Chapter 6

Scintigraphic Imaging of Vertebral Osteomyelitis With 111In-Biotin

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Study Design: Early diagnosis of vertebral infection (hematogenous or postsurgical) is necessary to choose a correct therapy and to minimize dramatic complications. All patients suspected to have vertebral infection underwent radiologic imaging and $^{111}$In-Biotin scintigraphy. **Objective:** Biotin is a growth factor used by many bacteria. The aim of our study is to use $^{111}$In-Biotin to diagnose vertebral infections. **Summary of Background Data:** Magnetic resonance imaging, even if endowed with fairly good sensitivity and specificity, shows some limitations in the study of the onset of pathology and in postsurgical conditions. Conventional scintigraphic imaging, like bone scintigraphy with $^{99m}$Tc-MDP, $^{67}$Ga-citrate scintigraphy, or Positron Emission Tomography with [18F]FDG, are limited by relatively low specificity; the use of Streptavidin/$^{111}$In-Biotin scintigraphy, based on aspecific uptake of tracer in the site of infection, shows good results in term of sensibility and specificity but the use of heterologous protein might engender immunogenic reactions. **Methods:** All patients (pts) ($n = 110$) of the study underwent $^{111}$In-biotin scintigraphy 2 hours after intravenous injection of the tracer, 71 pts were suspected to have hematogenous vertebral infection (Group I) and 39 pts were suspected to have postsurgical infection (Group II). The reference for final diagnosis was either bacterial cultures, histopathologic analysis, and/or clinical/imaging follow-up for at least 1 year. **Results:** $^{111}$In-biotin scintigraphy showed a sensitivity of 84% and specificity of 98% in Group I and a sensitivity of 100% and specificity of 84% in Group II. **Conclusions:** Our results showed that $^{111}$In-Biotin scintigraphy possess high diagnostic accuracy. This technique is easy to perform and requires short imaging time-point after intravenous tracer injection. Moreover if $^{111}$In-Biotin uptake is due only to high proliferation rate of bacteria presents in site of infection, it will be further investigated to discriminate definitely bacterial from sterile inflammation.

Key words: vertebral infection, $^{111}$In-Biotin scintigraphy, spine infection imaging.

Infections of the spine include vertebral osteomyelitis, discitis, and spondylodiscitis (SD). SD sometimes extends to the adjacent soft tissues and it can result in meningitis, epidural, subdural, paravertebral, retropharyngeal, mediastinic, or retroperitoneal abscesses. The most frequent site of vertebral infection is the lumbar spine (45%) followed by the dorsal (35%), and cervical (20%).1 Symptoms of spine infection are sometimes nonspecific, thus delaying diagnosis by as long as 6 weeks to 7 months.1–8 The course of SD is strongly affected by howearily is diagnosis made and is effective antibiotic therapy started.8 Undiagnosed spine infection or its delayed diagnosis leads to a mortality rate as high as 11%1.8 and to a neurologic impairment in about 20% of patients.7–9 SD most frequently occurs as a consequence of direct bacterial contamination due to open-wound trauma, spine
surgery, or other invasive medical procedures (spinal anesthesia, local infiltration for antalgic purposes),9–11 these conditions being defined as secondary SD. Purely hematogenous infections are instead less frequent, this condition being defined as primary SD. Pott disease is a special form of primary SD caused by *Mycobacterium tuberculosis*, extending frequently to paravertebral soft tissues12 and also presenting with multiorgan involvement.13,14 Back pain is the most frequent symptom in SD, followed by motor deficits and fever (usually low-grade).5,7 Blood chemistry abnormalities include moderately elevated C-reactive protein levels, erythrocyte sedimentation rate, and white blood cell count. Conditions predisposing to primary SD include drug addiction, prior implantation of vascular devices,2–6 prior severe infection at other sites and systemic metabolic diseases such as diabetes. Magnetic resonance (MR) is currently considered the imaging modality of choice in patients with possible spinal infection.15,16 The diagnostic accuracy of MR in the early phase of spinal osteomyelitis is high especially in primary SD, while postsurgical structural changes can hamper correct interpretation of MR imaging in secondary SDs.17 Furthermore, the diagnostic role of MR still remains controversial in the follow-up of patients,1,18,19 and several limitations have been reported.20 Bacterial culture from specimens obtained by computed tomography (CT)-guided biopsy has 100% diagnostic specificity, but its sensitivity has been reported to range between 58% and 91%,1,21 and this invasive procedure is not routinely employed.

### Table 1 Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>71</td>
<td>30</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>58.3 ± 14.9</td>
<td>53.7 ± 13.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>50</td>
<td>54</td>
</tr>
<tr>
<td>Range</td>
<td>16–86</td>
<td>25–76</td>
</tr>
<tr>
<td>Men/Women</td>
<td>36/35</td>
<td>11/20</td>
</tr>
<tr>
<td>Predisposing factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent infection</td>
<td>22 (30.9)</td>
<td>9 (29.0)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (9.9)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Non-spinal surgery</td>
<td>7 (9.9)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Spinal trauma</td>
<td>3 (4.2)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Others</td>
<td>7 (9.9)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Suspected site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumborum</td>
<td>39 (52.1)</td>
<td>21 (33.3)</td>
</tr>
<tr>
<td>L5–S1</td>
<td>16 (21.7)</td>
<td>14 (23.3)</td>
</tr>
<tr>
<td>Thoracic</td>
<td>10 (14.1)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>Cervical</td>
<td>1 (1.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Multiple levels</td>
<td>2 (2.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Antibiotic therapy</td>
<td>23 (32.3)</td>
<td>12 (20.0)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages

Prior studies have shown that 2-step streptavidin/\(^{111}\)In-biotin scintigraphy has high diagnostic accuracy in patients with various forms of osteomyelitis, including spinal infections. However, the predominant mechanism of streptavidin/\(^{111}\)In-biotin uptake
at the inflamed/infected site is related to the nonspecific accumulation of streptavidin (a 65 kDa protein) due to locally increased vascular permeability. $^{111}$In-biotin, accumulates at the inflamed/infected focus because of its extremely high affinity for streptavidin ($K_d \approx 10^{-15}$) and only a small fraction of $^{111}$In-biotin, not bound to streptavidin, is directly uptaken by growing bacteria.\textsuperscript{22,23} Biotin (also called vitamin H, molecular weight 224 Da) is a growth factor for human cells and for several bacteria. In particular, pyruvate carboxylase, a key metabolic pathway for producing energy by ATP cleavage, is biotin-dependent\textsuperscript{24} and bacterial Acetyl-coA carboxylase is a biotin-dependent enzyme implied in the first step of fatty acid synthesis.\textsuperscript{25} The potential of radiolabelled biotin as an infection-imaging agent per se has already been shown in an experimental animal model of infection using biotin labelled with fluorine-18\textsuperscript{26} as well as in a small group of patients with osteomyelitis using $^{111}$In-biotin.\textsuperscript{27} These considerations prompted us to explore the diagnostic potential of $^{111}$In-biotin alone to detect vertebral osteomyelitis in a large series of consecutive patients, in a relatively early stage of their disease.

Materials and Methods

Patients

Over a period of 4 years (2001–2004), we studied 114 consecutive patients with suspicion of spinal infection. The study was approved by our institutional review board, and informed consent was obtained from all patients. Inclusion criteria were (a) age>18 years; (b) presence of at least 2 of the following signs or symptoms: back pain, fever, radiologic findings suggesting spinal osteomyelitis, or positive bacterial culture from blood. Clinical data (including underlying condition and prior spinal surgery) and suspected site of spinal infection of the study population are given in Table 1. In addition to $^{111}$In-Biotin scintigraphy, all patients also underwent either magnetic resonance imaging (MRI) (n=94) or CT (n=20), as well as physical examination, routine biochemical analysis, and conventional radiograph, within 40 days after the onset of clinical signs or symptoms. Moreover, a detailed clinical history was obtained in all cases. Bacterial culture, histopathologic analysis, or clinical/imaging follow-up for at least 1 year were used as standard of reference for final diagnosis. Spinal infection was considered to be present on the basis of positive bacterial growth, characteristic findings on pathologic analysis, or in case of positive findings at clinical, laboratory, and imaging follow-up. Negative clinical/imaging signs and laboratory findings after 6 months without antibiotic therapy, as well as negative surgical findings or culture, were considered diagnostic for the absence of spinal infection. Four patients were excluded from the study because no follow-up data were available. The remaining 110 patients were divided into 2 groups on the basis of their clinical history: Group I included 71 patients with no history of prior spinal surgery, while Group II consisted of 39 patients with a
history of spine surgery within 2 years before the $^{111}$In-Biotin scan. Patients of Group I were significantly older than patients of Group II, while the proportion of women in Group II was significantly higher than in Group I. No significant differences were observed in the proportion of patients receiving antibiotic treatment at the time of $^{111}$In-Biotin imaging (2-tail Fisher exact test: $P=0.67$; $\chi^2$ test: $P=0.64$).

Radio pharmaceutical Preparation

Diethylene-triamine-penta-acetic acid (DTPA)-conjugated biotin [-bis(biocytinamide)], purchased from Sigma (St. Louis, MO), was diluted in sterile acetate buffer 0.2 M, pH 5.5. Aliquots containing 500 g/mL of DTPA-Biotin were then prepared and stored at 4°C for subsequent labeling with Indium-111. Just before administration, a 500 g DTPA-Biotin aliquot and $^{111}$In-chloride (111 MBq in about 1 mL) were mixed at room temperature for 15 minutes, and labeling efficiency was assessed by ascending chromatography (22,23); labeling efficiency was always >98%.

Scintigraphic Imaging

Scintigraphy was performed with a dual-head SPECT-camera (Millennium, GE Medical System, Milwaukee, WI), equipped with a medium-energy collimator, using 20% energy windows centered around the 173 and 247 keV energy peaks of $^{111}$In (matrix 128x128 pixels, with an 1.33 electronic zoom factor). Planar and single photon emission CT (SPECT) images of the site of suspected infection were acquired 2 hours after injection of the tracer in all patients. The single imaging time-point at 2 hours postinjection of $^{111}$In-Biotin was chosen because a previous study with streptavidin pretargeting had shown no additional diagnostic value of later acquisition times (18–24 hours).23 Anterior and posterior planar images were acquired for 2 million counts each; the kidneys and bladder (sites of physiologic excretion) were shielded when included in the imaging field of view. SPECT images were obtained in a step-and-shoot mode. The angular steps were 3° with a range of 180° per gamma camera head and a 30-second acquisition per step. The image matrix was 256x256 pixels, and images were reconstructed as 4.42-mm-thick sections by using an iterative algorithm. The patients suspected of having hematogenous infection underwent also total-body scan. The patients who showed definite focal uptake of $^{111}$In-Biotin in the spine were injected intravenous with 185 MBq of 99mTc-Nanocoll at the end of the $^{111}$In-Biotin acquisition. The images acquired 10 minutes later (without moving the patient) using a 20% energy window centered on the 140 keV energy peak of $^{99m}$Tc served to better identify the anatomic structure of the skeleton.

Image Interpretation

For the purpose of analysis, planar and SPECT images were evaluated randomly and independently by 2 experienced nuclear medicine physicians that were unaware
of the final diagnosis, as well as of the results of all other imaging methods. All areas with focal uptake of $^{111}$In-Biotin in the spine higher than background were noted and were graded as positive (presence of focal uptake) or negative (no focal uptake) for spinal infection. Equivocal readings were not permitted. Independent scores of the 2 readers were compared and discrepancies were resolved by consensus. In addition to providing better topographic localization, the radiocolloid scan was also helpful for confirming spinal infection, which presents as a photopenic area at the site with abnormally high accumulation of $^{111}$In-Biotin (28).

**Statistical Analysis**

Data were expressed as mean values ± standard deviation, and confidence intervals (CI) whenever appropriate. Sensitivity, specificity, accuracy, positive predictive value, and NPV of $^{111}$In-Biotin scintigraphy for the diagnosis of spinal infection were calculated for both the whole 110 patients’ population and separately for Group I and for Group II. Differences between Group I and Group II were assessed by unpaired-data t test, $\chi^2$, as well as with the McNemar test. Moreover, the relation of several demographic and clinical factors to vertebral infection was assessed with both univariate and multivariate nominal logistic regression analysis. The relative risks for significant predictors of the presence of vertebral infection were expressed by odds ratio with 95% confidence intervals. A 2-sided $P$ value of less than 0.05 was considered statistically significant. All statistical evaluations were performed using the SAS software (SAS Institute, Cary, NC).

**Results**

Final diagnosis of spinal infection was achieved in 51 of 110 patients by means of either positive tissue cultures from biopsy or surgical samples ($n = 33$), detection of a purulent mass at surgical inspection ($n = 8$) or positive clinical, laboratory, and imaging follow-up data ($n = 10$). The infectious agent was identified in 33 cases (64.7%), *Staphylococcus aureus* being the predominant germ. Nonpyogenic infective agents including *Mycobacterium tuberculosis* ($n = 6$) and *Candida albicans* ($n = 1$) (Table 2). In particular, spinal infection was diagnosed in 31 of 71 patients of Group I (43.7%). The 40 patients of Group I who did not have spine infection had either degenerative disease ($n = 12$), compression fracture ($n = 4$), spondylolisthesis ($n = 2$), ankylosing/rheumatoid spondylitis ($n = 3$), or malignancies ($n = 2$). Less common conditions (1 patient each) included pericarditis, vasculitis, anal abscess, psoas abscess, and lung empyema, while no certain pathologies were identified in 12 patients. Spinal infection was eventually diagnosed in 20 of 39 patients of Group II (51.3%). In these 20 patients, the interval between surgery and imaging ranged from 26 to 329 days (mean 127.2 ± 91.3 SD; median 102 days). In the remaining 19 cases no infection could be demonstrated. No statistically significant difference was found between Group I and Group II with regard to the incidence of vertebral...
infection ($P=0.44$). $^{111}$In-Biotin scintigraphy was positive in 50 of 110 patients and negative in 60 of 110 patients. Multisite foci of infection were detected in 2 cases. The diagnostic performance parameters of $^{111}$In-Biotin scintigraphy in terms of sensitivity, specificity, positive predictive value, negative predictive value (NPV), and accuracy in the detection of vertebral infection are reported in Table 3, stratified for Group I and Group II, as well as for the overall patients. In Group I, $^{111}$In-Biotin scintigraphy correctly identified 26 of 31 patients with infection (true-positive cases, see representative case in Figure 1), while there were 5 false-negative cases (Figure 2) and 1 false-positive case (Figure 3). The 5 false-negative cases included 4 patients with non-pyogenic spinal infection ($Mycobacterium tuberculosis$ in 3 cases, $Candida albicans$ in 1) and 1 patient with methicillin-sensitive $Staphylococcus aureus$ infection who had received high-dose antibiotic therapy for 8 days before imaging. The single false-positive $^{111}$In-Biotin scan was observed in a patient with compression fracture of a vertebral body. In Group II, $^{111}$In-Biotin imaging was positive in all 20 of 20 patients with spinal infection. However, 3 false-positive scans were observed in patients who had undergone surgery 32, 115, and 288 days, respectively, before scintigraphic imaging. No false-negative $^{111}$In-Biotin scans were recorded in patients of Group II. The McNemar test showed that the specificity of $^{111}$In-Biotin scintigraphy for the diagnosis of spinal infection in patients of Group I was significantly higher than in Group II ($P=0.01$), while $^{111}$In-Biotin scintigraphy was more sensitive in Group II, although at borderline of statistical significance ($P=0.058$).

Table 2. Results of Bacterial Cultures

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Staphylococcus aureus$</td>
<td>15</td>
<td>6</td>
<td>21 (29.4)</td>
</tr>
<tr>
<td>Multiresistant $staphylococci$</td>
<td>2</td>
<td>2</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td>Streptococci</td>
<td>1</td>
<td>1</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>1</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>$Mycobacterium tuberculosis$</td>
<td>6</td>
<td>—</td>
<td>6 (11.8)</td>
</tr>
<tr>
<td>$Candida albicans$</td>
<td>1</td>
<td>—</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>$Propionibacterium acnes$</td>
<td>—</td>
<td>1</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>1</td>
<td>1</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Bacterial growth, but non identified strains</td>
<td>4</td>
<td>9</td>
<td>13 (35.3)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.
Table 3. Diagnostic performance parameters of $^{111}$In-Biotin scintigraphy

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>$P$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.839 (0.699–0.969)</td>
<td>1.00 (1.00–1.00)</td>
<td>0.056</td>
<td>0.962 (0.823–0.994)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.972 (0.948–1.000)</td>
<td>0.942 (0.870–1.000)</td>
<td>0.011</td>
<td>0.982 (0.946–1.000)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.931 (0.895–0.962)</td>
<td>0.922 (0.899–1.000)</td>
<td>0.010</td>
<td>0.939 (0.903–0.959)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.921 (0.882–0.960)</td>
<td>0.920 (0.870–1.000)</td>
<td>0.015</td>
<td>0.920 (0.880–0.949)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.978 (0.930–0.994)</td>
<td>1.00 (1.00–1.00)</td>
<td>0.104</td>
<td>0.977 (0.930–0.995)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are 95% confidence interval.

Figure 1. Vertebral infection of L5 by *St. Aureus* in a patient of Group I. $^{111}$In-biotin scintigraphy (A) with tracer uptake in site of infection (TP) and corresponding $^{99m}$Tc-nanocolloids scintigraphy (B).

Figure 2. Lumbar vertebral infection by *Candida Albicans* in a patient of Group I. $^{111}$In-biotin scintigraphy (A) doesn’t show tracer uptake in site of infection (FN). Sagittal T1-weighted and
T2-weighted MRI shows confluent abnormal signal intensity involving L1-L2 disc and adjacent vertebral bodies with endplate erosions which are typical for SD (TP) (B).

Figure 3. Compression fracture of L5 in Group I patient. $^{111}$In-biotin scintigraphy shows mild tracer uptake in lumbar spine (FP) (A) corresponding on 99mTc-nanocolloids scintigraphy at L5 vertebra (B). False-positive scan of Group II, patient undergone surgery for L4-L5 disc hernia 115 days before $^{111}$In-biotin scintigraphy (C) and 99mTc-nanocolloids imaging (D)

**Discussion**

Diagnosing vertebral osteomyelitis early in the course of disease is crucial, considering that conventional radiology is sometimes negative within the first 4 weeks after the onset of clinical signs. Although MR imaging, especially with the fat suppression technique, is the method of choice to diagnose hematogenous vertebral infection, the diagnosis of spondylitis is not always simple. Some conditions, such as atypical site of infection, coexisting degenerative disease or myeloma-chordoma may hamper the radiologic diagnosis.16 These difficulties lead to discordant results
especially in secondary SD, when discriminating true infection from the normal postoperative degenerative disc may be challenging.29 Furthermore, MR cannot always discriminate between sterile inflammation and bacterial infection.17 Moreover, while both CT and MR only allow regional visualization of the body, whereas infection may actually be widespread, radionuclide scintigraphy offers the possibility of evaluating the whole body during a single imaging session. While the conventional radionuclide procedures proposed to complement MR in patients with suspected osteomyelitis (bone scintigraphy with $^{99m}$Tc-MDP, $^{67}$Ga-citrate scintigraphy, or PET with [18F]FDG) are generally highly sensitive, their specificity is widely variable.30–39 On the other hand, scintigraphy with autologous $^{99m}$Tc-HMPAO-leukocytes, a method widely applied to image infection in other sites of the body, is not suitable for spine infections.39,40 Among the most recent radiolabelled agents, $^{99m}$Tc-ciprofloxacin41,42 has demonstrated low specificity for spine infection, especially when evaluating recently operated patients.43 Radiolabelled small peptides as PEG-liposomes (44), IL-8 (45,46) and antimicrobial peptides (47,48) hold promise for diagnosing infection potential, but so far they have been employed only in experimental animal models. Therefore, their potential to distinguish infection from sterile inflammation remains to be totally validated in patients. All such limitations of radionuclide procedures for imaging spine infections have been overcome by 2-step Streptavidin/$^{111}$In-Biotin scintigraphy, proven to have superior diagnostic performance than either CT or MRI in patients with suspected spine infection.23 However, this approach raises the concerns that streptavidin, a heterologous protein, might elicit potentially dangerous immunogenic reactions in the patients undergoing such diagnostic procedure. These considerations prompted the present study based on administration of $^{111}$In-Biotin only, without streptavidin pre-targeting. The rationale of this simplified approach was that positivity of the scan would depend on direct uptake of $^{111}$In-Biotin by growing bacteria at the infection site rather than on nonspecific protein accumulation due to edema and leaking capillaries. The results obtained in this study show that singlestep $^{111}$In-Biotin scintigraphy presents high value of diagnostic accuracy (91.8%) in detecting spine infections, similar to that previously observed with the more complex 2-step Streptavidin/$^{111}$In-Biotin approach (23) in a different group of patients with suspected spine infection (94.5%). With $^{111}$In-Biotin scintigraphy we observed an especially high proportion of true-negative results in patients of Group I (39 of 40, or 97.5%), associated however, with a certain proportion of false-negative results (5 of 31, or 16.1%). The fact that 4 out of such 5 false negative scans were observed in patients with spine infection from either Mycobacterium tuberculosis (3 cases) or Candida albicans (1 case) raises an interesting issue. In particular, tuberculous infection is typically chronic and slow-evolving, the predominant cells are lymphocytes and
monocytes-macrophages, granulocytes are scanty, local vasodilatation is not pronounced, vascular permeability is only slightly altered, and endothelial activation is somewhat blunted. Furthermore, the replication rate of *Mycobacterium tuberculosis* is much slower than that of other bacteria, a feature that reduces the likelihood of biotin being actively concentrated at the infection site. On the other hand, biotin doesn’t seem to be an essential growth factor for fungi, such as *Candida albicans*. The results obtained in the present series are also consistent with a prior study in patients with spine infection undergoing 2-step Streptavidin/\[^{111}\text{In}-\text{Biotin}\] scintigraphy. In that study, the only 2 false-negative scans were observed in patients with tuberculous infection of the spine. As to the remaining patient with a false-negative \[^{111}\text{In}-\text{Biotin}\] scan, this occurrence might be linked to the long-term antibiotic therapy administered to this patient. As to the possibility of exploring with scintigraphy the whole body, the obvious clinical advantage offered by this feature versus CT/MR imaging became apparent in 2 patients of Group I (with hematogenic infection), in whom multifocal sites of vertebral infection were observed, some of which were previously unsuspected on clinical ground. In Group I, 39 of 71 patients underwent \[^{111}\text{In}-\text{Biotin}\] scintigraphy within a time range of 20 to 25 days after the onset of symptoms. Considering that scintigraphy correctly classified patients in this Group, we can conclude that \[^{111}\text{In}-\text{Biotin}\] scintigraphy has good diagnostic potential after 20 days from the onset of symptoms. Concerning the results obtained in patients of Group II (with suspected secondary SD), the especially high true-negative rate should be emphasized (16 of 16, or 100% NPV), while the only parameter limiting diagnostic accuracy was a non negligible rate of falsepositive scans (3 of 19, or 15.8%). Nevertheless, all such patients showed a somewhat weak focal accumulation of \[^{111}\text{In}-\text{Biotin}\] at the site of prior surgery, probably reflecting a locally altered vascular permeability due to surgical trauma rather than active tracer uptake. The false positive cases of this Group had undergone surgery 32, 115, and 288 days before imaging, respectively. Considering such a prolonged time range and the fact that true positive results were observed after 26 days from surgery lead to conclude that \[^{111}\text{In}-\text{Biotin}\] scintigraphy is expected to have good diagnostic potential starting from 25 days after surgery. Since \[^{111}\text{In}-\text{Biotin}\] does not appreciably accumulate in normal bone and/or bone marrow, the only anatomic landmarks in the scan are the sites of physiologic excretion, such as the kidneys when exploring the low-dorsal or lumbar spine. Instead, when exploring the high-dorsal or cervical spine, exact identification of the vertebral body harboring infection can be more problematic. Thus, when no SPECT/CT equipment is available for morphofunctional coregistered imaging, performing an additional 99mTc-nanocoll scintigraphy after completion of the \[^{111}\text{In}-\text{Biotin}\] scan greatly helps in better identifying the site of infection. Our study has
some limitations. Bone biopsy was not performed in all patients, and clinical/imaging follow-up data had to be used as a standard of reference in several cases introducing some potential verification bias. Moreover, selection of suspected spinal infection was based on clinical and radiologic or cultural findings and a selection bias thus may have been introduced. Nevertheless, we believe that our approach reflects daily clinical practice and, to minimize selection bias, we included patients consecutively. In conclusion, our results suggest that single-step $^{111}$In-Biotin scintigraphy is a safe, efficient imaging modality for detecting vertebral osteomyelitis within the first 40 days after the onset of clinical symptoms. The procedure is easier to perform than the 2-step Streptavidin/$^{111}$In-Biotin procedure previously described with the same type of clinical application, and does not entail any potential biologic hazard as administration of a heterologous protein such as Streptavidin might entail. Still, the 1-step method described here shows high diagnostic accuracy for both in hematogenous and postsurgical vertebral infection. However, further prospective studies comparing both $^{111}$In-Biotin scintigraphy and dynamic contrast-enhanced MRI with biopsy and culture findings in a larger series of patients are required for a more comprehensive elucidation of the diagnostic potential of this new scintigraphic tracer particularly in order to definitely discriminate bacterial infection from sterile inflammation.

Key Points
- $^{111}$In-Biotin uptake is due only to high proliferation rate of bacteria presents in site of infection.
- Single-step $^{111}$In-Biotin scintigraphy is a safe, efficient imaging modality for detecting vertebral osteomyelitis.
- $^{111}$In-Biotin scintigraphy will be further investigated to discriminate definitely bacterial from sterile inflammation.
References


