The Accuracy of Quantitative Analysis of Stress/Delayed Thallium-201 Myocardial Tomograms

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TO THE EDITOR: We have a few questions regarding a recent paper by Garcia et al. (*1*) describing a multicenter study for validating the accuracy of a new Emory program for quantitating reversibility of stress-induced thallium-201 myocardial perfusion images.

The first question concerns the definition of accuracy. The calculation of accuracy requires determining sensitivity and specificity (*2*). This study evaluates sensitivity but not specificity when comparing the new Emory program for detecting reversibility with visual interpretation of four experts. It would be important to know if there were patients, and if so how many, who demonstrated no reversibility according to the experts but did show reversibility with the new Emory program, i.e., what is the false-positive rate? Do the authors recommend diagnosing reversible ischemia if the new Emory program is positive and the images appear normal?

We also question this method when quantitating data in patients with balanced multi-vessel disease. Wouldn't one miss abnormal reversibility with disease involving the three major coronary vessels with this normalizing technique? In a previously described method of quantifying rotational thallium-201 myocardial tomography (*3*), the relative change in counts between stress and delayed images was handled by "multiplicative scale factors provided by commercial programs." Perhaps this relative method would detect multi-vessel disease, however, we would like to know how these scale factors were derived and validated since they were not discussed.

Our last question concerns the wisdom of recommending the use or this new program to train "diagnosticians with limited experience in interpreting thallium tomograms," since even the experts did not fully agree. There was a significant difference in performance of two of the four experts in diagnosing reversibility compared to the new Emory program (Table 2). One expert "tended to relate more subtle reversibility with significant ischemia." Furthermore, when there is disagreement between the experts and the new Emory program, there is no way to tell who or which is correct.

We belive these questions need to be addressed since the use of this new program will undoubtedly become widespread. The article will be very valuable to General Electric Medical Systems, one of the institutions participating in this study. We predict that they will heavily market this technology just as they have the previous Emory program.

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REPLY: In our multicenter validation, we chose to establish the accuracy of the method in terms of how well the results of the program agreed with those of experts in determining reversible versus fixed defects. This decision was based on the difficulty of establishing a gold standard to measure ischemia or infarction in vivo. Because of this choice and because in this analysis the absence of detecting reversibility did not mean a normal finding but rather a fixed defect, we chose to avoid terms like sensitivity and specificity. Nevertheless, it is not difficult to determine how many defects, or patients, were assessed as reversible by the program but demonstrated no reversibility by the expert's interpretations. Table 1 on our multicenter paper (*1*) gives a detailed comparison on a vascular territory basis. From the right column of this table, it can be determined that of 83 defects assessed to be fixed by the experts 15 were determined to be reversible by the new method. This comparison yields an 18% disagreement rate, which would correspond to what Lasher et al. call a false-positive rate. Since the purpose of this analysis is to determine how well the program's results agree with experts, it is clear that if there is a disagreement it is the program that is wrong. Previously, we established using five experts that there is only a 7% interobserver variability in the visual assessment of defect reversibility (*2*). The fact that experts disagree is not different from the fact that repeated measurements with any "gold standard" yield different results at least some of the time.

In addition to analyzing results in large populations, it is also important to analyze how to use the program in specific cases. One case of concern described by Lasher et al. is when the program suggests there is a reversible defect when the images appear normal to the physician. The program will show regions that change between stress and delayed imaging and the magnitude of the change. But as implemented, the program will not flag a region as reversible unless it was first determined to be associated with a stress-perfusion defect using the quantitative critera. Another case of concern is that the program will miss determining reversibility when the patient has balanced multivessel disease. If the flow reduction to all vascular beds is truly balanced (something we suspect happens rather infrequently), then no stress-perfusion defect will be detected since there is no myocardial region demonstrating a relative reduction in counts no matter what scale or normalization factor is used. This will confuse both the program and the expert into interpreting the scan as normal unless other markers of disease are used such as lung uptake of thallium-201 or slow washout of thallium-201 from the myocardium. One feature of our approach is that we continue to quantify the percent washout from the myocardium between stress and delayed imaging. Although we have not systematically analyzed how this independent parameter comple-

ments the measurement of reversibility, we suspect it will be of assistance in detecting ischemia.

We continue to believe that one of the best uses of this approach to data-based quantification is by diagnosticians with limited experience, since they can be reminded of the abnormal patterns of tracer concentration and change. In all cases, physicians should use the detection or characterization of abnormalities by these quantification programs as flags to point out regions of concern to be verified by the physician's own expertise. The development of these tools requires an extensive effort to make them as robust and accurate as possible and to understand their limitations. The ultimate reward for this effort is the widespread acceptance of the use of these tools by the nuclear medicine field.

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Tumor Imaging with Indium-Labeled Biotin

TO THE EDITOR: I read with interest the paper by Kalafonos et al. in which the authors reported positive imaging in 8 out of 10 lung tumors (1). A blinded observer found improved image quality in three of these eight when prior antibody conjugate had been given. Three were negative images with or without antibody-avidin conjugate. Rapid internalization of the conjugate is cited as the reason for nonspecific visualization (positive visualization but no improvement with Ab conjugate). A marginal difference in urine excretion, 71% ± 9% s.d. versus 83% ± 7% s.d., is presented as evidence for specific binding in patients receiving conjugate. It is reported that increasing the ^{111}In-biotin injected from 50 μg to 1000 μg did nothing to improve specific binding. The authors add that the positive images may be due "in part" to localization of labeled biotin in tumor.

Interestingly the authors have shown specific biotin uptake in the nude mouse tumor model and two tumor cell types in vitro over DTPA controls. This in itself may be a very important observation that must be carefully controlled. This control must now be applied to other interesting positive human tumor imaging results with the avidin-biotin systems reported recently (*1,2*).

However, I would like to put forward an alternate explanation for the lack of specific targeting not discussed by the authors. This is simply that in all cases there was not enough avidin administered to bind specifically more than 15% of the ^{111}In-biotin injected, if 100% of the conjugate had localized in the target. Since we know that at best only 1% or less of the injected antibody dose localizes in human tumors, this amount is reduced to 0.15% maximum. This calculation is based on the following assumptions: the 1 mg of protein injected was based on IgG MW = 150,000 and not the conjugate MW 210,000; bis-biotinyl DTPA MW = 1,102; Ab-avidin conjugate is a monomer (see Materials and Methods p. 1792) capable of binding one molecule of ^{111}In-bis-biotin DTPA (reduced from the native valency of 4 by steric hindrance due to the use of one site for conjugation, blocking one neighboring site, and the use of bis-biotin DTPA, which uses two sites to bind one ^{111}In. At the 50-μg biotin level, the molar ratio of biotin/streptavidin = 6.8/1. This ratio, which determines the amount of specific biotin binding possible, becomes much less favorable (136/1) if the amount of bis-biotin is increased to 1000 μg, as the authors did in an attempt to improve targeting. Under this condition, localization by specific binding becomes 0.74%–0.0074% maximum.

My main point is that pretargeting is a form of receptor binding, in which the concentration of receptor is very low, usually p*M*. In this situation, high-specific activity is mandatory for adequate specific localization of radiopharmaceuticals.

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REPLY: We wish to thank Dr. D.A. Goodwin for his thoughtful comments concerning our recent paper on patient imaging with ^{111}In-labeled biotin and streptavidin-conjugated anti-tumor antibodies (*1*). Dr. Goodwin has referred to our observation that specific targeting, as judged by image quality, was achieved in only three of eight patients. In addition to possible explanations for this phenomenon described in our report, Dr. Goodwin has added another: since only a limited concentration of streptavidin may be expected in tumor under the best of circumstances, the available biotin binding sites may have become saturated at the doses of biotin administered.

Dr. Goodwin is certainly correct that the localization of streptavidin-conjugated antibody in tumor is likely to be limited since tumor accumulation of this conjugate, as with any antibody, will be influenced by poor tumor perfusion, restricted vascular permeability, limited antigenic expression, etc. We also agree with his calculation that the 1 mg of conjugated antibody administered would bind approximately 5 μg of labeled biotin. By assuming a reasonable value for the percentage of administered antibody conjugate which localizes in tumor, it is possible to calculate the weight of labeled biotin required to achieve saturation of the biotin-binding sites therein. However, it is incorrect in our view to argue further that the biotin dose administered should be reduced to that which is approximately equivalent to this value. This ignores an important property of biotin, namely its rapid clearance from circulation. Since approximately 50% of administered biotin appears in urine in 1 hr (*1*), only a small fraction