Comparison of Indium-111 Nonspecific Polyclonal IgG with Indium-111-Leukocytes in a Canine Osteomyelitis Model

Donald S. Schauwecker, Kathy A. Carlson, Greg A. Miller, Lorrie A. Kalasinski, and Barry P. Katz

Department of Radiology and Laboratory for Diagnostic and Analytical Cytometry, Division of Biostatistics, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana

Osteomyelitis was surgically produced in the proximal tibia of ten dogs. A sham operation was performed on the other tibia. Early (3 hr) and late (20 hr) imaging was performed 1, 4, 7, 10, and 13 wk later, while the osteomyelitis progressed from acute to chronic. Indium-111-IgG had a significantly greater accumulation at the osteomyelitis site than ^{111}In-leukocytes, both during early ($p = 0.001$) and late ($p = 0.03$) imaging, and at each of the weeks studied ($p < 0.001$). During early imaging, both agents gave equivalent lesion to background ratios. On the late images, the ^{111}In-leukocytes gave significantly higher lesion-to-background ratios than ^{111}In-IgG ($p < 0.001$) and higher ratios than they did during the early images ($p < 0.001$). Both agents had greater accumulation in acute osteomyelitis than in chronic osteomyelitis ($p < 0.02$). Osteomyelitis in the surgical site can be distinguished from the uptake in the sham surgery site using ^{111}In-leukocytes, but not when using ^{111}In-IgG.

J Nucl Med 1991; 32:1394–1398

Recently Rubin, Fischman, and co-workers at Massachusetts General Hospital have developed a nonspecific polyclonal antibody against infection (IgG) (*1*). This IgG has been used in several animal and human studies and appears to be very effective (*1–5*). Presently, labeled leukocytes may be the best widely used radiotracer for the study of osteomyelitis (*6,7*). In this study, we compared ^{111}In-IgG with ^{111}In-leukocytes in Fitzgerald's canine model of osteomyelitis to determine the advantages and disadvantages of each agent.

MATERIALS AND METHODS

Canine Osteomyelitis Model

Closely following Fitzgerald's model, the proximal tibial metaphysis was exposed bilaterally through a posteromedial incision (*8*). A cortical window of about 1 cm^2 was removed and the underlying cancellous bone was curetted. Both intramedullary defects were filled with polymethylmethacrylate; the right was closed using sterile technique while the left was inocculated with 10^9 *Staphylococcus aureus* before filling with bone cement and closing. As documented by Fitzgerald, the neutrophilic response to this surgical treatment lasts about 2 wk and approximates the subacute osteomyelitis seen with an infected prosthesis (*8*). By 12 wk, the response is almost totally by lymphocytes and the model approximates chronic osteomyelitis (*8*). Ten 25–35 kg mixed sexed mongrel dogs were studied; five with ^{111}In-IgG and five with ^{111}In-leukocytes. No dog received both ^{111}In-IgG and ^{111}In-leukocytes.

Radiopharmaceuticals

The IgG, in kit form, was supplied by Doctors Rubin and Fischman. The ^{111}In-IgG was prepared and labeled with 0.5 mCi ^{111}In- as previously described (*4*). The ^{111}In-leukocytes were prepared by gravity sedimentation of approximately 47 ml of blood, followed by labeling with 0.5 mCi ^{111}In-tropolonate in plasma (*9*). Throughout the separation and labeling procedure, the leukocytes were constantly kept in plasma and never exposed to saline.

Imaging

Imaging was performed at approximately 3 and 20 hr (early and late images, respectively) following injection of the radiopharmaceutical. In a given dog, the same radiopharmaceutical was injected and imaging was performed on weeks 1, 4, 7, 10, and 13 following the surgical creation of osteomyelitis using a Picker 4/15 gamma camera and a medium-energy collimator. Data were acquired to an ADAC System III computer using a 128 × 128 × 16 matrix. Figure 1 shows typical images that were acquired for 900 sec. Typically, there were about 200,000 counts in an ^{111}In-leukocyte image and about 500,000 counts in an ^{111}In-IgG image.

Regions of interest were obtained in the left and right proximal and distal tibias. A region of interest also was drawn around a calibrated external source. These counts were corrected for differences in the number of pixels before ratios and percentages were calculated.

Originally, it was thought that the right proximal tibia (sham-surgery site) would be the best control for the osteomyelitis site. However, this proved to be a poor control site since inflammation from the surgery accumulated both radiopharmaceuticals as described in the Discussion section. Comparisons were made as

Received Jun. 19, 1990; revision accepted Dec. 31, 1990.

For reprints contact: Donald S. Schauwecker, PhD, MD, Division of Nuclear Medicine, Wishard Memorial Hospital, 1001 West 10th St., Indianapolis, Indiana 46202.

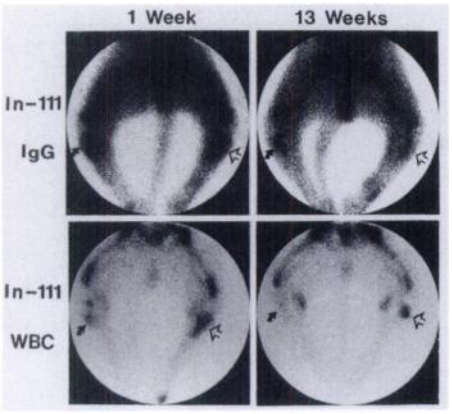

FIGURE 1. Anterior 24-hr ^{111}In-IgG (upper row) and ^{111}In-leukocyte (lower row) images obtained 1 wk (left column) and 13 wk (right column) after surgery. The open arrows identify the osteomyelitis site and the solid arrows identify the sham-surgery site.

follows: (1) between the osteomyelitis site and the ipsilateral distal tibia control site; (2) between the sham-surgery site and the ipsilateral distal tibia control site; (3) between the osteomyelitis site and external source; and (4) between the sham-surgery site and the external source.

Histologic Evaluation

At the completion of the last images, the animals were sacrificed and specimens were obtained for histologic evaluation. Plastic embedding was used because it preserves the tissue structure and cytologic detail more faithfully than does paraffin embedding (*10,11*). A board certified pathologist (GAM) prepared and analyzed all specimens. Half of the animals were evaluated. All of the sites infected with *Staphylococcus aureus* showed evidence of osteomyelitis, while the corresponding sham-surgery sites did not.

Statistical Analysis

Repeated measures analysis of variance (ANOVA) was used to examine differences among agents, weeks, and early versus late imaging for each of the comparisons seen in Figures 2–7 (*12*). In order to better answer the important questions about agents and imaging times, separate analyses were performed for early and late imaging, as well as for each agent. In the repeated measures ANOVA models for early and late imaging, week effect was the repeated factor, and agent was the grouping factor. The ANOVA for each agent considered day and week effects, both as repeated factors in the models. In addition, repeated measures ANOVAs were used to compare the osteomyelitis site and the sham-surgery site across weeks for each agent at each imaging time.

RESULTS

Osteomyelitis Site Versus Distal Tibia Control Site

Figure 2 is a plot of the uptake in the osteomyelitis site divided by the uptake in the distal tibia control site for the various weeks following surgery. The early images using ^{111}In-leukocytes and ^{111}In-IgG gave similar ratios ($p = 0.09$). The ratios for ^{111}In-IgG did not change from early to late imaging ($p = 0.14$). Notice that the ^{111}In-leukocytes gave significantly higher ratios during late imaging than

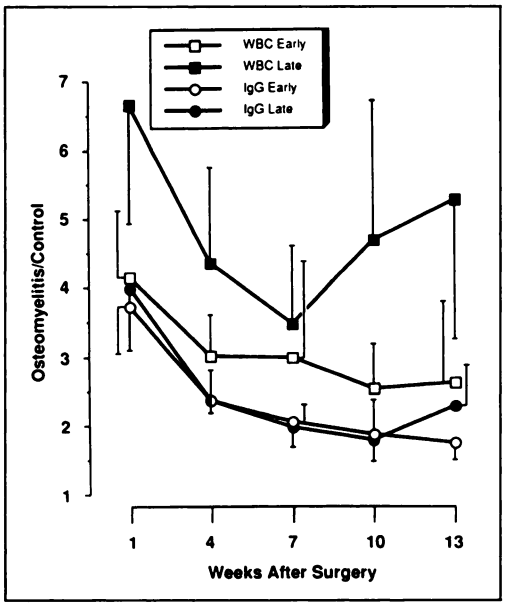

FIGURE 2. Plot of the counts at the osteomyelitis site divided by the counts at the ipsilateral distal tibia control site for the various weeks following surgery. The error bars represent one standard deviation.

during early imaging ($p = 0.001$). The late ^{111}In-leukocyte images provided significantly higher uptake ratios than those obtained with ^{111}In-IgG ($p < 0.001$). Finally, the uptake at week 1 was significantly greater than at any other week for both early and late imaging ($p = 0.01$).

Sham-Surgery Site Versus Distal Tibia Control Site

Figure 3 is a plot of the uptake in the uninfected sham-surgery site divided by the uptake in the distal tibia site

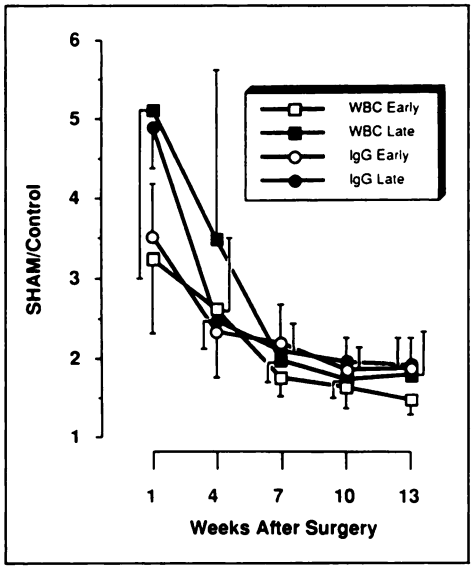

FIGURE 3. Plot of the counts at the sham-surgery site divided by the counts at the ipsilateral distal tibia control site for the various weeks following surgery. The error bars represent one standard deviation.

for the various weeks following surgery. The uptake at week 1 was greater than any subsequent week for both agents and for both early and late imaging ($p < 0.001$). There was no significant difference between agents or between early and late imaging at the sham-surgery site.

Osteomyelitis Site Versus Sham-Surgery Site Using ^{111}In-Leukocytes

Figure 4 replots the data from Figures 2 and 3 for ^{111}In-leukocytes only. The osteomyelitis site has higher ratios than the sham-surgery site in the early images ($p = 0.03$) and borderline higher ratios on the late images ($p = 0.06$). This may represent independent confirmation of the continued presence of osteomyelitis. If the osteomyelitis had disappeared, the osteomyelitis site and the sham-surgery site should give equivalent ratios.

Osteomyelitis Site Versus Sham-Surgery Site Using ^{111}In-IgG

Figure 5 replots the data from Figures 2 and 3 for ^{111}In-IgG only. There was no difference in osteomyelitis compared with sham ratios for either the early images ($p = 0.94$) or for the late images ($p = 0.19$).

Osteomyelitis Site Versus External Source

Figure 6 is a plot of the percent of the injected dose (%ID) localizing in the osteomyelitis site for the various weeks following surgery. On both early ($p = 0.002$) and late ($p = 0.03$) images, a significantly higher percent of the ^{111}In-IgG than of the ^{111}In-leukocytes went to the osteomyelitis site. In addition, the percentage of both ^{111}In-IgG ($p < 0.001$) and ^{111}In-leukocytes ($p = 0.004$) that went to the osteomyelitis site varied significantly across weeks. For both agents, the largest percentage occurred during Week 1. Finally, the %ID at the osteomyelitis site increased from early to late imaging using ^{111}In-leukocytes ($p = 0.008$), but did not change using ^{111}In-IgG ($p = 0.57$).

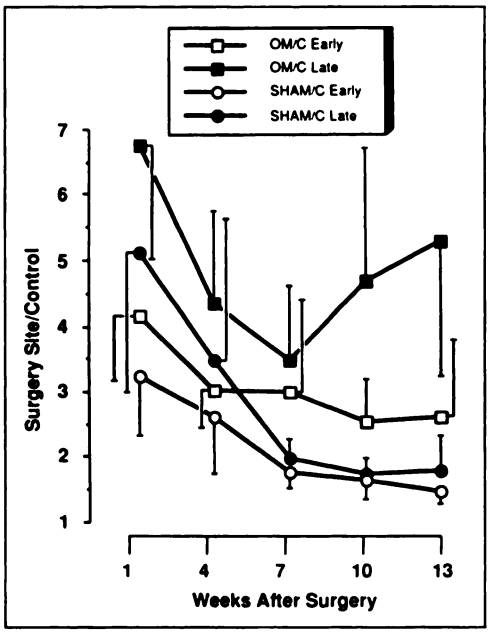

FIGURE 4. Plot of the ^{111}In-leukocyte osteomyelitis/control and sham/control data for the various weeks following surgery. The error bars represent one standard deviation.

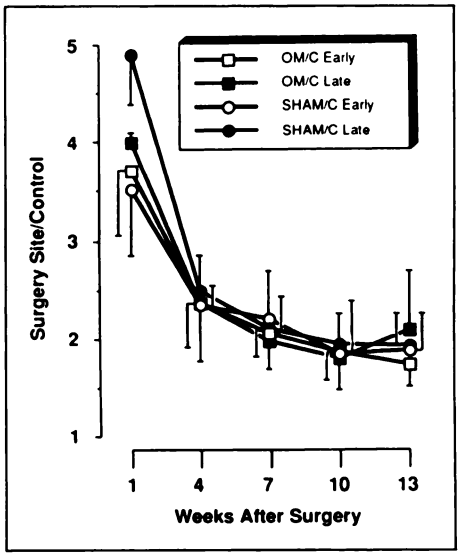

FIGURE 5. Plot of the ^{111}In-IgG osteomyelitis/control and sham/control data for the various weeks following surgery. The error bars represent one standard deviation.

Sham-Surgery Site Versus External Source

Figure 7 is a plot of the %ID localizing in the sham-surgery site for the various weeks following surgery. In both the early ($p < 0.001$) and late ($p < 0.001$) images, a significantly higher percent of ^{111}In-IgG than of ^{111}In-leukocytes went to the sham-surgery site. Uptake varied significantly across weeks for both ^{111}In-IgG ($p < 0.001$)

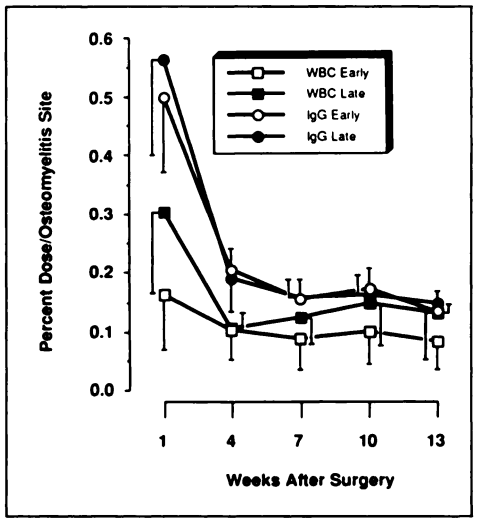

FIGURE 6. Percent of the injected dose localized at the osteomyelitis site obtained by comparing the activity at the osteomyelitis site to an external source for the various weeks following surgery. The error bars represent one standard deviation.

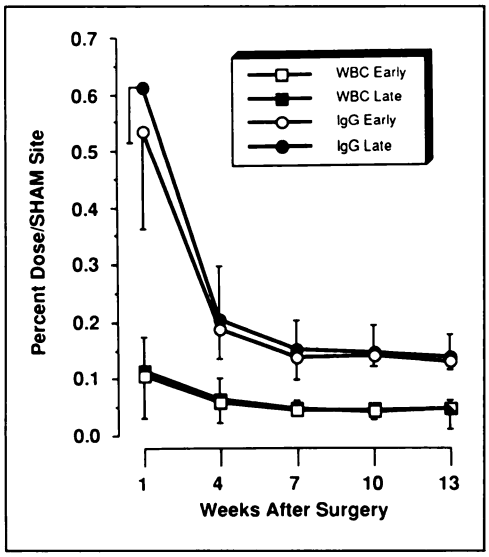

FIGURE 7. Percent of the injected dose localized at the sham-surgery site obtained by comparing the activity at the sham-surgery site to an external source for the various weeks following surgery. The error bars represent one standard deviation.

and ^{111}In-leukocytes ($p = 0.02$). The greatest uptake was observed in Week 1 for both agents. The %ID in the sham-surgery site did not change from early to late imaging using either ^{111}In-leukocytes ($p = 0.52$) or ^{111}In-IgG ($p = 0.07$).

Percent of the Injected Dose at the Osteomyelitis and Sham-Surgery Sites

Data in Figures 6 and 7 were evaluated to determine the %ID at the two sites. There were no significant differences in percentage at the two sites using ^{111}In-IgG for either early ($p = 0.54$) or late imaging ($p = 0.76$). With ^{111}In-leukocytes, there was greater %ID at the osteomyelitis site in the delayed images ($p = 0.03$), but not significantly more in the early images ($p = 0.11$).

DISCUSSION

A larger percentage of the injected ^{111}In-IgG dose than of the ^{111}In-leukocyte dose accumulates in the osteomyelitis site (Fig. 6). However, this is not equivalent to increased lesion-to-background ratios (Fig. 2). In actual clinical practice, the ^{111}In-IgG should be able to visualize osteomyelitis as well as the early ^{111}In-leukocyte images (Fig. 2). There is no improvement in the lesion-to-background ratio for ^{111}In-IgG between the early and late images (Fig. 2), and there was no additional accumulation of ^{111}In-IgG at the osteomyelitis site (Fig. 6). Therefore, the ^{111}In-IgG study could be started and completed in one day. There would be no advantage in having the patient return for delayed images when using ^{111}In-IgG as there is when imaging with ^{111}In-leukocytes.

Our results disagree slightly with those of Oyen et al. who noticed an approximately 20%–70% increase in uptake ratios on late IgG images as compared to the early images (*5*). His article failed to indicate if the observed increase was statistically significant. Two factors may account for these differences (*5*). First, Oyen studied humans, while our results are based on a canine model. Second, our ratios are based on control regions of interest in the ipsilateral limb, while his ratios are based on similar regions of interest in the unaffected contralateral limb.

Indium-111-leukocytes have a significantly higher lesion-to-background ratio for late images than for early images (Fig. 2). This is a real phenomenon that we have demonstrated in human patients using ^{111}In-granulocytes labeled with ^{111}In-tropolonate and ^{111}In-acetylacetone and with ^{111}In-mixed leukocytes labeled with ^{111}In-tropolonate (*9,13*). Figure 6 shows that additional accumulation of ^{111}In-leukocytes in the osteomyelitis site between the early and late images is responsible for most of this improvement.

If the improved lesion-to-background ratios were caused by a decrease in the background, then the sham-surgery site should show similar improvement. Figure 3 reveals some improvement in the lesion-to-background ratio for ^{111}In-leukocytes at the sham-surgery site, but not enough to be statistically significant ($p = 0.16$). Thus, the mechanism for the improved visualization of the lesion with delayed imaging using ^{111}In-leukocytes appears to arise primarily through continued accumulation of the labeled leukocytes at the osteomyelitis site.

Week 1 had the greatest percent absolute uptake (Fig. 6) and the highest osteomyelitis-to-background ratio (Fig. 2) for both ^{111}In-leukocytes and ^{111}In-IgG. As documented by Fitzgerald, the neutrophilic response lasts about 2 wk and approximates the subacute osteomyelitis seen with an infected prosthesis (*8*). By 12 wk, the response is almost totally by lymphocytes and the model approximates chronic osteomyelitis (*8*). In the more acute form of osteomyelitis, there appears to be greater inflow of the labeled cells and presumedly IgG, than in the more quiescent chronic form.

The sham-surgery site also accumulates the radiopharmaceutical (Fig. 7) and can be readily visualized (Fig. 3). This sham-surgery site may approximate the uptake seen in healing fractures, as documented by Van Nostrand et al. and Kim et al. (*14,15*). In Figure 4 there were significantly higher lesion-to-background ratios in the osteomyelitis site than the sham-surgery site using ^{111}In-leukocytes. However, there was no statistical difference in the sham-surgery site and the osteomyelitis site using ^{111}In-IgG (Fig. 5). Therefore, it may be more difficult to separate healing fractures from osteomyelitis using ^{111}In-IgG, than when using ^{111}In-leukocytes. Oyen studied four patients with fractures using ^{111}In-IgG and two had false-positive results (*5*).

Week 1 had the highest sham-to-background ratios (Fig. 3) and the highest %ID of both agents (Fig. 7). Following fracture, the greatest inflammation and healing would begin early, with a gradual diminution with the passage of

time. One would expect the highest inflow of ^{111}In-IgG and ^{111}In-leukocytes during the first week and less during later weeks. This may have been present in the clinical work of Van Nostrand et al., where the bulk of the false-positives using ^{111}In-leukocytes occurred relatively soon after the fracture (*14*). Similarly, of the four patients with fractures studied by Oyen, both patients with recent fractures (10 days and 3 wk) had false-positive ^{111}In-IgG studies, while both older fractures (26 mo) showed no increased ^{111}In-IgG accumulation (*5*).

In conclusion, ^{111}In-leukocytes have two advantages over ^{111}In-IgG. First, the higher lesion-to-background ratio seen on the delayed images (Fig. 2) could allow increased sensitivity. This, however, may not be the case if the lesion can be well-visualized on the early images. Using ^{111}In-granulocytes prepared with acetylacetone in saline, we found the late images more sensitive than the early images ($p = 0.01$) (*11*). However, when using ^{111}In-granulocytes prepared with tropolonate in plasma, no statistical difference could be found between the early and late images (*11*). Oyen et al. had good sensitivity and specificity using ^{111}In-IgG in 35 patients with bone and joint infections (*5*). Thus, the lower lesion-to-background ratios with ^{111}In-IgG may not be a significant problem. Second, ^{111}In-leukocytes should be superior to ^{111}In-IgG in diagnosing osteomyelitis in the presence of recent surgery or fractures.

On the other hand, ^{111}In-IgG has two advantages when compared with ^{111}In-leukocytes. First, the study can be completed within one day, which may lead to more rapid patient treatment. Second, IgG is available as a kit that is easy to prepare in comparison with the separation and labeling of ^{111}In-leukocytes.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Rubin and Fischman for supplying the IgG. They also thank Mrs. Katie Mae Natalie for her help in preparing this manuscript. This work was supported by NIH grant R01AR36460.

REFERENCES

1. Rubin RH, Young LS, Hansen, et al. Specific and nonspecific imaging of localized Fisher immunotype I pseudomonas aeruginosa infections with radiolabeled monoclonal antibody. *J Nucl Med* 1988;29:651–656.
2. Rubin RH, Fischman AJ, Needleman M, et al. Radiolabeled nonspecific polyclonal human immunoglobulin in the detection of focal inflammation by scintigraphy: comparison with gallium-67-citrate and technetium-99m-labeled albumin. *J Nucl Med* 1989;30:385–389.
3. Fischman AJ, Rubin RH, Khaw BA, et al. Detection of acute inflammation with In-111-labeled nonspecific polyclonal IgG. *Semin Nucl Med* 1988;18:335–344.
4. Rubin RH, Fischman AJ, Callahan RT, et al. In-111-labeled nonspecific immunoglobulin scanning in the detection of focal infections. *N Engl J Med* 1989;321:935–940.
5. Oyen WJG, Claessens RAMJ, Van Horn JR, et al. Scintigraphic detection of bone and joint infections with indium-111-labeled nonspecific polyclonal human immunoglobulin G. *J Nucl Med* 1990;31:403–412.
6. Schauwecker, DS, Park HM, Mock BH, et al. Evaluation of complicating osteomyelitis with Tc-99m-MDP, In-111-granulocytes and Ga-67-citrate. *J Nucl Med* 1984;25:849–853.
7. Seabold JE, Napola JV, Conrad GR, et al. Detection of osteomyelitis at fracture nonunion sites: comparison of two scintigraphic methods. *Am J Roentgenol* 1989;152:1021–1027.
8. Fitzgerald RH. Experimental osteomyelitis: description of a canine model and the role of depot administration of antibiotics in the prevention and treatment of sepsis. *J Bone & Joint Surg* 1983;65-A:371–380.
9. Schauwecker DS, Burt RS, Park HM, et al. Comparison of purified In-111-granulocytes and In-111-mixed leukocytes for imaging infections. *J Nucl Med* 1988;29:23–25.
10. Baron R, Vignery A, Neff L, et al. Processing of undecalcified bone specimens for bone histomorphometry. In: Recker RR, ed. *Bone histomorphometry: techniques and interpretation.* Orlando, FL: CRC Press; 1983:13–35.
11. Lumb G. Plastic embedding as a tool in surgical pathology diagnosis. *Ann Clin Lab Sci* 1983;13:393–399.
12. Winer BJ. Statistical principles in experimental design, second edition. New York: Wiley; 1971:514–603.
13. Schauwecker DS, Burt RW, Park HM, et al. Clinical comparison of In-111-acetylacetone and In-111-tropolone granulocytes. *J Nucl Med* 1986;27:1675–1679.
14. Van Nostrand D, Abrew SH, Callaghan JJ, et al. Indium-111-labeled white blood cell uptake in non-infected closed fracture in humans: a prospective study. *Radiology* 1988;167:495–498.
15. Kim EE, Pjura GA, Loury PA, et al. Osteomyelitis complicating fracture: pitfalls of ^{111}In-leukocyte scintigraphy. *Am J Roentgenol* 1987;148:927–930.