 10/41 patients with negative scans. All infections were nonfocal or located in the heart, liver region or urinary tract where physiological uptake obscures possible pathologic uptake. The overall sensitivity and specificity was 60% and 83%, respectively. **Conclusion:** In patients without PDCs for local inflammation, the diagnostic yield of scintigraphic techniques was quite low since no focal inflammation was observed. Therefore, ^{111}In-IgG scintigraphy should not be used as a second-step procedure in the work-up of these subgroup of patients with FUO. In patients with PDCs at local inflammation, ^{111}In-IgG is helpful in the diagnostic process in one-fourth of the patients. This diagnostic yield is comparable with that of the majority of other scintigraphic techniques used in the diagnostic process of patients with FUO.

Key Words: fever of unknown origin; indium-111-IgG scintigraphy

J Nucl Med 1997; 38:484–489

Petersdorf and Beeson (*1*) defined fever of unknown origin (FUO) as a febrile illness evolving over at least 3 wk, with documented temperature of at least 38.3°C (101°F) on three or more occasions and uncertain diagnosis after 1 wk of diagnostic work-up in the hospital.

Scintigraphic methods play an important role in the diagnostic process of these patients as instruments to demonstrate or exclude local inflammatory and infectious diseases. Scintigraphic imaging with ^{67}Ga, ^{111}In or ^{99m}Tc white blood cells (WBCs), ^{111}In labeled-immunoglobulin G (^{111}In-IgG) and ^{99m}Tc-labeled BW250/183, an antigranulocyte monoclonal antibody of murine origin, has been applied in patients with FUO to detect inflammatory foci (*2–7*). Some investigators believe that scintigraphy should be a second step as apposed to a last resort procedure in the evaluation of FUO (*2*). However, the diagnostic yield of scintigraphic methods in the diagnostic process of FUO is unknown, mainly because these previous studies were retrospective in nature.

We performed a prospective study on the utility of ^{111}In-IgG scintigraphy to ascertain the role and diagnostic yield of scintigraphy in patients with FUO without indices of inflammation. Indium-111-IgG scintigraphy has proven to be a promising technique in FUO in that it has technical advantages over other scintigraphic techniques and high diagnostic accuracy (*6,8*).

MATERIALS AND METHODS

Patients

From January 1992 through 1994, a prospective study on FUO, approved by all local ethical committees, was performed in all eight Dutch university hospitals. All immunocompetent patients fulfilling the classic criteria of FUO formulated by Petersdorf and Beeson (*1*) were entered into the study. All participants gave informed consent and 167 patients were included in our FUO protocol, which consisted of a standardized multiple choice history, physical examination and certain obligatory investigations (Table 1). Indium-111-IgG scintigraphy was performed in 58 of these 167 patients (33 women, 25 men; age range 21–87 yr, mean 55 yr).

Much consideration was given to the presence or absence of potentially diagnostic clues (PDCs), defined as all localizing abnormalities potentially pointing towards a diagnosis and the use of these PDCs in the diagnostic process. Misleading PDCs were defined as PDCs not leading to the definite diagnose. All data, including those on PDCs, were prospectively registered in a structured data collection form. In the presence of PDCs, appropriate investigations were performed. In the absence of PDCs or in the presence of only misleading PDCs, patients underwent a two staged screening diagnostic protocol (Table 1) which included ^{111}In-IgG scintigraphy in the first stage. This diagnostic protocol was discontinued when a definite diagnosis was made, PDCs appeared or fever subsided.

No PDCs or only misleading PDCs were present in 43 patients when prospectively studied. In these patients, the first stage of the diagnostic screening protocol was performed. Because this scintigraphic part of the study was not initiated until January 1993, only 23 of these 43 patients underwent ^{111}In-IgG scintigraphy. In the remaining 124 patients with PDCs, ^{111}In-IgG scintigraphy was performed in 35 patients because of suspected localized inflammation based on PDCs. Both groups are evaluated separately in this study.

Exclusion criteria for ^{111}In-IgG scintigraphy were agammaglobulinemia, selective IgA deficiency and a history of severe adverse reactions after intravenous or intramuscular administration of human IgG. Pregnant or lactating women were also excluded from this study. None of the patients had uremia, but this was not an exclusion criterion.

Received Jan. 18, 1996; revision accepted Jul. 5, 1996.

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TABLE 1
Diagnostic Protocol

Investigations Performed in all Patients after Study Inclusion
Sedimentation rate; hemoglobin; mean cellular volume;
platelet count; leukocyte count and differential count;
serum urea nitrogen; creatinine; sodium; potassium; protein; protein fractions;
alkaline phosphatase; aminotransferase; lactate dehydrogenase; creatine phosphokinase;
antinuclear antibodies; rheumatoid factors;
urinary analysis; faeces for occult blood;
blood cultures aerobic and anaerobic (three times); tuberculin test;
urine-, feces-, and sputum culture when indicated;
chest radiography; ultrasonography of upper abdomen
Phase 1: Diagnostic Protocol in Patients without PDCs
Pulse/temperature measurement with observer
Fundoscopy by an ophthalmologist
Calcium, phosphate, urate, amylase and TSH/T4
Immunoelectrophoresis of serum and urine
CRP, ACE, ANCA, anti-dsDNA, AST and cryoglobulin
C3, C4, CH50 and circulating immune complexes
Serology for *Cytomegalovirus*, *Epstein-Barr virus*, *Mycoplasma*, *Brucella*, *Toxoplasma*, *Borrelia*, *Coxiella*, *Treponema* and *Yersinia*
Blood cultures for more than a week, stools for worms, eggs, cysts
Blood, urine and gastric fluid cultures for tuberculosis
Bone marrow puncture and culture on *Mycobacteria, Brucella, Yersinia*
Indium-111-IgG scintigraphy
Radiography of teeth and sinus
Ultrasound of lower abdomen
Phase 2: Diagnostic Protocol in Patients without PDCs
Hepatitis B serology
Repeated PPD, when negative Merieux skin tests on anergy
Repeated chest radiography
IgD measurement
Liver biopsy and culture for *Mycobacteria* and other bacteria and fungi; IF on *Yersinia*
Crista biopsy and culture on *Mycobacteria*, *Brucella*, bacteria; IF on *Yersinia*
Ultrasound of the heart
CT abdomen and thorax
Colon radiography
Temporal artery biopsy if the patient is older than 55 yr

CRP = C-reactive protein; ACE = angiotensin converting enzyme; ANCA = antineutrophil cytoplasmatic antibody; AST = antistreptolysin titer; C = complement; CMV = cytomegalovirus; EBV = epstein-barr virus; IF = immunofluorescence; PPD = purified protein derivative.

When possible, the scintigraphic findings were verified microbiologically but in some cases verification was made by clinical, radiographic and ultrasonographic methods. The final diagnosis and prospective analysis of diagnostic clues were made by one of the authors of this article and the attending physicians.

Radiopharmaceuticals

Human nonspecific polyclonal IgG conjugated to diethylenetriaminepentaacetic bicyclic anhydride was prepared as a lyophilized kit for one step labeling with ^{111}In according to the manufacturer's instructions. A dose of 2 mg IgG labeled with 75 MBq of ^{111}In was injected intravenously.

Imaging Procedures

Scintigraphic images were obtained with a gamma camera connected to an image processor. All images were collected in digital format in a 256 × 256 matrix. A medium-energy, parallel-hole collimator was attached to the camera. Both ^{111}In peaks of 173 and 247 keV were used with 15% symmetric windows.

The ^{111}In-IgG images were acquired 4, 24 and 48 hr after injection for a preset time of 5, 7.5 and 10 min, respectively. At least once, 24 hr after injection, spot views of the total body were obtained. All images were interpreted by two observers who were blinded to the results of the verification procedures. Disagreements were resolved by consensus.

An ^{111}In-IgG scan was interpreted as positive only if consistent, focally increasing accumulation could be observed over time. An ^{111}In-IgG scan was considered true-positive only when this imaging procedure was considered helpful in the diagnosis.

Statistical Analysis

Differences between groups were analyzed using Fischer's exact test and Mann-Whitney U-test or Student's t-test.

RESULTS

Of the 58 patients who underwent ^{111}In-IgG scintigraphy, no diagnosis was established in 18 patients (31%), infection was found in 17 patients (29%), a neoplasm in 6 (10%), noninfectious inflammatory disease (NIID) in 14 patients (24%) and miscellaneous diseases in 3 (5%). For the following variables there were no significant differences between the group of patients with FUO who underwent ^{111}In-IgG scintigraphy (n = 58) and those who did not (n = 109): percentage of patients with no diagnosis, duration of diagnostic process, period of follow-up, age, percentage of patients with periodic fever and duration of hospitalization.

Fourteen of 35 (40%) patients (Table 2) with PDCs had positive scans as compared to 3 of 23 (13%) patients (Table 3) who had undergone ^{111}In-IgG scintigraphy as a screening procedure ($p = 0.04$).

In patients with PDCs, ^{111}In-IgG scintigraphy helped establish the final diagnosis in 9 of 35 (26%) patients (Table 2, Figs. 1, 2 and 3), whereas it was not helpful diagnostically in 23 patients (Table 3) who had the test as a screening procedure ($p = 0.03$).

In nine patients (16%), all patients with PDCs at local inflammation, ^{111}In-IgG scintigraphy was helpful in establishing a diagnosis. In eight patients (14%), a positive ^{111}In-IgG scintigram did not lead to the final diagnosis. In two of these patients, clinically suspected arthritis was confirmed by the ^{111}In-IgG scintigraphy, and in one patient, activity in the maxillary sinus was confirmed radiographically. However, a malignant lymphoma proved to be the cause of the fever. In the five remaining patients, ^{111}In-IgG scintigraphy was false-positive and resulted in several unnecessary tests. In one of the latter patients, focal activity was observed in the right iliosacral joint. Pathological abdominal activity was observed in two patients, in the right ankle in one patient and abnormal activity was observed in both lungs in the fifth patient. In four of these five patients, no definite diagnosis could be established.

The data on the 41 patients with negative ^{111}In-IgG scans are shown in Tables 2 and 3. In 14 of these patients, no diagnosis was established after extensive work-up. Overall follow-up after inclusion in the study varied from 33 to 1421 days (median 834 days). For patients without diagnosis, follow-up after study inclusion ranged from 362-1400 days (median 1053 days). In 10 patients, an infection was diagnosed. Urinary tract infections (n = 3), viral infections (n = 3), endocarditis, secondary syphilis, cholangitis due to sludge and chronic yersiniosis. Calculated overall sensitivity of ^{111}In-IgG scintigraphy in this study was 60% with a specificity of 83%.

DISCUSSION

In this study, we prospectively studied the utility of ^{111}In-IgG scintigraphy in patients with FUO. Sixteen percent of the

TABLE 2
Patient Characteristics of Indium-111 Scans Performed on Indication (n = 35)

Patient no.	Age (yr)	Clinical data	Localization uptake ^{111}In-IgG scan	Final diagnosis (follow-up from inclusion, d)	Additional investigations (plus obligatory investigations)
			True-Positive Scans		
1	65	Abdominal pain, diarrhea	Colon area	Diverticulitis	Coloscopy, abdominal CT
2	24	Rattling with normal x-ray	Right lung (Fig. 1)	Pleural empyema	CT, pleura puncture, course
3	26	Diffuse abdominal pain	Right lower abdomen	Right adnexitis	Laparoscopy, culture, course
4	72	Diffuse abdominal pain	Ascending colon	Diverticulitis	Colon radiography, abdominal US
5	72	Cervix cancer, tumor US	Low abdomen (Fig. 2)	Pelvic abscess	Laparotomy and culture
6	37	Pain wrist, sicca syndrome	Left arm	Granulomatous myositis	Muscle biopsy
7	33	Gartner's syndrome, abdominal pain	Desmoid tumor	Necrosis desmoid tumor	Abdominal CT, negative culture
8	62	Anemia, vascular graft	Colon	Ischemic colitis	Laparotomy
9	53	Heart murmur/*S. aureus*	Hip (Fig. 3)	Endocarditis, abscesses	Echocardiography
			Positive, Not Helpful Scans		
10	48	Vasculitis, breast cancer, arthritis	Many joints	Small metastasis	Protocol 1 plus 2*, lymph node biopsy
11	27	Abnormal liver biopsy, arthritis knee	Knee	Hepatitis C	Serology, puncture knee
12	52	IBD in past, abscess thoracic wall	Right iliosacral joint	Relapse IBD	MRI bony pelvis/2* coloscopy
13	69	Abdominal pain, diarrhea	Ascending colon	No diagnosis (362)	Coloscopy, colon radiography cultures
14	46	Lymphoma neck, abscess liver biopsy	Terminal ileum	No diagnosis (1067)	Protocol 1 plus 2*, no coloscopy
			Negative Scans		
15	78	Cystitis, cryoglobulinemia, dizziness	No activity	Mixed cryoglobulinemia	No infections, cryoglobulines
16	65	Erythrocyturia, heart murmur	No activity	Mixed cryoglobulinemia, glomerulonephritis	Biopsy kidney, cryoglobulines
17	77	Abnormal urinary analysis	No activity	Urinary tract infections	Third urine culture during antibiotics
18	87	Heart murmur, anemia, splenomegaly	No activity	Culture negative endocarditis	Echocardiography positive
19	67	Raynaud phenomena, valve disease	No activity	Drug fever	Clinical course
20	30	Tropical travels, gonorrhea past	No activity	Secondary syphilis	Serology, abdominal US/CT
21	42	Hematospermia, gonorrhea past	No activity	Recurrent prostatitis	Clinical course, response therapy
22	68	Mexican travel/diarrhea, dysuria	No activity	Urinary tract infection	Second urine culture/therapy typhus
23	70	Erythema nodosum, abdominal pain	No activity	Polymyalgia rheumatica	Abdominal US/CT, course
24	64	Heart murmur, lung atelectasis	No activity	Endocarditis *S. bovis*	Echocardiography/culture
25	65	Pain back, caries, breast cancer past	No activity	Temporal arteritis	Protocol 1 plus 2* (temporal biopsy)
26	55	Tropical travel, pain, smelly urine	No activity	Chronic yersiniosis	Protocol 1*, clinical course
27	31	Epididymitis, lesion spine MRI, rash	No activity	Nonclassifiable vasculitis	Protocol 1 plus 2*, spine biopsy
28	21	Wound contact mud, heart murmur	No activity	Reiter's syndrome	Exclusion endocarditis, course
29	21	Yersinia abscess spleen, aneurysms	No activity	Polyangiitis syndrome	Skin biopsy, thoracal DSA
30	39	Arthritis, heart murmur, urticaria	No activity	No diagnosis (1365)	Protocol 1*, joint radiography, US heart
31	31	Spitz-Holter drain, cough, blood stools	No activity	No diagnosis (1113)	Colon radiography, negative cultures
32	65	Heart murmur, hip prothesis	No activity	No diagnosis (1107)	Echocardiography, course
33	57	Lung infiltrate, paraprotein, osteolysis	No activity	No diagnosis (1142)	Protocol 1 plus 2*, bronchoscopy
34	32	Abdominal pain, polycystic ovarian disease	No activity	No diagnosis (854)	US, coloscopy, laparoscopy
35	18	Abdominal pain, cough	No activity	No diagnosis (627)	Protocol 1*, abdominal CT

*See Table 1.
IBD = inflammatory bowel disease; ANA = antinuclear antibody; RA = rheumatoid arthritis; T_4 = thyroxine; TSH = thyroid-stimulating hormone.

^{111}In-IgG scans were helpful in the diagnostic process. The percentage of scans helpful in the diagnostic process, as reported in literature, varied from 18% to 75% (Table 4), but in most studies the scintigraphic method was helpful in the diagnostic work-up in about one-quarter of the patients. This was also observed in our study, since ^{111}In-IgG scintigraphy had a diagnostic yield of 26% in a subgroup of 35 patients with PDC for local inflammation. The variation of diagnostic yield in literature probably depends on the degree of selection in the group of patients with FUO. All but one study was conducted retrospectively (*2*). Moreover, in most studies, a large percentage of postoperative patients were included.

No diagnosis could be made in 18/58 (31%) patients in our study. Our findings were similar to data presented in recent studies (*9,10*). In earlier studies, this percentage is even lower (*1,11*).

There are definitely some problems with the calculation of sensitivity and specificity of scintigraphic techniques in patients with FUO. First, since a final diagnosis is not established in all patients undergoing scintigraphy, the interpretation of the results of this procedure is hampered due to a lack of a golden standard. When additional investigations are negative and long-term follow-up does not reveal an infection in these patients, it is probably legitimate to presume that local inflammation is not the cause of fever in these patients. In 30% of patients in our study, no diagnosis could be made after a median follow-up of 2.5 yr. Second, in the subgroup of patients without PDC, no local inflammatory processes were found causing FUO. Thus, neither true-positive scans nor false-negative were found, making calculation of sensitivity and specificity impossible in this subgroup. Third, in patients with a negative scintigram, a variety of diseases were found that could not be diagnosed with

TABLE 3
Patients Characteristics of Indium-111-IgG Scans Performed as Screening (n = 23)

Patient no.	Age (yr)	Clinical data	Localization uptake ^{111}In-IgG scan	Final diagnosis (follow-up from inclusion, d)	Additional investigations (plus obligatory investigations)
			Positive, Not Helpful Scans		
36	70	None	Malleolus lateralis	No diagnosis (1169)	Ankle radiography, bone biopsy negative
37	57	Heart murmur/negative echocardiography, dyspnea with negative chest x-ray, RA	Both lungs	No diagnosis (1263)	Ventilation/perfusion scan
38	52	Abdominal lymphadenopathy	Paranasal sinuses	Malignant lymphoma	Sinus radiography, mucosal swelling
			Negative Scans		
39	37	Lymphadenopathy, erythema nodosa	No activity	No diagnosis (1400)	Protocol 1*
40	38	Changed defecation/normal coloscopy	No activity	No diagnosis (1269)	Protocol 1*
41	36	Cough, lymphadenopathy, splenomegaly	No activity	No diagnosis (1039)	Protocol 1*
42	46	Arthralgia, redness skin joint	No activity	No diagnosis (999)	Enteric radiography, coloscopy
43	67	Emphysema, liver function disturbance	No activity	No diagnosis (976)	Culture, US, liver biopsy
44	62	Prosthetic valves, right heart failure	No activity	No diagnosis (948)	Protocol 1 plus 2*
45	71	Lung lesion for 1 yr, thrombocytopenia	No activity	No diagnosis (868)	Chest radiography, bone marrow biopsy
46	21	Lymphadenopathy, splenomegaly, hemolysis	No activity	No diagnosis (904)	Protocol 1 plus 2*, hemolysis analysis
47	66	None	No activity	Mixed cryoglobulinemia	Protocol 1*
48	64	Generalized lymphadenopathy	No activity	AILD	Fourth lymph-node biopsy
49	25	Lymphadenopathy, abdominal pain	No activity	Takayasu's disease	Protocol 1 plus 2*, laparoscopy
50	33	Unexplained abundant diarrhea	No activity	Factitious fever	Proven laxative disuse
51	43	Urticaria, lymphadenopathy	No activity	Urticarial vasculitis	Protocol 1 plus 2*, skin biopsy
52	58	Liver function disorder, skin lesions	No activity	Cholangitis/sludge	Abdominal CT and US
53	29	Low back pain, diarrhea, iridocyclitis	No activity	Still's disease	Protocol 1*, clinical course
54	55	Sarcoidosis past, rash, lymphocytosis	No activity	Cytomegalovirus infection	Serology, ACE/chest x-ray
55	71	Urticarial vasculitis, monoclonal IgM	No activity	Schnitzler's disease	Protocol 1*, skin biopsy, course
56	42	Cardiac valve disease/negative US of heart, abdominal lymphadenopathy	No activity	Hodgkin's disease	Bone biopsy, histology spleen
57	44	Hepatosplenomegaly, lymphocytosis	No activity	Cytomegalovirus infection	Serology
58	65	Weight loss, dyspnea, heart failure, irregular heartbeat	No activity	Hyperthyroidism	T_4 and TSH

*See Table 1.

AILD = angioimmunoblastic lymphoma; ANA = antinuclear antibody; IBD = inflammatory bowel disease; RA = rheumatoid arthritis; T_4 = thyroxine; TSH = thyroid-stimulating hormone.

^{111}In-IgG scintigraphy because lesions were present in organs with relatively high physiologic uptake, such as the liver, heart and urinary tract. Nonfocal infections such as viral infections could not be excluded by ^{111}In-IgG scintigraphy. Despite these limitations of the technique, a negative scan did rule out focal infection or inflammation with a high degree of certainty.

Similar to ^{67}Ga, ^{111}In-WBCs and ^{99m}Tc-HMPAO-labeled WBCs, ^{111}In-IgG can be excreted in the bowel under physiological conditions (*5,12,13*). However, such excretion was not significant and hardly interfered with adequate evaluation of possible abdominal infections or inflammation (*14*). We observed in two patients only abnormal bowel activity. In six other patients, however, pathological activity in the abdomen led to the final diagnosis.

In contrast to Knockaert et al. (*2*), in our study the duration of hospitalization and diagnostic process of patients who underwent scintigraphy was not significantly longer than in patients who did not undergo scintigraphy. We performed ^{111}In-IgG scintigraphy as a secondary step in the diagnostic protocol for patients without PDCs, whereas Knockaert et al. (*2*) scheduled ^{67}Ga scintigraphy as a third step or last resort procedure when the source of fever remained unknown. Naturally, in this latter category, the chance of reaching a diagnosis is lower.

By prospectively separating patients without PDCs from those with PDCs for local inflammation, we found a strikingly low diagnostic yield of this technique when using it as a screening procedure in patients with FUO. Therefore, scinti-

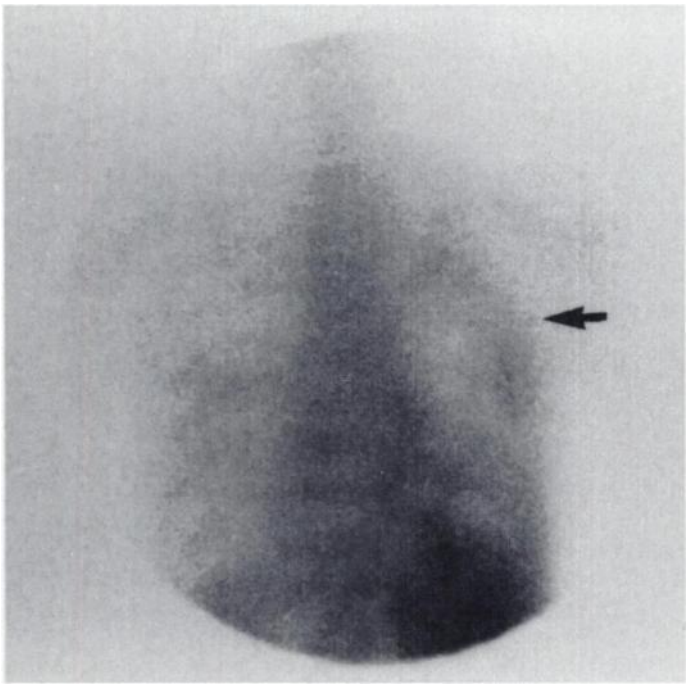

FIGURE 1. A 24-yr-old mentally disabled man presenting with fever and rattling respiration had a normal chest radiography. The ^{111}In-IgG scan shows abnormal activity in the right lung (posterior view). CT and pleural puncture proved pleural empyema caused by *S. pneumoniae*. After antibiotic therapy, fever and symptoms resolved (Patient 2).

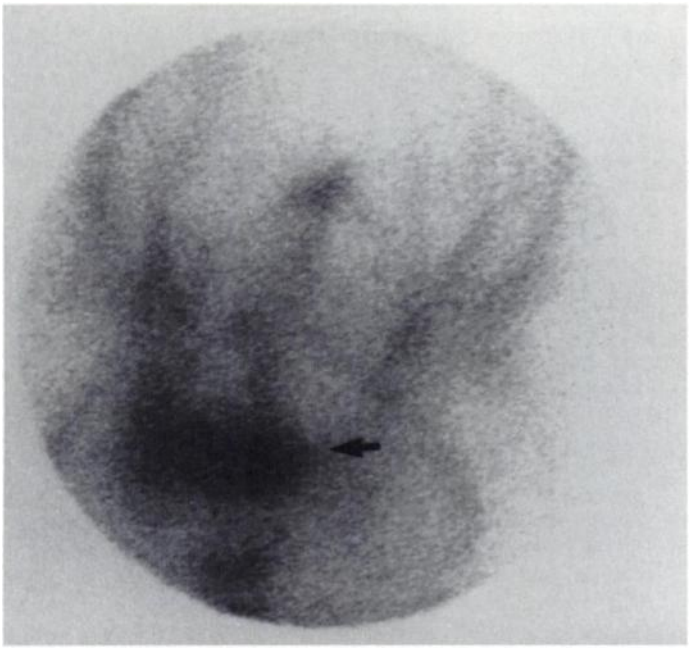

FIGURE 2. Cervical carcinoma was diagnosed in this 72-yr-old woman. Surgery was unsuccessful and radiotherapy was administered. After 3 mo, fever and abdominal pain developed. Abdominal US revealed a tumor consistent with abnormal uptake in the lower abdomen on ^{111}In-IgG scintigraphy. Laparotomy and culture revealed a pelvic abscess caused by *Peptococcus spp.* After surgery and antibiotic therapy, she recovered and her fever resolved (Patient 5).

graphic imaging should not be a second step procedure in the diagnostic work-up of this subcategory of patients with FUO.

CONCLUSION

During a 2-yr period, we prospectively investigated 167 patients with FUO. Of these patients, 58 underwent ^{111}In-IgG scintigraphy. These patients were prospectively separated in patients with or without PDCs. Overall sensitivity and specificity was 60% and 83%, respectively. In patients without PDCs for local inflammation, the diagnostic yield of scintigraphic techniques is quite low since no focal inflammation was observed. Therefore, ^{111}In-IgG scintigraphy should not be used as a second-step procedure in the work-up of these subgroup of patients with FUO. In patients with PDCs at local inflammation, ^{111}In-IgG is helpful in the diagnostic process in one-fourth of the patients. This diagnostic yield is comparable with that of the majority of other scintigraphic techniques used in the diagnostic process of patients with FUO.

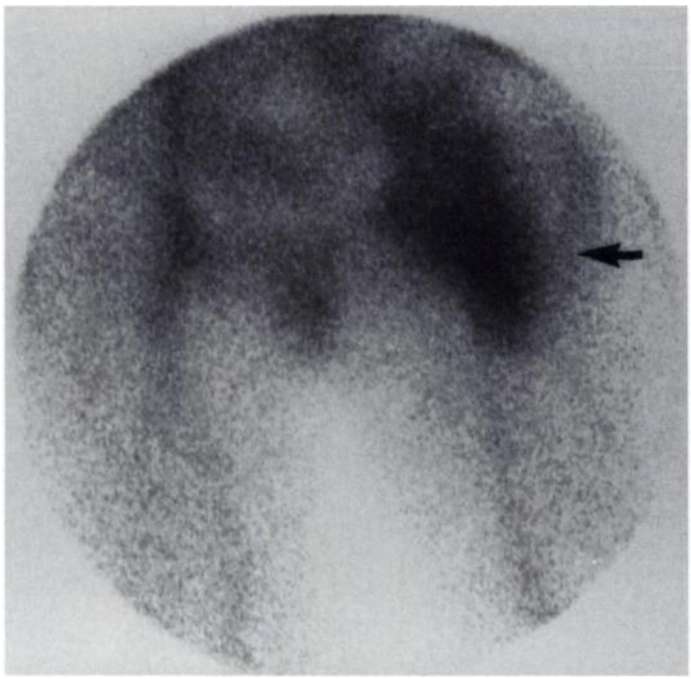

FIGURE 3. A 53-yr-old woman was referred from another hospital because of fever of more than 3 wk duration. She also had a painful hip. Blood cultures grew *S. aureus* and echocardiography revealed vegetations on the mitral valve. The ^{111}In-IgG scintigraphy revealed metastatic abscesses in hip femur, skull and chest. A culture of material obtained by puncture of the hip grew *S. aureus*. After antibiotic therapy, the patient underwent cardiosurgery for valve replacement. Thereafter, her fever disappeared (Patient 9).

TABLE 4
Diagnostic Utility of Scintigraphic Techniques in FUO in Literature

Investigators	No.	Scan	% helpful scans patients
Habibian et al. (*15*)	22	^{67}Ga	55
Hilson, Maisey (*16*)	67	^{67}Ga	75
Schmidt et al. (*4*)	32	^{111}In-oxine WBCs	22
Syrjälä et al. (*3*)	68	^{111}In-oxine WBCs	28
Roddie et al. (*5*)	17	^{99m}Tc-HMPAO WBCs	24
Macsweeney et al. (*17*)	25	^{111}In-tropolonate WBCs	24
Davies et al. (*18*)	28	^{111}In-tropolonate WBCs	18
Kelly et al. (*19*)	28	^{111}In-oxine WBCs	21
Suga et al. (*20*)	36	^{67}Ga	47
Becker et al. (*7*)	34	^{99m}Tc-anti NCA	24
Knockaert et al. (*2*)	145	^{67}Ga	29
Present study	58	^{111}In-IgG	16

NCA = nonspecific cross-reacting antigen.

ACKNOWLEDGMENTS

We thank the members of The Netherlands FUO Study Group for their contribution. This study was supported in part by The Netherlands Institute for internal medicine through a grant from Glaxo Inc. Zeist, The Netherlands and a grant from R.W. Johnson Pharmaceutical Research Institute, Spring House, PA. Members of the Netherlands FUO Imaging Group include: E.M.H.A. de Kleijn, J.W.M. van der Meer, W.J.G. Oyen, F.H.M. Corstens, University Hospital, St. Radboud, Nijmegen; H.G. Kreeftenberg and D.R. Piers, University Hospital, Groningen; P. Speelman and E.A. van Royen, University Hospital of the University of Amsterdam; S. de Marie and E.P. Krenning, University Hospital Rotterdam.

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Optimization of Technetium-99m-Labeled PEG Liposomes to Image Focal Infection: Effects of Particle Size and Circulation Time

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In previous studies we have shown that liposomes sterically stabilized with polyethylene glycol (PEG), preferentially localize in infectious and inflammatory foci. In this study, we further optimized the formulation of PEG liposomes for infection imaging in a rat model. **Methods:** The biodistribution and imaging characteristics of different liposomal formulations labeled with ^{99m}Tc were determined in rats with *S. aureus* infection of the left calf muscle. The influence of liposomal size (mean diameter varying from 90 nm to 220 nm) as well as circulation time (modulated by inclusion of 0–10 mole% phosphatidylserine) were studied. **Results:** The smallest liposomes displayed improved characteristics for infection imaging: 90-nm liposomes revealed the highest abscess uptake (1.6% ± 0.4% ID/g, 24 hr postinjection) in combination with the lowest splenic accumulation (6.9% ± 0.7% ID/g, 24 hr postinjection) as compared to the larger sized preparations. Enhanced abscess-to-blood ratios (4.0 versus 1.3 at 24 hr postinjection) were obtained by including 1.0 mole% phosphatidylserine in the lipid bilayer of the PEG liposomes. However, enhanced blood clearance of these liposomes reduced their absolute abscess uptake. **Conclusion:** These results indicate that the in vivo behavior of PEG liposomes can be modulated to optimize their characteristics for infection imaging.

Key Words: PEGylated liposomes; sterically stabilized liposomes; *S. aureus* infection

J Nucl Med 1997; 38:489–493

Liposomes are microscopic lipid vesicles consisting of one or more concentric lipid bilayers enclosing discrete aqueous spaces. Liposomes have been investigated extensively as carriers for drugs in attempts to achieve selective deposition and/or controlled release of the encapsulated contents (*1–5*). In addition, liposomes have been tested as vehicles to image infection and inflammation (*6,7*). However, conventional liposomes are rapidly taken up by cells of the mononuclear phagocyte system (MPS), which are primarily located in the liver and spleen (*8,9*). A decade ago, one of the major goals in liposome research was to enhance their circulatory residence time to allow enhanced targeting to non-MPS tissues. It has been demonstrated that small, neutral, cholesterol-rich liposomes composed of rigid phospholipids of high-phase transition temperature show prolonged circulation times at relatively high lipid doses (*10–12*). More recently, it was demonstrated that inclusion of polyethyleneglycol (PEG), conjugated to phosphatidylethanolamine in the bilayer increased the blood circulation time as well (*13,14*). This increment was at least as large as that observed with the rigid lipid composition but without the requirements of specific lipid composition, particle size and lipid dose (*15–17*). The prolonged circulation time of PEG liposomes, also referred to as sterically stabilized or Stealth® liposomes (Sequus Pharmaceuticals Inc., Menlo Park, CA), is caused by reduced recognition by the MPS, as reflected by delayed and diminished hepatic and splenic accumulation. The development of long-circulating liposomal formulations has offered several new applications for liposomes such as: (a) long-term controlled release of drugs in the circulation; (b) improved antibody-guided delivery of liposomes; and (c) enhanced targeting to non-MPS-related pathological sites such as tumors and inflammatory foci (*18,19*).

Our previous studies in rats have shown that PEG liposomes labeled with either ^{111}In or ^{99m}Tc may be excellent radiopharmaceuticals for imaging infectious and inflammatory foci (*1,2*). The aim of this study was to tailor the PEG-liposomal formulation for scintigraphic application in rats with focal *S. aureus* infection. The PEG-liposomal formulation we used in our previous studies was originally developed for controlled delivery of chemotherapeutics (*15,20,21*). In this study, we modified the size and lipid composition of the liposomes to optimize their in vivo behavior for imaging infection. Different liposome dispersions with a narrow size distribution were produced (mean size: 90, 120, 160 and 220 nm) and evaluated in vivo. In addition, the effects of enhanced blood clearance were investigated by incorporating increasing amounts of phosphatidylserine (PS) (0, 1 and 10 mole%) in the lipid bilayer. It has been shown that PS exposure strongly increases the recognition of PEG liposomes by macrophages, thereby causing enhanced blood clearance (*22,23*).

Received Apr. 16, 1996; revision accepted Jul. 3, 1996.
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