Radioimmunodetection of Medullary Thyroid Carcinoma Using Indium-111 Bivalent Hapten and Anti-CEA × Anti-DTPA-Indium Bispecific Antibody

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Pretargeting labeled bivalent hapten with bispecific antibodies has proven feasible in the clinic, and our earlier results have suggested the technique may be very sensitive for detecting small recurrences and metastases. Medullary thyroid carcinoma (MTC) is an example where this technique may be the most useful since local recurrences and isolated metastases are removed surgically when detected, and thyrocalcitonin provides a specific and sensitive tumor marker. In our current study, we evaluated pretargeted immunoscintigraphy in a larger number of MTC patients. **Methods:** Anti-carcinoembryonic antigen (CEA) × anti-diethylenetriaminepentaacetic acid (DTPA) indium bispecific antibody and ^{111}In-labeled bivalent DTPA hapten were administered sequentially (4–5 days apart) to 44 patients with elevated circulating calcitonin after resection of primary MTC. Immunoscintigraphy was performed 2, 5 and 24 hr after hapten injection and, when necessary, at longer time intervals. When available, a handheld gamma probe was used during surgery. **Results:** Fifteen patients had known tumor sites before immunoscintigraphy. Tumors were imaged in 12 (80%) of these patients, including 3 with liver metastases. Five unknown tumor sites were detected. For the 29 patients with occult disease, immunoscintigraphy detected high-activity uptake sites in 21 patients (72%), including 5 in the liver. Twelve were confirmed by surgery, 1 by guided morphologic imaging and 1 by venous catheterization. There were 2 false-positive patients. The other 5 patients have not yet been confirmed. All detected liver metastases were high-activity uptake areas. Radioimmunoguided surgery was used in 14 patients. It was considered helpful by the surgeon in 12 patients, including 4 patients where it determined the resection of small, not palpable nor visible, tumor-involved lymph nodes. Surgical resection resulted in a significant decrease (8 patients) or normalization (1 patient) of circulating calcitonin and CEA. **Conclusion:** This technique affords high sensitivity and specificity for detecting small tumor lesions including liver metastases. Its use for immunoscintigraphy and guided surgery should improve the therapeutic management of recurrent MTC.

Key Words: medullary thyroid carcinoma; anti-carcinoembryonic antigen antibody; calcitonin; pretargeting

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Surgery is the preferred first-line treatment option for medullary thyroid carcinoma (MTC). The extent of the surgical procedure has changed from partial thyroidectomy to total thyroidectomy and bilateral lymph node ablation (*1*). Nevertheless, persisting malignant tissue and recurrences remain frequent with local spreading in the cervical and mediastinal lymph nodes and distant metastases to liver, lungs or bones (*2*). Calcitonin provides a very sensitive tumor marker that becomes elevated well before any tumor lesion may be detected by palpation or morphological imaging (ultrasonography, CT or MRI) (*3–5*).

Tumor resection is indicated in the case of local recurrences or when only one isolated distant metastasis has been detected. It is important to localize any recurrent or metastatic disease as soon as possible after serum calcitonin concentration has increased. Selective venous catheterization for quantification of local calcitonin levels is a reliable procedure for localizing tumor sites, but the technique is invasive (*6*). Antibodies to carcinoembryonic antigen (CEA) (*7–11*) and radiopharmaceuticals such as metaiodobenzylguanidine (MIBG) (*12,13*), pentavalent ^{99m}Tc-dimercaptosuccinic acid (DMSA) (*14*) or somatostatin derivatives (*15,16*) have been proposed for localizing MTC recurrences by scintigraphy. These reagents have proven capable of detecting small recurrences of MTC, however, moderate tumor-to-background ratios still limit detection sensitivity. When these reagents are labeled with ^{111}In, the sensitivity of these methods for liver metastases, which are frequent in MTC, has been quite limited (*7,9,15,16*).

Using a pretargeting method, based on bispecific antibodies and low molecular weight radiolabeled bivalent haptens, which we refer to as the affinity enhancement system (AES) (*17,18*), we have been able to detect a large proportion of known tumor lesions expressing CEA in patients with colorectal cancer (*19,20*), non-small cell lung cancer (*21*) and MTC (*22,23*). Small unknown lesions have often been detected in these preliminary clinical studies. Here, we present the results of a clinical trial performed in five clinical centers in France. The aim of our study was to evaluate, on a larger number of patients, the sensitivity and the specificity of the AES method in recurrent and metastatic MTC. Additional objectives were to make a preliminary evaluation of the efficiency of AES immunoscintigraphy (IS) in patient management and, when available, to use a handheld gamma probe for surgical detection and oriented resection of tumor-involved lymph nodes.

PATIENTS

Forty-four patients (22 men, 22 women; age range 20–68 yr; mean 46 yr) with elevated calcitonin after previous surgery (total thyroidectomy and partial or complete resection of local lymph nodes) have been included. All these patients were considered in relapse and, as a consequence, all negative scintigraphies have been counted as false-negative. Informed consent to participate in the clinical study was obtained from all patients who met the following inclusion criteria: suspicion of recurrence (i.e., elevated circulating calcitonin after previous surgery), no radiotherapy, chemotherapy or immunotherapy

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within 3 mo before scintigraphy, negative pregnancy testing, no known allergy to murine proteins and more than 18 yr. All patients were in good, ambulatory clinical condition. The patients' body weights ranged from 40 to 105 kg (mean 64 kg).

Basal calcitonin varied between 34 and 35,100 pg/ml (median 796 pg/ml). CEA was often normal (below 5 ng/ml 13 cases) or moderately elevated (from 5 to 20 ng/ml 12 cases). Overall, CEA levels ranged from 1.1 to 819 ng/ml (median 13.5 ng/ml).

Sixteen of these patients had known tumor sites detected by palpation, ultrasonography, CT, MRI or bone scintigraphy. Surgery showed that the neck lesion of Patient 10, detected by ultrasonography, was a false-positive. The other 28 patients were included only on the indication of elevated circulating calcitonin. For IS performance evaluation, Patient 10 was counted as a patient with occult disease.

MATERIALS AND METHODS

CEA was monitored using commercial immunoassay kits from CIS Bio International (ELSA CEA or ELSA2 CEA, Gif-sur-Yvette, France) (normal range below 9 ng/ml) or Behringwerke AG (RIA-gnost CEA, La Defense, France) (normal range below 5 ng/ml). Calcitonin was monitored using immunoassay kits purchased from CIS Bio International (ELSA HCT, Gif-sur-Yvette, France). They were used according to the manufacturers' operating instructions. Human antimouse antibodies (HAMA) were measured by sandwich radioimmunoassay using bispecific antibody-coated tubes and ^{125}I-labeled bispecific antibody as a tracer. A single batch of goat antimouse immunoglobulin antiserum (Jackson Laboratories, West Grove, PA) was used as a standard. The sensitivity of this assay was 0.1 μg/ml.

The anti-CEA × anti-diethylenetriaminepentaacetic acid (DTPA) indium bispecific antibody was prepared according to the Good Manufacturing Practice (GMP) guidelines. The technique has been described previously (*17,18*). Briefly, the reduced Fab' fragment of the anti-CEA antibody (clone F6, CIS Bio International) was coupled to the Fab fragment of the anti-DTPA-indium antibody (clone 734, Immunotech S.A., Marseille, France) derivatized with the N-hydroxysuccinimide ester of ϵ-maleimidocaproic acid (EMCS, Sigma, Saint Quentin-Fallavier, France). The purified 100 kDa bispecific antibody was made 1 mg/ml (10 μM) in phosphate (10 mM) buffered saline (150 mM) and vialed in 5-ml glass vials. The preparation was checked for the absence of adventitious agents (bacteria, mycoplasma and viruses) and of pyrogens (limulus amaebocyte assay). The biochemical purity was checked by analytical size-exclusion chromatography and found to be better than 90%.

The bivalent hapten (Nα-(N,N,N′,N″-diethylenetriamine-tetraacetic acid-N″-acetyl)-L-tyrosyl-Nϵ-(N,N,N′,N″-diethylenetriamine-tetraacetic acid-N″-acetyl)-L-lysine: di-DTPA-tyrosyl-lysine) was prepared by reacting DTPA di-anhydride (Sigma, Saint Quentin-Fallavier, France) with L-tyrosyl-L-lysine (Bachem, Bubendorf, Switzerland). It was dissolved in acetate (100 mM) and citrate (10 mM) pH 5.0 (final concentration 10 μM) and distributed aseptically in 5-ml vials. A third series of 5-ml vials was prepared containing a sterile solution of nonradioactive indium trichloride (100 μM).

The radiolabeling of di-DTPA-tyrosyl-lysine was performed in the nuclear medicine departments of each clinical center as follows: 0.1 nmol of di-DTPA-tyrosyl-lysine per kg of body weight was transferred into a vial containing 100–200 MBq $^{111}InCl_3$ (CIS Bio International, Gif-sur-Yvette, France or Mallinckrodt, Bondoufle, France). The mixture was incubated for 20 hr at room temperature, then 300 μl nonradioactive indium-chloride were added to saturate all chelating groups of the bivalent hapten. The labeling efficiency was controlled by measuring the binding of a small aliquot of the labeled hapten on anti-DTPA-indium antibody-coated tubes. Usually, the immunoreactivity was better than 90%. Occasionally it was found in the 80%–90% range without apparent effect on tumor targeting.

The patients were injected intravenously with 0.1 mg of bispecific antibody per kg of body weight diluted in 100 ml normal saline by infusion over 0.5 hr. Four to 5 days later, the patients were given 100–200 MBq ^{111}In-labeled bivalent hapten (^{111}In-di-DTPA-tyrosyl-lysine).

An IS whole-body scan was performed 2 hr after hapten injection. Then, 5 hr after hapten injection, whole-body scan, SPECT and planar scintigraphy on the regions of interest were done. The same imaging protocol was repeated 24 hr and, when judged necessary by the investigator, 48 or 72 hr after hapten injection. Technetium-99m-labeled albumin, phytate or hexa-methylene-diphosphonate (HMDP) were injected for landmark (blood-pool, liver and bones) imaging. The patients were asked to urinate before each scintigraphy sequence to reduce bladder artifacts, but otherwise the patients received no treatment to increase the clearance of circulating radioactivity. Blood samples for HAMA detection were collected before injection and at 15 days, 1 mo and 3 mo after IS.

Twenty-four patients underwent surgery after IS. Radioimmunoguided surgery (RIGS) was performed in 14 patients, 3–7 days after injection of the labeled hapten. Four different handheld gamma probes were used (bismuth germanate, cadmium telluride, sodium iodide and thallium-doped cesium iodide). No difference in detection capability was observed. Results were considered positive when tumor to adjacent normal tissue ratios were more than 2.

The respective sensitivities of conventional diagnostic modalities, which included clinical examinations and morphologic imaging, were compared to IS to detect persistent or recurrent disease or metastases using a standard chi-square test. The distributions of tumor markers (CEA and calcitonin) in the general population and in the subpopulations of patients with and without known tumor sites were satisfactorily fitted to log-normal distributions with no significant skewness or kurtosis. Student's t-tests were then used to compare the geometric means.

RESULTS

The injection of the imaging agents was well tolerated in all cases with no side effects reported. However, HAMA was detected (>0.1 μg/ml in a specific sandwich assay) in a large fraction of the patients (61%) and was found higher than 1 μg/ml in 33% of the patients. HAMA was detected in the 15-day blood samples in 28% of the patients, while a significant number (17%) had detectable HAMA only at 3 mo. HAMA decreased slowly, and some patients were still HAMA-positive more than 1 yr after IS (data not shown).

After administration of the labeled hapten, serial images were recorded at 2, 5 and 24 hr. Images recorded 2 hr after hapten injection showed blood-pool distribution, although high-activity areas were detected in a few patients. In 24 of the 31 positive IS, tumor uptake showed up in the 5-hr images and was confirmed at 24 hr. SPECT was used to confirm whole-body or planar scintigraphy and obtain a more precise localization of the tumor. Late images (48 or 72 hr) were recorded when the 24-hr scans were negative or to improve the precision of tumor localization. These late images confirmed the 24-hr diagnostic except in one patient where a high-activity uptake area was detected only in the 72-hr images.

Patients' characteristics and IS findings are reported in Table 1. Patients with no known tumor sites are listed first followed by patients with known tumor localization in the order of increasing serum calcitonin levels. For the 29 patients with

TABLE 1
Immunoscintigraphy (IS) Results

Patient no.	Sex	Age (yr)	Tumor sites known before IS	IS findings	Confirmation	IS score
1	M	56	None (US, CT)	Abdomen	nc	FP
2	F	46	None (US, CT)	–	nc	FN
3	M	59	None (MRI)	Mediastinum	nc	nc
4	F	40	None (US, CT)	–	nc	FN
5	F	50	None (US, CT)	–	nc	FN
6	F	65	None (CT)	Mediastinum (upper left)	Surgery	TP
7	M	65	None (US, CT)	–	nc	FN
8	F	20	None (MRI)	–	nc	FN
9	F	67	None (US, CT)	Neck (retropharynx)	nc	nc
10	F	42	Left neck (US: false-positive)	–	Negative RIGS	FN
11	M	53	None (US, CT)	Mediastinum (2 sites)	RIGS, histology	TP
12	F	55	None (US, CT)	–	Negative liver MRI	FN
13	M	50	None (US, CT)	Hepatic metastasis	RIGS, histology	TP
14	F	41	None (US, CT)	Neck	RIGS, (determining)	TP
15	M	46	None (US, CT)	Neck	nc	nc
16	M	39	None (US, CT)	Left and right neck	RIGS, histology	TP
17	F	44	None (US, CT)	Right liver	nc	nc
18	M	47	None (US, CT)	Neck	Negative surgery, venous catheterization	TP
19	M	67	None (CT)	Left neck, liver	Surgery	TP
20	F	54	None (CT)	Neck	nc	nc
21	F	32	None (US, CT)	Right neck (imaging artifact)	Negative surgery	FP
22	M	47	None	Right and left neck	Surgery	TP
23	M	31	None (benign liver lesions*, MRI)	Left neck (2 sites)	Surgery, histology	TP
24	M	44	None	–	nc	FN
25	F	46	None (US, CT)	Upper mediastinum	Surgery	TP
26	M	33	None	Neck	RIGS, histology	TP
27	F	35	None	Mediastinum	Surgery, MRI	TP
28	F	59	None (US, CT)	Right neck, right liver	RIGS (determining), MRI, histology	TP
29	M	48	None (benign liver lesion*, MRI)	Right liver	MRI	TP
30	M	40	Neck (US, CT, MRI)	–	Negative RIGS, histology	FN
31	M	44	Liver (US, CT)	Liver	RIGS	TP
32	F	52	Neck (palpation), mediastinum (CT), liver (US, doubtful)	Neck, mediastinum, liver	CT	TP
33	M	60	Left neck (US), right neck (CT), liver (CT)	Left neck	RIGS (determining), histology	TP
34	M	32	Mediastinum (CT), lung (CT)	–	nc	FN
35	F	29	Mediastinum (left laterotracheal)	Neck (3 sites), mediastinum	Surgery, histology	TP
36	F	36	Thymus area (CT)	–	nc	FN
37	F	23	Neck (palpation, US), liver (US positive, CT negative)	Neck (multiple lesions), mediastinum (doubtful)	RIGS, histology	TP
38	F	43	Mediastinum (MRI, CT)	Mediastinum	RIGS (determining), histology	TP
39	M	54	Heterogeneous liver (CT), left neck and right mediastinum (CT)	Neck (2 sites), mediastinum, liver (3 sites)	Surgery, CT	TP
40	F	28	Neck (MRI), liver (doubtful)	Neck, liver (doubtful)	RIGS	TP
41	M	55	Supra-clavicular (US)	Supra-clavicular, bone metastases	Bone scintigraphy	TP
42	F	58	Vertebra (bone scintigraphy)	Vertebrae (2 sites)	Bone scintigraphy	TP
43	M	68	Neck (MRI, US), parietal bone (DMSA), liver (CT)	Neck, mediastinum	RIGS	TP
44	M	38	Mediastinum (CT)	Neck, mediastinum, liver (doubtful)	CT	TP

*MRI showed abnormal sites in the liver, which were interpreted as benign.

– = negative IS; RIGS = radioimmunoguided surgery; US = ultrasound; TP = true-positive; FP = false-positive; FN = false-negative; nc = not confirmed; DMSA = dimercaptosuccinic acid. The surgeon's judgment on the impact of the use of a gamma probe on surgical resection has been indicated (useful or determining).

occult disease, IS detected high-activity uptake sites interpreted as tumor localizations in 19 of them (65%) (Fig. 1), including 5 in the liver (Fig. 2). Of the 19 images interpreted as showing tumor sites, 14 were confirmed either by surgery (12 patients), guided morphologic imaging (1 patient) or venous catheterization (1 patient). The other 5 positive IS have not yet been confirmed because surgery was not performed. For 2 other patients (Patients 1 and 21), IS was first considered positive but,

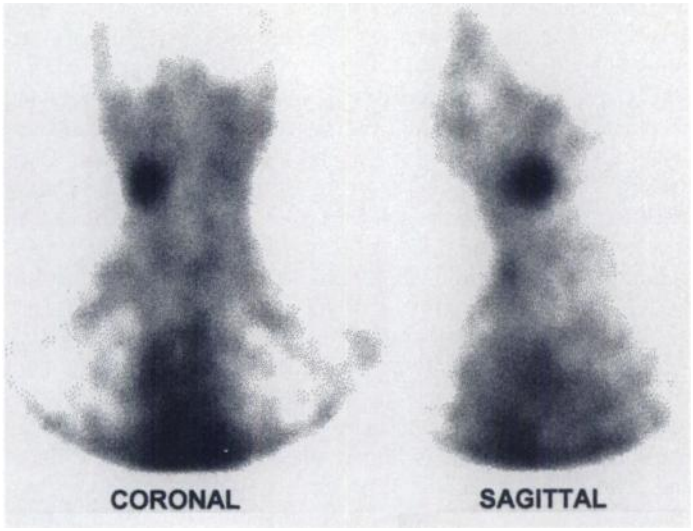

FIGURE 1. SPECT of neck and thorax of Patient 14 who had no tumor site known before IS. The coronal and sagittal views show highly contrasted spot in right upper neck.

on subsequent data examination, it was attributed in one case to a technical problem with the camera and in the other to slow intestinal transit of excreted activity. They are reported here as false-positives. Tumors were clearly imaged in 12 (80%) of the 15 patients with known tumor, including 3 patients with liver metastases that showed high tumor-to-normal-tissue contrast (Fig. 3). Five additional unknown tumor sites were detected and confirmed by surgery (3 patients) or bone scintigraphy (2 patients).

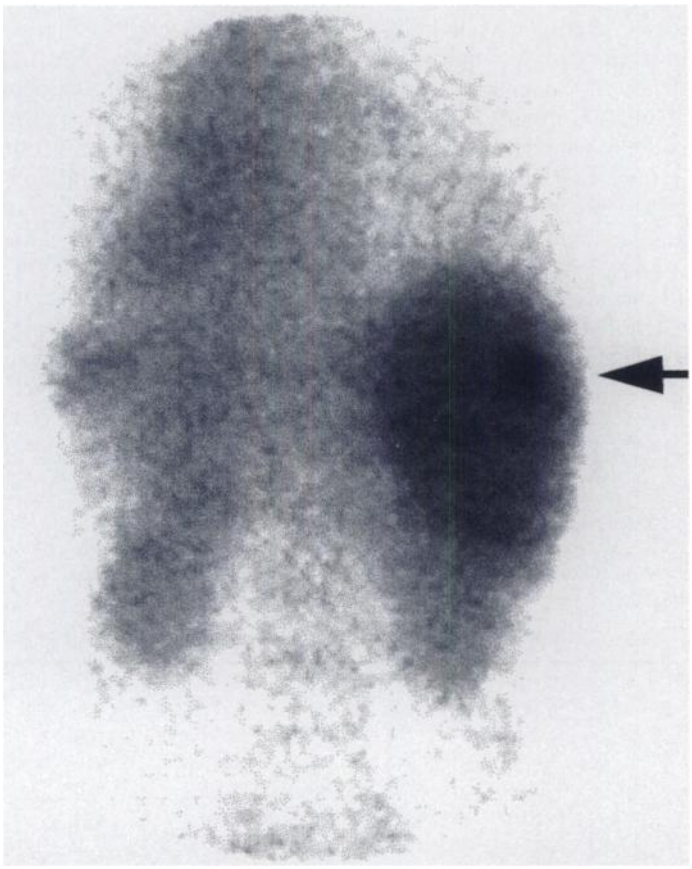

FIGURE 2. Planar scintigraphy (posterior view) of abdomen of Patient 28 who had no tumor site known before IS. Liver hot spot (arrow) was visualized and later confirmed by IS-directed MRI. IS also detected cervical recurrences that were removed surgically (not shown).

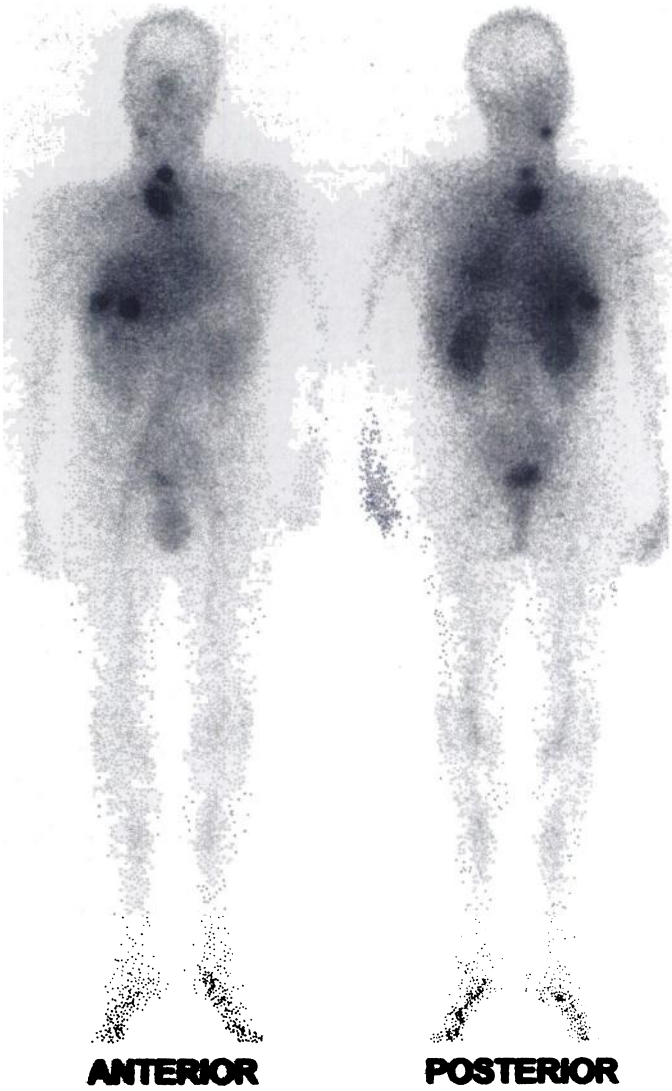

FIGURE 3. Whole-body scan of Patient 39 who had known recurrences of MTC in neck and upper mediastinum. Liver metastases were suspected. Whole-body image was recorded 24 hr postinjection labeled hapten. Multiple recurrences and liver metastases are clearly visualized in anterior and posterior views.

Table 2 summarizes the results of our imaging study. The negative IS studies (11 patients), including Patient 8 who had shown no change in CEA or calcitonin and no clinical evidence of tumor over 2 yr, have been counted as false-negative because of the elevated serum calcitonin levels. Overall, IS, with 11 false-negatives and 2 false-positives, appears more sensitive than conventional morphological imaging. The comparison of the proportions of positive results (including the not yet confirmed positive images) obtained before and after IS shows that IS is significantly more sensitive ($p = 0.06\%$, chi-square test). Specificity was high in both instances with only 1 and 2 false-positives, respectively, for conventional diagnostic and IS.

The tumor marker distributions (calcitonin and CEA) were well represented by log-normal distributions. The marker concentration difference between patients who scored negative or positive by conventional imaging or IS was highly significant for calcitonin ($p = 0.08\%$ and $p = 0.07\%$, respectively) but only marginally significant for CEA ($p = 8.5\%$ and $p = 6.8\%$, respectively) using Student's t-tests (Table 3). Patients found negative by conventional imaging had higher circulating calcitonin than those found negative by IS, although the difference was only marginally significant ($p = 8.6\%$). CEA and calcito-

TABLE 2
Summary of Immunoscintigraphy Results on a Per-Patient Basis

	Patients with known tumors (total = 15)	Patients with occult disease (total = 29)	Overall (total = 44)
True-positive	12 (80%)	14 (48%)	26 (59%)
Not confirmed	0	5 (17%)	5 (11%)
False-positive	0	2 (7%)	2 (5%)
False-negative	3 (20%)	8 (28%)	11 (25%)

nin showed no significant correlation, but patients with elevated CEA always had high calcitonin.

Overall, 24 patients underwent surgery after IS, either in the week after IS or later. One with occult disease (Patient 11) benefited from a dramatic decrease in calcitonin (from 410 to 9 pg/ml 6 mo after surgery) and CEA (from 79 to 5 ng/ml) serum concentrations, but a response to pentagastrin stimulation remained suggesting microscopic residual disease. At 12 mo after surgery, there was a discrete increase in calcitonin indicating disease progression (Table 4). Eight other patients (Patients 16, 19, 23, 25, 27, 28, 31 and 33) had calcitonin serum levels reduced by at least 50% within 6 mo after surgery. CEA decreased also in three of the patients (Patients 23, 27 and 31) who had high initial circulating CEA (Table 4). The other patients who underwent surgery showed stable calcitonin. The patients who did not undergo surgery showed either stable calcitonin levels or progression (Table 4).

The handheld gamma probe was used in 14 of these surgical operations. RIGS was negative in 2 patients. It was considered helpful by the surgeon, and in 4 patients it determined the resection of occult tumor sites (Table 1). The handheld gamma probe was used to decide, during surgery, if a given lymph node chain was involved by the tumor. In all patients, when the gamma probe detected a positive lymph node chain, the tumor involvement was confirmed by pathology. However, on a single node basis, there was some discrepancies between the handheld gamma probe result and pathology findings. In 8 patients, tumor samples and blood were counted at the time of surgery (3–5 days after administration of the activity). Tumor uptake was high: 5%–68% (median 21) of injected dose per kg of tumor. Blood activity was low: 0.2%–2.5% (median 0.9) of injected dose per liter. Tumor-to-blood ratios varied from 7 to 125 (median 27).

DISCUSSION

A relatively large proportion (66%) of patients with no known tumor sites have been included in our study. Positive imaging was obtained in 65% of these patients and 74% of these positive images have been confirmed by surgery, guided morphological imaging, venous catheterization or bone scintigraphy. Given the low number of false-positive IS in this study and in our experience (*19–23*), the nonconfirmed positive images were counted as true-positive in statistical analyses. Conversely, all negative cases were counted as false-negative because calcitonin was considered as a specific tumor marker in this patient population. Then, IS was found significantly more accurate than conventional imaging for detecting recurrences of MTC. It was remarkable that all clinical centers had very similar results in terms of sensitivity and specificity (not shown). The high sensitivity of the two-step IS is in line with previously published preliminary results in MTC (*22,23*). In several patients, liver metastases were detected as high-uptake areas in the liver. This is remarkable given the use of ^{111}In as the imaging radionuclide. With directly labeled antibody fragments and with ^{111}In-labeled somatostatin analogs, a normal liver usually shows high uptake, which considerably impairs tumor detection. In our study, 3 unknown liver metastases were detected by IS. The use of ^{111}In for IS allowed simultaneous imaging with ^{99m}Tc-labeled landmark radiopharmaceuticals. Technetium-99m-labeled albumin was found useful in most cases to distinguish vasculature from tumor-involved lymph nodes in the neck and in the mediastinum. Technetium-99m-labeled phytate and HMDP confirmed IS finding and in one patient (Patient 41) was indispensable for diagnosing a bone metastasis. Further studies would be required to definitely assess the influence of landmark imaging on the sensitivity and specificity of IS.

Patients were included on the basis of elevated calcitonin after thyroid ablation. A threshold for calcitonin level remains to be determined but 1 positive scan versus 3 negative were obtained with less than 100 pg/ml of circulating calcitonin and 28 of 33 patients (85%) had positive scans with calcitonin above 300 pg/ml. If high calcitonin levels correspond well to patients with detectable tumors, CEA was often found in the normal range in patients with positive scans (32%). Additionally, Patient 34 had high circulating CEA (819 ng/ml), but known tumor sites were missed by IS, presumably because soluble CEA interfered with tumor targeting. Conversely, of the 13 negative IS, eight patients had both normal CEA and calcitonin below 300 pg/ml. Only one patient (Patient 36) was IS negative with calcitonin above 300 pg/ml and normal CEA. Interference of circulating CEA may happen, but only for fairly high levels (*20*), and some tumors may express little CEA. The last situation seems rather rare (possibly Patient 36), and in this

TABLE 3
Distribution of Tumor Markers

		Conventional diagnostic evaluation		Immunoscintigraphy	
Marker		Negative	Positive	Negative	Positive
Calcitonin (pg/ml)	Minimum	34	135	34	67
	Maximum	8114	35,100	1457	35,100
	Median	503	1483	251	1100
	Mean*	481	1922	260	1219
CEA (ng/ml)	Minimum	1.1	1.1	1.1	1.3
	Maximum	240	819	819	479
	Median	12.9	41.0	2.7	18.9
	Mean*	12.0	29.2	8.1	21.8

*Geometric mean.
CEA = carcinoembryonic antigen.

TABLE 4
Tumor Marker Follow-Up

Patient no.	Tumor resection*	Calcitonin (pg/ml)			Carcinoembryonic antigen (ng/ml)		
		Before IS	Postsurgery (1–6 mo)	Follow-up (6–12 mo)	Before IS	Postsurgery (1–6 mo)	Follow-up (6–12 mo)
1	No surgery	34	40	47	1.1	2.0	1.5
2	No surgery	35	30	nd	2.3	3.0	nd
3	No surgery	67	133	60	5.9	6.0	5.9
4	No surgery	94	115	126	2.1	3.0	4.2
5	No surgery	111	130	125	3.1	3.4	nd
6	Satisfactory	176	nd	nd	18.9	nd	nd
7	No surgery	251	nd	320	2.7	nd	3.9
8	No surgery	253	752	nd	14.0	nd	nd
9	No surgery	257	nd	298	1.3	nd	nd
10	Negative	258	nd	nd	3.2	nd	nd
11	Satisfactory	410	9.0	25	79	5.0	5.5
12	No surgery	427	521	nd	27	26	22
13	Satisfactory	482	403	576	2.5	nd	nd
14	Satisfactory	491	450	nd	21	nd	nd
15	No surgery	503	508	672	11.8	11.6	8.5
16	Satisfactory	507	151	382	12.9	nd	nd
17	No surgery	564	1473	1300	40	49	57
18	Negative	566	440	695	4.4	7.0	8.1
19	Satisfactory	625	265	nd	6.8	7.9	nd
20	No surgery	688	656	628	21	22	24
21	Negative	905	639	670	37	17	20
22	Satisfactory	1023	nd	800	24	nd	nd
23	Satisfactory	1100	nd	191	14.4	nd	6.0
24	No surgery	1180	nd	921	165	nd	139
25	Satisfactory	1382	395	375	8.8	4.3	4.7
26	Satisfactory	1458	1393	nd	41	48	nd
27	Satisfactory	5300	1450	1075	240	29	20
28	Incomplete	6880	3019	4607	7.4	4.8	5.9
29	No surgery	8114	7660	nd	125	85	nd
30	Satisfactory	135	134	100	1.1	1.2	0.7
31	Satisfactory	610	300	320	41	14.6	17.9
32	No surgery	975	nd	nd	76	nd	nd
33	Satisfactory	1283	431	1072	1.4	nd	2.7
34	No surgery	1356	1886	1466	819	690	731
35	Satisfactory	1438	nd	nd	11.8	nd	nd
36	No surgery	1457	1445	nd	2.2	2.5	nd
37	Satisfactory	1483	1129	2197	13.1	8.9	13.8
38	Incomplete	>2000	nd	10160	170	nd	137
39	Satisfactory	2400	nd	nd	49.3	nd	nd
40	Satisfactory	2510	nd	nd	245	nd	nd
41	No surgery	3370	nd	nd	479	nd	nd
42	No surgery	3740	4288	3410	270	269	168
43	Incomplete	7790	7000	8600	3.5	nd	nd
44	No surgery	35100	nd	nd	7.9	nd	nd

*Surgery was scored satisfactory if tumor resection was macroscopically complete and confirmed by histology; incomplete when the tumor was not fully resected; or negative when no tumor site was found.
IS = immunoscintigraphy.

and other studies immunohistology in resected tumors and biopsies have found very high percentages of CEA expression (*10,11,22*). Overall, the indication of IS on the sole basis of elevated calcitonin serum levels seems warranted and the most frequent reason for negative IS studies is tumor masses below the detection capability of the gamma camera.

Fourteen patients were operated on with the help of a handheld gamma probe within 1 wk of the injection of the ^{111}In-labeled hapten. RIGS was negative in only 2 patients and it was determining for the resection of small tumor sites in 4 patients. This is also in agreement with our earlier results (*22*). A parallel study (*23*) on a small number of patients concentrated on using RIGS in a similar clinical setting. It concluded that the low non-tumor background provided by the two-step pretargeting technique conferred a high sensitivity and specificity to the use of the handheld gamma probe. Ten patients were operated on without the help of a gamma probe. Only 3 patients (Patients 10, 21 and 30) underwent surgery after negative IS and surgery was negative twice. Otherwise, surgery confirmed the presence of tumor at the sites detected by IS in all but one case. However, the number of patients who benefited from a normalization of calcitonin (1 patient) or at least from a significant reduction (8 patients), is rather low. In the above mentioned study (*23*), RIGS achieved more efficient tumor resection, but the tumor was not completely removed in many patients. In both studies, small tumor lesions close to larger

tumor sites, which were probably not imaged or not visualized separately from the larger tumor sites were found (especially when RIGS was used). It is quite possible that undetected small lesions were also present at distant sites not explored during surgery. In addition, diffuse bone marrow involvement, not likely to be detected with a gamma camera, has been reported in MTC (*24,25*).

These observations call for adjuvant therapy after surgical debulking. Radioimmunotherapy is one possible approach (*24–26*). With this perspective, HAMA induction is an important problem since, if their presence at the time of antibody administration is not associated with adverse side-effects, the targeting efficiency is usually dramatically reduced (*20,23*). We solved the problem of HAMA induction in part by improving the production and purification process to remove a small, but detectable, fraction of high molecular weight aggregates in the bispecific antibody preparations. Further reduction of immunogenicity should be obtained by humanization.

CONCLUSION

No attempt has yet been made to compare this IS approach with selective venous catheterization or scintigraphy with directly labeled antibodies, somatostatin analogs or pentavalent ^{99m}Tc-DMSA to assess more completely the potential of the two-step imaging approach. Nevertheless, this study fully confirmed our earlier results and shows that small tumor sites can be detected with a high level of specificity. Early detection of small recurrences and metastases of MTC using the AES reagents is possible and may be helpful for indicating the need for surgery. In addition, RIGS was useful in resecting small tumors. The AES reagents combine the advantages of using ^{111}In as an imaging radioisotope with high tumor uptake and low normal tissue background, including in the liver. Thus imaging and RIGS are possible within a time-frame of a few days as well as simultaneous use of ^{99m}Tc-labeled radiopharmaceuticals for landmark imaging. The demonstration of the clinical usefulness of this approach is likely to be validated further by optimal use of the handheld gamma probe during surgery performed within a few days of injecting of labeled hapten. This treatment should leave patients with a minimal tumor burden that may be amenable to radioimmunotherapy. Application of radioimmunotherapy to MTC using labeled anti-CEA antibodies has afforded clinical responses (*24,25*). The AES reagents, administered in higher amounts labeled with a beta-emitting radionuclide such as ^{131}I, are good candidates for this adjuvant therapy. After an initial dosimetry study (*26*), which demonstrated that high tumor exposure may be achieved for a reasonable injected dose (10–40 Gy/GBq), together with acceptable exposure of normal tissues, a Phase I dose escalation radioimmunotherapy trial is now in progress for evaluating toxicity and potential therapeutic efficacy.

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REFERENCES

1. Rossi RL, Cady B, Meissner WA, Wool MS, Sedgwick CE, Werver J. Non familial medullary thyroid carcinoma. *Am J Surg* 1980;139:554–560.
2. Jackson CE, Talpos GB, Kambouris A, Yott JB, Tashjian AH, Block MA. The clinical course after definitive operation for medullary thyroid carcinoma. *Surgery* 1983;94: 995–1001.
3. Tubiana M, Milhaud G, Coutris G, Lacour J, Parmentier C, Bok B. Medullary carcinoma and thyrocalcitonin. *Br Med J* 1968;4:87–89.
4. Goltzman D, Potts JT, Ridgway EC, Maloof F. Calcitonin as a tumor marker. Use of the radioimmunoassay for calcitonin in the postoperative evaluation of patients with medullary thyroid carcinoma. *N Engl J Med* 1974;290:1035–1039.
5. Block MA, Lackson CE, Tashjian AH. Management of occult medullary thyroid carcinoma evidenced only by serum calcitonin level elevations after apparently adequate neck operations. *Arch Surg* 1978;113:368–372.
6. Ben Mrad MD, Gardet P, Roche A, et al. Value of venous catheterization and calcitonin studies in the treatment and management of clinically inapparent medullary thyroid carcinoma. *Cancer* 1989;63:133–138.
7. Lumbroso J, Berche C, Mach JP, et al. Use of tomographic scintigraphy with radio-labelled monoclonal antibodies for detecting human digestive cancers and medullary cancers of the thyroid. *Bull Cancer (Paris)* 1983;70:96–102.
8. Reiners C, Eilles C, Spiegel W, Becker W, Borner W. Immunoscintigraphy in medullary thyroid cancer using a ^{123}I- or ^{111}In-labelled monoclonal antibody fragment. *Nuklearmedizin* 1986;25:227–231.
9. Baum RP, Hertel A, Lorenz M, Schwarz A, Encke A, Hor G. Technetium-99m-labelled anti-CEA monoclonal antibody for tumour immunoscintigraphy: first clinical results. *Nucl Med Commun* 1989;10:345–352.
10. Vuillez JP, Peltier P, Caravel JP, Chetanneau A, Saccavini JC, Chatal JF. Immunoscintigraphy using ^{111}In-labeled F(ab')$_2$ fragments of anticarcinoembryonic antigen monoclonal antibody for detecting recurrences of medullary thyroid carcinoma. *J Clin Endocrinol Metab* 1992;74:157–163.
11. Juweid M, Sharkey RM, Behr T, et al. Improved detection of medullary thyroid cancer with radiolabeled monoclonal antibodies to carcinoembryonic antigen. *J Clin Oncol* 1996;14:1209–1217.
12. Poston GJ, Thomas AM, MacDonald DW, et al. Imaging of metastatic medullary carcinoma of the thyroid with ^{131}I-meta-iodobenzyl guanidine. *Nucl Med Commun* 1986;7:215–221.
13. Baulieu JL, Guilloteau D, Delisle MJ, et al. Radioiodinated metaiodobenzylguadinine uptake in medullary thyroid cancer. A french cooperative study. *Cancer* 1987;60: 2189–2194.
14. Clarke SEM, Lazarus C, Mistry R, Maisey MN. The role of technetium-99m pentavalent DMSA in the management of patients with medullary carcinoma of the thyroid. *Br J Radiology* 1987;60:1089–1092.
15. Kwekkeboom DJ, Reubi JC, Lamberts SWJ, et al. In vivo somatostatin receptor imaging in medullary thyroid carcinoma. *J Clin Endocrinol Metab* 1993;76:1413–1417.
16. Dorr U, Sautter-Bihl ML, Bihl H. The contribution of somatostatin receptor scintigraphy to the diagnosis of recurrent medullary carcinoma of the thyroid. *Semin Oncol* 1994;21:42–45.
17. Le Doussal JM, Martin M, Gautherot E, Delaage M, Barbet J. In vitro and in vivo targeting of radiolabeled monovalent and divalent haptens with dual specificity monoclonal antibody conjugate: enhanced divalent hapten affinity for cell-bound antibody conjugate. *J Nucl Med* 1989;30:1358–1366.
18. Le Doussal JM, Gruaz-Guyon A, Martin M, Gautherot E, Delaage M, Barbet J. Targeting of indium-111-labeled bivalent hapten to human melanoma mediated by bispecific monoclonal antibody conjugates:imaging of tumors hosted in nude mice. *Cancer Res* 1990;50:3445–3452.
19. Le Doussal JM, Chetanneau A, Gruaz-Guyon A, et al. Bispecific monoclonal antibody-mediated targeting of an Indium-111-labeled DTPA dimer to primary colorectal tumors: pharmacokinetics, biodistribution, scintigraphy and immune response. *J Nucl Med* 1993;34:1662–1671.
20. Chetanneau A, Barbet J, Peltier P, et al. Pretargetted imaging of colorectal cancer recurrences using an ^{111}In-labelled bivalent hapten and a bispecific antibody conjugate. *Nucl Med Commun* 1994;15:972–980.
21. Vuillez JP, Moro D, Brichon PY, et al. Two-step immunoscintigraphy for non-small cell lung cancer staging using a bispecific anti-CEA/anti-indium-DTPA antibody and an indium-111-labeled DTPA dimer. *J Nucl Med* 1997;38:507–211.
22. Peltier P, Curtet C, Chatal JF, et al. Radioimmunodetection of medullary thyroid cancer using a bispecific anti-CEA/anti-Indium-DTPA antibody and an Indium-111-labeled DTPA dimer. *J Nucl Med* 1993;34:1267–1273.
23. De Labriolle-Vaylet C, Gruaz-Guyon A, Wioland M, et al. Radioimmunodétection de métastases de cancers médullaires de la thyroïde à l'aide d'une nouvelle méthode en deux temps [Abstract]. *J Méd Nucl* 1993;7/8:318.
24. Juweid M, Sharkey RM, Behr T, et al. Targeting and initial radioimmunotherapy of medullary thyroid carcinoma with ^{131}I-labeled monoclonal antibodies to carcinoembryonic antigen. *Cancer Res* 1995;55:5946s–5951s.
25. Juweid M, Sharkey RM, Behr T, et al. Radioimmunotherapy of medullary thyroid cancer with iodine-131-labeled anti-CEA antibodies. *J Nucl Med* 1996;37:905–911.
26. Bardiès M, Bardet S, Faivre-Chauvet A, et al. Bispecific antibody and iodine-131-labeled bivalent hapten dosimetry in patients with medullary thyroid carcinoma or small-cell lung cancer. *J Nucl Med* 1996;37:1853–1859.