Fractal Analysis of Nuclear Medicine Images Again: Validity and Interpretation of Results from New Analysis Methods

TO THE EDITOR: *The Journal of Nuclear Medicine* has recently published 2 closely related studies from a Japanese group, reporting the application of a fractal method to the analysis of SPECT images (*1,2*). Both studies used an intensity-thresholding approach to derive what these investigators termed the *fractal dimension,* a parameter that was claimed to quantify the spatially heterogeneous distribution of radioactivity. These 2 studies differ only in the application targets: one on pulmonary emphysema using a carbon particle radioaerosol (*1*) and the other on cerebral blood flow distribution in Alzheimer's disease with intravenous injection of ^{99m}Tc-labeled hexamethylpropyleneamine oxime (HMPAO) (*2*). Both studies suggested that a large fractal dimension indicates increased heterogeneity in the spatial distribution of radioisotopes and that the fractal dimension showed a statistically significant difference between patients and healthy volunteers.

We have evidence (*3,4*) that the fractal dimension obtained by the analysis method of Nagao et al. (*1,2*) is unrelated to spatial heterogeneity, nor does their result suggest the SPECT images to exhibit a fractal form as claimed in these articles. The main weakness of the studies performed by Nagao et al. lies in the fact that their so-called fractal dimension was obtained using an approach based on intensity thresholding. Therefore, the fractal dimension can be derived entirely from the image intensity histogram. Because the image intensity histogram is essentially the probability density function of the pixel values throughout the entire image region, the histogram naturally is unrelated to the heterogeneous spatial pattern of radioactivity distribution. Consequently, the fractal dimension as defined by Nagao et al. is unrelated to spatial heterogeneity of radioactivity as well.

In our previous publications (*3,4*) attempting a reinvestigation of the studies of Nagao et al. (*1,2*), we have demonstrated that the fractal dimension of Nagao et al. reflected the percentage area (or percentage volume, in the case of 3-dimensional images) of low radioactivity. In addition, the closely associated relationship between fractal dimension and area percentage was independent of imaging modality or the anatomy of interest. Thus, in the study on cerebral blood flow distribution in Alzheimer's disease with intravenous injection of ^{99m}Tc-labeled HMPAO (*2*), the fractal dimension of Nagao et al. is equivalent to the percentage volume of brain tissue showing less than 50% of peak cerebral blood flow. That is to say, an increased fractal dimension at most represents a "significant presence," rather than a "heterogeneous distribution," of local perfusion deficits.

Fractal dimension is not a term that can be arbitrarily defined. Demonstration of fractal behavior by a specific system entails measurements of a certain physical quantity with variations in ruler size over a wide range of scale, typically of at least one order of magnitude. In the study of Nagao et al. (*2*) using threshold values from 35% to 50% of maximum intensity, the variation in intensity scale spans less than only one-sixth of an order of magnitude. Furthermore, because the volume measured with intensity segmentation decreases with increasing threshold values for all images in the world, the conclusions of Nagao et al. on the fractal form of SPECT perfusion images would suggest that all kinds of images in the world exhibit a fractal form. Obviously, this claim would be entirely irrational. Therefore, the fact that the 3-dimensional cerebral blood flow maps obtained with SPECT look somewhat heterogeneous for patients with Alzheimer's disease does not mean that these images are suitable for all arbitrarily defined fractal dimension analyses. Nor does the success of previous reports by Kuikka et al. on the use of fractal approach based on relative dispersion analysis to quantify spatial heterogeneity (*5*) justify the appropriateness of the fractal analysis method of Nagao et al. We shall be attempting a theoretic comparison of the relative dispersion of Kuikka et al. and the histogram-based fractal analyses of Nagao et al. so that the readers of *The Journal of Nuclear Medicine* can be clear about the suitability of fractal analysis methods to quantify spatial heterogeneity.

Besides all the above pitfalls of the study of Nagao et al. (*2*), the fact that Nagao et al. had deliberately ignored our warnings on the weakness of their method surprised us. A letter from me to the editor of *The Journal of Nuclear Medicine* was published in the January 2001 issue, along with Nagao's reply (*4*). The letter was accepted on July 24, 2000. As explicitly stated in the most recent article of Nagao et al. (*2*), their manuscript was submitted to and received by *The Journal of Nuclear Medicine* on August 22, 2000, with revision accepted on May 29, 2001. As a consequence, Nagao et al. clearly were well aware of the deficiency of their approach before attempting to publish their study on cerebral blood flow distribution in Alzheimer's disease. In that article (*2*), however, not one of the potential weaknesses or the controversies was mentioned. In our opinion, any new analysis method claimed to be effective in clinical practice should undergo a solid validation process or should at least be allowed to face criticisms from the scientific society. The deliberate disregard of our warnings by Nagao et al. violates the ethics of honestly reporting their recognition of potential methodologic pitfalls, as mentioned above. This is an issue regarding not only scientific truth but also research honesty.

In conclusion, I would like to emphasize that a rigorous validation of any new analysis method is necessary before broadened clinical applications are attempted. In the case of fractal analysis proposed by Nagao et al. (*1,2*), a second investigation to prove its methodologic validity is strongly recommended.

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REPLY: Our 3-dimensional fractal analysis (3D-FA) was based on using an intensity threshold at different cutoff levels for the involved area. Because an increase in spatial heterogeneity on nuclear images could mean the presence of a region of abnormally high or low radioactivity, we suppose that a varying intensity in the involved area may be related to the heterogeneous distribution on nuclear images. 3D-FA measures the attenuation solution or the attenuation heterogeneity in a target organ. For example, an organ with larger variations in intensity is more heterogeneous. Our previous studies reported that attenuation heterogeneity in a target organ correlates with disease severity (pulmonary emphysema and Alzheimer's disease) (*1,2*).

The definition of spatial heterogeneity remains uncertain. Spatial heterogeneity depends strictly on the spatial scale, but we used the intensity threshold as a scale in 3D-FA. The fractal approach by Kuikka et al. was based on the dependency of relative dispersion on the size of the region (*3*). The relative dispersion fractal dimension is able to indicate the size of objects diffusively distributed in an image. 3D-FA measures a heterogeneity that depends on intensity. In contrast, the fractal approach by Kuikka et al. measures a heterogeneity that depends on spatial scale.

Chung et al. suggested that 3D-FA indicates only the percentage area of low radioactivity (*4*). In Technegas (^{99m}Tc-carbon particle radioaerosol; Daiichi Radioisotope Co. Ltd., Tokyo, Japan) SPECT images for 25 patients with pulmonary emphysema, the numbers of voxels at 15% and 35% cutoffs were 59,459 ± 33,090 (mean ± SD) and 19,311 ± 20,498, respectively, and the number of voxels decreased greatly with an increase in the cutoff level (*5*). Because patients with emphysema inhale a small amount of Technegas, the maximal pixel radioactivity is much smaller in patients with emphysema than in healthy volunteers. Hot spots, which were seen for patients with emphysema, had a high radioactivity with more than a 40% cutoff. Consequently, an area of relatively low radioactivity with a cutoff range of approximately 15%–35% is enough to account for the greatest ventilatory volume in patients with emphysema. This result indicates that 3D-FA is not just an indicator of the percentage area of low radioactivity.

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SOCIETY OF NUCLEAR MEDICINE

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^{99m}Tc-Ciprofloxacin Scintigraphy in Rabbit Model of Prosthetic Joint Infection

TO THE EDITOR: We read with interest the article by Sarda et al. (*1*). However, we would like to point out some major study design and data analysis flaws that undermine the quality of the work and the authors' conclusion about the value of ^{99m}Tc-ciprofloxacin in infection imaging.

The first flaw is the small sample size. With only 13 rabbits, 6 in the infected group (G1) and 7 in the uninfected group (G2), the study is heavily underpowered to demonstrate with a reasonable degree of confidence a statistically significant difference in imaging results between the 2 groups. Thus, although Figures 3 and 5 (these should have included error bars to indicate SDs) clearly demonstrate higher uptake of ^{99m}Tc-ciprofloxacin in infected knees than in uninfected knees, the difference was not statistically significant. Sarda et al. (*1*) acknowledge the small size in their study, but instead of accepting that this is a serious limitation and hence that the results should be interpreted with caution, they justify themselves by giving feeble excuses. Although we sympathize with their wish to use as few animals as possible for ethical reasons, they should have realized that such an approach may well compromise the scientific quality and validity of their study. Another justification they give is the good reproducibility of their animal model. However, as the title of their article states, the model was developed to study prosthetic joint infection due to methicillin-resistant *Staphylococcus aureus*. The model has not been validated for infection imaging studies with radionuclides.

The second flaw is a failure to compare like with like. The 2 arms of the study should have included equal numbers of rabbits matched for age, sex, and weight. The rabbits varied in age (and weight) between 74 and 120 d, and one was 6 mo old. This is important because ciprofloxacin is taken up by growing cartilage in young mammals. Hence, only mature animals should have been studied. A similar volume of inoculum should have been injected. Instead, 0.5 mL of 10^7 colony-forming units of methicillin-sensitive *S. aureus* was injected into G1 rabbits, and 1 mL of saline was injected into G2 rabbits. In addition, the suspension medium used to make the bacterial inoculum should have been used in the control arm; even better, a suspension of the dead bacteria could have been used as a control. Microbiologic evaluation should have been the same for both groups of rabbits. Quantitative bacterial counts of tissue samples were performed on 2 G1 rabbits but not on G2 rabbits. In G2 animals, only the knee exudates were cultured and found to be sterile. It is well known, and mentioned by the authors in their article, that culture of exudates lacks sensitivity for the diagnosis of prosthetic joint infection. Tissue samples generally yield higher culture positivity rates. Infection is an important and well-recognized complication of prosthetic joint surgery, the most common mechanism being contamination of the joint at the time of the operation, with rates of infection varying according to the type of joint (e.g., knee higher than hip). Infected and uninfected joints and rabbits should have been followed up identically with regard to the biodistribution, autoradiographic, and imaging studies.

The third flaw is use of ^{99m}Tc-ciprofloxacin for animal studies. ^{99m}Tc-ciprofloxacin has been standardized for human use only. For small-animal experiments, the preparation may have to be adjusted to give optimal results—for example, reducing the dose of ciprofloxacin from 2 mg to that appropriate for an adult rabbit weighing 2.9–3.5 kg.

Animal studies, when well designed and executed, may provide useful information, but care must be exercised when extrapolating results from these experiments directly to humans. The reasons given above are more credible explanations than those discussed by Sarda et al. (*1*) to account for the difference in results for

^{99m}Tc-ciprofloxacin imaging between their studies on animals and others' clinical studies on patients. In contrast with the study of Sarda et al. involving only 11 rabbits, we have studied the efficacy of ^{99m}Tc-ciprofloxacin in a much larger sample size, 879 patients (*2*). The study, under the auspices of the International Atomic Energy Agency (IAEA), was well designed and incorporated internationally recognized criteria for defining infection and agreed criteria for the interpretation of ^{99m}Tc-ciprofloxacin images. The overall sensitivity and specificity for detecting infection were 85.5% and 81.6%, respectively, but they varied according to the type of infection imaged. For example, the sensitivity and specificity in prosthetic joint infections were 96% and 91%, respectively (n = 194 patients). The IAEA study included 237 uninfected (control) patients, and hence the comment by Sarda et al. that we did not have control groups in our study is erroneous and should be retracted.

The statement of Sarda et al. (*1*) that an animal model of prosthetic joint infection closely mimics acute postoperative infection in humans sadly demonstrates a profound lack of knowledge and insight into the pathogenesis and pathobiology of this important disease in humans and hence an inability to evaluate critically the difference between the animal model and the human condition. The animal model of prosthetic joint infection is by its very nature artificial, designed to produce quick results and use the fewest animals possible (time and cost are often overriding factors, as well as or in addition to any ethical consideration)—for example, a study of the efficacy of different antibiotics as a prelude to clinical trials in humans. Hence, a large inoculum of organisms is used (10^7 colony-forming units in 0.5 mL by Sarda et al., i.e., a visible suspension of bacteria) to ensure an almost 100% infection rate (compared with up to a 5% infection rate after prosthetic joint surgery in humans) and a rapid onset of infection (clinically manifesting within a day in the model described by Belmatoug et al. (*3*) and used by Sarda et al.), which overwhelms the animal's defense system and sometimes results in death. By contrast, in human infections, the number of organisms contaminating the joint at the time of the operation (this period, not just the period after wound closure, is recognized as of greatest risk and was used in the animal model by Sarda et al. to inoculate the organism into the joint) is much smaller (hence, the operation is classified as a clean procedure). Thus, the time taken for the human infection to manifest clinically (i.e., the incubation period) is variable: usually a few weeks to a few months but sometimes longer than a year, depending on the type and virulence of the organisms and the host factors that determine susceptibility to and recovery from infection. The animal model is therefore atypical and does not represent well the spectrum and severity of infection occurring in real life and in real patients.

In addition, we would like to comment on the discussion of Sarda et al. (*1*). First, although quinolones might be taken up and concentrated inside neutrophils and macrophages, this effect must be slight with ^{99m}Tc-ciprofloxacin in vivo, as evidenced by the lack of uptake in bone and bone marrow (sites rich in these cells) in both human and animal studies, including that of Sarda et al. In addition, quinolones are not retained within these and other cells as long as are, for example, the newer macrolide antibiotics such as azithromycin. As the blood concentration drops, they leach out of the cells and tissues into the tissue fluid and then into the blood, to be excreted mostly in the urine. In healthy volunteers, the serum half-life of intravenous ciprofloxacin is 3.5–4.8 h, little remains at 12 h, and there is no accumulation. By contrast, ^{99m}Tc-ciprofloxacin remains bound to and is retained at sites of bacterial infection, giving a high target-to-background ratio, which is the basis of the bacterial specific imaging with this agent.

Second, the inhibitory effect of ciprofloxacin on growing cartilage has been well documented in beagle puppies given large doses of the antibiotic. This effect formed the basis of the contraindication for ciprofloxacin in children and pregnant women. However, ciprofloxacin has been and continues to be extensively and effectively used worldwide as an oral treatment for the troublesome and debilitating *Pseudomonas aeruginosa* infection in thousands of children with cystic fibrosis, for which no alternative oral treatment is available. The antibiotic has been well tolerated, and the adverse effect on cartilage growth seen in puppies has not been a problem in children. This is a classic example of the fact that for a variety of reasons, which include species difference, experimental data on animals may not always be directly applicable to humans. One must therefore be cautious about such extrapolation.

Our third comment regards inhibition of mammalian DNA gyrase by ciprofloxacin. This inhibition is unlikely to be significant with ^{99m}Tc-ciprofloxacin, which contains only a tracer dose of ciprofloxacin (2 mg, which is 1/200th of a single therapeutic intravenous dose of ciprofloxacin). Moreover, compared with bacterial DNA gyrase, the affinity of ciprofloxacin is 100–1,000 times lower for mammalian topoisomerase II, and the ready reversibility of the binding is compatible with ciprofloxacin pharmacokinetics in humans. Also important, the concentration of ciprofloxacin used in the studies cited by Sarda et al. (*1*) and similar published studies has varied from around to greatly above the therapeutic range. For example, a ciprofloxacin concentration of 5–50 mg/L was used in the study by Bryant and Mazza (the reference for which has not been given accurately by Sarda et al. and is provided here (*4*)), and a trovafloxacin concentration of 0.5–25 mg/L was used in the study by Pascual et al. (*5*), compared with a peak serum concentration of 2–4 mg/L 1 h after intravenous administration of the standard dose of 400 mg of ciprofloxacin. The therapeutic range is many times higher than is obtained by the administration of ^{99m}Tc-ciprofloxacin (assuming linear kinetics, the serum concentration of ciprofloxacin 1 h after intravenous administration of ^{99m}Tc-ciprofloxacin, which contains 2 mg of ciprofloxacin, is expected to be about 0.01–0.02 mg/L). Hence, the relevance of these studies to ^{99m}Tc-ciprofloxacin imaging is doubtful.

In conclusion, although interesting, the study of Sarda et al. (*1*) is fundamentally flawed by poor design. Regrettably, Sarda et al., instead of acknowledging the limitations of their study, try to justify their approach and support their results by uncritically citing the results of other investigators. This attempt is also reflected by their reference to the work of Welling et al., about which we have had many exchanges in letters to the editors of both *The Journal of Nuclear Medicine* and the *European Journal of Nuclear Medicine*. These authors have stated that their research has never been centered on ^{99m}Tc-ciprofloxacin but, rather, that they have used it as a control agent for their experiments with ^{99m}Tc-labeled cationic peptides for infection detection in animal experiments. There is, no doubt, much to be gleaned from animal experiments, but when poorly designed and evaluated they may yield misleading results and be misinterpreted, as well as causing a great deal of unnecessary suffering to and waste of animal life.

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REPLY: We read with interest the letter from Das et al. and wish to reply to each of their main points, beginning with the 3 flaws they perceived in our study.

First, the difference in ^{99m}Tc-ciprofloxacin uptake, whether significant or not, between the infected and uninfected prosthetic knees is not of major interest for the purpose of this study. The parameters that we wanted to determine were those of interest to clinical practice: sensitivity and specificity of ^{99m}Tc-ciprofloxacin imaging in prosthetic joint infections. To test the specificity of the tracer—not the difference in intensity of uptake between infected and uninfected prosthetic joints, but the presence or absence of significant uptake in the uninfected inflamed prosthetic knees by comparison with normal nonprosthetic knees—is relevant. We obtained positive findings on ^{99m}Tc-ciprofloxacin scans for up to 4 of 5 uninfected prosthetic joints. Therefore, we concluded that ^{99m}Tc-ciprofloxacin was not specific for the detection of bacterial infections, and we considered the waste of more animal lives neither necessary nor ethical. Activity ratios for the operated knee to the contralateral normal knee, being higher than 1 and increasing with time in uninfected prosthetic joints, were scored as additional indicators confirming qualitative analysis of the scintigrams. The fact that these ratios were higher in infected joints than in joints with postoperative inflammation is not surprising or even attended.

Second, all the rabbits were female. The variations in age and weight were small and not important, especially as far as ^{99m}Tc-ciprofloxacin uptake in growing-cartilage uptake is concerned, since in each rabbit the uptake in the operated knee was compared with that in the normal contralateral knee, each animal being its own control (activity ratios for the operated knee to the normal knee). The volumes of bacterial suspension (0.5 mL) and sterile saline (1 mL) were not exactly the same, but it would be surprising if the results obtained could depend on this parameter! The same suspension medium, sterile saline, was used for preparation of the bacterial inoculum and for control injections in the uninfected rabbits. We did not consider injections with dead bacteria, as this has no relevancy for the clinical situation, since the joints of humans without prosthetic infections would be most unlikely to contain dead bacteria. Quantitative bacterial counts were performed on tissue samples taken from infected rabbits to correlate the number of viable bacteria and ^{99m}Tc-ciprofloxacin tissue uptake. Counts were not performed on uninfected rabbits, for whom we had to verify only the sterility of the prosthetic joint. This verification was performed as in clinical practice, by culture of the froths of the proximal part of the prosthesis on agar plates. This intraoperative technique is completely different from culture of exudates after external needle joint aspiration and is much more sensitive. In pharmacologic studies performed on the same rabbit model, this technique is used as the gold standard to affirm complete recovery after antibiotic therapy.

Third, we agree that great care must be exercised when extrapolating results from animal experiments directly to humans, although we consider animal experiments necessary before a new tracer is studied in humans. However, our study results, which indicated nonspecific uptake of ^{99m}Tc-ciprofloxacin in sterile inflamed prosthetic joints, have recently been confirmed by other clinical studies (*1*). Additionally, we performed a prospective clinical study on 27 operated patients suspected of having osteoarticular infections or sterile mechanical osteoarticular diseases, and the results agreed with our findings for experimental animals: excellent sensitivity but poor specificity for ^{99m}Tc-ciprofloxacin (37.5%), which was taken in sterile pseudoarthrosis and sterile prosthetic loosening. Interestingly, groups that collaborate with Britton et al. also reported ^{99m}Tc-ciprofloxacin uptake in uninfected prosthetic joints, rheumatoid knees, extravascular hip necrosis, fibrous dysplasia, psoriatic arthritis, and nonunion fracture (*1*).

The clinical studies published by Britton et al. did not comprise well-defined control groups: No clear information was provided concerning eventual diseases in the uninfected patients included (how many of them had inflammatory disease?) (*2*). To affirm that ^{99m}Tc-ciprofloxacin is able to discriminate between bacterial infections and inflammation, one needs to select a well-defined control group, containing uninfected individuals having sterile inflammation. This is the reason that we took as a control group rabbits with no infection but with postoperative inflammation.

The animal model we used closely mimics human acute early postoperative infection, with an identical illness course, as confirmed by histologic and MRI findings. *S. aureus* is used in this model because it is most often involved in early acute infections. The onset of infection, which is not as rapid as stated by Das et al. (no clinical signs are visible until 5–10 d), is to our clinical experience comparable to that observed in humans. Moreover, the infection is better tolerated by the rabbits during the course of the experiments than would be anesthesia and surgery, which in some cases cause premature death in infected but also uninfected animals. It is not impossible that the number of inoculated bacteria is different from that found in humans (?), but this number is adequate to obtain high efficiency and reproducibility for the model, those parameters being essential to obtain valuable data. We did not pretend to respond to the problem of chronic prosthetic infection with this model. We chose this model of acute postoperative infection because it directly compares infection and sterile postoperative inflammation and therefore is accurate for testing the ability of the tracer to discriminate between infection and inflammation, the aim of our study.

We now reply to the 3 comments of Das et al. on the discussion section of our article.

First, the fact that quinolones are concentrated and transported by human neutrophils and macrophages by active processes has been demonstrated in vitro, and the mechanisms involved have been studied in detail (*3*). In vivo, quinolones have immunomodulating activities, by enhancing gene transcription of interleukins and growth factors in mature immune cells and myeloid progenitors in bone marrow (*4*). Ciprofloxacin uptake in activated neutrophils was previously shown to be 40 times superior to that observed in quiescent neutrophils, monocytes, and immature myeloid cells, because activation induces the use of a higher-affinity transport pathway (*3*). This finding is compatible with the fact that, in uninfected patients, in the absence of bacteria, we could visualize activated polymorphonuclear leukocytes in inflammatory lesions whereas quiescent polymorphonuclear leukocytes are not detectable, especially in bone marrow. The fact that we observed persistent ^{99m}Tc-ciprofloxacin uptake with no decrease in target-to-background ratio on 24-h images is the in vivo translation of binding to structures other than bacteria in uninfected but operated animals, probably including activated leukocytes, as previously suggested by Welling et al. in preclinical experiments (*5*).

Second, cartilage damage with quinolones has been demonstrated in mice, rats, dogs, and also rabbits, related to ciprofloxacin uptake by the chondrocytes on DNA–enzyme complexes (*6*). This fact is concordant with our findings showing intense ^{99m}Tc-ciprofloxacin uptake in growing plates of the rabbits. This is the reason that we pointed out chondrotoxicity by quinolones on growing cartilage in our discussion. Far from us was the idea that ^{99m}Tc-ciprofloxacin could have any chondrotoxicity in humans, because of the small amount administered. But as far as imaging is concerned, Dumarey et al. also showed intense ^{99m}Tc-ciprofloxacin uptake in the growth cartilage of humans, as in rabbits (*1*). About clinical practice, let us make it clear that the use of ciprofloxacin in children is strictly restricted to carefully selected indications, such as cystic fibrosis.

Third, the remarks of Das et al. about the kinetics of ciprofloxacin and its interaction with mammalian DNA gyrase and DNA ("linear kinetics") are hazardous in our view: Nothing is known about the parameters and kinetics of these interactions. It is surprising that Das et al. believe that the extrapolations of the data of Bryant and Pascual—showing ciprofloxacin uptake by mammalian DNA, phagocytes, and epithelial cells—were not scientifically correct, when their fundamental assumption that ^{99m}Tc-ciprofloxacin acts chemically and pharmacologically the same as unlabeled ciprofloxacin is not supported by any scientific or experimental evidence. Moreover, their group has never published significant data with ^{99m}Tc-ciprofloxacin in experimental settings to show its specificity in infection imaging.

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Total-Body and Red Marrow Dose Estimates

TO THE EDITOR: We read with great interest the article by Matthay et al. (*1*). We would like to comment on the total-body and red marrow dose estimates presented in this publication and discuss possible alternative methods of dosimetry analysis. These points highlight the need for the radionuclide-therapy community to continue to refine dosimetry models to be more patient specific.

The article states that "Ideally, the whole-body dose should correlate accurately with the activity per kilogram." Good correlation between these 2 quantities would be expected only for those agents that exhibit little or no interpatient variability in total-body clearance; the larger the variance in interpatient clearance rates for a given radiolabeled agent, the weaker the correlation. The authors correctly point out that the variance in clearance rate measured in their patients likely caused a reduced correlation between total-body dose and administered activity per kilogram. The fact that the S value for total body to total body (Eq. 7) was not adjusted to better represent individual patients through use of actual (or lean) body mass also likely contributed to a weaker correlation. This is a generally accepted correction needed to more accurately estimate dose. Thus, the less than ideal correlation (even though significant) between total-body dose and administered activity per kilogram, demonstrated in this article for ^{131}I-MIBG, was probably due primarily to the variability in total-body clearance between patients but also to the lack of correction for the mass of the patient.

Currently, a widely accepted approach for estimating red marrow dose uses a 2-component equation (*2*) for radionuclide therapy agents that do not bind to any blood, marrow, or bone elements in patients whose disease does not include significant bone marrow involvement or bone involvement. The first component reflects the red marrow dose contribution associated with activity distributed within the extracellular fluid space of red marrow because of circulating blood activity. The second component reflects the absorbed dose contribution associated with activity in the remainder of the body (RB), according to $D_{RM} = [\tilde{A}]_{blood}\,(CF)\,m_{RM}\,S(RM \leftarrow RM) + \tilde{A}_{RB} \times S(RM \leftarrow RB)$, where D_{RM} is the red marrow dose estimate; $[\tilde{A}]_{blood}$ is the concentration of cumulated activity in blood; CF is a correction factor for the marrow-to-blood activity concentration ratio; m_{RM} is the mass of the red marrow (multiplication of the last 3 terms, $[\tilde{A}]_{blood} \times CF \times m_{RM}$, results in the red marrow cumulated activity, $\tilde{A}_{RM}$); $S(RM \leftarrow RM)$ is the S value for red marrow to red marrow; $\tilde{A}_{RB}$ is the cumulated activity in the RB, obtained by subtracting the red marrow value, $\tilde{A}_{RM}$, from the total-body value, $\tilde{A}_{TB}$; and $S(RM \leftarrow RB)$ is the S value for RB to red marrow. Although it is not always done, additional distinguishable source organ contributions should also be included (*3*). The relative contribution of each of these 2 main components to the red

marrow dose estimate depends on the cumulated activity ratio for total body to blood. For ^{131}I and ratios of 1:1, 3:1, and 10:1, for example, the RB dose contribution can be shown to be approximately 25%, 50%, and 75%, respectively, of the total red marrow dose. Originally, the correction factor, CF, was set at unity (*2*), but other investigators have shown this value to be too conservative. CF is currently assigned either a fixed value of between 0.2 and 0.4 (*3*) or a value of 0.19/(1 − hematocrit) (*4*); given the observed range of patient hematocrit levels, the latter correction factor has been shown to vary by only ±15% from a mean of approximately 0.3.

Red marrow dosimetry for radiolabeled agents that bind to blood, marrow, or bone components, or in patients with significant bone marrow or bone metastases (as may be the case for patients given ^{131}I-metaiodobenzylguanidine [MIBG]), is much more complex and time consuming. In addition to the 2 components of red marrow absorbed dose described above as being due to the nonspecific presence of radioactivity in the extracellular fluid of the marrow and in the tissues of the body, specific uptake of radioactivity in marrow or bone elements may be other important sources of irradiation to the red marrow. To estimate the radiation contributions to red marrow from targeting of malignant (or normal) tissues in the marrow or bone, quantitative measurements involving imaging by scintillation camera are usually obtained to determine the biokinetics of these source regions (*3*). Estimating red marrow dose from image data, however, is admittedly still an uncertain science.

The red marrow dose was calculated in this study by multiplying the total-body cumulated activity by the S value for total body to bone marrow (Eq. 8). Use of the S value for total body to marrow is incorrect because this S value was calculated by assuming that the activity was distributed uniformly throughout the entire body, a scenario that is not true for ^{131}I-MIBG since there are individually distinguishable source organs. For ^{131}I-labeled agents and cumulated activity ratios of 1:1, 3:1, 5:1, and 10:1 for total body to blood, the authors' 1-component approach to estimating red marrow dose would result in an estimate that is a factor of approximately 0.45 too low, 0.90 too low, 1.10 too high, and 1.40 too high, respectively, compared with the widely accepted 2-component estimate of red marrow dose. Thus, for agents exhibiting ratios between approximately 3:1 and 5:1, the red marrow dose approach used by the authors could give reasonable results with an error on the order of 10%. However, given that the average cumulated activity ratio for total body to blood has been estimated to be approximately 1:1 for ^{131}I-MIBG (*5*) and, in addition, that the 2-component estimate of red marrow dose may be significantly lower than the actual absorbed dose because of specific targeting of marrow or bone elements, the approach taken by the authors may have significantly underestimated the red marrow dose and this underestimation may have contributed to the observed poor correlation between radiation dose and toxicity.

Hematologic toxicity is not easily predicted by pharmacokinetic and dosimetric variables, because in addition to the absorbed dose information, individuals' biologic response to radiation may vary because of inherent interpatient differences, decreased bone marrow reserve in some patients, and increased radiosensitivity due to prior chemotherapy or external-beam radiation.

We suggest that, ideally, patient-specific biokinetic parameters (including better image-based analyses) and dosimetric parameters should be used to calculate as accurate a radiation dose as possible, to ensure that the activity administered to the patient will deliver a radiation absorbed dose that effectively treats diseased tissues without harming healthy tissues. We believe that use of a simpler empiric method to determine the necessary therapeutic activity prescription (i.e., a fixed administered activity per unit body weight, or a 1-dose-fits-all approach), currently advocated by some physicians and scientists, is not in the overall best interest of the patient. More accurate and patient-specific models need to be developed, taking into account as many biokinetic, dosimetric, and biologic factors as necessary, such as cellularity, variable absorbed fraction, patient-specific CF (since there may be a significant interpatient variation), patient-specific mass adjustments, and differing bone marrow radiosensitivity. We believe that the radionuclide-therapy community should collect data and perform analyses to more accurately explain observed tumor response and normal-tissue toxicity and shed further light on the optimal treatment for patients receiving radionuclide therapy.

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REPLY: We thank Drs. Siegel, Stabin, and Sparks for their thoughtful and detailed comments on whole-body and red marrow dosimetry methods. If dosimetry is to be used as a prognostic tool, it is correct to assert that reasonable attempts should be made, when possible, both to refine the dosimetric models and to acquire accurate patient-specific data.

Our recent publication (*1*) was based on results dating back 15 years. We nonetheless considered the data of interest and published the results from the dosimetry methods in existence at the time. Current protocols for ^{131}I-metaiodobenzylguanidine (MIBG) therapy at the University of California, San Francisco (UCSF), have improved the accuracy of whole-body measurements in several ways. First, we have mounted a 5-atm digital ion chamber (model 451P; Inovision, Cleveland, OH) over the patient's bed.

This device, along with an Excel (Microsoft, Redmond, WA) add-in program, permits whole-body data acquisition at frequent desired intervals. We are now able to analyze the wash-in and washout curves using hundreds of data points. Second, a power function is used to interpolate S values for total body to red marrow on the basis of patient weight. The power function, mGy/GBq-s $= 0.03296 \times \text{kg}^{(-0.8917)}$, where $r = 0.99983$, was derived by fitting the MIRD S values for total body to red marrow versus the nominal whole-body mass of the 6 MIRD phantoms. Third, the 10%–25% of the administered activity excreted in the urine during the 2-h infusion is now measured in each patient. Subtracting this activity from the infused activity more accurately defines the y-intercept of the washout curve.

Regarding the comments on total-body dose, the above refinements will optimistically decrease both variability for patients who receive multiple ^{131}I-MIBG therapies and variability within a population of patients. Unfortunately, other factors are involved in the correlation between red marrow dose, hematologic toxicity, and administered activity. In addition, approximately 65% of ^{131}I-MIBG is associated with the remainder, and the contribution from the remainder to the red marrow based on the MIRDOSE3 and International Commission on Radiological Protection–53 biokinetic is 90.5% for a 5-y-old child. Also, the association between glomerular filtration rate, creatinine clearance, and whole-body ^{131}I-MIBG kinetics is well documented (*2*). Patients who have compromised renal function were not eligible for this study.

Identifying and understanding the numerous elements required to determine the red marrow dose that has a predictive value is beyond the scope of our clinical studies. However, we would like to respond to the comments on red marrow dose.

First, the correction factor of 0.2–0.4 that Drs. Siegel, Stabin, and Sparks cite is for 150-kDa antibodies (*3*), not 324-molecular-weight cations with a pKa of approximately 12. The equilibrium of ^{131}I-MIBG from the plasma space into the hematopoietic parenchyma and supporting stroma is unknown. In fact, quoting Siegel, "Pharmacokinetic principles indicate that traffic of molecules through the fenestrations of the endothelial layer depends at least on differences in electric charge between the endothelial layer and the molecule, molecular size. . . " (*3*). Furthermore, it is common knowledge that highly charged and polar molecules do not readily cross cell membranes.

Second, although we have never sampled red marrow from our pediatric patients to evaluate active uptake, we do know from viewing the marrow space in more than 3,000 diagnostic ^{123}I-MIBG scans since 1985 that, in the absence of disease, ^{123}I-MIBG does not concentrate in the red marrow. Our laboratory is anxiously awaiting the availability of ^{124}I-MIBG that will permit us to quantitatively address this issue using PET.

Third, our lack of a blood component was not an oversight. Blood samples taken 30 min after a bolus injection of ^{131}I-MIBG containing $<$0.1% free iodide, from patients with normal organ function, demonstrate a very small fraction of a percentage of the administered activity. Also, dynamic scintigraphic studies dramatically demonstrate clear images of normal organs and extensive kidney and bladder activity within minutes after a bolus injection of ^{123}I-MIBG. The above blood and scintigraphic data indicate an apparent first-pass plasma clearance of MIBG. This phenomenon has been previously reported (*4*).

As stated in our article (*1*), therapeutic amounts of ^{131}I-MIBG are infused over a 2-h period at a constant rate. Blood samples taken at 15-min intervals during the 2-h infusion and immediately afterward likewise demonstrated an extremely rapid blood clearance. For instance, the activity per gram of blood at 15, 30, 45, 60, 75, 90, 105, and 120 min during a constant rate infusion of 10.10 GBq was 46.99, 84.73, 88.80, 98.05, 120.62, 135.05, 136.90, and 144.67 kBq, respectively. At 1 and 2 h after the infusion, the activity was 49.58 and 18.50 kBq. The area under the curve of the 2-h infusion in this example was 196.10 kBq-h with a beta dose per gram of blood of approximately 2.0 cGy.

A plausible explanation for the rapid plasma clearance of ^{131}I-MIBG is as follows: First, ^{131}I-MIBG is a metabolically stable analog of norepinephrine; second, the concentration of catecholamines in both extracellular fluid and plasma is critical to body function; third, catecholamines are known to be highly polar chemicals that do not readily cross cell membranes; and fourth, there is a class of nonneuronal transporters that enable the body to rapidly clear and regulate catecholamine concentration in the blood (*5*). In humans, the organic cation transporters (OCT-1) are expressed primarily in the liver. The strategic localization of OCT-1 in an excretory organ is to eliminate specific substrates, of which ^{131}I-MIBG is one, into the bile. However, ^{131}I-MIBG is one of several substrates that the liver seems to lack the mechanisms to extract into the bile. A knockout mouse line that lacks functional OCT-1 demonstrated an approximately 4-fold reduction of ^{131}I-MIBG in the liver over functional controls (*5*).

Dynamic scintigraphic studies demonstrate clear images of normal organs, that is, extensive liver uptake within minutes after a bolus injection of ^{123}I-MIBG. They do not, with the exception of a momentary blush, demonstrate tumor. Blake et al. state that "Following the initial rapid clearance a quasistatic equilibrium is maintained between continuing rapid plasma clearance and ^{123}I-MIBG re-entering the circulation" (*2*). It is our conjecture that the liver functions as a margination pool for tumor ^{123}I-MIBG uptake.

The goal of our ^{131}I-MIBG treatment program has been to try to cure a highly malignant childhood cancer by giving the maximally effective doses. Ideally, pretherapy dosimetry should be used to predict both bone marrow toxicity and expected tumor absorbed doses for all radiotargeted isotope therapy. In fact, pretherapy dosimetry would also be ideal with all medical therapies to adjust them to individual pharmacokinetics and tolerance in each patient, but practicality and the goal of cure often dictate the use of the maximum tolerated dose determined in a phase I study, the approach that we took with ^{131}I-MIBG. Unfortunately, the many parameters inherent in tumor dosimetry and the as yet nonmeasurable biologic factors that may contribute to tumor response and to marrow toxicity make dosimetry a less than accurate exercise. We are well aware of these biologic variations in patient bone marrow reserve, ^{131}I-MIBG distribution and excretion, and tumor heterogeneity, after performing 139 high-dose ^{131}I-MIBG therapies here at UCSF. Nonetheless, we have initiated a new pretherapy dosimetry protocol for ^{131}I-MIBG to study early time-points and thus see if it is possible to improve the accuracy of predicting tumor and liver dose in each patient. As the imaging technology continues to improve, it is likely that dosimetric measurements may become more predictive of tumor response. However, the difficulty in estimating the impact on bone marrow stroma of prior therapy and infiltrating tumor will continue to confound toxicity predictions and make even the most accurate prediction of red marrow dose only an approximation for selecting a "safe" level of activity.

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