

## ORIGINAL ARTICLE

Imaging bacterial infection with  $^{99m}\text{Tc}$ -ciprofloxacin (Infecton)

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**Aims:** The diagnosis of deep seated bacterial infections, such as intra-abdominal abscesses, endocarditis, and osteomyelitis, can be difficult and delayed, thereby compromising effective treatment. This study assessed the efficacy of a new radioimaging agent, Tc-99m ciprofloxacin (Infecton), in accurately detecting sites of bacterial infection.

**Methods:** Eight hundred and seventy nine patients with suspected bacterial infection underwent Infecton imaging and microbiological evaluation. The sensitivity and specificity of Infecton in detecting sites of bacterial infection were determined with respect to Centres of Disease Control, World Health Organisation, and Dukes's criteria.

**Results:** Five hundred and seventy four positive and 295 negative images were produced. These included 528 true positives, 46 false positives, 205 true negatives and 90 false negatives, giving an overall sensitivity of 85.4% and a specificity of 81.7% for detecting infective foci. Sensitivity was higher (87.6%) in microbiologically confirmed infections.

**Conclusions:** Infecton is a sensitive technique, which aids in the earlier detection and treatment of a wide variety of deep seated bacterial infections. The ability to localise infective foci accurately is also important for surgical intervention, such as drainage of abscesses. In addition, serial imaging with Infecton might be useful in monitoring clinical response and optimising the duration of antimicrobial treatment.

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Infection is a major cause of mortality and morbidity not only in the developing countries but globally. Tuberculosis and multidrug resistant bacteria are increasing and provide diagnostic, therapeutic, and infection control challenges. Nuclear medicine techniques are often used in the context of fever of unknown origin or suspected bacterial infection to aid diagnosis and to establish the need for antimicrobial treatment. Existing methods of detecting infection by nuclear medicine techniques are relatively sensitive but are either non-specific, such as the bone scan or Gallium-67 citrate imaging, or inflammation specific, such as radiolabelled white blood cells or human immune globulin. A novel approach using a bacterially binding radiolabelled antibiotic, Tc-99m ciprofloxacin (Infecton)<sup>1–3</sup> was evaluated in a multinational trial as a bacterial specific imaging agent in comparison with these existing methods, and as an addition to the established radiological approaches. We report the results of Infecton imaging from this three year coordinated research programme between the International Atomic Energy Agency and hospitals in Argentina, Chile, Egypt, Greece, India, Indonesia, Singapore, and the UK.

"Tuberculosis and multidrug resistant bacteria are increasing and provide diagnostic, therapeutic, and infection control challenges"

## METHODS

## Patient selection

Eight hundred and seventy nine patients, suspected to have bacterial infection by the referring clinicians, were entered into our study. Table 1 shows the contribution of the different countries in our study to the types of infection imaged. Pregnant and lactating women or those with known hypersensitivity to quinolone antibiotics were excluded. Our study was

approved by the local ethics and radiation committees. After a full explanation of the study, informed signed consent was obtained from each patient.

## Preparation of Infecton

Infecton was produced by reducing 2 mg of ciprofloxacin (Bayer, Newbury, UK) with 500  $\mu\text{g}$  of stannous tartrate, at a buffered pH of 4.0, and radiolabelling with Technetium-99m up to 10 mCi (370 MBq). The agent was produced in house at St Bartholomew's Hospital, London, and supplied as a two phase kit formulation, requiring 10 minutes to prepare. Quality control was performed with ascending 5 cm chromatography in butanonone (methylethyl ketone) on Whatman's number 1 paper, which took 5–30 minutes and typically gave over 95% Tc-99m ciprofloxacin, with less than 5% free Tc-99m and reduced hydrolysed Tc-99m complex.

## Imaging protocol

For imaging, 10 mCi (370 MBq) of Tc-99m Infecton was injected intravenously over 40 seconds. The temperature, pulse, and blood pressure of each patient were recorded to monitor for adverse reactions. Three hundred to 500 Kcounts were collected by the local single or double headed  $\gamma$  camera, set with a low energy parallel hole general or high resolution collimator and peaked for 140 Kev with a 15% window. Images were captured as a 128  $\times$  128 or 256  $\times$  26 matrix. Anterior and posterior whole body static images were acquired at approximately one and four hours, and where indicated 24 hours after the injection.

**Abbreviations:** CDC, Centres for Disease Control; WHO, World Health Organisation

**Table 1** Recruitment of subjects by suspected infection and country of origin

Suspected infection	Argentina	Chile	Egypt	Greece	India	Indonesia	Singapore	UK	Total
Osteomyelitis	47	36	0	35	16	39	21	34	228
Orthopaedic prosthesis	124	41	0	11	6	0	6	6	194
Tuberculosis	0	0	30	1	69	24	0	6	130
Soft tissue	14	4	2	1	7	0	5	12	45
Abdominal	2	0	17	0	2	0	7	17	45
Surgical wound	5	4	0	0	0	0	1	17	27
Septic arthritis	2	6	0	2	0	5	6	6	27
Endocarditis	0	0	0	0	0	0	0	26	26
Primary bloodstream	1	1	2	0	0	3	5	14	26
Ear, nose, and throat	0	1	0	0	1	1	1	5	9
Genitourinary	0	0	0	0	1	0	5	5	11
Lower respiratory	0	2	3	0	0	3	11	9	28
Enteric	0	0	0	0	2	13	0	3	18
Intracranial	0	0	0	0	0	0	0	2	2
None	2	6	7	0	8	1	2	37	63
Total	197	101	61	50	112	89	70	199	879

**Criteria for diagnosis of infection**

Classification of the infection status of each patient imaged was undertaken by an independent microbiologist, blinded to the Infection imaging results and according to the following criteria:

- (1) The Centres for Disease Control and Prevention (CDC) criteria<sup>4</sup> for diagnosing infection. These rely on a combination of clinical, radiological, operative, microbiological, and histological findings, in addition to results of other laboratory tests, such as the white blood cell count, erythrocyte sedimentation rate, and C reactive protein value.
- (2) Dukes's criteria for infective endocarditis.<sup>5</sup>
- (3) The World Health Organisation (WHO) criteria for the diagnosis of tuberculosis.<sup>6</sup>

Table 2 shows the Dukes's and WHO criteria and table 3 shows the classification of the infection after application of all these criteria. A patient was considered to have definite infection if the criteria were met in full. A diagnosis of probable infection was made if the criteria were not met but the patient was treated as infected by the attending physician. A patient was considered to be not infected if the criteria were not fulfilled, the patient had responded fully to antibiotic treatment at the time of imaging, or an alternative diagnosis was subsequently made. Microbiological specimens, appropriate to the suspected infection—for example, swabs, tissue, blood, sputum—were obtained and cultured aerobically and anaerobically on appropriate media. Significant isolates were identified by conventional means in local laboratories. Wherever possible, isolates were tested for susceptibility to ciprofloxacin.

Each patient with definite or probable infection by the clinical criteria was then further classified as microbiology positive or negative.

**Criteria for the interpretation of Infection images**

Images were assessed as positive or negative by one to three nuclear medicine physicians, blinded to the clinical information and the diagnosis of infection by the above criteria, on a consensus or majority basis. Sequential and four hour images were mandatory for inclusion and interpretation. Twenty four hour images were used where doubt existed as to the specificity of uptake at four hours. If no consensus could be reached the image was classified as indeterminate.

The imaging results and the infection status of these patients were sent to the microbiologist at the coordinating hospital in London for interpretation of the imaging results as follows:

- (1) A true positive result was one in which the image showed an area of abnormal uptake of the agent together with evidence of focal infection at the same site within five days of the image. A positive scan in a patient with probable infection was considered true positive.
- (2) A false positive result was one in which the image findings were abnormal but there was no evidence of infection at the same site within five days of the image.
- (3) A true negative result was one in which imaging was negative and there was no evidence of focal infection within five days of imaging. This included patients whose infection had resolved with antibiotic treatment at the time of imaging.

**Table 2** Diagnostic criteria

- (1) **World Health Organisation criteria for tuberculosis:** "definite" cases are those confirmed by culture or supported by microscopy findings; "probable" cases are based on a clinical diagnosis of tuberculosis, combined with the intention to treat with a full course of anti-tuberculosis drugs
- (2) **Dukes's criteria for infective endocarditis (IE)**  
"Definite" IE
  - (a) **Pathological criteria:** microorganisms demonstrated in a vegetation/emboli/intracardiac abscess by culture or histology or pathological lesions of vegetation or intracardiac abscess present, confirmed by histology showing active endocarditis
  - (b) **Clinical criteria:** **2 major criteria** (positive blood cultures for IE and evidence of endocardial involvement—positive echocardiography or new valvular regurgitation) or **1 major and 3 minor criteria** (from: predisposition (heart condition or intravenous drug use); fever  $\geq 38^{\circ}\text{C}$ ; vascular phenomena, such as emboli, Janeway lesions; immunological phenomena, such as glomerulonephritis, Osler's nodes, Roth spots; microbiological evidence—positive blood culture but not meeting major criterion, or serological evidence; echocardiogram—consistent with IE but not meeting major criterion) or **5 minor criteria**

**Possible IE:** findings that fall short of "definite"

**Rejected IE:** alternative diagnosis; resolution with 4 days or less of antibiotic treatment; no pathological evidence at surgery or necropsy

**Table 3** Patient infection status by clinical and microbiological criteria and results of Infecton imaging

Infection status	Infecton +ve	Infecton -ve	Infecton indeterminate	Total
Definite infection Culture confirmed	236 (TP=236)	37 (FN=34, TN=3*)	3	276
Definite infection Culture unconfirmed	145 (TP=145)	12 (FN=6, TN=6)	0	157
Probable infection Culture confirmed	11 (TP=11)	1 (FN=1)	3	15
Probable infection Culture unconfirmed	138 (TP=136, FP=2*)	52 (FN=49, TN=3*)	4	194
Not infected	44 (FP=44)	193 (TN=193)	0	237
Total	574 (TP=528, FP=46)	295 (TN=205, FN=90)	10	879

\*These patients had responded to antibiotic treatment at time of imaging.  
FN, false negative; FP, false positive; TN, true negative; TP, true positive.

(4) A false negative result was one in which the image showed no abnormality but there was evidence of focal infection within five days of imaging. A negative scan in a patient with probable infection was considered false negative.

#### Statistical analysis

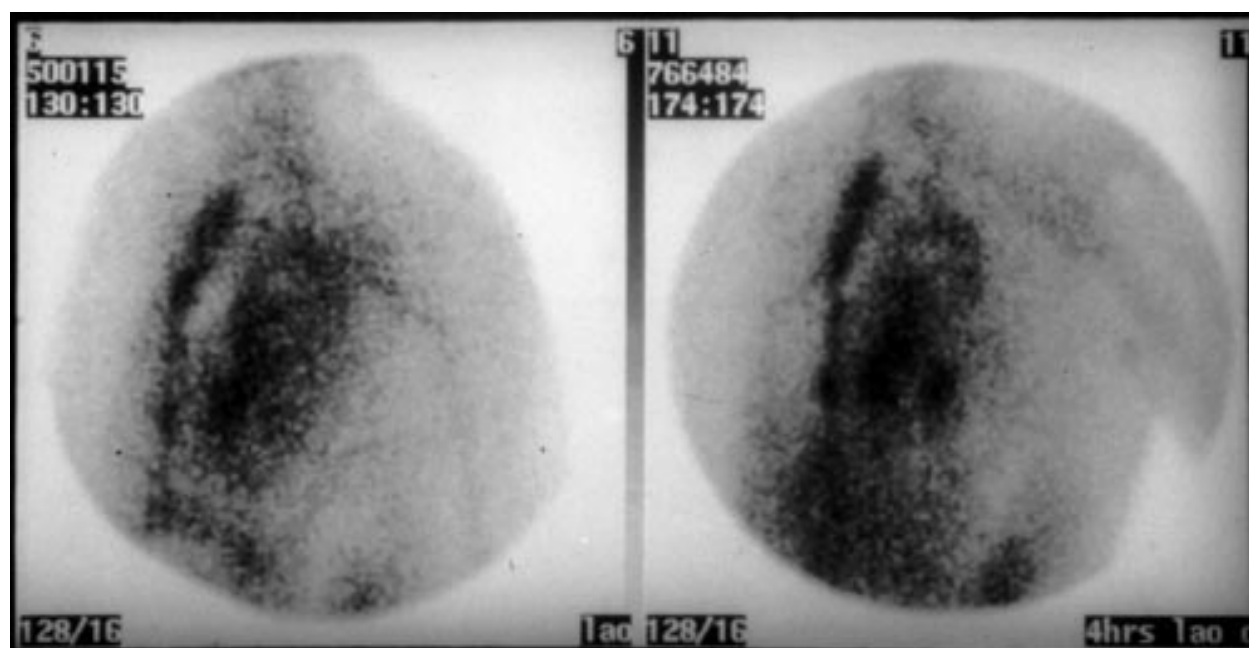
Data were double entered into the software package Epi Info 6 version 6.04b (CDC, Atlanta, USA), which allowed the sensitivity and specificity of Infecton imaging to be determined.

#### RESULTS

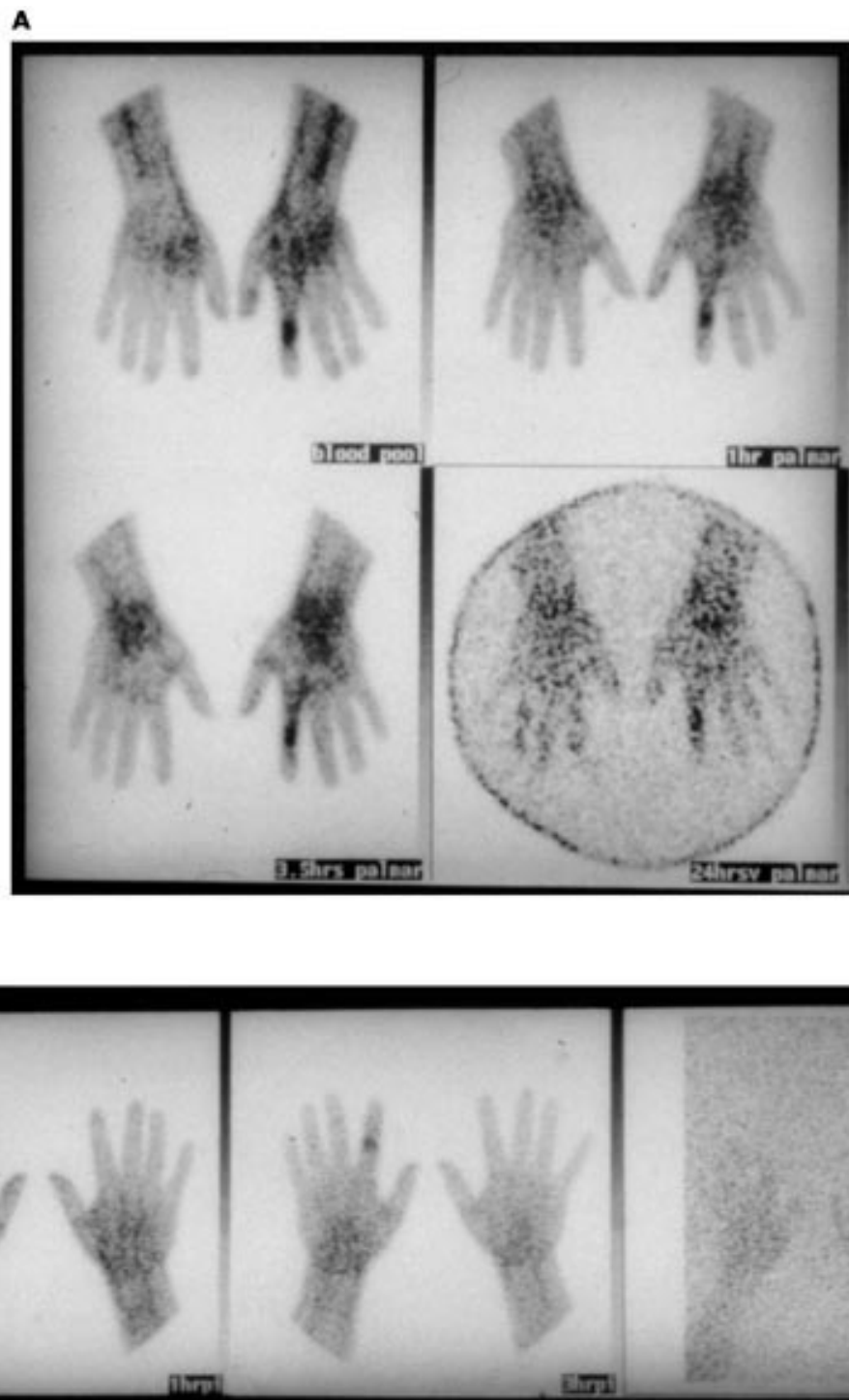
Eight hundred and seventy nine patients, 493 men and 386 women (mean age, 49.3 years; age range, 1–97), were evaluated. Five hundred and seventy four images were

positive, 295 were negative, and 10 were indeterminate. No adverse reactions occurred following the administration of Infecton to patients.

The normal Infecton image shows high uptake by the kidneys, with excretion to the urinary bladder, moderate uptake by the liver and spleen, and no uptake by bone or bone marrow. Early images show predominantly blood pool activity. The gall bladder may be seen occasionally and bowel activity is commonly seen at four hours in patients from the Asian sub-continent but rarely in Europeans or South Americans. An abnormal image, in addition to showing normal uptake, shows diffuse uptake at sites of bacterial infection. This is illustrated in fig 1: uptake in the sternum is seen in both images, indicating bacterial infection after sternotomy, which was confirmed microbiologically. The blood pool in the heart



**Figure 1** Patient with a surgical sternal split and mitral valve replacement developed fever, with positive blood cultures. Tc-99m Infecton images at one and four hours, left anterior oblique views of the heart. Left hand image: at one hour there is intense uptake in the sternum and a normal cardiac blood pool. Right hand image: at four hours there is still intense uptake in the sternum. Instead of the blood pool activity fading, there remains a focus of increased uptake in the mitral area. It was concluded that there is infection not only in the sternum but also in the prosthetic mitral valve. This was confirmed microbiologically after surgery.



**Figure 2** A man of 54 developed a painful swollen left index finger as a result of an infected interphalangeal joint. Tc-99m Infection imaging was performed on two occasions three months apart. (A) A set of blood pool, 1, 3.5, and 24 hour images show focally increased and persistent uptake in the left index finger. (B) Three months after antibiotic treatment, the early image still shows increased uptake in the left index finger. This is slightly less at four hours and has faded completely by 24 hours. It was concluded that the infection in the left index finger had resolved, leaving some residual inflammatory arthropathy. This demonstrates the importance of the 24 hour image to distinguish septic from inflammatory arthropathy.

at one hour should fade at four hours, but a focal area of increased uptake in the mitral area is seen and was confirmed to be an infected mitral valve prosthesis. Figure 2 shows septic arthritis of the left index finger imaged serially with Infectedon. Images of the hands at 10 minutes (blood pool), and 1, 3.5, and 25 hours show persistent uptake (fig 2A). Images of the same hand after successful antibiotic treatment show uptake

to be lower at three hours and faded at 24 hours (fig 2B), consistent with a residual non-infected arthropathy.

The results of Infectedon imaging in terms of certainty of diagnosis of infection according to the microbiological and clinical criteria referred to above are summarised in table 3. Table 4 shows the organisms grown from culture confirmed cases.

**Table 4** Organisms isolated from culture confirmed cases

Organism	Number
<i>Staphylococcus aureus</i>	114
Coagulase negative staphylococci	22
Streptococci	15
Enterococci	7
Other Gram positive organisms	6
Enterobacteriaceae	17
Non-fermentative Gram negative organisms	28
Other Gram negative organisms	7
Clostridia	4
Other anaerobes	1
<i>Mycobacterium tuberculosis</i>	52
Polymicrobial	17
<i>Nocardia asteroides</i>	1
Total	291

**Positive Infecton images**

Of the 574 positive images 528 were true positives. Forty six were considered false positives, two of these (one septic arthritis and one osteomyelitis) had responded fully to antibiotic treatment at the time of imaging. Twelve patients had inflammatory arthropathies, one carcinoma of the lung, and one disseminated candidiasis, one had an *Entamoeba histolytica* liver abscess, and one had *Pneumocystis carinii* pneumonia—these were classified as not infected because the organisms involved are not bacteria. The remaining 28 patients were referred as intrabdominal infection (three), orthopaedic prosthesis infection (three), osteomyelitis (15), septic arthritis (one), soft tissue infection (three), and tuberculosis (three), but were rejected by the CDC criteria and therefore classified as not infected. Three hundred and eighty one patients had definite infection by the CDC criteria, of whom 236 (62%) had positive microbiology as part of the CDC criteria. One hundred and forty nine patients did not fulfil the full CDC criteria but were treated as infected by the referring clinicians. In 11 (7%) of these cases an organism was cultured.

**Negative Infecton images**

Of the 295 negative images, 205 were true negative and 90 were considered false negative. However, only 40 (44%) of

these false negatives were definite infections by CDC criteria. Of these 40, 34 had microbiologically confirmed infections. False negatives occurring in CDC definite, microbiology positive cases included osteomyelitis (10), endocarditis (six), bloodstream infection (five), respiratory tract infection (five), tuberculosis (five), skin and soft tissue infection (five), intra-abdominal infection (two), genitourinary infection (one), and sinus infection (one).

From these results, it can be calculated that Infecton imaging has a sensitivity of 85.5% and a specificity of 81.6% for detecting infective foci. For cases of definite infection only the sensitivity was 90.5% and the specificity 82.1%. Sensitivity was 87.5% when only microbiologically confirmed cases were considered. Table 5 shows the sensitivity and specificity of Infecton in a wide range of conditions.

**Relation to antibiotic treatment**

Overall, 433 (82%) of the 528 infected patients with positive scans were on antibiotics at the time of imaging. In contrast, of the 102 infected patients with negative images, 80 (78.4%) were on antibiotics at the time of imaging. This difference was not significant ( $p > 0.05$ ).

**DISCUSSION**

This multicentre study shows that Tc-99m labelled ciprofloxacin (Infecton) is able to diagnose and localise a wide range of bacterial infections accurately. The worldwide distribution of the investigators confirmed the robustness of the preparation of the agent, the ease of imaging, and the absence of side effects. The infections detected included osteomyelitis, septic arthritis, prosthetic device infections, endocarditis, deep seated abscesses, and extrapulmonary tuberculosis. These are often difficult to diagnose early by other methods and result in delay in the institution of correct treatment, which may involve surgical intervention—for example, drainage of abscesses—in addition to antimicrobial treatment.

The evaluation of any new technique requires comparison with a standard. In assessing the efficacy of Infecton we have used agreed criteria for defining bacterial infections. Although the gold standard for diagnosing bacterial infection is culture, this may not be possible in many cases, either because no specimen can be obtained, or culture is influenced by concurrent antimicrobial treatment. Internationally recognised

**Table 5** Sensitivity and specificity of Infecton in imaging sites of bacterial infection

Suspected infection	Case definition	Sensitivity	Specificity
Osteomyelitis	Study criteria* (n=228)	90.5%	72.8%
	Culture confirmed cases (n=73)	86.9%	–
Orthopaedic prosthesis	Study criteria (n=194)	96%	91.6%
	Culture confirmed cases (n=63)	100%	–
Tuberculosis	Study criteria (n=131)	79.5%	76.9%
	Culture confirmed cases (n=52)	90.2%	–
Soft tissue	Study criteria (n=45)	82.4%	72.7%
	Culture confirmed cases (n=18)	94.4%	–
Abdominal	Study criteria (n=44)	87%	77%
	Culture confirmed cases (n=15)	93.3%	–
Surgical wound	Study criteria (n=27)	81%	100%
	Culture confirmed cases (n=13)	84.6%	–
Septic arthritis	Study criteria (n=27)	94.7%	75%
	Culture confirmed cases (n=7)	100%	–
Endocarditis	Study criteria (n=26)	62.5%	100%
	Culture confirmed cases (n=13)	61.5%	–

\*Infection was determined by Centres for Disease Control, Dukes's, or World Health Organisation criteria.

criteria,<sup>4,6</sup> which do not depend solely on culture but use other parameters, such as operative findings, histopathology, signs and symptoms of infection, laboratory tests, and other imaging techniques, were therefore used to evaluate the efficacy of Infecton. Despite the use of these criteria, on the whole sensitivity still rose slightly in the subgroup of patients with microbiologically confirmed infection. Many of the false positives occurred during the first year of this three year study, particularly in patients with active arthropathies in large joints such as rheumatoid arthritis, when only one and four hour images were taken. The value of the 24 hour images became apparent only after this early “learning phase” of the study. For example, the false positive rate in bone and joint diseases decreased from 5% to 0% in the UK arm of the study and from 4.1% to 2.5% in the Argentina arm of the study. In their study, Larikka *et al* also found a decreased false positive rate and greatly increased specificity (from 68% to 95%) in hip prostheses when 24 hour images were done, in addition to the standard one and four hour images.<sup>7</sup> Infecton is a small highly diffusible molecule, which is taken up initially at sites of inflammation. The uptake decreases as the blood concentration decreases. Non-specific uptake fades at four hours compared with the one hour image and fades further or disappears at 24 hours. On this basis, late (24 hours) images have reduced false interpretation in areas of high blood pool—for example, endocarditis, vascular prosthesis infection, and non-infective inflammatory disorders—and are useful in the distinction between inflammatory and septic arthritis. The lack of bone marrow uptake is a considerable advantage in assessing infection of the sternum after coronary artery bypass surgery. This is also true for some infections of the spine where an area of reduced uptake seen on white blood cell imaging was associated with an area of increased uptake in the Infecton scan in the presence of an infected discitis.<sup>8</sup>

Infecton was not taken up by the normal gastrointestinal tract during the first hour, this is probably because of biological barriers and partly the result of other unknown factors, which may include dormancy or resting gut flora. It is possible that the gut activity seen at four hours in Asian patients represents chronic asymptomatic gastrointestinal infection.

“The lack of bone marrow uptake is a considerable advantage in assessing infection of the sternum after coronary artery bypass surgery”

The overall sensitivity and specificity (85.4% and 81.7%, respectively) of Infecton for imaging sites of infection were good given that a wide variety of conditions in heterogeneous groups of patients across different countries were studied. This is apparent from table 5, which shows that the sensitivity and specificity of Infecton imaging varied according to the type of infection imaged. Future studies on Infecton will concentrate on more homogeneous groups of patients.

In our study, the most successful results were seen in osteomyelitis (sensitivity 90.5%, specificity 72.8%) and orthopaedic prosthesis (sensitivity 96%, specificity 91.6%), with good sensitivity in microbiology positive tuberculosis (90%), soft tissue (94.4%), and abdominal infections (93.3%), and excellent specificity in bacterial endocarditis (100%) and surgical wound infections (100%). In general, microbiologically confirmed infection showed only slightly better sensitivity than results using the CDC and the other clinical criteria without microbiology.

This imaging approach to bacterial infection avoids blood taking and labelling of blood components such as white blood cells, with the possibility of needle stick injury and viral transmission. It is applicable to the evaluation of infection in immunosuppressed and neutropenic patients, where culture is often negative and white blood cell imaging unreliable. We

### Take home messages

- Infecton gave an overall sensitivity of 85.4% and a specificity of 81.7% for detecting infective foci
- Sensitivity was higher (87.6%) in microbiologically confirmed infections
- Thus, Infecton is a sensitive technique, which could result in the earlier detection and treatment of a wide variety of deep seated bacterial infections
- The worldwide distribution of the investigators confirmed the robustness of the preparation of the agent, the ease of imaging, and the absence of side effects
- The ability to localise infective foci accurately is also important for surgical intervention, such as the drainage of abscesses
- Serial imaging with Infecton might be useful in monitoring clinical responses and optimising the duration of antimicrobial treatment

have previously shown that infections caused by quinolone resistant organisms can be imaged by Infecton as long as resistance in the organism is not caused by cell membrane impermeability, which prevents Infecton entering the bacterial cell (AV Hall *et al.*<sup>9</sup> <sup>99m</sup>Tc-Infecton. In vitro binding to bacteria and clinical efficacy in imaging sites of infection. 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, USA, September 15–18, 1996: D9).<sup>3</sup>

Because only a tracer dose of ciprofloxacin (2 mg, which is only one 200th of a single intravenous therapeutic dose of ciprofloxacin) is used in the labelling process, the risk of side effects from Infecton was expected to be very small, if any. This was borne out in our present study. In context, the incidence of side effects reported for a simple nuclear medicine bone scan is one in 1000, compared with one in 100 for a simple x ray using contrast. Similarly, the risk of clinically relevant resistance emerging is probably very small when ciprofloxacin is used at such low concentrations for diagnostic purposes.

More studies are currently under way to confirm whether Infecton can be used through serial imaging (conversion of positive to negative image) to monitor the response to and determine the requirements for stopping antimicrobial treatment or continuing it if no conversion has occurred. Thus, in addition to providing an alternative approach to conventional radiological and radionuclide imaging for detecting infections,<sup>9</sup> Infecton may play an important role in rationalising antimicrobial prescribing practices and thus contribute to the fight against antibiotic resistant bacteria.

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