Radioimmunodetection in Patients with Suspected Ovarian Cancer

N. Pateisky, K. Philipp, W. D. Skodler, K. Czerwenka, G. Hamilton, and J. Burchell

First Department of Obstetrics and Gynecology and First Department of Surgery, University of Vienna, Austria; and Imperial Cancer Research Fund, Lincoln's Inn Fields, London, England

Twenty-five patients, having either unilateral ovarian tumors of unknown etiology or suspected of having ovarian cancer recurrence were investigated by the method of immunoscintigraphy to rule out primary and/or metastatic tumor sites. Four-hundred micrograms of the tumor-associated monoclonal mouse antibody HMFG-2, raised against human milk fat globulin membranes and labeled with ^{123}I, were used for each patient to display the tumor sites by external scintigraphy. The dose ranged between 0.5 and 2.2 mCi, the specific activity between 1.25 and 5.5 mCi per mg of antibody. Nineteen of the patients underwent operations a few days after immunoscintigraphy. The remaining six patients were investigated by transmission computed tomography (TCT) to establish the presence or absence of tumor of the imaging. In 22 of the 25 cases the scintigraphic results correlated with the situation found at the subsequent operation, or by TCT, respectively, as well as with the histological diagnosis of the tumor type. Overall, there were just two false-negative and one false-positive scan report, the latter due to faulty reading of the scintigrams. Sixteen out of 18 tumor sites in 25 patients could be revealed by immunoscintigraphy, the smallest one being 1.5 cm in diam. In four of the patients immunoscintigraphy was the only noninvasive investigation method that could reveal the malignant tumor sites prior to the operation.

J Nucl Med 26:1369–1376, 1985

Malignant ovarian tumors provide one of the most frequent problems in gynecologic oncology. This is reflected by the fact that more than 60% of the patients with epithelial cancer of the ovary are diagnosed with advanced disease (*1*). Difficulties in the diagnosis, therapy control, and follow-up of ovarian cancer, results in a very poor prognosis for this disease. Since the first trials of tissue detection by external scintigraphy using radiolabeled antibodies (*2*) there has been hope of early cancer diagnosis by this noninvasive approach. Previous studies by different authors, mainly using polyclonal antibodies labeled by iodine-131 (^{131}I) demonstrated successfully the imaging of different cancers (*3–8*). The development of the hybridoma technique in 1975 (*9*) allows the production of antibodies with higher specificity against a given antigen. Monoclonal antibodies generated in this way should lead to an improvement of specificity in immunoscintigraphy of malignant lesions (*10,11*). The advantage of tumor-associated monoclonal antibodies over polyclonal antisera has been suggested in some model systems used in studying targeting of antibodies to human tumors (*12,13*). In this report we present our recent clinical experience with the method of immunoscintigraphy using ^{123}I-labeled tumor-associated monoclonal antibodies in patients with ovarian cancer.

Received Dec. 17, 1984; revision accepted Aug. 22, 1985.

For reprints contact: Norbert Pateisky, MD, First Department of Obstetrics and Gynecology, University of Vienna, Spitalgasse 23, A-1090 Vienna, Austria.

PATIENTS AND METHODS

Twenty-five patients between ages 32 and 74 yr entered our study after giving informed consent. Thirteen had a known history of carcinoma and underwent surgery as well as chemotherapy before their radioimmunoscintigraphy (RIS). The other 12 patients were hospitalized with suspicion of having uni- or bilateral ovarian tumors of unknown tumor type. At the time of clinical examination, malignancy was often suspected. Nineteen of the 25 patients studied underwent surgery a few days after the imaging procedure. The reports of the six remaining patients were compared with the findings of transmission computed tomography (TCT).

The suspicion of malignancy in the 12 patients with the ovarian tumors of unknown etiology was given by the patients' history, the clinical examination, serum tumor marker determination, ultrasound, and sometimes TCT.

ANTIBODY

The mouse monoclonal antibody HMFG-2*, raised against a component of the human milk fat globule membranes (*12*) reacts strongly with the lactating breast as well as with some other neoplasms of epithelial origin, such as adenocarcinoma of the ovary (*14*). After production in bulk by culturing hybridomas, the immunoglobulins were purified from culture supernatant by affinity chromatography using protein A coupled to Sepharose CL-4B+. The culture supernatant was applied to the column at pH 8.0 and the immunoglobulin separated from fetal calf serum components by sequential elution with 0.1 citrate buffer of decreasing pH. The antibody was then Millipore filtered into sterile ampules. Ten percent of the samples were tested for pyrogenicity (Pyrogent-Test), sterility, and toxicity before iodination and administration to the patients. More data about the HMFG-2 antibody are given in detail elsewhere (*15*).

IODINATION AND QUALITY CONTROL OF ANTIBODIES

Four-hundred micrograms of the purified and Millipore-filtered HMFG-2 antibody were labeled with ^{123}I by the Iodogen-method, using 1,3,4,6-tetrachloro-3a, 6a-diphenyl glycouril as iodogen (*16*). Before removing the free ^{123}I by a Sephadex-50 column, the labeling efficiency—always being ~70%—was determined by paper chromatography. The radioantibody solution thus prepared was then Millipore filtered again into sterile ampules. The range of specific activity was between 1.25 and 5.5 mCi per mg of antibody. The reactivity of the antibody preparation was tested before and after the iodination in an ELISA with whole T47D-cells acting as target cells. The antibodies were tested at sample concentrations of 10 μg/ml and dilutions up to 1/64 in quadriplicate. The range of immunoreactivity was between 70 and 95%.

APPLICATION AND IMAGING TECHNIQUE

Before imaging, the patients were skin-tested for hypersensitivity against mouse immunoglobulins of the IgG_1 subgroup by 10 μg of the antibody to be used later. The test was judged to be negative if there were no reactions of the skin (reddening and swelling) up to 15 min after application. Thyroid uptake of free and released ^{123}I was blocked by potassium iodine, 120 mg/day orally, starting 24 hr before the injection and continuing for 3 days. The radiolabeled antibody was given intravenously in a dose between 0.5 and 2.2 mCi dissolved in phosphate-buffered saline (PBS) containing 1% human serum albumin (HSA). To avoid false-positive findings due to an accumulation of radioactivity in the bladder, each patient received a permanent catheter before imaging. Four points on the patients' skin (navel, symphysis, ant. and sup. iliac spines) were marked by cobalt-57 sources to achieve improved location of the detected lesions. After the i.v. injection of the labeled antibody (400 μg in 5 ml PBS containing 1% HSA as a bolus injection), four static scintigrams were made at predetermined intervals (immediately, 4, 8, and 24 hr after dose). The images were recorded with a gamma camera‡ fitted with a high-resolution, low-energy collimator. The camera was linked to a computer.§ Anterior images were obtained from the abdomen, each accumulating 300,000 counts to make them comparable. The imaging time at 24 hr after application of the radioantibody ranged between 30 and 45 min. The highest tumor-to-nontumor ratio was usually achieved between 8 and 12 hr after antibody application. No subtraction technique was used to enhance image contrast. Scans from the side of the patients were performed in doubtful cases, to improve the localization of the detected lesions.

SCAN INTERPRETATION

The appearance of isolated hot spots (indicating a circumscript lesion) or diffuse activity accumulation (indicating widespread disease) within 24 hr after the i.v. application of the radioantibody were suggested to be a malignant lesion if the activity deposits remained through the end of the scanning procedure.

Image interpretation was performed without the knowledge of the other investigation results such as TCT, ultrasound, clinical investigation, serum tumor marker, etc. If the patient underwent surgery, the scan reports were additionally compared with the operation sites as well as with the histology report of the removed surgical biopsies.

RESULTS

All 25 imaging series were judged satisfactory from the technical point of view. Hypersensitive, allergic, or other reactions were not seen in any of the patients, either with skin testing before the i.v. dose of radioantibody or during the imaging procedure. Eighteen test results were considered positive; of these, 17 were true positives, with one false-positive. Of seven negative test results, five were true negatives, with two false-negatives. Seventeen of the 18 positive scintigrams correlated in detail with the findings at operation or by TCT. In each of these positive scans, the tumor-to-nontumor contrast was so high, that there was no need for any subtraction technique to enhance the image contrast. The one false-positive report (Case 12, Table 1) was due to faulty interpretation.

TABLE 1
Survey of Twenty-five Investigated Patients

Patient no.	Age	Clinical diagnosis	Histological diagnosis	RIS	Correlation OP	Correlation CT	Stage at primary diagnosis (FIGO)
1	58	Krukenberg tumor	Krukenberg tumor after stomach carcinoma	pos	Correct		—
2	68	Ovarian carcinoma	Undifferentiated ovarian carcinoma	pos	Correct		IV
3	44	Ovarian carcinoma	Low differentiated ovarian carcinoma	pos	Correct		III
4	64	Ovarian carcinoma	Undifferentiated ovarian carcinoma	pos	—	Correct	III
5	73	Ovarian tumor	Colonic carcinoma	pos	Correct		—
6	61	Ovarian tumor	Colonic carcinoma	pos	Correct		—
7	72	Sec. ovarian carcinoma, st. p. breast carcinoma	No tumor	neg	—	Correct	—
8	69	Recurrence of ovarian carcinoma	Adenocarcinoma of the ovary	neg	False		III
9	50	Ovarian tumor	Brenner tumor	pos	Correct		I
10	62	Recurrence of ovarian carcinoma	Cystadenocarcinoma of the ovary	pos	Correct		III
11	63	Recurrence of ovarian carcinoma	—	pos	—	Correct	III
12	32	St. p. ovarian ca. sec. look op.	No tumor	pos	False		IIb
13	64	Ovarian carcinoma	Differentiated ovarian carcinoma	pos	—	Correct	III
14	53	St. p. ovarian carcinoma	No tumor	neg	Correct		III
15	69	Ovarian tumor	Serous cystadeno-ca	pos	Correct		IIb
16	69	Ovarian tumor	Anaplastic ovarian carcinoma	pos	Correct		III
17	74	Ovarian tumor	Ben. ovarian tumor adenoma	neg	Correct		—
18	46	Sec. ovarian carcinoma, st. p. breast carcinoma	No tumor	neg	—	Correct	—
19	60	Ovarian tumor	Mucinous cystadenocarcinoma of the ovary	pos	Correct		Ia
20	71	St. p. granulosa cell tumor (op)	Granulosacelltumor	neg	(False)		III
21	56	Recurrence of ovarian carcinoma	Cystadenocarcinoma of the ovary (serous)	pos	Correct		III
22	69	Ovarian tumor	Adenocarcinoma of the ovary	pos	Correct		IV
23	72	Ovarian tumor	Serous cystadenocarcinoma of the ovary	pos	Correct		Ia
24	47	Ovarian tumor	Serous cystadenocarcinoma of the ovary	pos	Correct		III
25	53	Recurrence of ovarian carcinoma	Undifferentiated ovarian carcinoma	pos	—	Correct	III

One of the two false-negative findings was due to a small lesion (about 1.5 cm), attached to the pelvic wall, which microscopy revealed to be a mixture of scar tissue and some nests of malignant cells. It could not be determined whether the lesion represented a residue of the primary treatment (operation, irradiation, and chemotherapy) or recurrent disease. Direct binding tests of the histological sections were not performed in this study.

Table 1 summarizes some of the patients' data and the scintigraphic results, compared with the histological findings, the operation sites or the TCT report when an operation was not done. Five out of the 25 patients (9, 15, 16, 19, 23) suffered from an unilateral ovarian cancer. The contralateral healthy ovaries of these patients (confirmed by histology) failed to show any accumulation of radioactivity in the immunoscans. In Patient 4, with a negative image, no malignant tissue could be found at the following operation, which was carried out because of an ileus. A benign tumor in Case 17 also failed to accumulate antibody. The most interesting result was given by Case 20. As the HMFG-2 antibody is reported to be tumor-associated only for carcinomas (*12*), and it failed to detect

TABLE 2
Correlation of Surgical Findings and Other Imaging Procedures as Well as Clinical Examination

Patient no.	Operation		RIS		CT		US		Clin. ex.	
	a*	b†	a	b	a	b	a	b	a	b
1	1	+	1	+	?	–	0	–	0	–
2	2	+	2	+	2	+	1	+	?	+
3	2	–	2	–	0	–	0	–	0	–
5	1	–	1	–	1	–	?	–	1	–
6	1	–	1	–					1	–
8	1	–	0	–	?	–	0	–	1	–
9	1	–	1	–			1	–	1	–
10	2	+	2	+	1	–	?	?	?	?
12	0	–	1	–	0	–	0	–	0	–
14	0	–	0	–	0	–	?	–	0	–
15	1	–	1	–			1	–	1	–
16	1	+	1	–			1	–	1	–
17	0	–	0	–			1	–	1	–
19	1	–	1	–			1	–	1	–
20	1	+	0	–	1	+	1	–	?	+
21	1	–	1	–	?	–	?	–	0	–
22	1	+	1	+			1	–	1	–
23	1	–	1	–			1	–	1	–
24	1	+	1	+	1	–	1	–	1	–

*Indicates number of *malignant* tumor sites in columns operation, RIS, and CT and just number of tumor sites without judgment of dignity in columns US and Clin ex.

†Indicates suggestion of disseminated malignant disease.

the malignant germ-cell tumor of this patient. The tumor tissue by itself was not investigated concerning the absolute content of the HMFG-antigen.

Table 2 provides more details concerning the number of tumor sites in the 19 surgical patients. Whereas the RIS method and TCT permit a judgement of the tumor type, ultrasound and vaginal palpation were used only to screen for evidence of tumor sites without a judgement regarding malignancy, since the specificity of these methods is low. In eight patients with recurrent malignant disease (1, 3, 8, 12, 14, 20, 21, 24) all three imaging methods were used. Out of seven malignant lesions in these patients (confirmed by histologic report on the surgical specimens) five could be located and identified concerning their etiology by RIS, two by TCT and two were suggested by ultrasound. The lesion size ranged between 0.5 to 12 cm in diameter. The smallest malignant lesion we detected by the RIS method had a diameter of about 1.5 cm.

Overall, 17 of the 19 malignant tumor sites found in the 19 surgical patients were detected and classified by RIS (Table 3), yielding a sensitivity of 90%. The second column of Table 3 provides information about the suggested metastatic involvement of the abdominal cavity and the situation at the operation. Multiple intraoperative biopsies from different sites in the abdominal cavity were done in just five of the 19 surgical patients. The histologic report of the biopsies always confirmed the scan results.

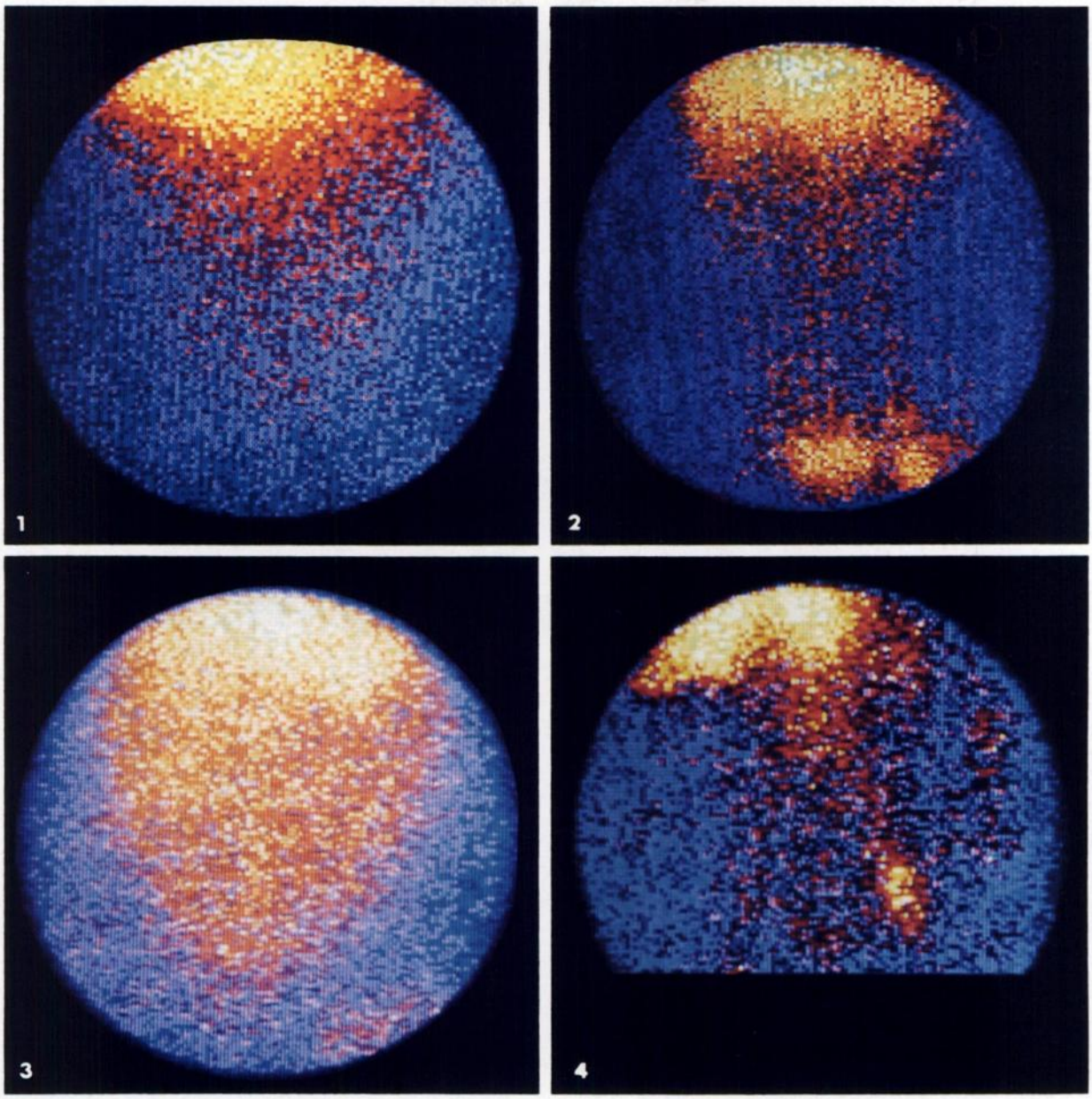
Figures 1, 2, and 3 reflect typical scan results. Figure 1 (Case 14) shows a true-negative scan of a patient without any microscopic or macroscopic malignant tumor sites. A urinary catheter prevents the buildup of the bladder activates, due to the free radioactive iodine excreted by the kidney. In Fig. 2, (Case 3) two clearly visualized hot spots in the region of the lesser pelvis. The subsequent operation removed two malignant tumor masses from exactly the indicated position. Figure 3 shows diffuse uptake in a patient (Case 2) bearing an inoperable ovarian cancer.

The tumor-to-nontumor count density ratios obtained from the 24-hr images of the patients with positive results ranged between 1.44 and 2.81. For a nontumor region we always chose an area in a part of the apparently tumor-free abdomen containing the same number of pixels as in the malignant tumor site. The counts obtained in the surgical tumor specimens of 11 patients, recorded in a well scintillation counter, were 3.5 to nine-fold higher than that in the respective adjacent healthy tissue (fat or muscle), but there was no clear correlation with the count density ratios. Because of the short half-life of ^{123}I, we were not able to measure radioactivity in all the surgically removed specimens.

The average uptake of ^{123}I-labeled HMFG-2 antibodies

TABLE 3
Imaging Results According to Tumor Sites

No. of cases	Prim. diagnosis	Prim. site	Sec. site	Total	Percent
n = 16	Ov. cancer	8/8	6/8	14/16	88%
n = 2	Colon-ca	2/2	—/—	2/2	100%
n = 1	Stomach-ca	—/—	1/1	1/1	100%
n = 19				17/19	90%



FIGURES 1–4

Figure 1: True-negative abdominal scan accumulated to 300,000 counts, showing no activity-accumulation except unspecific uptake by liver and spleen and some excretion by stomach mucosa. Notice absence of any activity in bladder region (lower part of scan). Figure 2: dominal scan accumulated to 300,000 counts showing two hot spots in region of true pelvis according to specific antibody accumulation in Case 3. Figure 3: Scan shows diffuse distribution of activity over whole peritoneal cavity in patient with inoperable ovarian carcinoma stage III (Case 2). Figure 4: Image reflects false-positive scan of Case 12. Lower part of scan shows activity accumulation in left pelvis region. Details are given in text

in ovarian carcinomas was determined and published by Epentetos et al. (*14*). The average value was ~0.6% of the injected amount. In our patients, 60% of the radioactivity was excreted by the kidneys into the bladder in the first 24 hr and appeared there as free ^{123}I. This was shown by paper chromatography of the urine samples.

In only three of the 25 patients studied did our findings and the surgical report or TCT fail to correlate. Two out of the 25 patients (7 and 18), who were not operated upon, had negative findings in both RIS and TCT. The value of the correlation in these two cases was slightly reduced because of the low TCT sensitivity in the assessment of ovarian cancer (17).

In Case 12 the reason for the false-positive finding was given by a moderate dysfunction of the left prevesical ureter, which had to be mobilized during the primary operation to remove an ovarian cancer stage III, and its lymphatic nodes completely. One year after the radical

operation and chemotherapy, the patient was immunoscintigraphed before the planned second look operation. On the scan (Fig. 4), a moderate prevesical dilation of the left ureter confirmed at the operation, mimicked specific activity accumulation due to a malignant lesion in the left lower abdomen. Additionally, investigation of this patient by single photon emission computed tomography (SPECT) (*8*), as we are now doing, would have probably helped to avoid the false interpretation of the planar scans in this case.

The false-negative scan in Case 8 was suggested to be due to the very tiny amount of tumor tissue. An additional reason may be poor delivery of the antibody to the available binding sites because of reduced tumor blood supply caused by embedding in scar tissue after the first operation. This as well as a number of other factors like density of binding sites, size, vascularity, or localization are important properties of the tumor, that may influence the imaging quality (*18*).

The false-negative results in Case 20 has to be judged from a special point of view. On one hand this germ cell tumor was a malignant lesion, but on the other hand it was not a carcinoma. Therefore it was not unexpected that there was no antibody-accumulation in this tumor.

DISCUSSION

The RIS method is described for various carcinomas, both in human and in animal, by a number of authors (*4–7,19–22*), the aim of our study was to confirm our initial results (*23*) and to present its clinical value in the diagnosis, therapy control and follow-up of patients with ovarian cancer. Information gained from patients with suspected current or recurrent ovarian cancer showed this noninvasive method to be of considerable value. In this disease, the percentage of tumor sites detected by other methods can easily be increased by findings of RIS, although we do need further information about each of the antibodies currently available. At the moment it seems that RIS is more favorable in revealing recurrent disease in patients with a known history of ovarian cancer than for the initial diagnosis. Perhaps there is a future role in locating the site of a supposed carcinoma after recognition of elevated blood levels of tumor-associated antigens, such as CA 12-5, by serum assays (*24*).

Although the aim of our study was to establish the method of RIS with an ^{123}I-labeled monoclonal tumor associated antibody (namely HMFG-2), and we therefore scanned almost all patients with obvious carcinomas at least in the very beginning of the study, it was surprising to find four patients (Nos. 3, 10, 11, 12) of the 25 where RIS could detect sites of lesions that were not found by the other routine methods for diagnosis and follow-up of ovarian cancer. In all four cases further therapy could be initiated at an optimal time. In Cases 3, 10, and 21, a second-look operation was performed, whereas Patient 11 underwent irradiation therapy.

In the past few years, most of the tumor-associated antibodies have been produced by the hybridoma technique (*9*). With this method it is possible to produce monoclonal antibodies of higher specificity than the polyclonals (*11,25*). This makes them favorable for immunoscintigraphy and helps to avoid false-positive results due to possible cross reactions. Another advantage of the monoclonal HMFG-2 antibody is that there is very little shedding of the HMFG-antigen into the blood stream. Therefore, there are very few circulating radioactive antibody-antigen complexes after the administration of the radioantibody, thus improving tumor-to-background ratio.

In the beginning of in vivo-RIS ^{131}I was usually used as radiolabel (*3,19,26–28*). The unfavorable energy-peak of the gamma rays limited the quality of the performed scans apart from the disadvantages of a long physical half-life and the beta-emission. The introduction of other radiotracers like ^{123}I or ^{111}In (*29,30*) in the method of RIS promises to overcome some of the problems caused by the use of ^{131}I. The good experience of other authors (*14,31,32*), the reduction of the radiation dose for the patients as well as the high photon yield, and therefore good imaging quality, encouraged the use of ^{123}I in our studies. Moreover, photons of this energy provide satisfactory tissue penetration, but with energy low enough to be easily collimated. A slight disadvantage is given by the short physical half-life of 13 hr in combination with the high excretion of the iodine ($\sim$ 60% in 24 hr) through the urine. Because of this the last scans were always performed not later than 24 hr after the i.v. application of the radioantibody. Another major advantage of using ^{123}I is given by the possibility to perform SPECT investigations (*18*) with good quality because of the high count rate.

Subtraction techniques are often used to reduce background activity, which is caused by the presence of labeled antibody and free radioactivity outside the target-tissue (*33*). The disadvantage of this method is the possibility of false-positive scans because of artifacts.

Using a second, cold antibody, directed against the hot one is another approach to lessen background activity and improve the tumor detection rate (*34*). Both the use of subtraction and second-antibody technique indicate that circulating antigen may sometimes prevent successful tumor imaging by RIS without the employment of additional methods to improve the image contrast.

However, we are convinced that the use of an antibody directed against an antigen, without remarkable blood levels like the HMFG-antigen, may contribute to a successful tumor imaging. Moreover, when the region of interest is the middle or lower abdomen, the low background advantage, together with the absence of major blood pools, make image contrast adequate without the use of subtraction methods to improve tumor-to-nontumor count density ratios.

The bladder, where the free radioactive iodine is excreted by the kidneys, is often referred to as a problem

region in RIS of the abdomen. It therefore seems important that an indwelling urinary catheter with a sodium chloride lavage during the scanning time helps to avoid any activity accumulation in the bladder, which is of major importance in patients with suspected ovarian carcinoma.

CONCLUSIONS

The clinical results of RIS in the management of ovarian cancer, as achieved by us to date, are much better than we ever expected them to be. Although there are a number of open questions, mainly concerning specificity and sensitivity of the antibody used, RIS has already shown remarkable practical value in the management of ovarian cancer. Further investigations, using tumor-associated and nonspecific antibodies simultaneously, are now being planned, to improve the method's value and to enlarge our knowlege about the in vivo behavior of the antibodies we used. This, as well as the assessment of different routes of administration (intravenous, subcutaneous, intraperitoneal) appropriate to the investigated areas, promises major progress in value for radioimmunoscintigraphy.

FOOTNOTES

*Producted and supplied to us by the Imperial Cancer Research Fund Laboratories, London.

†Pharmacia, Inc., Piscataway, NJ.

‡Picker Dyna Camera 4/15, Picker Intl, Northfort, CT.

§Digital PDP11, Digital Equipment Corp., Maynard, MA.

REFERENCES

1. Katz ME, Schwartz PE, Kopp DS, et al: Epithelial carcinoma of the ovaries:Current strategies. *Ann Intern Med* 95:98-111, 1981
2. Pressman D, Keighley G: The zone of activity of antibodies as determined by the use of radioactive tracers; the zone of activity of nephrotoxic anti-kidney serum. *J Immunol* 59:141-146, 1948
3. Goldenberg DM, DeLand FH: History and status of tumor imaging with radio-labeled antibodies. *J Biol Resp Modif* 1:121-136, 1982
4. DeLand FH, Kim EE, Casper S, et al: Radioimmunodetection of occult recurrent colonic carcinoma. *Am J Roentgenol* 138:145-148, 1982
5. Larson SM, Carrasquillo JAK, Kroku KA: Localization of I-131 labeled p 97-specific Fab fragments in human melanoma as a basis for radio-therapy. *J Clin Invest* 72:2101-2114, 1983
6. Belitsky PH, Ghose T, Aquino R, et al: Radionuclide imaging of primary renal-cell carcinoma by I-131-labeled antitumor antibody. *J Nucl Med* 19:427-430, 1978
7. Begent RHI, Stanuris G, Jones BE, et al: Radioimmunolocalisation of tumours by external scintigraphy after the administration of I-131 antibody to human chorionic gonadotropin: Preliminary communication. *J R Soc Med* 73:624-630, 1980
8. Berche C, Mach JP, Lumbroso A, et al: Tomoscintigraphy for detection of gastrointestinal and medullary thyroid cancers: First clinical results using radiolabeled monoclonal antibodies against CEA. *Br Med J* 285:1447-1451, 1982
9. Köhler G, Milstein C: Continous cultures of fused cells secreting antibody of predefined specifity. *Nature* 256:495-497, 1975
10. Manson DV, Williams AF: The kinetics of antibody binding to membrane antigen in solution and at the cell surface. *Biochem J* 187:1-20, 1980
11. Moshakis V, McIlkinney RAJ, Neville AM: Cellular distribution of monoclonal antibody in human tumours after i.v. administration. *Br J Cancer* 44:663-669, 1981
12. Taylor-Papadimitriou J, Peterson JA, Arklie J, et al: Monoclonal antibodies to epithelium-specific components of the human milk fat globuline membrane: Production and reaction with cells in culture. *Int J Cancer* 28:17-21, 1981
13. Kis M, McIlkinney V, Raghavam R, et al: Monoclonal antibodies to detect human tumors: An experimental approach. *J Clin Pathol* 34:314-320, 1981
14. Epenetos AA, Granovska M, Britton K, et al: Targeting of Iodine-123-labeled tumor-associated monoclonal antibodies to ovarian, breast and gastrointestinal tumours. *Lancet* ii:999-1004, 1982
15. Burchell J, Durbin H, Taylor-Papadimitriou J: Binding of monoclonal antibodies HMFG-1 and HMFG-2 to human breast cells. *J Immunol* 131:508-513, 1983
16. Miller WT, Smith JFG: Protein iodination using iodogen. *Int J Appl Radiat Isot* 34:639-641, 1983
17. Goldhirsch A, Triller JK, Greiner R, et al: Computed tomography prior to second-look operation in advanced ovarian cancer. *Obstet Gynecol* 62:630-634, 1983
18. Rankin EM, McVie JG: Radioimmunodetection of cancer: Problems and potential. *Br Med J* 287:1402-1404, 1983
19. Mach JP, Carrel S, Forni M, et al: Tumor localization of radiolabeled antibodies against CEA antigen in patients with carcinoma. *N Engl J Med* 303:5-10, 1980
20. DeLand FH, Goldenberg DM: In vivo radioimmunological lympho-scintigraphy in cancer. *J Can Assoc Radiol* 33:4-9, 1982
21. Goldenberg DM, Kim EE, Bennet SJ, et al: CEA radioimmunodetection in the evaluation of colorectal cancer and in the detection of occult neoplasms. *Gastroenterol* 84:524-532, 1983
22. Weinstein JN, Steller MA, Keenan AM, et al: Monoclonal antibodies in the lymphatics: Selective delivery to lymph node metastases of a solid tumor. *Science* 222:423-426, 1983
23. Pateisky N, Philipp K, Skodler WD, et al: First results in radioimmunodetection of malignant ovarian tumors using I-123 labeled monoclonal antibodies. *Cancer Detect Prevent* 6:625-631, 1983
24. Bast RC, Jr: A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med* 309:883-887, 1983
25. Ballou B, Levine G, Hakala TR, et al: Tumor location detected with radioactively labeled monoclonal antibody and external scintigraphy. *Science* 206:844-847, 1979
26. Hoffer PB, Beckermann C, Fang S, et al: Use of I-131-CEA antibody as a tumor scanning agent. *J Nucl Med* 15:323-327, 1974
27. Goldenberg DM, DeLand FH, Kim E, et al: Use of radiolabeled antibodies to CEA antigen for the detection and localization of diverse cancers by external photoscanning.

N Engl J Med 298:1384–1388, 1978
28. Goldenberg DM, Kim EE, DeLand FH, et al: Clinical radioimmunodetection of cancer with radioactive antibodies to human chorionic gonadotropin. *Science* 208:1284–1286, 1980
29. Hnatowich DJ, Layne WW, Childs RL, et al: Radioactive labeling of antibody: A simple and efficient method. *Science* 220:613–615, 1983
30. Fawwaz RA, Wang TST, Estabrook A, et al: Immunoreactivity and biodistribution of indium-111-labeled monoclonal antibody to human high molecular weight-melanoma associated antigen. *J Nucl Med* 26:488–492, 1985
31. Granovska M, Britton KE, Sheperd J: The detection of ovarian cancer using I-123 monoclonal antibody. *Radiobiol Radiother* 25:153–160, 1984
32. Davies JO, Davies ER, Howe K, et al: Radionuclide imaging of ovarian tumours with I-123-labeled monoclonal antibody (NDOG 2) directed against placental alkaline phosphatase. *Br J Obstet Gynecol* 92:277–286, 1985
33. DeLand FH, Kim EE, Goldenberg DM: Imaging approach in radioimmunodetection. *Cancer Res* 40:3046–3049, 1980
34. Goodwin D, Meares C, Diamanti C, et al: Use of specific antibody for rapid clearance of circulating blood background from radiolabeled tumor imaging proteins. *Eur J Nucl Med* 9:209–215, 1984