Detection of Malignant Melanoma with Iodine-123 Iodoamphetamine

Marvin B. Cohen, Romaine E. Saxton, Ralph R. Lake, Leslie Cagle, L. Stephen Graham, Anne Nizze, Leslie S. Yamada, Manju Gan, Gabriella Bronca, Karen Greenwell, and Donald L. Morton

Research Service and Nuclear Medicine Service, V.A. Medical Center, Sepulveda; Department of Surgery, University of California, Los Angeles, California

Iodoamphetamine (IMP) was shown by in vitro assay to have a high uptake by human melanotic melanoma cells, as compared to amelanotic melanoma cells. Eleven patients with proven malignant melanoma (MM) and 3 normal subjects were imaged at 2–4 hr and 16–24 hr after the i.v. injection 5 mCi (185 MBq) of [^{123}I]IMP. One patient had a recurrent tumor that was subsequently shown to be squamous cell carcinoma. The index lesion was not visualized in the three patients with amelanotic melanomas. The index lesion/lesions were visualized in six of the seven other patients, except for 4/16 nodules in one patient. The seventh patient had a large, necrotic melanotic tumor that was not visualized, but an unsuspected lesion in the iliac nodes was detected. Multiple unsuspected lesions were detected in a second patient. While many lesions were seen at 2–4 hr, all lesions (other than a patient with small bowel disease) were seen best at 16–24 hr. No eye uptake was observed in any patient or control subject. Testicular uptake was seen in all males at 16–24 hr. Iodine-123 IMP appears to be a useful agent for the detection and follow-up of patients with melanotic MM.

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Surgery remains the most effective treatment for malignant melanoma (MM), but it is often followed by tumor recurrence from undetected metastases in various parts of the body. Improved detection of lesions by tumor imaging techniques may improve survival in these patients. A number of radiopharmaceutical agents have been evaluated for the scintigraphic detection of MM including gallium-67 (^{67}Ga) citrate (*1–2*), iodine-131 (^{131}I) iodochloroquine (*3–4*), lead-203 (^{203}Pb) TRIS (*5–6*) and iodine-123 (^{123}I) methyltyrosine (*6*). Iodine-123 N-isopropyl p-iodoamphetamine (IMP) is used primarily for brain imaging and is associated with eye uptake in animals (*7*). Holman et al. (*8*) investigated the relationship of eye uptake of [^{123}I]IMP to melanin production and found slightly increased uptake of IMP in hypopigmented mouse melanoma cells but observed substantially higher uptake in pigmented melanoma cells in vitro. Wada et al. (*9*) have reported detection of clinical MM with [^{123}I]IMP in a single patient. More recently Ichise, Holman, and co-workers (*10*) detected metastatic MM in three of four patients using [^{123}I]IMP. Watanabe (*11*) also detected MM in four of eight patients using [^{123}I]IMP. We previously reported preclinical studies showing uptake of IMP in several human melanotic melanoma lines in cell culture (*12*). We now report successful planar clinical imaging with [^{123}I] IMP of melanotic lesions in a study performed on 11 patients with metastatic MM and three normal subjects.

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For reprints contact: Marvin B. Cohen, Nuclear Medicine Service, V.A. Medical Center, Sepulveda, CA 91343.

METHODS AND MATERIAL

Preclinical IMP uptake studies were performed with cultured human melanoma cell lines including UCLA-SO-M12, M14, RB, RS, and PK derived from surgical specimens of metastatic melanoma tumors. M12 and PK cells were melanotic, low passage cells of less than ten subcultures compared to the other melanoma lines. Controls included a human B lymphoblastoid cell line (LCL), pulmonary and colonic adenocarcinomas (P3 and SW6), and a malignant mesothelioma (MT1). One milligram of IMP (Medi-Physics, Inc., Richmond, CA) was labeled with 1 mCi of iodine-125 (DuPont Company, No. Billerica, MA) and was separated from unreacted ^{125}I by thin layer chromatography (*13,14*). The efficiency was only 10% for labeling this small amount of IMP and the specific activity was 100 μCi/mg. Subconfluent flask cultures of each type of tumor cell were trypsinized, washed in RPMI 1,640 medium, and resuspended at 5×10^5 cell/0.1 ml (hemocytom-

eter counts gave 95% viability by Trypan blue exclusion). All cells in 0.1 ml RPMI were transferred to duplicate 1.2 ml tubes and incubated at 37°C with 0.1 ml of RPMI containing 5×10^5 cpm [^{125}I]IMP for various times up to 24 hr. After incubation the cells were pelleted, washed three times in RPMI, and incorporated label was counted in a well counter (Beckman, Gamma 4000 spectrometer).

Eleven patients with proven MM and late metastases to any region except liver or lung were recruited from the John Wayne Clinic of the Jonsson-UCLA Comprehensive Cancer Center. Imaging studies were performed in the afternoon and the following morning. Patients with liver and lung metastases were excluded because of the high normal uptake of IMP or its labeled metabolites in these organs. The primary purpose of this pilot study was to determine the approximate number of proven index lesions detectable with [^{123}I]IMP. Written informed consent was obtained from these patients and 3 normal subjects (one male and two females). Iodine-123 (p, 5n) devoid of ^{124}I and other high energy nuclides was obtained from Crocker Laboratory, University of California at Davis and was used in our laboratory to exchange label N-isopropyl p-iodoamphetamine (Medi-Physics, Inc., Richmond, CA) by a modification of the method of Baldwin (*13–14*). Quality control procedures were performed as previously described (*14*). The thyroid was blocked by oral SSKI in normal subjects. Patients and subjects received ~1 mg of IMP labeled with 4.8 to 5.2 mCi (177–192 MBq) of ^{123}I. Anterior and posterior whole-body images (Technicare, Solon, OH, S-410) and spot planar images (Siemens, Inc., Iselin, NJ, Rotacamera) were obtained at 2–4 hours and 16–24 hr postinjection. The [^{123}I] IMP imaging studies were compared to the results obtained by clinical evaluation and radiologic examinations, including computed tomographic (CT) and nuclear magnetic resonance imaging. Tissue was obtained surgically in all patients for histologic examination and melanin staining.

RESULTS

A preliminary experiment was designed to test IMP incorporation by pigmented and nonmelanotic melanoma cell lines compared with several controls. Equal numbers of the human tumor cells were first incubated in assay tubes with [^{125}I]IMP, then washed, and the labeling yield quantitated in a gamma spectrometer. The [^{125}I]IMP uptake was 70–80% of maximum after 5 min incubation and reached peak values at 15–20 min in these tumor cells. After 60 min incubation a reduced IMP uptake was observed, which decreased further to only 5–10% of maximum by 24 hr. The results in Figure 1 show in vitro [^{125}I]IMP uptake by five human melanomas and four other cell lines after 20 min incubation time. Labeled IMP uptake was seen in all five melanomas (0.4–17.4% of input) compared with the B lymphoblastoid control cell line (0.1%). Labeled IMP uptake was greatest in the M12 and PK melanotic lines (5–17.4%) compared with the amelanotic melanomas (0.4–1.4%). However, increased [^{125}I] IMP uptake in the colon carcinoma line SW6 (4.1%)

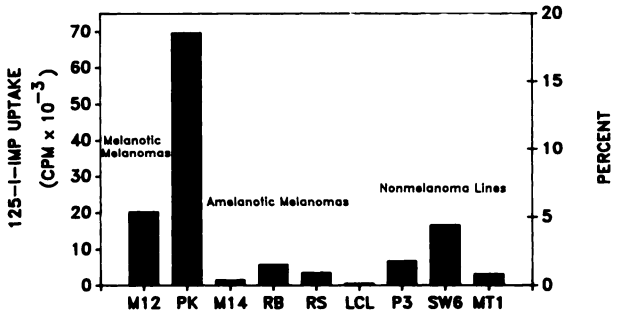

FIGURE 1
Uptake of [^{125}I]IMP by tumor and control cell lines. Five melanoma lines (M12, PK, M14, RB and RS), lymphoblastoid control line (LCL), two carcinoma lines (P3, SW6), and a mesothelioma (MT-1) were tested.

was comparable to M12, indicating that IMP incorporation is not restricted to melanoma cells in vitro.

Encouraged by this high level of [^{125}I]IMP uptake in melanotic melanomas in culture, we next tested clinical uptake of [^{123}I]IMP in three normal subjects and 11 patients referred with a diagnosis of metastatic melanoma. Iodine-123 IMP was rapidly cleared from the blood with uptake predominately in the lungs and with a small amount of uptake in the brain. As the [^{123}I]IMP cleared from the lungs, the liver uptake was increased. Uptake was not seen in the normal spleen. Most of the melanotic index lesions were detected by [^{123}I]IMP imaging as were several previously unknown lesions (Table 1). Amelanotic index lesions were not detected in three patients. The index lesion was not detected in a fourth patient shown later to have pathologic diagnosis of squamous cell carcinoma rather than metastatic MM. Many lesions were detectable on the early images, but were seen best on the delayed images. Eye uptake was not observed in any patient or normal subject. The thyroid was imaged in eight of the 11 patients. The testicles were visualized in all males on the delayed images, but were not seen on the 2–4 hr images. The ovaries were not visualized in any females at either imaging time.

The patient represented by the image shown in Figure 2 had three known index lesions; left axilla, left supraclavicular area, and subcutaneously in the right lower quadrant of the abdomen. These metastases were readily detected, as were several clinically unsuspected lesions in the spleen, left subclavian area and right mediastinum of this patient. The number of discrete lesions in the mediastinum could not be accurately determined in the IMP images and the nodes also appeared to be matted on CT scan, which corresponded to scintigraphic findings. It also was difficult and somewhat arbitrary to enumerate the lesions detected in the patient imaged in Figure 3. The patient had multiple 0.4 to 2.5 cm subcutaneous nodules in the head and neck area. These were sometimes contiguous and diffi-

TABLE 1
Clinical Data and Results of [^{123}I]IMP Imaging for Detection of MM

Patient no.	Age (yr)	Sex	Known lesions	Scan findings	Histology	Comments
1	34	M	L axilla-4 cm L supracl-5½ cm RLQ-abd-2½ cm	All known lesions plus subclav (2), rt mediastinum & spleen	"Moderate-marked pigment"	All lesions confirmed by autopsy
2	42	M	L scapula-2 cm L shin-1½ cm	Normal	Scapula "amelanotic" shin "lightly pigmented"	
3	71	F	L shin-1½ cm R femoral 1½ cm	Both seen on late image. Shin only at 2 hr	Shin "pigmented" femoral "Tan-white"	Femoral faintly visualized
4	31	M	R adrenal 9 × 15 cm	Left iliac	Iliac-"melanotic" adrenal "black" "large" areas of necrosis"	Iliac nodes and adrenal removed surgically
5	62	F	L abdomen (adrenal?) "Large"	Large left abdomenal mass	"Moderately melanotic"	Seen well at 1 hr but best at 24 hr
6	64	M	L-post auricular 6 cm	Normal	Thin needle: "compatible with melanoma" Surgical excision: "squamous CA"	Proven melanoma 5 yr prior
7	51	M	Small bowel multiple	Abnormal bowel left upper quadrant	"Moderate pigmented"	Seen best at 2 hr 30–50 small bowel lesions at surgery
8	43	F	L-neck-2 cm R supraclav-2 cm	Normal	"Amelanotic"	Paratracheal nodes found by CT
9	59	M	16 subcutaneous in head & neck 0.4–2 cm	10 discrete lesions plus matted area	"Moderately pigmented"	
10	42	M	Three peri-aortic nodes 2–4 cm	Normal	"Amelanotic"	
11	61	F	Peri-aortic nodes by CT scan	Two or more peri-aortic nodes	"Melanotic"	Faintly visualized

cult to count as separate lesions. We estimate that 12 of 16 lesions were detected by [^{123}I]IMP imaging in this patient. We also encountered problems in enumerating the number of lesions imaged in a patient with over 30 small bowel metastases found later at surgery (Figure 4). While it could not be definitely determined if all lesions were detected, the IMP images concurred with the CT images as to the extent of the disease. A large abdominal lesion was readily detected in Patient 5, but a similar size large index lesion (adrenal metastasis) was

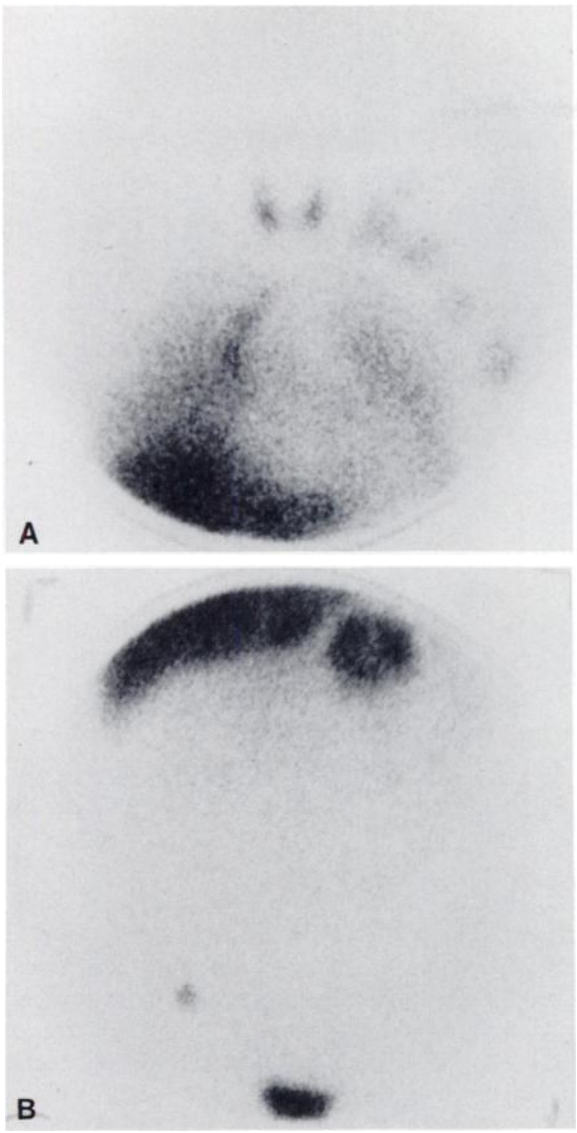

FIGURE 2
Patient 1. A: The normal lungs and the unblocked thyroid are well visualized at 16 hr. Only two of the lesions lateral to the thyroid were known at the time of [^{123}I]IMP imaging. The uptake in the right mediastinum also represented unknown lesions that were later confirmed by CT scan. B: The normal liver and urinary bladder were visualized at 16 hr. The index lesion in the right lower quadrant of the abdomen was detected, as was the unsuspected metastasis to the spleen.

not visualized in Patient 4 imaged in Figure 5. This index lesion was melanotic, but necrotic. However, an unknown area of metastasis was identified with [^{123}I] IMP in the left internal iliac nodes of this same patient. Some tracer activity also was seen in the bladder and the testicles were clearly visualized. We estimate the activity in the testicles in all subjects to be 0.25% of the activity remaining in the body at 24 hr. This is roughly equivalent to 0.4–0.5 rad delivered to the testicles from a 5 mCi (185 MBq) dose of (p,5n) ^{123}I.

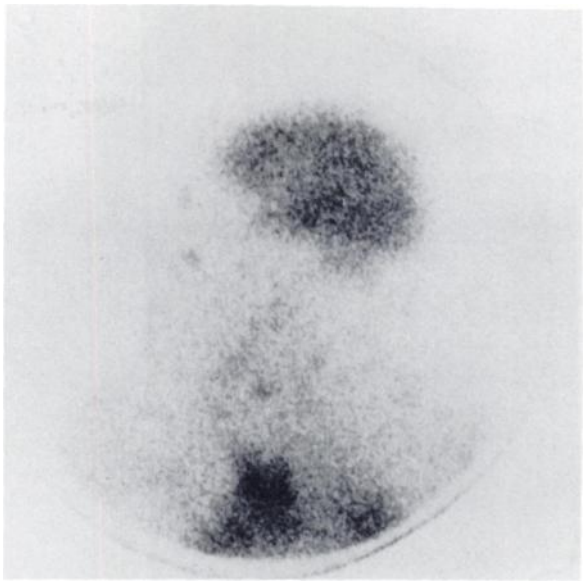

FIGURE 3
Patient 9. Multiple small and medium size subcutaneous nodules are seen. Many of these lesions tended to coalesce with adjacent lesions, which made it difficult to count the number of lesions directly on the scintigram.

DISCUSSION

Holman et al. (*8*) reported a positive correlation between increased IMP uptake and active production of melanin. These authors demonstrated significantly higher IMP uptake in pigmented mouse melanoma cells compared with amelanotic melanoma cells in culture. The uptake of IMP in the eye of black mice compared to albino mice was 18:1 at 2 hr and increased to 36:1 at 24 hr (*8*). B-16 mouse melanoma also has been detected scintigraphically in mice with [^{123}I]IMP by Watanabe (*11*). In addition, they detected nonpigmented Lewis lung tumors in mice, but three other tumor types were not visualized (*11*). We recently confirmed the high level of IMP uptake by two human melanotic malignant melanoma cell lines in cultures (*12*). The PK melanoma cell line established in culture from a highly melanotic tumor exhibited by far the strongest IMP uptake in our study. We also observed increased uptake of IMP in several human carcinoma cell lines in culture, but this also was seen in cultured amelanotic melanoma cell lines (Fig. 1). Amelanotic index lesions were not visualized in three of our patients.

Wada (*9*) and Ichise (*10*) reported successful imaging of melanoma patients 2 hr after the i.v. injection of 3–5 mCi of [^{123}I]IMP. Watanabe (*11*), on the other hand, imaged at 24 hr postinjection, but employed only 0.5–1 mCi of [^{123}I]IMP and detected MM in four of eight patients. Because histology was not reported in that study, it is not clear if the relatively low MM detection rate resulted from the low radionuclide scan dose, a

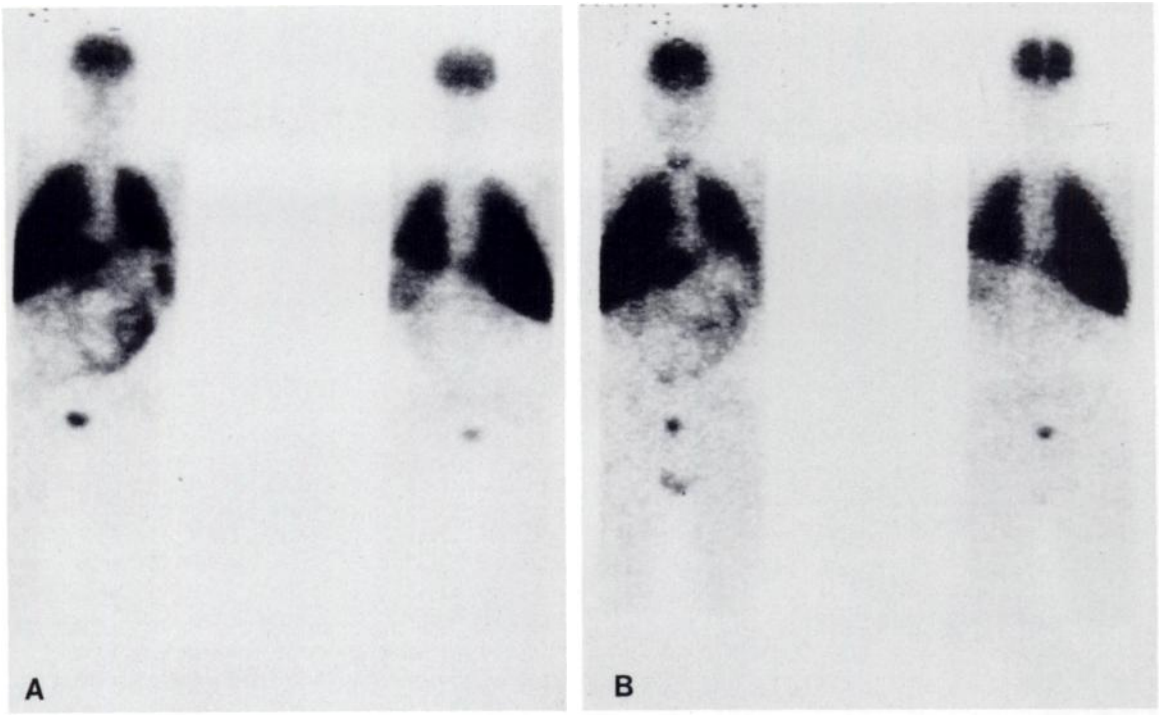

FIGURE 4
Patient 7. A: anterior (left) and posterior whole-body images at 2 hr. High uptake of tracer is seen in the bowel. B: Same patient at 16 hr, bowel uptake is again noted, but appears less prominent than on the early view. The testicles are seen on the late image.

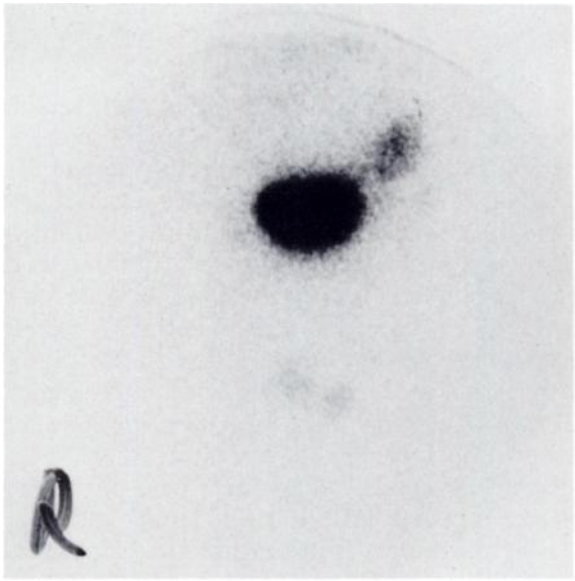

FIGURE 5
Patient 4. 9 × 15 cm index lesion not visualized, but unsuspected MM detected in lymph nodes around the left internal iliac vessels. The testicles are clearly visualized and there is residual activity in the bladder.

disproportionate number of amelanotic lesions, or a combination of both factors.

Lecouffe et al. (*15*) used IMP to image patients with benign and malignant skin lesions, ocular melanomas and metastatic melanoma, but did not find [^{123}I]IMP imaging to be useful in the management of their patients. However, the histology, melanin content and lesion size were not reported. Liewendahl (*16*) recently reported complete details on three MM patients imaged with both [^{123}I]IMP and technetium-99m monoclonal antibody fragments. A large melanotic metastasis in the neck was detected with both agents in one patient. No lesions were detected with either agent in another patient with more than 50 amelanotic subcutaneous nodules plus many internal metastases. Metastases to both thighs were detected with both agents in the third patient, even though the lesions were reported to be amelanotic. Patient 3 in our study had a melanotic metastasis in one area and an amelanotic metastatic lesion in the femoral region that was described as "Tan-white". Both were detected on the delayed image (Table 1).

The overall percentage of MM lesions detected by IMP imaging in our study was difficult to define accurately because of coalescence of skin lesions, matting of lymph nodes and the limited spatial resolution of planar imaging. The matted lymph nodes demonstrated by IMP images corresponded to the findings on CT scan in Patients 1, 7, and 11. Most of the lesions were detected with IMP in Patient 9, but four were not visualized. Detection of liver and lung metastases also may be difficult due to the high activity in these organs. For this reason patients with suspected lung or liver

MM metastases were excluded from our initial study. Use of ^{123}I devoid of ^{124}I and other longer lived and high-energy isotopes decreases background and facilitates imaging, especially on delayed images. Although many lesions were apparent by 2–4 hr after IMP injection, most MM lesions were more readily detected on the 16–24 hr image. One notable exception was a MM patient with multiple small bowel lesions detected by both the early and late images, but seen best on the early images (Fig. 4). However, Wada (*9*) noted the presence of IMP in normal bowel in his patient at 2 hr. In agreement with this finding, we also observed normal bowel uptake on the early planar images in 3/10 patients and 2/3 normal subjects. On the other hand, only faint bowel uptake was seen 1/10 patients and 0/3 normal subjects at 16–24 hr. Patient 7 in our study had multiple small bowel lesions and significant bowel uptake on both the early and late images. Both the MM patients and normal subjects were on an ad lib diet in this pilot study. The nonspecific uptake of IMP in the bowel apparently does occur and may interfere with the scintigraphic detection of tumor. The observation of normal bowel uptake of IMP is not fully understood and deserves further study.

Although surgery remains the treatment of choice in melanoma, successful surgical resection requires both early detection and precise localization of metastatic lesions. Recent studies suggest that patients with stage I MM have detectable levels of circulating tumor marker antigens (*17*). A study of the localization of MM using [^{123}I]IMP in patients with a positive blood test for circulating marker antigens to MM and a history of a melanotic primary lesion has not been performed. IMP imaging does not appear to be of clinical value in patients with amelanotic lesions. Imaging with [^{67}Ga] citrate or labeled monoclonal antibodies may be more useful in these patients (*2,6,18–21*). However, ^{67}Ga is not specific for melanoma, is prone to false-positive and false-negative images, but is able to equally detect melanotic and amelanotic MM (*6,10,19*). Liewendahl (*16*) recently compared [^{123}I]IMP with labeled monoclonal antibody in three patients for the detection of MM and reported that the antibody was superior. Clearly a larger study is needed to assess the clinical value of these two modalities. Nevertheless, the results of the present study provide evidence that Iodoamphetamine represents a clinically useful imaging agent for the detection of melanotic malignant melanoma.

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