Neutrophil-Specific $^{99m}$Tc-Labeled Anti-CD15 Monoclonal Antibody Imaging for Diagnosis of Equivocal Appendicitis

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We evaluated $^{99m}$Tc-labeled anti-CD15 immunoglobulin M monoclonal antibody (LeuTech) for diagnosing acute appendicitis in patients with an equivocal clinical presentation. LeuTech avidly binds to circulating and sequestered human polymorphonuclear neutrophils in vivo, eliminating in vitro cell labeling and blood handling. **Methods:** We studied 49 patients to evaluate the safety and efficacy of LeuTech imaging. $^{99m}$Tc-labeled LeuTech was prepared on site using a lyophilized kit, $^{99m}$Tc-labeled pertechnetate, and 2 different incubation techniques, 1 at room temperature and the other at 37°C. The abdomen was serially imaged for up to 3 h after the intravenous administration of 370–740 MBq $^{99m}$Tc-labeled LeuTech. Scans were read as positive or negative for acute appendicitis or other intra-abdominal infection. The institutional diagnosis was established by surgery, other diagnostic studies, or 1-mo clinical follow-up. **Results:** Scans were positive for appendicitis in all 49 patients with appendicitis, for a sensitivity of 100%, and negative for appendicitis in 19 of 23 patients without appendicitis, for a specificity of 83%. Accuracy, positive predictive value, and negative predictive value were 92%, 87%, and 100%, respectively. Results were not different between the LeuTech preparations. The rate of laparotomies with negative findings in patients who underwent surgery was 10%. The average time from injection to LeuTech visualization in the appendix for cases positive for appendicitis was 9 min. No serious adverse reactions occurred. **Conclusion:** LeuTech imaging is safe, rapid, and sensitive for diagnosis of appendicitis in equivocal cases. The potential advantages of LeuTech over currently available radiopharmaceuticals for infection imaging are ease of preparation, absence of blood handling, excellent image quality, no requirement for SPECT, and rapid diagnostic uptake.

**Key Words:** appendicitis; inflammatory diseases; infection imaging; neutrophils; $^{99m}$Tc-labeled anti-CD15 monoclonal antibody


Acute appendicitis is the most common surgical disease in the United States, resulting in approximately 250,000 appendectomies per year (1). Diagnosing acute appendicitis can be challenging, particularly early in the disease, at the extremes of age, and in pregnant individuals (2–5). Standard management for equivocal cases is hospital admission, frequent reexamination and sometimes early operation to avoid perforation in the highest-risk patients. When the diagnosis of appendicitis is delayed or missed; perforation, abdominal abscess, perforation, and death can occur (6). Conversely, a normal appendix is found in 15–40% of patients who undergo appendectomy (7–10). The relationship between perforation and negative laparotomy is reciprocal.

We previously reported that $^{99m}$Tc-labeled hexamethyl propyleneamine oxime (HMPAO) white blood cell (WBC) imaging is accurate in detecting acute appendicitis in patients with an equivocal clinical presentation (11). This technique proved to be highly sensitive for excluding appendicitis, thus allowing early discharge from the emergency room for those patients without appendicitis. However, radiolabeled WBCs have disadvantages. They are prepared by time-consuming and labor-intensive techniques requiring special equipment and trained personnel. In vitro WBC labeling techniques also require blood handling, carrying the risks of blood-borne infection and misadministration of radiolabeled WBCs. In practice, the additional costs and delays associated with radiolabeled WBC preparation have hindered its widespread use. A need exists for a faster, safer, and less expensive radioisotope method for imaging infection.

We evaluated the safety and efficacy of imaging with an anti-CD15 immunoglobulin M (IgM) murine monoclonal antibody, LeuTech (Palatin Technologies, Inc., Princeton, NJ), for diagnosing acute appendicitis in patients with an equivocal clinical presentation. LeuTech avidly binds to polymorphonuclear leukocytes (PMNs) and has been reported to display rapid diagnostic accumulation at sites of infection with high target-to-background ratios (12–15).
This study was designed to compare 2 preparations of 99mTc-labeled LeuTech for diagnostic efficacy in equivocal appendicitis, ability to allow a rapid diagnosis, scan quality as indicated by target-to-background count ratio, and safety.

MATERIALS AND METHODS

Patients

We selected 49 patients (24 females, 25 males; age range, 9–77 y; mean age, 29 y) with suspected acute appendicitis and an equivocal clinical presentation from November 19, 1997, through May 19, 1998, for inclusion in the trial. Sixteen patients were less than 18 y old. The attending surgeon evaluated each patient before LeuTech injection and established that the diagnosis was equivocal according to the following criteria: atypical history, atypical physical examination, normal WBC count, or absence of fever. The study protocol was approved by the investigational review board at Tri-City Medical Center, and informed consent was obtained from each patient or a parent or legal guardian before the procedure.

LeuTech Preparation and Administration

LeuTech was provided as a lyophilized kit containing 250 μg nonradioactive anti-CD15 IgM antibody and the reagents required for reconstitution and radiolabeling. Each vial was reconstituted with 0.25 mL saline containing 740–1480 MBq 99mTc-labeled sodium pertechnetate. Two preparations of LeuTech were tested. The first was incubated with 99mTc at room temperature for 15 min and was used in the first 18 patients (LeuTech-1); the second was incubated with 99mTc for 30 min in a 37°C water bath and was used in the next 31 patients (LeuTech-2). After incubation, 0.75 mL ascorbic acid (250 mg/mL) was added for stability and to increase the final volume to 1.0 mL. The administered intravenous dose was 0.3–0.5 mL (75–125 μg antibody), containing 370–740 MBq 99mTc-labeled LeuTech. The dose was scaled downward for children to 7.77 MBq per kilogram of body weight to a maximal dose of 740 MBq. Patients were observed for 1 h after injection, including measurement of vital signs.

LeuTech Imaging

Each patient was placed supine under a γ camera with a large field of view, with the bottom of the liver positioned at the top of the field. Immediately after LeuTech injection, a dynamic sequence of fifteen 4-min images for the first 11 patients and ten 4-min images for the last 38 patients was acquired for cine review. After patient ambulation and voiding, a 1 × 106 cpm anterior supine planar image of the abdomen was acquired. Posterior and 20°–25° right and left anterior oblique planar images were acquired for the same time. In some cases, anterior standing planar images were added. These were followed by a second dynamic sequence of eight 4-min images. The patients ambulated and voided again, after which a second set of planar images was acquired using the same technique as for the first set. LeuTech imaging was terminated at any time after the first dynamic sequence (40 min after injection) if the nuclear medicine physician determined that the scan showed unequivocally positive findings.

LeuTech Scan Interpretation

The normal biodistribution of LeuTech (Fig. 1) reflected renal excretion with immediate and persistent visualization of the kidneys and filling of the bladder. Adherence to protocol was necessary to avoid artifacts. In particular, voiding was necessary to empty the bladder and permit visualization of an abnormal, low-lying appendix. Activity in the ureters was infrequent but, when present, was generally intermittent and might be cleared with voiding, ambulation, and standing during imaging. Blood-pool activity was immediately visualized in the uterus and blood vessels but usually faded on images delayed beyond 60 min. Normal activity was present in the liver, spleen, and marrow but not in the gastrointestinal tract during imaging. Oblique views were necessary to distinguish an inflamed appendix from underlying iliac blood pool or pelvic bone marrow.

Scans were interpreted by the nuclear medicine physician on call and read as negative or positive for acute appendicitis and negative or positive for infection or inflammation other than appendicitis. Equivocal interpretations were not permitted. The criterion for reading a scan as positive for appendicitis was the presence of abnormal, persistent LeuTech accumulation within the appendix zone during imaging as outlined in Figure 1. Abnormal patterns of accumulation were found to be focal, linear, multifocal, or diffuse (Figs. 2 and 3). Scans showing abnormal uptake outside the appendicitis zone were read as positive for other infection or inflammation but negative for appendicitis. Negative scans for both appendicitis and other inflammation did not show any evidence of abnormal uptake within the abdomen or pelvis.

Quantitative Scan Analysis

Scans positive for appendicitis were analyzed for target-to-background count ratio. The first frame (0–4 min after LeuTech injection) and the 10th frame (36–40 min after LeuTech injection) of the initial dynamic set of images were included in the quantitative analysis. Regions of interest were tightly placed around the area of abnormal LeuTech accumulation in the appendix (target) and immediately adjacent to the appendix (background). Total counts within the regions were measured, and the ratio was calculated by dividing target counts by background counts.

The time from injection to the time the appendix first showed
was found abdomen turnaround and penumbilical without appendicitis. Abnormal pain, LeuTech. FIGURE 10,000, this inmaltyvlsuahzed anterior appendicitis surgery. 3. 2. FIGURE 13,800, of acute appendicitis was identified this image of anterior appendicitis atypical was found at surgery. Multifocal elongated accumulation was defined as acute appendicitis. Acute appendicitis was identified throughout right abdomen on this anterior image at 50 min (arrow). Acute appendicitis was found at surgery.

abnormal LeuTech accumulation was recorded for patients with appendicitis. The total imaging time after injection for patients without appendicitis was also recorded, as was the total study turnaround time, which was defined as the time the scan was requested to the time the results were reported to the referring surgeon.

Final Diagnosis
The final institutional diagnosis was established surgically in those patients undergoing laparotomy. Histopathologic results were used as the standard in instances of discordance between the surgical and histopathologic reports. All specimens of the appendix were cut into multiple sections and interpreted by a pathologist using routine hematoxylin-eosin staining. A diagnosis of early appendicitis was made when focal collections of neutrophils without transmural inflammation were seen in the appendiceal mucosa. The pathologist was unaware of the findings of LeuTech scanning.

The results of other diagnostic studies and a 1-mo follow-up phone call or office visit were used to determine the final diagnosis in patients not undergoing surgery. Nonspecific abdominal pain of unknown cause was a diagnosis of exclusion assigned to patients whose symptoms resolved without specific treatment and for whom a specific cause of abdominal pain was not found.

RESULTS
Final Diagnosis and Surgical Results
Appendectomy was performed on 29 patients with a preoperative diagnosis of acute appendicitis. Acute appendicitis was confirmed by histopathology for 26 patients. Among the 26 patients with appendicitis, 9 had surgical findings of perforated appendicitis and 5 had histopathologic findings of early appendicitis. Two patients underwent appendectomy and did not have surgical or histopathologic findings of acute appendicitis. One patient had surgical findings of acute appendicitis but negative histopathologic findings. The rate of laparotomies with negative findings was 3 of 29 (10.3%).

A diagnosis other than appendicitis was made for the remaining 23 patients. Three patients had other intra-abdominal infections, including Cytomegalovirus colitis (Fig. 4), mesenteric abscess, and enteritis. Four patients had noninfectious causes of abdominal pain, including a renal stone, mononucleosis, abdominal muscle strain, and sigmoid volvulus. A diagnosis of nonspecific abdominal pain was made in 16 patients.

LeuTech Safety
No serious adverse events were attributable to LeuTech injection. Vital signs did not significantly change after injection. One patient with a history of asthma had a brief episode of shortness of breath 1 h after injection that completely resolved without intervention. This occurrence was believed to be unrelated to the LeuTech injection because of timing and the manner in which it resolved.

LeuTech Efficacy
Appendicitis. No scans were false-negative for appendicitis. All 26 patients who had acute appendicitis diagnosed through surgery had a LeuTech scan positive for acute appendicitis. The sensitivity of LeuTech imaging for detection of acute appendicitis was 100% (Table 1). LeuTech scans showed negative findings in 19 of 23 patients without
clinical evidence of acute appendicitis and were false-positive for appendicitis in 4 patients. One patient with false-positive findings underwent exploratory laparotomy and resection of the right ovary for adhesions. Another patient had surgical findings consistent with acute appendicitis localized to the midline pelvis above the bladder. The location was the same as that shown on the scan, but histopathologic findings were negative for appendicitis. In the third patient with false-positive findings, no surgical or histopathologic evidence of acute inflammation corresponding to the site of abnormal LeuTech uptake was found. The fourth patient with false-positive findings had intense focal uptake in the right lower quadrant immediately after injection. This patient was treated with antibiotics and improved slowly for 2 wk but did not undergo surgery. Overall, the specificity of LeuTech imaging for appendicitis was 83%, accuracy was 92%, negative predictive value was 100%, and positive predictive value was 87%. For LeuTech-1 imaging, sensitivity, specificity, and accuracy were 100%, 86%, and 94%, respectively; and for LeuTech-2, 100%, 81%, and 90%, respectively. No statistically significant differences were seen between the 2 LeuTech preparations.

**Intraabdominal Infection.** Twenty-nine patients, including the 26 with acute appendicitis, had acute infection. The LeuTech scan was positive for infection in all 29 patients with acute infection, for a sensitivity of 100%. LeuTech scans were negative for infection in 16 of 20 patients without clinical evidence of acute infection and were false-positive for infection in 4 patients (the same patients who had false-positive findings for acute appendicitis). The specificity of LeuTech imaging for acute infection was 80%, accuracy was 92%, negative predictive value was 100%, and positive predictive value was 88%. No significant difference was seen between the 2 LeuTech preparations studied.

**Quantitative Analysis**

**Target-to-Background Ratio.** The average (±SD) target-to-background count ratio in the appendix for 0- to 4-min scans with true-positive findings was 1.5 ± 0.4 for LeuTech-1, 1.8 ± 0.7 for LeuTech-2, and 1.7 ± 0.7 for both LeuTech preparations. The ratios increased to 1.8 ± 0.6, 2.4 ± 1.0, and 2.1 ± 0.9, respectively, for the 36- to 40-min scans. Although no significant differences between LeuTech-1 and LeuTech-2 preparations were seen, LeuTech-2 scans showed a trend toward higher target-to-background count ratios at each time point studied.

**Time to Diagnosis.** For scans with positive findings, the average time from injection to initial visualization of the appendix was 15.5 ± 22.2 min for LeuTech-1, 6.0 ± 12.4 min for LeuTech-2, and 9.3 ± 16.8 min for both preparations. Sixteen of 26 (62%) scans with positive findings initially showed abnormal LeuTech uptake on the first dynamic image (0–4 min), and 26 of 26 (100%), by 60 min (Fig. 5). In comparisons of the 2 preparations, 6 of 11 (54%) scans with positive findings from LeuTech-1 and 10 of 15 (67%) from LeuTech-2 initially showed abnormal LeuTech uptake on the first dynamic frame (0–4 min).

For scans with negative findings, the average time to completion was 130 min, with a range of 110–180 min, as dictated by the protocol. Both preparations were associated with a 2-h average turnaround from the time the scan was requested to the time findings of acute appendicitis were reported to the surgeon. The turnaround time included radioisotope preparation, quality control, imaging, and interpretation.

**DISCUSSION**

We designed this study to evaluate the safety and efficacy of a new ⁹⁹mTc-labeled anti-PMN antibody, LeuTech, for diagnosing acute appendicitis in patients with an equivocal presentation. Although other methods have been used (barium enema, sonography, abdominal radiography), none is sufficiently sensitive to allow patients without appendicitis to be

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**TABLE 1**

LeuTech Scan Efficacy for Appendicitis

<table>
<thead>
<tr>
<th>Scan findings</th>
<th>Appendicitis</th>
<th>No appendicitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>19</td>
</tr>
</tbody>
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Sensitivity = 100%, specificity = 83%, and accuracy = 92%. LeuTech is manufactured by Palatin Technologies, Inc.
discharged from the emergency department, with the exception of $^{99m}$Tc-labeled HMPAO WBC imaging or, possibly, helical CT. Helical CT with oral and rectal contrast material has been used to diagnose acute appendicitis (16). Although this test is reported to be highly accurate, it has not been evaluated in equivocal cases in the outpatient setting before a decision is made to admit the patient for observation or surgery. Instillation of contrast material into the rectum makes this study somewhat invasive and adds to patient discomfort. Moreover, patients with appendicitis are frequently nauseated and have difficulty tolerating the volume of oral contrast material required for the test.

We reported favorable results for use of $^{99m}$Tc-labeled HMPAO WBC imaging in a series of 124 patients with an equivocal clinical presentation of acute appendicitis (11). The sensitivity for detecting appendicitis in this series was 98%, with a specificity of 87% and a negative predictive value of 98%. We found similar efficacy in children undergoing $^{99m}$Tc-labeled HMPAO WBC scanning for equivocal appendicitis (17). These results have been confirmed by others (18–21). $^{99m}$Tc-labeled HMPAO WBC imaging also affected patient management in our series (11). The high negative predictive value of this test reliably excluded an inflammatory cause of right lower quadrant pain; therefore, many patients (>90%) who would previously have required hospitalization and additional imaging were discharged from the emergency department and followed up as outpatients. The rate of laparotomies with negative findings in this equivocal group was reduced to 4% by imaging with $^{99m}$Tc-labeled HMPAO WBC.

Disadvantages of $^{99m}$Tc-labeled HMPAO WBC imaging are inherent in this clinical setting. The technique of radiolabeling WBCs is time consuming, labor intensive, expensive, and associated with a risk of misadministration and transmission of blood-borne infections. When an off-site pharmacy is used to prepare the radiolabeled WBCs, there is also a delay in obtaining results and a risk of damaging the cells. For these reasons, a similar infection imaging agent that avoids these pitfalls would be valuable for equivocal cases of appendicitis.

$^{99m}$Tc-labeled polyclonal and monoclonal antibodies have been evaluated for diagnosis of acute appendicitis. $^{99m}$Tc-labeled polyclonal human immune globulin was studied in 35 patients with suspected acute appendicitis (22). Despite the use of SPECT, the sensitivity was 91% and the negative predictive value was only 86%. Two monoclonal antibodies have been studied for use in acute appendicitis. Biersack et al. (23) evaluated antigranulocyte BW 250/183 (Cis Bio International, Gif Sur Yvette, France), a $^{99m}$Tc-labeled murine IgG1 antibody, in 32 patients with suspected appendicitis and showed a sensitivity of only 71% and specificity of 73%. This antibody has a relatively low association constant of $2 \times 10^{-9}$ mol/L and a PMN-bound activity of only 10%–20%. The $^{99m}$Tc-labeled Fab’ fragment (IMMU-MN3; Immunomedics, Inc., Morris Plains, NJ) has also been tested in patients with suspected acute appendicitis (24,25). IMMU-MN3 also has a relatively low PMN association constant of only $0.5 \times 10^{-8}$ mol/L compared with LeuTech. The sensitivity of planar imaging for detection of acute appendicitis was reported to be only 60%, and SPECT was required to obtain acceptable sensitivity (24). In a larger multicenter trial combining SPECT and planar imaging, this antibody had a sensitivity of 91% and a specificity of 85% for acute appendicitis (25). SPECT in this acute setting prolongs study turnaround time, potentially delaying treatment, and also increases the expense of scanning.

In this article, we report our experience with a new anti-PMN monoclonal antibody, LeuTech. This IgM murine monoclonal antibody specifically binds both to circulating PMNs and to PMNs sequestered at sites of infection, enabling relatively simple, rapid, and safe in vivo radiolabeling. LeuTech does not require blood handling, eliminating the risks of blood-borne infection and the potential for administration of radiolabeled cells in the wrong patient. Moreover, LeuTech can be prepared in a typical nuclear medicine department, reducing study turnaround time, eliminating additional costs associated with in vitro cell labeling, and reducing the risk of WBC dysfunction resulting from transport to and from an outside pharmacy. LeuTech has a high association constant of $10^{-12}$ mol/L for CD15 PMN.
receptors, permitting relatively strong binding compared with other antineutrophil monoclonal antibodies (12). More than 90% of the cell-bound activity in the blood is associated with PMNs. This high affinity for PMNs combined with rapid blood pool clearance and absence of physiologic bowel excretion during imaging produces excellent planar image quality with high target-to-background ratios. Moreover, we found that SPECT was not required for diagnosing appendicitis with LeuTech.

The clinical efficacy of LeuTech imaging for a wide variety of infections has been reported. Gratz et al. (15) reported favorable results in 17 patients with 23 sites of infection. The conclusion was that LeuTech performed better than \(^{99m}\text{Tc}\) labeled HMPAO WBC imaging in the same patient for detection of osteomyelitis, prostatic joint infection, and soft-tissue infection. Thakur et al. (14) showed that LeuTech imaging was diagnostic within 3 h of injection in 12 patients with known infection, including 5 with acute appendicitis.

We evaluated 2 LeuTech preparations, 1 using a 15-min incubation at room temperature and the other using a 30-min incubation in a 37°C water bath. Significant improvements in scan quality were seen with the LeuTech-2 preparation. Quantitatively, target-to-background count ratios were higher in cases of appendicitis, and qualitatively, significantly less renal excretion occurred, which was particularly noticeable by absence of ureters. On the basis of in vitro cell-binding assays, this is likely attributable to incubation in a 37°C water bath, which promotes stronger binding of the antibody to PMNs. The diagnostic efficacy of LeuTech imaging for acute appendicitis, however, was not significantly different between the 2 preparations.

We report no scans false-negative for appendicitis in the entire study population. Sensitivity was therefore 100%, indicating that LeuTech imaging identified all cases of acute appendicitis in these diagnostically challenging patients with equivocal clinical presentations. Moreover, early appendicitis was found in 5 of the 26 cases of acute appendicitis. The inflamed appendix was not seen by the surgeon in 2 of these cases and was reported to show early signs of inflammation in a third case. These findings suggest that LeuTech imaging is sensitive enough to detect early acute appendicitis before development of gross anatomic changes that can be seen at surgery. The negative predictive value in both groups was also 100%, indicating that negative findings reliably excluded the diagnosis of acute appendicitis and any other infectious or inflammatory process requiring surgery in the right lower quadrant. Our results using LeuTech imaging compare favorably with our previous results using \(^{99m}\text{Tc}\)-labeled HMPAO WBC imaging in a similar group of patients with an equivocal presentation of acute appendicitis.

In 4 patients, abnormal LeuTech accumulation in the appendicitis zone was not caused by appendicitis and led to false-positive scan results. Three of these scans led to unnecessary surgery, for a rate of 3 of 29 (10.3%) laparotomies with negative findings. The expected rate of negative laparotomy findings in a group of equivocal patients, however, would be expected to be much higher, possibly approaching 40%, depending on the population mix of female patients and children. In retrospect, the findings of the LeuTech scan of 1 of these patients should have been read as negative and would now be read as negative using the criteria established from this study. We retrospectively learned that for scans with true-positive findings, abnormal LeuTech uptake first appears within 60 min after injection. In this particular patient, delayed faint LeuTech uptake was first seen 120 min after injection, and the scan was misinterpreted as showing appendicitis. Therefore, we anticipate that with further experience in reading these scans, and using future established guidelines, the specificity may improve and the number of unnecessary laparotomies may decrease. A 100% specificity for appendicitis is unlikely, because other inflammatory conditions in the right lower quadrant will show abnormal LeuTech accumulation. However, surgeons use other clinical parameters, including repeated examinations, in addition to imaging before operating on patients with positive scan findings.

The ability to allow rapid diagnosis through fast turnaround time is an important prerequisite for an adjunctive imaging test for patients with suspected appendicitis. LeuTech met this criterion by allowing rapid diagnosis in the emergency setting. The average time to first reveal the appendix in cases of appendicitis after injection was 15.5 min for LeuTech-1 and 6.0 min for LeuTech-2. Abnormal LeuTech accumulation was seen in the first image frame 4 min after injection in 67% of LeuTech-2 scans and 54% of LeuTech-1 scans. The most significant time parameter for an emergency department physician or surgeon, however, is the total scan turnaround time from the initial request to receipt of results. In this study, the average turnaround time using either LeuTech preparation was 2.0 h for patients with appendicitis. This time is significantly less than the 2–4 h required to prepare for, and then the 1–2 h to perform, \(^{99m}\text{Tc}\)-labeled-HMPAO WBC imaging.

**CONCLUSION**

LeuTech imaging is sensitive for detection of acute appendicitis in patients with an equivocal clinical presentation in the emergency department. Use of LeuTech imaging has the potential to reduce the rate of laparotomies with negative findings and the number of unnecessary hospital admissions for observation. Acute appendicitis was imaged rapidly and safely in this equivocal group. The inflamed appendix was generally seen within 1 h after injection and frequently was seen within the first 4 min. High-quality planar images were produced with the second LeuTech preparation, eliminating the need for SPECT. We anticipate that LeuTech imaging will play a role in the diagnosis and management of equivocal appendicitis. Larger multicenter studies are required to confirm these results and to evaluate LeuTech imaging for general infections.
ACKNOWLEDGMENTS

This work was supported by Palatin Technologies, Inc., Princeton, NJ, under BB-IND 7358. The authors thank Jim Deitemeyer, Lisa Goldman, Tom Lewis, and Rudy Mohammmd, the Nuclear Medicine technologists at Tri-City Medical Center, who gave up many of their evenings and spent sleepless nights performing these studies on sick patients from the emergency room.

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