Dynamic Variable Background Subtraction: A Simple Means of Displaying Radiolabeled Monoclonal Antibody Scintigrams

Richard L. Wahl, Michael C. Tuscan,* and James M. Botti

Department of Internal Medicine, Division of Nuclear Medicine, The University of Michigan Medical Center, Ann Arbor, Michigan

Subtraction of a nonspecific radionuclide is frequently used to enhance visualization of tumors imaged by radiolabeled monoclonal antibodies. Determining the optimal amount of the nonspecific radionuclide image to subtract can be difficult. We have developed a computer program that generates a closed-loop cinematic display in which a continuously varying amount of the nonspecific radionuclide image is subtracted from the specific antibody image. Through examination of this dynamic display, a broad range of background-subtracted images can be viewed expeditiously in an effort to select the optimal level of background subtraction. This method may be useful in routinely displaying such background-subtracted studies.

J Nucl Med 27:545–548, 1986

Radiolabeled monoclonal antibodies hold considerable promise as specific imaging agents for tumors and some benign conditions (*1–5*). Although excellent diagnostic results have been reported by some investigators using polyclonal and monoclonal antibodies, these results have often been obtained through the use of background subtraction techniques (*6,7*). Such techniques are necessary because after i.v. injection, intact antibodies take many days to develop optimal tumor/nontumor ratios (*8,9*). Even when these ratios are achieved they often are relatively low (*9*). Although these ratios can be improved, and the time to optimal diagnostic image quality shortened by the use of antibody fragments such as the $F(ab')_2$ (*9*), background subtraction can further enhance images with this antibody fragment (*10*).

If background subtraction is used, the choice of the background subtracting agent and the amount of background activity to subtract are not always clear. Although a nonspecific, isotype-matched monoclonal antibody, or better yet, a nonspecific $F(ab')_2$ fragment (if imaging is being conducted with $F(ab')_2$'s) would seem the logical choice for subtraction, difficulties in labeling antibodies with agents that equally affect antibody localization and dosimetry questions have so far prevented this theoretically attractive approach from gaining popularity (*9,11*). In addition, differences in energy and thus differences in tissue attenuation between the two isotopes chosen can make background subtraction more difficult. The two agents most commonly used to date in clinical background subtraction studies have been technetium-99m-labeled human serum albumin, ([^{99m}Tc]HSA) and ^{99m}Tc-labeled red blood cells ([^{99m}Tc]RBCs).

Regardless of the agent chosen to represent nonspecific activity, the optimal amount of ^{99m}Tc activity to be removed by background subtraction is uncertain and frequently unclear in the literature (*6,7,12,13*). Although an initial first approximation is to normalize the iodine-131 (^{131}I) antibody and ^{99m}Tc images based on activity in the cardiac region on the anterior views of the chest obtained at the ^{131}I and the ^{99m}Tc photopeaks for equal imaging times, this is only a first approximation, as the biodistribution of [^{99m}Tc]HSA, [^{99m}Tc]RBCs, and ^{131}I-monoclonal antibodies differ in

Received Mar. 18, 1985; revision accepted Dec. 31, 1985.

For reprints contact: Richard L. Wahl, MD, University of Michigan Medical Center, Div. of Nuclear Medicine, BIG505 University Hospital, Box 0028, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0028.

* Present address: General Electric Medical Systems, P.O. Box 414, Milwaukee, WI 53201.

our experience. This difference is particularly obvious in the abdomen, where far more ^{99m}Tc activity is seen in the kidneys and bladder than labeled antibody and more ^{131}I-antibody may be seen in the bowel than ^{99m}Tc activity. While the indium-111 antibody may offer some advantage, its lower photon peak being very close to ^{99m}Tc's as well as its apparent entry into the bowel (possibly through the biliary tree) may make background subtraction (particularly in abdomen) difficult to optimize (*14*). Thus it is not always clear how much ^{99m}Tc activity should be subtracted from the ^{131}I-monoclonal antibody images.

To address this uncertainty, we have adapted a cinematic display to sequentially display increasingly more [^{99m}Tc]HSA image subtraction from the antibody images in an endless loop. This methodology easily allows viewing of the background subtracted images at multiple levels of subtraction. This eliminates the need to interpret a single image reflecting a single semi-empirical determination of the appropriate amount of background subtraction.

MATERIALS AND METHODS

Radiolabeled antibodies are given only after written informed consent is obtained. All antibodies and imaging protocols have human use committee approval and all antibodies have IND's on file with the Food and Drug Administration. All images are obtained with a large field-of-view gamma camera fitted with a high-energy (400 keV) collimator. Twenty-minute or 100,000-count images of the whole body are obtained daily at the ^{131}I energy peak (364 keV) with a 20% window after the i.v. injection of 1–2 mCi of the radiolabeled antibody of choice (500–1,000 μg). Background subtracted images are obtained at 48 and 120 hr. Technetium-99m HSA is separately or simultaneously imaged at the 140 keV photopeak with a 10% window. All image data are collected into a dedicated nuclear medicine computer. Downscatter from the ^{131}I to ^{99m}Tc window is determined on an anterior view of the chest obtained for 3 min immediately preceding the injection of the [^{99m}Tc]HSA. The patient is then given 1 mCi of [^{99m}Tc]HSA intravenously. The images over anatomic regions in which disease is suspected are obtained for 20 min at both the ^{131}I and ^{99m}Tc photopeaks. All images are stored in the computer. Six imaging positions are generally used including the anterior and posterior chest, abdomen, and pelvis.

After all images are acquired, the [^{99m}Tc]HSA images are corrected for the contribution from the ^{131}I downscatter. Generally, <5% of the total counts in the ^{99m}Tc window are due to ^{131}I downscatter. The resultant corrected ^{99m}Tc images for each projection are then used for the background subtractions.

A region of interest is drawn manually over the heart on the anterior downscatter corrected [^{99m}Tc]HSA view of the chest acquired 30–50 min after [^{99m}Tc]HSA was injected. This same region is drawn over the heart on the ^{131}I image obtained simultaneously. The total counts in this region on each set of images is determined and a ratio between I and Tc counts determined. The ratio is then multiplied times each scatter-corrected ^{99m}Tc image to form a "subtraction mask" for subtraction from the ^{131}I image. This initial subtraction mask should approximate the appropriate amount of background to be removed. As stated earlier, because of differences in energies and biodistributions of the two agents, as well as differences in time after injection among the [^{99m}Tc]HSA images, this provides only a first approximation of the appropriate amount of subtraction.

The final construction of the multiple background subtracted images that compose the frames of the cinematic loop is totally at the discretion of the operator. A typical approach would be to create 20 images in which 0.1–2.0 times the [^{99m}Tc]HSA background mask (by increments of 0.1) is subtracted from each of 20 identical ^{131}I antibody images. This is done automatically by a computer algorithm (will be supplied upon request). Thus a series of images with increasing ranges of background subtraction are generated, ranging from a minimal background subtraction to a subtraction that results in the loss of nearly all ^{131}I activity on the images.

A cinematic loop display is then achieved by sequentially displaying each of the incrementally background subtracted images in an endless loop. Coarser or finer increments can be selected, as well as nonarithmetic increases in the extent of background subtraction (e.g., exponential or sigmoidal) by altering the algorithm. The series of background-subtracted processed images when displayed cinematically initially shows the antibody image with only a small amount of nonspecific ^{99m}Tc activity removed. As more highly-subtracted images are displayed, more of the nonspecific agent's contribution is removed and the images should increasingly demonstrate the specific component of antibody localization. The number of frames used, increments of subtraction, and the speed of the cinematic display can be altered using the computer. This method allows many images with different information content to be viewed rapidly and easily. Persistent increased activity in a body structure seen before and well after cardiac blood-pool background activity has been subtracted suggests specific accumulation of labeled antibody in that region.

RESULTS

A representative illustration of this approach to background subtraction is seen in the following case. ^{131}I-labeled murine monoclonal antibody 5F9.3, which preferentially localizes to choriocarcinomas in nude mice (*15*), was injected intravenously into a patient suspected of having metastatic choriocarcinoma. This suspicion was due to a rising B-HCG level despite prior surgical excision of a pelvic and abdominal choriocarcinoma. A posterior 20-min acquisition view of the chest at the ^{131}I photopeak, shows considerable activity in the heart and upper abdomen (presumably related to blood-pool activity) persisting 120 hr postantibody injection. A hint of increased activity is seen in the left upper chest (Fig. 1A). The [^{99m}Tc]HSA image obtained at the same time shows extensive blood pool activity, without preferential localization to the left upper lung (Fig. 1B). Samples of images from the cinematic display, obtained with increasing levels of background subtraction, show the gradual disappearance of heart blood-pool activity but with persistent activity remaining in the left upper lung field (Fig. 1C–D, 2A–C). With maximal background subtraction (Fig. 2D) no patient radioactivity remains

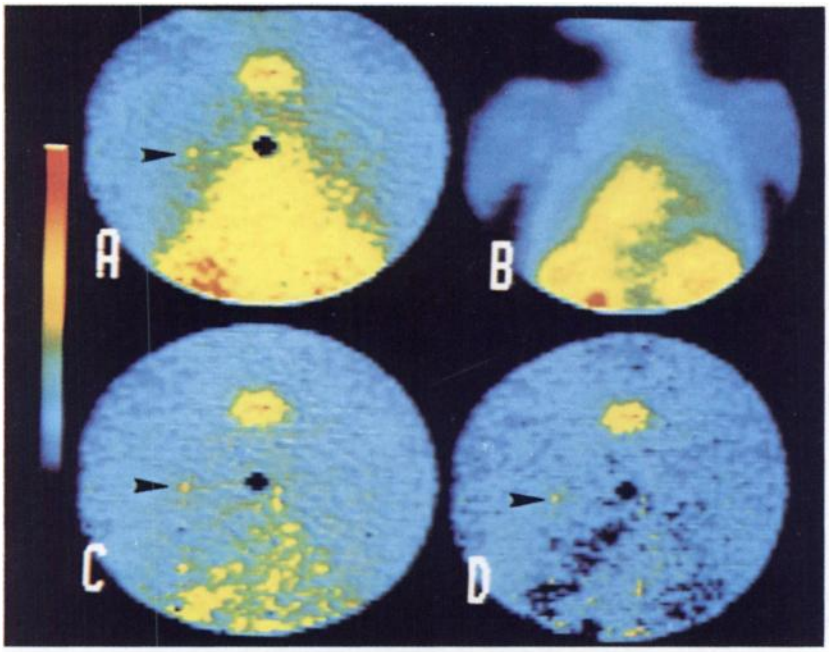

FIGURE 1
A: 120-hr ^{131}I monoclonal scintigram (5F9.3) of posterior chest. Note abundant blood-pool activity in heart and upper abdomen. Also note subtle increased uptake in left chest (arrow). Black dot in center of chest is due to computer artifact revealed on flood field exam. B: [^{99m}Tc]HSA scintigram acquired simultaneously with Fig. 1A. Note blood-pool activity in vascular organs. C, D: Background subtraction images with increasing amounts of [^{99m}Tc]HSA image removed from ^{131}I image (1C = 50% of 1B subtracted, 1D = 80% of 1B subtracted) (tumor focus indicated with arrow)

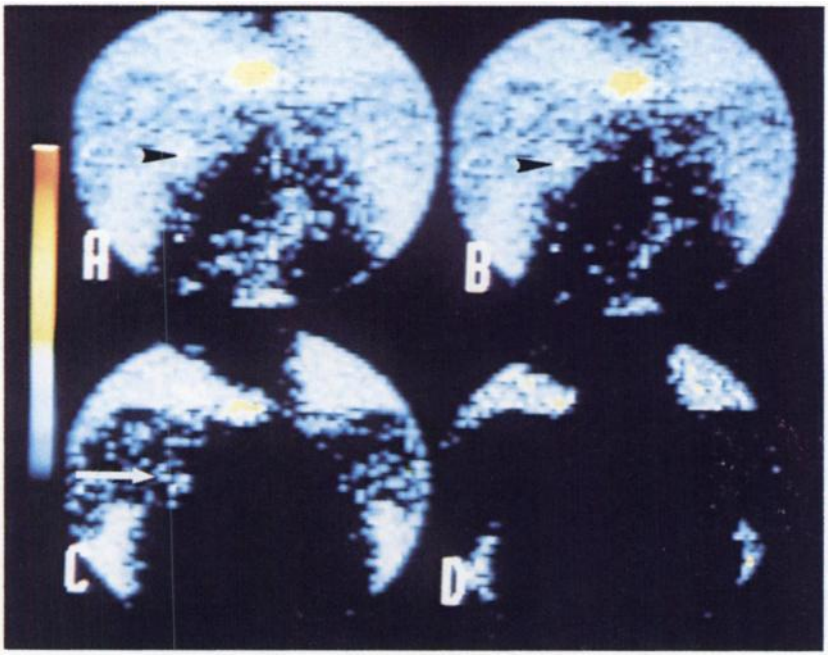

FIGURE 2
A–C: Further increasing amounts of [^{99m}Tc]HSA activity removed from ^{131}I image (2A = 90%, 2B = 110%, 2C = 150% of image 1B removed from 1A). Note residual ^{131}I activity in left upper chest (arrow) (A–C) corresponding to small focus of choriocarcinoma. D: Maximal background subtraction (200% of image 1B removed) shows all of ^{131}I activity in patient to now have been subtracted. Only ^{131}I scatter outside of patient remains. These and intervening images can be displayed in continuous loop cinematically to facilitate lesion detection

visualized. Activity extending outside of the chest on the most highly-subtracted images is due to the more extensive scattering of ^{131}I as compared with ^{99m}Tc.

When 15–30 of these images are sequentially displayed in an endless cinematic loop, the region of increased ^{131}I activity in the left chest stands out clearly. In this case, the increased left lung activity demonstrated was found at biopsy to be in a small focus of choriocarcinoma, which had been felt to represent stable pulmonary scarring until the antibody study was performed (*16*).

We have not as yet had an opportunity to perform a sufficient number of monoclonal antibody scans using this subtraction technique to determine if this method improves the sensitivity and specificity of these scans for lesion detection over examining a single background subtraction image.

DISCUSSION

Images obtained with radiolabeled monoclonal antibodies after i.v. administration can be suboptimal diagnostically due to relatively low tumor/background ratios and low absolute delivery of radioantibody to tumor. Background subtraction techniques can aid in making subtle lesions visible. This approach has some technical and theoretical limitations (*7,12*), that can make the exact amount of the nonspecific agent to be removed unclear. These limitations include differences in biodistribution of the background subtraction agents from the imaging agent and considerable differences in tissue attenuation between high and low energy isotopes

used for imaging. The cinematic mode we describe displaying incremental increases in background subtraction facilitates the detection of foci of disease. It allows the nuclear physician to examine quickly and easily a wide range of background subtraction magnitudes without making assumptions about the equivalency of biodistribution of the nonspecific component of each imaging agent.

In our limited clinical experience, we feel most comfortable diagnosing increased radioantibody uptake in a region if it persists well after cardiac blool-pool activity has been subtracted away. Certainly, limitations do apply as previously discussed, including excess iodine in the stomach, bowel, or bladder that could create false-positive images of normal routes of excretion in the abdomen. Thus, the techniques must still be used with caution. By appropriately selecting the subtraction parameters (subtraction algorithm curve), blood-pool activity will disappear near the middle of the background subtraction loop. While a prospective clinical trial will be necessary to evaluate this, the sensitivity and specificity of this technique, with its cinematic display node, greatly facilitates looking at a large number of views of a given region of interest in the body at increasing levels of background subtraction in comparison with selecting one level of subtraction or nonsubtraction and interpreting from those views. To examine static images at multiple levels of background subtraction, one might examine 90–300 separate static images (in a six-view study). This same data could be presented cinematically in just six cinematic display loops. This mode of display makes examining graded subtraction images logistically feasible and may save on film costs.

The ultimate widespread clinical utility of monoclonal antibodies as imaging agents will probably require better tumor/background ratios than are frequently achieved at present. It is possible that this may be achieved in the short-term by intralymphatic antibody delivery and other methods (*17,18*). Until this occurs, background subtraction of a nonspecific radiopharmaceutical such as [^{99m}Tc]HSA may be necessary in some cases to arrive at clinical diagnoses, particularly when intact monoclonal antibodies are used. A simple means to view and interpret such studies is through this dynamic graded subtraction image display.

ACKNOWLEDGMENTS

The authors thank Mrs. Michele Bell for her excellent secretarial assistance. This work was supported in part by NIH CA33802, NIH CA40497, and DE AC-02-76EV02031.

REFERENCES

1. Farrands PA, Pimm MV, Embletom MJ, et al: Radioimmunodetection of human colorectal cancer by an anti-tumour monoclonal antibody. *Lancet* 2:397–400, 1982
2. Larson SM, Brown JP, Wright PW, et al: Imaging of melanoma with I-131 labeled monoclonal antibodies. *J Nucl Med* 24:123–129, 1983
3. Khaw B-A, Matis JA, Melincoff G, et al: Monoclonal antibody to cardiac myosin: Imaging of experimental myocardial infarction. *Hybridoma* 3:11–23, 1984
4. Wahl RL, Parker CW: Monoclonal antibody radioimmunodetection of transplant rejection. *Transplantation* 40:451–454, 1985
5. Wahl RL, Fisher S: Radiolabeled anti-parathyroid hormone antibody for locating hyperplastic parathyroid glands. *Clin Res* 32:769A, 1984
6. Goldenberg DM, DeLand F, Kim E, et al: Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med* 298:1384–1388, 1978
7. Mach J-P, Carrel S, Forni M, et al: Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. *N Eng J Med* 303:5–10, 1980
8. Wahl RL, Philpott G, Parker CW: Monoclonal antibody radioimmunodetection of human-derived colon cancer. *Invest Radiol* 18:58–62, 1983
9. Wahl RL, Parker CW, Philpott GW: Improved radioimaging and tumor localization with monoclonal F(ab')$_2$. *J Nucl Med* 24:316–325, 1983
10. Moldofsky PJ, Powe J, Mulhern CB, et al: Metastatic colon carcinoma detected with radiolabeled F(ab')$_2$ monoclonal antibody fragments. *Radiology* 149:549–555, 1983
11. Goldenberg DM: Tumor imaging with monoclonal antibodies. *J Nucl Med* 24:360–362, 1983
12. DeLand FH, Kim E, Sissons G, et al: Imaging approach in radioimmunodetection. *Cancer Res* 40: 3046–3049, 1980
13. Beihn RM, Damvon JR, Hafner T: Subtraction technique for the detection of subphrenic abscesses using 67Ga and 99mTc. *J Nucl Med* 15:371–373, 1974
14. Halpern S, Dillman R, Witztum K, et al: Radioimmunodetection of melanoma utilizing In-111 96.5 monoclonal antibody: A preliminary report. *Radiology* 155:493–499, 1985
15. Khazaeli MB, Beierwaltes WH, LoBuglio AF: Radioimmunodetection of human embryonal carcinoma in nude mouse model utilizing monoclonal antibody. *Hybridoma* 3:89, 1984
16. Wahl RL, Jackson G, Laino L, et al: Monoclonal antibody radioimmunodetection of occult choriocarcinoma. *J Nucl Med* 26:P676, 1985 (abstr)
17. Weinstein JN, Steller MA, Keenan AM, et al: Monoclonal antibodies in the lymphatics: Selective delivery to lymph node metastases of a solid tumor. *Science* 222:423–433, 1983
18. Wahl RL, Jackson G, Laino L, et al: Improved radioimmunolocalization of human derived tumors by intralymphatic delivery of monoclonal antibodies. *Radiology* 153:316, 1984